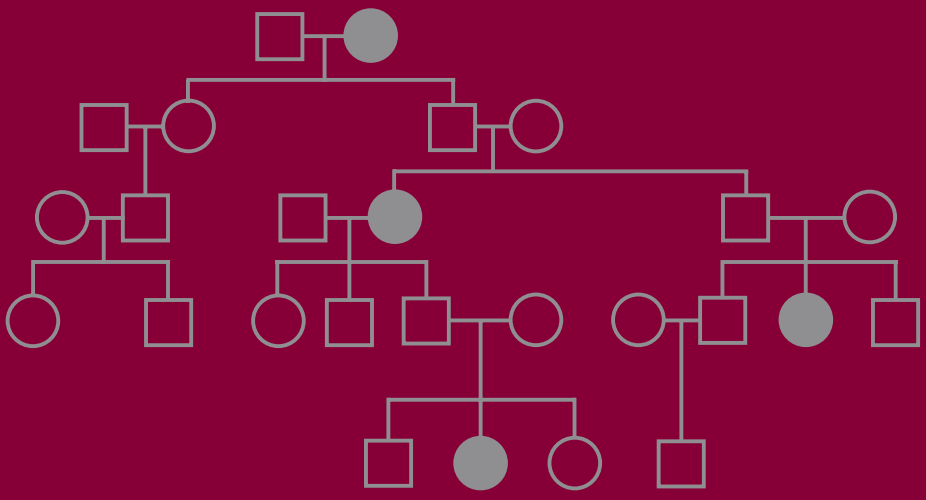
Hereditary Gynecologic Cancer

Risk, Prevention and Management



Edited by Karen H. Lu

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Edited by
Karen H. Lu
MD Anderson Cancer Center
Houston, TX, USA

informa
healthcare

New York London

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To my mom and dad, for showing me the wisdom and beauty of the family tree

Foreword

I am pleased to have been chosen to prepare the foreword for this book dealing with the gynecologic aspects of hereditary cancer-prone disorders, focusing heavily upon Lynch syndrome (LS) and hereditary breast–ovarian cancer (HBOC) syndrome, two syndromes which I described in the mid-1960s and early 1970s, respectively. The clinical and molecular genetic progress made in these disorders over this relatively short span has been truly remarkable.

The gynecologic components of these and several other hereditary cancer syndromes are featured in a series of sections, each of which has been written by world authorities. Their clinical and genetic linkage to gynecologic cancer is long overdue. In each case, an appropriate emphasis has been focused upon diagnosis, molecular genetic risk, prevention, and management. Attention to these areas of concern cannot be emphasized enough due to their general neglect in the overall clinical practice setting. In short, clinicians and genetic counselors must have a firm grasp of the genetics and natural history of hereditary cancer syndromes, given the reality of genetic risk assessment that has changed substantially from mere inference, based upon findings in a pedigree, to a high level of certainty of gynecologic cancer susceptibility in such disorders as HBOC and LS.

The book is arranged in five sections. Section 1 covers an overview of hereditary cancer where Johnathan Lancaster emphasizes the clinical relevance of hereditary ovarian cancer and the vital need to identify women who are at high risk. He addresses the fact that screening for ovarian cancer is wholly inadequate and, therein, the option for surgical prophylaxis should be offered to women who have completed their family, when a *BRCA* mutation is evidenced in HBOC or when a mismatch repair mutation is evidenced in an LS family.

Lancaster's overview is followed by Karen Lu's focus on endometrial cancer, a disease of particular importance when considering the LS. Endometrial

cancer has now been elevated to its rightful place in diagnosis and management of this disorder, thanks to Karen's recent stream of papers showing its role as a sentinel cancer in LS. The need for this emphasis dates to the early description of LS in the mid-1960s, where attention was focused almost exclusively on colorectal cancer, an approach, which followed in the footsteps of Aldred Warthin's 1913 report on "cancer families." The term "hereditary nonpolyposis colorectal cancer" (HNPCC) was subsequently coined; however, this term has been recognized as an inappropriate description of the syndrome. Specifically, although the disorder does not involve an excess of colonic polyps as found in familial adenomatous polyposis, nevertheless, it does involve colonic polyps at the rate expected for the general population. In addition to colorectal cancer, a variety of cancer types may be found, with particular importance given to carcinoma of the endometrium, the second most common lesion in the syndrome. Others are cancer of the ovary, stomach (especially in families indigenous to the Orient), small bowel, pancreas, upper uroepithelial tract, cutaneous sebaceous lesions in the Muir–Torre syndrome variant, and brain tumors (glioblastomas) in the Turcot syndrome variant.

Section 2 contains a series of chapters covering the pathology of *BRCA*-associated ovarian cancer by Chris Crum, the inadequacies of ovarian cancer screening, the hope of a cancer prevention, and the efficacy of risk-reducing surgery by Drs. Cass, Barnes, and Kauff, respectively. Attention is then given to breast cancer, which is the historical clue to the eventual diagnosis of the HBOC syndrome, which I initially described in the early 1970s, when it was clearly linked to a segregating pattern of both breast and ovarian cancer, hence the acronym HBOC.

Section 3 covers LS (HNPCC) with an overview of its molecular genetics and cancer risk by Eamon Sheridan, wherein he identifies a diagnostic and management pattern that is similar to the opening comments in section 1 on endometrial and ovarian cancer. This section concludes with Kathleen Schmeler's state-of-the-art chapter dealing with the option of prophylactic surgery for carcinoma of the endometrium and ovary in those women with the LS who have completed their families and where documentation of the disorder is fully established.

Strong and Walsh provide an overview of the Li–Fraumeni syndrome and Cowden syndrome in section 4.

Section 5 addresses genetic risk assessment, with the chapter by Sheri Babb covering ovarian cancer in *BRCA1*/*BRCA2* settings, and Molly Daniels covering testing and the use of molecular diagnostics in LS. Genetic discrimination, an unfortunate perception of many high-risk patients and which, unfortunately, may be a deterrent to disclosure of their history as well as to their coming forward for DNA testing, is covered by Patrick Lynch. Appropriately, Susan Peterson covers the psychological impact of genetic testing.

This book clearly is a wake-up call to both the clinical and basic science communities regarding the need to carefully assess the family history of cancer

and give appropriate attention to gynecologic cancer, as well as cancer of all anatomic sites, to establish a hereditary cancer syndrome diagnosis. Unfortunately, the mentioned evaluation of the family history remains one of the most neglected areas in the clinical workup of cancer patients. Further confounding this problem is the low rate of referral of high-risk patients for definitive molecular genetic evaluation, when indicated, thereby robbing such patients of highly targeted diagnostic, screening, and management opportunities. Careful attention to the contents of this book should help in the amelioration of these public health concerns.

Henry T. Lynch
Creighton University School of Medicine
Omaha, Nebraska

Preface

I was an obstetrics-gynecology resident when the news broke in 1993 that *BRCA1* had been cloned. While the name itself (BR: breast, CA: cancer gene) implied its association with hereditary breast cancers, those of us who cared for women with ovarian cancer understood that for many of these families, the ovarian cancer diagnosis was equally as devastating. Over the last 15 years, there have been large leaps in defining the specific cancer risks associated with *BRCA1* and *BRCA2*, in outlining specific management options for risk reduction, and in understanding the psychosocial issues surrounding the process of genetic testing. In addition, there have been major discoveries related to other hereditary cancer syndromes, including Lynch syndrome (germline mutation in DNA mismatch repair genes), Li–Fraumeni syndrome (germline mutation in p53), and Cowden syndrome (germline mutation in PTEN), all of which have gynecologic cancers as part of the spectrum of disease.

Where are we now with this young field of clinical cancer genetics and where do we need to go? How do we manifest the power of genetic testing to ultimately decrease the mortality and morbidity of gynecologic cancers? One of the fundamental paradigms associated with clinical genetic testing is that although the family member who may benefit most from genetic testing is the unaffected individual, the person who needs to undergo the testing first is the person with cancer. For a young woman who has witnessed her mother go through treatment for ovarian cancer and wants to undergo genetic testing to see if she is at risk, the genetic counselor will routinely say, "In order for the test to be meaningful, your mother who had ovarian cancer should have genetic testing first." If a mutation is identified in the mother with cancer, then the daughter, who is at 50% risk of inheriting the mutation, can be tested for that specific mutation. There is a very definite answer: yes or no. However, if the daughter who has not had cancer undergoes testing first, the interpretation of the results is more difficult. A positive result is positive, but a negative result could mean (*i*) her mother did not have a BRCA mutation, that is, she did not have a

hereditary form of ovarian cancer; (*ii*) her mother did have a BRCA mutation, but the daughter did not inherit it; and (*iii*) there is an as yet unidentified mutation in her family that the testing was unable to detect. Because of the importance of performing the genetic testing on a person with cancer, first we need to ask ourselves, How good are we at asking our cancer patients about family history? And how good are we about referring appropriate patients for genetic counseling and testing? My sense at my own institution and by speaking with my colleagues at other institutions is that we are not systematically screening our patients with ovarian and endometrial cancer for hereditary cancer syndromes.

Herein lies the purpose of this book. There is a need for practical education for physicians caring for women with gynecologic cancers to understand the role of the cancer doctor in identifying which patients may have a hereditary cancer syndrome. Today the implications of testing the ovarian cancer patient for a BRCA mutation include the ability to help not only the family members but also the patient herself. We know that having a BRCA mutation confers an improved survival in women with ovarian cancer. In addition, new therapies for the treatment of ovarian cancer are currently in clinical trials that are targeted toward women with ovarian cancer who have a BRCA mutation. The clinician needs to know how to identify which ovarian or endometrial cancer patients may have a hereditary predisposition, how to refer that person for genetic counseling, and how to manage that patient if her genetic test is positive. For clinicians including obstetrician-gynecologists, internists, family practitioners, and nurse practitioners, who care for women without cancer who are mutation carriers, this book provides education and information regarding risk-reducing strategies and options for screening and early detection. I would appreciate feedback from readers.

I have many people to thank for assisting me with this project. First, I am grateful to the authors of each chapter for delivering important information in a clear and approachable manner. Second, thanks to Molly Daniels, our GYN genetic counselor, for her helpful insights and continued partnership and to Jeannette Upshaw, who helped keep me and all involved with this project on track. Third, thanks to Dr. Gershenson and my colleagues at M.D. Anderson, who contribute so much to the work I do. Thanks to Robin Lacour, Shannon Westin, and Larissa Meyer, fellows who assisted in the writing of the Learning Points and Case Reports, and to all the fellows who have participated in my research. I owe a great deal to my patients, who inspire me in my research on hereditary cancers. Every patient has an awe-inspiring tale, and I never tire of hearing them. Finally, a word of deep gratitude to my husband Charlie and my children Ned, David, and Kate—thank you for your constant love and the joy you bring to my life.

Karen H. Lu

Contents

Contributors

Banu Arun Breast Medical Oncology and Clinical Cancer Prevention, and Clinical Cancer Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Sheri A. Babb Siteman Cancer Center at Barnes-Jewish Hospital and Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Mack N. Barnes Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Lynne Cohen Program for Prevention of Women's Cancer, The University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

Russell R. Broaddus Department of Pathology, Unit 85, University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Michael J. Callahan Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

Ilana Cass Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, and David Geffen School of Medicine at UCLA, Los Angeles, California, U.S.A.

Lee-may Chen Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California, U.S.A.

Christina S. Chu Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Christopher P. Crum Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

Molly S. Daniels Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Ann K. Folkins Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

David J. Gallagher Gynecologic Medical Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

Elke A. Jarboe Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

Noah D. Kauff Clinical Genetics Services, Department of Medicine, and Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

Kate M. Kraycirik Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Johnathan M. Lancaster Division of Gynecologic Surgical Oncology, Department of Interdisciplinary Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, U.S.A.

Sharyn N. Lewin Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

Karen H. Lu Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Henry T. Lynch Department of Preventive Medicine and Public Health, Creighton's Hereditary Cancer Institute, Creighton University School of Medicine, Omaha, Nebraska, U.S.A.

Patrick M. Lynch Department of GI Medicine, Nutrition, and Hepatology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Susan K. Peterson Department of Behavioral Science, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Miguel A. Rodriguez-Bigas Department of Surgical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Stephen C. Rubin Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Kathleen M. Schmeler Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Eamonn Sheridan St. James's University Hospital, Leeds, U.K.

Louise C. Strong Department of Cancer Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Thuy M. Vu Department of Surgical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Christine S. Walsh Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, California, U.S.A.

1

Clinical Relevance of Hereditary Ovarian Cancer

Johnathan M. Lancaster

Division of Gynecologic Surgical Oncology, Department of Interdisciplinary Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, U.S.A.

KEY POINTS

- The most important risk factor for the development of ovarian cancer is family history.
- Hereditary ovarian cancer is most commonly associated with hereditary breast/ovarian cancer–associated mutations (*BRCA1*, *BRCA2*). To a lesser extent, hereditary nonpolyposis colorectal cancer–associated mutations (*MLH1, MSH2, MSH6, PMS2*) are associated with hereditary ovarian cancer.

When assessing familial risk, remember the following:

- Adoption limits interpretation of family history.
- Small families may not manifest low-penetrance genes.
- Families with few female relatives may underrepresent female cancers despite the presence of a predisposing family mutation.
- Males can transmit gynecologic cancer predisposing genes.
- Hysterectomy and/or oophorectomy at a young age in multiple family members can mask a hereditary gynecologic cancer predisposition.
- Family histories change over time and should be reassessed regularly.

It is important to emphasize that hereditary cancer risk assessment is a *process* that

- includes assessment of risk, education, and counseling;
- is conducted by a physician, genetic counselor, or other provider with expertise in cancer genetics;
- may include genetic testing if desired after appropriate counseling and consent is obtained.

- General obstetrician/gynecologists and primary care physicians as well as gynecologic oncologists should be aware of the referral guidelines for hereditary cancer risk assessment.

BACKGROUND AND HISTORY

The concept that human disease can be inherited has been recognized for many centuries. In the 19th century, the emergence of hemophilia in the offspring of British Queen Victoria was one of the most notable early examples of a familial disease trait. The subsequent appearance of the disease in Royal Houses of Spain, Russia, and Prussia illustrated how inherited disease transmission can occur as well as the impact it can have on successive generations of affected individuals. In the 21st century, inherited cancer susceptibility has become one of the most well-recognized familial traits. Research focused on the molecular basis of inherited cancer predisposition promises to not only enhance our ability to tailor care for individuals with familial cancer syndromes but also to shed light on the biologic underpinnings of sporadic cancer.

Familial breast/ovarian cancer has become one of the best characterized of the hereditary syndromes and has been the focus of considerable public interest, as evidenced by the media focus on the identification of the BRCA1 and BRCA2 breast/ovarian cancer susceptibility genes in the early 1990s (1,2). Since the localization and subsequent cloning of these two genes, the scientific community's understanding of hereditary cancer susceptibility has increased dramatically (1–4). Many of the scientific, clinical, and socioeconomic challenges associated with genetic disease traits have been faced for the first time in the context of hereditary breast/ovarian cancer (HBOC) syndrome, such that the disease has become something of a vanguard for genetic testing and management of patients with familial cancer predisposition in general.

In light of the public awareness of hereditary cancer as well as the options for genetic evaluation and risk-reducing strategies, it is becoming increasingly important for gynecologists and other physicians who provide care to women to be familiar with the nuances of hereditary cancer syndromes. In this chapter we will examine the clinical relevance of hereditary ovarian cancer, including a review of the genes that have been implicated in hereditary ovarian cancer, the clinical features that may be used to identify women who might benefit from a

genetic evaluation and possibly genetic testing, and the clinical management options available for women with hereditary ovarian cancer susceptibility.

THE GENETIC BASIS TO HEREDITARY OVARIAN CANCER

Despite the host of factors, including inheritance, environment, hormones, and behavior, that have been linked to the development of cancer, a single unifying theory to explain human carcinogenesis remains elusive. It is clear, however, that cancer is fundamentally a genetic disease, and as research technology rapidly evolves, the number of cancers in which distinct genetic defects are identifiable continues to increase.

The total number of cells present in a tissue is dependent on a critical balance between cell proliferation, senescence, and apoptosis. Ovarian cancers exhibit a high degree of genetic disruption that is manifest at both the chromosomal and molecular levels, and the genetic alterations that underlie the malignant transformation of ovarian surface epithelium primarily target genes involved in the control of these processes (5,6). Thus, development of an ovarian cancer can result from inactivation of tumor suppressor genes or activation of oncogenes so that disruption of complex regulatory pathways occurs, with the net effect being an increased number of cells (7). Mutations that inactivate DNA repair genes accelerate the accumulation of other cancer-causing mutations.

Tumor suppressor genes encode proteins that normally inhibit proliferation, and inactivation of these genes plays a role in the development of most cancers. Most hereditary cancer syndromes are due to transmission of germline mutations in tumor suppressor genes. Knudson's "two-hit" model established the paradigm that both alleles must be inactivated in order to exert a phenotypic effect on tumorigenesis (8). In the case of hereditary cancer susceptibility, the initial "hit" is the inheritance of an inactivating (germline) mutation in one copy of the gene. Later, somatic events (frequently large chromosomal losses) result in the second hit and complete loss of tumor suppressor function. In contrast, "sporadic" cancers arise through the accumulation of genetic changes that are acquired throughout the life of an organism. The mechanism of inactivation of tumor suppressor genes, whether germline or somatic, may vary from one cancer to the next. Frequently, mutations in tumor suppressor genes alter the base sequence resulting in the production of a premature stop codon (TAG, TAA, or TGA) and truncated protein product. Several types of mutational events can result in the creation of such premature stop codons, including nonsense mutations, in which a single-base substitution changes the nucleotide sequence from one that codes for a specific amino acid to one that produces in a stop codon. In addition, microdeletions or insertions of one or several nucleotides that disrupt the reading frame of the DNA (frameshifts) also lead to the generation of downstream stop codons. In some cases, missense mutations occur that change only a single amino acid in the encoded

protein. The functional significance of such a change depends on the amino acid alteration and the location within the gene. A mutation in one allele, whether germline or somatic, is revealed following somatic inactivation of the corresponding wild-type allele, typically by deletion of part or all of the chromosome. This loss of heterozygosity has become recognized as the hallmark of tumor suppressor gene inactivation. Tumor suppressor genes may also be inactivated by promoter region methylation.

Oncogenes encode proteins normally involved in stimulating proliferation. However, when these gene products are overactive, they contribute to the process of malignant transformation. Activation of oncogenes can occur via amplification of the number of gene copies, by point mutations, or by translocation from one chromosomal location to another.

HEREDITARY OVARIAN CANCER: GENES AND DISEASE PATTERNS

Although many factors influence a woman's risk of developing ovarian cancer, family history is believed to be the most important predictor of risk for the disease. Overall, approximately 10% of human cancers develop in individuals with family history consistent with the presence of an autosomal dominant susceptibility allele (9,10). Thus, in the United States, inherited risk may contribute to the development of more than 2000 of the 22,000 new cases of ovarian cancer each year (11–13).

Traditionally, inherited ovarian cancer largely falls into two clinically defined syndromes: (*i*) HBOC syndrome (including site-specific ovarian cancer and breast/ovarian cancer predisposition) and (*ii*) hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome. Although the majority of hereditary breast cancers are *BRCA1* or *BRCA2* related, individuals with Li-Fraumeni syndrome (caused by germline TP53 gene mutations) and Cowden syndrome (caused by germline mutations in the PTEN gene) also have an increased risk of breast cancer (3,14–16).

Genetic linkage studies suggest that the majority of site-specific ovarian cancer families and breast/ovarian cancer families are due to alterations in the BRCA1 gene and that familial site-specific breast cancer is due to alterations in *BRCA1* (approximately 45% of families) or *BRCA2* (approximately 35% of families) (4,14,17). However, in a Gynecologic Oncology Group (GOG) study of patients with ovarian cancer who also had a family history of breast and/or ovarian cancer, only 12 out of 26 eligible patients were found to have deleterious *BRCA1* or *BRCA2* mutation, 8 in *BRCA1* and 4 in *BRCA2,* suggesting that mutations in *BRCA1* are responsible for twice the number of hereditary ovarian cancers than *BRCA2* and also raising the possibility that additional susceptibility alleles exist that contribute to the familial ovarian cancer phenotype (18). Supporting this theory more recently, sequence and large genomic rearrangement analysis of 283 ovarian cancer families in the United Kingdom and the United States identified mutations in *BRCA1* or *BRCA2* in only 37% and 9% of families,

respectively (19). Interestingly, in this study, the frequency of *BRCA1* or *BRCA2* mutation was lower in families with fewer cases of breast cancer. Mutations were identified in 81% of families containing three or more ovarian cancer and one or more breast cancer (<60 years of age) cases, whereas the mutation frequency dropped to 27% in families containing only two ovarian cancer cases and no breast cancer. This comprehensive analysis of *BRCA1* and *BRCA2* in ovarian cancer families provides further support for the view that additional hereditary ovarian cancer genes may exist. In this regard, mutations in the DNA mismatch repair genes, *MLH1* and *MSH2,* have been shown to be responsible for a small proportion of hereditary ovarian cancer cases as a component of the HNPCC/Lynch syndrome (20–22). Although mutational inactivation of additional dominant susceptibility genes may account for many of those *BRCA1*- or *BRCA2*-negative ovarian and breast/ovarian cancer families, it is also possible that the low-penetrance susceptibility alleles, including single-nucleotide polymorphisms, also contribute to a subset of hereditary ovarian cancer families.

RISK ASSOCIATED WITH MUTATIONS IN OVARIAN CANCER SUSCEPTIBILITY GENES

The *BRCA1* and *BRCA2* genes are located on chromosomes 17q and 13q, respectively (3,4). Both are large genes, containing more than 20 exons, producing transcripts in excess of 7,000 basepairs (1,2). Inactivating mutations have been identified throughout the entire coding sequence of both genes (18,19). Individuals carrying germline mutations in the *BRCA1* cancer susceptibility gene have up to 69% risk of breast cancer and 46% risk of ovarian cancer by age 70 (23,24). Germline *BRCA2* mutations are associated with 74% and 12% risks of breast and ovarian cancer by age 70, respectively (23,24). Individuals with mutations in DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*) that underlie Lynch/HNPCC syndrome have approximately 42% to 60% and 9% to 12% risks of endometrial and ovarian cancer, respectively, by age 70 (25,26). Women and men with HNPCC also have up to a 60% lifetime risk of colorectal cancer.

IDENTIFICATION OF WOMEN AT RISK FOR HEREDITARY OVARIAN CANCER

Traditionally, the hallmarks of a hereditary cancer syndrome include the presence of multiple family members affected with the disease, an early age of cancer development, and the presence of multiple and/or bilateral primary cancers (27–29). Although such clinical markers are well recognized, recent advances in our understanding of molecular genetics have made it possible to define some of the genetic alterations that predispose individuals to inherited cancers (1,2,30–34), making it possible to provide women with a more quantified and individualized assessment of inherited ovarian cancer risk as well as options for tailored screening and prevention strategies that may reduce morbidity from

the disease (35–44). In this context, it is important to discriminate between hereditary cancer risk assessment and genetic testing. Hereditary cancer risk assessment is a process that includes assessment of risk, education, and counseling, and *may* include a genetic testing component if desired after appropriate counseling and consent is obtained.

Unfortunately, many women who might benefit from such an assessment are not identified as being at increased risk by their primary care provider, gynecologist, or oncologist. One of the greatest impediments to enhancing care for women at risk of inherited ovarian cancer is appropriate referral to a physician, genetic counselor, or other provider with expertise in cancer genetics such that the patient may undergo a comprehensive hereditary cancer risk assessment. This may, in part, be due to an incomplete understanding of the clinical parameters that should be considered as indicators for referring patients for hereditary cancer risk assessment, and highlights the need for increased genetic education efforts at graduate and postgraduate training levels.

Hereditary Cancer Risk Assessment and the Oncologist

Review of the parameters listed in Table 1 highlights the influence that a personal history of cancer has on individual risk related to HBOC syndrome. Thus, during active therapy or posttreatment surveillance, medical, surgical, and radiation oncologists will likely encounter patients with hereditary cancer risk more frequently than other providers. Clearly, the likelihood of identifying a deleterious alteration in *BRCA1* or *BRCA2* increases with the number of early-onset breast and/or ovarian cancer cases in a family, such that, in general, women with a personal history of breast cancer diagnosed before age 40 (or older in the presence of additional risk factors); ovarian, fallopian, or primary peritoneal cancer at any age; bilateral or multiple-primary breast cancer; or a known family *BRCA1* or *BRCA2* mutation are candidates for referral to assessment of inherited *BRCA1* or *BRCA2* risk. Similarly, patients with endometrial or colon cancer diagnosed before age 50 (or older in the presence of additional risk factors), endometrial or ovarian cancer with synchronous or metachronous colon cancer, or a Lynch/HNPCC-related tumor in the presence of a known Lynch/HNPCC mutation in the family should be considered for genetic evaluation for Lynch/HNPCC syndrome. It is important to emphasize that clinical parameters, in general, are simply a guide to facilitate the identification of women who may have an increased likelihood of carrying a mutation that predisposes to ovarian cancer development and should not be viewed as rigid requisites for inclusion or exclusion of patients for referral to genetic evaluation.

Gynecologic oncologists caring for women with ovarian, fallopian, or primary peritoneal cancer encounter significant numbers of women who carry germline mutations in *BRCA1* or *BRCA2*. Although it is generally accepted that approximately 10% of human cancers have an inherited component (9–13), recent data suggests that 16% of women with invasive, nonmucinous, ovarian

Table 1 Clinical Parameters That Should Be Considered as Guides for Referring Patients to a Provider with Expertise in Hereditary Cancer Risk Assessment for HBOC Syndrome

Consider hereditary cancer risk assessment for HBOC syndrome caused by mutations in BRCA1 or BRCA2 genes, if:
Affected individual with at least one of the following:
Breast cancer at ≤40 yr
Premenopausal breast cancer (≤50 yr) and a close relative[a] with premenopausal breast cancer (≤50 yr)
Premenopausal breast cancer (≤50 yr) and a close relative[a] with ovarian, male breast, or pancreatic cancer at any age
Postmenopausal breast cancer (>50 yr) with two close relatives[a] diagnosed with breast cancer at any age (particularly if at least one cancer was diagnosed at ≤50 yr)
Breast cancer at ≤50 yr and Ashkenazi Jewish descent
Postmenopausal breast cancer (>50 yr), Ashkenazi heritage, and at least one close relative[a] diagnosed with breast cancer at any age (particularly if diagnosed at ≤50 yr)
Ovarian, fallopian, or primary peritoneal cancer at any age
Cancer at any age and a known familial mutation
Two breast primaries, including bilateral disease
Ovarian, fallopian, or primary peritoneal cancer and breast cancer at any age
Unaffected individual with:
A first- or second-degree relative who meets any of the above criteria

[a]A close relative is defined as a first-degree (one who is one meiosis away from a particular individual in a family, such as a parent, sibling, offspring), second-degree (one who is two meioses away from a particular individual in a pedigree, such as a grandparent, grandchild, uncle, aunt, nephew, niece, half-sibling), or third-degree relative (one who is three meioses away from a particular individual in a pedigree, such as a great-grandparent, biologic first cousin).
Abbreviation: HBOC, hereditary breast/ovarian cancer.

cancer may have mutations in either *BRCA1* or *BRCA2* (43). In a population-based study of 232 incident cases of epithelial ovarian cancer, full sequencing of *BRCA1* and *BRCA2* and rearrangement testing of *BRCA1* revealed 32 (13.8%) mutations, 20 (8.6%) in *BRCA1* and 12 (5.2%) in *BRCA2* (45). No mutations were identified in 23 borderline or 13 mucinous tumors, such that the *BRCA1* or *BRCA2* mutation frequency in invasive, nonmucinous, ovarian cancer was 16.3%. More than 40% of *BRCA2* mutations were outside the ovarian cancer cluster region. In this population-based study, it is important to note that 31% of *BRCA1* or *BRCA2* mutation carriers had no first- or second-degree family history of breast or ovarian cancer. The frequency of *BRCA1* or *BRCA2* mutation identified in this study as well as the lack of family history in many mutation carriers illustrates why family history cannot be relied on as a clinical indicator of *BRCA1* or *BRCA2* risk in patients with ovarian cancer, and underscores why

genetic evaluation should be considered for all patients with a personal history of the disease.

Gynecologic oncologists also encounter hereditary ovarian cancer in the context of HNPCC/Lynch syndrome, an autosomal dominant disease caused by mutations in DNA mismatch repair genes, including *MLH1, MSH2, MSH6, PMS1* or *PMS2*. The syndrome is characterized by a predisposition to a spectrum of cancers such as colorectal, endometrial, upper gastrointestinal, urinary tract, as well as ovarian. As noted previously, the risk (by age 70) of endometrial and ovarian cancers are approximately 42% to 60% and 9% to 12%, respectively (36,37), such that the presence of either of these two diseases in a patient with concurrent or previous HNPCC/Lynch-related cancers should be viewed as an indication for possible genetic evaluation.

Hereditary Cancer Risk Assessment and the Obstetrician/Gynecologist and Primary Care Provider

Many patients who might benefit from a hereditary cancer risk assessment do not have a personal history of cancer or may be cancer survivors and hence are no longer under the care of an oncologist. Opportunities to identify and appropriately refer these women are, therefore, seen most frequently by primary care providers and general obstetricians and gynecologists, requiring that such clinicians have familiarity with—and be watchful for—the features of hereditary cancer syndromes. As previously stated, the presence of multiple family members affected with breast and/or ovarian cancer (or other Lynch/HNPCC-linked cancers), an early age of cancer development, and the presence of multiple and/or bilateral primary cancers should be viewed as an indicator for the possible presence of a hereditary cancer syndrome (27–29). However, specific clinical parameters exist (Table 1) that can be used to guide referrals to providers with expertise in hereditary cancer risk assessment. These clinical features highlight the importance of considering both personal and family history in a comprehensive evaluation, and underscore the significance of age of disease onset, ethnicity, and presence or absence of multiple and/or bilateral primary cancers in both the patient and family member. While these specific criteria identify the majority of individuals that meet thresholds for genetic evaluation, there are some patients who may not meet the specific criteria, but may still benefit from genetic risk assessment. These individuals include members of families with few female relatives, resulting in an underrepresentation of female cancers despite the presence of a predisposing family mutation (46,47); families in which multiple members underwent hysterectomy and/or oophorectomy at a young age, thus potentially masking a hereditary gynecologic cancer predisposition (48); and families that include adoption within the lineage.

It should be noted that when evaluating a family for possible transmission of a deleterious mutation, it is most efficient to start by testing an affected individual.

QUESTIONS RELATED TO THE BENEFITS AND RISKS OF GENETIC EVALUATION

As obstetricians and gynecologists, primary care providers, and oncologists become more proactive in identification of individuals who might benefit from hereditary cancer risk assessment, they will increasingly be faced with questions from patients regarding the process and implications of genetic assessment and genetic testing. As such, clinicians should be equipped to provide an overview of the process, including the limitations, benefits, and risks.

CLINICAL BENEFITS AND MANAGEMENT OPTIONS FOLLOWING GENETIC ASSESSMENT

Hereditary cancer risk assessment allows physicians to provide individualized and quantified assessment of risk, as well as options for tailored screening and prevention strategies that may reduce morbidity from the disease (35–44). In this regard, several strategies have been demonstrated to improve outcome for individuals at increased risk, including magnetic resonance imaging breast screening (38,39), HNPCC/Lynch colorectal cancer screening with colonoscopy (40), and prophylactic surgery (41–44). Preliminary studies suggest that prophylactic surgery reduces gynecologic cancer risk by more than 90% in some cases (35–37). Though not proven to impact outcome, additional commonly employed management strategies include screening with mammography, serum tumor markers (such as CA125), transvaginal sonography, and endometrial biopsy. For patients at risk for HBOC, approaches to chemoprevention include oral contraceptive pill to reduce ovarian cancer risk and selective estrogen receptor modulators, such as tamoxifen, to reduce breast cancer risk (49–53).

RISKS AND LIMITATIONS OF GENETIC EVALUATION

Genetic testing for inherited cancer susceptibility requires informed consent that should include education and counseling (pre- and posttest), concerning the risks, benefits, and limitations of testing. Such information should include the implications of both positive and negative genetic test results, including psychologic stress, changes to family dynamics, and the potential for social, economic, educational, and insurance discrimination. Although there is potential for insurance and/or employment discrimination, there is little evidence that this has occurred to date (53–55). Furthermore, while legal protection against discrimination remains incomplete, the 1996 Health Insurance and Portability and Accountability Act prohibits a genetic test result from being classified as a preexisting condition, in the absence of symptoms (56). Despite this, many patients may be reluctant to seek reimbursement from their health insurance company for genetic services. Current charges for a comprehensive *BRCA1* or *BRCA2* screen are in excess of $3000,

whereas single site (known family mutation) and multisite (three Ashkenazi Jewish founder mutations) are less than $400 and $500, respectively. HNPCC testing currently costs approximately $2000.

In addition to information on cost, pretest counseling should also include education on the limitations of current genetic testing technology and the subsequent risks of false-negative results, as well as the uncertainties associated with genetic variants of uncertain significance. Although genetic testing errors associated with failure to detect missense mutations and small insertions or deletions in *BRCA1* or *BRCA2* are thought to be low (<1%), large structural rearrangements that are not as easily identified may represent a significant proportion of undetected mutations in some populations (56–58). Posttest counseling should include education on risk-reduction strategies as outlined above.

The risk of developing breast, ovarian, or endometrial cancer in a woman under age 21 is low, even in individuals carrying mutations in inherited cancer susceptibility genes. Thus, a genetic test result for HBOC or Lynch/HNPCC would change the clinical management of very few women under the age of 21. In light of this fact and the potential negative consequences of genetic testing, genetic testing of women under age 21 for HBOC or Lynch/HNPCC is not recommended in the absence of a family history of extremely early-onset cancer.

CASE REPORT

J.F. was first diagnosed with left breast cancer in January 2001, at the age of 50, and then developed ductal carcinoma in situ of the right breast in October 2002. In January 2003, she was noted to have a pelvic mass at the time of her annual gynecologic examination and was subsequently diagnosed with stage IIIC ovarian cancer. Her family history was significant for a paternal grandmother with postmenopausal breast cancer; three of six paternal aunts with breast cancer, diagnosed at age 43, 46, and 54; and three paternal cousins with breast cancer, diagnosed at age 35, 42, and 50. For her personal and family histories, she was referred to genetic counseling in February 2005 and had BRCA testing performed in May 2005, which revealed a *BRCA1* mutation.

LEARNING POINTS

- Upon the diagnosis of breast, ovarian, fallopian tube, or primary peritoneal cancer, family history should be reviewed and referral for genetic counseling should be considered, if appropriate.
- A high index of suspicion for a genetic abnormality should exist in patients who develop two separate primary breast cancers or both breast and ovarian cancers.

REFERENCES

1. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994; 266(5182):66–71.
2. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995; 378(6559):789–792.
3. Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990; 250:1684–1689.
4. Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994; 265(5181):2088–2090.
5. Gallion HH, Powell DE, Smith LW, et al. Chromosome abnormalities in human epithelial ovarian malignancies. Gynecol Oncol 1990; 38:473–477.
6. Iwabuchi H, Sakamoto M, Sakunaga H, et al. Genetic analysis of benign, low-grade, and high-grade ovarian tumors. Cancer Res 1995; 55:6172–6180.
7. Bishop JM. Cancer: the rise of the genetic paradigm. Genes Dev 1995; 9:1309–1315.
8. Knudson AG. Hereditary predisposition to cancer. Ann N Y Acad Sci 1997; 833:58–67.
9. Lynch HT, Bewtra C, Wells IC, et al. Hereditary ovarian cancer: clinical and biomarker studies. In: Lynch HT, Kullander S, eds. Cancer Genetics in Women. Boca Raton: CRC Press, Inc., 1987:49–97.
10. Claus EB, Thompson N, Risch N. Genetic analysis of breast cancer and steroid hormone study. Am J Hum Genet 1991; 48:232–242.
11. American Cancer Society. Cancer Facts & Figures 2007. Atlanta: American Cancer Society, 2007.
12. Whittemore AS. Characteristics relating to ovarian cancer risk: implications for prevention and detection. Gynecol Oncol 1994; 55:S15–S19.
13. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001; 68(3):700–710.
14. Easton DF, Bishop DT, Ford D, et al. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1993; 52(4):678–701.
15. Garber JE, Goldstein AM, Kantor AF, et al. Follow-up study of twenty-four families with Li-Fraumeni syndrome. Cancer Res 1991; 51(22):6094–6097.
16. Tsou HC, Teng DH, Ping XL, et al. The role of MMAC1 mutations in early-onset breast cancer: causative in association with Cowden syndrome and excluded in BRCA1-negative cases. Am J Hum Genet 1997; 61(5):1036–1043.
17. Gayther SA, Mangion J, Russell P, et al. Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. Nat Genet 1997; 15(1):103–105.
18. Reedy M, Gallion H, Fowler JM, et al. Contribution of BRCA1 and BRCA2 to familial ovarian cancer: a gynecologic oncology group study. Gynecol Oncol 2002; 85(2):255–259.
19. Ramus SJ, Harrington PA, Pye C, et al. Contribution of BRCA1 and BRCA2 mutations to inherited ovarian cancer. Hum Mutat 2007; 28(12):1207–1215.
20. Narod S, Ford D, Devilee P, et al. Genetic heterogeneity of breast-ovarian cancer revisited. Breast Cancer Linkage Consortium. Am J Hum Genet 1995; 57(4): 957–958.

21. Frank TS, Manley SA, Olopade OI, et al. Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. J Clin Oncol 1998; 16(7):2417–2425.
22. Bewtra C, Watson P, Conway T, et al. Hereditary ovarian cancer: a clinicopathological study. Int J Gynecol Pathol 1992; 11(3):180–187.
23. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003; 72(5): 1117–1130.
24. King MC, Marks JH, Mandell JB, and New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302(5645):643–646.
25. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6:105–110.
26. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81:214–218.
27. Garber JE, Offit K. Hereditary cancer predisposition syndromes. J Clin Oncol 2005; 23:276–292.
28. Wooster R, Weber BL. Breast and ovarian cancer. N Engl J Med 2003; 348:2339–2347.
29. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003; 348:919–932.
30. Lancaster JM, Wooster R, Mangion J, et al. BRCA2 mutations in primary breast and ovarian cancers. Nat Genet 1996; 13:238–240.
31. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993; 75:1027–1038.
32. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993; 75:1215–1225.
33. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994; 368:258–261.
34. Papadopoulos N, Nicolaides NC, Wei YF, et al. Mutation of a mutL homolog in hereditary colon cancer. Science 1994; 263:1625–1629.
35. King MC, Marks JH, Mandell JB, and New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302:643–646.
36. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6:105–110.
37. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81:214–218.
38. Kriege M, Brekelmans CT, Boetes C, et al. Efficacy of MRI and mammography for breast-cancer screening in women with a familial or genetic predisposition. N Engl J Med 2004; 351:427–437.
39. Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. JAMA 2004; 292:1317–1325.
40. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000; 118:829–834.

41. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002; 346:1609–1615.
42. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002; 346:1616–1622.
43. Meijers-Heijboer H, van Geel B, van Putten WL, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2001; 345:159–164.
44. Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006; 354:261–269.
45. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005; 104(12):2807–2816.
46. Weitzel JN, Lagos VI, Cullinane CA, et al. Limited family structure and BRCA gene mutation status in single cases of breast cancer. JAMA 2007; 297:2587–2595.
47. Kauff ND, Offit K. Modeling genetic risk of breast cancer. JAMA 2007; 297: 2637–2639.
48. Kramer JL, Velazquez IA, Chen BE, et al. Prophylactic oophorectomy reduces breast cancer penetrance during prospective, long-term follow-up of BRCA1 mutation carriers. J Clin Oncol 2005; 23:8629–8635.
49. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2. National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. J Am Med Assoc 2001; 286:2251–2256.
50. Gronwald J, Tung N, Foulkes WD, et al., and Hereditary Breast Cancer Clinical Study Group. Tamoxifen and contralateral breast cancer in BRCA1 and BRCA2 carriers: an update. Int J Cancer 2006; 118:2281–2284.
51. Weitzel JN, Robson M, Pasini B, et al. A comparison of bilateral breast cancers in BRCA carriers. Cancer Epidemiol Biomarkers Prev 2005; 14:1534–1538.
52. Burke W, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. JAMA 1997; 277(12):997–1003.
53. Burke W, Petersen G, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. JAMA 1997; 277(11):915–919.
54. Hall MA, Rich SS. Laws restricting health insurers' use of genetic information: impact on genetic discrimination. Am J Hum Genet 2000; 66:293–307.
55. Armstrong K, Weber B, FitzGerald G, et al. Life insurance and breast cancer risk assessment: adverse selection, genetic testing decisions, and discrimination. Am J Med Genet A 2003; 120:359–364.
56. United States Department of Labor—Employee Benefits Security Administration. Available at: http://www.dol.gov/ebsa/faqs/faq_consumer_hipaa.html. Accessed August 10, 2007.
57. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA 2006; 295(12): 1379–1388.
58. Agata S, Viel A, Della Puppa L, et al. Prevalence of BRCA1 genomic rearrangements in a large cohort of Italian breast and Breast/Ovarian cancer families without detectable BRCA1 and BRCA2 point mutations. Genes Chromosomes Cancer 2006; 45(9):791–797.

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Clinical Relevance of Hereditary Endometrial Cancer

Karen H. Lu

Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- Approximately 5% of all endometrial cancers are due to an inherited predisposition.
- Lynch syndrome is the main endometrial cancer–inherited predisposition syndrome. Women with Lynch syndrome have a 40% to 60% lifetime risk of developing endometrial cancer, a 40% to 60% lifetime risk of developing colon cancer, and a 10% to 12% lifetime risk of developing ovarian cancer.
- Clinical criteria including young age of onset, personal history of a prior colon cancer, and family history of colon and endometrial cancer can be "red flags" for identifying an endometrial cancer patient as having Lynch syndrome.
- Tumor studies, including immunohistochemistry for MLH1, MSH2, MSH6, and PMS2, and the microsatellite instability assay may be helpful prior to performing genetic testing for Lynch syndrome.

INTRODUCTION

In 2008, there will be an estimated 40,100 cases of endometrial cancer and 7470 deaths from the disease in the United States (1). The majority of endometrial cancers are due to obesity. Approximately 5% of all endometrial cancers are due

to a hereditary disposition (2). The most common hereditary syndrome related to endometrial cancer is Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. Less commonly, endometrial cancer is seen in individuals with Cowden syndrome (see chap. 15). While much attention on hereditary gynecologic cancers has been focused on BRCA1- and BRCA2-related ovarian cancer, Lynch syndrome–associated endometrial cancer is also important for the gynecologic oncologist and gynecologist. There are two key reasons to identify women with endometrial cancer as having Lynch syndrome. First, women with endometrial cancer and Lynch syndrome have a high risk of developing a second cancer, i.e., a synchronous or metachronous colon cancer. These women should be offered screening colonoscopy, which has been shown to be effective in the prevention and early detection of colon cancer (3). Second, clinical genetic testing is available for these women. Once, a Lynch syndrome–associated mutation is identified, unaffected family members can then undergo predictive genetic testing. This chapter will highlight characteristics or red flags for clinicians to use to identify women with endometrial cancer as possibly having Lynch syndrome.

WHAT IS LYNCH SYNDROME?

Lynch syndrome, or HNPCC syndrome, is a hereditary cancer syndrome characterized by early onset colon cancer and endometrial cancer (4). In the past, Lynch syndrome was divided into Lynch I and Lynch II, with Lynch I characterizing families with multiple cases of colon cancer and Lynch II characterizing those with both colon and other extracolonic cancers, including endometrial cancer. However, with the discovery that the underlying germline molecular defect in all of these individuals is a mutation in the DNA mismatch repair gene family (MLH1, MSH2, MSH6, or PMS2), there is now simplification of the nomenclature to "Lynch syndrome." In the last decade, multiple studies have been performed that have clarified the cancer risks associated with having a Lynch syndrome mutation. In addition, criteria have been developed to assist clinicians in identifying which colon or endometrial cancer patients need to be referred for a genetics evaluation. Finally, ongoing studies are defining effective cancer screening and prevention strategies.

Compared with the individuals in the general population, individuals with Lynch syndrome face staggering risks of colon and endometrial cancer (Fig. 1). For men, lifetime risk of colon cancer is approximately 80%, and for women, it is 40% to 60%. In addition, women with Lynch syndrome have a 40% to 60% lifetime risk of endometrial cancer (5,6). These risks are significantly higher than the 4% to 5% risk of colon cancer and the 3% risk of endometrial cancer in individuals in the general population. Other cancer risks for individuals with Lynch syndrome include ovary (12% lifetime risk), small bowel (<5% lifetime risk), stomach (13% lifetime risk), renal pelvis and ureteral cancers (4% lifetime

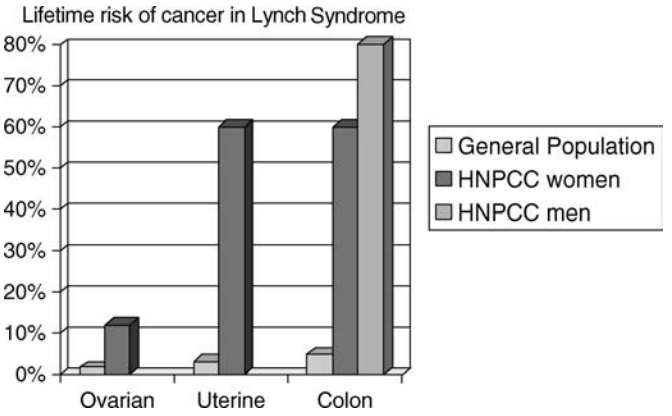

Figure 1 Lifetime risk of colon, endometrial, and ovarian cancers in men and women with Lynch syndrome compared with general population risk.

risk), biliary tract (2% lifetime risk), and brain (4% lifetime risk) (5,6). Individuals may also have sebaceous adenomas/carcinomas and keratoacanthomas, and this variant of Lynch syndrome is referred to as Muir–Torre syndrome.

Dr. Alfred Warthin, a pathologist at the University of Michigan, described the original family in 1913. Dr. Warthin's seamstress described to him that an excessive number of her family members died of gastric and uterine cancers, some at young ages. In fact, his seamstress eventually developed and died of endometrial cancer. This family, referred to as Family G, was reported by Dr. Warthin in the Archives in Internal Medicine in 1913 (7). The original pedigree of the seamstress' family was updated by Dr. Henry Lynch in 1971 in Cancer and again in 2005 after the specific molecular defect in the family had been identified (8,9). Despite the recognition, even early on, of the importance of endometrial cancer in this hereditary cancer syndrome, much of the subsequent research has been focused on colon cancer risk.

IDENTIFICATION OF INDIVIDUALS WITH LYNCH SYNDROME

Before the discovery of the underlying genetic defect, the diagnosis of Lynch syndrome was based on clinical criteria called the Amsterdam criteria. If a family had three individuals in a lineage with colon cancer, two in successive generations, and one who developed colon cancer under the age of 50, the term HNPCC or Lynch syndrome was applied. While initially focused on colon cancer, the Amsterdam criteria were subsequently revised to include all Lynch syndrome–associated cancers (Table 1) (10). The easiest way to recall the Amsterdam II criteria is the 3-2-1 rule: *three* or more relatives with Lynch-associated cancers in a lineage, including cancer of the colon, endometrium, ovary, small bowel, stomach,

Table 1 Amsterdam II Criteria (Patient Must Meet ALL of the Following)

- Three or more relatives with a histologically verified HNPCC-associated cancer or cancer of the endometrium, small bowel, ureter, or renal pelvis, one of whom is a first-degree relative of the other two; familial adenomatous polyposis (FAP) should be excluded
- HNPCC-associated cancer involving at least two generations
- One or more HNPCC-associated cancer cases diagnosed before the age of 50

Abbreviation: HNPCC, hereditary nonpolyposis colorectal cancer.

renal pelvis, biliary tract, or brain; *two* in successive generations; *one* or more Lynch-associated cancer diagnosed before the age of 50 years.

In the early 1990s, the underlying genetic defect of Lynch syndrome was found to occur in one of several members of the DNA mismatch repair gene family. Families with Lynch syndrome were found to have specific defects in MLH1, MSH2, MSH6, or PMS2 (11–14). Mutations in MLH1 and MSH2 account for more than 90% of cases of Lynch syndrome (15). Families with MSH6 have a higher incidence of endometrial cancers with later age of onset of both colon and endometrial cancers (16–18). Individuals who have Lynch syndrome have inherited one allele of a defective mismatch repair gene. Subsequent somatic loss of function of the corresponding normal allele results in defective DNA mismatch repair. DNA mismatch repair proteins are necessary to fix errors that commonly occur during DNA replication. The specific gene mutation in a family is inherited in an autosomal dominant fashion, with each child having a 50% risk of inheriting the mutation. Not all individuals who inherit a Lynch syndrome mutation will have cancer, and this is called incomplete penetrance. Overall, Lynch syndrome accounts for approximately 3% of all colon cancers and 3% of all endometrial cancers (19). In the general population, it is estimated that Lynch syndrome mutations occur in 1/500 to 1/1000 individuals, similar to the rate of BRCA1 and BRCA2 mutations in the general population.

Unlike with BRCA1 and BRCA2, tumor studies can be performed that allow clinicians an intermediate step prior to performing germline mutational analysis in evaluating individuals who potentially may have Lynch syndrome. These tumor studies can be performed on paraffin-embedded tissue. Immunohistochemistry (IHC) for MLH1, MSH2, MSH6, and PMS2 is a relatively inexpensive study. Loss of the respective protein expression by IHC (e.g., loss of staining of MSH2) suggests that there may be a germline mutation of the gene (germline mutation in MSH2). Therefore, genetic testing can be targeted for the MSH2 gene. However, for MLH1 loss by IHC, the cause can be either from germline mutation or from somatic hypermethylation of the MLH1 promoter. A more specialized test can be performed to rule out hypermethylation of the MLH1 promoter.

An additional tumor test that can be performed is called microsatellite instability (MSI) (20). MSI is a marker for an abnormally functioning DNA mismatch repair system. Tumors that develop in individuals with Lynch syndrome have the characteristic phenotype MSI. However, for both colon and endometrial cancers, MSI can be the result of either a germline mutation (i.e., Lynch syndrome) or a nongermline mutation or somatic change. As mentioned above, the somatic change most frequently associated with MSI is hypermethylation of the MLH1 promoter. Microsatellites are regions of the DNA in which there are single, di-, tri-, or quadranucleotide repeats (e.g., CACACACA). By comparing normal tissue with tumor tissue in an individual, the MSI assay identifies tumors that have an abnormally functioning DNA mismatch repair system. The National Institutes of Health has specified a panel of six microsatellite regions that can be tested for instability: BAT25, BAT26, BAT40, D5S346, D2S123, and D17S250. By convention, if a tumor has allelic shift in two or more of the six microsatellites, the tumor is designated microsatellite instability–high (MSI-H). Additional details of these two tumor studies are provided in chapter 11 by Russell Broaddus.

IDENTIFYING INDIVIDUALS WHO HAVE LYNCH SYNDROME

Traditionally, gastroenterologists, GI surgeons, and GI medical oncologists have played a great role in identifying individuals with Lynch syndrome. However, women with Lynch syndrome have an equal lifetime risk of colon and endometrial cancer, and gynecologic oncologists and gynecologists also need to be aware of the red flags identifying a woman with endometrial cancer as having Lynch syndrome. There are two key reasons to identify women with endometrial cancer as having Lynch syndrome. The first reason is that genetic testing is most helpful when it is performed on the cancer patient first. The gynecologic oncologist caring for a young endometrial cancer patient may be the first physician to note the possibility of Lynch syndrome in a family. If the endometrial cancer patient undergoes genetic testing and a mutation is identified, then other unaffected family members can be tested for the specific mutation. In a study of women with Lynch syndrome who had a history of both gastrointestinal cancer and gynecologic cancer, the gynecologic cancer (usually endometrial) was the "sentinel" cancer in over 50% of the cases (21). The second reason is that for the patient with endometrial cancer, there is a high likelihood of a synchronous or metachronous colon cancer if she has Lynch syndrome. Both of these issues were highlighted in a recent New England Journal of Medicine case report, Case 13-2007, in which a 40-year-old woman with a preoperative diagnosis of endometrial cancer was found intraoperatively to have a colon cancer (22). Ultimately, her pathology revealed three primary tumors, including a stage 1B endometrial adenocarcinoma, stage 1B clear cell adenofibromas of borderline malignancy with endometriosis in both ovaries, and a Dukes' stage B1 colon adenocarcinoma. Prior to the patient's diagnosis, there was a family history of

uterine cancer at age 50 in the patient's mother, and colon cancer at age 55 in the maternal grandfather. No previous diagnosis of Lynch syndrome had been made in the family. With the diagnosis of synchronous endometrial and colon cancers, the patient was referred for genetic counseling. Tumor studies (MSI and immunohistochemical studies) were performed on the colon and endometrial cancers, both tumors demonstrated MSI and loss of the MSH2 protein. The patient underwent germline mutation testing of MSH2 and was found to have a mutation. The patient's mother has also tested positive for the mutation, and two unaffected sisters are interested in testing.

Published criteria have been developed to assist physicians caring for colon cancer patients in identifying patients as having Lynch syndrome. These are called the Bethesda criteria and were revised in 2004 (Table 2) (23). The revised Bethesda criteria address four broad criteria: (*i*) young age at onset, (*ii*) synchronous or metachronous cancers, (*iii*) specific histologic findings of the tumor, and (*iv*) family history. There are new Society of Gynecologic Oncology guidelines for identifying a woman with endometrial cancer as having Lynch syndrome (Tables 3 and 4) (24). As a general rule, these three criteria are red flags that a gynecologic oncologist can use to identify an endometrial cancer patient as having Lynch syndrome: (*i*) young age of onset, (*ii*) synchronous or metachronous cancers, and (*iii*) family history.

Table 2 The Revised Bethesda Guidelines for Testing Colorectal Tumors for MSI

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors,[a] regardless of age.
3. Colorectal cancer with the MSI-H[b] histology[c] diagnosed in a patient who is less than 60 years of age.[d]
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

[a]HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome, and carcinoma of the small bowel.
[b]MSI-H in tumors refers to changes in two or more of the five National Cancer Institute–recommended panels of microsatellite markers.
[c]Presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.
[d]There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines.
Abbreviations: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer; MSI-H, microsatellite instability–high.

Table 3 Patients with Greater Than Approximately 20% to 25% Chance of Having an Inherited Predisposition to Endometrial, Colorectal, and Related Cancers and for Whom Genetic Risk Assessment is Recommended

- Patients with endometrial or colorectal cancer who meet the revised Amsterdam criteria (29) as listed below:
  - At least 3 relatives with a Lynch/HNPCC-associated cancer (colorectal cancer, cancer of the endometrium, small bowel, ureter, or renal pelvis) in one lineage
  - One relative should be a first-degree relative of the other two
  - At least 2 successive generations should be affected
  - At least 1 HNPCC-associated cancer should be diagnosed before age 50
- Patients with synchronous or metachronous endometrial and colorectal cancer with the first cancer diagnosed prior to age 50
- Patients with synchronous or metachronous ovarian and colorectal cancer with the first cancer diagnosed prior to age 50
- Patients with colorectal or endometrial cancer with evidence of a mismatch repair defect (i.e., MSI or immunohistochemical loss of expression of MLH1, MSH2, MSH6, or PMS2)
- Patients with a first- or second-degree relative with a known mismatch repair gene mutation

Abbreviations: HNPCC, hereditary nonpolyposis colorectal cancer; MSI, microsatellite instability.

Table 4 Patients with Greater than Approximately 5% to 10% Chance of Having an Inherited Predisposition to Endometrial, Colorectal, and Related Cancers and for Whom Genetic Risk Assessment May Be Helpful

- Patients with endometrial or colorectal cancer diagnosed prior to age 50
- Patient with endometrial or ovarian cancer with a synchronous or metachronous colon or other Lynch/HNPCC-associated tumor[a] at any age
- Patients with endometrial or colorectal cancer and a first-degree relative with a Lynch/HNPCC-associated tumor[b] diagnosed prior to age 50
- Patients with colorectal or endometrial cancer diagnosed at any age with two or more first- or second-degree relatives[b] with Lynch/HNPCC-associated tumors,[a] regardless of age
- Patients with a first- or second-degree relative[b] who meets the above criteria

[a]Lynch/HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome, and carcinoma of the small bowel.

[b]First- and second-degree relatives are parents, siblings, children, aunts, uncles, nieces, nephews, grandparents, and grandchildren.

Abbreviation: HNPCC, hereditary nonpolyposis colorectal cancer.

Age Under 50

Two studies have specifically examined the likelihood of identifying a Lynch syndrome mutation in women with endometrial cancer under the age of 50. The first study, by Berends et al., tested 63 women in Finland with endometrial cancer under age 50 for germline mutations in MLH1, MSH2, and MSH6 (25). The authors found an 8% (5/63) rate of finding a Lynch syndrome mutation, with one MLH1, three MSH2, and one MSH6 mutation. Having a first-degree relative with a Lynch syndrome–associated cancer increased the likelihood of finding a Lynch syndrome mutation to 23% in these young women.

Our group recently published a prospective study of 100 women with endometrial cancer presenting to three gynecologic oncology centers (26). We found 9% (9/100) of the women had a Lynch syndrome mutation, with one MLH1, seven MSH2, and one MSH6 mutation. Two additional women had molecular studies consistent with Lynch syndrome. Similar to the Berends study, having a first-degree relative with a Lynch syndrome–associated cancer was highly predictive of identifying a mutation. We also found that the mutation carriers had a significantly lower body mass index (BMI) compared with the noncarriers. The combination of a BMI greater than 30 and a negative family history was highly predictive of not having a Lynch syndrome mutation in our cohort of women with endometrial cancer under age 50. These data will need to be confirmed in larger studies, but can be helpful to clinicians.

In a large, population based study of endometrial cancer performed in Ohio, Hampel et al. reported that in the subset of 81 women under age 50, four women (4.9%) were found to have a Lynch syndrome mutation (19). Overall, these rates in endometrial cancer patients reported by Berends et al., Lu et al., and Hampel et al. are similar to those rates for patients with colon cancer under age 50 (27). In summary, age under 50 years for women with endometrial cancer can be a consideration for additional tumor studies to evaluate individuals for Lynch syndrome.

Synchronous or Metachronous Cancers

A patient with endometrial cancer who has a history of colon cancer has a high likelihood of having Lynch syndrome. Millar et al. studied 40 women who had a history of both colon and endometrial cancer and found that 18% (7/40) had a germline MLH1 or MSH2 mutation (28). Therefore, gynecologic oncologists can use a history of colon cancer as a strong red flag for referring their endometrial cancer patients to genetic counseling.

Our group was interested in determining whether women with Lynch syndrome who have a history of both gastrointestinal and gynecologic cancer developed their gastrointestinal cancer or their gynecologic cancer first. We identified 117 women with Lynch syndrome who had both gastrointestinal and gynecologic cancer and found that 16 women had the cancers diagnosed

simultaneously, and of the remaining 101 women, 52 women (51%) were first diagnosed with an endometrial or ovarian cancer and 49 women (49%) had a colorectal cancer diagnosed first (21). In the group of women who had an endometrial cancer first, there was a median time of 11 years before the diagnosis of the gastrointestinal cancer. Gynecologic oncologists clearly need to play a crucial role in identifying an endometrial cancer patient as having Lynch syndrome. In addition, there appears to be time to refer the patient to genetic counseling and testing and subsequently to institute screening recommendations to decrease colon cancer risk.

Synchronous endometrial and ovarian cancers are not uncommon and occur in about 10% of all ovarian cancers and in 5% of all endometrial cancers. Given that both endometrial and ovarian cancers are found in Lynch syndrome, what is the likelihood that a patient with these cancers has a germline Lynch syndrome mutation? Case reports such as the one reported above demonstrate that patients with Lynch syndrome have been found to have synchronous ovarian and endometrial cancers. However, no detailed studies have examined the ovarian histologies in these cases, and overall, few studies have adequately described the ovarian cancers in women with Lynch syndrome. Soliman et al. examined a group of 102 women with synchronous ovarian and endometrial cancers and found that only 7% met either clinical or molecular criteria for Lynch syndrome (29). Therefore, a patient with synchronous endometrial and colon cancers is much more likely than a patient with synchronous endometrial and ovarian cancers of having Lynch syndrome.

Family History

The third red flag has to do with family history. In the revised Bethesda criteria, one criterion includes an individual with colon cancer who has one or more first-degree relatives with colon cancer or other Lynch syndrome–associated cancer when one is under the age of 50. An additional criterion includes an individual with colon cancer who has two or more first- or second-degree relatives with colon cancer or other Lynch syndrome–associated cancer, regardless of age. These criteria can reasonably be applied to women with endometrial cancer.

Tumor Histology

In the revised Bethesda criteria, there are certain tumor histologies or tumor characteristics for colon cancer that are associated with Lynch syndrome. These include presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern (23). For Lynch syndrome–associated endometrial cancer, there are few studies that have addressed this specific issue and it is unclear whether Lynch syndrome–associated endometrial cancers exhibit these or other unique

pathologic findings. In a larger clinical-pathologic study of 50 Lynch syndrome–associated endometrial cancers by Broaddus et al., the distribution of stage and histology mirrored that of the general population (30); 78% were stage 1, 10% were stage 2, and 12% were stage 3 or 4. Eighty-six percent were of endometrioid histology with 44% of grade 1, 39% of grade 2, and 16% of grade 3.

Using Red Flags in Everyday Practice

In an individual who has young age of onset, synchronous or metachronous colon and endometrial cancer and/or multiple individuals in a lineage with Lynch syndrome–associated cancers, a gynecologic oncologist should strongly consider referral of that patient to genetic counseling. In certain institutions, molecular screening is currently being performed on the basis of clinical criteria, including young age of onset. When an identified germline mutation is found, predicted genetic testing can be performed in unaffected family members. Screening and prevention measures for both colon and endometrial cancer have been published by consensus groups. Later chapters in this book will discuss methods for screening and prevention, including risk-reducing surgery for Lynch syndrome–associated cancers.

OVERALL SURVIVAL

Do women with Lynch syndrome–associated endometrial cancer have an improved or worse survival compared to those with sporadic endometrial cancer? A study by Boks et al. examined survival of endometrial cancer patients who had Lynch syndrome and compared them with age- and stage-matched women with sporadic endometrial cancer (31). The overall five-year cumulative survival rates were similar, with 88% for women who had Lynch syndrome and 82% for women who had sporadic endometrial cancer. For Lynch syndrome–associated colon cancer, overall survival appears more favorable (32,33). In addition, there is data to support that 5-fluorouracil-based chemotherapy, which is given for stage 2 and 3 colon tumors, may not be as helpful in patients with MSI-H phenotype compared with patients whose tumors are microsatellite stable (34,35). Clearly, additional studies are necessary to determine if endometrial cancer associated with Lynch syndrome has a more favorable survival as compared with sporadic endometrial cancer.

SUMMARY

While there has been much attention focused on BRCA1- and BRCA2-related ovarian cancer in the gynecologic cancer community, there has been less attention focused on Lynch syndrome–associated endometrial cancer. Understanding the red flags to identify women with endometrial cancer as having

Lynch syndrome is crucial and can benefit the patient in preventing a second cancer. In addition, by identifying a specific deleterious germline mutation in a woman with endometrial cancer, unaffected family members have the opportunity to undergo predictive testing. Later chapters in this book outline the management options for screening and prevention of endometrial cancer in these high-risk, unaffected women.

CASE REPORT

TG is a 39-year-old female with a history of colorectal carcinoma who presented with a new diagnosis of endometrial cancer. She was initially diagnosed at age 38 with a right-sided colorectal carcinoma after she presented with abdominal pain and vaginal discharge. She underwent right hemicolectomy, appendectomy, and resection of the terminal ileum.

After receiving standard chemotherapy, she continued to complain of vaginal discharge. Six months after the completion of her primary chemotherapy regimen, she underwent a screening Pap smear, which demonstrated adenocarcinoma. Subsequent endometrial biopsy confirmed diagnosis of high-grade adenocarcinoma. At the time of her staging hysterectomy and bilateral salpingoophorectomy, she was found to have stage 3C uterine papillary serous adenocarcinoma with involvement of the para-aortic lymph nodes. She was treated with a paclitaxel-based chemotherapy regimen.

Of note, TG fulfilled the Amsterdam II criteria for Lynch syndrome. Her father died of colorectal carcinoma at age 52. Colorectal carcinoma was also diagnosed in her paternal grandfather and paternal uncle at ages 59 and 35, respectively. Finally, a paternal aunt had the diagnoses of both endometrial and colorectal carcinoma. Germline mutation testing revealed a mutation in MSH2.

LEARNING POINTS

- Women with Lynch syndrome have an equal risk of developing colorectal or endometrial carcinoma.
- Detailed family history should always be obtained in a woman who presents with colorectal or endometrial carcinoma at age younger than 50 years.

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007; 57(1):43–66.
2. Gruber SB, Thompson WD. A population-based study of endometrial cancer and familial risk in younger women. Cancer and Steroid Hormone Study Group. Cancer Epidemiol Biomarkers Prev 1996; 5(6):411–417.

3. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000; 118(5):829–834.
4. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003; 348(10):919–932.
5. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214–218.
6. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6(1):105–110.
7. Warthin A. Heredity with reference to carcinoma as shown by the study of the cases examined in the pathological laboratory of the University of Michigan. Arch Intern Med 1913; 12:546–555.
8. Douglas JA, Gruber SB, Meister KA, et al. History and molecular genetics of Lynch syndrome in family G: a century later. JAMA 2005; 294(17):2195–2202.
9. Lynch HT, Krush AJ. Cancer family "G" revisited: 1895–1970. Cancer 1971; 27(6): 1505–1511.
10. Vasen HF, Watson P, Mecklin JP, et al. New clinical criteria for hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999; 116(6):1453–1456.
11. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994; 368(6468):258–261.
12. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993; 75(5):1027–1038.
13. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993; 75(6):1215–1225.
14. Hendriks YM, Jagmohan-Changur S, van der Klift HM, et al. Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). Gastroenterology 2006; 130(2):312–322.
15. Peltomaki P, Vasen HF. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. Gastroenterology 1997; 113(4):1146–1158.
16. Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 2004; 127(1):17–25.
17. Miyaki M, Konishi M, Tanaka K, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997; 17(3):271–272.
18. Wijnen J, de Leeuw W, Vasen H, et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. Nat Genet 1999; 23(2):142–144.
19. Hampel H, Panescu J, Lockman J, et al. Comment on: screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2007; 67(19):9603.
20. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop Microsatellite Instability for cancer detection and familial predisposition: development criteria for the determination of microsatelitte instability in colorectal cancer. Cancer 1998; 58:5248–5257.

21. Lu KH, Dinh M, Kohlmann W, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. Obstet Gynecol 2005; 105(3):569–574.
22. Seiden MV, Patel D, O'Neill MJ, et al. Case records of the Massachusetts General Hospital. Case 13-2007. A 46-year-old woman with gynecologic and intestinal cancers. N Engl J Med 2007; 356(17):1760–1769.
23. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96(4):261–268.
24. Lancaster JM, Powell CB, Kauff ND, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. Gynecol Oncol 2007; 107(2):159–162.
25. Berends MJ, Wu Y, Sijmons RH, et al. Toward new strategies to select young endometrial cancer patients for mismatch repair gene mutation analysis. J Clin Oncol 2003; 21(23):4364–4370.
26. Lu KH, Schorge JO, Rodabaugh KJ, et al. Prospective determination of prevalence of Lynch syndrome in young women with endometrial cancer. J Clin Oncol 2007; 25(33):5158–5164.
27. Pinol V, Castells A, Andreu M, et al. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA 2005; 293(16):1986–1994.
28. Millar AL, Pal T, Madlensky L, et al. Mismatch repair gene defects contribute to the genetic basis of double primary cancers of the colorectum and endometrium. Hum Mol Genet 1999; 8(5):823–829.
29. Soliman PT, Broaddus RR, Schmeler KM, et al. Women with synchronous primary cancers of the endometrium and ovary: do they have Lynch syndrome? J Clin Oncol 2005; 23(36):9344–9350.
30. Broaddus RR, Lynch HT, Chen LM, et al. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. Cancer 2006; 106(1):87–94.
31. Boks DE, Trujillo AP, Voogd AC, et al. Survival analysis of endometrial carcinoma associated with hereditary nonpolyposis colorectal cancer. Int J Cancer 2002; 102 (2):198–200.
32. Watson P, Lin KM, Rodriguez-Bigas MA, et al. Colorectal carcinoma survival among hereditary nonpolyposis colorectal carcinoma family members. Cancer 1998; 83(2):259–266.
33. Sankila R, Aaltonen LA, Jarvinen HJ, et al. Better survival rates in patients with MLH1-associated hereditary colorectal cancer. Gastroenterology 1996; 110(3):682–687.
34. de Vos tot Nederveen Cappel WH, Meulenbeld HJ, Kleibeuker JH, et al. Survival after adjuvant 5-FU treatment for stage III colon cancer in hereditary nonpolyposis colorectal cancer. Int J Cancer 2004; 109(3):468–471.
35. Jover R, Zapater P, Castells A, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut 2006; 55(6):848–855.

3

Pathology of BRCA-Associated Ovarian Cancers, Including Occult Cancers

Elke A. Jarboe, Ann K. Folkins, and Christopher P. Crum

Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

Michael J. Callahan

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

KEY POINTS

- Despite a negative preoperative work-up, occult malignancies are found in 2.3% to 17% of patients undergoing risk-reductive bilateral salpingo-oophorectomy (average 5–6%).
- In patients with known *BRCA* mutations or at high risk for hereditary breast and ovarian cancer who are undergoing risk-reduction surgery, special attention should be paid to the pathological processing of specimens. Pathology requisition forms should detail the clinical history and request complete microsectioning of both ovaries and fallopian tubes.
- Early-stage fallopian tube cancers involving the distal fimbriated end are diagnosed more frequently in asymptomatic women with *BRCA1*/*BRCA2* undergoing risk reduction surgery, suggesting a model of possible tumorigenesis.

INTRODUCTION

The purpose of this chapter is to describe the pathology of pelvic epithelial malignancies associated with known mutations in the tumor suppressor genes BRCA1 and BRCA2. This discussion will address the following aspects of this disease, including (*i*) histologic type, (*ii*) grade, (*iii*) stage, and (*iv*) distribution and compare these parameters with sporadic pelvic carcinomas. Finally, we detail recent advances in the detection of precursor lesions that have come to light from studies of women undergoing prophylactic salpingo-oophorectomy. For the purpose of this discussion, the term "pelvic (serous) carcinoma" is preferred, but unless otherwise specified, is synonymous with the term "ovarian carcinoma." The term "BRCA positive (BRCA+)" refers to women with a germline mutation in the BRCA1 or BRCA2 gene.

GENERAL ASPECTS OF BRCA MUTATION–ASSOCIATED PELVIC CANCER

In the United States, epithelial ovarian cancer has the highest mortality rate of any malignancy of the female genital tract (1). Most of these cases are diagnosed at an advanced stage when the opportunity for cure is markedly diminished, reflecting the absence of effective screening strategies. In the United States, the average lifetime risk of developing ovarian cancer is approximately 1.4% (2). This risk increases in women carrying germline mutations in BRCA1 or 2 from 16% to 54% (3–6). Prior studies have demonstrated up to a 96% reduction in the risk of ovarian cancer development in at-risk women undergoing prophylactic surgery (7,8). Occult carcinomas will be identified in 2.3% to 17% of cases, averaging 5–6% (7–15). The risk of developing pelvic cancer increases as a function of age in these women, beginning around age 40 (16,17). Approximately 10% to 15% of women with pelvic serous carcinomas have germline BRCA mutations. Some studies have recorded higher frequencies of BRCA mutations in women with tubal and primary peritoneal carcinomas, although most of the literature has similar prevalences of tubal and ovarian carcinomas in both BRCA+ and BRCA– women (11–14).

HISTOPATHOLOGIC FEATURES AND STAGE OF PRESENTATION FOR HEREDITARY PELVIC CANCERS

Ovarian epithelial carcinomas are subdivided into a wide range of histologic types, the most common of which are serous, clear cell, endometrioid, mucinous and, less commonly, transitional or mixed (18). These tumors can be divided into two general groups according to their likelihood of being discovered in the ovary. The first group includes mucinous, clear cell, and endometrioid carcinomas. They are strongly associated with an ovarian origin, presumably arising in either inclusion cysts or endometriosis. These tumors are often associated with

benign epithelial neoplasms. Endometrioid and mucinous tumors are also more likely to be confined to the ovary at the time of presentation, not withstanding some risk of bilateral involvement.

The second group includes serous carcinomas in which greater than 80% of the carcinomas are discovered on the peritoneal surfaces when diagnosed. Although some of these tumors appear to arise in benign ovarian lesions such as endometriosis or benign cystadenomas, the majority are devoid of a tangible starting point (Fig. 1). This fact has fostered theories that these tumors arise from the ovarian surface epithelium or elsewhere, such as the fallopian tube or peritoneal surfaces (primary peritoneal carcinoma). This will be discussed further below.

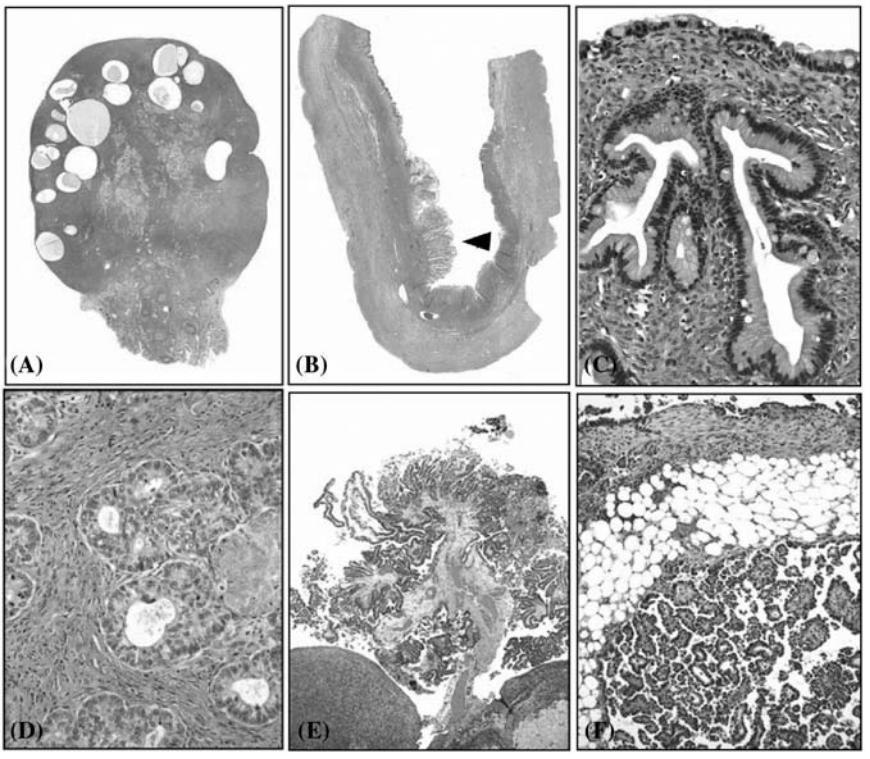

Figure 1 Origins of ovarian epithelial carcinoma. Epithelial inclusions in the ovarian cortex (**A**) presumably are the source of many ovarian epithelial carcinomas. Endometrioid carcinomas arise within endometriotic cysts (**B**) and may have both low- (**C**) and high- (**D**) grade histopathology. In contrast, many serous carcinomas involve the ovarian surface at the time of diagnosis (**E**) and have already metastasized to the omentum (**F**), a pattern characteristic of most malignancies associated with inherited mutations in the BRCA1 or BRCA2 genes.

Table 1 Comparison of Ovarian Carcinomas Associated with BRCA(+) Women and Either BRCA(–) Women or Population Controls

Parameter	BRCA1 and/or BRCA2(+)	BRCA(—) or controls
Serous carcinoma	63–86%	44–59%
Endometrioid carcinoma	6–12%	7–14%
Mucinous carcinoma	0–6%	9–23%
Other carcinoma[a]	7–8%	6%
Grade 1–2	21–38%	38–40%
Grade 3	63–74%	48–58
Stage I	12–17%	21–43%
Stage II	2–19%	8–17%
Stage III–IV	72–81%	40–71%

[a]Includes undifferentiated carcinomas and less common subtypes.
Source: From Ref. 19–22.

The frequency of individual tumor histotypes associated with BRCA+ women and controls is summarized in Table 1 and reveals two differences between BRCA+ and sporadic carcinomas. Overall, in BRCA mutation–negative women, the frequency of serous carcinomas ranges from 46% to 70%. The balance consists mainly of endometrioid and mucinous tumors, which collectively account for between 15% and 46% of ovarian carcinomas (19–21,23). In contrast, serous carcinomas comprise 70% to 80% of BRCA+ malignancies, the remainder distributed among clear cell and undifferentiated carcinomas. From 0% to 10% are classified as mucinous or endometrioid (19–23). The reader can readily appreciate that the majority of epithelial malignancies associated with BRCA+ women are of the type (serous) that is most poorly understood in terms of pathogenesis.

In keeping with the fact that serous carcinomas are typically high-grade malignancies relative to endometrioid and mucinous tumors, the proportion of high-grade (grade 3) carcinomas in the BRCA+ group is significantly higher than noncarriers (69–84% vs. 48–68%) (19–21,23). While several investigators have reported high grade to be more frequent in BRCA+ than in BRCA– cases (19–21,23), a minority have reported a similar distribution of grades between the two groups (24,25).

Reports of difference in stage of disease also vary. Over 60% of BRCA+ and negative carcinomas present as stage III or IV, with values as high as 94% for the BRCA+ group (19–21,23). In most series, ovarian cancers in the setting of BRCA1/BRCA2 were diagnosed at a high stage (stage III and IV) more frequently than those in patients without BRCA mutations (19,20,23,24). However, two studies found no significant difference in ovarian cancer stage between BRCA carriers and patients developing sporadic cancers (21,25).

In addition to the differences in histologic phenotype between malignancies found in carriers and noncarriers of BRCA mutations, there is a

difference in association with borderline serous ovarian neoplasms. Werness reported 11 borderline tumors in 134 tumors from 79 non-BRCA families, in contrast to none of 85 tumors from 47 BRCA1- or BRCA2-positive families (Table 1) (19). Rubin et al. reported just three borderline tumors in their series (22). Equally significant is the difference in proportions of borderline versus malignant serous neoplasms associated with BRCA mutations. In one study, 4.3% of borderline and 24.2% of early-stage malignant ovarian carcinomas were BRCA+ (26) This is consistent with the association between borderline epithelial tumors and malignancies of the mucinous and endometrioid phenotype and further distinguishes the two patient groups.

BRCA1 mutations make up from 60% to 89% of all BRCA mutation–associated pelvic cancers. There is no appreciable difference between the pathology associated with BRCA1 versus BRCA2 mutations (19–21,23,27).

SITE OF ORIGIN FOR BRCA+ MALIGNANCIES

Current classification systems for designating the site of origin for serous carcinomas are inherently imprecise. A diagnosis of a fallopian tube carcinoma requires the presence of an intraepithelial carcinoma and a prominent tubal tumor mass. Tumors designated as ovarian or peritoneal must have the larger tumor masses in these respective sites, primarily because, with the exception of occasional coexisting cystadenomas or endometriosis, precursor lesions have not been demonstrated with any regularity in either to confirm the source of the tumor.

One of the most intriguing aspects of familial ovarian cancer is the emerging paradox between the classification of malignancies that are symptomatic versus those discovered incidentally in women undergoing risk-reduction prophylactic salpingo-oophorectomy. Until recently, virtually all of the pelvic malignancies of BRCA+ women described in the literature have been ovarian (19,20,28). One study of a consecutive group of pelvic serous carcinomas classified 90% as ovarian (28). However, beginning in the late 1990s, investigators reported occasional fallopian tube carcinomas in BRCA+ women. What becomes apparent in analyzing the data is the sharp distinction between the sites of origin depending on whether the cancer was symptomatic or asymptomatic at time of diagnosis. Figure 2 illustrates the interesting contrast between three studies of symptomatic women and three studies that focused largely on asymptomatic women undergoing risk-reduction salpingo-oophorectomy (8–10). Four recent studies of the latter totaled 352 cases in which the majority underwent careful analysis of the ovary and fallopian tubes (8–10,17). Of these, 26 (7.4%) were found to have a malignancy, and of these, 19 (73%) were attributed to the distal fallopian tube. The proportion of tumors designated as primary tubal malignancies ranged from 60% to 100%. In summary, the majority of symptomatic patients are diagnosed with ovarian cancer. In contrast, for asymptomatic individuals, there is a preponderance of fallopian tube carcinomas.

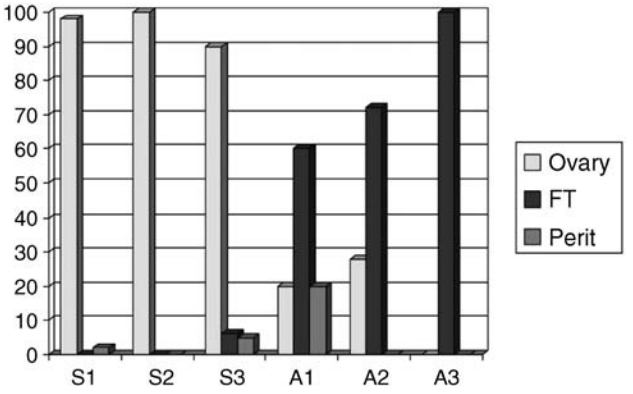

Figure 2 Effect of clinical presentation on classification of primary site. Differences in classification of pelvic carcinomas detected in three studies each of symptomatic (S) (19,20,29) and asymptomatic (A) (8,9,30) (i.e., following risk-reducing surgery) BRCA+ women. Small malignancies diagnosed in asymptomatic BRCA+ women typically involve the fimbria and are classified as tubal rather than ovarian in origin, suggesting a greater than expected origin in the distal tube.

INTRAEPITHELIAL CARCINOMA OF THE DISTAL FALLOPIAN TUBE

The obvious paradox between the observed frequency of symptomatic ovarian cancers and asymptomatic tubal malignancies in women with germline BRCA mutations might be explained by the location and dynamics of tumor development in the fimbria. Recently, at least three reports have shown that virtually every fallopian tube carcinoma documented in either BRCA+ or BRCA– women originates in or near the fimbria (8,30,31). The close proximity between these fimbrial tumors and either the ovarian cortex or peritoneal surface will largely explain how a tumor arising in the distal tube can be mistaken for a primary ovarian or pelvic peritoneal malignancy once it has spread to these organs. Central to this hypothesis is the existence of a noninvasive entity that is capable of metastasizing without invading into the subepithelial stroma of the distal salpinx. Such an entity, termed "(serous) tubal intraepithelial carcinoma" (STIC), has been well documented in both BRCA+ and sporadic carcinomas and is analogous to similar superficial serous carcinomas of the endometrium, which are known to metastasize without stromal invasion (Fig. 3).

Colgan et al. originally described occult carcinoma in 5 of 60 (8.3%) high-risk women undergoing prophylactic salpingo-oophorectomy (11). In two of these cases (BRCA+), the occult carcinoma was present in the fallopian tube and included one STIC (15). In both cases, the fallopian tubes were grossly unremarkable. Paley et al. reported occult carcinomas in the fallopian tubes of two BRCA+ women, including one STIC. Positive peritoneal cytology was present

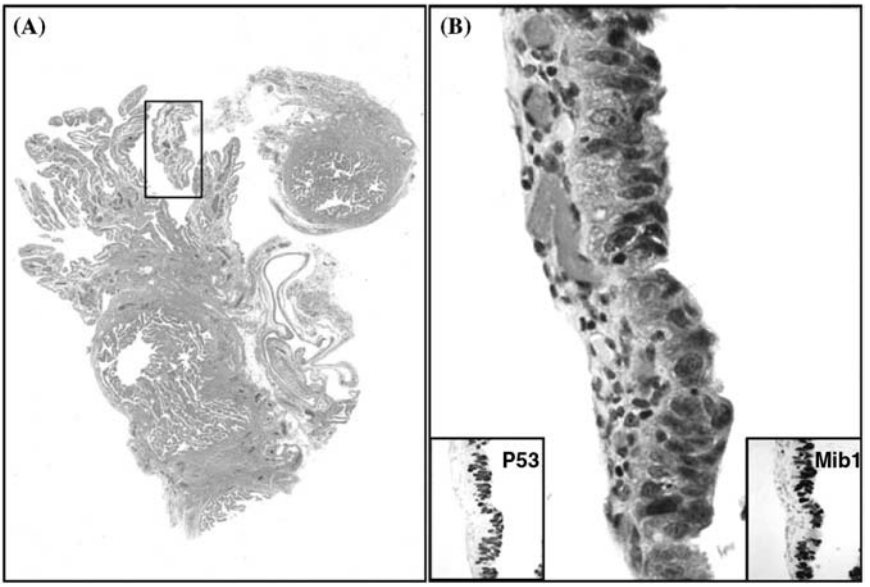

Figure 3 STIC. Histopathology of STIC arising in a small focus in the distal fallopian tube (*boxed area*) (**A**). Higher magnification discloses cytologic atypia and disorganized growth (**B**), highlighted by a high p53 and Mib1- (proliferation) staining index (*insets*). *Abbreviation*: STIC, serous tubal intraepithelial carcinoma.

in both cases (32). Leeper et al. detailed findings in 30 BRCA+ women undergoing prophylactic surgery; the fallopian tubes had been extensively sectioned in most cases. Five (17%) patients were noted to have occult cancers, including three (10%) with primary lesions in the fallopian tube. In two cases, the lesions were STICs, one of which was described in association with positive peritoneal cytology. All of these women were asymptomatic and had unremarkable preoperative workups (9). Agoff et al. described four cases of early fallopian tube carcinoma in high-risk women undergoing prophylactic procedures. Three patients had STICs, and two of these had positive peritoneal cytology (33). These reports demonstrate the propensity of STICs to shed malignant cells; thus, patients with STICs may be candidates for adjunctive chemotherapy.

STICs are distinguished from normal salpingeal mucosa by one or more of the following parameters that typify malignancy, including (*i*) epithelial stratification, (*ii*) loss of cell polarity; (*iii*) a more homogeneous appearance to the cell population, with abundant and prominent nucleoli; (*iv*) a tendency for the nuclei to become more rounded in appearance; (*v*) small fracture lines in the epithelium; and (*vi*) exfoliation of small epithelial cell clusters from the surface, with or without degenerative changes (Fig. 3) (34).

Eighty percent of serous carcinomas exhibit accumulation of p53 protein in the tumor cell nuclei, and a high percentage harbor mutations in the p53 tumor suppressor gene, estimated to be altered in over 80% of cases (35,36). Consequently, most tumors with these mutations accumulate mutated p53 and exhibit intense nuclear staining with these antibodies (Fig. 3). Similarly STICs can be identified by the accumulation of both p53 (a consequence of p53 mutations) and Ki-67, a proliferative marker that is identified with the MiB1 antibody (37). The significance of the latter will be discussed below. Typically, virtually every tumor cell nucleus is positive for p53, excepting cases that have undergone deletion mutations that preclude detection of the protein. Because the morphologic features of STICs vary in degree, immunostaining with these biomarkers occasionally is helpful in confirming the diagnosis. However, the pathologist relies almost entirely on the conventionally stained slide to verify the presence of STIC.

An anticipated question is whether STICs are really the origin of extratubal pelvic serous malignancies or simply signify a second independent tumor with no relationship to the ovarian or peritoneal neoplasm. Historically, the majority of pelvic serous carcinomas are assumed to be monoclonal based on the presence of the same p53 mutation(s) in tumors at different sites (34). Some tumors appear to be multiclonal, however, this does not exclude the tubal hypothesis inasmuch as multifocal STICs in the fimbria have been described (34,36). Recently, one study profiling a series of STICs and coincident ovarian serous carcinomas showed that in all cases a p53 mutation was shared between the two sites, suggesting a causal relationship (36).

A CARCINOGENIC SEQUENCE IN THE DISTAL TUBE (THE P53 SIGNATURE)

Why the fimbria is a preferred location for early tubal carcinogenesis is unclear. The fimbria is exposed to the peritoneal cavity, is in close proximity to the ovarian surface, and merges with the serosal mesothelium, forming a "Mullerian-mesothelial" junction. This region also exhibits epithelial plasticity, often harboring reserve cells or nests of transitional metaplasia (Walthard cell rests). Benign tumors, including serous cystadenomas and cystadenofibromas, also reside in this site. Moreover, the same factors implicated in ovarian carcinogenesis, such as ovulation, are in close proximity to the distal fallopian tube. It remains to be determined whether topography imposes a greater degree of biologic or "genetic stress" on the fimbrial mucosa in genetically susceptible individuals.

Numerous studies have attempted to identify a serous carcinogenic sequence in the upper genital tract, specifically by identifying evidence of molecular alterations in normal epithelium. This has been reported in the ovarian surface epithelium or inclusion cysts in women with ovarian cancer and in the endosalpinx, but without a universally accepted and readily identifiable precursor lesion (28,38,39). In a recent study, systematic p53 immunostaining of

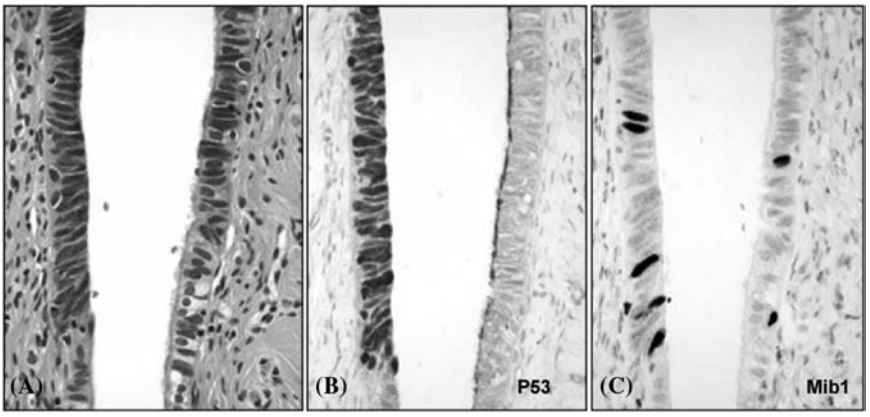

Figure 4 Histopathology of the "p53 signature." These occur in benign-appearing epithelium in the fimbria (**A**) and are defined as strong accumulations of nuclear p53 protein (**B**) (*left*), often with p53 mutations. In contrast to tubal intraepithelial carcinomas, p53 signatures have a low proliferative (Mib1) index (**C**, compare with 3**B**). p53 signatures are candidate early precursors to tubal carcinoma.

fallopian tubes from both BRCA+ women and women without a history of pelvic cancer disclosed small linear p53-positive foci in the fimbria (37). These foci, termed "p53 signatures," were present in approximately one-third of women from both groups. Importantly, p53 signatures share many features with serous carcinomas in this site, including cell type involved (secretory), evidence of DNA damage, and in many cases, reproducible p53 mutations (Fig. 4). p53 signatures localize to the same region of the tube (fimbria) that serous carcinomas are derived from, are more common in women with tubal carcinomas, and in some instances, can be found in physical continuity with malignant epithelium. Because of their association with STICs, p53 signatures are presumed to be an early precursor.

A MODEL FOR PELVIC SEROUS CARCINOGENESIS IN THE BRCA+ WOMAN

On the basis of recent studies, a provisional (and somewhat theoretical) model for high-grade serous ovarian carcinoma in the BRCA+ woman can be developed that takes into account the more recently proposed tubal pathway, which is summarized in Figure 5 (34,37). This model does not exclude the ovary as a source of pelvic carcinomas in BRCA+ women, via inclusion cysts or endometriosis. Rather, this model is proposed as the most common pathway to malignancy in the BRCA+ population, based on the data accrued from careful examination of prophylactic oophorectomy specimens. Although endometrioid carcinomas are occasionally seen in the distal tube in this population, p53

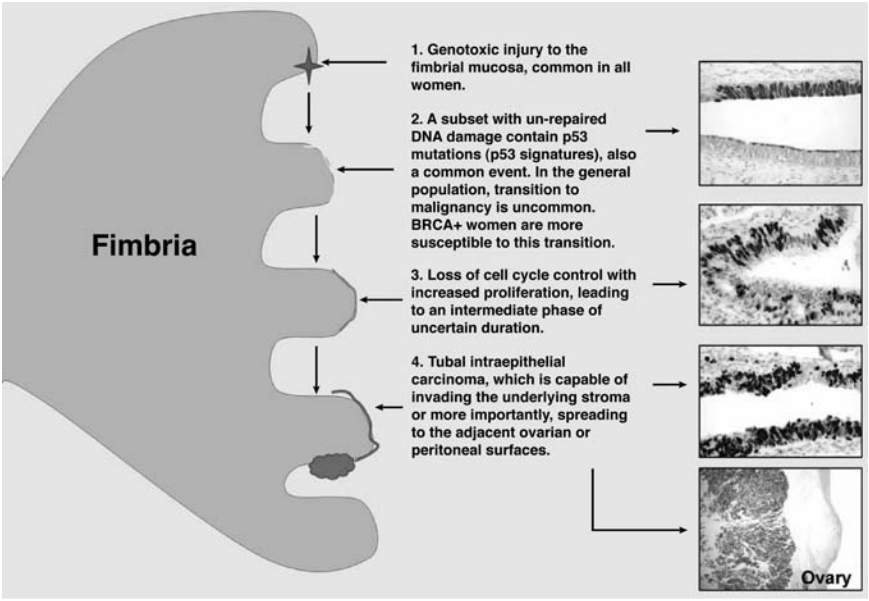

Figure 5 Schematic of pathway to tubal carcinoma (highlighted by p53 immunostaining). In this model, the p53 signature occurs commonly in women, irrespective of BRCA status. However, progression to increased proliferation and ultimately intraepithelial malignancy (TIC) or worse is more common in BRCA+ women. Thus, a preexisting BRCA mutation (and possibly, additional BRCA mutations) ultimately functions as a promotor in this system. *Abbreviation*: TIC, tubal intraepithelial carcinoma.

mutations may or may not be involved in the development of these tumors. We concentrate on the pathway in Figure 5 because there is morphologic evidence to support it. The initial phases of this pathway would be the same for all women irrespective of BRCA status. The presence of a BRCA mutation would increase the risk of completion of the pathway to the endpoint of malignancy.

The first step in this pathway would entail oxidative stress to the secretory epithelial cells of the tube, leading to unrepaired DNA damage, cell cycle arrest, and, in some, p53 mutations. Some of these events likely lead to cell death, while others permit limited clonal expansion of the population containing p53 mutations (p53 signatures). The next major step would be reinitiation of cell growth by an unknown mechanism that overrides cell cycle arrest following DNA damage, leading to proliferation producing a condition intermediate between a p53 signature and a frank malignancy. We have identified lesions in this category but do not know the duration of this phase. The next step would entail development of a tubal intraepithelial carcinoma, capable of escaping the tube via exfoliation onto the pelvic or ovarian surfaces (and in some cases onto endometrium) or directly invading of fimbrial submucosa.

An appreciation of the above process brings to light at least five variables that influence serous tumor development and spread, including the following:

1. Location of susceptible epithelium. Some ovaries have abundant inclusions, others endometriosis, and others still exhibit minimal or no epithelial activity. Because these are sites for tumor development, the mechanism(s) by which they develop in these extraovarian sites is important (40).
2. Type of epithelium. There is an increasing body of evidence indicating that target cell type has a major role in tumorigenesis (41). Moreover, the molecular events that impose a risk of cancer are also cell type specific. The evolution of endometrioid and low-grade serous and mucinous tumors of the ovarian cortex involves a series of molecular events that are distinct from serous carcinoma and are associated with a mixed rather than strictly secretory cell type.
3. Genotoxic injury. The target epithelium, whether it is on the ovarian surface or the fimbrial mucosa, must be exposed to a genotoxic insult whether it is the result of ovulation, hormonal fluctuations, or carcinogen exposure (42).
4. Risk factors for progression from precursor to early carcinoma. The dominant known risk factor is a BRCA mutation. There is no consistent evidence that the fallopian tubes or ovaries from BRCA+ women differ in their appearance from controls. Preliminary evidence also indicates that the risk of finding an early precursor (p53 signature) is similar in both BRCA+ women and controls (37). Thus, in this model, the BRCA mutation is a mitigating factor in the evolution from precursor to carcinoma.
5. Patterns of tumor growth and expansion. Because serous tumors have a high propensity for implantation and growth on the peritoneal surfaces, it is likely that small tumors arising in the tube would explain a proportion of tumors otherwise classified as ovarian or primary peritoneal (43). This is supported by the outcome of early occult carcinomas in the tubes of BRCA+ women (15). A similar argument could be made for tumors arising on the ovarian surface, although a serous carcinogenic sequence in this site is less easily demonstrated.

ARE BRCA+ PELVIC CANCERS DIFFERENT FROM SPORADIC TUMORS?

The knowledge gained from the recent studies of the fallopian tube in BRCA+ women has strengthened the hypothesis of the fimbria as the origin of the malignancies in these women. However, the number of cases to date of early carcinoma is too small to establish that every pelvic serous malignancy in BRCA+ women arises in the distal fallopian tube. What is apparent, however, is that the BRCA+ population is susceptible to a subset of serous malignances that have a strong connection with the distal tube.

In a consecutive series of pelvic serous carcinomas, the majority of which did not have a documented BRCA mutation, Kindelberger et al. showed that nearly three in four cases of presumed ovarian carcinoma involved the endosalpinx and nearly one-half contained a documented tubal intraepithelial carcinoma (36). Similarly, Kindelberger et al. found that approximately one-half of "primary peritoneal serous carcinomas" were associated with tubal intraepithelial carcinoma (36,44). This is a compelling evidence that a significant proportion of serous carcinomas originates in the distal fallopian tube. This does not exclude the ovarian cortex as a source of serous carcinomas but implies that in the + population, this pathway is less prominent, either because of a lower frequency of risk factors (such as endometriosis or cystadenomas) for primary ovarian cancer development in these women or because the predominance of factors promoting early tubal cancer in BRCA+ women renders this pathway more conspicuous. In either case, studies of the distal fallopian tube in BRCA+ women will enable investigators to more efficiently define ovarian and tubal pathways to pelvic serous cancer.

CLINICAL CONSIDERATIONS

Given the increased risk for pelvic serous carcinomas, BRCA+ women undergoing risk-reducing surgery should be counseled of the risk of discovering occult malignancy and the need for further surgery for staging. The surgical specimens of women with BRCA or other germline mutations that create a predisposition to ovarian cancer who undergo risk-reducing surgery should undergo special pathologic review. Ovaries and fallopian tubes that are removed prophylactically from women in this high-risk population should be processed in their entirety and examined closely not just for obviously neoplastic lesions but also for more subtle morphological abnormalities of the surface epithelium or the epithelium lining cortical inclusion cysts. The appropriate patient history and indication for surgery should be communicated to the pathologist so that microsectioning of the entire specimen is performed.

CASE REPORT

O.C. is a 60-year-old Caucasian woman who developed invasive breast cancer at the age of 45. She has no known Ashkenazi Jewish ancestry. Her mother was diagnosed with ovarian cancer at the age of 45 and colon cancer at age 74. A paternal aunt and a sister of her paternal grandmother were diagnosed with postmenopausal breast cancer (see pedigree). Approximately 14 years after her diagnosis of breast cancer, she self-referred herself for genetic counseling. Testing revealed a BRCA1 mutation.

At 32 years, a hysterectomy was performed for a history of endometriosis and menorrhagia, but the ovaries were left in situ. Given her increased risk for developing ovarian cancer, she was counseled and agreed to have a bilateral salpingo-oophorectomy for risk reduction.

Preoperatively, a pelvic ultrasound revealed a 1.1 × 1.2 × 0.8 cm right ovary, and the left ovary was not visualized. A CA-125 level was less than 7.0 cm. A laparoscopic bilateral salpingo-oophorectomy was performed. Final pathology showed a high-grade carcinoma involving the epithelium of the right fallopian. A pelvic washing was negative for malignant cells. She subsequently underwent a staging procedure, which revealed no further disease.

LEARNING POINTS

- Patients should be counseled preoperatively that occult malignancies are found in 2.3% to 17% of patients (average 5–6%).
- Patients should be consented preoperatively for a possible more extensive surgery/staging if frozen section of suspicious lesions diagnoses a malignancy.
- Patients should be aware that further surgery/staging may be necessary at a later date if final pathology reveals occult malignancy.
- Predictors of occult neoplasia in women undergoing risk-reducing salpingo-oophorectomy include those aged > 40 years and having a *BRCA1*/*BRCA2* mutation (8).
- Complete microsectioning of tubes and ovaries should be requested on all high-risk specimens.

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA: A Cancer Journal for Clinicians 2007; 57(1):43–66.
2. Cannistra SA. Cancer of the ovary. N Engl J Med 2004; 351(24):2519–2529.
3. Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994; 343(8899):692–695.
4. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997; 336(20):1401–1408.
5. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998; 62(3):676–689.
6. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302(5645):643–646.
7. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002; 346(21):1616–1622.
8. Finch A, Shaw P, Rosen B, et al. Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. Gynecol Oncol 2006; 100(1):58–64.
9. Leeper K, Garcia R, Swisher E, et al. Pathologic findings in prophylactic oophorectomy specimens in high-risk women. Gynecol Oncol 2002; 87(1):52–56.

10. Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005; 23(1):127–132.
11. Colgan TJ, Murphy J, Cole DE, et al. Occult carcinoma in prophylactic oophorectomy specimens: prevalence and association with BRCA germline mutation status. Am J Surg Pathol 2001; 25(10):1283–1289.
12. Risch HA, McLaughlin JR, Cole DEC, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001; 68:700–710.
13. Levine DA, Argenta PA, Yee CJ, et al. Fallopian tube and primary peritoneal carcinomas associated with BRCA mutations. J Clin Oncol 2003; 21:4222–4227.
14. Aziz S, Kuperstein G, Rosen B, et al. A genetic epidemiology study of carcinoma of the fallopian tube. Gynecol Oncol 2001; 80:341–345.
15. Lu KH, Garber JE, Cramer DW, et al. Occult ovarian tumors in women with BRCA1 or BRCA2 mutations undergoing prophylactic oophorectomy. J Clin Oncol 2000; 18(14):2728–2732.
16. Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 Mutation. JAMA 2006; 296(2):185–192.
17. Callahan MJ, Crum CP, Medeiros F, et al. Primary fallopian tube malignancies in BRCA-positive women undergoing surgery for ovarian cancer risk reduction. J Clin Oncol 2007; 25:3985–3990.
18. Scully RE. Classification of human ovarian tumors. Environ Health Perspect 1987; 73:15–24.
19. Werness BA, Ramus SJ, DiCioccio RA, et al. Histopathology, FIGO stage, and BRCA mutation status of ovarian cancers from the Gilda Radner Familial Ovarian Cancer Registry. Int J Gynecol Pathol 2004; 23(1):29–34.
20. Shaw PA, McLaughlin JR, Zweemer RP, et al. Histopathologic features of genetically determined ovarian cancer. Int J Gynecol Pathol 2002; 21(4):407–411.
21. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005; 104(12):2807–2816.
22. Rubin SC, Benjamin I, Behbakht K, et al. Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1. N Engl J Med 1996; 335(19):1413–1416.
23. Werness BA, Ramus SJ, Whittemore AS, et al. Histopathology of familial ovarian tumors in women from families with and without germline BRCA1 mutations. Hum Pathol 2000; 31(11):1420–1424.
24. Zweemer RP, Shaw PA, Verheijen RM, et al. Accumulation of p53 protein is frequent in ovarian cancers associated with BRCA1 and BRCA2 germline mutations. J Clin Pathol 1999; 52(5):372–375.
25. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. JAMA 2000; 283(17):2260–2265.
26. Gotlieb WH, Chetrit A, Menczer J, et al. Demographic and genetic characteristics of patients with borderline ovarian tumors as compared to early stage invasive ovarian cancer. Gynecol Oncol 2005; 97(3):780–783.
27. Sekine M, Nagata H, Tsuji S, et al. Mutational analysis of BRCA1 and BRCA2 and clinicopathologic analysis of ovarian cancer in 82 ovarian cancer families: two

common founder mutations of BRCA1 in Japanese population. Clin Cancer Res 2001; 7(10):3144–3150.
28. Piek JM, van Diest PJ, Zweemer RP, et al. Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. J Pathol 2001; 195(4):451–456.
29. Piek JM, Torrenga B, Hermsen B, et al. Histopathological characteristics of BRCA1- and BRCA2-associated intraperitoneal cancer: a clinic-based study. Fam Cancer 2003; 2(2):73–78.
30. Medeiros F, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006; 30(2):230–236.
31. Cass I, Holschneider C, Datta N, et al. BRCA-mutation-associated fallopian tube carcinoma: a distinct clinical phenotype? Obstet Gynecol 2005; 106(6):1327–1334.
32. Paley PJ, Swisher EM, Garcia RL, et al. Occult cancer of the fallopian tube in BRCA-1 germline mutation carriers at prophylactic oophorectomy: a case for recommending hysterectomy at surgical prophylaxis. Gynecol Oncol 2001; 80(2):176–180.
33. Agoff SN, Garcia RL, Goff B, et al. Follow-up of in situ and early-stage fallopian tube carcinoma in patients undergoing prophylactic surgery for proven or suspected BRCA-1 or BRCA-2 mutations. Am J Surg Pathol 2004; 28(8):1112–1114.
34. Mok CH, Tsao SW, Knapp RC, et al. Unifocal origin of advanced human epithelial ovarian cancers. Cancer Res 1992; 52(18):5119–5122.
35. Singer G, Stohr R, Cope L, et al. Patterns of p53 mutations separate ovarian serous borderline tumors and low- and high-grade carcinomas and provide support for a new model of ovarian carcinogenesis: a mutational analysis with immunohistochemical correlation. Am J Surg Pathol 2005; 29:218–224.
36. Kindelberger DW, Lee Y, Miron A, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. Am J Surg Pathol 2007; 31(2):161–169.
37. Lee Y, Miron A, Drapkin R, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol 2007; 211(1):26–35.
38. Feeley KM, Wells M. Precursor lesions of ovarian epithelial malignancy. Histopathology 2001; 38(2):87–95.
39. Deligdisch L, Einstein AJ, Guera D, et al. Ovarian dysplasia in epithelial inclusion cysts. A morphometric approach using neural networks. Cancer 1995; 76(6):1027–1034.
40. Zweemer RP, van Diest PJ, Verheijen RH, et al. Molecular evidence linking primary cancer of the fallopian tube to BRCA1 germline mutations. Gynecol Oncol 2000; 76(1):45–50.
41. Ince TA, Cviko AP, Quade BJ, et al. p63 Coordinates anogenital modeling and epithelial cell differentiation in the developing female urogenital tract. Am J Pathol 2002; 161(4):1111–1117.
42. Murdoch JW. Carcinogenic potential of ovulatory genotoxicity. Biol Reprod 2005; 73(4):586–590.
43. Lee Y, Medeiros F, Kindelberger D, et al. Advances in the recognition of tubal intraepithelial carcinoma: applications to cancer screening and the pathogenesis of ovarian cancer. Adv Anat Pathol 2006; 13(1):1–7.
44. Carlson J, Miron A, Jarboe E, et al. Serous tubal intraepithelial carcinoma (STIC): Its potential role in primary peritoneal serous cancer (PPSC) and serous cancer prevention. J Clin Oncol (in press).

4

Ovarian Cancer Screening

Ilana Cass

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, and David Geffen School of Medicine at UCLA, Los Angeles, California, U.S.A.

KEY POINTS

- Effective screening for ovarian cancer is limited by the low prevalence of the disease in the general population, the uncertainty of a preclinical state, and the unknown timeline of ovarian carcinogenesis.
- The use of CA 125 as a screen for the early detection of ovarian cancer is limited by the possibility of both false-positive and false-negative results.
- Risk models with algorithms that include serial CA 125 measurements and patient age or a combination of serum markers, which includes CA 125, may be better predictors than CA 125 alone, and continue to be investigated.
- Multimodal screening (CA 125 plus transvaginal ultrasound) may have more utility in high-risk populations compared with low-risk populations. However, BRCA-associated cancers may be fallopian tube or peritoneal in origin, limiting the success of ultrasound detection.

CHALLENGES FOR AN OVARIAN CANCER SCREENING TEST

Ovarian cancer remains the most lethal gynecologic malignancy due in large part to the advanced stage of disease at which patients are diagnosed. For the minority of patients whose disease is localized to the pelvis, five-year survival

rates approach 70% to 90%, and cure is a tenable goal of therapy. At present, 55% of patients present with advanced stage disease with five-year survival rates of 35% to 50% (1). Early detection of ovarian cancer, therefore, is an important goal to improve women's survival.

Efforts to screen for ovarian cancer have been hindered by the relatively low prevalence of disease, nonspecific symptoms, and uncertainty as to the existence of a preclinical state that would allow for earlier disease detection. An additional obstacle is the unknown timeline of ovarian carcinogenesis. The natural history of ovarian cancer is not directly observable. The advanced stage at which the majority of women present suggests that the disease has a rapid course that challenges the concept of a predictable progression from early-stage disease confined to the ovary to widespread metastasis. A stochastic model using mathematic modeling and expert opinion were combined to approximate the timeline of progression of ovarian cancer. Given the range of duration and the coefficient of variation of duration for each stage of disease, this model estimates that the sequence from preclinical state to stage IV disease is approximately 28.5 months. Given the best estimate of mean duration in each stage of disease and median CA 125 values at clinical detection, the authors suggest that approximately 44% of cases could be detected earlier using a yearly screening program (2).

An ideal screening test or program should combine high sensitivity (the probability of a test being positive in individuals with the disease) and high specificity (the probability of a test being negative in those patients without the disease). The screening tools should be inexpensive, noninvasive, and result in the reduction of disease-associated morbidity and mortality. An increase in sensitivity, by decreasing the cutoff value, will lead to a decrease in the specificity and vice versa. Specificity is a significant concern in screening women for ovarian cancer, especially among high-risk women, because the majority of women who test positive will require surgical intervention with its attendant cost, morbidity, and anxiety. Neither patients nor physicians will accept a large number of surgeries to detect a single ovarian cancer. For example, among the general population for whom the risk of ovarian cancer is approximately 1.8%, even a test with 98% specificity would result in 50 false-positive results with potential surgical intervention to find a single case of ovarian cancer. A screening tool for this population requires a 99.6% specificity to yield a positive predictive value of 10% (10 surgeries to detect one ovarian cancer). By convention, a 10% positive predictive value is considered reasonable justification for a screening test (3). Lower specificity may be acceptable in high-risk populations, such as BRCA mutation carriers, where the incidence of ovarian cancer approaches 40%. In these populations, a test with 90% specificity would result in a positive predictive value of 10% (4–6).

AVAILABLE SERUM TUMOR MARKERS

To date a variety of ovarian tumor markers have been studied, but CA 125 has been the most commonly reported in clinical practice. Since its discovery more than 25 years ago, the CA 125 tumor antigen has been the standard for monitoring the response of ovarian cancer patients to therapy and for surveillance in recurrent disease. CA 125 is a monoclonal antibody directed against the CA 125 antigen, a glycoprotein expressed in coelomic epithelium during embryonic development and in the majority of nonmucinous ovarian carcinomas. Only 1% of 888 healthy people and 6% of 143 patients with nonmalignant disease had serum level greater than 35 U/mL (7,8). The specificity of CA 125 is, however, limited by numerous nonmalignant and malignant conditions that cause elevated CA 125, including fibroids, endometriosis, menses, endometrial and breast cancer. Age also affects specificity of CA 125. Higher specificity is achieved using a CA 125 cutoff of 35 U/mL in women >50 years of age compared with women <50 years, 98.5% versus 94.5% (9). Any disease that irritates the mesothelium-derived surface (10) can cause an elevated CA 125, including lupus, cirrhosis, congestive heart failure, diverticulitis, and pancreatitis (7). The performance of CA 125 as a screening tool for early detection and risk prediction has been limited by both false-positive and false-negative results (11).

The merit of CA 125 is the observation that mildly elevated values can precede the clinical diagnosis of ovarian cancer by 10 to 60 months. A retrospective analysis of the JANUS serum bank collected up to 18 months before an ovarian cancer diagnosis found that a reference CA 125 value of 30 U/mL had a 50% sensitivity to detect ovarian cancer (12,13). A single CA 125 value has a positive predictive value of approximately 2% to detect ovarian cancer in asymptomatic postmenopausal women (14–16). In one of the largest ovarian cancer screening studies including 22,000 women, a single CA 125 value had a sensitivity of 58% and a specificity of 98.5%. As such, CA 125 has not been a very useful tool in screening for ovarian cancer to date (16).

One strategy to improve the early detection of ovarian cancer is a more sophisticated approach to the measurement and interpretation of CA 125. The CA 125 II radioimmunoassay has largely replaced CA 125 due to its better correlation with CA 125 and lower false-positive rate. CA 125 II uses a high-affinity antibody with enhanced resolution and a 50% reduction in the assay variability (14).

Another potential refinement of CA 125 II is based on the observation that CA 125 levels correlate with tumor volume and have been noted to rise exponentially in the early phase of tumor growth. In contrast, CA 125 elevations due to other nonmalignant causes would be expected to remain constant over time (Fig. 1). The trend of serial CA 125 values over time would be expected to better

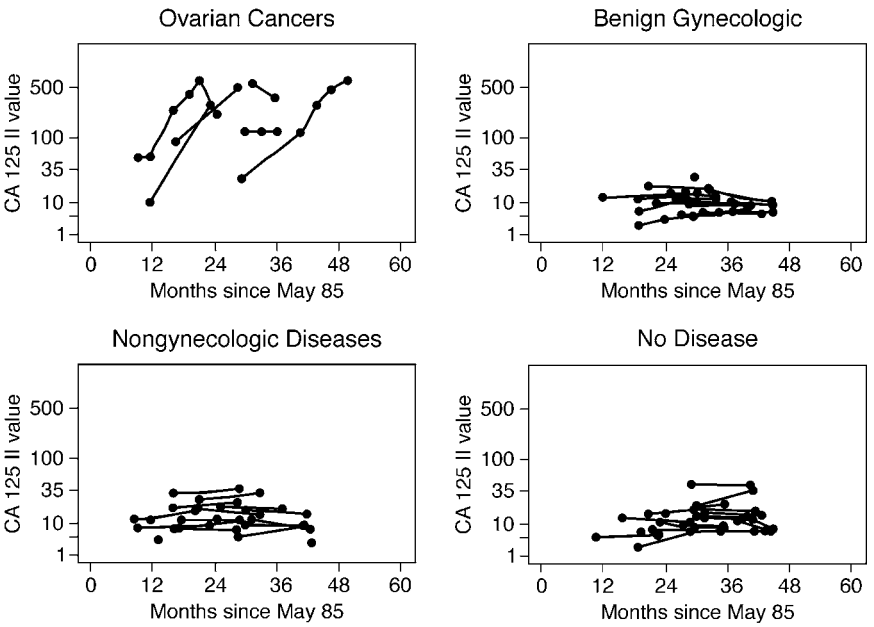

Figure 1 CA 125 II levels of women with ovarian cancer versus women with benign or no disease. *Source*: From Ref. 14.

identify women with occult ovarian cancer rather than single values. Skates and colleagues tested this hypothesis by reanalyzing sera from a cohort of 175 women with initially elevated CA 125 who had participated in the Stockholm screening study of 5550 women over the age of 40. Elevated CA 125 levels prompted more intensive follow-up, including additional CA 125 determinations every three months. A group of 175 women matched by age were similarly followed to partially blind the clinician to the CA 125 value. The serum bank was combined with the Swedish Tumor Registry and the Hospitalization Registry to provide comprehensive follow-up and diagnoses for the study group of women.

By analyzing the longitudinal CA 125 values on a log scale, a linear regression line for each patient was created. The intercept is the best estimate of the log(CA 125) at the initial screen, and the slope is the change from the intercept over one year. A computer algorithm calculates the probability of ovarian cancer by incorporating the normal distribution for the slope and intercept of women with and without cancer and the known incidence of ovarian cancer based on patient age. This method uses a scatter plot of slope versus intercept to determine the likeliness of a point segregating the patients who have cancer versus those who do not have cancer (Fig. 2). The ratio of these quantities

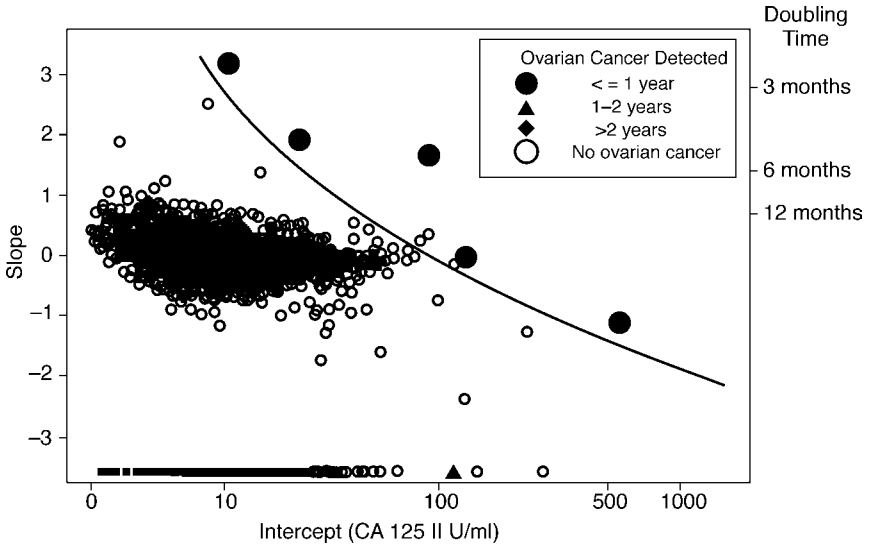

Figure 2 CA 125 II slope versus intercept for the validation set. *Source*: From Ref. 14.

multiplied by the odds of ovarian cancer based on patient age result in the odds of a patient having ovarian cancer. In this retrospective analysis, the risk of ovarian cancer algorithm (ROCA) resulted in a specificity of 99.7%, sensitivity of 83%, and a positive predictive value of 16% when tested in a separate validation set. A potential application of the ROCA strategy for ovarian cancer screening would require that patients with initially elevated CA 125 values have repeat measurements over a short period, although the ideal cutoff value might be lower than the standard 35 U/mL depending on patient age (14).

The ROCA algorithm has been incorporated into the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOS), one of the largest prospective randomized studies designed to recruit 200,000 postmenopausal women (Fig. 3) (6). Study groups include 50,000 women monitored with CA 125 algorithm, 50,000 followed by annual ultrasound, and a control group of 100,000. The study is projected to follow women for seven years. Women in the CA 125 arm are segregated into risk groups based on their CA 125 value: low-risk women return for annual CA 125, intermediate-risk women return for repeat CA 125 in three months, and high-risk women are directly referred for transvaginal ultrasound. Results from the pilot study of 13,000 women confirmed that the ROCA has high specificity and positive predictive value of 99.8% and 19%, respectively; 144 women had elevated risk, based on an elevated initial value ($n = 91$) or a rising value ($n = 44$) and 16 patients had surgery to detect 5 ovarian malignancies (17).

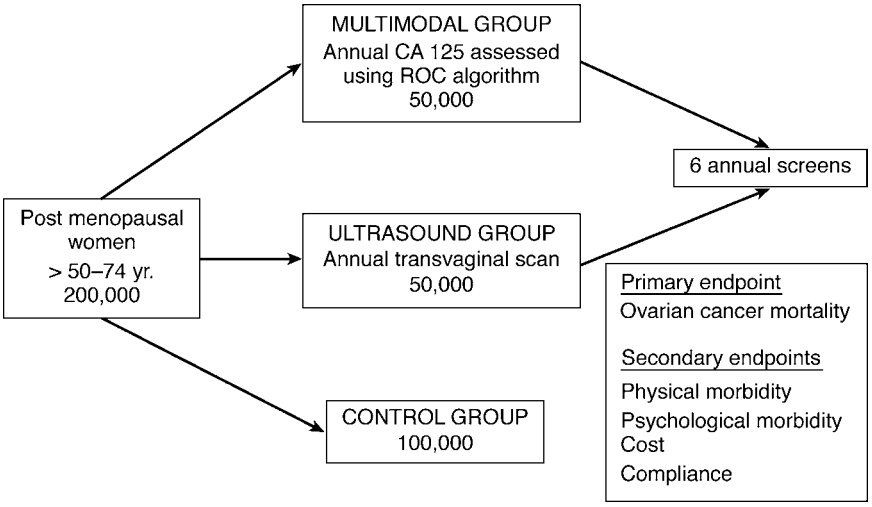

Figure 3 UKTCOS trial design. All women will be followed up for seven years via "flagging" through the Office of National Statistics and via postal questionnaires. *Source*: From Ref. 6.

Preliminary results of a prospective, multicenter screening trial in high-risk women replicate the high positive predictive value of the ROCA in this population; 2343 high-risk women have enrolled and 38 have had study indicated surgery. ROCA identified two of the three incident cases in early stage and all three of the prevalent cases for a positive predictive value of 13% and sensitivity of 83%. The remaining three incident cases were found at prophylactic salpingo-oophorectomy. Ongoing study combining ROCA possibly with additional markers in larger populations is necessary to confirm these results (18).

An alternative algorithm that relies on serial CA 125 values has been used in high-risk women in the Netherlands. In this model, the patient's serial values over a median of 12 months were analyzed by plotting the log of the relative change in CA 125 from the serial visits plotted against the absolute CA 125 value. Each patient, therefore, acts as her own control. This model has obvious advantages in a population of younger women whose menstrual cycle can compromise the validity of a single threshold value. Cases, 388 women at hereditary high risk for cancer and 370 age-matched controls had serial CA 125 values before surgery. The average age of 388 women at hereditary high risk in this study was 40 years and 75% were premenopausal. The group included 89 women who had prophylactic bilateral salpingo-oophorectomy, of whom 60 were documented BRCA mutation carriers. CA 125 levels were generally higher among premenopausal women, although both serial and absolute values predicted ovarian cancer. Dysplasia of the fallopian tube or ovary was predicted by a CA 125 of 14 U/mL among prophylactic cases, although the overlapping range of values limits the clinical significance of this finding. The authors concluded

that CA 125 levels do not behave differently in women at high risk for ovarian cancer compared with controls, and are similarly influenced by age and menopausal status (11,19).

NOVEL TUMOR MARKERS

An alternative strategy is to develop novel markers that complement or replace CA 125. A panel of markers seems a more logical approach given the heterogeneity of tumor marker expression seen in healthy women and in ovarian cancer patients. Crump and colleagues characterized the behavior of five serum tumor markers in a high-risk group of women for over six years of participation in an ovarian cancer screening program. Serial measurements of CA 125, HER-2/neu, urinary gonadotropin peptide, lipid-associated sialic acid, and Dianon marker 70/K were measured during six years of follow-up of 1237 healthy high-risk women. These markers have been shown to be elevated in other tumor types as well as ovarian carcinoma. Substantial heterogeneity was observed among women in the behavior of each marker, especially CA 125, and markers behaved independently. The observed independence of markers further supports the concept of a panel of markers to enhance specificity and minimize the likelihood of false positives (20).

Skates and colleagues combined CA 125 with other tumor markers, CA 15-3, CA 72-4, and macrophage colony-stimulating factor (M-CSF), used in other solid cancers to test alternative statistical analysis models to enhance the detection of early-stage ovarian cancer. The mixture discriminant analysis (MDA) model most efficiently combined information using the markers to improve preoperative detection of early-stage disease. The MDA model incorporates some of the variability of biomarker distribution of ovarian cancer, such as tumor histology, and estimates the proportion of cancers of each histology. By combining CA 125, CA 72-4, and M-CSF, the sensitivity for detection of early-stage disease was 70% at 98% specificity (21).

One approach to identify novel tumor markers includes immunizing a mouse with human ovarian carcinoma tissue to obtain tumor-specific antibodies. Murine monoclonal antibodies have been made against mesothelin and M-CSF to detect antigens in the sera of patients with ovarian cancer (22,23). Another approach to finding potential biomarkers is the identification of genes that are overexpressed in ovarian cancers compared with normal ovarian tissue using microarray techniques. The WFDC2 (HE4) gene is amplified in ovarian cancer, and HE4 protein serum levels were better able to discriminate benign from malignant disease than CA 125 (24). Other promising candidate markers include prostasin, human kallikreins, and osteopontin (25–28). Correlation between the overexpression in tissue and the serum levels of the shed antigens in the circulation requires further validation in larger prospective studies.

Part of the inconsistency in tumor marker expression among ovarian cancer patients may result from the varied histology of ovarian carcinomas. CA

125 tissue expression varies by histology and has more consistent expression in serous and endometrioid carcinomas than mixed mullerian tumors or mucinous carcinomas. Lu and colleagues (29) examined the role of histology in gene expression in ovarian cancer. Using Affymetrix assays, they compared a group of 42 ovarian cancers of different stages and histologies with five pools of normal ovarian epithelial tissue scrapings. mRNA expression of the upregulated genes using reverse transcription correlated well with array data and was further confirmed using immunohistochemical staining of protein overexpression of the upregulated genes in the ovarian carcinomas. A combination of three protein markers CLDN3 (Claudin 3), CA 125, and MUC1 were present in 157 of 158 cancers (99.4%), and all tumors demonstrated the combination of CLDN3, CA 125, and vascular endothelial growth factor (VEGF). Claudins are part of a family of membrane proteins that play a role in the cell's tight junction permeability.

Another limitation of the CA 125 tumor marker is the clinical observation that half of patients with early-stage disease and 20% of patients with advanced stage disease have normal CA 125 levels (7). Preoperative serum levels of CA 125 do not necessarily correlate with CA 125 staining intensity within tissue (30). To study markers that might complement CA 125, tissue microarrays from ovarian carcinomas with little to absent CA 125 expression have been studied. Kallikrein 6, 10, osteopontin, and claudin 3 were expressed by 100% of the CA 125–deficient ovarian carcinomas. A smaller subset of these nonstaining CA 125 ovarian carcinomas also expressed DF3, VEGF, MUC1, mesothelin, HE4, and CA 19-9 (5,30). Further correlation of potential biomarker expression in tumor tissue and serum levels is necessary to develop an optimal panel of biomarkers.

Mor and colleagues examined serum samples from 86 women, including 28 healthy controls, 18 newly diagnosed, and 40 recurrent advanced stage ovarian cancer patients using antibody screening microarray analysis. Of 169 proteins, 4 proteins, leptin, prolactin, osteopontin, and insulin-like growth factor, appeared to distinguish controls from cancer patients. The four proteins were further evaluated in a cross-validation study of 106 healthy controls and 100 patients with ovarian cancer. While no single protein could reliably identify patients with cancer, the combination of all four achieved a sensitivity, specificity, and positive predictive value of 95%, with a negative predictive value of 94% (31).

Analysis of the serum proteins using mass spectrometry is the newest technology that has been applied to screening for ovarian cancer. Mass spectrometry has been used to identify distinctive peptide patterns that can differentiate women with ovarian cancer from healthy controls. Initial data suggested that proteomic spectra correctly differentiated women with cancer from controls, including women with early-stage disease (32). Subsequent investigation has optimized techniques to sort artifact from tumor-associated proteins and has further refined the algorithms used to analyze the protein spectra (5,6). More recently, investigation has identified candidate serum proteins in ovarian cancer. Kozak and colleagues (33,34) described a biomarker panel that correctly identified 21 of 22 invasive ovarian cancers, including 10 of 11 early-stage

cancers. Subsequently, the authors characterized the proteins as transthyretin, β-hemoglobin, apolipoprotein AI, and transferrin. The biomarker panel improved detection of ovarian carcinomas and specifically improved detection of mucinous ovarian carcinomas compared with standard CA 125 testing. In a multicenter case-control study of 153 ovarian cancer patients, serum proteomic expressions were studied to identify potential biomarkers specific to early-stage ovarian cancer. Results comparing early-stage disease patients to controls were analyzed independently and cross-validated at two other centers. Three biomarkers were identified: apolipoprotein A1, a truncated form of transthyretin (both downregulated in cancer), and a fragment of inter-α-trypsin heavy chain H4 (upregulated in cancer). The sensitivity of the three markers with CA 125 in a multivariate model was superior to CA 125 alone, 74% versus 65% with a fixed specificity of 95% (35).

Current prospective trials are testing proteomic technology in an ovarian cancer screening trial. Results from these studies will add to our understanding of the best techniques to study proteomics and further the development of the optimal panel of ovarian cancer biomarkers.

OVARIAN CANCER SCREENING IN THE LOW-RISK POPULATION

Thus far, few prospective, randomized studies of ovarian cancer screening have demonstrated a decrease in mortality—the gold standard of efficacy for any screening test in the general population of low-risk women. Jacobs and colleagues (36) conducted a prospective, randomized controlled study of multimodal screening using CA 125 and ultrasound in women at population risk. Postmenopausal women were randomized to annual screening for three years (10,958 women) using CA 125 as the primary screen versus control (10,997 women). In the screening group, 29 patients underwent surgery for abnormal test results. Sixteen ovarian cancers were detected: 6 index cancers detected by the first screen and 10 developed intercurrent ovarian cancers during eight years of follow-up. Median survival for the 16 screen-detected ovarian cancers was better than that of the 20 ovarian cancers in the control group, 72.9 months versus 41.8 months ($p = 0.011$), although the study was not adequately powered to detect a reduction in mortality. Although more of the screen detected cancers were early stage compared with the control group, there was no significant shift in the stage of disease between groups.

Another ongoing study is the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer trial in the United States. This prospective, multicenter, randomized, controlled trial was designed to study the efficacy of screening for ovarian cancer using CA 125 II and transvaginal ultrasound in a low-risk population. The screening arm included 39,115 women randomized to intervention of CA 125 annually for six years and transvaginal ultrasound for four years between November 1993 and December 2001. To date, 25,403 women have completed the first four years of test requirements. Thus far, screening has detected 63 of the 95 ovarian cancers diagnosed in women randomized to

intervention; 1166 biopsies have been performed during the first four years of the trial. Although the percentage of biopsies finding ovarian cancer rose from 3.5% in the first year to 9.6% in the fourth year, the yield is still very low. Abnormal ultrasounds led to more biopsies than abnormal CA 125 values. While most of the cancers detected by abnormal CA 125 turned out to be advanced stage, abnormal ultrasound results detected 10 of 13 early-stage ovarian cancers in women with normal CA 125 tests. Abnormal ultrasound tests had a positive predictive value of 0.54% to 0.99%, while abnormal CA 125 had a positive predictive value of 2.4% to 4.4%.

Outcomes for the 39,000 unscreened women in the control arm remain blinded, and investigators do not know how many cancers have been diagnosed in these patients. In sum, 95% of the tests were normal and the majority of patients were diagnosed with advanced stage disease. It is premature to determine the impact of ovarian cancer screening on mortality in the general population based on these interim results. Therefore, the current ovarian cancer screening guidelines outlined by the U.S. Preventative Services Task Force from 1996, stating that "routine screening for ovarian cancer using ultrasound, serum tumor markers or pelvic examination is not recommended," remains the best practice of care (15,37).

OVARIAN CANCER SCREENING IN THE HIGH-RISK POPULATION

Current recommendations for BRCA mutation carriers include twice yearly screening with CA 125 and transvaginal ultrasound, beginning between the ages of 30 and 35 or 5 to 20 years earlier than the earliest age, if it is the first diagnosis of ovarian cancer in the family (38–40). The timing of initiation of screening and interval are largely based on expert opinion and common sense rather than any clear understanding of the natural history of ovarian carcinogenesis (41). The goal of screening is to detect more early-stage ovarian cancers and improve patient outcome. Compliance with these guidelines to date has not conclusively achieved either goal.

Several points of caution are necessary when interpreting the results of some of the larger and more current screening trials in high-risk women (Table 1). Studies have variable inclusion criteria, patient populations, and lack comprehensive genetic testing for BRCA mutations. Earlier studies were initiated prior to development of current risk assessment tools used to quantify risk of BRCA mutations, while other studies used modified criteria to identify high-risk patients (42–45). Many studies draw from hereditary cancer clinics with family members of patients with known mutations, while other studies draw from self-referral populations. The definition of high-risk patients and corresponding inclusion criteria in each study are different and include a family history of ovarian cancer, a personal history of breast cancer, multiple first-degree relatives with ovarian cancer, and patients with documented BRCA mutations (19,46–52). The variable inclusion criteria affect the proportion of

BRCA mutation carriers within each study that directly impacts the prevalence of disease and performance of screening tools. Studies with the highest proportion of documented BRCA mutation carriers (19,43,48,49,52–54) would be expected a priori to have more ovarian cancers, which might improve the success of their screening programs.

To date, only one study provides data regarding survival outcome for high-risk patients with screen-detected ovarian cancer. Van Nagell and colleagues recently reported the success of annual transvaginal ultrasound screening in 25,000 women to detect early-stage ovarian cancer. A family history of ovarian cancer in a first- or second-degree relative was present in only 23% of patients, and genetic testing was not routinely performed. CA 125, morphology indexing and color Doppler were used as a second-tier tool following a persistent initial ultrasound abnormality. Of the 364 patients with persistent abnormal screening tests, 35 primary invasive cancers, 9 borderline ovarian tumors, and 7 cancers metastatic to the ovary were detected. Nine women had ovarian cancer diagnosed within 12 months of a normal screening test. Transvaginal ultrasound had a sensitivity of 85%, specificity of 98.7%, and a positive predictive value of 14%. Excluding nonepithelial and borderline cancers, more of the screened patients had early-stage disease, 22 of 30 (73%), compared with 34% of historical controls. Five-year survival rates were significantly better for screened patients compared with controls, even when limiting analysis to invasive, epithelial cancers, 77.2% versus 48.7%, respectively ($p < 0.001$). Extrapolation of these findings to high-risk women is difficult; however, the authors recommend that an increased screening frequency for high-risk women would be more appropriate (46).

Another issue to consider in reported success of screening programs is to distinguish between incident and prevalent cases of screen-detected ovarian cancers. The value of screening may best be analyzed from incident cases given that these ovarian cancers are detected during surveillance following a normal prior screen. Prevalent cases would be expected to have more advanced stage disease and a longer preclinical phase (41). Cases of occult cancer noted at the time of prophylactic risk-reducing salpingo-oophorectomy (RRSO) should not be included in the analysis. Ideally, only surgeries performed for diagnostic purposes in the face of abnormal screening tests should be evaluated and used to determine the positive predictive value.

On further inspection of the screen-detected ovarian cancers outlined in Table 1, it is apparent that invasive, epithelial ovarian cancers are not the only cancers discovered at surgery. While some data regarding exact histology may be incomplete, 15 of the cancers identified were borderline (14) or granulosa cell tumors (1). These tumors tend to present as stage I disease and are often asymptomatic. Removal of these types of ovarian tumors would not generally be expected to contribute much to the reduction in mortality from ovarian cancer as compared with invasive, epithelial ovarian cancer based on the different natural history of these entities. Further speculation regarding the possible benefit of removing benign ovarian lesions that may progress to invasive ovarian

Table 1 Ovarian Cancer Screening in High-Risk Women

Author	Year	Number of patients	Inclusion criteria	Screening protocol	Number of diagnostic surgeries	Prevalent cases	Incident cases	Interval cases
Bourne (45)	1994	1502	FH first-degree relative	TVS ± CA 125	62	(3) IA LMP (2) IA (1) III	(1) IIA	(1) IIB (3) III (2) peritoneal
Karlan (42)	1999	1261	FH first-degree relative	TVS, CA 125 twice a year until 1995, then annually	?	(1) IB LMP	(1) IB LMP (1) IC (3) IIIC peritoneal	(4) IIIC peritoneal
Dorum (51)	1999	803	FH first-degree relative or breast cancer	TVS, CA 125 annually	?	(3) I LMP (1) I (1) II (9) III	(1) I LMP (1) III	
Liede (44)	2002	290[a]	Jewish with BRCA testing	TVS, CA 125 twice a year until 1995, then annually	24		(1) IC (1) IIC (1) IIIC tubal/ peritoneal (2) IIIc peritoneal	(3) IIIC
Scheuer (53)	2002	Subgroup 62	BRCA mutation carriers	TVS, CA 125 biannually	10	(1) IC (1) IIC peritoneal (1) IIC (1) Unstaged	(1) IA	
Tailor (54)	2003	2500	FH first-degree relative	TVS, CA 125 if abnormal TVS	104	(2) IA LMP	(2) IA LMP (1) IA granulose (2) IA, (1) IC (1) IIB (2) IIB	(2) IIIC peritoneal (7) IIIC

Vasen (52)	2005	138	Known BRCA mutation in family	TVS, CA 125 annually	?	(2) III	(2) III (1) IV	(1) III
Stirling (50)	2005	1110	Moderate risk (4–10% lifetime risk), High risk (>10% lifetime risk)	TVS, CA 125 (CA 125 done if abnormal TVS 1 center)	39		(1) IC LMP (1) IC (1) IC tubal (1) IIB, (1) IIC (1) IIIB, (3) IIIC (1) IV	(1) IIIA, (1) IIIC (1) IV
Fishman (47)	2005	4526	At least FH first known personal or family BRCA mutation	TVS	49		(1) IIIA peritoneal (2) IIB peritoneal (1) IIIC peritoneal (4) IIIC tubal (2) IIIC	
Olivier (48)	2006	312	Known personal or family BRCA mutation	TVS, CA 125 annually	49		(1) IC (1) IIIB (1) IV	(1) IV
Bosse (43)	2006	676	At least FH first known personal or family BRCA mutation	TVS, CA 125 biannually	10		(1) IC	
Oei (49)	2006	512	At least FH first known personal or family BRCA mutation	TVS, CA 125 annually	24		(1) IIIC	
Hermsen (19)	2007	388	>2 relatives breast/ovarian cancer	TVS, CA 125 annually			(1) IC (1) IIB (3) IIIC	

[a]These subjects may include some reported in Ref. 42. *Abbreviations*: FH, family history; TVS, transvaginal ultrasound.

carcinoma remains unknown. Limited data suggests that mucinous epithelial ovarian carcinoma may progress through the histologic continuum of cystadenoma to borderline to invasive carcinoma, although mucinous carcinomas are much less common among BRCA mutation carriers (41,55,56).

The question remains as to whether patient survival will be dramatically altered by the discovery of screen-detected ovarian cancers. Excluding the borderline and granulosa cell tumors, the actual proportion of early-stage invasive, epithelial ovarian cancers is similar to the distribution of disease seen in nonscreened populations and would not be expected to significantly improve patient survival. Of the 70 invasive epithelial ovarian carcinomas detected in patients with screening, 17 (24%) patients had stage I/IIB disease (Table 2) (1). It is possible that patients with screen-detected advanced stage disease have less tumor burden than comparable advanced stage patients who present with symptoms that may translate into better survival, but this remains to be seen.

Part of the challenge for early detection screening programs in high-risk patient is the phenotype of BRCA-associated gynecologic carcinomas. BRCA mutation carriers appear to have an increased risk of tubal and peritoneal carcinomas, which may not lend themselves to early detection as readily as ovarian cancer (44,57–59). The estimated incidence of primary peritoneal carcinoma in the general population is approximately one tenth the frequency of ovarian cancer. Among high-risk populations, the proportion of primary peritoneal carcinomas may be twice as high (57). Liede and colleagues calculated the cumulative risk of primary peritoneal carcinoma to be 20% over 10 years for

Table 2 Summary of Early Detection in Ovarian Cancer Screening Trials

Author	Number of screen-detected ovarian cancers	Number of stage I/IIB screen-detected ovarian cancers (% of total cases)	Number of screen-detected invasive epithelial ovarian cancers	Number of stage I/IIB screen-detected invasive epithelial ovarian cancers (% of total invasive epithelial cases)
Bourne (45)	7	6 (86)	4	2 (50)
Karlan (42)	6	3 (50)	4	1 (25)
Dorum (51)	16	6 (38)	12	2 (16)
Liede (44)	5	1 (20)	5	1 (20)
Scheuer (53)	5	2 (40)	5	2 (40)
Tailor (54)	11	7 (63)	6	2 (33)
Vasen (52)	5	0	5	0
Stirling (50)	10	4 (40)	9	3 (33)
Fishman (47)	10	0	10	0
Olivier (48)	3	1 (33)	3	1 (33)
Bosse (43)	1	1 (100)	1	1 (100)
Oei (49)	1	0	1	0
Hermsen (19)	5	2 (40)	5	2 (40)
Total			**70**	**17 (24)**

BRCA1 mutation carriers on the basis of population frequencies in their study (44). Of the 111 cancers described in Table 1, 19 (17%) were primary peritoneal carcinomas. By convention, primary peritoneal carcinomas have minimal ovarian enlargement with the majority of tumor distribution seen in the omentum and peritoneal surfaces (42). Similarly, primary tubal carcinoma can present with extensive disseminated disease with small tubal masses or lesions (60). As such, a screening test like transvaginal ultrasound, which relies on the identification of an adnexal mass as an early event in cancer progression, may have limited success.

Prospective trials with more uniform inclusion criteria are needed to determine the best screening strategy for women at high risk for ovarian cancer. A randomized study design that assigns this population of women to no screening would be unethical. The optimal screening tools and screening interval for high-risk women is unknown based on existing studies. Because the UKCTOS general population study excluded women at high risk of ovarian cancer, there is a corollary UKCTOS for women with a lifetime risk of ovarian cancer >10% called the U.K. Familial Ovarian Cancer Screening Study. This prospective study includes annual CA 125 and transvaginal ultrasound, with four-monthly sera sample collection for future retrospective analysis and discovery of future biomarkers. Investigators hypothesize that a distinct familial ROCA index will result from these analyses that can be validated in a prospective screening program for high-risk women. Anticipated accrual should be complete by 2007 with study results available in 2012 (6,10).

The Gynecologic Oncology Group (GOG) is currently evaluating the optimal strategies for risk reduction among women at increased genetic risk of ovarian cancer. This prospective study (GOG #199) compares RRSO and longitudinal CA 125 screening. One goal of the study is to quantify the positive predictive value and specificity of the ROCA based on every three month serial CA 125 measurements and annual ultrasound for women who opt for surveillance. To be eligible, patients must have a documented BRCA deleterious mutation or a family or personal history that carries a high probability (>20%) of carrying a BRCA mutation. Thus far, patient characteristics and genetic testing history are known for 2503 of the 2593 evaluable patients, of whom 38% have opted for RRSO. A longitudinal serum, plasma, and tissue repository will facilitate future evaluation of promising new biomarkers in this cohort of high-risk women. Between these two large prospective trials, certain progress will be made to determine the optimal screening program, time to begin screening, and interval between screens for high-risk women.

OVARIAN CANCER SCREENING PROGRAM ACCEPTABILITY AMONG HIGH-RISK WOMEN

Screening programs have consistently reported high levels of acceptability and compliance among high-risk women. Despite the inconvenience, anxiety, and mild discomfort of pelvic exams and ultrasounds, patient dropout rates are less

than 23% in longitudinal studies (6,18). Drawing on compliance rates in the general population for annual mammography and cervical cancer screening, it would appear that high-risk women are generally a highly motivated, proactive population of women who are diligent in seeking out care.

A single institution study from MD Anderson Cancer Center examined factors that were associated with the selection of risk-reducing strategies among 554 women who underwent genetic testing between 2001 and 2005. The majority of women were Caucasian, 12.5% were Jewish, and 64% had a prior history of breast cancer, of whom 74% were diagnosed before the age of 50; 387 (69.9%) of women opted for surveillance, 9.4% chose both mastectomy and oophorectomy, and 5.4% had oophorectomy. Among the 132 BRCA mutation carriers, 63% had prophylactic surgery compared with 32% of non-BRCA mutation carriers. Factors associated with the selection of prophylactic surgery were BRCA mutation, a history of breast cancer or previous breast biopsies, or family history of ovarian cancer (61).

In a similar study, Schwartz and colleagues followed 289 women referred for genetic testing due to family history and utilization of risk-reducing strategies in the 12 months following genetic testing. Similar to the prior study, the majority of patients were Causcasians (94%) and college educated (77%). Seventy-nine patients had deleterious BRCA mutations and 27% of these women opted for RRSO. Factors that predicted RRSO included a family history of ovarian cancer, perceived risk of ovarian cancer, and baseline ovarian cancer worry. The authors suggest that preexisting risk perception can strongly influence how patients interpret information provided at genetic counseling. Intervention to correct inaccurate baseline risk perception should enhance the outcomes and satisfaction with genetic counseling and testing (62).

Extrapolation of these findings to other patient populations in our society may be limited by the underutilization of genetic testing and counseling and unknown prevalence of BRCA-associated ovarian carcinoma among certain ethnic groups. Studies have shown that factors associated with patient referral to genetic testing include higher education, younger age at diagnosis of breast or ovarian cancer, Jewish heritage, and a recent health care interaction with a gynecologist rather than a primary care physician. African-Americans were less likely to be referred for genetic counseling. The racial disparity was not explained by the likelihood of a BRCA mutation, socioeconomic status, cancer risk perception, or attitudes toward testing (40).

CONCLUSION

Despite many limitations, the early detection of ovarian cancer among high-risk women is an important goal that mandates ongoing research and resources. Significant progress has been made based on prior studies and experience through improved clinical trial design and improved technology in biomarker

discovery. With several, large prospective trials currently underway, we are poised to better understand the ovarian carcinogenesis and to identify earlier alterations in the molecular pathways that lead to ovarian cancer. Improved detection of early-stage disease would have enormous implications for BRCA mutation carriers, given their significant lifetime risk of developing ovarian cancer, as well as the general population.

CASE REPORT

M.B., a 63-year-old female, presented to the high-risk ovarian screening clinic, given her family history of a sister who was diagnosed with ovarian cancer at the age of 66 and a niece who was diagnosed with breast cancer at the age of 40. On her initial screening ultrasound, she was noted to have a slightly enlarged right ovary with a normal CA 125. She opted to undergo a prophylactic total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAHBSO), at which time she was diagnosed with occult stage IC ovarian cancer. After her diagnosis, she proceeded with genetic testing and was found to have a BRCA1 variant of uncertain significance.

LEARNING POINTS

- The goal of ovarian cancer screening is detection of early-stage disease.
- Currently, ovarian cancer screening in many high-risk populations involves serum CA 125 and transvaginal ultrasound every six months.
- CA 125 may be normal in up to 50% of patients with early-stage disease.

REFERENCES

1. Heintz AP, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 2006; 95(suppl 1):S161–S192.
2. Skates SJ, Singer DE. Quantifying the potential benefit of CA 125 screening for ovarian cancer. J Clin Epidemiol 1991; 44(4–5):365–380.
3. Jacobs I, Oram D. Screening for ovarian cancer. Biomed Pharmacother 1988; 42(9): 589–596.
4. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302(5645):643–646.
5. Bast RC Jr., Badgwell D, Lu Z, et al. New tumor markers: CA125 and beyond. Int J Gynecol Cancer 2005; 15(suppl 3):274–281.
6. Rosenthal AN, Menon U, Jacobs IJ. Screening for ovarian cancer. Clin Obstet Gynecol 2006; 49(3):433–447.
7. Bast RC Jr., Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983; 309 (15):883–887.

8. Zurawski VR Jr., Knapp RC, Einhorn N, et al. An initial analysis of preoperative serum CA 125 levels in patients with early stage ovarian carcinoma. Gynecol Oncol 1988; 30(1):7–14.
9. Einhorn N, Sjövall K, Knapp RC, et al. Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer. Obstet Gynecol 1992; 80(1):14–18.
10. Jacobs IJ, Mackay J, Menon U, et al. Familial ovarian screening—effective or ineffective? Br J Cancer 2006; 95(8):1124; author reply 1126–1127.
11. Karlan BY, McIntosh M. The quest for ovarian cancer's Holy Grail: can CA-125 still be the chalice of early detection? J Clin Oncol 2007; 25(11):1303–1304.
12. Zurawski VR Jr., Orjaseter H, Andersen A, et al. Elevated serum CA 125 levels prior to diagnosis of ovarian neoplasia: relevance for early detection of ovarian cancer. Int J Cancer 1988; 42(5):677–680.
13. Zurawski VR Jr., Sjovall K, Schoenfeld DA, et al. Prospective evaluation of serum CA 125 levels in a normal population, phase I: the specificities of single and serial determinations in testing for ovarian cancer. Gynecol Oncol 1990; 36(3):299–305.
14. Skates SJ, Xu FJ, Yu YH, et al. Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers. Cancer 1995; 76(10 suppl):2004–2010.
15. Buys SS, Partridge E, Greene MH, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial. Am J Obstet Gynecol 2005; 193(5):1630–1639.
16. Jacobs I, Davies AP, Bridges J, et al. Prevalence screening for ovarian cancer in postmenopausal women by CA 125 measurement and ultrasonography. BMJ 1993; 306(6884):1030–1034.
17. Menon U, Skates SJ, Lewis S, et al. Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer. J Clin Oncol 2005; 23(31):7919–7926.
18. Skates SJ, Drescher CW, Isaacs C, et al. A prospective multi-center ovarian cancer screening study in women at increased risk. J Clin Oncol 2007; 25(18S):5510.
19. Hermsen BB, von Mensdorff-Pouilly S, Berkhof J, et al. Serum CA-125 in relation to adnexal dysplasia and cancer in women at hereditary high risk of ovarian cancer. J Clin Oncol 2007; 25(11):1383–1389.
20. Crump C, McIntosh MW, Urban N, et al. Ovarian cancer tumor marker behavior in asymptomatic healthy women: implications for screening. Cancer Epidemiol Biomarkers Prev 2000; 9(10):1107–1111.
21. Skates SJ, Horick N, Yu Y, et al. Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA 15-3, CA 72-4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions. J Clin Oncol 2004; 22(20):4059–4066.
22. Scholler N, Fu N, Yang Y, et al. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. Proc Natl Acad Sci U S A 1999; 96(20):11531–11536.
23. Xu FJ, Ramakrishnan S, Daly L, et al. Increased serum levels of macrophage colony-stimulating factor in ovarian cancer. Am J Obstet Gynecol 1991; 165(5 pt 1): 1356–1362.
24. Hellstrom I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003; 63(13):3695–3700.
25. Diamandis EP, Scorilas A, Fracchioli S, et al. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma. J Clin Oncol 2003; 21(6):1035–1043.

26. Diamandis EP, Yousef GM, Petraki C, et al. Human kallikrein 6 as a biomarker of Alzheimer's disease. Clin Biochem 2000; 33(8):663–667.
27. Mok SC, Chao J, Skates S, et al. Prostasin, a potential serum marker for ovarian cancer: identification through microarray technology. J Natl Cancer Inst 2001; 93(19): 1458–1464.
28. Kim JH, Skates SJ, Uede T, et al. Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA 2002; 287(13):1671–1679.
29. Lu KH, Patterson AP, Wang L, et al. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res 2004; 10(10):3291–3300.
30. Rosen DG, Wang L, Atkinson JN, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol 2005; 99(2):267–277.
31. Mor G, Visintin I, Lai Y, et al. Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci U S A 2005; 102(21):7677–7682.
32. Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 2002; 359(9306):572–577.
33. Kozak KR, Amneus MW, Pusey SM, et al. Identification of biomarkers for ovarian cancer using strong anion-exchange ProteinChips: potential use in diagnosis and prognosis. Proc Natl Acad Sci U S A 2003; 100(21):12343–2348.
34. Kozak KR, Su F, Whitelegge JP, et al. Characterization of serum biomarkers for detection of early stage ovarian cancer. Proteomics 2005; 5(17):4589–4596.
35. Zhang Z, Bast RC Jr., Yu Y, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Res 2004; 64(16): 5882–5890.
36. Jacobs IJ, Skates SJ, MacDonald N, et al. Screening for ovarian cancer: a pilot randomised controlled trial. Lancet 1999; 353(9160):1207–1210.
37. Partridge E, Kreimer AR, Buys S, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian Screening Trial: Results from 4 years of annual screening in a randomized trial. Gynecol Oncol 2007; 104(3 suppl):S14.
38. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast and Ovarian cancer. 2006. Accessed at www.nccn.org/professionals/physician_gls/PDF/genetics_screening.pdf version 1.
39. Burke W, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer: II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. JAMA 1997; 277(12):997–1003.
40. Armstrong K, Micco E, Carney A, et al. Racial differences in the use of BRCA1/2 testing among women with a family history of breast or ovarian cancer. JAMA 2005; 293(14):1729–1736.
41. Hogg R, Friedlander M. Biology of epithelial ovarian cancer: implications for screening women at high genetic risk. J Clin Oncol 2004; 22(7):1315–1327.
42. Karlan BY, Baldwin RL, Lopez-Luevanos E, et al. Peritoneal serous papillary carcinoma, a phenotypic variant of familial ovarian cancer: implications for ovarian cancer screening. Am J Obstet Gynecol 1999; 180(4):917–928.
43. Bosse K, Rhiem K, Wappenschmidt B, et al. Screening for ovarian cancer by transvaginal ultrasound and serum CA125 measurement in women with a familial predisposition: a prospective cohort study. Gynecol Oncol 2006; 103(3):1077–1082.
44. Liede A, Karlan BY, Baldwin RL, et al. Cancer incidence in a population of Jewish women at risk of ovarian cancer. J Clin Oncol 2002; 20(6):1570–1577.

45. Bourne TH, Campbell S, Reynolds K, et al. The potential role of serum CA 125 in an ultrasound-based screening program for familial ovarian cancer. Gynecol Oncol 1994; 52(3):379–385.
46. van Nagell JR Jr., DePriest PD, Ueland FR, et al. Ovarian cancer screening with annual transvaginal sonography: findings of 25,000 women screened. Cancer 2007; 109(9):1887–1896.
47. Fishman DA, Cohen L, Blank SV, et al. The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. Am J Obstet Gynecol 2005; 192(4): 1214–1221; discussion 1221–1222.
48. Olivier RI, Lubsen-Brandsma MA, Verhoef S, et al. CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer. Gynecol Oncol 2006; 100(1):20–26.
49. Oei AL, Massuger LF, Bulten J, et al. Surveillance of women at high risk for hereditary ovarian cancer is inefficient. Br J Cancer 2006; 94(6):814–819.
50. Stirling D, Evans DG, Pichert G, et al. Screening for familial ovarian cancer: failure of current protocols to detect ovarian cancer at an early stage according to the international Federation of gynecology and obstetrics system. J Clin Oncol 2005; 23 (24):5588–5596.
51. Dorum A, Heimdal K, Løvslett K, et al. Prospectively detected cancer in familial breast/ovarian cancer screening. Acta Obstet Gynecol Scand 1999; 78(10):906–911.
52. Vasen HF, Tesfay E, Boonstra H, et al. Early detection of breast and ovarian cancer in families with BRCA mutations. Eur J Cancer 2005; 41(4):549–554.
53. Scheuer L, Kauff N, Robson M, et al. Outcome of preventive surgery and screening for breast and ovarian cancer in BRCA mutation carriers. J Clin Oncol 2002; 20(5): 1260–1268.
54. Tailor A, Bourne TH, Campbell S, et al. Results from an ultrasound-based familial ovarian cancer screening clinic: a 10-year observational study. Ultrasound Obstet Gynecol 2003; 21(4):378–385.
55. Jordan S, Green A, Webb P. Benign epithelial ovarian tumours-cancer precursors or markers for ovarian cancer risk? Cancer Causes Control 2006; 17(5):623–632.
56. Cass I, Baldwin RL, Varkey T, et al. Improved survival in women with BRCA-associated ovarian carcinoma. Cancer 2003; 97(9):2187–2195.
57. Nossov V, Shapiro A, Li A, et al. Jewish women are at higher risk for primary peritioneal carcinoma. Gynecol Oncol 2007; 104(3):S39.
58. Levine DA, Argenta PA, Yee CJ, et al. Fallopian tube and primary peritoneal carcinomas associated with BRCA mutations. J Clin Oncol 2003; 21(22):4222–4227.
59. Brose MS, Rebbeck TR, Calzone KA, et al. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst 2002; 94 (18):1365–1372.
60. Crum CP, Drapkin R, Kindelberger D, et al. Lessons from BRCA: the tubal fimbria emerges as an origin for pelvic serous cancer. Clin Med Res 2007; 5(1):35–44.
61. Uyei A, Peterson SK, Erlichman J, et al. Association between clinical characteristics and risk-reduction interventions in women who underwent BRCA1 and BRCA2 testing: a single-institution study. Cancer 2006; 107(12):2745–2751.
62. Schwartz MD, Kaufman E, Peshkin BN, et al. Bilateral prophylactic oophorectomy and ovarian cancer screening following BRCA1/BRCA2 mutation testing. J Clin Oncol 2003; 21(21):4034–4041.

5

Current Concepts in Chemoprevention of Ovarian Cancer

Mack N. Barnes

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Lynne Cohen Program for Prevention of Women's Cancer, The University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

KEY POINTS

- Given the difficulty in developing effective screening strategies, further research into effective chemoprevention of ovarian cancer in high-risk individuals is needed.
- Oral contraceptive use appears to modify the risk of developing ovarian cancer in both high-risk and control populations.
- Chemoprevention with nonsteroidal anti-inflammatory drugs, acetaminophen, and retinoid derivatives remains investigational.

INTRODUCTION

Ovarian carcinoma is the fourth leading cause of death from cancer in the female population and the most fatal gynecologic malignancy. Approximately 70% of patients with a diagnosis of ovarian carcinoma will present as stage III or IV. Modern surgery and cytotoxic chemotherapy will produce a complete clinical response in 70% of patients (1). However, the majority of patients will experience relapse, and the response to "salvage" treatments is often brief (2). These results

are not surprising as adjuvant therapy for most advanced stage solid tumors rarely yields durable response rates. Therefore, treatment of advanced ovarian carcinoma has yielded little improvement in long-term survival over the past 30 years.

This chapter will give a brief overview of ovarian cancer screening in the general population and the historical observations on the pathogenesis of ovarian carcinoma. Chemoprevention strategies for the general population and high-risk populations will be discussed. Lastly, developmental models of spontaneous ovarian carcinogenesis and experimental methods for evaluating chemopreventive agents will be explored.

OVARIAN CANCER SCREENING IN THE GENERAL POPULATION

Recognizing that therapeutic intervention of any advanced stage solid tumor is unlikely to produce a durable cure rate, attention has been given to screening to identify early-stage disease amenable to curative resection. The fundamental flaw in this strategy centers on the relatively low prevalence of this disease in the general population. As such, the effectiveness of any screening strategy will be severely hindered by a low positive predictive value. Given an estimated prevalence of 50 cases per 100,000 population, a test with 99% specificity and 100% sensitivity would yield only 1 in 21 women undergoing surgical intervention with ovarian cancer (3). The screening trials performed to date would support these problematic statistics. A representative study by Jacobs et al. used screening with CA-125 and ultrasound in 22,000 subjects (4). These authors identified 41 women with positive screening results, of which 11 were noted to have cancer. Importantly, 70% of the identified cancers were stage III or IV. Results such as these have led an National Institutes of Health consensus conference to conclude that "there is no evidence available yet that the current screening modalities of CA-125 and transvaginal ultrasonography can be effectively used for widespread screening to reduce mortality from ovarian cancer . . ." (3).

Strategies that focus on prevention may, therefore, provide the most rational approach for meaningful reductions in deaths attributable to ovarian carcinoma. Increasing knowledge of inheritable genetic lesions in cohorts of patients allows for the identification of high-risk populations. Moreover, while the molecular events leading to the development of ovarian cancer are unknown, a carcinogenic pathway has been suggested that involves uninterrupted ovulation in a growth-stimulating hormonal milieu, leading to increased probability of genetic lesions and expansion of tumorigenic clones (5).

HISTORICAL PERSPECTIVES AND OBSERVATIONS

Pregnancy is a physiologic state associated with prolonged periods of anovulation and accompanied by high levels of circulating progesterone. Epidemiologic studies have documented that multiparity is associated with decreased risk of ovarian cancer (5). Specifically, compared with nulligravidous women with a

relative risk of 1.0, women with a single pregnancy have a relative risk of 0.6 to 0.8, with each additional pregnancy lowering risk by about 10% to 15% (5).

Several studies have also shown a reduced risk of ovarian cancer with the use of oral contraceptives (OCs) (Table 1). A reduction in risk is apparent after only a few months usage, but the apparent protection is greatest among long-term users (5). The reduction in the risk of women who have used combination estrogen-progestin OCs for at least three years is approximately 40% (6). The reduction in risk appears to persist for a number of years after discontinuation (6).

The proposition that environmental carcinogens may play a role in the development of ovarian carcinoma is supported by data on the perineal use of talc as well as surgical interventions that occlude the physiologic pathway (i.e., the oviduct) from the environment to the ovary (5,7,8). Observational studies have demonstrated a reduced risk of ovarian carcinoma following tubal ligation and hysterectomy even when the ovaries are left in situ (5).

Historically, theories regarding the pathogenesis of ovarian carcinoma have centered on the process of incessant ovulation. In theory, ovulation through the epithelial lining of the ovary with subsequent repair occurs in a hormonal milieu conducive to induction and growth of a dysregulated clone. If a patent oviduct (pathway not occluded by hysterectomy or tubal ligation) is present, potential

Table 1 Oral Contraceptives and Risk of Ovarian Cancer

Author	Date	Cases	Controls	Relative risk	95% CI
Ness (11)	2001	727	1360	0.6	0.5–0.8
Siskind (12)	2000	794	853	0.57	0.4–0.82
Narod[a] (13)	1998	207	161	0.5	0.3–0.8
Vessey (14)	1995	42	N.S.[b]	0.3	0.1–0.7
Hankinson (15)	1995	260	N.S.	0.65	0.4–1.05
Rosenberg (16)	1994	441	2065	0.6	0.4–0.8
John (17)	1993	251	114	0.62	0.24–1.6
Parazzini (18)	1991	505	1375	0.7	0.5–1.0
Franceschi (19)	1991	971	2258	0.6	0.4–0.8
Parazzini (20)	1991	91	237	0.6	0.2–1.4
Gwinn (21)	1990	436	3833	0.5	0.5–0.7
CASH GRP (22)	1987	546	4228	0.6	0.5–0.7
Tzonou (23)	1984	150	250	0.4	0.1–1.1
La Vechia (24)	1984	209	418	0.6	0.3–1.0
Rosenberg (25)	1982	136	187	0.6	0.4–0.9
Cramer (26)	1982	144	139	0.11	0.04–0.33
Willett (27)	1981	47	464	0.8	0.4–1.5
Weiss (28)	1981	112	552	0.57	N.S.

[a]Study population comprises carriers of BRCA1 and BRCA2 mutations.
[b]Not stated.

carcinogens could gain entry, thus influencing the carcinogenic potential of early transformed cells. Incessant ovulation therefore increases the probability of mutational events that lead to propagation of an initiated cell; this may lead to additional events associated with the transformation to a clinically relevant cancer. The proposed protective benefit of both pregnancy and the use of OCs has centered on reduction of ovulatory events leading to decreased probability of genetically damaged cells. However, recent investigations have suggested that progestins may influence apoptosis leading to the demise of cells that are molecularly damaged and thus may become malignant (9,10).

CHEMOPREVENTION STRATEGIES

Oral Contraceptives in the General Population

OCs have been demonstrated repeatedly to reduce the subsequent risk of ovarian carcinoma in observational studies (Table 1) (11–28). Historically, the effect has been attributed to reduction in the number of ovulatory events associated with regular use of OCs. More recent data, however, suggest that the protective effect of OCs may be more complex. An innovative study supporting the use of progestins as chemopreventive agents in ovarian carcinoma was recently published by Rodriguez et al. In a randomized design, these authors examined the effect of levonorgestrel on ovarian epithelium in 130 ovulatory macaque monkeys (9). This progestin was administered for over 35 months, at the end of which the animals were sacrificed and the ovarian epithelium was examined for apoptosis using immunohistochemistical techniques. These authors demonstrated significantly increased apoptotic cell counts in the ovarian epithelium of animals exposed to progesterone and hypothesized that progestin-induced apoptosis of the ovarian epithelium is responsible for the chemopreventive effect of OCs. This idea is a deviation from the widely accepted theory that suppression of incessant ovulation is responsible for reduced risk of ovarian cancer (9). Moreover, they theorized that by increasing the apoptotic tendency of ovarian epithelial cells that have incurred genetic damage, OC progestins may function to enhance the apoptotic death of aberrant cells that are not yet neoplastic, thereby decreasing the risk of ovarian cancer.

Several studies have suggested that the degree of protection is associated with the duration of use of OCs (16,18,19,29–31). The length of protection appears to be strongly correlated with duration of use. Prolonged risk reduction has been reported when OCs are used longer than four to six years, and minimal benefit is observed if its use is restricted to six months to two years (16,18,30,31). Moreover, the protective benefit of OCs is diminished with time and returns to baseline approximately 15 years after last regular use of OCs (16,18,19).

The influence of the estrogen/progestin content of a particular OC on subsequent ovarian cancer risk is an issue that needs further study. Ness et al. demonstrated identical risk reduction for OCs with high-estrogen/high-progesterone

content when compared to low-estrogen/low-progesterone content pills (32). However, a recent observational study by Schildkraut et al. suggested that low-progesterone OC formulations were associated with a significantly higher risk of ovarian cancer when compared with high-progesterone potency OC formulations (33).

The protective effect of OCs would appear to be consistent across races as John et al. demonstrated a reduction in risk of 0.6 in African-American women with OC use of six years or more (17).

Oral Contraceptive Use in a High-Risk Population

One of the strongest risk factors for the subsequent development of ovarian cancer is a family history of multiple affected members. Studies by Gross and Schlesselman and Tavani et al. have demonstrated a risk reduction with OC use in women with strong family histories (34,35). These results have led Tavani et al. to suggest that five years of OC use in "high-risk" women can reduce ovarian cancer risk to the level observed in studies of low-risk women and those who never used OCs but have parity as a protective factor (35). Several studies have been performed in women with known BRCA mutations. While an initial study by Narod et al. of 207 women with confirmed BRCA1/BRCA2 mutations demonstrated a statistically significant risk reduction by 50% with OC use (13), this finding was not confirmed in a subsequent study of 244 women by Modan et al. where a risk reduction was present but not statistically significant (36). Further studies have supported a reduction in risk from OC use in this high-risk population. Whittemore et al. suggested that long-term OC use reduced the risk of ovarian cancer among women who carry mutations of BRCA1 or BRCA2 (37). In their case ($N = 147$) control ($N = 304$) study, use of OCs for six years was associated with an odds ratio of 0.62, although the confidence interval did include 1.0 (0.35–1.09). The largest study to date involving women with deleterious mutations in BRCA1 or BRCA2 is contributed by McLaughlin et al. (38). These authors examined 799 carriers with a history of ovarian cancer compared with 2424 control women. Use of OCs significantly reduced the risk of ovarian cancer in carriers of BRCA1 mutation (OR = 0.56, CI = 0.45–0.71) and BRCA2 mutation (OR = 0.39, CI = 0.23–0.66).

Nonsteroidal Anti-inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) have generated significant enthusiasm as chemoprevention agents, particularly in the area of colon carcinoma (39). While some observational studies of ovarian carcinoma suggest a risk reduction with the use of some NSAID derivatives, the rational for their use as chemopreventive agents has been lacking. However, recent animal studies examining the effects of NSAIDs on normal ovulation shed light into the potential mechanisms that may be operative. Foremost, several classes of

NSAIDs have been demonstrated to inhibit ovulation across multiple species of vertebrates (40). Indomethacin appears to be a potent inhibitor of ovulation in a dose-dependent fashion (40–42). In vitro analysis has suggested that interference with local prostaglandins may interfere with apoptosis of surface epithelial cells required for surface rupture of the dominant follicle (40). NSAIDs have also been demonstrated to result in potent growth inhibition and apoptosis in ovarian cancer cells (43). More recently, Roland et al. have suggested an association between overexpression of COX-2 and subsequent loss of ovarian epithelial basement membrane as a possible neoplastic precursor event (44). As such, these authors postulate that the beneficial effect of COX-2 inhibition may prevent this basement membrane loss.

Interesting evidence for an antigonadotropic effect in animals also exists for acetominophen. Acetaminophen has a phenol ring, similar to estradiol, and an acetyl group similar to progesterone, indicating a potential sex steroid antagonist property (45). Evidence of this antigonadotropic property was suggested by toxicology studies demonstrating uterine, ovarian, and testicular atrophy in rats fed at 25,000 ppm (46). In this study, the frequency of ovarian cysts was 23% in mice exposed to 3000 to 6000 ppm acetominophen compared with 38% of mice either not exposed or minimally exposed (46).

Several observational studies have been performed focusing on the association of analgesic use and risk of ovarian cancer with inconclusive results (Table 2). Cramer et al. demonstrated a trend toward reduced risk of 0.75 with at least weekly use of aspirin over a six-month period, while Tavani et al. demonstrated a reduced risk of 0.72 in "former users of aspirin" (45,47). One should interpret these results with caution; however, as the 95% confidence intervals included 1.0 and data from a study by Moysich et al. demonstrated no evidence of reduced risk in aspirin users (48).

Table 2 Analgesic Utilization and Risk of Ovarian Cancer

Author/Analgesic	Cases	Controls	Relative risk	95% CI
Moysich (48)				
Aspirin	547	1094	1.0	0.73–1.39
Acetominophen	547	1094	0.56	0.34–0.86
Tavani (47)				
Aspirin	749	898	0.72	0.35–1.47
Rosenberg (50)				
Aspirin	780	2570	0.5	0.2–0.9
Acetominophen	780	2570	0.9	0.6–1.4
Cramer (45)				
Aspirin	563	523	0.75	0.52–1.1
Ibuprofen	563	523	1.03	0.64–1.64
Acetominophen	563	523	0.52	0.31–0.86

Epidemiologic evidence also exists for acetominophen. Cramer et al. demonstrated a reduction of risk to 0.39 (95% CI, 0.21–0.74) when acetominophen was used on a daily basis (45). Similarly, Moysich et al. observed a risk reduction of 0.32 (95% CI, 0.27–0.97) in women with "greatest frequency" of use (48). Rodriguez et al. also reported a 45% lower death rate from ovarian cancer in women using acetominophen daily; however, the confidence interval included one (49). Reports suggesting a potential protective benefit of NSAIDs are countered by a case-control study of Rosenberg et al. examining the potential protective benefit of regular acetominophen use (50). These authors found little evidence of an ovarian cancer risk reduction associated with this analgesic. However, taken together, current data presents a rational argument for the continued study of these agents in clinical and preclinical investigations.

Retinoid Derivatives

Theoretically, an agent used for chemoprevention should have the capacity to cause cellular differentiation leading to apoptosis in an initiated cell destined to become frankly malignant. Experimental evidence does exist for growth inhibition and promotion of cellular differentiation by retinoid derivatives in ovarian cancer cells. As such, in vitro experiments using retinoids in cancer cells have demonstrated growth inhibition after application of all-trans-retinoic acid in CAOV3 cells (51). Additionally, Caliero et al. and Brooks et al. have demonstrated increased induction of cytokeratins in ovarian cancer cells when exposed to retinoic acid, suggesting a role in the differentiation of cells (52,53). Finally, Supino et al. exposed ovarian cancer cell lines to fenretinide and observed apoptosis in A2780 ovarian cancer cells (54). The ability of retinoid derivatives to prevent ovarian carcinoma is also suggested by an observational study by Veronisi et al. and De Palo et al. (55,56). These authors reported a phase III trial of fenretinide for the prevention of second breast cancers. Subgroup analysis demonstrated a significantly lower incidence of development of ovarian cancer in the treatment group. These results, however, must be interpreted with caution to the limited number of ovarian cancer cases and the statistical pitfalls inherent in subgroup analyses. These studies helped define the basis for a clinical trial (GOG190) examining the tissue effects of administration of fenretidine in women undergoing prophylactic oophorectomy due to high familial risk of ovarian cancer. This study unfortunately closed secondary to poor accrual.

DEVELOPMENT OF MODELS OF SPONTANEOUS OVARIAN CARCINOGENESIS

Rodent-Based Models

Investigators have attempted to develop epithelial ovarian cancer models in rodents. The major problems, however, are that the ovarian cancer incidence is low, the

cancers induced are not primarily of epithelial origin, the latency period of the cancers is extremely long, or the carcinogen administration is time consuming (i.e., requiring surgery or breeding of animals). A rodent model that overcomes these problems would allow the rapid screening of new compounds for their chemopreventive activity and permit the measurement of biomarkers for the early detection of this cancer. Recent work by Dinulescu et al. describes an intriguing murine model of ovarian carcinogenesis incorporating a K-ras gene manipulation that may be amenable to further study of novel compounds directed at preventing ovarian carcinoma (57). Mice with activated K-ras are crossed with mice that have a PTEN gene flanked by stretches of DNA targeted by recombinase. Following injection of Cre recombinase construct into the ovarian bursa of these mice, with resultant expression of K-ras and inactivation of PTEN, metastatic endometrioid ovarian adenocarcinoma is observed.

Avian Hen Model

While studies investigating "induced" carcinomas have been performed, they are hindered by biologic differences between induced and spontaneous tumor formation. Identification of spontaneous ovarian carcinogenesis in the laying hen (*Gallus domesticus*) may provide the answer to this dilemma. As detailed in a report by Fredrickson, at a mean age of four years, 19% of hens had the spontaneous development of a histologically confirmed ovarian adenocarcinoma (58). Fredrickson examined 466 hens with an age range of two to seven years (58). This author noted an overall incidence of ovarian tumors of 32%. A trend toward increasing incidence was noted with increasing age; 12% at mean age 3.9 years, 32% at mean age 4.2 years, and 50% at mean age 6.1 years. Histologic confirmation of adenocarcinoma was obtained. This finding is supported by the pathologic investigation of 1000 chickens by Papsolomontos et al. where ovarian adenocarcinoma was common in older birds (59). An attractive aspect regarding these "model" tumors is that they arise spontaneously without use of exogenous chemical carcinogens. Moreover, the postulated etiology giving rise to these tumors is incessant ovulation (laying hens ovulate every 28 hours), recapitulating the theoretical instigating event in human ovarian adenocarcinoma.

We have performed necropsy on 200 two-year-old hens. Of these hens, nine were thought to have gross evidence of metastatic ovarian carcinoma and ascites (60). These specimens were then submitted to a University of Alabama at Birmingham pathologist for histologic review. Eight of nine specimens were documented to be papillary serous carcinomas of the ovary. While the purpose of this study was not to identify the absolute rate of ovarian cancer in two-year-old hens, the 4% rate of grossly identifiable ovarian cancer in these young hens is consistent with the report by Frederickson where the rate increases with the age of the hen. Furthermore, the histologic appearance of these tumors is consistent with a papillary serous adenocarcinoma that is similar in microscopic appearance to human epithelial ovarian cancers. The most important finding in this study is

the identification of known human ovarian cancer tissue biomarkers that are reactive across species and can be detected in avian tumors. Cross-reactive biomarkers included cytokeratin, AE1/AE3, EGFr, erbB-2, Lewis Y, CEA, and TAG-72 (60). Using this model, a second study suggested decreased risk of development of genital tract adenocarcinoma associated with decreased ovulatory activity induced by administration of Depo-Provera (61). In an animal model of spontaneous ovarian carcinoma, manipulation of these biomarkers may yield clues as to the etiology of ovarian carcinoma and the biologic effects of preventive compounds. More importantly, alterations in biomarker expression induced by chemopreventive agents in animal studies can then be targeted when applied to human clinical trials of putative chemopreventive compounds.

EVALUATION OF CHEMOPREVENTIVE AGENTS IN INDIVIDUALS AT RISK FOR OVARIAN CARCINOMA

To date, the precise precancerous lesion that subsequently undergoes malignant transformation leading to the clinical syndrome of ovarian carcinoma has not been identified. However, an important study by Salazar et al. yielded clues as to what the early pathogenic changes might be leading to ovarian cancer in a high-risk population of women (62). These authors examined the ovaries resected prophylactically from 20 women deemed to be at high familial risk for the subsequent development of ovarian cancer. As noted previously, the ovaries from these patients demonstrated a statistically increased incidence of histologic changes considered to be associated with the progression to carcinoma.

Important in the evaluation of tissues is the immunohistochemical assessment of potential surrogate endpoint biomarkers that allow for the detection of genetically induced cellular alterations that are associated with the development of subsequent cancer. Markers that have potential utility include alterations in epidermal growth factor, erbB-2, transforming growth factor, vascular endothelial growth factor (VEGF), proliferation markers, and apoptosis assays. The value of identification of these markers results from the ability to detect how putative chemopreventive agents might affect them and, therefore, alter the course of progression to cancer.

These concepts have been united in a clinical model to evaluate the effects of chemopreventive agents on noncancerous ovarian epithelial tissues. In this model, patients considered to have an increased risk for the development of ovarian carcinoma by virtue of a documented genetic alteration or an inherited "familial" risk are exposed to a chemopreventive agent prior to undergoing prophylactic oophorectomy. The resected ovaries are then examined for morphologic changes and biomarker alterations relative to "normal" control ovaries. A feasibility study has been performed using this patient population (63). Enrollment of patients was completed in 16 months. Of 29 eligible patients, 20 enrolled onto study. One patient from each group did not complete surgical intervention. No significant differences were observed in the enrollment

characteristics between the groups. No occult cases of ovarian cancer were identified and no differences in the presence of follicular cysts, hemorrhagic cysts, or inclusion cysts were noted on initial pathologic review. While the mean serum VEGF levels obtained following administration of a COX-2 inhibitor were lower than preadministration in five of six patients, statistical significance in this difference was not observed ($p = 0.359$). However, this is most likely due to the small number of serum samples available. Certainly, further studies in this population are needed.

Current Clinical Practice of Chemoprevention of Ovarian Cancer in High-Risk Populations

For patients who decline risk-reducing surgery, OC pills remain the most well studied and effective chemopreventive agent to date. Given the current low-dose formulations available today, OC should be offered to women at high risk for ovarian cancer. However, in this high-risk population, risk of breast cancer must also be considered and discussed with patients. In a matched case-control study by Narod et al., use of OC in women with a germline BRCA1 mutation was associated with a moderately increased risk of breast cancer (OR = 1.2, CI = 1.02−1.40) (64). The associated risk increased among women with BRCA1 mutations if they used OC for at least five years (OR = 1.20, CI = 1.11−1.60); if they first used OCs before 1975 (OR = 1.42, CI = 1.17–1.75); and if they used OC prior to age 30 (OR = 1.29, CI = 1.09−1.52). For women with a BRCA2 mutation, there was no association with an increased risk (OR = 0.94, CI = 0.72−1.24). However, the data for BRCA2 carriers was limited and the confidence interval crosses one.

A study by Milne et al. found that there was no increased risk to BRCA1 carriers, but in fact a slightly decreased risk (OR = 0.22, CI = 0.10–0.49, $p < 0.001$) if the women used OC for at least one year (65). There was an increased risk in BRCA1 positive women, although not statistically significant for women who used OC before 1975. Again, data from BRCA2 positive women must be interpreted with caution, given the small numbers and inclusion of 1.0 in the 95% confidence interval. In this population, OC use was associated with an OR of 1.02, CI of 0.34 to 3.09. Further studies of the association between OCs and subsequent breast cancer risk in women with BRCA1 and BRCA2 mutations are necessary to clarify this issue.

CONCLUSIONS

To date, prevention of ovarian carcinoma represents an extremely neglected field of study. However, continued developments in molecular biology, biologic therapeutics, and pathogenesis/carcinogenesis are creating a solid rationale to explore prevention as a rational approach to reduce deaths attributable to ovarian carcinoma. It is our intention that by disseminating information regarding recent

advances in the prevention of ovarian cancer, including chemoprevention, new investigational endeavors will be stimulated.

CASE REPORT

C.P. is a 32-year-old gravida 0 with a known BRCA2 mutation and no personal history of cancer. She initially presented for genetic counseling two years ago after a sibling was diagnosed with a *BRCA2* mutation. Her father was diagnosed with breast cancer at age 72. Two of her four sisters had premenopausal breast cancer, diagnosed at the age of 44 and 48, respectively. No other relatives on her paternal or maternal side have a history of cancer.

C.P. had menarche at age 18 and a past history of four years of oral contraceptive use. Prior to the knowledge of her *BRCA* status, she performed monthly self-breast examinations and had yearly mammography and clinical breast and pelvic examinations.

C.P. was counseled that bilateral salpingo-oophorectomy would reduce breast cancer risk by 50% as well as reduce ovarian cancer risk by greater than 90%. As she desired to retain her childbearing potential, C.P. opted not to undergo risk-reductive surgery, declining bilateral salpingo-oophorectomy. She was further counseled regarding chemoprevention strategies, but she chose the option of risk reduction through oral contraceptive pills.

LEARNING POINTS

- Although the greatest reduction in risk of developing ovarian cancer comes from a bilateral salpingo-oophorectomy, for patients who decline surgery, chemoprevention with oral contraception pills is currently the most effective option.
- The use of low-dose oral contraceptive for five years or less has not been associated with an appreciable increase in the risk of breast cancer in carriers of *BRCA1/BRCA2*.

REFERENCES

1. McGuire W, Hoskins W, Brady M. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Eng J Med 1996; 334:1–6.
2. Partridge E, Barnes M. Epithelial ovarian cancer: prevention, diagnosis, and treatment. CA Cancer J Clin 1999; 49:297–316.
3. NIH Consensus Development Panel on Ovarian Cancer. Ovarian cancer; screening, treatment and follow-up. JAMA 1995; 273:491–497.
4. Jacobs I, Davies A, Bridges J, et al. Prevalence screening for ovarian cancer in postmenopausal women by CA125 measurement and ultrasonography. BMJ 1993; 306:1030–1034.

5. Engstrom P, Meyskens F. Cancer prevention. In: Hoskins W, Perez C, Young R, eds. Principles and Practice of Gynecologic Oncology. Philadelphia: Lippincott-Raven Publishers, 1997.
6. Hankinson S, Colditz G, Hunter D, et al. A quantitative assessment of oral contraceptive use and risk of ovarian cancer. Obstet Gyencol 1992; 80:708–714.
7. Longo D, Young R. Cosmetic talc and ovarian cancer. Lancet 1979; 2:349–351.
8. Wong C, Hempling R, Piver M, et al. Perineal talc exposure and subsequent epithelial ovarian cancer: a case control study. Obstet Gynecol 1999; 93:372–376.
9. Rodriguez G, Walmer D, Cline M, et al. Effect of progestin on the ovarian epithelium of macaques: cancer prevention through apoptosis. J Soc Gynecol Invest 1998; 5:271–276.
10. Risch H. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst 1998; 90:1774–1786.
11. Ness R, Grisso J, Vergona R, et al. Oral contraceptives, other methods of contraception, and risk reduction for ovarian cancer. Epidemiology 2001; 12:307–312.
12. Siskind V, Green A, Bain C, et al. Beyond oral contraceptives and epithelial ovarian cancer. Epidemiology 2000; 11:106–110.
13. Narod S, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. Hereditary ovarian cancer clinical study group. N Engl J Med 1998; 339:424–428.
14. Vessey M, Painter R. Endometrial and ovarian cancer and oral contraceptives-findings in a large cohort study. Br J Cancer 1995; 71:1340–1342.
15. Hankinson S, Colditz G, Hunter D, et al. A prospective study of reproductive factors and risk of epithelial ovarian cancer. Cancer 1995; 76:284–289.
16. Rosenberg L, Palmer J, Zauber A, et al. A case control study of oral contraceptive use and invasive epithelial ovarian cancer. Am J Epidemiol 1994; 139:654–661.
17. John E, Whittemore A, Harris R, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of seven US case control studies. Epithelial ovarian cancer in black women. J Natl Cancer Inst 1993; 85:142–147.
18. Parazzini F, La Vechia C, Negri E, et al. Oral contraceptive use and the risk of ovarian cancer: an Italian case control study. Eur J Cancer 1991; 27:594–598.
19. Franceschi S, Parazzini F, Negri, et al. Pooled analysis of 3 European case control studies of epithelial ovarian cancer. Oral contraceptive use. Int J Cancer 1991; 49:61–65.
20. Parazzini F, Restelli C, La Vechia C, et al. Risk factors for epithelial ovarian tumors of borderline malignancy. Int J Epidemiol 1991; 20:871–877.
21. Gwinn M, Lee N, Rhodes P. Pregnancy, breast feeding, and oral contraceptives and the risk of ovarian cancer. J Clin Epidemiol 1990; 43:559–568.
22. Cancer and Steroid Hormone (CASH) Group. The reduction of risk of ovarian cancer associated with oral contraceptive use. N Engl J Med 1987; 316:650–655.
23. Tzonou A, Day N, Trichopoulos D, et al. The epidemiology of ovarian cancer in Greece: a case control study. Eur J Cancer Clin Oncol 1984; 20:1045–1052.
24. La Vechia C, Franceschi S, Decarli A. Oral contraceptive use and the risk of epithelial ovarian cancer. Br J Cancer 1984; 50:31–34.
25. Rosenberg L, Shapiro S, Slone S, et al. Epithelial ovarian cancer and combination oral contraceptives. JAMA 1982; 247:3210–3212.
26. Cramer D, Hutchinson G, Welch W. Factors affecting the association of oral contraceptives and ovarian cancer. N Engl J Med 1982; 307:1047–1051.
27. Willett W, Bain C, Hennekens C. Oral contraceptives and risk of ovarian cancer. Cancer 1981; 48:1684–1687.

28. Weiss N, Lyon J, Liff J, et al. Incidence of ovarian cancer in relation to the use of oral contraceptives. Int J Cancer 1981; 28:669–671.
29. Wu M, Whittemore A, Paffenbarger R, et al. Personal and environmental characteristics related to epithelial ovarian cancer. Reproductive and menstrual events and oral contraceptive use. Am J Epidemiol 1988; 128:1216–1227.
30. Gross T, Schlesselman J, Stadel B. The risk of epithelial ovarian cancer in short term users of oral contraceptives. Am J Epidemiol 1992; 136:46–53.
31. Hartge P, Whittemore A, Itnyre J et al. Rates and risks of ovarian cancer in subgroups of white women in the Unites States. Obstet Gynecol 1994; 84:760–764.
32. Ness R, Grisso J, Klapper J, et al. Risk of ovarian cancer in relation to estrogen and progestin dose and use characteristics of oral contraceptives. Am J Epidemiol 2000; 152:233–241.
33. Schildkraut J, Calingaert B, Marchbanks P, et al. Impact of progestin and estrogen potency in oral contraceptives on ovarian cancer risk. J Natl Cancer Inst 2002; 94:32–38.
34. Gross T, Schlesselman J. The estimated effect of oral contraceptive use on the cumulative risk of ovarian cancer. Obstet Gynecol 1994; 83:419–424.
35. Tavani A, Ricci E, La Vechia C, et al. Influence of menstrual and reproductive factors on ovarian cancer risk in women with and without family history of breast or ovarian cancer. Int J Epidemiol 2000; 29:799–802.
36. Modan B, Hartge P, Hirsh-Yechezkel G, et al. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a BRCA1 or BRCA2 mutation. N Engl J Med 2001; 345:235–240.
37. Whittemore A, Balise R, Pharoe P, et al. Oral contraceptive use and ovarian cancer risk among carriers of BRCA1 or BRCA2 mutations. Br J Cancer 2004; 91:1911–1915.
38. McLaughlin J, Risch H, Lubinski J, et al. Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case control study. Lancet Oncol 2007; 8:26–34.
39. Ahnen D. Colon cancer prevention by NSAIDs: what is the mechanism of action? Eur J Surg 1998; 582s:111–114.
40. Murdoch W, Lund S. Prostaglandin independent anovulatory mechanism of indomethacin action: inhibition of tumor necrosis factor alpha induced sheep ovarian cell apoptosis. Biol Reprod 1999; 61:1655–1659.
41. Espey L, Khonda H, Mori T, et al. Rat ovarian prostaglandin levels and ovulation as indicators of the strength of non-steroidal anti-inflammatory drugs. Prostaglandins 1988; 36:875–879.
42. Ando M, Kol S, Irahara M, et al. Non-steroidal anti-inflammatory drugs (NSAIDs) block the late, prostanoid-dependent/ceramide independent component of ovarian IL1 action: implications for the ovulatory process. Mol Cell Endocrinol 1999; 157: 21–30.
43. Burford C, Barnes M, Oelschlager D, et al. Effects of non-steroidal anti-inflammatory agents (NSAIDS) on ovarian carcinoma cell lines: preclinical evaluation of NSAIDs as chemopreventive agents. Clin Cancer Res 2002; 8:202–209.
44. Roland I, Yang W, Yang D, et al. Loss of surface and cyst epithelial basement membranes and preneoplastic morphologic changes in prophylactic oophorectomies. Cancer 2003; 98:2607–2623.
45. Cramer D, Harlow B, Titus-Ernstoff L, et al. Over the counter analgesics and risk of ovarian cancer. Lancet 1998; 351:104–107.

46. National Toxicology Program. Toxicology and carcinogenesis studies of acetominophen in F344/N rats and B6C3F1 mice. Technical report series no 394. NIH publication no 93-2849, Research Triangle Park, North Carolina, USA, 1993.
47. Tavani A, Gallus S, La Vechia C, et al. Aspirin and ovarian cancer: an Italian case control study. Ann Oncol 2000; 11:1171–1173.
48. Moysich K, Mettlin C, Piver M, et al. Regular use of analgesic drugs and ovarian cancer risk. Cancer Epidemiol Biomarkers Prev 2001; 10:903–906.
49. Rodriguez C, Henley S, Calle E, et al. Paracetamol and risk of ovarian cancer mortality in a prospective study of women in the USA. Lancet 1998; 352:1354–1355.
50. Rosenberg L, Palmer J, Rao R, et al. A case-control study of analgesic use and ovarian cancer. Cancer Epidemiol Biomarkers Prev 2000; 9:933–937.
51. Wu S, Zhang D, Donigan A, et al. Effects of conformationally restricted synthetic retinoids. J Cell Biochem 1998; 68:378–388.
52. Caliero M, Marmouget C, Guichard S, et al. Response of four human ovarian carcinoma cell lines to all-trans retinoic acid: relationship with induction of differentiation and retinoic acid receptor expression. Int J Cancer 1994; 56:743–748.
53. Brooks S, Timmerman J, Lau C, et al. Effect of differentiation agents on expression of CA125, alkaline phosphatase, and cytokeratins in human ovarian adenocarcinoma cells (OVCA 433). Gynecol Oncol 1991; 42:265–272.
54. Supino R, Crosti M, Clerici M, et al. Induction of apoptosis by fenretinide (4HPR) in human ovarian carcinoma cells and its association with retinoic acid receptor expression. Int J Cancer 1996; 65:491–497.
55. Veronisi U, De Palo G, Marubini E, et al. Randomized trial of fenretidine to prevent second breast malignancy in women with early breast cancer. J Natl Cancer Inst 1999; 91:1847–1856.
56. De Palo G, Veronesi U, Camerini T, et al. Can fenretidine protect women against ovarian cancer? (letter). J Natl Cancer Inst 1995; 87:146–147.
57. Dinulescu D, Ince T, Quade B, et al. Role of K-ras and Pten in the development of mouse models of endometriosis and endometroid ovarian cancer. Nat Med 2005; 11: 63–70.
58. Fredrickson T. Ovarian tumors of the hen. Environ Health Perspect 1987; 73:35–51.
59. Papsolomontos P, Appleby E, Mayor O. Pathologic findings in condemned chickens: a survey of 1000 carcases. Vet Rec 1969; 85:459–464.
60. Burford C, Barnes M, Berry W, et al. Immunohistochemical expression of molecular markers in an avian model: a potential model for preclinical evaluation of agents for ovarian cancer chemoprevention. Gynecol Oncol 2001; 81:373–379.
61. Barnes M, Berry W, Straughn M, et al. A controlled trial of ovarian cancer chemoprevention using medroxyprogesterone acetate in an avian model of spontaneous ovarian carcinogenesis. Gynecol Oncol 2002; 87:57–63.
62. Salazar H, Godwin A, Daly M, et al. Microscopic benign and invasive malignant neoplasms and a cancer prone phenotype in prophylactic oophorectomies. J Natl Cancer Inst 1996; 88:1810–1820.
63. Barnes MN, Chheing DF, Dreher M, et al. Feasibility of performing chemoprevention trials in women at elevated risk of ovarian carcinoma: initial examination of celecoxib as a chemopreventive agent. Gynecol Oncol 2005; 98(3):376–382.
64. Narod SA, Dubé MP, Klijn J, et al. Oral contraceptives and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2002; 94(23):1773–1779.
65. Milne RL, Knight JA, John EM, et al., Oral contraceptive use and risk of early-onset breast cancer in carriers and noncarriers of BRCA1 and BRCA2 mutations. Cancer Epidemiol Biomarkers Prev 2005; 14(2):350–356.

6

Risk-Reducing Salpingo-Oophorectomy for the Prevention of Inherited Breast and Ovarian Cancer

Sharyn N. Lewin

Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

Noah D. Kauff

Clinical Genetics Services, Department of Medicine, and Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

KEY POINTS

- A risk-reducing salpingo-oophorectomy reduces the risk of ovarian cancer by 85% to 90%.
- A risk-reducing salpingo-oophorectomy reduces the risk of breast cancer by 40% to 70%.
- The risk of developing primary peritoneal cancer after a risk-reducing salpingo-oophorectomy is 1% to 6%.
- Surgical technique should remove the entire fallopian tubes and ovaries.
- The surgical team must communicate the indication for surgery to the pathologist to ensure complete and serial sectioning of the ovaries and fallopian tubes to rule out occult cancers.
- Hysterectomy may be performed as part of the risk-reducing surgery. However, no cases of fallopian tube cancer developing within the intramural (uterine) segment of the fallopian tube have been reported.

INTRODUCTION

Heritable mutations in one of the *BRCA* genes cause approximately 8% to 13% of epithelial ovarian cancers (1–3). Women with mutations in *BRCA1* have a 35% to 60% chance of developing a *BRCA*-associated gynecologic (ovarian, fallopian tube or primary peritoneal) cancer by age 70, corresponding to a relative risk of 30 to 45 times that of women in the general population (4–6). Similarly, women with mutations in *BRCA2* have a 10% to 27% chance of developing a *BRCA*-associated gynecologic cancer by age 70, corresponding to a relative risk of 6 to 20. Women with mutations in either of these genes are also at tremendously increased risk of breast cancer, with 56% to 84% of mutation carriers developing breast cancer by age 70 (4–7). Over the past decade, a great deal has been learned about the efficacy of risk-reducing strategies in women with an inherited predisposition secondary to a mutation in one of these genes. Unfortunately, currently available ovarian cancer screening modalities have not proven to be effective for women with an inherited risk of ovarian cancer. While chemoprevention with oral contraceptives may reduce the risk of ovarian cancer, both the incomplete prevention conferred against ovarian cancer as well as possible deleterious impact on breast cancer risk limit their use as a risk-reduction strategy in isolation. Given these issues, risk-reducing salpingo-oophorectomy (RRSO) has become one of the cornerstones of risk reduction for women with an inherited risk of ovarian cancer and should be considered in all women who harbor a deleterious germline mutation in *BRCA1* or *BRCA2*. In this chapter, the sentinel studies supporting RRSO will be reviewed as well as the salient issues surrounding both pre- and postoperative counseling.

THE HISTORY BEHIND RISK-REDUCING SALPINGO-OOPHORECTOMY

From a historical perspective, the role of salpingo-oophorectomy in breast cancer prevention and treatment long predates its role in ovarian cancer prevention. In 1889, salpingo-oophorectomy was first suggested for breast cancer treatment by the German surgeon Schinizer. Reports of the procedure first being performed, however, did not surface until seven years later [reviewed by Love and Philips (8)]. In 1968, Feinleib reported that premenopausal oophorectomy decreased subsequent breast cancer occurrence (9), and in 1982, Brinton proposed that prophylactic oophorectomy may be beneficial in reducing breast cancer risk in women with a strong family history of the disease (10).

The notion of oophorectomy to prevent the development of ovarian cancer was first proposed in 1950 by a pathologist, A.F. Liber (11). He described a family of a mother and her five daughters who all developed pathologically confirmed ovarian cancer and suggested that other family members may want to consider oophorectomy before ovarian cancer could develop. Over the next 30 years, oophorectomy was commonly performed in women with a family history of the disease. In 1982, however, Tobacman reported on a series of three

women from 16 families who developed disseminated adenocarcinoma histologically indistinguishable from ovarian cancer (now referred to as primary peritoneal cancer) after undergoing prophylactic oophorectomy due to a strong family history of the disease (12).

Following this initial report, in 1993, Piver et al. analyzed data from 931 families with at least two first- or second-degree relatives with ovarian cancer. In this series, Piver and colleagues found six cases of primary peritoneal cancer after prophylactic oophorectomy in 324 women who had undergone the procedure (13). In 1995, Struewing reanalyzed data from Tobacman's 16 hereditary ovarian cancer families and examined the incidence of cancer in the first-degree relatives of the individuals with ovarian cancer and compared this to the incidence of ovarian cancer that would be expected in the general population (14). In this study, Struewing demonstrated a 24-fold increased risk of ovarian cancer in 436 women who had not undergone oophorectomy compared with a 13-fold increased risk of "ovarian" cancer in 44 women who had undergone oophorectomy. Importantly, this was not a statistically significant difference.

In 1997, the Cancer Genetic Studies Consortium reviewed the available evidence and stated, "There is insufficient evidence to recommend for or against prophylactic oophorectomy as a measure for reducing ovarian cancer risk. Women with *BRCA1* mutations should be counseled that this is an option available to them. Those considering prophylactic oophorectomy should be counseled that cancer has been documented to occur after the procedure" (15).

RISK-REDUCING SALPINGO-OOPHORECTOMY IN *BRCA1* AND *BRCA2* MUTATION CARRIERS

With this background, a group from Memorial Sloan-Kettering Cancer Center (MSKCC) initiated a prospective follow-up study to ascertain the efficacy of RRSO in individuals with deleterious *BRCA* mutations (16). From 1995 to 2001, 173 women with a germline mutation in *BRCA1* or *BRCA2* who were 35 years of age or older and had ovaries in situ were enrolled onto one of three prospective follow-up studies. Of these 173 women, 101 (58%) elected RRSO, a median of 3.6 months after receiving the results of genetic testing, while 72 (42%) chose surveillance. At the time of RRSO, three patients were found with occult ovarian (2) or fallopian tube (1) cancers. After excluding these women from the actuarial analysis, during two years of follow-up, one peritoneal and three breast cancers were diagnosed in the 98 women who underwent RRSO (at 16.3 and a mean of 10.3 months after RRSO, respectively). This was compared to five *BRCA*-associated gynecologic and eight breast cancers in the 72 women electing surveillance. In this series, RRSO was associated with a 75% reduction in the combined risk of breast and gynecologic cancer (HR = 0.25; 95% CI, 0.08–0.74) (16).

Concurrently with this report, a retrospective study from the Prevention and Observation of Surgical Endpoints (PROSE) study group also demonstrated

Table 1 Studies Evaluating the Impact of Risk-Reducing Salpingo-Oophorectomy on Breast or *BRCA*-Associated Gynecologic Cancer Risk in Carriers of *BRCA1* and *BRCA2* Mutations

Study	Design	*N* (RRSO)	Gynecologic cancer	Breast cancer
Kauff et al. (16) NEJM 2002	Prospective	98	HR = 0.15 (95% CI, 0.02–1.31)	HR = 0.32 (95% CI, 0.08–1.20)
Rebbeck et al. (17) NEJM 2002	Retrospective	259	HR = 0.04 (95% CI, 0.01–0.16)	HR = 0.53 (95% CI, 0.33–0.84)
Rutter et al. (18) JNCI 2003	Retrospective	251	OR = 0.29 (95% CI, 0.12–0.73)	
Eisen et al. (19) J Clin Oncol 2005	Retrospective	1439		OR = 0.46 (95% CI, 0.32–0.65)
Domchek et al. (20) Lancet Oncol 2006	Prospective	155	HR = 0.11 (95% CI, 0.03–0.47)	HR = 0.36 (95% CI, 0.20–0.67)
Finch, et al. (21) JAMA 2006	Combined	1045	HR = 0.20 (95% CI, 0.07–0.58)	
Kauff et al. (22) J Clin Oncol 2008	Prospective	509	HR = 0.12 (95% CI, 0.03–0.41)	HR = 0.53 (95% CI, 0.29–0.96)

Source: Adapted from Ref. 23.

that RRSO was associated with a significant reduction in both breast (HR = 0.47; 95% CI, 0.29–0.77) and *BRCA*-associated gynecologic cancer (HR = 0.04; 95% CI, 0.01–0.16) (17). Of the 259 individuals in the surgery group, two patients developed primary peritoneal cancer following salpingo-oophorectomy, compared with 58 ovarian or primary peritoneal cancers developing in 292 controls who did not undergo risk-reducing surgery. Since the time that these two studies were reported, at least five additional studies have been published describing the impact of RRSO on subsequent *BRCA*-associated cancer risk (18–23). These are summarized in Table 1.

Surgical Technique

While limited direct evidence exists regarding the impact of RRSO on life expectancy, decision analyses reveal an incremental increase in life expectancy of 2.6 years for *BRCA* mutation carriers who undergo this procedure (24). Furthermore, RRSO can be performed laparoscopically in most patients, with discharge home occurring the same day in the majority of cases. Further advantages of a minimally invasive approach include decreased recovery time and morbidity as well as potentially less adverse impact on body image. In most cases, it is therefore reasonable to start the procedure using a laparoscopic approach. Conversion to a minilaparotomy may occur in the event of technical difficulties due to significant adhesions or body habitus. Similarly, an open

approach would be required if cancer is discovered. Patients who undergo a laparoscopic RRSO should receive preoperative counseling regarding the potential need for conversion to laparotomy. This discussion as well as the subsequent risk of primary peritoneal cancer, namely 1% to 6% after RRSO, should be clearly documented in the surgical consent. The incidence of surgical complications, including bleeding, infection, and injury to surrounding organs such as bowel, bladder, and ureters, is low (16). These risks and benefits should all be thoroughly discussed with patients prior to the surgical procedure.

On entering the abdomen, pelvic washings should be taken for cytologic evaluation. Malignant cells have been discovered in pelvic washings from patients undergoing RRSO. In one report, one of 35 women undergoing RRSO had positive cytology even when no primary ovarian cancer could be identified on detailed sectioning of the entire surgical specimen (25). Additionally, as the entire ovaries and fallopian tubes are at risk for malignant transformation, it is imperative that all of the at-risk tissue be removed. The ovarian vessels should be transected at least 2 cm proximal to the ovary. This requires opening the pelvic sidewall peritoneum to expose the retroperitoneal space, identifying the ureter and isolating the infundibular pelvic ligament that contains the ovarian blood supply. Any adhesions also need to be carefully excised. Such a technique is necessary to minimize the possibility of an ovarian remnant (26). While there are no documented reports of the development of ovarian cancer in an ovarian remnant in a *BRCA* mutation carrier, there are at least five reports in the literature of ovarian cancer occurring in an ovarian remnant after oophorectomy (27–31).

Similarly, during an RRSO, as much of the fallopian tube as possible needs to be removed. Controversy exists as to whether this requires concomitant removal of the uterus, as a small intramural portion of fallopian tube will be left within the uterine cornua if a hysterectomy is not performed. While this residual fallopian tube tissue is at theoretic risk for malignant transformation, this has never been reported to occur following RRSO in a *BRCA* mutation carrier (23). Additionally, in the largest clinical-pathologic study of fallopian tube cancers to date, 92% of cancers for which the origin could be identified originated in the distal or mid-portion of the tube (32).

Hysterectomy at the Time of RRSO

An unanswered question is whether women with *BRCA* mutations should have a concurrent hysterectomy at the time of RRSO. Regarding the aforementioned issues related to the residual intramural fallopian tube at the uterine cornua, there have been no reported cases of malignant transformation. Additionally, in the largest series of primary peritoneal carcinomas occurring after bilateral salpingo-oophorectomy (BSO), all six peritoneal cancers occurred after BSO was performed in conjunction with hysterectomy (13). Other arguments for concomitant hysterectomy at the time of RRSO include (*i*) simplifying hormone replacement, (*ii*) eliminating the possible increased risk of endometrial cancer associated with

tamoxifen use in *BRCA* mutation carriers, and (*iii*) eliminating the possible increased risk of serous carcinoma of the uterus in *BRCA* mutation carriers.

While hormone replacement therapy (HRT) after hysterectomy with RRSO may only require estrogen as opposed to estrogen plus progesterone, no data is available in *BRCA* mutation carriers regarding differences in efficacy, tolerability, or impact on subsequent breast cancer risk of one hormone replacement regimen over the other (23). Additionally, it is not clear if it is appropriate to extrapolate from the results of the Women Health Initiative (WHI) trials, as there is ample reason to suggest that HRT use in women at an inherited risk undergoing a premature surgical menopause may have significantly different effects than HRT use in asymptomatic women in their 60s taking these medications for possible cardioprotective benefit.

Several studies have assessed the incidence of endometrial cancer in *BRCA* mutation carriers in an attempt to address the question of whether or not hysterectomy should be performed at the time of RRSO. Results, to date, have been inconclusive. One epidemiologic study found a 2.6-fold increase in endometrial cancer risk in *BRCA1* mutation carriers (33). Another study from MSKCC, however, found no increased prevalence of *BRCA* mutations in 200 Ashkenazi Jewish endometrial cancer patients (34). A recent case-control study suggested that *BRCA* mutation carriers harbor an increased risk of endometrial cancer primarily in association with tamoxifen use (35). However, limitations of this report include that two of the four *BRCA* mutation carriers who took tamoxifen and developed endometrial cancer took the medication for 8 to 13 years, which is substantially longer than that recommended in current practice.

Recently, a report out of Israel has suggested an association between *BRCA* mutations and serous carcinomas of the uterus (36). However, a Canadian study including 56 women with serous carcinoma of the uterus could not confirm this association (37).

In sum, hysterectomy may be reasonable at the time of RRSO, but, based on current data, is definitely not required. While some assert that the morbidity between laparoscopic BSO and laparoscopic-assisted vaginal hysterectomy, for example, is minimal, a study from Duke revealed the largest predictor of complications with laparoscopic procedures was the inclusion of hysterectomy (38). Whether the potential morbidity outweighs the unproved benefits remains to be seen. Women with mutations considering RRSO should be apprised of the relative risks and benefits and make an informed decision in concert with their surgeon (39).

Pathologic Evaluation of RRSO Specimens

Some reports have described occult invasive cancers in 2% to 10% of *BRCA* mutation carriers who undergo RRSO (16,17,40–42). Additionally, as previously noted, malignant cytology has been found in peritoneal washings from women undergoing RRSO, even in the absence of occult ovarian or fallopian tube cancer.

This data supports serially sectioning the entire ovary and fallopian tube in 2- to 3-mm sections. It is also critical to communicate with the pathologist that the specimens are from an RRSO. When specimens are obtained laparoscopically, they should not be morcellated, but rather placed in a laparoscopic bag for retrieval. This preserves the ovarian and fallopian tube tissue for sectioning by the pathologist.

Management Following RRSO

An important issue following RRSO is the treatment of the effects of premature surgical menopause, including vasomotor symptoms, vaginal dryness and increased bone loss along with the potential impact of surgical menopause on libido, mood, sleep, and cardiovascular disease risk. While some of these issues may not impact oncologic outcome or life expectancy, in one report, sexual symptomatology was the most important predictor of satisfaction with the decision to undergo RRSO (43).

For women without a personal history of breast cancer, short-term systemic HRT may be an option. Rebbeck et al. studied the effect of HRT on post-RRSO breast cancer risk reduction in women with *BRCA* mutations. While use of short-term HRT following RRSO was associated with a nonsignificant increase in breast cancer risk compared with no HRT use (HR = 1.35; 95% CI, 0.16–11.58), patients who used HRT had a profound reduction in subsequent cancer risk compared with women who did not undergo RRSO (HR = 0.37; 95% CI, 0.14–0.96) (44). The authors caution, however, that a larger sample size with longer follow-up is necessary to further examine the interplay between RRSO and HRT.

Given the results of the Hormonal Replacement Therapy After Breast Cancer—Is It Safe (HABITS) trial, systemic HRT would likely be considered contraindicated in most women with a history of breast cancer (45). Options for treating menopausal symptoms in women with a personal history of breast cancer can include selective serotonin reuptake inhibitors (SSRIs) for vasomotor symptoms or nonhormonal moisturizers for vaginal symptomatology. SSRIs have been shown to decrease the severity and frequency of vasomotor symptoms in roughly two-thirds of women with breast cancer (46). Nonhormonal vaginal moisturizers have also been shown to alleviate vaginal symptoms in some women who have undergone premature surgical menopause (47).

Low-dose vaginal estrogen may also be more reasonable in women with a prior history of breast cancer, given the much lower systemic absorption, but this remains an off-label indication. In the setting of aromatase inhibitor therapy, particular caution is needed as there has been one report that low-dose vaginal estrogen can appreciably elevate serum estradiol levels when used in concert with an aromatase inhibitor (48). Given this report, some authors have advocated periodic monitoring of serum estradiol levels in women who are using both an aromatase inhibitor and a low-dose vaginal estrogen preparation (23).

Impact of RRSO on Other Health Risks

As premature menopause affects osteoporosis risk (49) and may also impact cardiovascular disease risk (50), these issues should also be addressed in women undergoing RRSO. An assessment of osteoporosis risk by bone densitometry should likely be performed within the first year postoperatively (23). Management can then be guided by objective criteria. As women with a premature surgical menopause may also be at increased risk of cardiovascular disease, women who have undergone RRSO should be assessed for modifiable cardiovascular risk factors, including hypercholesterolemia, hypertension, diabetes, and tobacco use, such that these risk factors can be appropriately addressed and minimized (23,39).

Timing of RRSO

Several factors should be considered when discussing the optimal timing of RRSO, including the woman's fertility desires, timing of both gynecologic and breast cancer risks, and additional risk-reduction strategies taken by the patient. Clearly, RRSO should be deferred until childbearing is complete. However, as women delay childbearing into their late 30s and 40s, women with *BRCA1* mutations may be exposed to a significant risk of ovarian cancer (11–21% by age 50) (51–53). For women with *BRCA2* mutations, the risk of ovarian cancer does not appear to increase until approximately a decade later, and these women only have a 2% to 3% chance of developing ovarian cancer by age 50 (51–53). Given this, in the setting of a *BRCA2* mutation, it may be reasonable to defer RRSO until around the time of natural menopause. However, women with a *BRCA2* mutation who pursue this option need to be aware that they will lose the substantial benefit that RRSO confers against subsequent breast cancer (22,23,39).

RRSO IN WOMEN WITHOUT A DEMONSTRABLE *BRCA1* OR *BRCA2* MUTATION

Up until this point, this chapter has dealt exclusively with women having documented mutations in *BRCA1* or *BRCA2*. Is there a role for RRSO in women from hereditary breast cancer families who have undergone genetic testing and in whom no deleterious mutation has been identified? In addressing this issue, one should probably deal separately with women from site-specific hereditary breast cancer families and hereditary breast cancer families with ovarian cancer in the lineage. In 1998, the linkage consortium published data from 237 families with at least four cases of breast cancer diagnosed prior to age 60 (5). If there was just one case of ovarian cancer in the lineage, 90% of the families showed linkage to *BRCA1* or *BRCA2* even if a mutation could not be identified on sequencing. Given this data, families with multiple cases of early-onset breast cancer and even one case of ovarian cancer likely should be managed as if an occult *BRCA1*

or *BRCA2* mutation is present. However, in hereditary breast cancer families with no ovarian cancer in the lineage, only about half of these families are explained by mutations in *BRCA1* or *BRCA2*. Is there role for RRSO in women from these *BRCA*-negative families? In order to address this question, a group from MSKCC prospectively evaluated the incidence of breast and ovarian cancer in 165 *BRCA*-negative, site-specific hereditary breast cancer families and compared the observed incidence to the expected incidence derived from SEER rates. During 3.4 years of follow-up, women from these families had a threefold increased risk of breast cancer compared with population rates (SIR 3.13; 95% CI, 1.88–4.89). However, there was not a significantly increased risk of ovarian cancer in this cohort (SIR 1.52; 95% CI, 0.02–8.46) (54). While this data is preliminary, if confirmed, it suggests that there may not be a role of RRSO for gynecologic cancer prevention in *BRCA*-negative site-specific breast cancer families. It is important to remember, however, a retrospective study has suggested that RRSO is protective against breast cancer at all levels of risk, so there still may be a role of RRSO for breast cancer prevention in these families (55).

CONCLUSIONS AND FUTURE DIRECTIONS

The lifetime risk of ovarian cancer increases from a baseline of 1.5% to approximately 39% to 46% in *BRCA1* mutation carriers and 10% to 27% in *BRCA2* mutation carriers. Given limitations in currently available screening and chemopreventive approaches, RRSO should be discussed with all women with an inherited mutation in either *BRCA1* or *BRCA2*. Although reduction in both breast and ovarian cancer incidence has been clearly demonstrated, there are multiple other physical, psychologic, and sexual issues that need to be addressed simultaneously in women considering this procedure. These are best discussed by a team of individuals experienced in the management of women with an inherited risk.

In terms of the future, while RRSO is currently the most efficacious method of ovarian cancer risk reduction in women with *BRCA* mutations, it remains a suboptimal approach. Only continued research in screening, prevention, and the basic pathogenesis of inherited ovarian cancer will allow us to make the prophylactic removal of healthy organs obsolete and is a goal that we need to strive toward.

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CASE REPORT

N.B., a 42-year-old woman with two children, elected to undergo a risk-reducing salpingo-oophorectomy after both she and her mother tested positive for a deleterious *BRCA1* mutation. Her mother, who had been diagnosed with breast cancer at the age of 36, pursued hereditary cancer risk assessment and genetic testing at the age of 64, when she was diagnosed with ovarian cancer. N.B.'s family history was also significant for her grandmother having been diagnosed with ovarian cancer at the age of 62 and her sister being diagnosed with breast cancer at the age of 40. N.B. had no personal cancer diagnosis.

At the time of risk-reducing salpingo-oophorectomy, N.B.'s ovaries and fallopian tubes appeared grossly normal. However, on final pathology, there was noted to be a 2-mm invasive serous carcinoma present in the distal fallopian tube. N.B. was then referred to a gynecologic oncologist for recommendations and follow-up.

LEARNING POINTS

- Risk-reducing salpingo-oophorectomy is most effective when performed as soon as childbearing is complete.
- In risk-reducing surgery, the surgeon must make an effort to remove all possible portions of the ovaries and fallopian tubes.
- The pathologic assessment of the ovaries and fallopian tubes should include complete serial sectioning of the ovaries and fallopian tubes in their entirety.
- Patients with *BRCA1* and *BRCA2* mutations, who have normal-appearing ovaries, have a 2% to 10% risk of an occult invasive ovarian or fallopian tube cancer being found at the time of careful pathologic review.
- Patients with occult findings at risk-reducing salpingo-oophorectomy should be referred to a gynecologic oncologist.

REFERENCES

1. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline *BRCA1* and *BRCA2* mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001; 68:700–710.
2. Rubin SC, Blackwood MA, Bandera C, et al. *BRCA1, BRCA2* and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: relationship to family history and implications for genetic testing. Am J Obstet Gynecol 1998; 178:670–677.
3. Pal T, Permuth-Wey J, Betts JA, et al. *BRCA1* and *BRCA2* mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005; 104:2807–2816.

4. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. N Engl J Med 1997; 336:1401–1408.
5. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998; 62:676–689.
6. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003; 72:1117–1130.
7. King MC, Marks JH, Mandell JB, and New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2*. Science 2003; 302:643–646.
8. Love RR, Philips J. Oophorectomy for breast cancer: history revisited. J Natl Cancer Inst 2002; 94:1433–1434.
9. Feinleib M. Breast cancer and artificial menopause: a cohort study. J Natl Cancer Inst 1968; 41:315–329.
10. Brinton LA, Schairer C, Hoover RN, et al. Menstrual factors and risk of breast cancer. Cancer Invest 1988; 6:245–254.
11. Liber AM. Ovarian cancer in a mother and five daughters. Arch Pathol 1950; 49: 280–290.
12. Tobacman JK, Greene MH, Tucker MA, et al. Intra-abdominal carcinomatosis after prophylactic oophorectomy in ovarian-cancer-prone families. Lancet 1982; 2:795–797.
13. Piver MS, Jishi MF, Tsukada Y, et al. Primary peritoneal carcinoma after prophylactic oophorectomy in women with a family history of ovarian cancer. A report of the Gilda Radner Familial Ovarian Cancer Registry. Cancer 1993; 71:2751–2755.
14. Struewing JP, Watson P, Easton DF, et al. Prophylactic oophorectomy in inherited breast/ovarian cancer families. J Natl Cancer Inst Monogr 1995:33–35.
15. Burke W, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. *BRCA1* and *BRCA2*. Cancer Genetics Studies Consortium. JAMA 1997; 277:997–1003.
16. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a *BRCA1* or *BRCA2* mutation. N Engl J Med 2002; 346:1609–1615.
17. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of *BRCA1* or *BRCA2* mutations. N Engl J Med 2002; 346:1616–1622.
18. Rutter JL, Wacholder S, Chetrit A, et al. Gynecologic surgeries and risk of ovarian cancer in women with *BRCA1* and *BRCA2* Ashkenazi founder mutations: an Israeli population-based case-control study. J Natl Cancer Inst 2003; 95:1072–1078.
19. Eisen A, Lubinski J, Klijn J, et al. Breast cancer risk following bilateral oophorectomy in *BRCA1* and *BRCA2* mutation carriers: an international case-control study. J Clin Oncol 2005; 23:7491–7496.
20. Domchek SM, Friebel TM, Neuhausen SL, et al. Mortality after bilateral salpingo-oophorectomy in *BRCA1* and *BRCA2* mutation carriers: a prospective cohort study. Lancet Oncol 2006; 7:223–229.
21. Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a *BRCA1* or *BRCA2* Mutation. JAMA 2006; 296:185–192.

22. Kauff ND, Domchek SM, Friebel TM, et al. Risk-reducing salpingo-oophorectomy for the prevention of *BRCA1* and *BRCA2* associated breast and gynecologic cancer: a multi-center, prospective study. J Clin Oncol 2008; 26:1331–1337.
23. Kauff ND, Barakat RR. Risk-reducing salpingo-oophorectomy in patients with germline mutations in *BRCA1* or *BRCA2*. J Clin Oncol 2007; 25:2921–2927.
24. Grann VR, Jacobson JS, Thomason D, et al. Effect of prevention strategies on survival and quality-adjusted survival of women with *BRCA1*/2 mutations: an updated decision analysis. J Clin Oncol 2002; 20:2520–2529.
25. Colgan TJ, Boerner SL, Murphy J, et al. Peritoneal levage cytology: as assessment of its value during prophylactic oophorectomy. Cancer Res 2002; 85:397–403.
26. Nezhat CH, Seidman DS, Nezhat FR, et al. Ovarian remnant syndrome after laparoscopic oophorectomy. Fertil Steril 2000; 74:1024–1028.
27. Fueyo J, Garces JM, Soriano JC, et al. Adenocarcinoma of the ovary in the ovarian remnant syndrome. Rev Clin Esp 1990; 186:415–416.
28. Bruhwiler H, Luscher KP. Ovarian cancer in ovarian remnant syndrome. Geburtshilfe Frauenheilkd 1991; 51:70–71.
29. Kazadi Buanga J, Laparte Escorza MC, Lopez Garcia G. Ovarian remnant syndrome. A case report of a malignancy. J Gynecol Obstet Biol Reprod 1992; 2:769–772.
30. Glaser D, Burrig KF, Mast H. Ovarian cancer in ovarian remnant syndrome? Geburtshilfe Frauenheilkd 1992; 52:436–437.
31. Narayansingh G, Cumming G, Parkin D, et al. Ovarian cancer developing in the ovarian remnant syndrome. A case report and literature review. Aust N Z J Obstet Gynaecol 2000; 40:221–223.
32. Alvarado-Cabrero I, Young RH, Vamvakas EC, et al. Carcinoma of the fallopian tube: a clinicopathological study of 105 cases with observations on staging and prognostic factors. Gynecol Oncol 1999; 72:367–379.
33. Thompson D, Easton DF. Breast Cancer Linkage Consortium. Cancer Incidence in *BRCA1* mutation carriers. J Natl Cancer Inst 2002; 94:1358–1365.
34. Levine DA, Lin O, Barakat RR, et al. Risk of endometrial carcinoma associated with *BRCA* mutation. Gynecol Oncol 2001; 80:395–398.
35. Beiner ME, Finch A, Rosen ME, et al. The risk of endometrial cancer in women with *BRCA1* and *BRCA2* mutations. A prospective study. Gynecol Oncol 2007; 104:7–10.
36. Lavie O, Hornreich G, Ben-Arie A, et al. *BRCA* germline mutations in Jewish women with uterine serous papillary carcinoma. Gynecol Oncol 2004; 92:521–524.
37. Goshen R, Chu W, Elit L, et al. Is uterine papillary serous adenocarcinoma a manifestation of the hereditary breast-ovarian cancer syndrome?Gynecol Oncol 2000; 79: 477–481.
38. Havrilesky LJ, Peterson BL, Dryden DK, et al. Predictors of clinical outcomes in the laparoscopic management of adnexal masses. Obstet Gynecol 2003; 102:243–251.
39. Society of Gynecologic Oncologists: Clinical Practice Committee. Statement on Prophylactic Salpingo-Oophorectomy. Gynecol Oncol 2005; 98:179–181.
40. Lu KH, Garber JE, Cramer DW, et al. Occult ovarian tumors in women with *BRCA1* or *BRCA2* mutations undergoing prophylactic oophorectomy. J Clin Oncol 2000; 18:2728–2732.
41. Colgan TJ, Murphy J, Cole DE, et al. Occult carcinoma in prophylactic oophorectomy specimens: prevalence and association with *BRCA* germline mutation status. Am J Surg Pathol 2001; 25:1283–1289.

42. Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in *BRCA* mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005; 23:127–132.
43. Robson M, Hensley M, Barakat R, et al. Quality of life in women at risk for ovarian cancer who have undergone risk-reducing oophorectomy. Gynecol Oncol 2003; 89:281–287.
44. Rebbeck TR, Friebel T, Wagner T, et al. Effect of short-term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in *BRCA1* and *BRCA2* mutation carriers: the PROSE Study Group. J Clin Oncol 2005; 23:7804–7810.
45. Holmberg L, Anderson H, and HABITS Steering and Data Monitoring Committees. HABITS (hormonal replacement therapy after breast cancer—is it safe?), a randomised comparison: trial stopped. Lancet 2004; 363:453–455.
46. Nelson HD, Vesko KK, Haney E, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA 2006; 295:2057–2071.
47. Loprinzi CL, Abu-Ghazaleh S, Sloan JA, et al. Phase III randomized double-blind study to evaluate the efficacy of a polycarbophil-based vaginal moisturizer in women with breast cancer. J Clin Oncol 1997; 15:969–973.
48. Kendall A, Dowset M, Folkerd E, et al. Caution: vaginal estradiol appears to be contraindicated in postmenopausal women on adjuvant aromatase inhibitors. Ann Oncol 2006; 17(4):584–587.
49. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA 2001; 285:785–795.
50. Colditz GA, Willett WC, Stampfer MJ, et al. Menopause and the risk of coronary heart disease in women. N Engl J Med 1987; 316:1105–1110.
51. Satagopan JM, Boyd J, Kauff ND, et al. Ovarian cancer risk in Ashkenazi Jewish carriers of *BRCA1* and *BRCA2* mutations. Clin Cancer Res 2002; 8:3776–3781.
52. Kauff ND, Barakat RR. Surgical risk-reduction in carriers of *BRCA* mutations: where do we go from here?Gynecol Oncol 2004; 93:277–279.
53. King MC, Marks JH, Mandell JB, and New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2*. Science 2003; 302:643–646.
54. Kauff ND, Mitra M, Robson ME, et al. Risk of ovarian cancer in *BRCA1* and *BRCA2* mutation negative hereditary breast cancer families. J Natl Cancer Inst 2005; 97: 1382–1384.
55. Olsen JE, Sellers TA, Iturria SJ, et al. Bilateral oophorectomy and breast cancer risk reduction among women with a family history. Cancer Detect Prev 2004; 28(5): 357–360.

7

Risk Management of Hereditary Breast Cancer

Banu Arun

Breast Medical Oncology and Clinical Cancer Prevention, and Clinical Cancer Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- The American Cancer Society recommends annual MRI screening and mammography for women with a known BRCA mutation and certain other women whose lifetime risk of developing breast cancer is 20% to 25% or greater. These tests can be staggered so that women have imaging every six months.
- Tamoxifen may decrease the risk of developing invasive breast cancer as much as 49% in high-risk individuals and should be offered as chemoprevention. The benefit in BRCA1 carriers is controversial.
- For individuals with germline BRCA mutations, risk-reducing mastectomy decreases risk of developing breast cancer by 90% to 95% and risk-reducing oophorectomy decreases breast cancer risk by 45% to 50%.

INTRODUCTION

Since the identification of the BRCA1 gene, genetic testing for breast cancer susceptibility has been incorporated into the clinical practice of oncology. During this process, individuals who are at increased risk are identified and appropriate risk management options are discussed. This chapter will review risk

management options for individuals who are at increased hereditary risk for developing breast cancer.

SCREENING

The aim of screening is to identify breast cancer at a stage when a surgical cure is likely, ideally small breast cancers that are node negative. The recommendations for screening and follow-up of individuals with an inherited predisposition to breast cancer, as recommended by the Cancer Genetics Studies Consortium, include monthly self-breast examination beginning at age 18 to 21 years, annual or semiannual clinical breast examination beginning at age 25 to 35 years, and annual mammography beginning at age 25 to 35 years (1). These recommendations for optimal screening modality and frequency are largely based on expert opinion and have not been validated in prospective studies with mortality endpoints (2,3).

Brekelmans et al. recently reported on the surveillance of 128 individuals with known BRCA1 and BRCA2 mutations, which were followed at least with annual mammograms, annual clinical breast examinations, and monthly self-breast examinations (3). Within a median follow-up time of three years, nine breast cancers developed in mutation carriers, of which four were interval cancers, not detected during the course of screening. Another recent study also reported on 165 BRCA1 and BRCA2 mutation carriers who were followed with monthly self-breast examination, clinical breast examination two to four times per year, and annual mammograms (4). At a mean follow-up of 24 months, 12 breast cancer cases developed. Of those, six (50%) were interval cancers. In five cases, the breast mass was detected by the patient and in one case by the physician. The remaining six cases were detected by routine mammograms. Three of the breast cancer cases were invasive cancers, and three were ductal carcinoma in situ (DCIS). The finding of DCIS in this and two other recent studies (5,6) is of interest, since this implies the presence of a noninvasive phase of disease in a subset of patients that can be identified by radiological screening.

Nevertheless, at this point, with standard annual screening, the development of interval cancers remains an important problem. The reasons interval cancers occur include dense breast tissue, which does not allow the detection of an already existing malignant process, or an aggressive tumor with a high growth rate that occurred since the last screening mammogram. Whether increasing the frequency of mammograms to every six months is better or new screening modalities need to be developed remains unclear. A number of studies have suggested that screening with magnetic resonance imaging (MRI) may benefit women at high risk (5–7). In one study (8), 236 women with BRCA1 or BRCA2 mutation underwent annual 1–3 mammography, ultrasound, MRI, and clinical breast examinations every six months. Twenty-two cancers were identified, and MRI was found to be more sensitive for detecting breast cancers than ultrasound, mammogram, or clinical breast examination alone. In another study (9), 1909 high-risk women, including 358 with germline mutations, were screened with

yearly mammogram, MRI, and clinical breast examinations every six months. The sensitivity for detecting invasive breast cancer was higher for MRI compared with mammogram or clinical breast examinations.

On the basis of these studies, in April 2007, the American Cancer Society (ACS) released their recommendations for breast MRI screening (10). The ACS recommended annual MRI screening for women with a known BRCA mutation; women who are first-degree relatives of an individual with a known BRCA mutation, but have not pursued testing themselves; or women whose lifetime risk of developing breast cancer is 20% to 25% or greater, as defined by BRCAPRO or other models that are largely dependent on family history. In some cases, data from screening MRI studies did not provide sufficient evidence for recommendations. Therefore, the ACS relied on available inferential evidence and expert consensus opinion to recommend annual MRI screening for women who have received radiation to the chest between age 10 and 30 years; women with Li-Fraumeni syndrome, Cowden syndrome, and Bannayan-Riley-Ruvalcaba syndrome; and first-degree relatives of those known to have these syndromes. However, whether MRI improves survival remains unanswered.

CHEMOPREVENTION

Selective Estrogen Receptor Modulators

Currently, the selective estrogen receptor modulators (SERMs), tamoxifen and raloxifene, are approved for the risk reduction of breast cancer in high-risk individuals (11,12). The study that led to the approval of tamoxifen was the phase III National Surgical Adjuvant Breast and Bowel Project (NSABP) chemoprevention trial (BCPT-P1), which randomized 13,388 women at high risk for breast cancer to tamoxifen versus placebo (11). Eligible women had to be 60 years or older, or between ages 35 and 59 and have a diagnosis of LCIS, or a projected five-year risk of developing breast cancer greater than 1.66%, according to the modified Gail model (13). After a median follow-up of 54 months, a 49% reduction in the incidence of invasive breast cancer ($p < 0.00001$) and a 50% reduction of noninvasive cancer ($p < 0.0001$) occurred among those receiving tamoxifen. However, tamoxifen did not reduce the occurrence of ER-negative breast cancers. Another study, the IBIS-I trial randomized 7152 women, aged 35 to 70 years, who were at increased risk of breast cancer, to receive either tamoxifen or placebo for five years (14). At a median follow-up of 50 months, a 32% reduction in the odds of developing breast cancer in the tamoxifen group was found. However, two other studies, the Royal Marsden (15) and the Italian study, did not show a significant difference in breast cancer incidence between women given tamoxifen and those given placebo (16).

Side effects of tamoxifen in the NSABP trial included an increased risk of endometrial cancer; the relative risk in the tamoxifen group was 2.5 and increased to 4.01 in women aged 50 years or older. Deep-vein thrombosis and pulmonary

emboli were also seen more often in the tamoxifen group, with women aged 50 years or older again at higher risk (relative risk was 1.71 for deep-vein thrombosis and 3.00 for pulmonary emboli) (11). An apparent increase in the risk of stroke among women taking tamoxifen did not reach statistical significance. A marginally significant increase in the occurrence of cataract formation and the risk of requiring cataract surgery was noted for the tamoxifen group (11). Other side effects included increased hot flushes and vaginal discharge.

The other SERM raloxifene was evaluated in a large phase III trial, STAR, or NSABP-P2, against tamoxifen. The eligibility criteria were the same, except that women had to be postmenopausal (12). Raloxifene was as effective as tamoxifen in reducing the incidence of invasive breast cancer and had a slightly better toxicity profile. However, it was less effective than tamoxifen in reducing the incidence of noninvasive breast cancer, including DCIS.

While it is important to discuss the benefit and risk ratio of SERMs for high-risk women, the impact of SERMs on women with high genetic risk, such as BRCA1 or BRCA2 mutation carriers, is currently not well defined. In this effort, BRCA1 and BRCA2 gene sequencing was performed on all breast cancer cases ($n = 288$) in women who participated in the NSABP-P1 trial, and 19 cases were found to have the BRCA1 or BRCA2 mutation (17). Five out of 8 patients with BRCA1 received tamoxifen, and 3 out of 11 patients with BRCA2 mutations received tamoxifen; 83% of BRCA1 breast tumors were ER-negative, whereas 76% of BRCA2 breast tumors were ER-positive. This study suggests that tamoxifen reduces breast cancer incidence in BRCA2 carriers, but not in BRCA1 carriers; however, firm conclusions cannot be drawn as the sample size is low. In contrast, another study showed that tamoxifen reduces the risk of contralateral breast cancer in women with BRCA1 or BRCA2 mutation (18). Two-hundred and nine women with a BRCA1 or BRCA2 mutation and bilateral breast cancer were compared with 384 women with unilateral breast cancer and BRCA1 or BRCA2 mutation in a matched case-control study, where history of tamoxifen use for first breast cancer was obtained. Their results revealed that tamoxifen use reduced the risk of contralateral breast cancer by 50% in women with a BRCA1 or BRCA2 mutation. Furthermore, studies have also shown that bilateral prophylactic oophorectomies also reduce the risk of breast cancer in BRCA1 or BRCA2 mutation carriers, indicating again the efficacy of antihormonal intervention (19,20). To summarize, it remains unknown whether tamoxifen can reduce the risk of breast cancer in BRCA1 mutation carriers. Currently, there is no efficacy data with raloxifene in BRCA mutation carriers.

New Potential Agents

Aromatase inhibitors are a group of potential agents that can be considered for the use of chemoprevention. The aromatase inhibitors block the conversion of androgens to estrogens. Aromatase activity, via increasing local estrogen synthesis, may play an early role in breast cancer carcinogenesis (21). In vivo

models have shown that aromatase expression in breast tissue can induce the development of premalignant lesions (22). Recently, results of three adjuvant hormonal trials with anastrozole, letrozole, or exemestane have demonstrated a 50% to 58% reduction in primary contralateral breast cancer in women treated with the aromatase inhibitors versus tamoxifen (23–25). The NSABP-B35 is currently investigating anastrozole versus tamoxifen in patients with DCIS, and the IBIS-II study is evaluating anastrozole versus placebo in high-risk women. However, as in the case of SERMs, aromatase inhibitors will most probably be effective in reducing the incidence of ER-positive breast cancers and might not be effective in high-risk women with BRCA1 mutations.

Other potential agents that are currently being investigated might be more effective for ER-negative breast cancer prevention that would also include BRCA1-associated breast cancer. One of the potential nonhormonal agent is the selective cyclooxygenase-2 (COX-2) inhibitor, celecoxib (26,27), which is currently under investigation in phase II breast cancer chemoprevention trials (28). Other promising agents for the prevention of ER-negative breast cancers include polyamine biosynthesis inhibitors, difluoromethylornithine (DFMO) (29), vitamin D analogues, retinoids (30), cyclin-dependent kinase inhibitors (31), telomerase inhibitors (32), isoflavonoids (33), and molecular chemopreventive approaches including targeted gene therapy for BRCA1 mutation carriers (34).

PROPHYLACTIC MASTECTOMY

Reduction of breast cancer risk by prophylactic mastectomy has been studied in retrospective and prospective studies (4,35–38). Despite a major risk reduction, development of cancer can still be seen after surgery, mostly due to the fact that prophylactic surgery does not technically remove all glandular tissue. Even though prophylactic mastectomies and oophorectomies are usually considered in genetically high-risk individuals, there could be certain circumstances where an average risk individual might consider surgery, such as having a history of multiple prior breast biopsies or unreliable physical and/or radiological examination because of nodular and dense breast tissue. Most studies evaluating the benefit of prophylactic surgeries have been carried out in individuals with familial breast/ovarian syndromes. In one study Hartman et al. studied 639 women with a family history of breast cancer who underwent bilateral prophylactic mastectomies. Among those, 214 women were considered high risk and 425 as moderate risk. Breast cancer incidence in the high-risk group was compared with a control group consisting of the probands' sisters ($n = 403$) who had not undergone prophylactic mastectomy. The results of the study showed a 90% reduction in the incidence of breast cancer in the prophylactic mastectomy group (35). The same investigators reported later on the effect of bilateral mastectomy in a subset of 26 women who were found to be BRCA1 or BRCA2 mutation carriers. At a median follow-up of 13.4 years none of them developed breast cancer (36). A prospective study reported recently on 76 women with

BRCA1 or BRCA2 mutation who underwent prophylactic mastectomy and 63 women with BRCA1 or BRCA2 mutation who opted for surveillance. At 2.9 years of follow-up, no breast cancer occurred in the women who had prophylactic mastectomy, whereas eight breast cancers occurred in the surveillance group (37). Finally, another prospective study reported on the effect of prophylactic mastectomy in 194 individuals with a BRCA1 or BRCA2 mutation, of whom 29 opted for prophylactic mastectomy. Even though the follow-up was short (mean 24 months) none of these individuals developed breast cancer, whereas 12 breast cancers were identified in the remaining group who opted for surveillance (4). Another study evaluated 483 women with germline BRCA1/2 mutations at a mean follow-up of 6.4 years, breast cancer was diagnosed in 2 (1.9%) of 105 women who had bilateral prophylactic mastectomy and in 184 (48.7%) of 378 matched controls who did not have the procedure. Bilateral prophylactic mastectomy reduced the risk of breast cancer by approximately 95% in women with prior or concurrent bilateral prophylactic oophorectomy and by approximately 90% in women with intact ovaries (38).

PROPHYLACTIC OOPHORECTOMY

The efficacy of prophylactic oophorectomies in breast cancer risk reduction for the general population has been previously shown in several studies. Brinton et al. reported a 45% reduction in breast cancer risk in women who underwent prophylactic oophorectomy before the age 40 years, compared with women who underwent natural menopause (39). Parazzini et al. reported a 20% risk reduction after prophylactic oophorectomy in premenopausal women (40). Another study reported a 50% reduction in the risk of breast cancer with prophylactic oophorectomy in women aged less than 50 years; risk reduction was not seen in women aged 50 years and older (41). One study reported reduction in breast cancer with oophorectomy performed premenopausally, even with the use of hormonal replacement therapy (42).

The effect of prophylactic oophorectomy has also been studied in genetically high-risk patients (43). In a small cohort, Rebbeck et al. (44) reported that breast cancer risk was reduced by at least 50% in women with a BRCA1 mutation ($n = 43$) who underwent prophylactic oophorectomy, compared with women who did not undergo surgery ($n = 79$). A recent, multicenter retrospective study revealed a 53% risk reduction in individuals with a BRCA1 or BRCA2 mutation who underwent prophylactic oophorectomy (45). Out of 99 women who underwent prophylactic oophorectomy, 21 developed breast cancer, compared with 60 breast cancers out of 142 matched controls. Recently, the results of a prospective study in BRCA1 and BRCA2 mutation carriers, with a mean follow-up of 24.2 months was reported (46). Three breast cancers occurred in 69 individuals who had prophylactic salpingo-oophorectomy group, compared with 8 breast cancers in 62 individuals who opted for surveillance.

A recent study analyzed 1439 patients with breast cancer and 1866 matched controls derived from a registry of BRCA1 and BRCA2 carriers to estimate the odds ratios of breast cancer for having had a bilateral oophorectomy. It was shown that a previous history of oophorectomy was associated with a significant reduction in breast cancer risk of 56% for BRCA1 carriers (OR = 0.44; 95% CI, 0.29–0.66) and of 46% (OR = 0.57; 95% CI, 0.28–1.15) for BRCA2 carriers. It appeared that the risk reduction was greater if the oophorectomy was performed before age 40 and that the protective effect was evident for 15 years post-oophorectomy (47).

INTEGRATION OF GENETIC RISK INFORMATION INTO BREAST CANCER MANAGEMENT

Currently, the multidisciplinary team involved in the treatment of breast cancer includes surgical oncologist, medical oncologists, and radiation oncologists. Recently, genetic risk assessment has become an important aspect of this multidisciplinary team approach. Not only is the likelihood of finding a mutation higher in affected individuals, if incorporated early in the management, genetic testing results may affect treatment options. For example, because of the 32% estimated 10-year risk of contralateral breast cancer in BRCA1 carriers (48), some women with stage I or II breast cancer may choose to undergo prophylactic (risk-reducing) oophorectomy and/or contralateral mastectomy as part of their initial surgical treatment plan. There is less information available regarding the impact of radiation therapy on local recurrence in BRCA mutation carriers. It is thought that ionizing radiation may pose a special hazard for women with *BRCA* mutations, who are deficient in their ability to repair radiation-induced DNA breaks (49). Some studies have shown that ipsilateral recurrence rates are similar in mutation carriers and women without mutations in large clinic-based studies (48,50). Metcalfe et al. (48) estimated the 10-year cumulative incidence of ipsilateral recurrence to be 34% in *BRCA* carriers with breast conserving surgery who did not receive radiotherapy, but was only 9% in those who did ($p = 0.01$ for difference; ipsilateral recurrences include both local recurrences and new primary ipsilateral cancers). Similar findings were observed in studies of unselected Ashkenazi women undergoing lumpectomy and radiation therapy (51). However, other studies have suggested a late risk of ipsilateral second primary malignancies (52,53).

Even though, currently, patients with *BRCA1*-associated breast cancers receive the same type of adjuvant chemotherapy as non-BRCA-associated breast cancer, it has been suggested that *BRCA1*-associated tumors are highly sensitive to certain chemotherapy agents such as mitomycin (54) and platinum (41,55,56). Furthermore, new targeted agents, such as poly (ADP-ribose) polymerase (PARP-1) inhibitors are currently being investigated as single or combination agents for the treatment of BRCA1-associated breast cancer (57).

PSYCHOSOCIAL ASPECTS

Psychosocial aspects of risk assessment and counseling and the decision process for risk reduction strategies for breast cancer are mostly relevant for individuals who are genetically at high risk. One aspect is, for example, the reaction to a positive genetic test result. One study showed that individuals who underestimated their emotional response and could not accurately predict their emotional response to test result disclosure, experienced greater psychological distress at six months (58). Another study evaluating the adverse psychological effects in members of BRCA1- and BRCA2-linked families who declined genetic testing revealed that the presence of cancer-related stress symptoms at baseline was strongly predictive of the onset of depressive symptoms in family members who were invited but declined testing. The depression rate of these individuals was not only increased compared to noncarriers but also compared to mutation carriers who had decided to be tested, pointing out that dealing with uncertainty is more difficult than knowing about a positive test result (59).

Risk-reduction options for women at high risk basically include either a more conservative approach with screening or a more aggressive approach with chemoprevention or risk-reduction surgeries. The decision process is mainly affected by risk perception and breast cancer worry. In one study, for example, 19% of 333 women stated that they would consider prophylactic mastectomy if they tested positive and 54% reported being unsure. Variables correlating with the potential decision for mastectomy included age, risk estimate, and breast cancer anxiety; younger women with higher risk and higher levels of anxiety were more likely to consider prophylactic mastectomy (60). Another study in 554 high-risk women, including 142 BRCA mutation carriers showed that women who were BRCA carriers, or women who had a history of breast cancer, DCIS, breast biopsy, or had a family history of ovarian cancer were more likely to undergo surgery for cancer risk reduction (61). Other studies evaluated the perception of prophylactic mastectomy in BRCA1 or BRCA2 mutation carriers. In two studies, the acceptance for prophylactic mastectomy was 3% to 8% (62,63), whereas a study from Rotterdam in 139 unaffected BRCA1 or BRCA2 mutation carriers reported an acceptance of 55% (37). Finally, a recent study in 194 BRCA1 or BRCA2 mutation carriers reported that 29 (15%) women opted for prophylactic mastectomy (4). The differences in the acceptance rate in these studies are unclear, but cultural differences most probably play an important role. Furthermore, advances in autologous reconstruction with skin-sparing mastectomy have made consideration of this option for patients with a high risk for breast cancer, a much more acceptable option for some women.

CONCLUSIONS

More than a decade after *BRCA1* was cloned, we have started identifying individuals at the highest hereditary risk for cancer who have served as a model to investigate strategies for prevention or early detection of breast malignancies.

Current risk management options for women at hereditary high risk range from screening to chemoprevention to prophylactic surgeries. The molecular pathogenesis of *BRCA1*- and *BRCA2*-associated breast tumors are currently being studied, which will ultimately lead to tailored treatments for women with newly diagnosed breast cancer and *BRCA* mutations. Other areas of research include individual risk estimates for women carrying *BRCA* mutations, based on consideration of the particular mutation inherited and also on the presence of modifying genetic and environmental factors.

CASE REPORT

MO is a 62-year-old survivor of breast cancer. Given her family history of breast and ovarian cancer, she was referred to genetic counseling where she was diagnosed with a germline BRCA1 mutation. Although she was taking Arimidex, MO desired further action to decrease her risk of a second breast cancer or new ovary cancer. She recently underwent laparoscopic risk-reducing salpingo-oophorectomy and will soon be undergoing a bilateral mastectomy with reconstruction for further risk reduction.

LEARNING POINTS

- Risk management options for this patient include monthly self-breast examinations along with annual MRI and mammogram, chemoprevention with tamoxifen, semiannual CA125 and transvaginal ultrasound, and risk-reducing mastectomy or salpingo-oophorectomy.
- Risk-reducing mastectomy will decrease her risk of breast cancer by at least 90%, and salpingo-oophorectomy will decrease her breast cancer risk by 50%.

REFERENCES

1. Burke W, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. JAMA 1997; 277:997–1003.
2. Eisinger F, Alby N, Bremond A, et al. Recommendations for medical management of hereditary breast and ovarian cancer: the French National Ad Hoc Committee. Ann Oncol 1998; 9:939–950.
3. Brekelmans CT, Seynaeve C, Bartels CC, et al. Effectiveness of breast cancer surveillance in BRCA1/2 gene mutation carriers and women with high familial risk. J Clin Oncol 2001; 19:924–930.
4. Scheuer L, Kauff N, Robson M, et al. Outcome of preventive surgery and screening for breast and ovarian cancer in BRCA mutation carriers. J Clin Oncol 2002; 20:1260–1268.
5. Stoutjesdijk MJ, Boetes C, Jager GJ, et al. Magnetic resonance imaging and mammography in women with a hereditary risk of breast cancer. J Natl Cancer Inst 2001; 93:1095–1102.

6. Warner E, Plewes DB, Shumak RS, et al. Comparison of breast magnetic resonance imaging, mammography, and ultrasound for surveillance of women at high risk for hereditary breast cancer. J Clin Oncol 2001; 19:3524–3531.
7. Kuhl CK, Schmutzler RK, Leutner CC, et al. Breast MR imaging screening in 192 women proved or suspected to be carriers of a breast cancer susceptibility gene: preliminary results. Radiology 2000; 215:267–279.
8. Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. JAMA 2004; 292:1317–1325.
9. Kriege M, Brekelmans CT, Boetes C, et al. Efficacy of MRI and mammography for breast-cancer screening in women with a familial or genetic predisposition. N Engl J Med 2004; 351:427–437.
10. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007; 57:75–89.
11. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998; 90:1371–1388.
12. Vogel VG, Costantino JP, Wickerham DL, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA 2006; 295: 2727–2741.
13. Gail M, Brinton L, Byar D, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989; 81:1879–1886.
14. Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer—96-month follow-up of the randomized IBIS-I trial. J Natl Cancer Inst 2007; 99:272–282.
15. Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. Lancet 1998; 352:98–101.
16. Veronesi U, Maisonneuve P, Rotmensz N, et al. Italian randomized trial among women with hysterectomy: tamoxifen and hormone-dependent breast cancer in high-risk women. J Natl Cancer Inst 2003; 95:160–165.
17. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. JAMA 2001; 286:2251–2256.
18. Narod SA, Brunet JS, Ghadirian P, et al. Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. Hereditary Breast Cancer Clinical Study Group. Lancet 2000; 356:1876–1881.
19. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002; 346:1609–1615.
20. Rebbeck TR. Inherited predisposition and breast cancer: modifiers of BRCA1/2-associated breast cancer risk. Environ Mol Mutagen 2002; 39:228–234.
21. Bulun SE, Price TM, Aitken J, et al. A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription. J Clin Endocrinol Metab 1993; 77:1622–1628.

22. Tekmal RR, Ramachandra N, Gubba S, et al. Overexpression of int-5/aromatase in mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities. Cancer Res 1996; 56:3180–3185.
23. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. Lancet 2002; 359:2131–2139.
24. Goss PE, Ingle JN, Martino S, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 2003; 349:1793–1802.
25. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer 10.1056/NEJMoa040331. N Engl J Med 2004; 350:1081–1092.
26. Arun B, Goss P. The role of COX-2 inhibition in breast cancer treatment and prevention. Semin Oncol 2004; 31:22–29.
27. Howe LR, Subbaramaiah K, Brown AM, et al. Cyclooxygenase-2: a target for the prevention and treatment of breast cancer. Endocr Relat Cancer 2001; 8:97–114.
28. Arun B, Logan C, Yin G, et al. Phase II prevention trial of celecoxib in women at increased risk for breast cancer. Proc Am Soc Clin Oncol 2007 (abstr 1501).
29. Meyskens FL Jr., Gerner EW. Development of difluoromethylornithine (DFMO) as a chemoprevention agent. Clin Cancer Res 1999; 5:945–951.
30. Arun B, Mohsin S, Miller A, et al. Acquisition of breast tissue in a biomarker modulation study using bexarotene in women at high risk of breast cancer. Proc Am Soc Clin Oncol 2005; 23:1002 (abstr).
31. Brown PH, Lippman SM. Chemoprevention of breast cancer. Breast Cancer Res Treat 2000; 62:1–17.
32. Herbert BS, Wright AC, Passons CM, et al. Effects of chemopreventive and antitelomerase agents on the spontaneous immortalization of breast epithelial cells. J Natl Cancer Inst 2001; 93:39–45.
33. Barnes DM, Dublin EA, Fisher CJ, et al. Immunohistochemical detection of p53 protein in mammary carcinoma: an important new independent indicator of prognosis? Hum Pathol 1993; 24:469–476.
34. Fan S, Wang J, Yuan R, et al. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 1999; 284:1354–1356.
35. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 1999; 340: 77–84.
36. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. J Natl Cancer Inst 2001; 93: 1633–1637.
37. Meijers-Heijboer H, van Geel B, van Putten WL, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2001; 345:159–164.
38. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 2004; 22:1055–1062.
39. Brinton LA, Schairer C, Hoover RN, et al. Menstrual factors and risk of breast cancer. Cancer Invest 1988; 6:245–254.

40. Parazzini F, Braga C, La Vecchia C, et al. Hysterectomy, oophorectomy in premenopause, and risk of breast cancer. Obstet Gynecol 1997; 90:453–456.
41. Schairer C, Persson I, Falkeborn M, et al. Breast cancer risk associated with gynecologic surgery and indications for such surgery. Int J Cancer 1997; 70:150–154.
42. Meijer WJ, van Lindert AC. Prophylactic oophorectomy. Eur J Obstet Gynecol Reprod Biol 1992; 47:59–65.
43. Struewing JP, Watson P, Easton DF, et al. Prophylactic oophorectomy in inherited breast/ovarian cancer families. J Natl Cancer Inst Monogr 1995; 17:33–35.
44. Rebbeck TR, Levin AM, Eisen A, et al. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. J Natl Cancer Inst 1999; 91: 1475–1449.
45. Rebbeck TR. Prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers. J Clin Oncol 2000; 18:100S–103S.
46. Kauff ND, Perez-Segura P, Robson ME, et al. Incidence of non-founder BRCA1 and BRCA2 mutations in high risk Ashkenazi breast and ovarian cancer families. J Med Genet 2002; 39:611–614.
47. Eisen A, Lubinski J, Klijn J, et al. Breast cancer risk following bilateral oophorectomy in BRCA1 and BRCA2 mutation carriers: an international case-control study. J Clin Oncol 2005; 23:7491–7496.
48. Metcalfe K, Lynch HT, Ghadirian P, et al. Contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. J Clin Oncol 2004; 22:2328–2335.
49. Narod S, Lubinski J. Re: Roles of radiation dose, chemotherapy, and hormonal factors in breast cancer following Hodgkin's disease. J Natl Cancer Inst 2003; 95:1552.
50. Pierce LJ, Strawderman M, Narod SA, et al. Effect of radiotherapy after breast-conserving treatment in women with breast cancer and germline BRCA1/2 mutations. J Clin Oncol 2000; 18:3360–3369.
51. Chappuis PO, Nethercot V, Foulkes WD. Clinico-pathological characteristics of BRCA1- and BRCA2-related breast cancer. Semin Surg Oncol 2000; 18:287–295.
52. Turner BC, Harrold E, Matloff E, et al. BRCA1/BRCA2 germline mutations in locally recurrent breast cancer patients after lumpectomy and radiation therapy: implications for breast-conserving management in patients with BRCA1/BRCA2 mutations. J Clin Oncol 1999; 17:3017–3024.
53. Haffty BG, Harrold E, Khan AJ, et al. Outcome of conservatively managed early-onset breast cancer by BRCA1/2 status. Lancet 2002; 359:1471–1477.
54. Moynahan ME, Cui TY, Jasin M. Homology-directed DNA repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. Cancer Res 2001; 61:4842–4850.
55. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. JAMA 2000; 283:2260–2265.
56. Warner E, Trudeau M, Holloway C. Sensitivity of BRCA-1-related breast cancer to neoadjuvant chemotherapy: practical implications. Breast J 2003; 9:507–508.
57. De Soto JA, Wang X, Tominaga Y, et al. The inhibition and treatment of breast cancer with poly (ADP-ribose) polymerase (PARP-1) inhibitors. Int J Biol Sci 2006; 2: 179–185.
58. Dorval M, Patenaude AF, Schneider KA, et al. Anticipated versus actual emotional reactions to disclosure of results of genetic tests for cancer susceptibility: findings from p53 and BRCA1 testing programs. J Clin Oncol 2000; 18:2135–2142.

59. Lerman C, Hughes C, Lemon SJ, et al. What you don't know can hurt you: adverse psychologic effects in members of BRCA1-linked and BRCA2-linked families who decline genetic testing. J Clin Oncol 1998; 16:1650–1654.
60. Meiser B, Butow P, Friedlander M, et al. Intention to undergo prophylactic bilateral mastectomy in women at increased risk of developing hereditary breast cancer. J Clin Oncol 2000; 18:2250–2257.
61. Uyei A, Peterson SK, Erlichman J, et al. Association between clinical characteristics and risk-reduction interventions in women who underwent BRCA1 and BRCA2 testing: a single-institution study. Cancer 2006; 107:2745–2751.
62. Wagner TM, Moslinger R, Langbauer G, et al. Attitude towards prophylactic surgery and effects of genetic counselling in families with BRCA mutations. Austrian Hereditary Breast and Ovarian Cancer Group. Br J Cancer 2000; 82:1249–1253.
63. Lerman C, Narod S, Schulman K, et al. BRCA1 testing in families with hereditary breast-ovarian cancer. A prospective study of patient decision making and outcomes. JAMA 1996; 275:1885–1892.

8

Management of BRCA Mutation-Negative Patients

David J. Gallagher

Gynecologic Medical Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

Noah D. Kauff

Clinical Genetics Services, Department of Medicine, and Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

KEY POINTS

- The majority of families with multiple cases of both ovarian cancer and early-onset breast cancer have an identifiable *BRCA1* or *BRCA2* mutation. However, almost half of families with multiple cases of breast cancer only (site-specific breast cancer families) do not have an identifiable *BRCA1* or *BRCA2* mutation.
- Reasons why families with multiple cases of breast cancer only may not have an identifiable *BRCA1* or *BRCA2* mutation include (*i*) the cluster of cancers is a chance event, (*ii*) the individual tested may be a phenocopy (i.e., the tested individual has a sporadic cancer unrelated to an inherited familial predisposition), (*iii*) the inherited predisposition is due to a mutation in an as yet undiscovered cancer predisposition gene, or (*iv*) currently used mutation techniques are unable to detect a mutation that is present within *BRCA1* or *BRCA2*.

- In one study, women from *BRCA*-negative site-specific breast cancer families (with at least 3 cases of breast cancer and at least 1 case of breast cancer diagnosed prior to age 50) were not at increased risk of ovarian cancer.

INTRODUCTION

Increased public awareness of the availability of genetic testing, coupled with stronger evidence for the efficacy of risk-reducing strategies in women with an inherited risk, has led to a greater demand for *BRCA* testing. However, even in the setting of a clear, autosomal-dominant, inherited cancer susceptibility, sequencing of *BRCA1* and *BRCA2* will not identify a deleterious mutation in many families. In particular, families with multiple cases of breast cancer, but no cases of ovarian cancer (site-specific breast cancer families), do not have an identifiable *BRCA1* or *BRCA2* mutation almost half of the time (1,2). Such negative testing in the setting of a suspicious family history can lead to considerable anxiety for both patients and their family members. Additionally, while treatment paradigms have been created for mutation carriers of highly penetrant syndromes (3–5), there is limited guidance available for individuals who have evidence of a cancer predisposition but have wild-type genetic test results. In this chapter, we discuss the reasons for negative genetic testing in families with features of autosomal-dominant inherited predisposition and consider the management of these individuals.

CAUSES OF *BRCA*-NEGATIVE HEREDITARY BREAST CANCER

There are a number of reasons why clusters of multiple family members with breast cancer might not be explained by identifiable mutations in *BRCA1* or *BRCA2*, including (*i*) the cluster of cancers is a chance event, (*ii*) the individual tested may be a phenocopy (i.e., the tested individual has a sporadic cancer unrelated to an inherited familial predisposition), (*iii*) the inherited predisposition is due to a mutation in an as yet undiscovered cancer predisposition gene, or (*iv*) currently used mutation techniques are unable to detect a mutation that is present within *BRCA1* or *BRCA2*.

Coincidental Clustering

Breast cancer is the single most frequent female carcinoma with one in eight to one in nine women developing it in their lifetime (6). On the basis of its frequency alone, there is a possibility that breast cancer could cluster together within a given family by chance, particularly if there are a number of females in a lineage who live to an advanced age. However, the development of breast cancer is much less common at younger ages, with only 1 in 211 women developing breast cancer by age 40, and 1 in 54 women developing breast cancer by age 50 (6). Ovarian cancer

is much less common with only 1 in 79 women developing this disease by age 85. While it is certainly possible that several cases of later onset breast cancer and/or ovarian cancer could cluster together in a given family by chance, it becomes much less likely that multiple individuals ($\geq$3) in a single lineage have early-onset breast cancer (i.e., prior to age 50) and/or ovarian cancer are by chance alone.

The Individual Tested Is a Phenocopy

Given that breast cancer is the single most common cancer in women, it is certainly possible that it could develop in an individual whose family has an inherited predisposition, but in whom the individual affected women did not inherit the familial predisposition. Such a possibility is more likely if the individual tested is older, and therefore more likely to have breast cancer irrespective of underlying inherited risk. It is for this reason that it is frequently most informative to test the individual who was youngest at time of diagnosis to minimize the possibility that a phenocopy is being tested.

Other Breast Cancer Susceptibility Genes

It is now more than a decade since the discovery of the *BRCA1* and *BRCA2*. It was initially assumed that a number of other high-penetrance cancer susceptibility genes, associated with a markedly increased breast cancer risk, would be found. This hope, however, has not been realized to date. While several other high-penetrance cancer susceptibility genes, such as TP53 and PTEN, have been identified, these are found in a very small number ($<$1–2%) of *BRCA*-negative inherited breast cancer families (7). A number of low-penetrance cancer susceptibility genes such as ATM and CHEK2 have also been identified, but given that these are associated with relatively small modification of breast cancer risk (relative risks generally less than 2–3), it is not clear that mutations in any of these low-penetrance genes in isolation can explain families with clearly autosomal-dominant breast cancer susceptibility (8). Additionally, environmental factors that cluster in families are unlikely to explain all the remaining predisposition within families (9). It is more likely that these cancer susceptibilities are mediated through mutations in many genes, each conferring a moderate risk of disease.

Polygenic Model

This hypothesis is supported by twin studies, which suggest that a large amount of genetic susceptibility follows a polygenic model (10,11). The principal methods used for detecting further susceptibility genes are linkage analysis and association studies (12). Linkage analysis depends on the cosegregation of a gene and a phenotype through a pedigree. This approach, however, has been unsuccessful in identifying additional high-penetrance susceptibility regions, providing

support for a polygenic model. Two mathematical models based on segregation data have been proposed to explain the pattern of inheritance in families that cluster cases of breast cancer but are *BRCA1/2* negative. The first by Cui et al. suggested a mixed pattern of inheritance, including both a recessive and a polygenic component (13). This study was limited by older screening techniques and may have included some *BRCA1/2*-positive patients. A more recent study found no evidence for another major gene, but instead proposed a polygenic model where a number of genes with small effect combine multiplicatively (14). These lines of evidence suggest that cancer in *BRCA*-negative families may, at least in some instances, result from variations at multiple alleles that are inherited in high-penetrance combinations.

Association studies are case-control studies that compare frequency of variants among cancer cases and controls. If variations being studied are associated with a small relative risk of breast cancer, adequately powered studies to evaluate these variations can require hundreds to thousands of subjects. Public databases have been established to facilitate sharing of information with the aim of expediting the results of this approach. Genome-wide association studies, a newer technique that has identified novel low-penetrance breast cancer susceptibility loci, is well suited to identifying frequently occurring variants associated with lower breast cancer risk, but does not appear to be as well suited to identifying rare variants with moderate risk (15,16).

Undetectable Mutations in *BRCA1* and *BRCA2*

While much mutation detection in *BRCA1* and *BRCA2* is done by direct sequencing, one shortcoming of this approach is that it will miss structural rearrangements, such as large deletions, insertions, duplications, or inversions. Additionally, noncoding mutations in promoters, enhancers, and other regulatory regions are not detected by conventional sequencing (17,18). These limitations explain why direct sequencing detects only 63% to 85% of mutations in *BRCA1* and *BRCA2* (1,19). In one recent study, genomic rearrangements were found in 35 (12%) of 300 hereditary breast ovarian cancer families in which no deleterious mutation was detected by sequencing (20). Several other studies have seen similar rates of previously occult mutations (17,21). Importantly, even in these studies, no rearrangements were detected in the majority of families with an autosomal-dominant pattern of inheritance, suggesting the possibility of either undetected noncoding *BRCA* mutations or the presence of mutations in other as yet unidentified cancer susceptibility genes.

CLINICAL MANAGEMENT

Before discussing the clinical management of individuals with a negative *BRCA* mutation testing, it is important to emphasize the difference between families with site-specific breast cancer (with up to 4 or 5 cases of female breast cancer,

but no ovarian or male breast cancer) and those with hereditary breast-ovarian cancer. The linkage data discussed above demonstrated that over 90% of hereditary breast-ovarian cancer (in which there are multiple cases of early-onset breast cancer and at least one case of ovarian cancer) is attributable to mutations in *BRCA1* and *BRCA2* (1). However, the same study also demonstrated that approximately half of site-specific breast cancer families are not linked to these two genes. Specifically, in families with four or five cases of female breast cancer diagnosed prior to age 60 and no ovarian cancer, only 33% showed linkage to *BRCA1* or *BRCA2*. Even in families with six or more case of female breast cancer diagnosed prior to age 60, 19% did not demonstrate linkage to *BRCA1* or *BRCA2*.

Extrapolating from this data, families with multiple cases of breast cancer prior to age 60 *and* a family history of ovarian cancer or multiple other *BRCA*-associated (pancreatic, prostate, melanoma) cancers in a specific lineage should likely be treated as if there may be an occult *BRCA* mutation. Similarly, an occult *BRCA* mutation should be suspected in site-specific breast cancer families with six or more breast cancers prior to the age of 60.

For families meeting either of these criteria, affected individuals should be managed in similar manner to women where a *BRCA* mutation has been detected. Treatment options including prophylactic mastectomy, prophylactic salpingo-oophorectomy, chemoprevention, and surveillance appropriate for *BRCA1* and *BRCA2* mutation carriers should be discussed; specifics of these management strategies are addressed in detail in other chapters.

How unaffected individuals within these families should be optimally managed is less clear. Where there is an autosomal-dominant pattern of inheritance, these individuals will have a 50% chance of inheriting the unidentifiable predisposition that is within the family. They may, however, not be at any increased risk of cancer. Given these management challenges, such individuals are best managed by a multidisciplinary team experienced in the care of individuals who may be at inherited risk.

Site-Specific Breast Cancer Families

Given the data cited above suggesting that less than one-third of families, in which there are five or less cases of early-onset (diagnosed prior to age 60) breast cancer and no ovarian or male breast cancer, segregates a *BRCA1* or *BRCA2* mutation, it is not clear that *BRCA*-negative women in these families should be managed in the same manner as if a *BRCA1* or *BRCA2* mutation has been identified.

Certainly, women in these families remain at increased risk for breast cancer, and incremental breast cancer risk reduction (i.e., increased surveillance, chemoprevention, and risk-reducing mastectomy) should be discussed. At Memorial Sloan-Kettering Cancer Center, recommended screening for these individuals includes monthly self-examination, clinical breast examination two

times per year, and annual mammography starting 5 to 10 years prior to the earliest age of breast cancer in the family (but not before age 25). Additionally, we discuss that the American Cancer Society recommends screening MRI for women with a 20% to 25% or greater lifetime risk of breast cancer (5); however, we also review the limitations of currently available risk models (22).

Whether women from these families are at increased risk of ovarian cancer is more controversial. Studies in ungenotyped women with a personal and family history of breast cancer suggested that these women were at a greater risk of ovarian cancer than the general population (23,24). If this is the case, then it may be appropriate to manage all women in these families with incremental gynecologic risk-reduction approaches including, in select cases, risk-reducing surgeries. In order to provide data relevant to this issue, Kauff et al. recently conducted a prospective study to examine the risk of breast and ovarian cancer in *BRCA*-negative, site-specific breast cancer kindreds (25). In this study, there were 165 *BRCA*-negative, site-specific hereditary breast cancer kindreds identified in which there were at least three cases of breast cancer (mean 4.14, range 3–9) in a lineage with at least one breast cancer diagnosed prior to age 50. All probands had undergone *BRCA* mutation screening by either full sequencing or, in individuals of exclusively Ashkenazi Jewish heritage, founder mutation testing, as this has been shown to detect approximately 95% of detectable mutations in this population (26,27). Probands, along with their first- and second-degree relatives, were followed prospectively for a mean of 3.4 years to determine the incidence of new breast and ovarian cancer in these kindreds. The observed rates of breast and ovarian cancer were then compared with the expected population rates obtained from SEER.

As expected, a threefold increased risk of subsequent breast cancer was observed in this cohort (SIR 3.13; 95% CI, 1.88–4.89; $p < 0.001$). However, there was no increased risk of ovarian cancer observed in 2534 women years of follow-up with 1 case observed and 0.66 expected (SIR 1.52; 95% CI, 0.02–8,46; $p = 0.04$). These results, if confirmed, suggest that women from *BRCA*-negative site-specific breast cancer families may not be at significantly increased risk of ovarian cancer.

CONCLUSION

Evaluation of genetic predisposition to cancer is now a widely accepted component of cancer care. Specific management paradigms have been defined for carriers of genetic mutations associated with highly penetrant syndromes. However, much less information is available regarding the management of individuals with a negative genetic test in a family where there is considerable evidence for a genetic predisposition to cancer. In this chapter, the potential reasons for a wild-type result in these families as well as management strategies for women from these families were discussed. Unfortunately, definitive data to guide us is limited, and prospective studies evaluating management strategies in

women from *BRCA*-negative hereditary breast cancer families are urgently needed to improve management of women from these families.

CASE REPORT

NC is a 38-year-old woman diagnosed with right-sided invasive lobular breast carcinoma. Her family history was significant for a mother and sister with breast carcinoma. She underwent right-sided therapeutic mastectomy and left-sided prophylactic mastectomy. The patient was referred for genetic counseling and underwent *BRCA* testing in which no *BRCA* mutation was identified. Despite her negative *BRCA* testing, the patient desired a prophylactic oophorectomy to reduce her risk of subsequent ovarian cancer. While data is limited, her physician informed her that she did not likely have an increased risk of ovarian cancer.

LEARNING POINTS

- Women with a history suggestive of site-specific inherited breast cancer often do not demonstrate an identifiable *BRCA* mutation.
- While data are limited, there is no known increase in the risk of ovarian cancer among women with *BRCA*-negative, site-specific breast cancer; therefore, risk-reduction strategies for ovarian cancer are currently not recommended for most women in these families.

REFERENCES

1. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998; 62(3):676–689.
2. Rodriquez E, Domchek SM. The prevention of hereditary breast cancer. Semin Oncol 2007; 34(5):401–405.
3. National Comprehensive Cancer Network. Clinical practice guidelines in oncology, version 1.2007: Genetic/familial high-risk assessment—Breast and ovarian cancer. Available at: http://www.nccn.org/professional/physician_gls/PDF/genetics_screening.pdf.
4. Society of Gynecological Oncologists Clinical Practice Committee statement on prophylactic salpingo-oophoectomy. Gynecol Oncol 2005; 98:179–181.
5. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007; 57(2): 75–89.
6. SEER 17 Incidence and Mortality, 2000-2003. National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006. Available at: www.cdc.gov/nchs.
7. Robson ME, Boyd J, Borgen PI, et al. Hereditary breast cancer. Curr Probl Surg 2001; 38(6):387–480.

8. Oldenburg RA, Kroeze-Jansema K, Kraan J, et al. The CHEK2*1100delC variant acts as a breast cancer risk modifier in non-*BRCA1*/*BRCA2* multiple-case families. Cancer Res 2003; 63(23):8153–8157.
9. Hopper JL. The epidemiology of genetic epidemiology. Acta Genet Med Gemellol (Roma) 1992; 41(4):261–273.
10. Peto J, Mack TM. High constant incidence in twins and other relatives of women with breast cancer. Nat Genet 2000; 26(4):411–414.
11. Swerdlow AJ, De Stavola BL, Floderus B, et al. Risk factors for breast cancer at young ages in twins: an international population-based study. J Natl Cancer Inst 2002; 94(16):1238–1246.
12. March RE. Gene mapping by linkage and association analysis. Mol Biotechnol 1999; 13(2):113–122.
13. Cui J, Antoniou AC, Dite GS, et al. After *BRCA1* and *BRCA2*-what next? Multifactorial segregation analyses of three-generation, population-based Australian families affected by female breast cancer. Am J Hum Genet 2001; 68(2):420–431.
14. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003; 72(5): 1117–1130.
15. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; 447:1087–1093.
16. Antoniou AC, Easton DF. Risk prediction models for familial breast cancer. Future Oncol 2006; 2(2):257–274.
17. Gad S, Caux-Moncoutier V, Pagès-Berhouet S, et al. Significant contribution of large *BRCA1* gene rearrangements in 120 French breast and ovarian cancer families. Oncogene 2002; 21(44):6841–6847.
18. Montagna M, Dalla Palma M, Menin C, et al. Genomic rearrangements account for more than one-third of the *BRCA1* mutations in northern Italian breast/ovarian cancer families. Hum Mol Genet 2003; 12(9):1055–1061.
19. Unger MA, Nathanson KL, Calzone K, et al. Screening for genomic rearrangements in families with breast and ovarian cancer identifies *BRCA1* mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. Am J Hum Genet 2000; 67(4):841–850.
20. Walsh T, Casadei S, Hale Coats K, et al. Spectrum of Mutations in *BRCA1*, *BRCA2*, *CHEK2*, and *TP53* in Families at High Risk of Breast Cancer. JAMA 2006; 295:1379–1388.
21. Buffon A, Capalbo C, Ricevuto E, et al. Prevalence of *BRCA1* and *BRCA2* genomic rearrangements in a cohort of consecutive Italian breast and/or ovarian cancer families. Breast Cancer Res Treat 2007; 106(2):289–296.
22. Kauff ND, Offit K. Modeling genetic risk of breast cancer. JAMA 2007; 297: 2637–2639.
23. Bergfeldt K, Rydh B, Granath F, et al. Risk of ovarian cancer in breast-cancer patients with a family history of breast or ovarian cancer: a population-based cohort study. Lancet 2002; 360(9337):891–894.
24. Lorenzo Bermejo J, Hemminki K. Risk of cancer at sites other than the breast in Swedish families eligible for *BRCA1* or *BRCA2* mutation testing. Ann Oncol 2004; 15(12):1834–1841.

25. Kauff ND, Mitra N, Robson ME, et al. Risk of ovarian cancer in *BRCA1* and *BRCA2* mutation-negative hereditary breast cancer families. J Natl Cancer Inst 2005; 21; 97(18): 1382–1384.
26. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in *BRCA1* and *BRCA2*: analysis of 10,000 individuals. J Clin Oncol 2002; 20(6):1480–1490.
27. Kauff ND, Perez-Segura P, Robson ME, et al. Incidence of non-founder *BRCA1* and *BRCA2* mutations in high risk Ashkenazi breast and ovarian cancer families. J Med Genet 2002; 39(8):611–614.

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Therapy and Prognosis of BRCA-Associated Ovarian Cancer

Christina S. Chu and Stephen C. Rubin

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

KEY POINTS

- Ovarian cancer patients with germline BRCA mutations may have a survival advantage over those with sporadic ovarian cancer; however, contradictory reports exist.
- Survival advantage in ovarian cancer patients with germline BRCA mutations is proposed to be due to increased sensitivity to chemotherapy conferred by BRCA mutations and deficient DNA repair mechanisms.
- Targeted therapies, such as poly-adenosine diphosphate-ribose polymerase (PARP) inhibitors, that exploit this defect in DNA repair are being studied in ovarian cancer.

INTRODUCTION

Only about 10% of cases of epithelial ovarian cancer (EOC) are due to an inherited predisposition (1–5), with a majority of these attributable to alterations in the BRCA1 and BRCA2 genes and a small contribution due to mutations in the DNA mismatch repair genes involved in the Lynch II or hereditary nonpolyposis colorectal carcinoma syndrome. For BRCA mutation carriers, the lifetime risk for ovarian cancer is estimated to be between 20% and 40%.

THE ROLE OF BRCA1 AND BRCA2 IN OVARIAN CANCER

Clearly, mutations in BRCA1 and BRCA2 are important factors in hereditary ovarian cancer, with prevalence varying widely depending on ethnicity, personal, and family history. For example, for those of Ashkenazi Jewish heritage, 90% of the cases of breast and ovarian cancers are linked to three well-documented founder mutations (the 185delAG and 5382insC mutations in BRCA1 and the 6174delT mutation in BRCA2) (6,7). In these women, the reported prevalence of genetic alternations has been noted to be as high as 2.5% (6,7). With the added factor of a personal history of breast cancer, the prevalence may rise to 10% (8,9); for those with a personal history of ovarian cancer, the rate is as high as 40% (10,11). Even in the general population, a given individual with breast cancer may possess a 3% risk for mutation and the addition of a family history of ovarian cancer may increase the risk to 22.8% (12). In high-risk families with multiple cases of breast and/or ovarian cancer, individual women may have a risk as high as 40% of carrying a mutation in either BRCA1 or BRCA2 (12).

In the general population, patients with sporadic EOC, BRCA1, and BRCA2 were felt to play a lesser role. Previous studies have documented BRCA1 mutations in only 1 of 800 people in the general population and in only 3% to 6% of all patients with epithelial ovarian carcinoma (13,14). Newer evidence suggests that the prevalence of BRCA mutations in unselected patients with ovarian cancer may be higher than previously reported. Risch et al. (15) examined a population series of 1171 unselected patients from Ontario, Canada, with ovarian cancer. BRCA1 and BRCA2 mutation screening was performed utilizing testing for common variants, protein truncation testing of long exons, and denaturing gradient gel electrophoresis or denaturing high-performance liquid chromatography for the remainder of the genes. Although the estimated carrier frequencies of mutations in the general population were 0.32% for BRCA1 and 0.69% for BRCA2, the authors noted a 13.2% frequency of BRCA1 and BRCA2 mutations among the 977 patients with invasive ovarian cancer. For women with BRCA1 mutations, the calculated cumulative incidence of ovarian cancer was 24% and that of breast cancer was 90% to age 80. Buller et al. (16) examined 250 consecutive women with ovarian cancer at a single institution and noted that 40 of 250 (16%) demonstrated mutations in BRCA1. For those with BRCA2 mutations, the cumulative incidences were 8.4% and 41%, respectively. Pal et al. (17) conducted a population-based study of 209 patients with unselected invasive ovarian cancer patients in Florida and noted that 15.3% of women had mutations in either BRCA1 or BRCA2. Their findings concluded that relying on family history alone to trigger genetic testing would miss more than 30% of BRCA-associated ovarian cancers. Likewise, only 41% of mutation carriers reported a family history of breast or ovarian cancer in a Polish population-based study (18). A significant number of ovarian cancer patients with potential undocumented mutations in BRCA genes, especially in the absence

of family history, may have important implications for a given individual's prognosis as well as for direction of future targeted therapeutic strategies.

PROGNOSIS OF BRCA-ASSOCIATED OVARIAN CANCER

The effect of mutation status, itself, on prognosis is somewhat controversial. Several studies have attempted to clarify the relationship between BRCA mutation and prognosis (Table 1). While most have demonstrated a significantly more favorable outcome in comparison to sporadic ovarian cancers (10,19–24), others have not been able to confirm this finding (16,25,26).

Evidence for an Improved Prognosis

Buller et al. (27) was the first to provide indirect evidence of improved prognosis. In 1993, even prior to the cloning of BRCA1, the authors reported on 11 members of 4 families affected by hereditary ovarian cancer—each family demonstrated two or more first-degree relatives with a diagnosis of ovarian cancer. Of these 11 patients, 1 was stage II, 6 were stage III, 3 were stage IV, and one was unstaged. The authors found a 67% five-year survival for the 11 study patients, compared with only 17% in a selected comparison group of 34 consecutively treated stage III patients of similar age ($p < 0.04$).

In 2000, additional epidemiologic evidence suggested improved prognosis for patients with familial ovarian cancer. A large population-based U.S. Surveillance, Epidemiology, and End Results (SEER) database study identified 824 white women with EOC and a prior diagnosis of breast cancer (28). These patients had an overall estimated five-year survival of 49% compared with only 45% among women without a history of prior breast cancer. Among women diagnosed over the age of 55 and those with advanced disease, the improvement in prognosis was even more pronounced. Given the approach, the usual pitfalls of large, population-based studies were apparent, including lack of data on standard prognostic factors such as disease residual and type of adjuvant treatment. However, the main strength was the ability to minimize selection bias, and these findings provided further indirect evidence that BRCA mutations conferred a survival advantage when compared with patients with sporadic cancer. Although the patients did not undergo formal genetic testing, prior calculations have estimated that 88% of women with both breast and ovarian cancer are carriers of BRCA1 mutations (29).

The first study of survival in ovarian cancer patients with documented mutations in the BRCA genes was reported in 1996 by Rubin et al. (19). Fifty-three advanced stage patients with germline mutations in BRCA1 were compared with age- and stage-matched controls unselected for family history. BRCA1 mutation carriers demonstrated a marked improvement in median survival of 77 months compared with only 29 months for controls ($p < 0.001$). Though the study did not elaborate on important clinical factors, including

Table 1 Survival in Ovarian Cancer Patients with Mutations in BRCA1 and BRCA2

Author	Year	Location	Mutation	Population	Number of mutation patients	Number of control patients	Stage	5-Yr survival cases	5-Yr survival controls	*p* value
Rubin (19)	1996	Multiple centers, United States	BRCA1	Unselected for family history	43	43 (case matched)	III, IV	77 mo (median survival)	43 mo (median survival)	<0.001
Aida (21)	1998	Japan	BRCA1	Breast-ovarian cancer families	13	29 (case matched)	III	78.6%	30.0%	<0.05
Johannsson (25)	1998	Sweden	BRCA1	Breast-ovarian cancer families, national tumor registry	38	112	I–IV	32%	37%	NS
Pharoah (26)	1999	United Kingdom	BRCA1, BRCA2	Breast-ovarian cancer families, national cancer registry	151	552	I–IV	21% BRCA1, 25% BRCA2	30%	0.005
Boyd (10)	2000	New York, United States	BRCA1, BRCA2	Consecutive cases diagnosed at single institution, patients of Jewish origin	81	101	III, IV	45%	25%	0.004
Ben David (22)	2002	Israel	BRCA1, BRCA2	Unselected for family history, Jewish women from national study	234	549	I–IV	65.8 (3-yr survival)	51.9% (3-yr survival)	<0.001
Buller (16)	2002	Iowa, United States	BRCA1	Single institution, consecutive patients	59	59 (case matched)	I–IV	4.1 yr (median survival)	3.5 yr (median survival)	NS
Cass (23)	2003	California, United States	BRCA1, BRCA2	Tumor registry, Jewish women	29	25	III, IV	65%	48%	0.046
Majdak (24)	2005	Poland	BRCA1, BRCA2	Consecutive cases from single institution	34 (18 pathogenic, 16 unclassified variants)	171	I–IV	77% (3-yr survival[a])	31% (3-yr survival)	0.019

[a]For pathogenic mutation carrier.

potential differences in treatment, and was criticized for possible selection biases in formulating the control group, this was the first report of a dramatic improvement in prognosis for documented mutation carriers. This finding was confirmed in 1998 by Aida et al. (21) in a smaller Japanese study of 13 patients with germline mutations in BRCA1. They selected age- and treatment-matched controls for comparison and noted a five-year survival of 78.6% versus 30.3%, respectively ($p < 0.05$), as well as a significant advantage in median disease-free interval (91.4 months vs. 40.9 months, respectively, $p < 0.05$).

In 2000, Boyd et al. (10) performed a retrospective cohort study and identified 189 Jewish women with ovarian cancer from among 933 consecutively treated patients at Memorial Sloan-Kettering Cancer Center. Of these patients, 88 were found to have mutations in either BRCA1 or BRCA2. Those with documented mutations had significantly improved five-year survival (45% vs. 25%, $p = 0.004$), as well as longer median time to recurrence (7 months vs. 14 months, $p < 0.001$), and gained a 25% reduction in the relative risk of death compared with those without mutations. Among patients with stage III disease, altered BRCA was noted to be an independent factor influencing prognosis. The authors minimized selection bias by using archival material from a large consecutive series of ovarian cancer patients to eliminate preferential inclusion of living mutations carriers. In addition, given that all patients and controls were cared for at the same institution over the same period, treatment differences between the groups were minimized.

Several other reports have also supported a more favorable prognosis for patients with mutations in BRCA1 and BRCA2 (22–24). Ben David et al. (22) performed a large study of 234 mutation carriers identified from a nationwide study and noted improved three-year survival when compared with 549 mutation-negative controls (65.8% vs. 51.9%, respectively, $p = 0.001$). The difference persisted even after controlling for the younger age of the mutation carriers. Similarly, a smaller study by Cass et al. (23) found a more favorable five-year survival (65% vs. 48%), disease-free survival (49 months vs. 19 months, $p = 0.16$), and improved response rate to therapy (72% vs. 36%, $p = 0.01$) for 34 Jewish mutation carriers compared with 35 women with sporadic tumors. Finally, Majdak et al. (24) identified 34 patients with mutations in BRCA1 and BRCA2, 16 were unclassified variants and 18 were pathogenic. On multivariate analysis, pathogenic mutation in BRCA1, but not unclassified variant mutation, was an independent factor in predicting a decreased risk of recurrence and improved survival.

Evidence for a Poorer Prognosis

Despite the findings of these initial reports, other investigators soon published data showing either no significant survival advantage or even worse survival for patients with BRCA mutations. Johannsson et al. (25) identified 38 patients with ovarian cancer from a population-based registry of breast cancer families in

southern Sweden. Of these 38 patients, 7 also had a diagnosis of breast cancer. The authors concluded that while survival in the first years after diagnosis appeared better for BRCA mutation carriers than for age- and stage-matched controls, long-term survival was similar. In fact, multivariate analysis even showed a statistically worse survival for BRCA1 patients than controls. Similarly, Pharoah et al. (26) also noted a significantly worse prognosis for ovarian cancer patients with BRCA1 and BRCA2 mutations compared with sporadic cases. These authors conducted a large study of 151 patients from 57 families with documented BRCA1 and BRCA2 and also examined 199 patients from 62 families in which a BRCA1 or BRCA2 mutation was not found after genetic testing. For controls, they selected 552 age-matched cases from the general population. The authors noted that overall survival in familial ovarian cancer cases as a whole was significantly worse than for population controls; five-year survival was 21% in patients from BRCA1 families, 25% from BRCA2 families, 19% from families with no identified mutation, versus 30% in population controls ($p < 0.005$). The results may have been biased given that patients in the familial ovarian cancer groups had a significantly higher incidence of advanced, stage III and IV disease at presentation (83% vs. 56% for population controls, $p < 0.001$). Another significant weakness was the lack of direct mutation testing among individual patients in the study. The authors merely assumed that all patients suffering from ovarian cancer in families with a previously documented mutation were automatic carriers of a mutation themselves. Finally, Buller et al. (16) were also unable to detect any difference in survival between patients with BRCA1 inactivation compared with matched controls. In their analysis, the authors examined 59 cancers with presumed BRCA1 dysfunction based on an identified mutation or absent BRCA1 mRNA because of promoter hypermethylation. They used rigorously selected controls from the same population, matched for p53 mutation type, age, stage, grade, disease site, and the presence of BRCA1 mRNA. No significant differences were noted in median survival for those with BRCA1 dysfunction compared with controls (4.1 years vs. 3.5 years, respectively).

It remains unclear why different investigators have noted such varying effects of BRCA alteration on survival. One theory proposes that differing mechanisms of BRCA inactivation may influence patient outcome. Indeed, in 2002, Buller et al. (16) examined survival in patients with ovarian cancer based on the mechanism of BRCA1 dysfunction. Although median survivals were nearly identical to those of case-matched controls without abnormalities in BRCA, it was notable that patients with germline mutations exhibited a median survival nearly twice that of patients with other mechanisms of BRCA1 dysfunction (4.5 years for germline mutations, 2.8 years for somatic mutations, or 2.3 years for promoter silenced cancers). These findings were confirmed in 2006 by Chiang et al. (30) who compared survival of patients with ovarian cancer from a hospital-based tumor bank to examine the outcome of patients with BRCA1 silencing due to promoter hypermethylation. Tumors were classified as having a wild-type BRCA gene, a BRCA mutation, or a methylated BRCA.

Patients with a methylated BRCA1 promoter were noted to have significantly decreased median disease-free interval (9.8 months vs. 35.6 months, $p = 0.04$) as well as median overall survival (35.6 months vs. 78.6 months, $p = 0.02$) when compared with BRCA1 mutation carriers.

MECHANISMS FOR BRCA-ASSOCIATED SURVIVAL ADVANTAGE

The mechanism for the purported survival advantage conferred by BRCA mutation is not entirely clear. Some speculate that BRCA-associated tumors grow more slowly than their sporadic counterparts. Another theory posits greater susceptibility to chemotherapy. In fact, several reports have cited prolonged disease-free intervals after surgery and chemotherapy (10,23), and another has documented a significantly higher growth fraction in BRCA-associated malignancies (31), suggesting that increased proliferation may contribute to improved chemosensitivity.

Further evidence that BRCA mutations may increase sensitivity to chemotherapy relates to the functions of the genes themselves. BRCA1 has been implicated in a broad number of cellular functions, including DNA repair, the maintenance of genomic integrity, and cell-cycle checkpoint control (32–36). However, the main role of BRCA2 appears to involve interaction with RAD51 in homologous recombination DNA repair (37). Cells with mutated BRCA proteins may therefore be rendered less capable of repairing chemotherapy-induced DNA damage, potentially leading to an improved response to treatment. Support for this theory was provided by Husain et al. (38) who noted increased levels of BRCA1 protein in cisplatin-resistant breast and ovarian cancer cell lines. In the ovarian cancer cell line SKOV-3 CDDP/R, DNA damage repair was correspondingly improved. The investigators showed that the effects could be reversed by antisense inhibition of BRCA1, which induced a decreased efficiency of DNA repair, enhanced apoptosis, and restoration of cisplatin sensitivity. Data by Cass et al. (23) also noted that in vitro chemosensitivity testing was predictive of response to treatment with platinum and paclitaxel among patients with hereditary cancers, but not in patients with sporadic tumors.

THERAPY FOR BRCA-ASSOCIATED OVARIAN CANCER

To date, despite recognized differences in tumor biology, therapy of BRCA-associated ovarian cancers has essentially consisted of the same standard surgery and adjuvant chemotherapy that is utilized for sporadic malignancies. Patients with early-stage disease undergo surgery, then chemotherapy with three to six cycles of a taxane and a platinum agent depending on clinicopathologic risk factors. Patients with advanced disease generally undergo cytoreductive surgery followed by six to eight cycles of a combination taxane/platinum-based regimen. In an effort to limit systemic toxicity, recent efforts in cancer therapy have increasingly focused on targeted approaches, which usually aim to inhibit a

specific factor driving tumor growth. Recent successes have included imatinib mesylate for chronic myelogenous leukemia and gastrointestinal stromal tumors, gefitinib for non–small cell lung cancer, and trastuzumab for breast cancer (39). However, in the case of breast and ovarian cancers associated with BRCA mutations, given the known role of BRCA in the repair of double-stranded DNA breaks, investigators have attempted to exploit a weakness in the tumor cell to enhance antitumor therapy (39).

Bryant et al. (40) and Farmer et al. (41) speculated that cells deficient in the ability to repair double-stranded DNA breaks might be susceptible to therapies aimed at increasing the number of breaks. Prior work by Conde et al. (42) showed that mice engineered to lack the poly-adenosine diphosphate-ribose polymerase 1 (PARP1) enzyme exhibited large numbers of unrepaired single-stranded DNA breaks. During routine DNA replication, these single-stranded breaks are converted to double-stranded breaks and then repaired. However, in the presence of BRCA dysfunction, the addition of PARP inhibition appears to lead to fatal accumulation of unrepairable DNA breaks that would normally undergo repair via homologous recombination in the presence of wild-type BRCA. In vitro assays by Bryant et al. (40) and Farmer et al. (41) both demonstrated that even low-dose inhibition of PARP1 resulted in cell death in cell lines lacking either BRCA1 or BRCA2. In contrast, BRCA wild-type cells were unaffected by PARP1 inhibition and continued with routine growth. This effect of PARP1 inhibitors was confirmed in murine tumor models. Though PARP1 is normally involved in repair of DNA breaks and attraction of other repair proteins to the site of injury, it does not appear to be required for survival given that mice deficient in the enzyme appear to be healthy and fertile, and those mice treated with PARP inhibitor appeared otherwise healthy. This promising novel approach to anticancer therapy involves no cytotoxic or DNA-damaging agent, but solely acts to selectively inhibit DNA repair in susceptible tumor cells. Clinical trials of inhibitors of PARP are currently under way in patients with BRCA-related breast and ovarian cancers.

CONCLUSION

Patients with ovarian cancer associated with mutations in BRCA1 and BRCA2 clearly demonstrate some important clinical differences from those with sporadic malignancies. The majority of reports have identified a survival advantage for those with mutations, though several investigators have found otherwise, perhaps pointing to a more complex model where the specific mechanism of BRCA dysfunction may also be an important predictor of outcome. Given that BRCA mutated cells are deficient in certain DNA repair mechanisms, these tumors may be more sensitive to traditional chemotherapy. Newer targeted therapies aimed at exploiting this deficiency in DNA repair, such as the use of PARP1 inhibitors, are on the horizon.

CASE REPORT

J.S. was diagnosed with stage III ovarian cancer in April 2000, at the age of 63. At the time of her initial presentation it was documented that her twin sister had been diagnosed with breast cancer at the age of 36. Additionally, four paternal aunts and one maternal aunt had been diagnosed with postmenopausal breast cancer.

J.S. was treated with the standard surgery, followed by adjuvant therapy with six cycles of carboplatin and paclitaxel. At the end of her treatment, she was without evidence of disease. She was followed with routine examinations, and in July 2005, with the addition of a daughter and a cousin recently diagnosed with premenopausal breast cancer, her family history now prompted a referral to genetic counseling. She was counseled and tested in February 2006 and received her BRCA 1–positive results in April 2006, having been disease free for six years since her diagnosis of ovarian cancer. She proceeded to undergo bilateral prophylactic mastectomies for breast cancer risk reduction and remains in remission seven years after her diagnosis of ovarian cancer, having had no treatment after the initial chemotherapy.

LEARNING POINTS

- Germline BRCA mutations, resulting in deficiencies in DNA repair, may make ovarian cancer more responsive to cytotoxic chemotherapy.
- Advantages in progression-free and overall survival are believed to be related to the chemoresponsiveness of BRCA-associated tumors.

REFERENCES

1. Schildkraut JM, Risch N, Thompson WD. Evaluating genetic association among ovarian, breast, and endometrial cancer: evidence for a breast/ovarian cancer relationship. Am J Hum Genet 1989; 45(4):521–529.
2. Houlston RS, Collins A, Slack J, et al. Genetic epidemiology of ovarian cancer: segregation analysis. Ann Hum Genet 1991; 55(pt 4):291–299.
3. Bewtra C, Watson P, Conway T, et al. Hereditary ovarian cancer: a clinicopathological study. Int J Gynecol Pathol 1992; 11(3):180–187.
4. Narod SA, Madlensky L, Bradley L, et al. Hereditary and familial ovarian cancer in southern Ontario. Cancer 1994; 74(8):2341–2346.
5. Lynch HT, Lynch JF, Conway TA. Hereditary ovarian cancer. In: Rubin SC, Sutton GP, eds. Ovarian Cancer. New York: McGraw-Hill, 1993:189–217.
6. Malone KE, Daling JR, Thompson JD, et al. BRCA1 mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history. JAMA 1998; 279(12):922–929.
7. Couch FJ, Hartmann LC. BRCA1 testing—advances and retreats. JAMA 1998; 279(12): 955–957.

8. Robson M, Levin D, Federici M, et al. Breast conservation therapy for invasive breast cancer in Ashkenazi women with BRCA gene founder mutations. J Natl Cancer Inst 1999; 91(24):2112–2117.
9. Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994; 343(8899):692–695.
10. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. JAMA 2000; 283(17):2260–2265.
11. Moslehi R, Chu W, Karlan B, et al. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. Am J Hum Genet 2000; 66(4): 1259–1272.
12. Newman B, Mu H, Butler LM, et al. Frequency of breast cancer attributable to BRCA1 in a population-based series of American women. JAMA 1998; 279(12): 915–921.
13. Schildkraut JM, Thompson WD. Familial ovarian cancer: a population-based case-control study. Am J Epidemiol 1988; 128(3):456–466.
14. Takahashi H, Behbakht K, McGovern PE, et al. Mutation analysis of the BRCA1 gene in ovarian cancers. Cancer Res 1995; 55(14):2998–3002.
15. Risch HA, McLaughlin JR, Cole DE, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl Cancer Inst 2006; 98(23):1694–1706.
16. Buller RE, Shahin MS, Geisler JP, et al. Failure of BRCA1 dysfunction to alter ovarian cancer survival. Clin Cancer Res 2002; 8(5):1196–1202.
17. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005; 104(12):2807–2816.
18. Menkiszak J, Gronwald J, Gorski B, et al. Hereditary ovarian cancer in Poland. Int J Cancer 2003; 106(6):942–945.
19. Rubin SC, Benjamin I, Behbakht K, et al. Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1. N Engl J Med 1996; 335(19):1413–1416.
20. Boyd J. Molecular genetics of hereditary ovarian cancer. In: Rubin SC, Sutton GP, eds. Ovarian Cancer. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2001:3–22.
21. Aida H, Takakuwa K, Nagata H, et al. Clinical features of ovarian cancer in Japanese women with germ-line mutations of BRCA1. Clin Cancer Res 1998; 4(1):235–240.
22. Ben David Y, Chetrit A, Hirsh-Yechezkel G, et al. Effect of BRCA mutations on the length of survival in epithelial ovarian tumors. J Clin Oncol 2002; 20(2):463–466.
23. Cass I, Baldwin RL, Varkey T, et al. Improved survival in women with BRCA-associated ovarian carcinoma. Cancer 2003; 97(9):2187–2195.
24. Majdak EJ, Debniak J, Milczek T, et al. Prognostic impact of BRCA1 pathogenic and BRCA1/BRCA2 unclassified variant mutations in patients with ovarian carcinoma. Cancer 2005; 104(5):1004–1012.
25. Johannsson OT, Ranstam J, Borg A, et al. Survival of BRCA1 breast and ovarian cancer patients: a population-based study from southern Sweden. J Clin Oncol 1998; 16(2):397–404.
26. Pharoah PD, Easton DF, Stockton DL, et al. Survival in familial, BRCA1-associated, and BRCA2-associated epithelial ovarian cancer. United Kingdom Coordinating Committee for Cancer Research (UKCCCR) Familial Ovarian Cancer Study Group. Cancer Res 1999; 59(4):868–871.

27. Buller RE, Anderson B, Connor JP, et al. Familial ovarian cancer. Gynecol Oncol 1993; 51(2):160–166.
28. McGuire V, Whittemore AS, Norris R, et al. Survival in epithelial ovarian cancer patients with prior breast cancer. Am J Epidemiol 2000; 152(6):528–532.
29. Frank TS, Manley SA, Olopade OI, et al. Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. J Clin Oncol 1998; 16(7):2417–2425.
30. Chiang JW, Karlan BY, Cass L, et al. BRCA1 promoter methylation predicts adverse ovarian cancer prognosis. Gynecol Oncol 2006; 101(3):403–410.
31. Levine DA, Federici MG, Reuter VE, et al. Cell proliferation and apoptosis in BRCA-associated hereditary ovarian cancer. Gynecol Oncol 2002; 85(3):431–434.
32. Xu X, Weaver Z, Linke SP, et al. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol Cell 1999; 3(3):389–395.
33. Yang H, Jeffrey PD, Miller J, et al. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science 2002; 297(5588):1837–1848.
34. Gretarsdottir S, Thorlacius S, Valgardsdottir R, et al. BRCA2 and p53 mutations in primary breast cancer in relation to genetic instability. Cancer Res 1998; 58(5):859–862.
35. Xu X, Wagner KU, Larson D, et al. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. Nat Genet 1999; 22(1):37–43.
36. Scully R, Anderson SF, Chao DM, et al. BRCA1 is a component of the RNA polymerase II holoenzyme. Proc Natl Acad Sci U S A 1997; 94(11):5605–5610.
37. Gudmundsdottir K, Ashworth A. The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene 2006; 25(43):5864–5874.
38. Husain A, He G, Venkatraman ES, et al. BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum(II). Cancer Res 1998; 58(6):1120–1123.
39. Brody LC. Treating cancer by targeting a weakness. N Engl J Med 2005; 353(9): 949–950.
40. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005; 434(7035):913–917.
41. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005; 434(7035):917–921.
42. Conde C, Mark M, Oliver FJ, et al. Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. EMBO J 2001; 20(13):3535–3543.

10

Molecular Genetics and Cancer Risks in Lynch Syndrome

Eamonn Sheridan

St. James's University Hospital, Leeds, U.K.

KEY POINTS

- The mismatch repair system maintains the integrity of the genome through correction of base pair mismatches in newly synthesized DNA.
- Lynch syndrome can result from inherited defects in the mismatch repair system. These tumors will display the classic MSI+ phenotype.
- Germline mutations in MLH1, MSH2, MSH6, or PMS2 are the most common inherited causes of Lynch syndrome.
- MSI can be caused by nonhereditary mechanisms, most frequently methylation of the MLH1 promoter.
- Lynch syndrome confers an equal lifetime risk of endometrial and colorectal carcinoma of 40% to 60%, and a 12% lifetime risk of ovarian carcinoma.

INTRODUCTION

In 1895, the pathologist Aldred Warthin first became aware of the concerns of his seamstress about the large number of cancers in her family. In 1913, he reported his observations on the family in the Archives of Internal Medicine (1). Sadly the seamstress died of metastatic endometrial cancer anyway and the report was filed away on the shelves of medical libraries, despite a follow-up report in 1925 (2). In the 1960s, the family was reinvestigated by Henry

Lynch in response to concerns expressed by a later member of the family (3). Following extensive work on this and other families, in 1971, Lynch and Krush proposed criteria for the "Cancer Family Syndrome" (4), which is now known as Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC). Despite its particular association with colorectal cancer, apparent from Lynch's 1966 paper, from its earliest description gynecological malignancy has been a part of this condition. In 1991, clinical criteria were established for Lynch syndrome, this greatly facilitated efforts to identify the causative genes (5).

Following the original clinical descriptions, the biological causes of Lynch syndrome were identified as dominantly inherited mutations in DNA mismatch repair (MMR) genes (6–10). Finally, in 2000, the causative mutation in Warthin's original family was reported (11), time to report in this case being 105 years!

An understanding of the nature of the mutations that cause this condition requires some comprehension of the processes of DNA replication and of the mechanisms that repair damaged DNA. A brief introduction to this topic follows.

DNA REPLICATION

The structure of DNA is a double helix in which complementary bases are held together by weak hydrogen bonds (Fig. 1). The bases are attached to a backbone consisting of alternating sugar residues and phosphate groups. The phosphate group links the 3′ carbon atom on one sugar to the 5′ carbon atom of the next sugar (Figs. 2 and 3). It follows therefore that at one end of the molecule there is a sugar residue in which the 5′ carbon atom is not linked to a neighboring molecule. At the other end there will be a sugar residue in which the 3′ carbon atom is not linked to a neighboring 5′ carbon atom. DNA sequences are normally

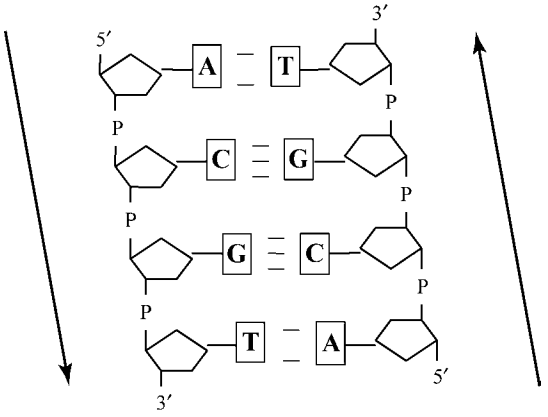

Figure 1 The two strands of the DNA double helix run in opposite (anti-parallel) directions.

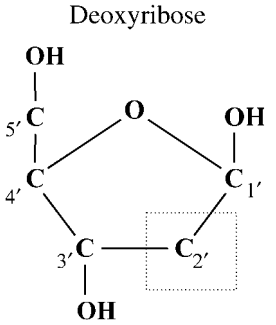

Figure 2 Structure of deoxyribose showing the position of each of the carbon atoms.

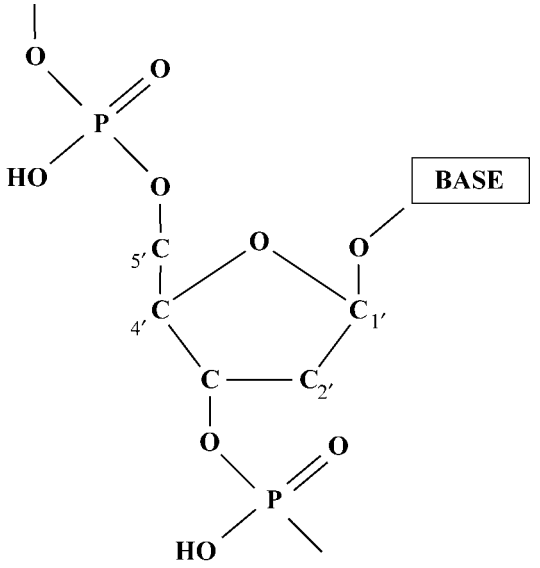

Figure 3 Phosphate bonds are formed between carbon atom number 3 of one deoxyribose and number 5 carbon of the next deoxyribose.

written in the direction 5′ to 3′, from the base with the free 5′ carbon to the base with the free 3′ carbon atom (Fig. 4). The two strands of DNA run in opposite directions to each other, referred to as anti-parallel (Fig. 1).

When DNA is replicated, the double helix is unwound by a helicase enzyme and each DNA strand acts as a template for the production of complementary daughter strands. As the two parent strands of DNA run in opposite directions to each other (5′>3′ and 3′>5′), the daughter strands are synthesized in opposite directions as well. One strand is extended in a 5′>3′ direction, this is referred to as the leading strand, the other in a 3′>5′ direction referred to as the

- DNA sequences are written in a 5′-3′ direction
- This is the direction in which DNA and RNA are synthesized

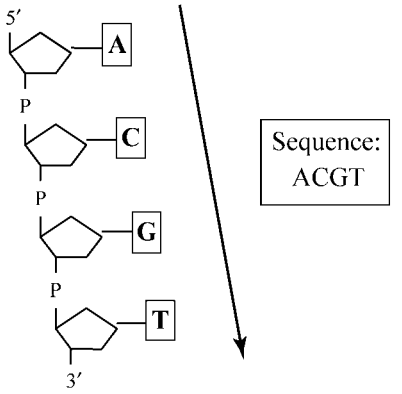

Figure 4 DNA sequences are written in the 5′ (5 prime) to 3′ (3 prime) direction.

lagging strand. When replication occurs only the leading strand will have a free 3′ carbon at which to attach the 5′ carbon of the next sugar residue, hence replication extends from this point in the same 5′>3′ direction. On the lagging strand, extension also occurs in 5′>3′ direction, in the opposite direction to that of the sequence itself. Thus, the lagging strand is sythesized in a series of discontinuous small fragments often referred to as Okazaki fragments, orientated in a 5′ to 3′ direction. A single-strand nick is produced between the fragments, which is subsequently repaired to produce a continuous strand.

MISMATCH REPAIR

The integrity of the genome is maintained by a variety of sophisticated mechanisms that repair damaged DNA. The MMR system is one of the best characterized of these, it corrects base pair mismatches in newly synthesized DNA. The major DNA polymerase in eukaryotes, polymerase δ has a very efficient 5′>3′ proofreading activity. However, mistakes still occur. The primary function of the MMR system is to eliminate base-base mismatches and insertion/deletion loops, which arise during DNA replication (12). Insertion/deletion loops classically result in the shortening or lengthening of repetitive sequences in microsatellites.

MICROSATELLITE INSTABILITY

Microsatellites are multiple tandem repeats that consist of a short number of usually mono-, di-, or tri-nucleotides; these are particularly prone to slippage and inefficient proofreading by DNA polymerase. If the MMR system is inactivated

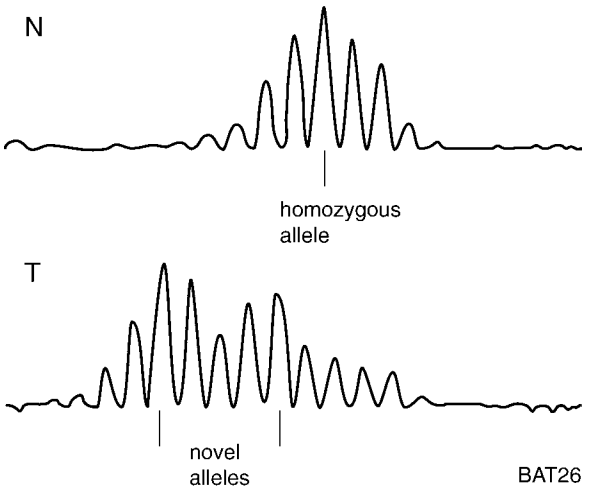

Figure 5 Microsatellite instability at the BAT-26 locus in tumor compared with homozygous allele in constitutional DNA. The BAT-26 microsatellite contains an intronic run of 26 adenosines. Long noncoding mononucleotide repeats such as Bat-26 (26 adenosines) tend to be shortened by the multistep cumulative unstable process. The upper electropherogram shows the trace from an individual essentially homozygous for an allele at the BAT-26 locus. The lower electropherogram shows the trace from tumor material from the same patient; there is an additional shorter allele seen in the tumor due to slippage at the BAT-26 locus.

then these microsatellites typically undergo shortening, e.g., a run of 10 adenines is shortened to 9 adenines. This is termed microsatellite instability (MSI) and is seen in tumor cells (Fig. 5), which harbor biallelic MMR mutations. MMR in humans depends on homologues of the bacterial MutS and MutL proteins, which function as heterodimers (12). The MutSα complex is a heterodimer of MSH2/MSH6 and is the most abundant species. There are lesser amounts of the MutSβ complex that consists of a heterodimer of MSH2/MSH3. The MutS complex initiates DNA repair by mismatch recognition. Interaction between this recognition complex and downstream repair proteins is dependent on MutL-like activity. An MLH1/PMS2 heterodimer (MutLα) is the major species providing MutL-like MMR activity in human cells (12). The MMR system can be thought of as a surveillance system that keeps an eye on newly synthesized DNA. When it detects a mismatch, the error is excised and DNA polymerase is recruited to resynthesize the damaged fragment. There are four stages to the MMR process: (*i*) mismatch identification, (*ii*) recruitment of repair enzymes, (*iii*) removal of a patch of sequence around and including the mismatch, and (*iv*) resynthesis of the correct sequence (Fig. 6). The original strand of DNA is used as the template in this last process.

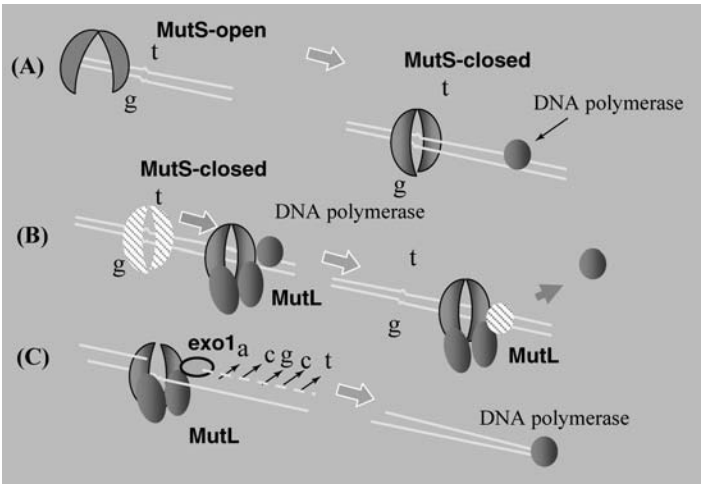

Figure 6 MMR repair. (**A**) The MSH2/MSH6 (MutS) heterodimer in an open configuration surveys the genome for mismatches; a g/t mismatch is identified. The heterodimer binds to the mismatch, a configuration change takes place and it adopts a closed structure. (**B**) The MSH2/MSH6 (MutS) heterodimer migrates along the DNA molecule, the MLH1/PMS2 heterodimer (MutL) is recruited to the repair complex. Migration occurs, the DNA polymerase complex is encountered and displaced from the DNA. (**C**) MutL recruits further components, including exonuclease 1, which excises the DNA strand back to and beyond the mismatch. The DNA polymerase complex then resynthesizes the DNA using the parent strand as the template.

THE MMR SYSTEM

Mismatch Identification

The human homologue of the bacterial MutS complex is responsible for mismatch recognition. MutSα is the predominant species, it recognizes base-base mismatches and 1 base-pair (bp) insertion/deletion loops. The lesser abundant species MutSβ recognizes longer (1–4 bp) insertion/deletion loops. In bacteria, the MutS complex scans newly synthesized DNA for mismatches. When one is encountered, the complex binds to the DNA in a reaction requiring the exchange of ADP for ATP, this brings about a structural change in the heterodimer, converting it from an open to a closed configuration, this latter closes upon the DNA (13). The complex forms a sliding clamp that can travel laterally along the double-stranded DNA molecule. One model for MutSα function suggests that migration along the DNA strand occurs until the complex reaches a single-strand nick in the newly synthesized DNA, thus enabling MutSα to distinguish between the newly synthesized strand and the parent strand (14).

Recruitment of Repair Enzymes

The DNA/MutSα complex recruits MutLα, which is a heterodimer consisting of hhMLH1 and hPMS2. MutLα itself does not seem to actively engage in the repair process. Rather it appears to displace the DNA polymerase from the newly synthesized DNA and then recruits further repair enzymes that directly cut out the damaged strand of DNA. Despite its indirect role in MMR, abrogation of hMLH1 activity results in MSI, as shown by epigenetic silencing of hMLH1 in sporadic colorectal cancers (14).

Removal of Damaged DNA

Exonuclease 1 (Exo1) is recruited by MutLα and cuts out the daughter strand of DNA from the single-strand nick right back to the mismatch. This excised strand of DNA can be up to 1000 nucleotides long and may extend to about 150 bp past the site of the mismatch (15). Exo1 appears to play a crucial role in excision, although cooperation with other enzymes including replication proteins A and C (RPA and RPC) as well as proliferating cell nuclear antigen (PCNA) is also required. The precise partners involved in strand excision seem to depend on the direction of excision, whether it proceeds in a 5′ or a 3′ direction (16).

Resynthesis of the Correct Sequence

In bacteria, the crucial enzymes involved are DNA polymerase III and DNA polymerase δ (17). There is a clear requirement for DNA pol δ in humans, but it may be that other components of the DNA polymerase system are also needed.

MMR GENES AND APOPTOSIS

Along with their crucial role in repairing damaged DNA, there is strong experimental evidence that MMR gene products also play a role in the initiation of apoptosis in response to DNA damaging agents. Human cell lines deficient in hMLH1 (18) and hMSH2 and hMSH6 (19) showed resistance to the cell-killing effects of the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine tolerance. The resistance was reversed by whole chromosome complementation resulting in active hMLH1, hMSH2, and hMSH6 proteins. These experiments clearly identified a role for MMR genes in the initiation of apoptosis. Further experiments in mice have shown that a failure to initiate apoptosis is important in tumorigenesis in MMR-deficient animals (20,21). How MMR promotes the initiation of apoptosis remains obscure. It is also unclear which apoptotic pathways are required, since both p53-dependent and independent pathways seem to be required (20). Since many of the agents used in these experiments have a role in chemotherapy, it may be clinically valuable to understand the mechanisms involved.

MMR DYSFUNCTION

Dysfunction of the MMR system is a consequence of genetic alterations that lead either to abrogation of protein expression or the expression of a protein that is functionally impaired. These alterations can be the result of classical mutational events that produce an alteration in the DNA sequence; these can be either inherited or acquired. They can also result from somatic events that do not alter the DNA sequence. These latter are termed epigenetic, the commonest is silencing of the MLH1 expression by methylation of its promoter sequences.

GERMLINE MUTATIONS

Germline mutations in the MMR genes cause dominantly inherited Lynch syndrome. Germline mutations are classically point mutations within the coding sequence, but larger genomic deletions also occur (22).

Point mutations can be silent (no change in amino acid sequence), or create nonsense mutations (introducing a stop codon and resulting in a truncated protein that may be unstable) or missense mutations (changing one amino acid). Nonsense mutations are generally regarded as pathogenic or disease causing, it can be far harder to be sure about the likely effects of missense mutations. These latter sequence variations may result in a protein with no biological activity or to an unstable or variably stable protein with some residual activity. Missense variants are therefore difficult to classify and many diagnostic laboratories will not report them as disease causing.

The International Society for Gastrointestinal Hereditary Tumors (InSiGHT; www.insight-group.org) curates a database of mutations in the *hMLH1*, *hMSH2*, *hPMS2*, *hPMS1*, *hMSH6*, and *hMLH3* genes. At last count, there were 659 mutations recorded. This is a submission database, there is no obligation to report to it, so until recently there was no catalogue of published mutations. However Woods et al. recently made available a database in which they had actively assembled all published mutations in *hMLH1*, *hMSH2*, and *hMSH6* (http://www.med.mun.ca/MMRvariants/) (23). The accompanying paper also detailed published mutations in *hPMS2*. This database holds details of all published sequence variants, including those where the pathogenic effect is unknown. Details of some 1224 variants were recorded at the time of writing.

Mutations in *hMLH1* and *hMSH2* account for the majority of the mutations reported to the InSiGHT database and in the literature generally. Certain mutations do occur commonly and some of these are seen in specific ethnic groups. The deletion of exons 1-6 in *hMSH2* is seen in North American populations (24) and the exon 16 *hMLH1* deletion is seen in the Finnish population (25); these are associated with possible founder events. Other common mutations such as the intron 5 splice site mutation in *hMHS2* do not appear to be due to founder effects (26,27). Genomic deletions constitute an appreciable proportion of all Lynch syndrome mutations and it is important that diagnostic laboratories perform assays that detect such mutations (22).

Outcome of Germline Mutations in MMR Genes

The phenotype associated with heterozygous mutations in *hMLH1*, *hMSH2*, and *hMSH6* is of classical Lynch syndrome. The phenotype associated with heterozygous mutations in *hPMS2* is similar but the penetrance of *hPMS2* mutations appears to be lower (28). Penetrance is a statistical concept, which refers to the frequency with which a genotype manifests itself in a given phenotype, in this case features of Lynch syndrome. About 50% of Lynch syndrome is due to mutations in *hMLH1*, 40% due to mutations in *hMSH2*, and about 10% due to mutations in *hMSH6* (29). Mutations in *hPMS2* have been recorded in only a small number of families. Mutations in *EXO1*, hMLH3, and *TGFbRII* have been recorded as the cause of Lynch syndrome in isolated families, but they do not play a major role in the pathogenesis of Lynch syndrome.

The lifetime risks of cancer in gene carriers are high but vary between studies (Table 1). A wide variety of other cancers are seen, particularly of the brain, ureter, and bile ducts. Small bowel cancers occur at a frequency of about 4% to 7% in Lynch syndrome (30–32); however, a recent population-based study identified mutations in *hMLH1*, *hMSH2*, or *hMSH6* in more than 80% of cases (33). A strong argument can be made regarding small bowel cancers as a feature of Lynch syndrome.

Mutations in *hMLH1* and *hMSH2*

The original clinical reports of Lynch syndrome indicated a high incidence of endometrial and ovarian cancer (34,35). After the *hMLH1* and *hMSH2* genes were identified as the cause, analysis of disease risk in families bore out these clinical observations. In a survey of 67 *hMLH1* or *hMSH2* gene carriers, Dunlop et al. estimated that the risk of endometrial cancer by age 70 in female gene carriers was 42% (36) compared with a risk of colorectal cancer of only 30%. No ovarian cancers were seen in this study. In a Finnish study of 360 *hMLH1* or *hMSH2* mutation carriers, the cumulative incidence of endometrial cancer by age 70 was 60% and that of ovarian cancer was 12%. The incidence of colorectal cancer in women in this study was 54% (30); 47 of the families in this study had

Table 1 Lifetime Cancer Risk in Lynch Syndrome

Colorectal (men)	>80%
Colorectal (women)	40%
Endometrial	43–60%
Ovarian	9–12%
Gastric	5–10%
Urinary tract	4–6%
Renal cell	3.3%
Bile duct/gallbladder	2–3%
Small bowel	1–4%

mutations in the *hMLH1* gene, of whom 30 carried the exon 16 deletion referred to above. Only three families had mutations in the *hMSH2* gene. More recent studies have indicated possibly lower risks associated with mutations in *hMLH1* and *hMSH2*. In 2001, Vasen et al. published data on 79 families, 34 with mutations in *hMLH1*, 40 in *hMSH2*, and 5 in *hMSH6*. The cumulative incidence of endometrial cancer by age in *hMLH1* mutation carrier was 20% and in *hMSH2* mutation carriers 37% (32). In a series of 348 French patients with mutations in either *hMLH1* or *hMSH2*, the cumulative lifetime incidence of endometrial cancers was 45% in *hMLH1* carriers and about 60% in *hMSH2* carriers (37). Quehenberger et al. recently reported on 84 families of Dutch origin, 39 with mutations in *hMLH1* and 45 with mutations in *hMSH2*. There was no significant difference in risk at either locus, the risk of endometrial cancer by age 70 was 31.5% and of colorectal cancer 22.4%. In this study, the risk of colorectal cancer in males was 26.7% (38). These later studies have included smaller families than those seen in the initial studies, many of which had been used in gene identification. The earlier studies applied Kaplan–Meier estimations to cohorts ascertained on the basis of multiple affected cases to determine risks. The most recent study tried to make a correction for the size of the families ascertained (38). While the precise disease estimates vary, there is little doubt that mutations in *hMLH1* and *hMSH2* carry significant risks of endometrial cancer and ovarian cancer.

hMSH6 Mutations

The *hMSH6* gene product forms a heterodimer with hMSH2, the combination is particularly involved in the repair of single nucleotide mismatches (39). However, the frequency of *hMSH6* mutations in families fulfilling the Amsterdam criteria is low (40). Furthermore, tumors in *hMSH6* null mice do not display the characteristic MSI-high (MSI-H) phenotype seen in their *hMLH1* and *hMSH2* null counterparts (20). Wu et al. detected 4 pathogenic *hMSH6* mutations in 18 patients with suspected HNPCC and MSI-low (MSI-L) tumors, one patient with an MSI-H tumor phenotype was found to have an *hMSH6* mutation. However, this latter patient also had an *hMLH1* frameshift mutation, making interpretation of the *hMSH6* mutation extremely difficult (10). Berends et al. subsequently investigated in depth 25 index cases and 8 relatives with *hMSH6* variants (41). These were ascertained from a cohort of 316 individuals suspected as having Lynch syndrome. Seven patients shared a common mutation (650insT, 651_652insT) in exon 4a. Haplotype analysis indicated this was likely to represent a founder effect in the Dutch population under study. Five other different truncating mutations were found and there were another 10 unclassified variants in this study; 14 out of 26 colorectal and endometrial cancers in this study were MSI-L (41). A common mutation (p.F1088fsX1092 c.3261_3262ins C) has been reported in a German population. The frequency of the Amsterdam criteria I or II positive families in this latter study was only 37%, an observation similar to that

made in the previously mentioned reports. Nineteen of 27 tumors from patients with truncating *hMSH6* mutations in this study were MSI-H; however, these investigators used an expanded panel of markers to test for MSI, which may explain the higher frequency reported. Endometrial cancer was reported in this study as occurring at a similar frequency in *hMSH6* mutation carrier as in *hMSH2* or *hMLH1* mutation carriers (42).

Endometrial cancer and atypical hyperplasia have been reported at high frequency in *hMSH6* mutation carriers (40,41,43); 73% of *hMSH6* mutation carriers were found to have endometrial cancer or atypical hyperplasia in one early study (40).

Goodfellow et al. reported the frequency of germline *hMSH6* mutations in an unselected series of endometrial cancers as 1.6% (44). In a series of 519 Finnish patients with endometrial cancer, Ollikainen identified 23 families with site-specific familial endometrial cancer. Only one family harbored *hMSH6* mutation while one further family had a likely pathogenic *hMSH2* mutation. In this series, a further nine families ascertained by endometrial cancer in the index case had mutations in either *hMLH1* or *hMSH2* (45).

Mutations in the *hMSH6* gene undoubtedly confer a high risk of endometrial cancer, and the early studies suggest that this risk is may be higher than for women with *hMLH1* or *hMSH2* mutation (40,43,45).

PROMOTER METHYLATION

The term gene is often used to refer only to a segment of DNA that is transcribed into RNA. However, in the classical view of a gene, the term would refer to the gene control region as well. The control region refers to the whole expanse of DNA involved in regulating transcription of a gene, including the promoter, where transcription factors and polymerases assemble, and all of the sequences to which gene regulatory proteins bind. A minority of the cytosine residues in human DNA are methylated; those that are methylated are found in the CpG dinucleotide (that is, the methylated cytosines are almost always ones whose 3′ carbon atom is linked by a phosphodiester bond to the 5′ carbon atom of a guanine). Overall, the density of CpGs in vertebrate DNA is lower than expected; however, there are stretches of DNA with relatively higher levels of CpG. These regions are referred to as CpG islands and are often associated with the promoter regions of transcriptionally active genes. Methylation of cytosine residues in CpG is an epigenetic mechanism that plays an important part in mammalian gene control, acting as a general method of maintaining repression of transcription. Hypermethylation of *hMLH1* was first described in the majority of MSI+ sporadic colorectal cancers (14,46) and was associated with abrogation of *hMLH1* protein expression as determined by immunohistochemistry (14). Similar observations have subsequently been made in endometrial cancer; this is the major mechanism giving rise to MMR dysfunction in endometrial cancer (47).

CARCINOGENIC EFFECTS OF MMR DYSFUNCTION

The development of a mutator phenotype with increased mutation rates for insertion/deletion mismatches and base-base mismatches is thought to be the principal mechanism whereby MMR dysfunction results in cancer. As pointed above, however, MMR proteins also participate in other cellular processes particularly in the initiation of apoptosis in response to DNA damage (13). Dysfunction of these aspects of MMR protein function might also contribute to carcinogenesis. The mutator phenotype model is, however, the one which has received most attention.

MSI as originally described referred to anonymous microsatellite sequences used in laboratory experiments. The definition of the MSI-H phenotype was in terms of the proportion of microsatellites mutated in tumors, at first this tended to mean whatever markers happened to be lying around in the laboratory fridges. However, subsequently the marker sets to be used and the reporting were standardized (48,49). It is important to realize that this is a global instability phenomenon affecting microsatellite repetitive sequences. The standardized marker sets that are used to classify the MSI phenotype in colorectal cells consist of mono- and di-nucleotide markers in the noncoding genomic DNA.

MSI may, however, also affect coding region microsatellites with deleterious consequences for gene expression by the introduction of frameshift mutations that provide a growth advantage or an immune escape mechanism to affected cells. This subset of coding microsatellites defines critical targets promoting MSI-dependent carcinogenesis. These are referred to as Real Common Mutations by Duval et al. (50).

The prototype for this effect is the *TGFβRII* gene in colorectal cancer. This has a 10 adenine repeat in its coding region. Frameshift alterations were confirmed to be inactivating mutations by functional studies showing a loss of the TGFβRII tumor suppressor function (51). Subsequently, attempts have been made to catalogue genes whose coding regions contain microsatellites and which might therefore be regarded as targets for functionally significant MSI (51–54).

At first the proposal was that all genes would be affected by inactivating frameshifts, like *TGFβRII*, and this did indeed prove the case for a variety of important targets such *IGFIIR*, *BAX*, *Caspase-5*, *hMSH3*, and *hhMSH6.* In some cases, however, frameshift mutations can alter coding repeats located downstream of important functional gene domains or upstream of others. Mutations in the *Axin* gene are thought to have a dominant-negative effect. Mutations in *TCF-4* abrogate its ability to bind to CtBP, one of its transcriptional repressors, thus enhancing levels of *TCF-4* production. The same mutational mechanism results in both loss of function or gain of function, the end result is a selection advantage for the cells concerned.

Further studies on MSI have indicated that targets may be tissue specific. For example, *TGFbRII* mutations are seen in about 80% of MSI-H colorectal

cancers; however, they occur in only about 20% of MSI-H endometrial cancers (50). Woerner and colleagues developed a model indicating *TGFbRII*, *BAX*, *TCF-4*, *MSH3*, *ACVR2*, *PTHL3*, *HT001*, *AC1*, and *SLC23A1* represent Real Common Targets in colorectal cancers, while *TAF1B*, *AIM2*, *and SLC23* were targets in endometrial cancer (55). Duval et al. also demonstrated differences between targets in endometrial and colorectal cancers (50). Tissue specificity does appear to be a key feature of targets of MSI likely to confer a growth advantage.

GERMLINE MMR GENE MUTATION COMPARED WITH *hMLH1* METHYLATION IN GYNECOLOGICAL TUMORS

In a large series of endometrial cancer, approximately 20% of all endometrial cancers harbor MMR gene defects as evidenced by the presence of MSI (44,56–60). The vast majority of these cases will not be a consequence of germline mutations in MMR genes, rather they result from somatically acquired hypermethylation of the *hMLH1* promoter (44,61). It is not clear if there are differences in the clinicopathological features of MSI+ endometrial cancers due to germline mutations and those due to *hMLH1* hypermethylation. Only one study of reasonable size has compared MSI+ endometrial carcinomas in germline MMR mutation carriers with MSI+ cancers in which the underlying mechanism is *hMLH1* hypermethylation (62). In this study of 50 women with germline mutations and 26 women with sporadic *hMLH1* mutations, age of onset was later in the hypermethylation group, and the group had significantly fewer grade 1 tumors and more grade 3 tumors. The *hMLH1* methylated group alone had significantly fewer nonendometrioid tumors than was seen in overall endometrial cancer population presenting to the institution that reported this study. Of the 50 MMR mutation carriers in this study, 47 had mutations in *hMSH2* and only 3 *hMLH1* mutation carriers. Black et al. reported on a series of 93 MSI+ endometrial cancers, previous studies would indicate that the bulk of the tumors in this report were MSI+ due to promoter hypermethylation (56). There were significant associations between MSI status and myometrial invasion, advanced stage, and endometrioid histology. It is difficult to compare this study with that of Broaddus et al. (62), but the *hMLH1* hypermethylated group in that study appear to have broadly similar stage at presentation and degree of myometrial invasion. However, as there were differences in the overall reported rates of lymphovascular invasion and tumor grade, it is difficult to compare the data in the two papers to determine if there are clear differences between MSI+ tumors due to *hMLH1* hypermethylation and those due to germline mutations. In other studies of smaller numbers, there were no associations between MSI status and stage, grade, or subtype (58,61,63). There have been other reports of a significant association between MSI and advanced stage (59,64).

SURVIVAL IN TUMORS WITH DEFECTIVE MMR

Improved survival was reported in the initial studies on MSI+ colorectal cancer patients (65) and this seemed to be confirmed in subsequent reports (37,66–68). However, there are reports that have failed to confirm the prognostic significance of MSI in colorectal cancer (69,70). In the case of endometrial cancer, the majority of studies that reported outcome found no significant advantage associated with MSI+ tumors (58–60,64,71). However, Maxwell et al. reported that five-year survival was 77% in a series of 29 MSI+ tumors compared with only 48% in 102 MSI− tumors, this difference was significant at the $p = 0.03$ level (63). Only three polymorphic markers were used to define MSI in this study, one of which was part of the NIH-approved panel. In a more recent study, the original panel of NIH markers was used to define MSI. Significant differences in both disease-free and overall survival between the 93 patients with MSI+ tumors and the 380 patients with MSI− tumors were reported by Black et al. (56).

In a series of 50 patients with endometrial cancer from families harboring a germline mutations in MMR genes, there was no significant difference in survival compared with 100 age- and stage-matched patients with sporadic endometrial cancer (72).

Similar to the case with colorectal cancer, the data accumulating suggest that survival may be better in women with MSI+ endometrial cancers.

OVARIAN CANCER

Overall, about 2% of all ovarian cancer is due to germline mutations in MMR genes (73,74). The lifetime risk of ovarian cancer in MMR mutation carriers is generally estimated to be 8% to 15%. The c.1346T>C *hMSH6* mutation has been reported as carrying a 33% lifetime risk of ovarian cancer; all mutations may not convey equivalent risks, though good data in this area are sparse. Between 12% and 16% of reasonably sized series of ovarian cancers assessed by the NCI consensus panel (73,75–78) have shown MSI+. This is similar to the rate seen in sporadic colorectal and endometrial cancers. There is little data on the impact of MSI status in ovarian cancer. A single study from the Dutch registry indicated no difference in survival between 26 women with ovarian cancer from HNPCC families and an age- and stage-matched group of 52 controls (79).

CONCLUSIONS

Germline mutations in the MMR genes result in classic Lynch syndrome with tumors displaying the characteristic MSI+ phenotype. The commonest cause of MSI+ in gynecological tumors is however not germline mutations but hypermethylation of the *hMLH1* promoter. MSI positivity results in the development of a mutator phenotype in clones of neoplastic cells. Deleterious mutations occur particularly in coding microsatellites in important genes involved in growth

regulation and apoptosis. These confer a growth advantage on these cells resulting in the development of clinically significant tumors. There is evidence that MSI+ tumors may have a better overall outcome than MSI− tumors, although larger studies are probably needed to define this further. There is also accumulating evidence that MSI+ tumors respond differently to chemotherapeutic agents. An understanding of the way that the MMR dysfunction influences tumor cell growth and survival will be an important contributor to improving outcome in patients with MSI+ cancers.

CASE REPORT

MS is a 49-year-old woman who initially presented to her primary physician with irregular vaginal bleeding. Pelvic ultrasound revealed a thickened endometrial stripe. Subsequent dilation and curettage demonstrated a high-grade endometrioid endometrial carcinoma. Hysterectomy and staging procedures revealed a stage IIIc endometrial carcinoma. She was subsequently treated with chemotherapy and radiation.

Although a detailed family history revealed no family history of Lynch syndrome–associated cancers, her diagnosis before the age of 50 was suggestive of Lynch syndrome. MS was referred for genetic counseling and tested positive for a germline mutation in MSH6. She is pursuing periodic screening with colonoscopy given her high risk of colorectal carcinoma.

REFERENCES

1. Warthin A. Heredity with reference to carcinoma as shown by the study of the cases examined in the pathological laboratory of the University of Michigan, 1895–1913. Arch Intern Med 1913; 12:546–555.
2. Warthin A. The further study of a cancer family. J Cancer Res 1925; 9:279–286.
3. Lynch HT, Shaw MW, Magnuson CW, et al. Hereditary factors in cancer. Study of two large midwestern kindreds. Arch Intern Med 1966; 117(2):206–212.
4. Lynch HT, Krush AJ. Cancer family "G" revisited: 1895-1970. Cancer 1971; 27(6): 1505–1511.
5. Vasen HF, Mecklin JP, Khan PM, et al. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991; 34(5):424–425.
6. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993; 75(6):1215–1225.
7. Nicolaides NC, Papadopoulos N, Liu B, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994; 371(6492):75–80.
8. Papadopoulos N, Nicolaides NC, Wei YF, et al. Mutation of a mutL homolog in hereditary colon cancer. Science 1994; 263(5153):1625–1629.
9. Papadopoulos N, Nicolaides NC, Liu B, et al. Mutations of GTBP in genetically unstable cells. Science 1995; 268(5219):1915–1917.

10. Wu Y, Berends MJ, Sijmons RH, et al. A role for MLH3 in hereditary nonpolyposis colorectal cancer. Nat Genet 2001; 29(2):137–138.
11. Yan H, Papadopoulos N, Marra G, et al. Conversion of diploidy to haploidy. Nature 2000; 403(6771):723–724.
12. Harfe BD, Jinks-Robertson S. DNA mismatch repair and genetic instability. Annu Rev Genet 2000; 34:359–399.
13. Fishel R. The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: revising the mutator hypothesis. Cancer Res 2001; 61(20):7369–7374.
14. Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A 1998; 95(12):6870–6875.
15. Genschel J, Bazemore LR, Modrich P. Human exonuclease I is required for 5′ and 3′ mismatch repair. J Biol Chem 2002; 277(15):13302–13311.
16. Umar A, Boyer JC, Thomas DC, et al. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J Biol Chem 1994; 269(20):14367–14370.
17. Chang DK, Ricciardiello L, Goel A, et al. Steady-state regulation of the human DNA mismatch repair system. J Biol Chem 2000; 275:29178.
18. Koi M, Umar A, Chauhan DP, et al. Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-methyl-N′-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. Cancer Res 1994; 54(16):4308–4312.
19. Umar A, Koi M, Risinger JI, et al. Correction of hypermutability, N-methyl-N′-nitro-N-nitrosoguanidine resistance, and defective DNA mismatch repair by introducing chromosome 2 into human tumor cells with mutations in MSH2 and MSH6. Cancer Res 1997; 57(18):3949–3955.
20. Edelmann L, Edelmann W. Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. Am J Med Genet C Semin Med Genet 2004; 129(1):91–99.
21. Shimodaira H, Yoshioka-Yamashita A, Kolodner RD, et al. Interaction of mismatch repair protein PMS2 and the p53-related transcription factor p73 in apoptosis response to cisplatin. Proc Natl Acad Sci U S A 2003; 100(5):2420–2425.
22. Taylor CF, Charlton RS, Burn J, et al. Genomic deletions in MSH2 or MLH1 are a frequent cause of hereditary non-polyposis colorectal cancer: identification of novel and recurrent deletions by MLPA. Hum Mutat 2003; 22(6):428–433.
23. Woods MO, Williams P, Careen A, et al. A new variant database for mismatch repair genes associated with Lynch syndrome. Hum Mutat 2007; 28(7):669–673.
24. Wagner A, Barrows A, Wijnen JT, et al. Molecular analysis of hereditary nonpolyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene. Am J Hum Genet 2003; 72(5):1088–1100.
25. Percesepe A, Borghi F, Menigatti M, et al. Molecular screening for hereditary nonpolyposis colorectal cancer: a prospective, population-based study. J Clin Oncol 2001; 19(19):3944–3950.
26. Desai DC, Lockman JC, Chadwick RB, et al. Recurrent germline mutation in MSH2 arises frequently de novo. J Med Genet 2000; 37(9):646–652.

27. Froggatt NJ, Green J, Brassett C, et al. A common MSH2 mutation in English and North American HNPCC families: origin, phenotypic expression, and sex specific differences in colorectal cancer. J Med Genet 1999; 36(2):97–102.
28. Truninger K, Menigatti M, Luz J, et al. Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterology 2005; 128(5): 1160–1171.
29. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol 2003; 21(6):1174–1179.
30. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214–218.
31. Rodriguez-Bigas MA, Vasen HF, Lynch HT, et al. Characteristics of small bowel carcinoma in hereditary nonpolyposis colorectal carcinoma. International Collaborative Group on HNPCC. Cancer 1998; 83(2):240–244.
32. Vasen HF, Stormorken A, Menko FH, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001; 19(20):4074–4080.
33. Schulmann K, Brasch FE, Kunstmann E, et al. HNPCC-associated small bowel cancer: clinical and molecular characteristics. Gastroenterology 2005; 128(3):590–599.
34. Vasen HF, Watson P, Mecklin JP, et al. The epidemiology of endometrial cancer in hereditary nonpolyposis colorectal cancer. Anticancer Res 1994; 14(4B):1675–1678.
35. Watson P, Vasen HF, Mecklin JP, et al. The risk of endometrial cancer in hereditary nonpolyposis colorectal cancer. Am J Med 1994; 96(6):516–520.
36. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6(1):105–110.
37. Parc Y, Boisson C, Thomas G, et al. Cancer risk in 348 French MSH2 or MLH1 gene carriers. J Med Genet 2003; 40(3):208–213.
38. Quehenberger F, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the hMLH1 and hMSH2 gene: correction for ascertainment. J Med Genet 2005; 42(6):491–496.
39. Drummond JT, Anthoney A, Brown R, et al. Cisplatin and adriamycin resistance are associated with MutLalpha and mismatch repair deficiency in an ovarian tumor cell line. J Biol Chem 1996; 271(33):19645–19648.
40. Wijnen J, de Leeuw W, Vasen H, et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. Nat Genet 1999; 23(2):142–144.
41. Berends MJ, Wu Y, Sijmons RH, et al. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. Am J Hum Genet 2002; 70(1):26–37.
42. Plaschke J, Engel C, Kruger S, et al. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations: the German Hereditary Nonpolyposis Colorectal Cancer Consortium. J Clin Oncol 2004; 22(22):4486–4494.
43. Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 2004; 127(1):17–25.
44. Goodfellow PJ, Buttin BM, Herzog TJ, et al. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. Proc Natl Acad Sci U S A 2003; 100(10):5908–5913.

45. Ollikainen M, Abdel-Rahman WM, Moisio AL, et al. Molecular analysis of familial endometrial carcinoma: a manifestation of hereditary nonpolyposis colorectal cancer or a separate syndrome? J Clin Oncol 2005; 23(21):4609–4616.
46. Kane MF, Loda M, Gaida GM, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res 1997; 57(5):808–811.
47. Simpkins SB, Bocker T, Swisher EM, et al. MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. Hum Mol Genet 1999; 8(4):661–666.
48. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58(22):5248–5257.
49. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96(4):261–268.
50. Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. Cancer Res 2002; 62(9):2447–2454.
51. Markowitz S, Wang J, Myeroff L, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 1995; 268(5215):1336–1338.
52. Park J, Betel D, Gryfe R, et al. Mutation profiling of mismatch repair-deficient colorectal cancers using an in silico genome scan to identify coding microsatellites. Cancer Res 2002; 62(5):1284–1288.
53. Woerner SM, Gebert J, Yuan YP, et al. Systematic identification of genes with coding microsatellites mutated in DNA mismatch repair-deficient cancer cells. Int J Cancer 2001; 93(1):12–19.
54. Woerner SM, Kloor M, Mueller A, et al. Microsatellite instability of selective target genes in HNPCC-associated colon adenomas. Oncogene 2005; 24(15):2525–2535.
55. Woerner SM, Benner A, Sutter C, et al. Pathogenesis of DNA repair-deficient cancers: a statistical meta-analysis of putative Real Common Target genes. Oncogene 2003; 22(15):2226–2235.
56. Black D, Soslow RA, Levine DA, et al. Clinicopathologic significance of defective DNA mismatch repair in endometrial carcinoma. J Clin Oncol 2006; 24(11):1745–1753.
57. Caduff RF, Johnston CM, Svoboda-Newman SM, et al. Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol 1996; 148(5):1671–1678.
58. Macdonald ND, Salvesen HB, Ryan A, et al. Molecular differences between RER+ and RER− sporadic endometrial carcinomas in a large population-based series. Int J Gynecol Cancer 2004; 14(5):957–965.
59. Muresu R, Sini MC, Cossu A, et al. Chromosomal abnormalities and microsatellite instability in sporadic endometrial cancer. Eur J Cancer 2002; 38(13):1802–1809.
60. Orbo A, Eklo K, Kopp M. A semiautomated test for microsatellite instability and its significance for the prognosis of sporadic endometrial cancer in northern Norway. Int J Gynecol Pathol 2002; 21(1):27–33.
61. Salvesen HB, MacDonald N, Ryan A, et al. Methylation of hMLH1 in a population-based series of endometrial carcinomas. Clin Cancer Res 2000; 6(9):3607–3613.
62. Broaddus RR, Lynch HT, Chen LM, et al. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. Cancer 2006; 106(1):87–94.

63. Maxwell GL, Risinger JI, Alvarez AA, et al. Favorable survival associated with microsatellite instability in endometrioid endometrial cancers. Obstet Gynecol 2001; 97(3):417–422.
64. Baldinu P, Cossu A, Manca A, et al. Microsatellite instability and mutation analysis of candidate genes in unselected sardinian patients with endometrial carcinoma. Cancer 2002; 94(12):3157–3168.
65. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science 1993; 260(5109):816–819.
66. Benatti P, Gafa R, Barana D, et al. Microsatellite instability and colorectal cancer prognosis. Clin Cancer Res 2005; 11(23):8332–8340.
67. Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000; 342(2):69–77.
68. Sinicrope FA, Rego RL, Halling KC, et al. Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology 2006; 131(3):729–737.
69. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006; 354(26):2751–2763.
70. Bertario L, Russo A, Sala P, et al. Survival of patients with hereditary colorectal cancer: comparison of HNPCC and colorectal cancer in FAP patients with sporadic colorectal cancer. Int J Cancer 1999; 80(2):183–187.
71. Wong YF, Ip TY, Chung TK, et al. Clinical and pathologic significance of microsatellite instability in endometrial cancer. Int J Gynecol Cancer 1999; 9(5):406–410.
72. Boks DE, Trujillo AP, Voogd AC, et al. Survival analysis of endometrial carcinoma associated with hereditary nonpolyposis colorectal cancer. Int J Cancer 2002; 102(2): 198–200.
73. Malander S, Rambech E, Kristoffersson U, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol 2006; 101(2):238–243.
74. Rubin SC, Blackwood MA, Bandera C, et al. BRCA1, BRCA2, and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: relationship to family history and implications for genetic testing. Am J Obstet Gynecol 1998; 178(4):670–677.
75. Cai KQ, Albarracin C, Rosen D, et al. Microsatellite instability and alteration of the expression of hMLH1 and hMSH2 in ovarian clear cell carcinoma. Hum Pathol 2004; 35(5):552–559.
76. Geisler JP, Goodheart MJ, Sood AK, et al. Mismatch repair gene expression defects contribute to microsatellite instability in ovarian carcinoma. Cancer 2003; 98(10): 2199–2206.
77. Singer G, Kallinowski T, Hartmann A, et al. Different types of microsatellite instability in ovarian carcinoma. Int J Cancer 2004; 112(4):643–646.
78. Sood AK, Holmes R, Hendrix MJ, et al. Application of the National Cancer Institute international criteria for determination of microsatellite instability in ovarian cancer. Cancer Res 2001; 61(11):4371–4374.
79. Crijnen TE, Janssen-Heijnen ML, Gelderblom H, et al. Survival of patients with ovarian cancer due to a mismatch repair defect. Fam Cancer 2005; 4(4):301–305.

11

Pathology of Lynch Syndrome-Associated Gynecological Cancers

Russell R. Broaddus

Department of Pathology, Unit 85, University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- The histology of endometrial and ovarian carcinomas arising in the setting of Lynch syndrome is heterogeneous.
- Complex atypical hyperplasia is the precursor lesion to endometrioid-type endometrial carcinomas arising in Lynch syndrome.
- Lynch syndrome endometrial carcinoma is characterized by high levels of microsatellite instability. Currently, there are no distinct pathological features that accurately predict the presence of microsatellite instability.
- At the time of prophylactic surgery, the uterus and ovaries should be evaluated by frozen section followed by routing pathological and microscopic evaluation for unsuspected carcinoma.
- Tissue testing for Lynch syndrome includes microsatellite instability analysis as well as immunohistochemical testing for MLH1, MSH2, MSH6, and PMS2.

INTRODUCTION

There is a tremendous amount of literature regarding the pathology of colon carcinoma associated with Lynch syndrome. Unfortunately, there is much less information available for Lynch syndrome–associated endometrial cancer and

even less for Lynch syndrome–associated ovarian cancer. The bulk of this chapter will therefore focus on what is known regarding endometrial cancer in Lynch syndrome. Some attention will be devoted to ovarian cancer as well. Finally, a tissue-based approach to identifying endometrial and ovarian cancer patients with Lynch syndrome will be discussed.

PATHOLOGY OF ENDOMETRIAL CARCINOMA

Before focusing on Lynch syndrome–associated endometrial cancers, it is instructive to briefly review the classification of uterine neoplasms in the general population. There are a wide variety of tumors that can arise from the uterus, including carcinomas derived from the epithelium of the endometrium and mesenchymal tumors derived from stromal components of the uterus, such as smooth muscle tumors (leiomyomas and leiomyosarcomas) and stromal tumors (endometrial stromal nodule and endometrial stromal sarcoma). To date, mesenchymal tumors have not been associated with Lynch syndrome. Therefore, the remainder of this section will be devoted to endometrial carcinoma.

Endometrial carcinoma is a heterogeneous disease at the microscopic and clinical levels. Broadly, endometrial carcinoma can be divided into two categories, endometrioid and nonendometrioid. In the general population, approximately 75% to 80% of endometrial carcinoma is the endometrioid subtype, with nonendometrioid histologies representing the balance. The endometrioid tumors are graded on a three-tier system according to glandular differentiation present microscopically. Well-differentiated endometrioid adenocarcinomas are composed almost entirely of well-formed glands and are considered FIGO (International Federation of Gynecology and Obstetrics) grade 1. Poorly differentiated, FIGO grade 3 endometrioid adenocarcinoma is composed predominantly of solid sheets of malignant cells microscopically, with very little gland formation. FIGO grade 2 endometrioid adenocarcinoma has a more intermediate level of differentiation. Endometrioid adenocarcinoma can be associated with the presence of a precursor lesion, endometrial complex hyperplasia with atypia. The nonendometrioid carcinoma group is a diverse set of tumors primarily composed of uterine papillary serous carcinoma (UPSC), malignant mixed mullerian tumor (MMMT), and clear cell carcinoma (CCC). Representative photomicrographs of the endometrioid and nonendometrioid tumors are presented in Figure 1.

Microscopic recognition of the subtypes of endometrial carcinoma is important, as the different subtypes are associated with different biological behavior. In general, well-differentiated, grade 1 endometrioid adenocarcinoma is associated with early stage at diagnosis and a good prognosis. Patients with these tumors are often cured by hysterectomy alone. Unopposed exposure to high doses of estrogen is associated with endometrioid-type adenocarcinomas, especially the grade 1 tumors. In contrast, the nonendometrioid tumors, particularly UPSC and MMMT, are associated with advanced stage at diagnosis. Patients with these uterine tumors often require surgery and adjuvant

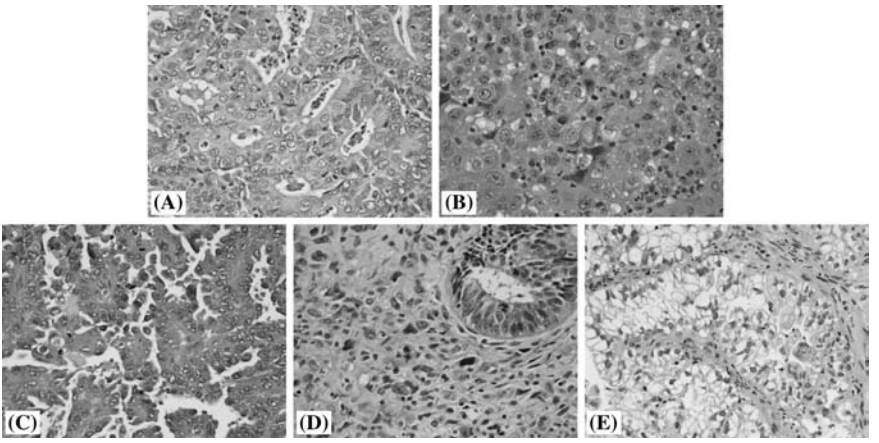

Figure 1 Photomicrographs of different histotypes of endometrial carcinoma (H&E, 200×). (**A**) Grade 1 endometrioid adenocarcinoma composed of well-formed neoplastic glands that make up the majority of this tumor microscopically. (**B**) Grade 3 endometrioid adenocarcinoma composed primarily of solid sheets of tumor cells with only occasional gland formation. Grade 2 endometrioid adenocarcinoma (not shown) has a mixture of well-formed glands and solid areas microscopically. (**C–E**) The nonendometrioid tumors uterine papillary serous carcinoma (**C**), malignant mixed mullerian tumor (**D**), and clear cell carcinoma (**E**). The nonendometrioid tumors typically have a poor prognosis compared with the well-differentiated, grade 1 endometrioid tumors.

chemotherapy and/or radiation treatment to control their disease. Even with such added therapy, the prognosis for patients with these tumors is usually poor. CCC is a more rare type of nonendometrioid endometrial carcinoma. It commonly can coexist with UPSC. Although CCC in the ovary is associated with an especially poor prognosis, the biological behavior of these tumors in the uterus is more heterogeneous.

PATHOLOGY OF ENDOMETRIAL CARCINOMA IN LYNCH SYNDROME

The molecular hallmark of a defect in DNA mismatch repair, as is seen in Lynch syndrome, is high levels of microsatellite instability (MSI-high) measured in tumor DNA compared with DNA from normal tissues. MSI-high can be due to Lynch syndrome (mutation of *MLH1, MSH2, MSH6, PMS2*, or other less common genes) or methylation with subsequent transcriptional silencing of the *MLH1* gene promoter. MSI-high due to *MLH1* methylation has been well described in the literature to occur in 15% to 20% of sporadic endometrial and colon carcinoma. Much of what is known regarding MSI-high colon and endometrial carcinoma is, therefore, pertinent to sporadic tumors; the relevance to the Lynch syndrome–associated tumors is not clear.

To clarify any possible differences between sporadic MSI-high endometrial carcinoma and Lynch syndrome–associated MSI-high endometrial carcinoma, we recently completed a large study comparing the pathological features of these groups (1). In this study, we analyzed 50 endometrial carcinomas from women with known Lynch syndrome mutations. For comparison, we studied 42 sporadic endometrial carcinomas from women younger than 50 years of age, as the mean age of the Lynch syndrome endometrial cancer patients was 46.8 years. These women were proven to be negative for *MLH1* and *MSH2* mutations by formal genetic testing. An additional comparison group consisted of 26 sporadic MSI-high endometrial carcinomas with methylation of *MLH1* and loss of MLH1 protein by immunohistochemistry. The group of sporadic MSI-high tumors was derived from analysis of a larger group of 128 endometrial carcinomas (85 endometrioid, 19 UPSC, and 24 MMMT). Remarkably, one of the clearest differences between these three groups was that the sporadic younger than 50 group (41/42, 97.6%) and the sporadic *MLH1* methylation group (25/26, 96.2%) were almost entirely composed of tumors with endometrioid histology. In contrast, the Lynch syndrome group was more heterogeneous, with 43 of 50 (86%) tumors with endometrioid histotype. Among the three groups, there were no statistical differences in myometrial invasion, presence of lymphatic/vascular invasion, or stage. Importantly, 22% of Lynch syndrome endometrial carcinomas were stage II, III, or IV, implying the need for adjuvant chemotherapy and/or radiation therapy in addition to hysterectomy. It has been suggested that endometrial carcinoma is important in women with Lynch syndrome, because it can act as a "sentinel cancer," preceding the diagnosis of colorectal cancer in 51% of these women by a median time of 11 years (2). However, with nearly a quarter of endometrial cancers in Lynch syndrome requiring some type of adjuvant therapy beyond surgery, it is clear that endometrial cancer itself is an important cancer in women with Lynch syndrome.

Compared with endometrial cancers in the Lynch syndrome and sporadic younger than 50 groups, endometrial cancers in the sporadic *MLH1* methylated group had a greater percentage of grade 2 and grade 3 endometrioid tumors and more advanced stage tumors overall (1). Additionally, a subset of the *MLH1* methylated group had a distinctive microscopic morphology that was not observed in the other two groups. This distinctive histology was characterized as "undifferentiated," in that the tumor cells were monotonous and small-to-medium sized, larger than histocytes but smaller than usual endometrioid carcinoma cells (1). The undifferentiated tumor cells grew in solid, discohesive sheets with no gland formation (Fig. 2). By immunohistochemistry, the undifferentiated carcinomas were weakly positive for pancytokeratin and entirely negative for estrogen receptor and progesterone receptor. In contrast, more usual grade 3 endometrioid carcinoma is strongly positive for pancytokeratin and has at least focal positive expression of hormone receptors.

In the general population, nonendometrioid endometrial carcinoma is typically diagnosed in older women with a mean age of 65 to 68 years (3–7).

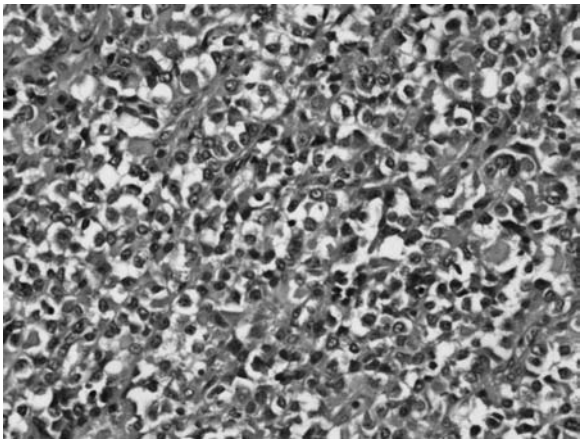

Figure 2 "Undifferentiated" endometrial carcinoma (H&E, 200×). Similar to grade 3 endometrioid adenocarcinoma, undifferentiated carcinoma microscopically consists of sheets of cells with no or little gland formation. However, the tumor cells of undifferentiated carcinoma are smaller than those of grade 3 endometrioid tumors. We have observed the undifferentiated endometrial carcinomas only in association with MSI-high due to MLH1 methylation, not in the MSI-high endometrial cancers associated with Lynch syndrome.

However, in Lynch syndrome, we have found that the mean age of diagnosis of nonendometrioid tumors is 46.4 years, similar to the mean age of endometrial cancer diagnosis in the Lynch syndrome group overall (46.8 years) (1). CCC, mixed UPSC + CCC, and MMMT have been identified as nonendometrioid tumors in Lynch syndrome. Carcangiu et al. published, in abstract form, a study in which they identified a preponderance of CCC from their Italian cohort of women with Lynch syndrome (8). This study has not yet been published in the peer-reviewed literature. From our experience, CCC has certainly been observed in women with Lynch syndrome, but it does not make up the majority of the endometrial carcinomas. Interestingly, from our previous study, we found that all of the nonendometrioid tumors arose in women with MSH2 mutations (1). In the population-based study of Hampel et al. (9) and subsequent follow-up (10), two Lynch syndrome–associated nonendometrioid endometrial carcinomas were identified, both in women with *MSH6* mutations. In our subsequent studies, we have identified only one woman with an *MLH1* mutation and a nonendometrioid endometrial carcinoma. This suggests that there may be a genotype-phenotype relationship in which MSI due to loss of *MLH1*, either by methylation of the promoter or due to gene mutation, is almost exclusively associated with higher-grade endometrioid tumors and undifferentiated tumors. In contrast, MSI due to defects in the *MSH2/MSH6* pair can result in a more varied spectrum of endometrial carcinoma histology. More

studies including nonendometrioid tumors will be needed to verify this possible genotype-phenotype relationship.

Although not common, we have also identified a smaller subset of women with Lynch syndrome endometrial cancer who have tumors arising in, and centered on, the lower uterine segment. Because of the younger age of these patients and tumor protruding from the cervix, some of these women had been previously diagnosed clinically as having cervical cancer. Endometrial and endocervical adenocarcinoma can usually be readily distinguished by microscopic examination, as endometrial adenocarcinoma is typically estrogen receptor positive, vimentin positive, and CEA negative by immunohistochemistry, while endocervical adenocarcinoma is negative for estrogen receptor and vimentin, but positive for CEA (11). It is interesting to note that a very informal survey of familial cancer databases at other institutions has yielded a number of women with Lynch syndrome with a diagnosis of "cervical cancer." If possible, it would be highly informative to reexamine the pathology slides from these cases to determine if any of these represent endometrial carcinoma arising in the lower uterine segment.

Synchronous primary tumors of the endometrium and ovary occur in approximately 10% of women with ovarian carcinoma and 5% of women with endometrial carcinoma (12). According to the revised Bethesda guidelines, women with synchronous endometrial and ovarian carcinomas should be evaluated for Lynch syndrome (13). In a large study of 102 women with synchronous endometrial and ovarian carcinomas, only 7 had molecular criteria (MSI-high with immunohistochemical loss of MLH1, MSH2, or MSH6 protein expression) or clinical criteria (Amsterdam family history) for a diagnosis of Lynch syndrome (14). Therefore, the vast majority of women with synchronous endometrial and ovarian tumors do not have Lynch syndrome.

For the purposes of endometrial cancer prevention, it would be very useful to know if endometrial complex atypical hyperplasia (CAH), the immediate precursor to sporadic endometrioid-type endometrial carcinoma, is also a precursor for Lynch syndrome–associated endometrioid carcinoma. There is very limited experience with endometrial hyperplasia in Lynch syndrome. In clinical trials being conducted at M.D. Anderson Cancer Center, we have encountered two women with CAH at baseline endometrial biopsy. At hysterectomy, both of these women had grade 1 endometrioid adenocarcinoma associated with CAH. Thus, we believe that CAH is indeed a precursor lesion for endometrioid-type endometrial adenocarcinomas arising in Lynch syndrome. Because of the defect in DNA mismatch repair, it has been hypothesized that colon adenomas, particularly proximal ones, are more likely to progress to colonic adenocarcinoma, and progress more rapidly, than adenomas in the general population (15–17). This hypothesis would be extremely difficult to test in Lynch syndrome–associated endometrial cancer, as it is well established in the general population that CAH and grade 1 endometrioid adenocarcinoma frequently coexist (18).

MICROSCOPIC FEATURES OF MSI-HIGH ENDOMETRIAL CARCINOMA

There is a considerable amount of literature on the presence or absence of distinctive microscopic features in MSI-high colorectal carcinoma. Some of the microscopic features that have been associated with the presence of MSI-high include poor differentiation, mucinous features, signet ring cell differentiation, mixed tumor histology, tumor cells growing in a medullary-type pattern, increased tumor infiltrating lymphocytes, and a Crohn's-like inflammatory infiltrate at the tumor periphery (19). Most of these studies have not distinguished between sporadic MSI-high due to *MLH1* methylation versus MSI-high due to germline mutation of a DNA mismatch repair gene. It is therefore unclear if there are microscopic differences between these two MSI-high groups. It must be noted, however, that these distinctive microscopic features may not be present in a substantial subset of colorectal carcinoma. Up to 40% of colorectal carcinomas do not have such distinguishing microscopic characteristics (19). Therefore, microscopic features alone cannot be used to determine which colorectal cancer patients should be evaluated for Lynch syndrome.

Microscopic features of MSI-high endometrial carcinoma have also been studied, but not to the extent of that for MSI-high colorectal carcinoma (20,21). As is the case for colorectal cancer, the source of the MSI (*MLH1* methylation vs. germline mutation of a DNA mismatch repair gene) was not delineated in these studies. One study found that MSI-high endometrial cancers were associated with higher tumor grade, presence of squamous metaplasia, deeper myometrial invasion, presence of lymphatic/vascular invasion, and extrauterine spread (20). The Memorial Sloan-Kettering group found that high numbers of tumor infiltrating lymphocytes and the presence of peritumoral lymphocytes were associated with MSI-high (21). At the higher levels of tumor infiltrating lymphocytes (40 lymphocytes per 10 high-power fields), these counts had a sensitivity of 85% in predicting MSI-high status but a specificity of only 46%. Although the published data for endometrial cancer is limited, it is our opinion that microscopic features of endometrial cancer are not sufficiently sensitive and specific to be used as accurate predictors of the presence of high levels of MSI.

OVARIAN CANCER IN LYNCH SYNDROME

The literature on ovarian cancer in Lynch syndrome is even more limited than that for endometrial cancer. Sporadic ovarian cancer, similar to sporadic endometrial cancer, is an extremely heterogeneous disease. Pathologically, tumors of the ovary can be divided into epithelial, sex cord/stromal, and germ cell types, with the epithelial tumors the most common. The most common epithelial type of tumor is high-grade serous carcinoma, but other subtypes include CCC, MMMT, mucinous carcinoma, and transitional cell carcinoma. Watson et al. (22) have compiled the largest published study on ovarian cancer in Lynch syndrome. In this retrospective study, the clinical records of 79 women with ovarian cancer

from 14 registries in 11 different countries were analyzed. Forty-four of the women were from families with known Lynch syndrome mutations, while the remainder had family histories consistent with Lynch syndrome. A wide variety of epithelial tumors were identified, including serous carcinoma, mucinous carcinoma, endometrioid carcinoma, CCC, and mixed histology carcinomas. Interestingly, five nonepithelial ovarian tumors were also identified, including granulosa cell tumor (x2), sex cord tumor, endodermal sinus tumor, and dysgerminoma. Neither immunohistochemistry for MLH1, MSH2, MSH6, and PMS2 nor MSI analysis was performed on any of these tumors, so it is not yet certain whether these nonepithelial tumors are truly associated with Lynch syndrome. Other published reports have examined immunohistochemistry of DNA mismatch repair gene products and MSI analysis in ovarian cancer (23–28). These studies have reported a similar wide variety of epithelial histotypes associated with MSI-high, including MMMT, CCC, mucinous carcinoma, endometrioid carcinoma, and carcinomas with mixed histologies. From these previous studies, it is not clear if *MLH1* methylation is associated predominantly with the endometrioid histotype, as is the case in endometrial carcinoma with MLH1 methylation. Also, from these studies, it is not clear how often pure high-grade serous carcinoma is associated with the presence of MSI-high, whether due to *MLH1* methylation or germline mutation of a DNA mismatch repair gene. In a large study, Rosen et al. (26) found no cases of MSI-high in 168 pure high-grade serous carcinomas of the ovary. In this same study, MSI-high was detected in ovarian MMMT, CCC, endometrioid carcinoma, and tumors with mixed histology (including serous). MSI-high, however, was detected in small numbers of ovarian high-grade serous carcinoma in another study (23). From the above studies, it appears clear that the presence of a defect in DNA mismatch repair can result in a wide variety of epithelial histotypes of ovarian carcinoma. This is in sharp contrast to the situation with hereditary *BRCA1* or *BRCA2* mutations, which are almost exclusively associated with one histotype of ovarian cancer, high-grade serous carcinoma (29–31).

HANDLING OF THE PROPHYLACTIC SURGERY SPECIMEN

Prophylactic hysterectomy and bilateral salpingo-oophorectomy is a rational and effective cancer prevention option in a woman with Lynch syndrome, especially if she has finished childbearing (32). Such prophylactic surgery may occur during a colectomy or partial colectomy for a previously diagnosed colon carcinoma. Because of the possibility of the presence of an unexpected endometrial or ovarian carcinoma (33,34), a prophylactic total hysterectomy should ideally be performed by a gynecologic oncologist who could perform a surgical staging procedure if necessary. The hysterectomy specimen should be examined intraoperatively by a pathologist who has been informed that the patient has Lynch syndrome. The endometrial cavity and the bilateral ovaries should be carefully examined and any suspicious masses should be analyzed microscopically by

frozen section. Particular attention should be devoted to the lower uterine segment, as tumors arising here can be small. If an occult ovarian or endometrial carcinoma is detected at the time of frozen section analysis, a staging procedure could then be performed if necessary. If no abnormalities are detected by the pathologist at the time of intraoperative consultation, then routine pathological sampling and microscopic examination of the ovaries and endometrium are sufficient. If the endometrial cavity and bilateral ovaries show no gross abnormalities, there is no evidence to support the microscopic examination of the entire endometrium or ovaries and fallopian tubes. For women with *BRCA1* or *BRCA2* mutations, it has been well documented that microscopic, occult carcinomas can be present, especially in the fimbriated end of the fallopian tube (35). Therefore, for these women undergoing prophylactic hysterectomy, the entire ovaries and fallopian tubes are microscopically examined. To date, such microscopic carcinomas have not been described in the ovaries or endometrium of women with Lynch syndrome.

TISSUE TESTING FOR IDENTIFYING WOMEN WITH LYNCH SYNDROME

Tissue testing (immunohistochemistry and MSI analysis) has emerged as a practical first step in the evaluation of women thought to be at risk for having Lynch syndrome. At our institution, we perform immunohistochemistry for MLH1, MSH2, MSH6, and PMS2 and MSI analysis from formalin-fixed, paraffin-embedded tissues. The above antibodies are commercially available. Importantly, frozen tissues or special handling of tissues are not necessary for these analyses. For the immunohistochemistry tests, it is important to choose sections of tumor that have normal cells present. Such nontumor cells serve as extremely useful internal controls. Figure 3A demonstrates the typical strong, positive

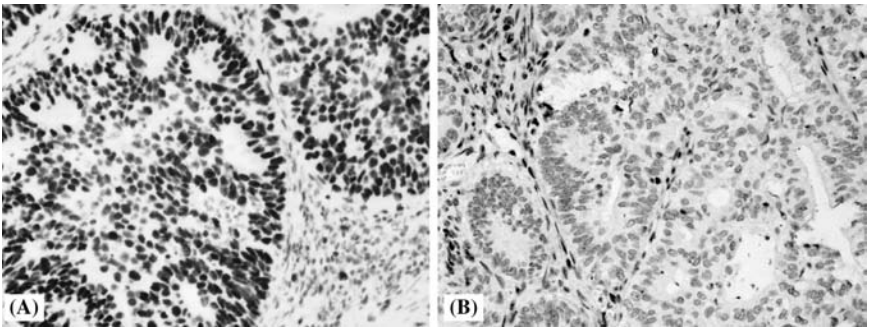

Figure 3 Immunohistochemistry for MSH2 (**A**) and MLH1 (**B**) in a representative endometrial carcinoma (200×). This tumor demonstrates strong, positive nuclear expression for MSH2 in a majority of the tumor cells (dark nuclear staining). However, the tumor cell nuclei are entirely negative for MLH1 expression, with adjacent nonneoplastic stromal cells having intact positive nuclear expression for MLH1.

nuclear expression of MSH2 protein observed in an endometrial adenocarcinoma with an intact *MSH2* gene. In Figure 3B, the tumor cells show no nuclear expression of MLH1, while adjacent stromal cells and inflammatory cells are positive for MLH1. Therefore, this tumor would be considered positive for MSH2, but negative for MLH1.

We perform MSI analysis in parallel with the immunohistochemistry. For MSI analysis, tumor and normal nontumor tissues are required. Any normal tissues from the hysterectomy specimen can be used, including cervix, benign fallopian tube, or benign lymph nodes. The pathologist maps on H&E stained slides the tumor and the normal areas to be microdissected. The tumor and normal areas are then carefully scraped from the unstained tissue sections and placed into Eppendorf tubes for DNA extraction and PCR amplification using fluorescent primers. For larger tumors, 5 to 10 unstained slides of normal and tumor usually yield sufficient DNA for the PCR-based MSI analysis. A panel of seven markers recommended by the National Cancer Institute (36) (BAT25, BAT26, BAT40, D2S123, D5S346, D173250, and TGF-βR2) is used to detect changes in the number of microsatellite repeats in the tumor compared with normal tissue. The amplified DNA is analyzed on an ABI Genetic Analyzer using capillary electrophoresis. Tumors with allelic shift in two or more microsatellites in the panel are considered MSI-high. Tumors with no allelic shift in all seven microsatellites are considered microsatellite-stable. Tumors with allelic shift in only one microsatellite are considered MSI-low. The significance, if any, of MSI-low in endometrial and ovarian tumors is not known. A sample chromatogram for one of the microsatellites is shown in Figure 4. Here, the tumor (lower tracing) has more peaks than the normal nontumor tissue from the same patient (upper tracing). Therefore, this would be considered allelic shift for this microsatellite.

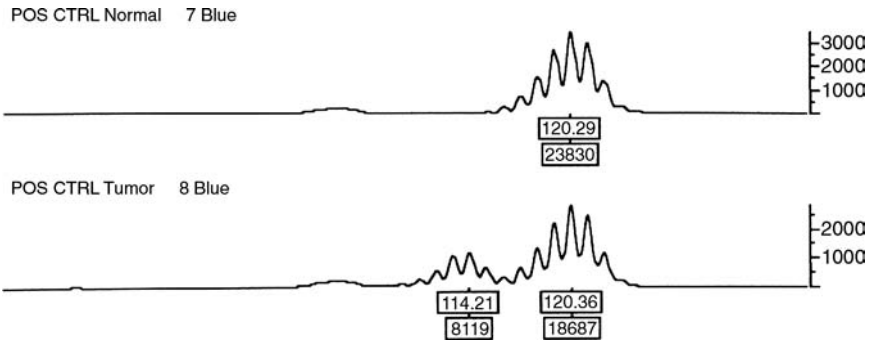

Figure 4 Representative MSI chromatogram for one microsatellite. The tumor DNA (*lower tracing*) demonstrates an increased number of peaks compared with the DNA extracted from nonneoplastic tissue from the same patient (*upper tracing*). Therefore, there is allelic shift in this microsatellite. If a tumor exhibits such allelic shift in two or more of the panel of seven microsatellites, the tumor is considered MSI-high.

For MSI-high tumors with loss of MLH1 by immunohistochemistry, we also perform a PCR-based assay to detect for possible methylation of the *MLH1* promoter. If methylation is present, it is much more likely that the patient has a sporadic carcinoma rather than a Lynch syndrome–associated tumor. The *MLH1* methylation assay can be performed using the same DNA as was extracted for the MSI analysis. In the *MLH1* methylation analysis, however, the DNA must be treated with bisulfite to convert any methylated cytosines to uracil. Then, the bisulfite-treated DNA is amplified using primers that are specific for the methylated and unmethylated *MLH1* gene. Similar to the MSI test, the amplified DNA is analyzed on an ABI Genetic Analyzer using capillary electrophoresis.

It has been noted that a subset of extracolonic carcinomas from patients with Lynch syndrome may not exhibit the usual high levels of MSI invariably observed in the colon carcinomas from these patients (37,38). In one large study, 23% of the endometrial carcinomas demonstrated no MSI, even when an extended panel of 12 markers was used (38). Such microsatellite-stable or MSI-low tumors can occur even with documented immunohistochemical loss of a DNA mismatch repair gene product. The reason for the differing patterns in MSI between colon carcinomas and extracolonic carcinomas is not clear at this point.

CASE REPORT

LG is a 41 year old woman who presented to her physician with vaginal bleeding and abdominal pain. Endometrial biopsy revealed high grade endometrioid endometrial carcinoma. She underwent laparoscopic hysterectomy, bilateral salpingoophorectomy, and staging procedures. She was found to be stage IIIc secondary to pelvic lymph node involvement and was treated with chemotherapy and radiation treatment.

Unfortunately, LG was estranged from her family, so no family history was available. Given her young age, immunohistochemical testing and microsatellite instability analysis was performed on her tumor tissue. She was found to have loss of MSH2 and high levels of microsatellite instability in her tumor. Subsequent mutation testing confirmed the presence of a MSH2 mutation and, therefore, Lynch Syndrome. This information proved to be useful, as LG has 2 daughters and 1 son, all younger than 20 years of age. Colon cancer screening can be initiated in LG, and her offspring can be offered genetic counseling and possible testing for the MSH2 mutation.

LEARNING POINTS

- The histology of endometrial and ovarian carcinoma arising in the setting of Lynch Syndrome is heterogeneous.
- Lynch Syndrome endometrial carcinoma is characterized by high levels of microsatellite instability. Currently, there are no distinct pathologic features which accurately predict the presence of microsatellite instability.

- MSI-High endometrial cancer secondary to Lynch Syndrome can be pathologically distinct from sporadic MSI-High endometrial cancer secondary to *MLH1* methylation.
- At the time of prophylactic surgery, the uterus and ovaries should be evaluated by intra-operative frozen section analysis to evaulate for an unsuspected endometrial or ovarian carcinoma.
- Tissue testing for Lynch Syndrome includes microsatellite instability analysis, *MLH1* promoter methylation assay, and immunohistochemistry for MLH1, MSH2, MSH6, and PMS2.

REFERENCES

1. Broaddus RR, Lynch HT, Chen L, et al. Pathologic features of endometrial carcinoma associated with HNPCC. Cancer 2006; 106:87–94.
2. Lu KH, Dinh M, Kohlmann W, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. Obstet Gynecol 2005; 105:569–574.
3. Abeler VM, Kjorstad KE. Clear cell carcinoma of the endometrium: a histopathological and clinical study of 97 cases. Gynecol Oncol 1991; 40:207–217.
4. Christopherson W, Alberhasky R, Connelly P. Carcinoma of the endometrium II. Papillary adenocarcinoma: a clinicopathological study of 46 cases. Am J Clin Pathol 1982; 77:534–540.
5. Webb GA, Lagios MD. Clear cell carcinoma of the endometrium. Am J Obstet Gynecol 1987; 156:1486–1491.
6. Doss LL, Llorens AS, Henriquez EM. Carcinosarcoma of the uterus: a 40-year experience from the state of Missouri. Gynecol Oncol 1984; 18:43–53.
7. Olah KS, Dunn JA, Gee H. Leiomyosarcomas have a poorer prognosis than mixed mesodermal tumours when adjusting for known prognostic factors: the results of a retrospective study of 423 cases of uterine sarcoma. Br J Obstet Gynaecol 1992; 99: 590–594.
8. Carcangiu ML, Dorji T, Radice P, et al. HNPCC-related endometrial carcinomas show a high frequency of non-endometrioid types and of high FIGO grade endometrioid carcinomas. Mod Pathol 2006; 19(suppl 1):173A (abstr).
9. Hampel H, Frankel W, Panescu J, et al. Screening for lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 206; 66:7810–7817.
10. Hampel H, Panescu J, Lockman J, et al. Comments on: screening for lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2007; 67:9603.
11. McCluggage WG, Sumathi VP, McBride HA, et al. A panel of immunohistochemical stains, including carcinoembryonic antigen, vimentin, and estrogen receptor, aids the distinction between primary endometrial and endocervical adenocarcinomas. Int J Gynecol Pathol 2002; 21:11–15.

12. Zaino R, Whitney C, Brady M, et al. Simultaneously detected endometrial and ovarian carcinomas-a prospective clinicopathologic study of 74 cases: a Gynecologic Oncology Group Study. Gynecol Oncol 2001; 83:355–362.
13. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96:261–268.
14. Soliman PT, Broaddus RR, Schmeler KM, et al. Women with synchronous primary cancers of the endometrium and ovary: do they have Lynch syndrome? J Clin Oncol 2005; 23:9344–9350.
15. Jass JR, Smyrk TC, Stewart SM, et al. Pathology of hereditary non-polyposis colorectal cancer. Anticancer Res 1994; 14(4B):1631–1634.
16. Jass JR, Cottier DS, Jeevaratnam P, et al. Diagnostic use of microsatellite instability in hereditary non-polyposis colorectal cancer. Lancet 1995; 346:1200–1201.
17. Rijcken FEM, Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. Gut 2002; 50:382–386.
18. Trimble CL, Kauderer J, Zaino R, et al. Concurrent endometrial carcinoma in women with a biopsy diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. Cancer 2006; 106:812–819.
19. Alexander J, Watanabe T, Wu TT, et al. Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 2001; 158:527–535.
20. Honore LH, Hanson J, Andrew SE. Microsatellite instability in endometrioid endometrial carcinoma: correlation with clinically relevant pathologic variables. Int J Gynecol Cancer 2006; 16:1386–1392.
21. Shia J, Black D, Hummer AJ, et al. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. Hum Pathol 2008; 39:116–125.
22. Watson P, Butzow R, Lynch HT, et al. The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2001; 82:223–228.
23. Sood AK, Holmes R, Hendrix MJ, et al. Application of the National Cancer Institute criteria for determination of microsatellite instability in ovarian cancer. Cancer Res 2001; 61:4371–4374.
24. Gras E, Catasus L, Arguelles R, et al. Microsatellite instability, MLH-1 promoter hypermethylation, and frameshift mutations at coding mononucleotide repeat microsatellites in ovarian tumors. Cancer 2001; 92:2829–2836.
25. Cai KQ, Albarracin C, Rosen D, et al. Microsatellite instability and alteration of the expression of hMLH1 and hMSH2 in ovarian clear cell carcinoma. Hum Path 2004; 35:552–559.
26. Rosen DG, Cai KQ, Luthra R, et al. Immunohistochemical staining of hMLH1 and hMSH2 reflects microsatellite instability status in ovarian carcinoma. Mod Pathol 2006; 19:1414–1420.
27. Malander S, Rambech E, Kristoffersson U, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol 2006; 101:238–243.
28. Komanska K, Malander S, Masback A, et al. Ovarian cancer at young age: the contribution of mismatch-repair defects in a population-based series of epithelial ovarian cancer before age 40. Int J Gynecol Cancer 2007; 17:789–793.

29. Shaw PA, McLaughlin JR, Zweemer RP, et al. Histopathologic features of genetically determined ovarian cancer. Int J Gynecol Pathol 2002; 21:407–411.
30. Piek JMJ, Torrenga B, Hermsen B, et al. Histopathological characteristics of *BRCA1*- and *BRCA2*-associated intraperitoneal cancer: a clinic based study. Fam Cancer 2003; 2:73–78.
31. Werness BA, Ramus SJ, DiCioccio RA, et al. Histopathology, FIGO stage, and *BRCA* mutation status of ovarian cancers from the Gilda Radner Familial Ovarian Cancer Registry. Int J Gynecol Pathol 2004; 23:29–34.
32. Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch Syndrome. N Engl J Med 2006; 354:261–269.
33. Chung L, Broaddus RR, Crozier M, et al. Unexpected endometrial cancer at prophylactic hysterectomy in a woman with hereditary nonpolyposis colon cancer. Obstet Gynecol 2003; 102:1152–1155.
34. Pistorius S, Nagel M, Kruger S, et al. Combined molecular and clinical approach for decision making for surgery in HNPCC patients: a report on three cases in two families. Int J Colorect Dis 2001; 16:402–407.
35. Fabiola M, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006; 30:230–236.
36. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58:5248–5257.
37. Broaddus RR, Lynch PM, Lu KH, et al. Unusual tumors associated with the hereditary nonpolyposis colorectal cancer syndrome. Mod Pathol 2004; 17:981–989.
38. Kuismanen SA, Moiso AL, Schweizer P, et al. Endometrial and colorectal tumors from patients with hereditary nonpolyposis colon cancer display different patterns of microsatellite instability. Am J Pathol 2002; 160:1953–1958.

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Endometrial and Ovarian Cancer Screening and Prevention in Women with Lynch Syndrome

Lee-may Chen

Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California, U.S.A.

KEY POINTS

- Individuals from Lynch/hereditary nonpolyposis colorectal cancer families are at increased lifetime risk of several malignancies and are often diagnosed at an earlier age.
- Current screening recommendations for gynecologic surveillance in this high-risk patient population include annual pelvic examination, pap smear, pelvic ultrasound, and endometrial biopsy.
- Any gynecologic complaint such as abnormal bleeding should prompt a thorough workup including endometrial biopsy.
- Chemoprevention, although not proven in this population, may include oral contraception.

INTRODUCTION

The first description of a family with Lynch syndrome/hereditary nonpolyposis colorectal cancer (Lynch/HNPCC) was published by Dr. Alfred Warthin in 1913. The initial proband was Dr. Warthin's seamstress who was concerned about her

personal risk of endometrial cancer and indeed later succumbed to this disease. Family G was later included in the work of Dr. Henry Lynch describing a cancer family syndrome, which was notable for a preponderance of colorectal, endometrial, stomach, ovarian, small bowel, and other adenocarcinomas. With the advent of molecular genetics, germline mutations in family of DNA mismatch repair genes, including MLH1, MSH2, MSH6, and PMS2, have been found to be the underlying defect in Lynch/HNPCC families (1–5). The population prevalence of these genes is about 1/1000 to 1/3000 (6). About 2% of colorectal, endometrial, and ovarian carcinomas are felt to be attributable to Lynch/HNPCC (7,8).

Individuals from Lynch/HNPCC families are at significantly increased lifetime risk of several malignancies and are often diagnosed at an earlier age. The hallmark cancer for Lynch/HNPCC is colorectal cancer. However, for women, the lifetime risks of endometrial cancer and colorectal cancer are almost equal at 40% to 60% (9). There may be a difference in the cancer phenotype, depending on which mismatch repair gene is affected. The risk of ovarian cancer for individuals with germline mutations in MLH1 and MSH2 is substantially higher than in the general population, with a lifetime risk of 9% to 12%. MSH2 may be associated with a higher risk of developing endometrial cancer than MLH1 (35–79% vs. 25–31%), and MSH6 may be associated with a higher risk (71–73%) of endometrial cancer that presents at a later age (10–13).

Identification of women from a Lynch/HNPCC family allows them the opportunity to participate in genetic counseling and other familial cancer programs where they can be educated about their genetic risks and provided coordinated, multidisciplinary care. Management of gynecologic cancer risks in this high-risk population involves a three-pronged approach of surveillance, chemoprevention, and risk-reducing surgery.

SURVEILLANCE

Goals of Surveillance

The objective of a cancer-screening program is to detect tumors at a stage earlier than it would present naturally so that treatment is more likely to be successful. Ideally, a screening test must be noninvasive and inexpensive. The prototypical example of a successful screening test is the Papanicolaou smear, which has been instrumental in the early detection and prevention of invasive cervical cancer. Screening for endometrial cancer in the general population is not cost-effective given the relative low frequency of disease, and generally early disease at the time of diagnosis. Screening for ovarian cancer in the general population is also not effective, as our current tests of CA125 and ultrasound lack the sensitivity and specificity needed to detect early disease with an appropriate positive predictive value.

In Lynch/HNPCC, a successful screening test has been identified through studies of colonoscopy, which has been demonstrated to decrease mortality (14).

A prospective cohort study by Jarvinen et al. followed 133 individuals from 22 Lynch/HNPCC families with screening every three years by either colonoscopy or flexible sigmoidoscopy and barium enema and compared outcomes with 119 controls who declined surveillance or could not be contacted (15). Over 15 years, the incidence of colorectal cancer was 8/133 (6%) in the screening group, compared with 19/119 (16%) in the control group, $p = 0.014$. The relative risk of death was reduced to 0.344 (95% confidence interval, CI 0.172–0.683). For gynecologic cancers, there is less definitive data to support screening in this high-risk population.

HNPCC/Lynch Pathology Characteristics

Characterization of the pathology and clinical outcomes of Lynch/HNPCC-related endometrial cancer suggests that it has a similar biology to sporadic endometrial cancer (16). In a cohort of 50 women with Lynch/HNPCC and endometrial cancer, there were no significant differences in the distribution stage, grade, or histology when compared with cases of sporadic endometrial cancer. Most women did present with early-stage disease (78% stage I, 10% stage II, and 12% stage III/IV). However, the women with Lynch/HNPCC were younger, with a mean age of 47 at presentation. In another study comparing women with Lynch/HNPCC endometrial cancer to controls matched for age and stage, the mean age was 50, and there was no difference in five-year survival (88% vs. 82%, $p = 0.59$) (17). In consideration of potential screening and chemoprevention recommendations, Lynch/HNPCC-associated endometrial cancer can be predicted to present and behave as sporadic endometrial cancer would, albeit at a younger age.

In contrast, Lynch/HNPCC-related ovarian cancer is less well understood. The largest series of 80 patients included cases from 1936 to 1997 and demonstrated a preponderance of early-stage disease (61% stage I, 23% stage II, 14% stage III, and 2% stage IV) (18). The mean age at diagnosis was 43. Approximately 90% of tumors were invasive epithelial carcinomas, with a similar distribution of histologies and grades to the population-based comparison group. Twenty-two percent of ovarian cancers in patients with Lynch/HPNCC presented with a synchronous endometrial cancer. A separate case-control study matched for stage, age, and year of diagnosis found similar five-year survival rates of 64.2% for Lynch/HNPCC ovarian cancer, compared with 58.1% for sporadic ovarian cancer ($p = 0.59$) (19).

While Lynch/HNPCC-related endometrial cancer appears to behave similarly to sporadic endometrial cancer, Lynch/HNPCC-related ovarian cancer seems to present at an earlier stage and often with a synchronous endometrial cancer. A better understanding of the pathology and clinical course of individuals with Lynch/HNPCC will help elucidate the best screening strategies in this high-risk population.

Criteria for Screening

Many unanswered questions remain in the management of gynecologic screening in Lynch/HNPCC. Current cancer-screening guidelines for women with Lynch/HNPCC are outlined in Table 1 (20). For gynecologic screening, annual endometrial sampling and ultrasound are recommended, without strong evidence for either.

Identifying a high-risk population at risk for cancers associated with Lynch/HNPCC is challenging. Women fall into several categories: known mutation carriers, members of a family with a known mutation, members of Amsterdam II families (see Appendix), members of HNPCC-like families, women diagnosed with colon cancer at a young age, and young women with atypical endometrial hyperplasia. Certainly, those women with a mutation or strong family history should undergo surveillance and be offered risk-reducing surgery.

Another important question is when to begin the screening process for gynecologic malignancies. The age to begin screening can be ascertained from the cumulative cancer incidence of ovarian and endometrial cancer from pedigree analysis. A retrospective review of 90 Lynch/HNPCC families based on Amsterdam II criteria in the Royal Melbourne Hospital registry compared the age of initial cancer diagnosis and cumulative cancer incidence with population cancer incidence data (21). The mean age of endometrial cancer diagnosis was 47.9, with a cumulative incidence of 0.3% by age 30, 0.3% by age 35, and 0.9% by age 40. The mean age of ovarian cancer diagnosis was 48.3, with a cumulative incidence of 0.2% by age 30, 0.5% by age 35, and 0.7% by age 40. Initiation of a gynecologic screening program at age 3 to 35 would leave 3% to

Table 1 Recommended Management for At-risk Women in Lynch/HNPCC Families

Intervention	Recommendation
Colonoscopy	Every 1–2 yr beginning at age 20–25 yr or 10 yr younger than the youngest age at diagnosis in the family, whichever comes first. For MSH6 families, begin at age 30 yr
Endometrial sampling	Every year beginning at age 30–35 yr
Transvaginal ultrasound	Every year beginning at age 30–35 yr
Urinalysis with cytology	Every 1–2 yr beginning at age 25–35 yr
History and examination, with review of systems, education, counseling	Every year beginning at age 21 yr
Colorectal resection	Generally not recommended for primary prophylaxis, but if cancer diagnosed, subtotal colectomy is favored
Hysterectomy or oophorectomy	Discuss as option after childbearing is complete

Source: From Ref. 20.

7% of gynecologic cancers to occur before the commencement of surveillance. The authors conclude that this would be comparable to the 3% of colorectal cancers that occur before the commencement of screening colonoscopy as recommended at age 25.

Surveillance Studies

The evidence supporting surveillance to detect early endometrial or ovarian cancer in Lynch/HNPCC is limited. There are four notable surveillance and screening studies for gynecologic cancer in Lynch/HNPCC. The first study is a brief description of the experience of two familial cancer centers in the United Kingdom and the Netherlands (22). Two hundred and ninety-two women (171 satisfying Amsterdam criteria) between the ages of 25 and 65 were followed prospectively and offered annual or biennial pelvic ultrasound. Between 1994 and 1999, 522 ultrasounds were performed over 826 patient-years. There were no cases of endometrial carcinomas detected by screening. There were two cases of interval endometrial cancers that presented at 5 and 27 months after their last scans. These women, age 46 and 57, respectively, presented with abnormal bleeding and were diagnosed with stage I disease.

The second study describes the 10-year experience at the University of Groningen in the Netherlands where 41 women with Lynch/HNPCC between the ages of 27 and 60 were offered annual examination, ultrasound, and CA125 level over 197 patient-years (23). Endometrial sampling was performed for a thickened endometrium of >12 mm in premenopausal woman and >5 mm in a premenopausal woman. An insufficient office biopsy was followed by a hysteroscopy with dilatation and curettage. Thirty-five women were premenopausal. Four women reported clinical symptoms on annual examination. On further evaluation, one woman was found to have a benign endometrial polyp. Of 179 ultrasounds, only 17 (in 11 patients) were abnormal. Further evaluation by tissue sampling of the 11 patients with abnormal ultrasounds resulted in the diagnosis of atypical endometrial hyperplasia in three women. There were no abnormal CA125 levels and no ovarian cancers diagnosed in this cohort. One woman, aged 61, was diagnosed with an interval stage I endometrial carcinoma after she presented with postmenopausal bleeding 8 months after a normal ultrasound.

While the first two studies evaluated transvaginal ultrasound as a screening modality, the third study evaluated the addition of endometrial biopsy to the screening protocol. In this Finnish study, 175 women with germline mutations in either MLH1, MSH2, or MSH6 participated in a surveillance program that entailed endometrial sampling and ultrasound every 2 to 3 years after ages 30 to 35 (24). Fifty-three women attended only one screening session. In total, there were 503 visits for 759 patient-years. All visits included a clinical examination. Additionally, 94% of visits included an ultrasound, 74% included endometrial sampling, and 28% included a CA125 level.

Twenty-five (5%) women had an abnormal endometrial biopsy, including 11 (2%) who were diagnosed with endometrial cancer. One of the endometrial cancer patients also had an abnormal ultrasound, while another also had an abnormal Pap smear. Surveillance endometrial biopsies also identified 14 patients with endometrial hyperplasia; 4 with complex atypical hyperplasia, 8 with complex hyperplasia, and 2 with simple hyperplasia. Forty-three (25%) women had risk-reducing surgery. One case each of occult adenocarcinoma and complex hyperplasia were diagnosed in the surgical specimens from prophylactic hysterectomies. Neither case had a preoperative endometrial biopsy. Additionally, two women were diagnosed with interval cancers—both stage I—3 and 31 months after surveillance.

The 11 screen-detected endometrial cancer patients were compared with 83 women from the same families who were diagnosed with symptom-detected endometrial cancer (Table 2). Because of the small numbers, there was no significant difference in long-term outcomes. Overall, 10-year survival was 100% in the screened group and 92% in the unscreened group. However, there appeared to be evidence of stage migration with 7% of women in the surveillance group presenting with stage III/IV disease versus 17% of women who presented symptomatically.

Six women were diagnosed with an abnormal CA125, one of whom was eventually diagnosed with ovarian cancer. Overall, there were four women diagnosed with ovarian cancer—two interval cancers (stage I and stage III) diagnosed two and five months after normal surveillance visits—and two occult cancers (both stage I) diagnosed at the time of risk-reducing surgery.

The fourth study reports on the addition of hysteroscopy to endometrial sampling (25). Fifty-seven women with Lynch/HNPCC were monitored through annual hysteroscopy and endometrial biopsy over 91 patient-years. Outpatient flexible diagnostic hysteroscopy was attempted 91 times, with 81 (89%) successful procedures. Thirty-four (42%) of 81 hysteroscopies were described as normal, 12 (15%) showed an endometrial polyp, 11 (14%) showed atrophy, 10

Table 2 Comparison of Screen-Detected Endometrial Cancer Versus Unscreened Endometrial Cancer in Lynch/HNPCC

	Screen-detected endometrial cancer	Unscreened endometrial cancer
Median age	52 (range 36–71)	50 (range 27–85)
Stage at diagnosis		
Stage I	86%	81%
Stage II	7%	2%
Stage III	7%	13%
Stage IV	0%	4%
10-yr survival	100%	92%

Source: From Ref. 24.

(12%) showed hypertrophy, 7 (9%) showed fibroids/adenomyosis, and 2 were suspicious for malignancy. Endometrial biopsy was attempted 86 times, with 75 (88%) successful procedures. Fifty-three (71%) of 75 endometrial biopsies were normal (14 atrophic, 12 proliferative, 27 secretory), 6 (8%) showed an endometrial polyp, 3 showed endometrial hyperplasia without atypia, 2 showed endometrial cancer, and 11 (14%) were nondiagnostic. Despite the recommendation to perform the endometrial biopsy during the first part of the menstrual cycles, the majority of normal biopsies showed secretory endometrium. Twenty-four operative hysteroscopy procedures were performed, but no additional cases of atypical hyperplasia or cancer were identified. The two hysteroscopies suspicious for cancer were confirmed by endometrial biopsy. After definitive surgery, the patients were diagnosed with stage IB grade 3 and stage IC grade 2 endometrioid carcinoma, respectively. Both women with endometrial cancer also had a history of abnormal vaginal bleeding, even though neither presented for medical care complaining of these symptoms. In addition to the two women with endometrial cancer, three other women underwent hysterectomy based on hysteroscopy/biopsy findings—two were confirmed to have hyperplasia without atypia, while one had benign secretory endometrium. In total, four women were diagnosed with endometrial hyperplasia without atypia—three were diagnosed by endometrial biopsy, and the fourth was diagnosed by operative hysteroscopy to evaluate a polyp seen on diagnostic hysteroscopy.

In each of the surveillance studies performed in this high-risk population, the sensitivity of ultrasound and CA125 screening for endometrial and ovarian cancer remains poor. However, the available evidence suggests that endometrial sampling is more effective, supported by the improved ability to detect precancerous conditions such as atypical complex hyperplasia and stage migration in the screened population. Hysteroscopy may be feasible, but may not add much to endometrial sampling. To date, there are no adequate data to support ovarian cancer screening, even in this high-risk population.

Compliance With Screening

The current rates of gynecologic surveillance in Lynch/HNPCC are low. Forty-four individuals at increased risk for colorectal cancer at the Dana Farber Cancer Institute were surveyed about their cancer surveillance practices (26). Of this group, 16 were women with Lynch/HNPCC. Three of the 16 women were less than age 35 and thus not included in the current screening recommendations. Similarly, one woman had previously undergone hysterectomy and so was not eligible for surveillance. Of the 12 women with uterus in situ, only 3 (25%) were undergoing endometrial surveillance by either ultrasound or biopsy. Ten (63%) of Lynch/HNPCC patients had reported seeing their gynecologist within the past 12 months. However, only half of them underwent appropriate endometrial cancer screening, defined for the purposes of this study as annual transvaginal ultrasound or endometrial biopsy starting at age 35.

In a survey of 27 mutation carriers from a hereditary colorectal cancer risk program, 11 of 16 (69%) women with a uterus in situ reported surveillance by either ultrasound or biopsy (27). Among 21 women with ovaries, 13 (62%) reported surveillance by ultrasound or CA125. Among six women with colorectal cancer but no gynecologic disease, the prevalence of surveillance was only 50%.

Since the procedures of ultrasound and endometrial biopsy may be uncomfortable and unappealing to women, further studies illustrating attitudes toward screening and surveillance may help increase compliance with screening for gynecologic malignancies. Additionally, physician's awareness of current screening recommendations for this high-risk population may play a role.

Physician and Patient Awareness

The awareness of gynecologic cancer risk and screening may be promoted through gynecologists, other primary care providers, and genetic counselors. Taking a family history and educating patients of gynecologic cancer risks when appropriate should be part of routine care in the gynecologic examination. Twenty-two of 41 (81%) of women had seen their gynecologist after receiving their Lynch/HNPCC diagnosis, but only 12% of women report hearing about their gynecologic cancer risks from their gynecologist, in comparison with 48% of women hearing this information from a genetic counselor. While limited by small numbers, these data suggest that women with colorectal cancer alone may be less likely to follow through with gynecologic surveillance, possibly due to a lower awareness of their gynecologic cancer risks.

Women with Lynch/HNPCC often present to care after the diagnosis of a related malignancy. In a study of women with metachronous endometrial and colorectal cancer, 49% of women presented with the colon cancer first, at a median age of 40. The median time before developing their next cancer was eight years (28). The time interval after the diagnosis of their sentinel cancer allows the opportunity for additional education and reinforcement of recommended screening procedures by colorectal surgeons, medical oncologists, gynecologists, and other health care providers.

It is unknown if monitoring for symptoms may lead to equivalent diagnoses or outcomes, but given the number of interval cancers diagnosed in each of the screening studies, education about symptoms is also important.

CHEMOPREVENTION

There are no studies looking specifically at chemoprevention for Lynch/HNPCC. However, in the general population there are large case-control studies using the Cancer and Steroid Hormone (CASH) data collected by the Surveillance, Epidemiology, and End Results (SEER) program that demonstrated a 50% reduction in the risk of endometrial and ovarian cancer with the use of oral contraceptive pills (29,30). The pathology of Lynch/HNPCC endometrial cancers is similar to

sporadic endometrial cancer with regard to stage, grade, and histology, so that in the absence of chemoprevention data specific to the Lynch/HPNCC population, the potential for prevention can be extrapolated. In the treatment of endometrial hyperplasia, a variety of progestins with varying potencies have been shown to be effective at arresting disease progression (31–33). Ongoing research is evaluating the role of levonoregestrel oral contraceptive pills versus depomedroxyprogesterone acetate as possible agents for chemoprevention in women with Lynch/HNPCC.

Lynch/HNPCC-associated ovarian cancer may be a biologically different disease when compared with the general population, so the benefit of oral contraceptive pills in reducing the risk of ovarian cancer is less clear. Using the model of BRCA1/2-associated ovarian cancer as another high-risk population, oral contraceptive pills are still considered favorably as the data for hormonal chemoprevention, suggesting potential benefit and at least no apparent increased cancer risk (34–36).

CONCLUSIONS

Our personal recommendation, as well as recommendations by the National Comprehensive Cancer Network (NCCN) and the Journal of the American Medical Association (JAMA) for surveillance and risk reduction of gynecologic malignancies of women with Lynch/HNPCC, is to educate women about their health risks and symptoms of endometrial cancer (20,37). They should be considered for risk-reducing surgery through hysterectomy with oophorectomy at age 35 to 40 or on completion of childbearing. Endometrial sampling should begin annually around ages 30 to 35. Neither ultrasound nor CA125 is effective enough for routine screening; better ovarian cancer screening test is needed. In the absence of better screening methods for ovarian cancer, the NCCN recommends transvaginal ultrasound and CA125 levels every 6 to 12 months (37). Oral contraceptive pills should be considered for women who choose not to undergo risk-reducing surgery, but the actual risk reduction in women with HNPCC/Lynch has yet to be quantified. Alternatives for endometrial cancer chemoprevention may include other forms of progestin therapy, but this awaits validation from future studies.

CASE REPORT

E.B. is a 31-year-old gravida 1 para 1 who was recently diagnosed with stage I colon cancer. She has a family history significant for colon cancer and endometrial cancer. Her mother was diagnosed with colon cancer at age 43 and then endometrial cancer at age 47. Her maternal grandfather and great grandfather were diagnosed with colon cancer. E.B. was referred for genetic counseling and tested positively for a germline mutation in MSH2.

The patient has been taking oral contraceptive pills for six years. She has regular menses and no breakthrough bleeding, postcoital spotting, or dyspareunia. For gynecologic surveillance, she is followed with yearly pelvic examination, pap smear, pelvic ultrasound, and endometrial biopsy. Although she is still seeking fertility, she is contemplating undergoing a total abdominal hysterectomy and bilateral salpingo-oophorectomy in the next few years after she is finished childbearing.

LEARNING POINTS

- Individuals with Lynch/HNPCC are often diagnosed with cancer at a significantly younger age than unaffected individuals.
- Individuals with Lynch/HNPCC are at an increased lifetime risk for multiple malignancies.
- Screening for gynecologic malignancies in this high-risk population should be performed annually.
- Hysterectomy and oophorectomy should be offered as an option for risk reduction.

REFERENCES

1. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994; 368(6468):258–261.
2. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993; 75(5):1027–1038.
3. Miyaki M, Konishi M, Tanaka K, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997; 17(3):271–272.
4. Nicolaides NC, Papadopoulos N, Liu B, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994; 371(6492):75–80.
5. Mitchell RJ, Farrington SM, Dunlop MG, et al. Mismatch repair genes hMLH1 and hMSH2 and colorectal cancer: a HuGE review. Am J Epidemiol 2002; 156(10):885–902.
6. Dunlop MG, Farrington SM, Nicholl I, et al. Population carrier frequency of hMSH2 and hMLH1 mutations. Br J Cancer 2000; 83(12):1643–1645.
7. Hampel H, Stephens JA, Pukkala E, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 2005; 129(2):415–421.
8. Malander S, Rambech E, Kristoffersson U, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol 2006; 101(2):238–243.
9. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214–218.
10. Vasen HF, Stormorken A, Menko FH, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001; 19(20):4074–4080.

11. Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 2004; 127(1):17–25.
12. Green J, O'Driscoll M, Barnes A, et al. Impact of gender and parent of origin on the phenotypic expression of hereditary nonpolyposis colorectal cancer in a large Newfoundland kindred with a common MSH2 mutation. Dis Colon Rectum 2002; 45(9):1223–1232.
13. Wijnen J, de Leeuw W, Vasen H, et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. Nat Genet 1999; 23(2):142–144.
14. Johnson PM, Gallinger S, McLeod RS. Surveillance colonoscopy in individuals at risk for hereditary nonpolyposis colorectal cancer: an evidence-based review. Dis Colon Rectum 2006; 49(1):80–93; discussion 94–95.
15. Jarvinen HJ, Aarnio M. Surveillance on mutation carriers of DNA mismatch repair genes. Ann Chir Gynaecol 2000; 89(3):207–210.
16. Broaddus RR, Lynch HT, Chen LM, et al. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. Cancer 2006; 106(1):87–94.
17. Boks DE, Trujillo AP, Voogd AC, et al. Survival analysis of endometrial carcinoma associated with hereditary nonpolyposis colorectal cancer. Int J Cancer 2002; 102(2):198–200.
18. Watson P, Bützow R, Lynch HT, et al. The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2001; 82(2):223–228.
19. Crijnen TE, Janssen-Heijnen ML, Gelderblom H, et al. Survival of patients with ovarian cancer due to a mismatch repair defect. Fam Cancer 2005; 4(4):301–305.
20. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006; 296(12):1507–1517.
21. Brown GJ, St John DJ, Macrae FA, et al. Cancer risk in young women at risk of hereditary nonpolyposis colorectal cancer: implications for gynecologic surveillance. Gynecol Oncol 2001; 80(3):346–349.
22. Dove-Edwin I, Boks D, Goff S, et al. The outcome of endometrial carcinoma surveillance by ultrasound scan in women at risk of hereditary nonpolyposis colorectal carcinoma and familial colorectal carcinoma. Cancer 2002; 94(6):1708–1712.
23. Rijcken FE, Mourits MJ, Kleibeuker JH, et al. Gynecologic screening in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2003; 91(1):74–80.
24. Renkonen-Sinisalo L, Bützow R, Leminen A, et al. Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. Int J Cancer 2007; 120(4):821–824.
25. Lecuru F, Metzger U, Scarabin C, et al., Hysteroscopic findings in women at risk of HNPCC. Results of a prospective observational study. Fam Cancer 2007; 6(3):295–299.
26. Stoffel EM, Garber JE, Grover S, et al. Cancer surveillance is often inadequate in people at high risk for colorectal cancer. J Med Genet 2003; 40(5):e54.
27. Yang K, Allen B, Conrad P, et al. Awareness of gynecologic surveillance in women from hereditary non-polyposis colorectal cancer families. Fam Cancer 2006; 5(4): 405–409.
28. Lu KH, Dinh M, Kolhmann W, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. Obstet Gynecol 2005; 105(3):569–574.

29. Oral contraceptive use and the risk of endometrial cancer. The Centers for Disease Control Cancer and Steroid Hormone Study. JAMA 1983; 249(12):1600–1604.
30. The reduction in risk of ovarian cancer associated with oral-contraceptive use. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. N Engl J Med 1987; 316(11):650–655.
31. Randall TC, Kurman RJ. Progestin treatment of atypical hyperplasia and well-differentiated carcinoma of the endometrium in women under age 40. Obstet Gynecol 1997; 90(3):434–440.
32. Wildemeersch D, Dhont M. Treatment of nonatypical and atypical endometrial hyperplasia with a levonorgestrel-releasing intrauterine system. Am J Obstet Gynecol 2003; 188(5):1297–1298.
33. Horn LC, Schnurrbusch U, Bilek K, et al. Risk of progression in complex and atypical endometrial hyperplasia: clinicopathologic analysis in cases with and without progestogen treatment. Int J Gynecol Cancer 2004; 14(2):348–353.
34. Narod SA, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. Hereditary Ovarian Cancer Clinical Study Group. N Engl J Med 1998; 339(7):424–428.
35. McLaughlin JR, Risch HA, Lubinski J, et al. Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study. Lancet Oncol 2007; 8(1):26–34.
36. Modan B, Hartge P, Hirsh-Yechezkel G, et al. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a BRCA1 or BRCA2 mutation. N Engl J Med 2001; 345(4):235–240.
37. Colorectal Screening: Clinical Practice Guidelines in Oncology © 2006 National Comprehensive Cancer Network, Inc. 2007 1/26/2007 [cited 2007 August 8]; Version 1.2007. Available at: http://www.nccn.org/professionals/physician_gls/PDF/colorectal_screening.pdf.

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Colon Cancer and Other Lynch Cancers: Screening and Prevention

Thuy M. Vu and Miguel A. Rodriguez-Bigas

Department of Surgical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- Individuals with Lynch syndrome are at increased lifetime risk of colorectal cancer, small bowel cancer, and transitional cell carcinoma of the kidney/ureter, and other cancers.
- Surveillance by colonoscopy every 1 to 2 years, starting at age 20 to 25 has been proven to decrease mortality from colorectal cancer in individuals with Lynch syndrome.
- Guidelines for screening for malignancies in the upper gastrointestinal tract and urologic system are less clear and should be individualized on the basis of personal and family history.

INTRODUCTION

Individuals identified to be at risk for Lynch syndrome, by detection of either a germline mutation in one of the mismatch repair (MMR) genes (*hMLH1*, *hMSH2*, *hMSH6*, or *hPMS2*) or suspected carrier of an MMR gene mutation by informative tumor studies (high levels of microsatellite instability and/or loss of MMR protein expression), are recommended to undergo high-risk surveillance of the colon and rectum and other extracolonic sites. These recommendations also

apply to either individuals suspected of being at risk or affected members of Lynch families based on their family history. This chapter will review the literature regarding screening recommendations for colorectal, stomach, small bowel, and urinary tract tumors.

SURVEILLANCE AND PREVENTION

Colon Cancer

Individuals with Lynch syndrome have significantly increased lifetime risk for colorectal cancer (CRC), which is estimated to be as high as 70% by age 70 years (2). Men with Lynch syndrome have a higher risk than women to develop CRC (3–5). Approximately two-thirds of the cancers occur in the proximal colon with the average age of onset in the mid 40s (2). Additionally, these individuals have a high risk for development of synchronous and metachronous colorectal tumors. Approximately 7% to 18% of individuals with Lynch syndrome present with synchronous CRCs, and the risk for metachronous colorectal tumors is up to 50% within 15 years of the first (6–8). Despite the high cancer risks, it has been noted, however, that individuals with Lynch syndrome–associated CRC have more favorable outcome than those with sporadic CRC (9,10).

Adenomas and cancer are more commonly identified in families with Lynch syndrome (11,12). The adenoma-carcinoma sequence in Lynch syndrome patients appears to be more accelerated, with carriers of MMR mutations developing adenomas at earlier ages than noncarriers (13,14). In addition, a significant difference was noted between size, location, and histology of Lynch-associated adenomas than sporadic adenomas. Lynch adenomas tend to be proximally located, and despite being smaller in size than sporadic adenomas, they are often more highly dysplastic (15). Lynch adenomas >5 mm in both the proximal colon and rectum were frequently more dysplastic than large sporadic adenomas.

Since adenomas in individuals with Lynch syndrome appear to progress to CRC faster than in the general population and because of the occurrence of interval cancers following negative surveillance colonoscopy, the frequency of colonoscopic surveillance in patients with Lynch syndrome should be at shorter intervals and begin decades earlier compared with the general population (16,17). A study in the Netherlands revealed that patients who underwent surveillance at intervals of two years or less were more likely to have localized cancers if a cancer was detected at surveillance colonoscopy compared with those who underwent colonoscopy at longer intervals (18). Similarly, a study in Finland demonstrated that colonoscopy was effective in decreasing CRC incidence and mortality in Lynch syndrome patients. In this study, a 62% decrease in CRC incidence and a 65% reduction in overall death rate in hereditary nonpolyposis colorectal cancer (HNPCC) at-risk individuals who underwent surveillance with flexible sigmoidoscopy and barium enema or colonoscopy every 3 years over a 15-year period was reported (19). Since the majority of neoplasms in Lynch

syndrome patients will occur proximal to the splenic flexure, flexible sigmoidoscopy has no role in surveillance in the Lynch syndrome unless the patient has had an abdominal colectomy.

The evidence in support of the frequency and age at which to begin colonoscopic surveillance demonstrates significant benefits for improved health outcomes (20). CRC-specific 10-year survival was greater in individuals who pursued surveillance compared with individuals who did not (21). On the basis of the clinical data-proving efficacy of colorectal screening, the current recommendation is to repeat surveillance at an interval of 1 to 2 years, beginning at age 20 to 25 years (8,18,20,22).

High-resolution colonoscopy with chromoendoscopy has been shown to improve the detection of adenomas in individuals with Lynch syndrome (23,24). In two studies, significantly more adenomas and a significantly higher number of flat adenomas were detected by chromoendoscopy than by conventional colonoscopy (24). Clinicians may consider adopting this strategy as part of the high-risk surveillance plan for individuals who have a high risk for colorectal carcinoma.

The role of prophylactic colectomy in Lynch syndrome is controversial. However, this procedure should be discussed with each patient and should potentially be offered as an option to MMR gene mutation carriers. Factors that would lend support for a prophylactic colectomy is the high lifetime risk for CRC, the rapid progression from adenoma to carcinoma, the inability to perform a complete colonoscopy, a patient who refuses colonoscopic surveillance, and cancer phobia in a patient. It must be kept in perspective that even though the risk for CRC in Lynch syndrome patients is high, it is not a certainty. The CRC penetrance in Lynch syndrome patients is incomplete; therefore, approximately 15% to 40% of mutation carriers would undergo major surgery for prophylactic colectomy despite having the potential to never develop CRC (18). Additionally, individuals with Lynch syndrome have 10% to 30% risk for the development of extracolonic tumors as well as rectal carcinoma, so undergoing such a procedure would not eliminate the need for continued cancer surveillance, including rectal cancer surveillance (25). Mathematical models have been developed to quantify the potential benefit of prophylactic colectomy versus endoscopic surveillance (26,27). In decision model analysis, when estimates of health-related quality of life were considered, surveillance was preferred over prophylactic colectomy (26). However, while both models show greater life expectancy for prophylactic colectomy over surveillance, the gain was very modest (26,27).

On the basis of review of the literature, a recent joint statement from the American Society for Clinical Oncology and Society of Surgical Oncology supported prophylactic colectomy in certain circumstances (28). This includes individuals for whom colonoscopic surveillance is not technically feasible or who refuse to comply with the frequent colonoscopic surveillance. In clinical practice, few individuals are recommended to undergo prophylactic colectomy, and the majority undergo regular colonoscopy for the management of CRC risk in Lynch syndrome.

In a patient with Lynch syndrome, who has been diagnosed with CRC, the increased risk for synchronous and metachronous CRC justifies aggressive surgical management. The surgical treatment options should include segmental resection, total or subtotal colectomy, and total proctocolectomy. The initial workup should include complete colonoscopy, given the proximal location of CRC in Lynch syndrome and the 7% to 18% incidence of synchronous CRC in Lynch syndrome patients. Depending on the location of multiple colon and/or rectal tumors, total colectomy may be the best surgical treatment for some patients (25). Total colectomy may also be considered at the time of diagnosis as rationale for prevention of metachronous colon cancer, which is 40% in 10 years (7,8,29). For patients with index segmental resection, diagnosis of a metachronous CRC would necessitate additional colorectal surgery, including completion of total colectomy.

Although it would not be possible to conduct a clinical trial evaluating survival outcomes between the surgical treatment options, mathematical models suggest that subtotal colectomy would provide the most benefit to younger individuals with early-stage cancers (30). However, the risk of rectal cancer still exists following such a procedure (31,32). The lifetime risk of developing cancer in the rectum following abdominal colectomy has been reported as 3% for every 3 years during the first 12 years after colectomy, or 6% to 20% (25,31). Therefore, continued surveillance of the rectal stump after abdominal colectomy is essential.

In summary, colonoscopic surveillance has been demonstrated to decrease CRC incidence and mortality in Lynch syndrome patients. Therefore aggressive surveillance should be undertaken in these patients. The surgical options in Lynch syndrome depend on the location of the tumor, age of the patient, and the clinical stage of the tumor. All of these factors must be taken into consideration and the therapy individualized at the time of surgery for CRC in Lynch syndrome patients.

Gastric Cancer

The clinical management of extracolonic cancers in Lynch syndrome is less well defined. The risk for gastric cancer varies substantially depending on the geographical region of ascertainment. Studies from the Finnish registry of HNPCC reported a cumulative risk of 13% for gastric cancer compared with data from the Dutch population where a 2.1% to 4.3% cumulative risk was reported (4,5). The rates of gastric cancer are even higher in Asian Lynch syndrome populations. After CRC, gastric cancer was the most commonly observed extracolonic cancer in the Chinese population (44%) (33). Data from the Korean population showed relative risk of 3.2-fold and 11.3-fold increase of gastric cancer as compared with the reference population and in younger age groups, respectively (34). The majority of gastric cancers are of the intestinal type, and consistent with other Lynch syndrome neoplasms, the age of onset of gastric carcinoma is earlier (34,35).

Systemic surveillance has been extensively evaluated for gastric cancer in Lynch syndrome, with varying opinions. The recommendation of the International Society of Gastrointestinal Hereditary Tumors (InSiGHT) is for surveillance of the stomach if there is a positive family history, with initiation of surveillance at 30 to 35 years and frequency of 1 to 2 years (36). The German HNPCC Consortium recently reported that only 26% of gastric cancer cases observed in their series had a family history of gastric cancer, and the majority of the cancers were diagnosed after the age of 35 years (37). Thus, they recommend upper GI endoscopy including the duodenum, beginning at age 35 years, regardless of a positive family history. However, a Finnish group concluded that surveillance gastroscopy may not be beneficial in Lynch syndrome when comparing the results of screening between mutation-positive and mutation-negative family members (38). In this study, no cases of premalignant dyspalsia or early cancer were detected. A duodenal cancer was detected and it was advanced (38).

There is no data on efficacy of gastric cancer screening; however, clinicians in both North America and Europe recommend upper GI endoscopy for individuals with a positive family history of gastric cancer or in areas with a high incidence of gastric cancer (20,39). A discussion between physician and patient should include the risks and benefits, costs, and lack of demonstrated efficacy for gastric cancer screening.

Small Bowel Cancer

The risk for small bowel cancer (SBC) is significantly increased in Lynch syndrome patients; however, it is poorly characterized. The lifetime risk for SBC ranges from 1% to 4%, which is >100 times the risk in general population (27,40). The majority occur in the proximal small bowel (41–43). Park et al. conducted a survey among members of InSiGHT, providing the largest collection of data to date on the clinical features of SBC (34). The data was ascertained from 85 individuals in 78 families documenting 90 SBC. The mean age of diagnosis was 48 years, with 10% of the affected individuals presenting before age 30 years. Small bowel carcinoma occurred slightly more often in men (60%) (34). Data from the three large SBC surveys revealed that 34% to 57% of the individuals presented with SBC as their first Lynch-associated malignancy, 22% to 33% had SBC as the only Lynch-associated cancer, and few individuals had a positive family history (6–17%) (41–43).

Evidence-based medicine is lacking for surveillance of SBC in HNPCC. Most of the small bowel tumors occur in the proximal small bowel, and Park et al. remark that approximately 43% of SBCs could have been detected by endoscopy surveillance, supporting the argument for routine surveillance of SBC (41). The German HNPCC Consortium recommends duodenoscopy or push enteroscopy starting at the age of 30 years as beneficial for early detection of proximal SBC (43). However, efficacy of such screening has not been proven for cost-effective utility, and others advise against screening based on the low

Table 1 High-Risk Colon and Extracolonic Cancer Surveillance Recommendations for Lynch Syndrome

Tumor risk	Screening modality	Frequency	Age at initiation
Colon	Colonoscopy	1–2 yr	20–25 yr
Gastric[a]	EGD	1–2 yr	30–35 yr
Urinary tract[a]	Abdominal ultrasound, urinalysis, and urine cytology	1 yr	30–35 yr

[a]If there is a positive family history.
Abbreviation: EGD, esophagogastroduodenoscopy.

relative risk and lack of sensitive imaging modalities (27). Prospective studies are needed to assess the clinical utility and risk and benefits of surveillance for SBCs in Lynch syndrome.

Urothelial Cancer

Transitional cell carcinoma of the renal pelvis and the ureter occur at increased frequency in Lynch syndrome. The relative risk of developing transitional cell cancer of the renal pelvis or ureter is estimated to be 14, with a cumulative risk of $<10\%$ (4,40,44). Vasen et al. observed that MSH2 mutation carriers have a significantly increased risk of developing urinary tract carcinoma than MLH1 mutation carriers (5). Familial clustering has been observed, and women are equally affected (44–46). Two large multigeneration pedigrees of families with documented MMR mutation have been reported with multiple individuals with upper urological malignancies and individuals with metachronous ureteral and renal pelvis tumors (44,45).

Recommendations for surveillance of upper urinary tract carcinomas have been made despite the lack of data-proving clinical efficacy. These recommendations include renal ultrasound, urinalysis, and urine cytology, with further evaluation of presence of hematuria if the family history is positive for urinary tract carcinoma (20,36,39). Physicians and patients should acknowledge the lack of clinical data in support of the benefits of urinary tract cancer screening, despite use of annual urinalysis and urine cytology as a relatively inexpensive and non-invasive screening method. A study from Denmark, presented in abstract form, revealed no benefit from performing urine cytology in the detection of urinary tract cancers in over 900 Lynch syndrome patients (47). Currently no data exists on efficacy of screening in this population, and prospective study is needed (20,44).

SUMMARY

Individuals with Lynch syndrome should consider participating in an intensive surveillance program for the increased cancer risks. Table 1 provides a summary of the current colon and extracolonic cancer surveillance recommendations.

Colonoscopic surveillance of frequency every 1 to 2 years, beginning at age 20 to 25 years, has been proven to prevent CRC-related deaths and reduce mortality (19,48). The effects of a surveillance program showed decrease in CRC mortality over time (49). Surveillance for extracolonic cancers, including gastric, small bowel, and upper urinary tract is less clear (20,39). Gastric and proximal SBC screening may be considered in the context of a positive family history, with limited data on clinical cost-effectiveness. This would include regular upper endoscopy beginning at 30 to 35 years. Urinary tract screening, by annual urinalysis with cytology, is noninvasive and relatively inexpensive, with no benefit proven in review of a large registry cohort (47). Additional studies are required to evaluate the efficacy of most extracolonic cancer screening in Lynch syndrome patients; however, large registries and collaborative efforts may be required given the rarity of Lynch syndrome.

CASE REPORTS

P.F. is a 42-year-old G1P1 without a personal history of cancer. Her family history is significant for multiple family members with uterine, colon, and renal cancer. A mutation in MSH2 was identified in a cousin with colon cancer. P.F. then underwent predictive genetic testing and tested positive for the same mutation.

P.F. began a cancer surveillance program tailored to individuals with Lynch syndrome. On her first colonoscopy, a pedunculated polyp was found in the transverse colon. Pathology revealed a tubular adenoma. The following year, two small polyps were found in the ascending and descending colon. Pathology revealed hyperplastic lymphoid nodules. She continues to undergo yearly colonoscopy screening.

LEARNING POINTS

- Colonoscopy effectively identifies precancerous polyps and has been shown to decrease mortality from colon cancers in individuals with Lynch syndrome.
- Consensus groups such as the National Comprehensive Cancer Network (NCCN) and Cancer Genetics Consortium recommend that colonoscopy should begin at age 20 to 25 years or 10 years prior to the youngest age at diagnosis in the family and repeat surveillance every 1 to 2 years (1).

REFERENCES

1. NCCN Clinical Practice Guidelines in Oncology: Genetic/Familial High-Risk Assessment: Breast and Ovarian. Rockledge, PA: National Comprehensive Cancer Network, 2006.
2. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003; 348(10):919–932.

3. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6(1):105–110.
4. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214–218.
5. Vasen HF, Stormorken A, Menko FH, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001; 19(20):4074–4080.
6. Fante R, Roncucci L, Di Gregorio C, et al. Frequency and clinical features of multiple tumors of the large bowel in the general population and in patients with hereditary colorectal carcinoma. Cancer 1996; 77(10):2013–2021.
7. Fitzgibbons RJ Jr., Lynch HT, Stanislav GV, et al. Recognition and treatment of patients with hereditary nonpolyposis colon cancer (Lynch syndromes I and II). Ann Surg 1987; 206(3):289–295.
8. Burke W, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. JAMA 1997; 277(12):997–1003.
9. Sankila R, Aaltonen LA, Jarvinen HJ, et al. Better survival rates in patients with MLH1-associated hereditary colorectal cancer. Gastroenterology 1996; 110(3):682–687.
10. Watson P, Lin KM, Rodriguez-Bigas MA, et al. Colorectal carcinoma survival among hereditary nonpolyposis colorectal carcinoma family members. Cancer 1998; 83(2):259–266.
11. Lanspa SJ, Lynch HT, Smyrk TC, et al. Colorectal adenomas in the Lynch syndromes. Results of a colonoscopy screening program. Gastroenterology 1990; 98(5 pt 1): 1117–1122.
12. Dove-Edwin I, Sasieni P, Adams J, et al. Prevention of colorectal cancer by colonoscopic surveillance in individuals with a family history of colorectal cancer: 16 year, prospective, follow-up study. BMJ 2005; 331(7524):1047.
13. Lindgren G, Liljegren A, Jaramillo E, et al. Adenoma prevalence and cancer risk in familial non-polyposis colorectal cancer. Gut 2002; 50(2):228–234.
14. De Jong AE, Morreau H, Van Puijenbroek M, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 2004; 126(1):42–48.
15. Rijcken FE, Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. Gut 2002; 50(3): 382–386.
16. Lynch HT, Smyrk T, Jass JR. Hereditary nonpolyposis colorectal cancer and colonic adenomas: aggressive adenomas? Semin Surg Oncol 1995; 11(6):406–410.
17. Vasen HF, Nagengast FM, Khan PM. Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome). Lancet 1995; 345(8958):1183–1184.
18. de Vos tot Nederveen Cappel WH, Nagengast FM, Griffioen G, et al. Surveillance for hereditary nonpolyposis colorectal cancer: a long-term study on 114 families. Dis Colon Rectum 2002; 45(12):1588–1594.
19. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000; 118(5):829–834.
20. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006; 296(12):1507–1517.

21. Renkonen-Sinisalo L, Aarnio M, Mecklin JP, et al. Surveillance improves survival of colorectal cancer in patients with hereditary nonpolyposis colorectal cancer. Cancer Detect Prev 2000; 24(2):137–142.
22. de Jong AE, Nagengast FM, Kleibeuker JH, et al. What is the appropriate screening protocol in Lynch syndrome? Fam Cancer 2006; 5(4):373–378.
23. Lecomte T, Cellier C, Meatchi T, et al. Chromoendoscopic colonoscopy for detecting preneoplastic lesions in hereditary nonpolyposis colorectal cancer syndrome. Clin Gastroenterol Hepatol 2005; 3(9):897–902.
24. Hurlstone DP, Karajeh M, Cross SS, et al. The role of high-magnification-chromoscopic colonoscopy in hereditary nonpolyposis colorectal cancer screening: a prospective "back-to-back" endoscopic study. Am J Gastroenterol 2005; 100(10): 2167–2173.
25. Scaife CL, Rodriguez-Bigas MA. Lynch syndrome: implications for the surgeon. Clin Colorectal Cancer 2003; 3(2):92–98.
26. Syngal S, Weeks JC, Schrag D, et al. Benefits of colonoscopic surveillance and prophylactic colectomy in patients with hereditary nonpolyposis colorectal cancer mutations. Ann Intern Med 1998; 129(10):787–796.
27. Vasen HF, Wijnen JT, Menko FH, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology 1996; 110(4):1020–1027.
28. Guillem JG, Wood WC, Moley JF, et al. ASCO/SSO review of current role of risk-reducing surgery in common hereditary cancer syndromes. Ann Surg Oncol 2006; 13(10):1296–1321.
29. Van Dalen R, Church J, McGannon E, et al. Patterns of surgery in patients belonging to Amsterdam-positive families. Dis Colon Rectum 2003; 46(5):617–620.
30. de Vos tot Nederveen Cappel WH, Buskens E, Van Duijvendijk P, et al. Decision analysis in the surgical treatment of colorectal cancer due to a mismatch repair gene defect. Gut 2003; 52(12):1752–1755.
31. Rodriguez-Bigas MA, Vasen HF, Pekka-Mecklin J, et al. Rectal cancer risk in hereditary nonpolyposis colorectal cancer after abdominal colectomy. International Collaborative Group on HNPCC. Ann Surg 1997; 225(2):202–207.
32. Lee JS, Petrelli NJ, Rodriguez-Bigas MA. Rectal cancer in hereditary nonpolyposis colorectal cancer. Am J Surg 2001; 181(3):207–210.
33. Cai SJ, Xu Y, Cai GX, et al. Clinical characteristics and diagnosis of patients with hereditary nonpolyposis colorectal cancer. World J Gastroenterol 2003; 9(2):284–287.
34. Park YJ, Shin KH, Park JG. Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea. Clin Cancer Res 2000; 6(8):2994–2998.
35. Aarnio M, Salovaara R, Aaltonen LA, et al. Features of gastric cancer in hereditary non-polyposis colorectal cancer syndrome. Int J Cancer 1997; 74(5):551–555.
36. Weber T. Clinical surveillance recommendations adopted for HNPCC. Lancet 1996; 348(9025):465.
37. Goecke T, Schulmann K, Engel C, et al. Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC Consortium. J Clin Oncol 2006; 24(26):4285–4292.
38. Renkonen-Sinisalo L, Sipponen P, Aarnio M, et al. No support for endoscopic surveillance for gastric cancer in hereditary non-polyposis colorectal cancer. Scand J Gastroenterol 2002; 37(5):574–577.
39. Vasen HF, Moslein G, Alonso A, et al. Guidelines for the clinical management of Lynch syndrome (HNPCC). J Med Genet 2007; 44(6):353–362.

40. Aarnio M, Mecklin JP, Aaltonen LA, et al. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Int J Cancer 1995; 64(6):430–433.
41. Park JG, Kim DW, Hong CW, et al. Germ line mutations of mismatch repair genes in hereditary nonpolyposis colorectal cancer patients with small bowel cancer: International Society for Gastrointestinal Hereditary Tumours Collaborative Study. Clin Cancer Res 2006; 12(11 pt 1):3389–3393.
42. Rodriguez-Bigas MA, Vasen HF, Lynch HT, et al. Characteristics of small bowel carcinoma in hereditary nonpolyposis colorectal carcinoma. International Collaborative Group on HNPCC. Cancer 1998; 83(2):240–244.
43. Schulmann K, Brasch FE, Kunstmann E, et al. HNPCC-associated small bowel cancer: clinical and molecular characteristics. Gastroenterology 2005; 128(3):590–599.
44. Sijmons RH, Kiemeney LA, Witjes JA, et al. Urinary tract cancer and hereditary nonpolyposis colorectal cancer: risks and screening options. J Urol 1998; 160(2): 466–470.
45. Lynch HT, Taylor RJ, Lynch JF, et al. Multiple primary cancer, including transitional cell carcinoma of the upper uroepithelial tract in a multigeneration HNPCC family: molecular genetic, diagnostic, and management implications. Am J Gastroenterol 2003; 98(3):664–670.
46. Wagner A, Hendriks Y, Meijers-Heijboer EJ, et al. Atypical HNPCC owing to MSH6 germline mutations: analysis of a large Dutch pedigree. J Med Genet 2001; 38(5): 318–322.
47. Myrhøj T, Anderson MB, Bernstein I. Screening for urinary tract cancer with urin cytology in Lynch syndrome and familial colorectal cancer. Fam Cancer 2008 Apr 4. [E-pub ahead of print]
48. Jarvinen HJ, Mecklin JP, Sistonen P. Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 1995; 108(5): 1405–1411.
49. de Jong AE, Hendriks YM, Kleibeuker JH, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006; 130(3):665–671.

14

Endometrial and Ovarian Cancer Risk-Reducing Surgery in Women with Lynch Syndrome

Kathleen M. Schmeler

Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Henry T. Lynch

Department of Preventive Medicine and Public Health, Creighton's Hereditary Cancer Institute, Creighton University School of Medicine, Omaha, Nebraska, U.S.A.

KEY POINTS

- Risk-reducing hysterectomy should be recommended for women with Lynch syndrome, aged 35 years or older, who have completed childbearing.
- If a woman with Lynch syndrome is undergoing surgery for colorectal carcinoma, consideration should be made for the concurrent performance of a hysterectomy and bilateral salpingo-oophorectomy.
- Women with Lynch syndrome undergoing prophylactic hysterectomy may have an occult malignancy; therefore, preoperative endometrial biopsy and CA-125 should be considered.
- Patients undergoing prophylactic hysterectomy for Lynch syndrome should have an intraoperative evaluation of the uterus and ovaries.

INTRODUCTION

Lynch syndrome/hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant inherited cancer susceptibility syndrome caused by a germline mutation in one of the DNA mismatch repair genes (*MSH2*, *MLH1*, *MSH6*, *PMS2*) (1–4). It is associated with early onset of cancer (age below 50 years) and the development of multiple cancer types including cancer of the colon/rectum, endometrium, ovary, small bowel, ureter, renal pelvis, as well as glioblastoma multiforma in the Turcot's syndrome variant and sebaceous neoplasms in the Muir–Torre syndrome variant. Women with Lynch syndrome have a 40% to 60% lifetime risk of developing endometrial cancer. The risk of endometrial cancer equals or exceeds the risk of developing colorectal cancer. In addition, women with Lynch syndrome have a 10% to 12% lifetime risk of developing ovarian cancer (5,6). There is a great deal of genetic and phenotypic heterogeneity in Lynch syndrome. Families with *MSH6* mutations appear to have lower expression of colorectal cancers but an excess of endometrial cancers (7,8).

There is currently limited information on the efficacy of surveillance in reducing endometrial and ovarian cancer risk in women with Lynch syndrome (9,10). The current gynecologic cancer screening guidelines include annual endometrial sampling and transvaginal ultrasonography beginning at age 30 to 35 years (11,12). These recommendations are based on expert opinion alone, as there have been no controlled studies demonstrating the efficacy of these screening modalities in young, premenopausal women with Lynch syndrome.

Another option for women with Lynch syndrome is risk-reducing gynecologic surgery. In 1997, the Cancer Genetics Studies Consortium reviewed the available evidence regarding prophylactic hysterectomy and bilateral salpingo-oophorectomy (BSO) and published a consensus statement concluding that there was insufficient evidence to recommend for or against prophylactic surgery to reduce gynecologic cancer risk in women with Lynch syndrome (11). Despite the lack of evidence, several authors suggested that prophylactic hysterectomy and BSO were reasonable options for this group of women following the completion of childbearing (13–15). Recent studies have provided evidence for the efficacy of risk-reducing gynecologic surgery in women with Lynch syndrome and will be reviewed in this chapter (16,17).

RISK-REDUCING SURGERY FOR ENDOMETRIAL AND OVARIAN CANCER

Schmeler et al. (16) performed a retrospective cohort analysis of 315 women with germline *MLH1*, *MSH2*, or *MSH6* mutations. Women who had undergone prophylactic hysterectomy with or without BSO were compared with those who had not. Sixty-one women who had undergone hysterectomy for preventive reasons or benign conditions were matched with 210 controls of similar age. In

addition, 47 women who had a BSO performed at the time of their hysterectomy were matched with 223 controls of similar age.

No endometrial or ovarian cancers developed in those who had surgery, whereas 33% of those who did not have surgery developed endometrial cancer and 5.5% developed ovarian cancer. In this cohort, 100% of potential new endometrial (Fig. 1) and ovarian cancer (Fig. 2) cases were prevented with prophylactic surgery. This reduction was significant for endometrial cancer but not for ovarian cancer; however, power was limited for the latter by the small number of ovarian cancers diagnosed in the cohort.

The median age at diagnosis was 46 years for endometrial cancer and 42 years for ovarian cancer. Six percent of endometrial cancers and 17% of ovarian cancers were diagnosed in women younger than 35 years. These findings were consistent with previous studies of women with Lynch syndrome that reported the mean age at endometrial cancer diagnosis to be 48 to 49 years (18,19) and the mean age at ovarian cancer diagnosis to be 42 years (20).

The findings by Schmeler et al. (16) support performing prophylactic hysterectomy and BSO in women with Lynch syndrome after the age of 35 or once childbearing is complete. Lindor et al. (12) recently published updated recommendations for the care of individuals with an inherited predisposition to Lynch syndrome. The authors performed a systematic review of the existing literature and provided recommendations for the clinical management of affected families based on available evidence and expert opinion. The recommendations

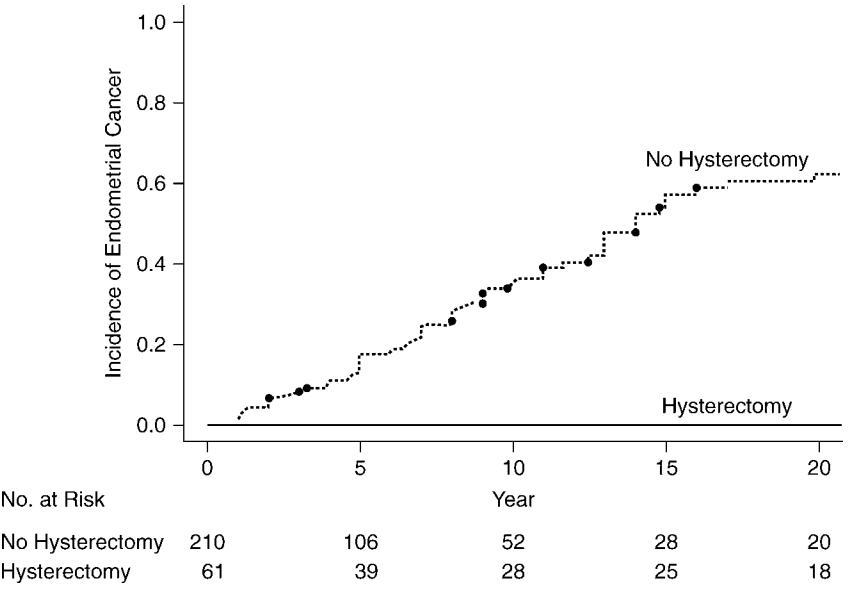

Figure 1 Cumulative incidence of endometrial cancer among women with Lynch syndrome who underwent prophylactic hysterectomy and those that did not. *Source*: From Ref. 16.

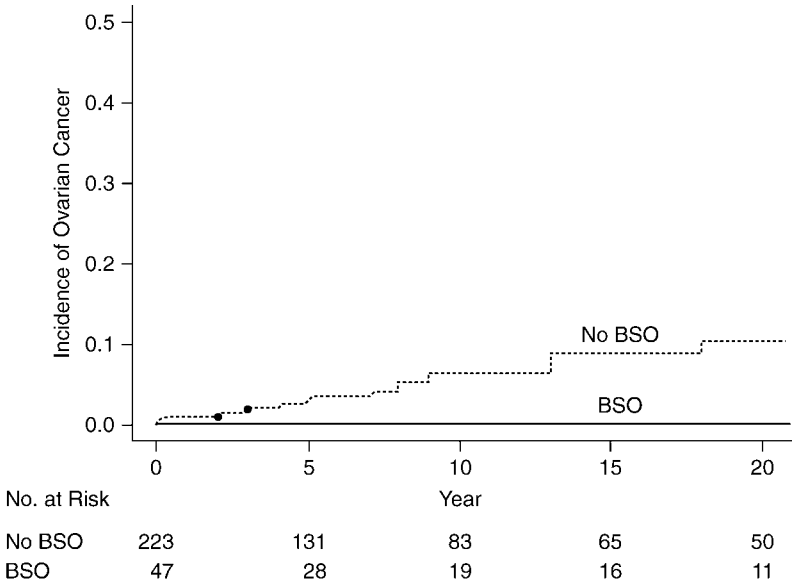

Figure 2 Cumulative incidence of ovarian cancer among women with Lynch syndrome who underwent prophylactic BSO and those that did not. *Source*: From Ref. 16.

include that prophylactic hysterectomy and BSO should be offered to women aged 35 years or older who do not want to preserve fertility. However, this should follow genetic counseling with a careful discussion of the risks, benefits, and limitations of the procedure.

A recent study by Chen et al. (17) compared management strategies for the prevention of gynecologic cancers in women with Lynch syndrome using a theoretical cohort of 10,000 women. They developed a three-arm decision analytic model comparing (*i*) annual gynecologic examination, (*ii*) annual screening with ultrasonography, endometrial biopsy, and CA-125 levels, and (*iii*) prophylactic hysterectomy with BSO. When comparing prophylactic surgery with screening, the authors reported that one would need to perform 75 surgeries to save one woman's life. For cancer prevention, however, only 28 and 6 prophylactic surgeries would need to be performed to prevent one case of ovarian and one case of endometrial cancer, respectively. These findings provide evidence that prophylactic hysterectomy and BSO decreases cancer-specific mortality and cancer treatment morbidity in women with Lynch syndrome.

Occult Cancers at the Time of Prophylactic Surgery

In the study by Schmeler et al. (16), three women (5%) who underwent prophylactic hysterectomy were found to have occult endometrial carcinomas. This finding

emphasizes the need for maintaining a high index of suspicion during prophylactic surgery in women with Lynch syndrome. Previous studies of *BRCA* mutation carriers have reported that 2% to 10% of women undergoing prophylactic BSO have occult ovarian carcinomas diagnosed at the time of surgery (21–26).

Preoperative assessment with endometrial biopsy, transvaginal ultrasound, and CA-125 level should be considered. At the time of surgery, the uterus and ovaries should be carefully assessed. The pathologist should be advised of the high risk of endometrial and ovarian cancer, and the specimens carefully examined intraoperatively with frozen sections performed if indicated. In addition, the surgeon should be prepared to perform a complete staging operation in the case of occult carcinoma.

Primary Peritoneal Cancer Following Prophylactic BSO

To date, there have been no reported cases of primary peritoneal cancer following prophylactic BSO in women with Lynch syndrome. Previous studies in women with *BRCA* mutations have reported an incidence of primary peritoneal cancer following prophylactic BSO of 0.8% to 1.0% (22,27). Longer follow-up and further study is necessary to determine the risk of primary peritoneal cancer in women with Lynch syndrome following prophylactic BSO.

Synchronous and Metachronous Colorectal and Endometrial or Ovarian Cancer

Women with Lynch syndrome are at high risk for developing synchronous or metachronous cancers (15,28,29). A woman with Lynch syndrome who survives colon cancer has a high likelihood of developing endometrial or ovarian cancer. Similarly, a woman who survives an endometrial or ovarian cancer is at high risk for developing colon cancer. Lu et al. (15) reported on 117 women with Lynch syndrome with dual primary cancers. In 16 women (14%), the colorectal and gynecologic cancers (endometrial or ovarian) were diagnosed simultaneously. Of the remaining 101 women, 52 (51%) had their endometrial or ovarian cancer diagnosed first and 49 women (49%) had their colon cancer diagnosed first.

In the study by Schmeler et al. (16), 41 women (13%) were diagnosed with synchronous (3 patients) or metachronous (38 patients) colorectal and endometrial or ovarian cancers. In 21 of these 41 women (51%), the gynecologic cancer was diagnosed following treatment for colorectal cancer. The median time between the diagnoses of colon cancer and gynecologic cancer was five years. The gynecologic malignancies in these women could have been prevented if prophylactic hysterectomy and BSO had been performed at the time of their surgery for colorectal cancer. Strong consideration should be given to concurrent prophylactic hysterectomy and BSO in women undergoing surgery for colorectal cancer.

Disadvantages of Prophylactic Surgery

The disadvantages of prophylactic hysterectomy and BSO include surgical complications and premature menopause. The most common surgical complications associated with hysterectomy and BSO are bleeding, infection, and injuries to the urinary tract and bowel. These complications have been reported to occur in 1% to 9% of women undergoing hysterectomy and BSO for benign conditions (16,30–32).

In premenopausal women, prophylactic BSO results in premature menopause. Symptoms may include hot flashes, vaginal dryness, sexual dysfunction, and sleep disturbances. In addition, these women are at increased risk for osteoporosis (33–35). Many of these conditions can be managed with hormonal or nonhormonal medications (36). Unlike *BRCA* mutation carriers, there are no specific or unique contraindications to hormone replacement therapy in women with Lynch syndrome.

An additional consideration is the finding that ovarian conservation at the time of hysterectomy for benign disease conferred a survival advantage in women younger than 65 years. Using a Markov decision analysis model, Parker et al. (35) compared women who underwent oophorectomy with women who had ovarian conservation. In women at average risk for ovarian cancer, a prophylatic oophorectomy before age 55 years was calculated to confer an excess mortality of 8.58% at 80 years of age. The implications of these findings in women at higher risk for carcinoma secondary to Lynch syndrome is unclear; therefore, this information should simply be included in the preoperative counseling of every patient.

SUMMARY

Risk-reducing hysterectomy and BSO are reasonable options for women with Lynch syndrome. Given the average age at diagnosis of gynecologic cancers, it should be offered to women aged 35 years or older who do not want to preserve fertility. Preoperative counseling should address the trade-offs between cancer risk reduction and the risks and side effects of surgery. The uncertainties regarding gynecologic cancer surveillance as a potential alternative approach must also be discussed. In addition, when a woman is undergoing surgery for colorectal cancer, consideration should be given to performing a concurrent prophylactic hysterectomy and BSO.

Further research is necessary to determine the efficacy of screening methods compared with prophylactic surgery for reduction of endometrial and ovarian cancer morbidity and mortality in women with Lynch syndrome. Additional study is also needed to assess the effect of prophylactic surgery on survival and gynecologic cancer-related deaths.

CASE REPORT

S.N. is a 48-year-old woman who underwent genetic testing at the age of 38, following her father's diagnosis of Lynch syndrome. She was found to have an *MSH2* mutation. She underwent extensive counseling regarding her endometrial and colorectal cancer risk. Initially, she chose periodic screening with colonoscopy and endometrial biopsy. However, after her first endometrial biopsy, she declined further endometrial screening secondary to discomfort. At the age 48, she chose to undergo a prophylactic laparoscopically assisted vaginal hysterectomy and bilateral salpingo-oophorectomy.

The surgery was uncomplicated, and no unusual intraoperative findings were noted. However, final pathology demonstrated mixed clear cell and endometrioid adenocarcinoma, invading 5 of 12 mm of myometrium with microscopic involvement of the endocervical glands. The bilateral adnexae were normal. Given the finding of unexpected cancer, the patient underwent a staging procedure three months after her initial surgery. This consisted of peritoneal washings, omental biopsy, as well as pelvic and para-aortic lymph node dissection. All specimens were free of tumor, resulting in a diagnosis of stage IIa endometrial cancer.

REFERENCES

1. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993; 75(6):1215–1225.
2. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993; 75(5): 1027–1038.
3. Papadopoulos N, Nicolaides NC, Wei YF, et al. Mutation of a mutL homolog in hereditary colon cancer. Science 1994; 263(5153):1625–1629.
4. Kolodner RD, Tytell JD, Schmeits JL, et al. Germ-line msh6 mutations in colorectal cancer families. Cancer Res 1999; 59(20):5068–5074.
5. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214–218.
6. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6(1): 105–110.
7. Plaschke J, Engel C, Krüger S, et al. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations: the German Hereditary Nonpolyposis Colorectal Cancer Consortium. J Clin Oncol 2004; 22(22):4486–4494.
8. Lynch HT, Boland CR, Gong G, et al. Phenotypic and genotypic heterogeneity in the Lynch syndrome: diagnostic, surveillance and management implications. Eur J Hum Genet 2006; 14(4):390–402.

9. Dove-Edwin I, Boks D, Goff S, et al. The outcome of endometrial carcinoma surveillance by ultrasound scan in women at risk of hereditary nonpolyposis colorectal carcinoma and familial colorectal carcinoma. Cancer 2002; 94(6):1708–1712.
10. Rijcken FE, Mourits MJ, Kleibeuker JH, et al. Gynecologic screening in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2003; 91(1):74–80.
11. Burke W, Petersen G, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. JAMA 1997; 277(11):915–919.
12. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006; 296(12):1507–1517.
13. Bertagnolli MM. Surgical prevention of cancer. J Clin Oncol 2005; 23(2):324–332.
14. Lynch HT, Watson P, Shaw TG, et al. Clinical impact of molecular genetic diagnosis, genetic counseling, and management of hereditary cancer. Part II: Hereditary nonpolyposis colorectal carcinoma as a model. Cancer 1999; 86(11 suppl):2457–2463.
15. Lu KH, Dinh M, Kolhmann W, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. Obstet Gynecol 2005; 105(3):569–574.
16. Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006; 354(3):261–269.
17. Chen LM, Yang KY, Little SE, et al. Gynecologic cancer prevention in lynch syndrome/hereditary nonpolyposis colorectal cancer families. Obstet Gynecol 2007; 110(1):18–25.
18. Vasen HF, Stormorken A, Menko FH, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001; 19(20):4074–4080.
19. Boks DE, Trujillo AP, Voogd AC, et al. Survival analysis of endometrial carcinoma associated with hereditary nonpolyposis colorectal cancer. Int J Cancer 2002; 102(2): 198–200.
20. Watson P, Bützow R, Lynch HT, et al. The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2001; 82(2):223–228.
21. Rebbeck TR, Prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers. Eur J Cancer 2002; 38(suppl 6):S15–S17.
22. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002; 346(21): 1609–1615.
23. Lu KH, Garber JE, Cramer DW, et al. Occult ovarian tumors in women with BRCA1 or BRCA2 mutations undergoing prophylactic oophorectomy. J Clin Oncol 2000; 18(14):2728–2732.
24. Colgan TJ, Murphy J, Cole DE, et al. Occult carcinoma in prophylactic oophorectomy specimens: prevalence and association with BRCA germline mutation status. Am J Surg Pathol 2001; 25(10):1283–1289.
25. Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005; 23(1):127–132.
26. Schmeler KM, Sun CC, Bodurka DC, et al. Prophylactic bilateral salpingo-oophorectomy compared with surveillance in women with BRCA mutations. Obstet Gynecol 2006; 108(3 pt 1):515–520.

27. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002; 346(21):1616–1622.
28. Lynch HT, Harris RE, Lynch PM, et al. Role of heredity in multiple primary cancer. Cancer 1977; 40(4 suppl):1849–1854.
29. Mecklin JP, Jarvinen HJ. Clinical features of colorectal carcinoma in cancer family syndrome. Dis Colon Rectum 1986; 29(3):160–164.
30. Kovac SR. Hysterectomy outcomes in patients with similar indications. Obstet Gynecol 2000; 95(6 pt 1):787–793.
31. Weber AM, Lee JC. Use of alternative techniques of hysterectomy in Ohio, 1988–1994. N Engl J Med 1996; 335(7):483–489.
32. Goodno JA Jr., Powers TW, Harris VD. Ureteral injury in gynecologic surgery: a ten-year review in a community hospital. Am J Obstet Gynecol 1995; 172(6): 1817–1820; discussion 1820–1822.
33. Prior JC, Vigna YM, Wark JD, et al. Premenopausal ovariectomy-related bone loss: a randomized, double-blind, one-year trial of conjugated estrogen or medroxyprogesterone acetate. J Bone Miner Res 1997; 12(11):1851–1863.
34. Graziottin A, Basson R. Sexual dysfunction in women with premature menopause. Menopause 2004; 11(6 pt 2):766–777.
35. Parker WH, Broder MS, Liu Z, et al. Ovarian conservation at the time of hysterectomy for benign disease. Obstet Gynecol 2005; 106(2):219–226.
36. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004; 291(14):1701–1712.

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Other Syndromes

Christine S. Walsh

Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, California, U.S.A.

Louise C. Strong

Department of Cancer Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- Peutz–Jeghers syndrome, Cowden syndrome, and Li–Fraumeni syndrome are other genetic syndromes with associated gynecologic malignancies.
- Peutz–Jeghers syndrome is characterized by pigmented lip lesions and multiple gastrointestinal polyps. There is an associated increased risk for ovarian sex cord–stromal tumors with annular tubules and adenoma malignum of the cervix. Germ line mutations in STK11/LKB1 have been identified in the majority of individuals with Peutz–Jeghers syndrome.
- Cowden syndrome, caused by germline PTEN mutations, is associated with mucocutaneous lesions, hamartomatous gastrointestinal polyps, macrocephaly, thyroid disease, benign breast disease, breast cancer, and endometrial cancer.
- Li–Fraumeni syndrome, caused by germline mutation in the p53 tumor suppressor gene, is characterized by young-onset breast cancer, soft tissue and bone sarcomas, adrenal cortical tumors, brain tumors, and multiple primary cancers in an individual. Ovarian tumors have been reported in women with Li–Fraumeni syndrome.

INTRODUCTION

Peutz–Jeghers syndrome (PJS) and Cowden syndrome (CS) are familial lentiginoses syndromes (1) that deserve mention due to their gynecologic manifestations. Both syndromes are characterized by distinctive dermatologic manifestations, gastrointestinal polyposis, and an elevated predisposition toward various benign and malignant growths (2). (Table 1) Li–Fraumeni syndrome (LFS) is characterized by multiple diverse neoplasms, including soft tissue sarcomas and young-onset breast cancer (3,4). These hereditary cancer syndromes result from the autosomal dominant inheritance of a mutated tumor suppressor gene in the germline DNA, causing the mutant allele to be present in every cell of the body. Cancer arises in different tissues through a series of genetic events, including the inactivation of the second, normal allele. Early recognition of these genetic syndromes provides the opportunity for specialized care and an aggressive approach to cancer screening.

PEUTZ–JEGHERS SYNDROME

Background and History

PJS is characterized by pigmented lesions on the lips and buccal mucosa and multiple gastrointestinal polyps. This association was first recognized in a Dutch family by Peutz in 1921 and definitively reported in descriptive detail by Jeghers et al. in 1949 (5,6). The eponym "Peutz–Jeghers syndrome" was first used in a

Table 1 Clinical Characteristics of Peutz–Jeghers and Cowden Syndromes

	Peutz–Jeghers syndrome	Cowden syndrome
Susceptibility gene	STK11/LKB1 (19p13.3) in 60%	PTEN (10q23.31) in 80%
Inheritance pattern	Autosomal dominant	Autosomal dominant
Dermatologic manifestations	Pigmentation of lips and buccal mucosa, which develops during childhood and fades with age	Hamartoma • Facial trichilemmomas • Acral keratoses • Papillomatous papules
Gastrointestinal polyposis	Hamartomatous polyposis	Hamartomatous polyposis
Genital tract tumors	SCTAT, cervical adenoma malignum	Endometrial cancer, uterine leiomyomata
Other malignancies	Gastrointestinal (colon, stomach, small intestine), pancreas, breast, lung, thyroid, lymphatic	Primarily breast, thyroid, RCC
Clinical morbidity	Gastrointestinal bleeding, intussusception, cancer	Cancer

Abbreviations: RCC, renal cell carcinoma; SCTAT, sex cord tumors with annular tubules.

report published in 1954 (7). Numerous reports have since emerged, detailing the various clinical manifestations.

Genetics

The PJS susceptibility gene was discovered in 1998. The susceptibility locus was mapped to the distal part of chromosome 19p through comparative genomic hybridization, targeted linkage analysis, and loss of heterozygosity analysis (8). Mutations in a novel human gene in this locus, encoding the serine/threonine kinase *STK11*, were found to segregate with the syndrome in three generations of an affected PJS family (9). The function of *STK11*, also known as *LKB1*, has not yet been fully elucidated, but it is hypothesized to be a tumor suppressor gene that controls cell polarity (10). A haplotype analysis of cancer tissue arising in this syndrome has found loss of heterozygosity with retention of the mutated germline STK11 allele, providing further support of the *STK11*'s role as a tumor suppressor gene (11).

Mutations in *STK11* are found in about 60% of cases of PJS, suggesting genetic heterogeneity and the presence of other, yet undiscovered, genes that cause this disorder (12). Germline mutations in *STK11* have been found to be dispersed throughout the gene, with most causing null alleles (13). It has been suggested that genetic mutation screening strategies that combine DNA and RNA approaches may be advantageous (13).

PJS–Associated Tumors

Skin

Characteristic skin findings exist in more than 95% of patients with PJS. Flat, pigmented macules that look like freckles occur in distinctive areas, such as on the lips, inside the mouth, and on the dorsal and volar aspects of hands and feet. These small spots are 1 to 5 mm in size, bluish-gray to brown in color, and exist most commonly on the lips and perioral region (94%), hands (74%), buccal mucosa (66%), and feet (62%) (14). They have also been described around the nostrils, perianal area, and occasionally on the rectal mucosa (5). Biopsy of these areas reveals the presence of increased melanocytes at the epidermal-dermal junction and increased melanin in the basal cells (6). Malignant degeneration is not a typical feature.

This distinguishing pigmentation appears during the first two years of life and progressively becomes more prominent over the first decade of life, with the macules increasing in number and size. After puberty, the pigmentation characteristically fades (15), which can lead to difficulty in making the diagnosis at older ages. However, the macules on the buccal mucosa characteristically persist (5,6,16), making this an important place to examine when considering PJS in the differential diagnosis.

Gastrointestinal Tract

Gastrointestinal polyps are the source of major clinical morbidity in PJS. They begin to grow in the first decade of life (14) and are found throughout the gastrointestinal tract, but most commonly in the small intestine, particularly the jejunum (14,16,17). They are classified as hamartomas, which are disorganized proliferations of the mature cell types that are normally found within the organ (17).

Symptoms include acute and chronic gastrointestinal bleeding and abdominal pain due to recurrent intussusceptions (18). The average age of diagnosis based on gastrointestinal symptoms is around 22 to 26 years (14,17), with patients presenting with intestinal obstruction (42%), abdominal pain (23%), rectal bleeding (13%), or rectal extrusion of a polyp (7%) (14). Intussusception occurs in about 47% of patients, and about half of the patients will require operation (14). Patients have been reported to undergo multiple surgeries and can be left with the complications of short bowel syndrome (19).

Genital Tract

PJS is associated with rare tumors of the ovary, testis, and cervix (18). Females with the syndrome are at risk for developing ovarian sex cord–stromal tumors with annular tubules (SCTAT), which have a histology intermediate between granulosa cell and Sertoli cell tumors (20). Males with the syndrome can develop testicular tumors with a histology intermediate between sex cord tumor with annular tubules and large cell calcifying Sertoli cell tumors (21). In both genders, these stromal tumors can be hormonally active, causing signs of hyperestrogenism. Boys can develop gynecomastia, girls can present with isosexual precocious puberty, and reproductive-age or postmenopausal women can demonstrate signs of menstrual irregularity or postmenopausal bleeding (21–27).

The ovarian SCTAT is a rare tumor that is distinctly associated with PJS (28). These lesions are typically benign, multifocal, bilateral, and small, potentially microscopic in size (22). They are usually found incidentally and rarely undergo malignant conversion (29). In contrast, SCTAT tumors that occur sporadically are usually unilateral, large, and more frequently become malignant (22). Other ovarian pathology reported in PJS includes granulosa cell tumor (30,31), Sertoli–Leydig cell tumor (32), borderline tumors (33), and mucinous tumors (34,35).

A rare cancer of the cervix has also been found to occur in patients with PJS. Adenoma malignum, or minimal deviation adenocarcinoma, is notoriously difficult to diagnose (36,37). This mucinous neoplasm demonstrates a deceptive well-differentiated histology, but is associated with an aggressive clinical course (37,38). Several reports have described the detection of this malignancy in PJS with various imaging modalities, such as ultrasound, CT, and MRI (39,40), with the tumor appearing as a multicystic, hyperechoic, endocervical mass.

Malignancies

Patients with PJS have an elevated lifetime risk of 22% to 38% for developing malignancies in various sites (41,42), with one meta-analysis suggesting a cumulative risk for all cancers of 93% by age 64 (43). Compared with the general population, the relative risk for a PJS patient developing cancer was estimated to be elevated ninefold (RR 9; 95% CI, 4.2, 17.3), with the risk being elevated 13-fold for the development of a gastrointestinal malignancy (RR 13; 95% CI, 2.7, 38.1) (42). In a more recent series, the risk of developing cancer was higher in PJS females (RR 18.5; 95% CI, 8.5, 35.2), than in males (RR 6.2; 95% CI, 2.5, 12.8), and the risk was particularly elevated for the development of breast and gynecologic malignancies (RR 20.3; 95% CI, 7.4, 44.2) (41). The chance of a PJS patient dying from cancer by the age of 57 was estimated at 48% (42). In a follow-up report of the original Dutch family reported by Peutz, only 17 of 22 affected family members survived into adulthood. The average age of death was 38 for affected family members, compared with 69 in unaffected family members (44).

Gastrointestinal malignancies have been reported in the colon, duodenum, stomach, esophagus, ileum, jejunum, and stomach (45–47). While hamartomas are benign lesions, there is evidence to suggest that they can undergo adenomatous and neoplastic changes (11,19,47,48), progressing through a hamartoma-adenoma-carcinoma sequence.

Malignancies have also been reported in the pancreas, lung, breast, endometrium, kidney, thyroid, gallbladder, bile duct, blood stem cells (6,41,43,46,49,50), and female reproductive tract (detailed above). One study estimated the risk of developing breast cancer to be 32% by the age of 60 (51).

Clinical Recommendations

The clinical management of PJS should address the morbidity caused by the multiple gastrointestinal hamartomas as well as the elevated risk of various malignancies. Patients suspected to have PJS should be offered genetic counseling and testing (52). The early introduction of appropriate screening strategies is predicted to improve prognosis (53). Various management algorithms have been proposed, but none have been validated, and the optimal treatment strategies remain unknown.

In the first two decades of life, the gastrointestinal polyps are the major source of clinical morbidity. Several groups have recommended beginning upper endoscopy and small bowel series at the age of eight and to continue every two to three years if polyps are encountered (54). Endoscopic or surgical removal of the polyps can be performed and may lead to reduced risk of acute or chronic bleeding, intussusception, or need for emergent surgery for bowel obstruction (55,56). It has been suggested that the rate of new polyp formation may slow with age (57).

Cancer surveillance can be performed keeping in mind the reported sites of potential malignancy, including the breast, colon, pancreas, stomach, small bowel, ovaries, uterus, cervix, and testicles. One group has suggested a screening strategy that introduces colonoscopy, upper endoscopy, and small bowel series every 2 to 3 years beginning at age 18 and endoscopic ultrasound of the pancreas ±CT scan and CA19-9 every 1 to 2 years beginning at the age of 25. Males are also at risk for testicular tumors and should have testicular ultrasound every two years starting at birth. Females are at risk for ovarian, cervical, and breast cancers (58) and should add monthly breast self-examination at age 18, yearly pelvic examination and pap smear at age 21, clinical breast examination every six months at age 25, and yearly breast mammogram or MRI, transvaginal ultrasound, and CA125 blood test at age 25 (54). These remain expert recommendations that have not yet been validated as effective screening strategies.

COWDEN SYNDROME

Background and History

CS is an autosomal dominant disorder that was first described in 1963 in the family of Rachel Cowden (59). Also known as multiple hamartoma syndrome, the disease demonstrates high penetrance in both sexes (60) and is characterized by the development of multiple hamartomas, distinctive dermatopathologic manifestations, and a predisposition toward various malignancies (2,61). The incidence was originally estimated to be about one per million (62), but it is now believed to be much more common, as the variable expression of the disease can lead to subtle clinical signs and underdiagnosis. The incidence is now believed to be closer to 1 per 200,000 (63), but this may also prove to be an underestimate.

The most commonly reported features include mucocutaneous lesions, macrocephaly, thyroid abnormalities, fibrocystic breast disease, breast and thyroid cancer, and early-onset uterine leiomyoma (60,61,63–65). More recently, endometrial cancer has been recognized to be a feature of this syndrome (66,67). More than 90% of patients with CS are believed to manifest clinical signs by the age of 20 and 99% will develop mucocutaneous changes by the age of 29 (60,68).

Genetics

In a search for the CS susceptibility gene, an autosomal genome scan using DNA markers in 12 affected families found a maximum lod score at a marker on 10q22-q23 (68). The following year, a novel tumor suppressor gene, PTEN (protein tyrosine phosphatase with homology to tensin), was found in this location and demonstrated to be mutated in sporadic brain, breast, and prostate cancer (69,70). The PTEN gene was demonstrated to be the CS susceptibility gene with germline mutations segregating with disease in four of five CS families (71). Subsequent studies have confirmed germline mutations in about 80% of probands with CS with mutations scattered throughout the gene (62,72).

Germline mutations in PTEN have also been found in 60% of patients with the related Bannayan-Riley-Ruvalcaba syndrome (BRRS), which is characterized by lipomatosis, macrocephaly, and a speckled penis (73,74).

PTEN encodes for a ubiquitously expressed, multifunctional phosphatase that removes phosphate groups from tyrosine and serine residues and from lipids (74). One of its main functions is to inhibit the PI3'-kinase/AKT cell survival pathway (75,76). Loss of PTEN inhibition allows for unchecked AKT-mediated cell survival, cell motility, and resistance to apoptotic signals (74,77,78).

The importance of PTEN's tumor suppressor activity is highlighted by the finding of somatic PTEN mutations in multiple sporadic tumors, including cancers of the thyroid, endometrium, prostate, and brain (71,79–81). A heterozygous PTEN mouse model provides additional support for the role of PTEN in tumor suppression. The PTEN+/– mice experience a high rate of breast and endometrial cancers as well as loss of heterozygosity at the PTEN locus in the tumor tissues (82).

Diagnostic Criteria

In 1995, investigators from North America and Europe who were interested in localizing the CS susceptibility gene formed the International Cowden Syndrome Consortium. They generated consensus diagnostic criteria based on expert opinion that have subsequently been tested and found to be robust (66). On the basis of emerging information, the criteria have periodically been revised, and they now include endometrial cancer and RCC as diagnostic entities. The U.S.-based National Comprehensive Cancer Network (NCCN) has adopted these criteria (83). The most recent guidelines are listed in Table 2 and are available at the NCCN website (www.nccn.org) in the NCCN Clinical Practice Guideline in Oncology—Genetic/Familial High-Risk Assessment: Breast and Ovarian V.1.2007 algorithm (84).

CS–Associated Tumors

Skin

CS patients will develop characteristic mucocutaneous changes in the second and third decades of life (85). Hamartomatous lesions, such as facial trichilemmomas, acral keratoses, and papillomatous papules, are defined to be pathognomonic diagnostic criteria. Trichilemmomas are benign tumors that arise from the outer root sheath epithelium of hair follicles and are characteristically found on the face in CS (60,86). Acral keratoses are smooth or verrucous growths found on the hands and feet (65,85). Papillomatous papules are small, solid, epithelial elevations that can occur on the skin or mucosal membranes. A coalescence of this process in the mouth can lead to a cobblestone appearance of the tongue or a furrowing known as scrotal tongue (64). These mucocutaneous changes generally precede the development of internal malignancies (85).

Table 2 NCCN Diagnostic Criteria for CS

Pathognomonic criteria	Adult LDD
	Mucocutaneous lesions
	• Trichilemmomas, facial
	• Acral keratoses
	• Papillomatous papules
Major criteria	Breast cancer
	Thyroid cancer, especially follicular thyroid carcinoma
	Macrocephaly (megalocephaly) (i.e., $\geq$97th percentile)
	Endometrial cancer
Minor criteria	Other thyroid lesions (e.g., adenoma, multinodular goiter)
	Mental retardation (i.e., IQ $\leq$ 75)
	Gastrointestinal hamartomas
	Fibrocystic disease of the breast
	Lipomas
	Fibromas
	GU tumors (especially renal cell carcinoma)
	GU structural manifestations
	Uterine fibroids
Operational diagnosis in an individual	Any single pathognomonic criterion, but mucocutaneous lesions if meeting specific criteria two or more major criteria one major and $\geq$3 minor criteria $\geq$4 minor criteria
Operational diagnosis in a family where one relative is diagnostic for CS	Individuals must have one or more of the following:
	A pathognomonic criterion
	Any one major criteria with or without minor criteria
	Two minor criteria
	History of BRRS

Abbreviations: LDD, Lhermitte–Duclos disease; GU, genitourinary; IQ, intelligence quotient; CS, Cowden syndrome; BRRS, Bannayan-Riley-Ruvalcaba syndrome.
Source: From Ref. 84.

The presence of specific numbers or combinations of these mucocutaneous lesions is sufficient to make the diagnosis of CS, even in the absence of other clinical findings (84). Other dermatologic findings, which are included as minor diagnostic criteria, include lipomas or fibromas (84).

Gastrointestinal Tract

Hamartomatous polyps of the intestine affect approximately 40% to 60% of patients with CS (60,87). Polyps have been described in the esophagus, stomach, small and large intestine, colon, and anus. The malignant potential of these polyps is low, and only isolated cases of colon cancer have been reported (88).

The presence of gastrointestinal hamartomas is a minor diagnostic criterion (84).

Central Nervous System

Macrocephaly is the most common extracutaneous manifestation of CS, occurring in 80% of patients (60). Progressive macrocephaly and mild to moderate mental retardation may be important diagnostic signs in young children, as they are present before the development of mucocutaneous changes (61). Lhermitte–Duclos disease (LDD), which is characterized by macrocephaly, ataxia, and cerebellar hamartomas (dysplastic cerebellar gangliocytomatosis), cosegregates with a subset of CS families (71).

The presence of LDD alone is sufficient to make the diagnosis of CS. Macrocephaly ($\geq$ 97th percentile) is a major diagnostic criteria, and mental retardation (i.e., IQ $\leq$ 75) is a minor diagnostic criteria (84).

Neck

Thyroid disease occurs in 62% of CS patients, encompassing a spectrum of benign (goiter, benign adenomas, thyroglossal duct cysts) and malignant (particularly follicular thyroid carcinoma) pathology (59,60). Multiple papillomas on lingual tonsils, epiglottis, and surrounding structures have also been observed in a patient with CS. These polyps were the source of airway obstruction during induction of general anesthesia for breast cancer surgery (89).

Thyroid cancer is a major diagnostic criterion, and other thyroid lesions (e.g., adenoma, multinodular goiter) are minor diagnostic criteria for CS (84).

Breasts

Benign changes, such as fibrocystic breast disease, occur in 75% of female CS patients. The spectrum of benign breast disease includes fibrocystic changes, fibroadenomas, benign ductal hyperplasia, intraductal papillomatosis, adenosis, lobular atrophy, breast hamartomas, and densely fibrotic hyalinized nodules (90).

Breast cancer is the most common malignancy to occur in CS, affecting over 20% of patients, with a mean age around 40 to 45 years (range 14–65 years) (60,64). The histopathology is most commonly ductal, but also includes lobular carcinomas (90). Cases of male breast cancer have also been reported (91).

Breast cancer is a major diagnostic criterion, and fibrocystic disease of the breast is a minor diagnostic criterion for CS (84).

Genitourinary Tract

Patients with CS can develop a spectrum of benign and malignant changes in the genitourinary tract. Endometrial cancer is now recognized to be within the spectrum of pathology in this disorder, and it has been suggested that the presence of endometrial cancer in a family might increase the likelihood of discovering a causative germline mutation (66,67). Other pathology described in the female genital tract include multiple, early-onset uterine leiomyomata (60).

In the urinary tract, structural malformations, benign ureteral polyps, and tumors such as renal cell carcinomas (RCCs) have been described (63).

Endometrial cancer is a major diagnostic criterion. Genitourinary tumors (RCCs), genitourinary structural manifestations, and uterine fibroids are all minor diagnostic criteria (84).

Malignancies

CS is a hereditary cancer syndrome. Breast cancer, thyroid cancer (especially follicular carcinoma), and endometrial cancer are all among the major diagnostic criteria and genitourinary tumors (especially RCCs) are among the minor diagnostic criteria (84). All of these malignancies occur at a higher than expected frequency among affected individuals. Other tumors that have been observed in patients with CS include cancers of the liver, pancreas, colon, ovary, bladder, brain, lung, bone, and skin (62,67,85,92–94).

Clinical Recommendations

Individuals who meet the diagnostic criteria for CS (Table 2) should be offered genetic counseling. Genetic testing for germline PTEN mutations has recently become available. Recognition and diagnosis of this syndrome provides the opportunity for heightened cancer surveillance. The NCCN guidelines for CS management are listed in Table 3 (84). Endometrial cancer screening via endometrial biopsy is recommended, but there is no data to support its efficacy.

LI–FRAUMENI SYNDROME

Background and History

LFS was originally described in 1969 as a familial clustering of soft tissue sarcomas, young-onset breast cancers, and other diverse neoplasms in families, with etiology unknown (3,4). Follow-up of these and additional families, plus findings from other investigators, confirmed this familial cancer aggregation as a distinct syndrome with elevated cancer risk (95–97). Additional studies provided statistical evidence for a likely genetic causation (98). In 1988, Li et al. summarized the longitudinal follow-up data on 24 families and established clinical criteria for the syndrome (Table 4) (99). These criteria included a proband with a sarcoma occurring before 45 years of age, with a first-degree relative with any cancer before 45 years of age and an additional first- or second-degree relative in the same lineage with any cancer before 45 years of age or a sarcoma at any age. Further, they defined the component tumors of the syndrome that were in significant excess in individuals under the age of 45 in their kindreds. These included bone and soft tissue sarcomas, breast cancer, brain tumors, leukemias, and adrenocortical carcinomas. Their overall summary showed a high frequency of multiple primary tumors occurring in the

Table 3 NCCN Guidelines for CS Management

Recommended screening tests	When to begin screening
Men and women	
Annual comprehensive physical examination, attention to breast, and thyroid examination	Age 18 yr or 5 yr earlier than earliest cancer diagnosed in family
Annual urinalysis	Consider annual urine cytology and renal ultrasound if family history of renal cancer
Baseline thyroid ultrasound, consider repeating annually	Age 18 yr
Annual dermatologic examination	
Education regarding signs and symptoms of cancer	
Advise about possible inherited cancer risk to relatives and consideration of genetic consult and/or testing	
Women	
Breast self-examination, monthly breast self-examination	Age 18 yr
Semiannual clinical breast examination	Age 25 yr or 5–10 yr earlier than earliest breast cancer in family
Annual mammography, breast MRI	Age 30–35 yr or 5–10 yr earlier than earliest breast cancer in family
Blind endometrial biopsies	Age 35–40 yr or 5 yr earlier than earliest endometrial cancer in family
Annual endometrial ultrasound	Postmenopausal
Discuss option for risk-reducing mastectomy	

Abbreviations: NCCN, National Comprehensive Cancer Network; CS, Cowden syndrome; MRI, magnetic resonance imaging.
Source: From Ref. 84.

syndrome, with sarcomas and breast cancers the most common tumors, as initial and as subsequent tumors. The syndrome differed significantly from most familial or hereditary cancer syndromes described at that time by the absence of any stigmatizing anomalies, the diversity of observed cancer types, and the high frequency of multiple primary tumors.

Genetics

Using a candidate gene approach, in 1990, deleterious germline mutations in the tumor suppressor gene p53 were identified in several LFS families (100,101), and mutations were shown to segregate with the cancer phenotype (102). Given that

Table 4 Diagnostic criteria for Li-Fraumeni syndrome and Li-Fraumeni-like syndrome

Li-Fraumeni syndrome (99) 1988	Birch Criteria (Li-Fraumeni like syndrome) (104) 1994	Chompret Criteria (105, 106) 2000
Proband <45 years with a sarcoma Plus First degree relative <45 years with any cancer Plus Additional first-or second degree relative in the same lineage aged <45 years with any cancer or sarcoma at any age	Proband with any childhood tumor, or sarcoma, brain tumor, or adrenocortical tumor <45 years Plus First-or second degree relative in the same lineage with typical LFS tumor at any age or any cancer <45 years Plus Another first-or second degree relative in the same lineage with any cancer <60 years	Proband with sarcoma, brain tumor, breast cancer or adrenocortical carcinoma <36 years Plus At least one first-or second degree relative affected by sarcoma, brain tumor, breast cancer or adrenocortical carcinoma (other than breast cancer if the proband is affected by breast cancer) <46 years or multiple primary tumors OR Proband with multiple primary tumors two of which are sarcoma, brain tumor, breast cancer or adrenocortical carcinoma and the first of which occurred before <36 years OR Proband with adrenocortical carcinoma whatever the age of onset and family history

somatic mutations in p53 were known to be associated with a variety of human tumors, it seemed reasonable that germline p53 mutations might underlie this hereditary syndrome of diverse neoplasms (103). Although not all families that meet the classic LFS criteria have p53 germline mutations, and not all families with p53 germline mutations have classic LFS criteria, for the purpose of this chapter, LFS and germline mutation in p53 have been considered equivalent.

LFS is regularly cited as rare, accounting for only a small fraction of most cancer types, excluding those rare sites mentioned above. Until recently, there have been little data on overall population incidence. However, from their U.K. population-based study of breast cancer occurring before age 31 in which all

patients were genotyped for BRCA1/2 and p53, Lalloo et al. estimated that the birth prevalence of p53 germline mutations may be 1 in 5000, a higher frequency than this might have been expected, suggesting that many cases are likely not detected clinically (104,105).

The penetrance for cancer in germline p53 mutation carriers has been estimated in several populations. Chompret et al. and Hwang et al. observed an earlier age of cancer onset and higher cumulative cancer risk in female (90–100%) as compared with male (70%) mutation carriers (106,107). Breast cancer accounted for the majority of the female cancers, with the highest risk occurring between the ages of 20 and 45 years.

LFS is also associated with a high risk of multiple primary tumors. Hisada et al. reported on follow-up of the original 24 LFS kindreds (not all were available for p53 mutation testing) (108). In that series, the cumulative risk of a second cancer at 30 years after first cancer diagnosis was 57% (±10%), with the highest risks occurring in those with the youngest age at first cancer. The majority of subsequent cancers were of the previously defined component tumor spectrum.

LFS-Associated Tumors

Component Tumors

With the advent of genetic testing, it was important to clarify further the spectrum of tumor types and cancer risks associated with carrying a p53 germline mutation, as well as the criteria for LFS and for genetic testing. To determine whether there might be a broader spectrum of tumors or range of cancer risks not detected by the classic criteria, less rigorous criteria were developed by Birch et al. and more recently by Chompret et al. (Table 4) (106,109,110). Importantly, the criteria of Chompret allowed for the potential of de novo mutations, relying on the proband's affection status, regardless of the family history of cancer (106,110). These criteria were predicted to identify a mutation in about 20% of patients meeting the criteria.

As there has been more germline p53 testing, the cancer risk has become more clear. Many studies have examined the frequency of p53 germline mutations in specific cancer types. These studies have identified rare tumors with an extremely high-risk probability of being associated with a p53 germline mutation, such as childhood adrenal cortical carcinomas (50–80%), choroid plexus tumors, and embryonal rhabdomyosarcoma before the age of three years (111–114). The high frequency of young-onset breast cancer in p53 mutation carriers led to many studies to determine the overall contribution of p53 to young onset or familial breast cancer. However, the frequency of mutations in familial breast cancer, bilateral breast cancer, breast cancer with multiple primary tumors, and breast cancer below the age of 40 years is low (115). Even selection

for patients with breast cancer occurring before 31 years of age and not attributable to BRCA1/2 mutations yields only a 4% to 5% incidence of a p53 germline mutation (104,105). Clearly, none of the guidelines offer both high predictive value and sensitivity for detection of LFS.

LFS and Gynecologic Cancer

Gynecologic cancers have never been cited as component tumors in the LFS criteria. However, p53 mutations are commonly observed as somatic mutations in ovarian and other gynecologic cancers; thus, it is not surprising that gynecologic tumors are occasionally reported in LFS, both in systematic series and case reports (107,108,116–125). In a survey of p53 somatic mutations in ovarian cancers, Kupryjanczyk et al. noted that 2 of 20 patients whose tumor had a p53 mutation also had a p53 germline mutation (116). However, in most systematic studies of LFS, no excess of gynecologic tumors has been noted (100,120–122). To date, no specific clusters of gynecologic cancers have been observed in LFS kindreds, and no specific genotype or phenotype correlation for gynecologic tumors noted. Nevertheless, the most frequently reported gynecologic tumor is ovarian cancer that occurs at a strikingly earlier age of onset in LFS (39.5 years) than in the general population (64.3 years) (122).

Given the above data, surprisingly Hwang et al. noted a highly significant, four ovarian cancers in 29 female p53 mutation carriers in families ascertained through a systematic series of childhood sarcoma patients (107). The familial cohort was not selected for familial cancer or multiple primary tumors and had been followed longitudinally for many years. Only two of the ovarian tumors had occurred when the families were ascertained, the other two developed during follow-up. In an expansion of that series in which ascertainment was not related to gynecologic tumors (Strong et al., unpublished data), we have now observed 7 ovarian tumors in 93 female p53 mutation carriers, such that 7.5% of female mutation carriers have developed a gynecologic tumor (1 in 13). This is a fivefold higher risk than the lifetime risk of 1.4% reported for women born today, or 1 in 71 (126). The gynecologic tumor types observed were variable, including an ovarian leiomyosarcoma, ovarian choriocarcinoma, both high-grade (2) and low malignant potential (1) papillary serous ovarian carcinomas, and a juvenile granulosa cell ovarian tumor. The range of ages at diagnosis was 13 to 62 years, with all but one of the gynecologic tumors occurring following one or more other cancers. The average age at onset for the epithelial tumors was 48 years, significantly earlier than in the general population in which 60% occur over the age of 50 years (126). Interestingly these numbers are similar to those of Hisada et al. who reported follow-up data on the original 24 LFS kindreds in which there were 8 gynecologic cancers (6 ovarian, 2 uterine) in 104 women, including two occurring after previous cancers (108). So although ovarian cancers are reported to represent only 1.7% of tumors in the IARC p53 TP53 Mutation Database, they

may still be an important source of morbidity and mortality in LFS patients (127). Few other gynecologic tumors have been observed.

Given the high frequency of young-onset breast cancer in LFS, it is likely that most LFS kindreds with ovarian cancers will present to the gynecologist as possible hereditary breast-ovarian cancer syndrome. However, if negative for BRCA1/2, depending on the further personal and family history, p53 mutation testing may be indicated. Walsh et al. identified germline p53 mutations in a few breast cancer families (123). Key indicators of risk would include those criteria outlined in Table 4 in terms of personal and family cancer history, in particular the occurrence of at least one of the component LFS tumors, in addition to young onset breast cancer. However, we cite a cautionary note regarding the reliability of cancer family history for LFS. Lalloo et al. conducted a population-based study of women diagnosed with breast cancer aged 30 years or younger in the United Kingdom (104,105). In this study of 99 patients who were tested for mutations in BRCA1/2 and TP53, four probands with p53 germline mutations were identified. Although two ultimately were found to have a cancer family history consistent with LFS, they had not been recognized at the time of diagnosis. The other two had been considered nonfamilial, with one shown to be a de novo mutation. Overall, of those young breast cancer patients negative for BRCA mutations, p53 mutations were observed in 5%. The study demonstrated the difficulty in obtaining a clear LFS family history, even when it was present, as has been noted previously; however, more importantly, the study also indicated that the LFS patients accounted for most of the young breast cancer patients who developed multiple primary cancers (128). Given the frequent occurrence of additional cancer in radiation-treated sites in LFS patients, the investigators recommended avoiding radiation therapy in cancer treatment, when feasible, providing a strong rationale for identifying those at-risk patients.

Clinical Recommendations

Given the rarity of experience with gynecologic tumors in LFS patients, there are no data regarding distinct prognosis or treatment, other than the recommendation to avoid radiation treatment, if feasible. The only specific screening recommendations for p53 germline mutation carriers are the American Cancer Society breast screening guidelines based on level of risk; the guidelines include annual screening with MRI as an adjunct to mammography for high-risk women (>20–25% lifetime risk), and the Expert Consensus Opinion panel included p53 mutation carriers in this group (129).

As somatic p53 mutations are common among many human cancers, many new therapies are being developed to target tumors with p53 mutations, some of which presumably may especially benefit LFS patients (125,129–135). Such approaches are consistent with the heralding of a new era of personalized medicine (136).

PEUTZ–JEGHERS SYNDROME CASE REPORT

J.P. is a 20-year-old woman with obvious clinical manifestations of Peutz–Jeghers syndrome, including pigmented macules on her lips and inside her mouth as well as hamartomatous gastrointestinal polyps. She had been diagnosed at the age of 14 without genetic testing. She presented for counseling to discuss screening for gynecologic malignancies. The options for genetic testing were discussed. Although the patient had a definite clinical diagnosis, the implications of testing for the benefit of her siblings and future children were addressed. The decision was made not to pursue testing. After consultation with gynecologists and gastroenterologists, there was no clear consensus on screening. Eventually, despite several differing opinions, it was recommended that J.P. undergo colonoscopy and upper gastrointestinal screening every two to three years, a yearly gynecologic examination including a pap smear and a transvaginal ultrasound, a clinical breast examination every six months, and a yearly mammogram.

LEARNING POINTS

- Peutz–Jeghers syndrome has unique clinical manifestations and may be diagnosed without genetic testing.
- While women with Peutz–Jeghers syndrome are at risk for gastrointestinal malignancies, they are also at risk for gynecologic malignancies. The characteristic gynecologic tumors are cervical adenoma malignum, which is an aggressive cancer, and the ovarian SCTAT, which is benign.
- Currently, there are no validated effective screening strategies. Therefore, screening examinations, beginning between the ages of 18 and 25, should focus on malignancies for which individuals with Peutz–Jeghers syndrome are most at risk.

COWDEN SYNDROME CASE REPORT

C.L. is a nulliparous, 42-year-old women with newly diagnosed endometrial cancer, after having presented with intermenstrual bleeding. On physical examination, her physician notes numerous abnormal growths on the mucous membranes of her mouth and nose, as well as several wart-like growths surrounding her mouth and nose. C.L. states that these growths began to appear when she was approximately 30 years old. She never saw a doctor for these lesions because her mother had similar growths and they were not causing her any problems. She is an only child and her medical history is significant for fibrocystic breast disease. Of note, her mother had been diagnosed with breast cancer at the age of 38 and has a thyroid goiter.

C.L. was treated with a laparoscopic hysterectomy and bilateral salpingo-oophorectomy for her stage Ia, grade I endometrial cancer and was referred to a dermatologist and a genetic counselor on the basis of her physical examination

findings and family history. A biopsy of one of C.L.'s skin lesions was performed, and she and her mother were informed that the skin lesions that they had were "trichilemmomas," which are a pathognomonic sign of Cowden syndrome. They were also counseled on their risk for developing other cancers and the importance of regular breast, thyroid, skin, and colon screening.

LEARNING POINTS

- Cowden syndrome has unique dermatologic manifestations, some of which are pathognomonic for the disease.
- Women with Cowden syndrome are at risk for breast, thyroid and uterine malignancies.
- Benign manifestations of Cowden syndrome include mucocutaneous lesions, thyroid goiter or adenoma, uterine leiomyoma, fibrocystic breast disease, breast fibroadenomas, lipomas, ileal and colonic hamartomatous polyps.
- Women diagnosed with Cowden syndrome should actively participate in breast, thyroid, colon, and skin screening.

While endometrial screening has been recommended, there are no data to support its efficacy.

REFERENCES

1. Stratakis CA, Kirschner LS, Taymans SE, et al. Carney complex, Peutz-Jeghers syndrome, Cowden disease, and Bannayan-Zonana syndrome share cutaneous and endocrine manifestations, but not genetic loci. J Clin Endocrinol Metab 1998; 83(8): 2972–2976.
2. Schreibman IR, Baker M, Amos C, et al. The hamartomatous polyposis syndromes: a clinical and molecular review. Am J Gastroenterol 2005; 100(2):476–490.
3. Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med 1969; 71(4):747–752.
4. Li FP, Fraumeni JF Jr. Rhabdomyosarcoma in children: epidemiologic study and identification of a familial cancer syndrome. J Natl Cancer Inst 1969; 43(6): 1365–1373.
5. Peutz JLA. Very remarkable case of familial polyposis of mucous membrane of intestinal tract and nasopharynx accompanied by peculiar pigmentations of skin and mucous membrane (Dutch). Nederl Maandschr Geneesk 1921; 10:134–146.
6. Jeghers H, Mc KV, Katz KH. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits; a syndrome of diagnostic significance. N Engl J Med 1949; 241(26):1031–1036.
7. Bruwer A, Bargen JA, Kierland RR. Surface pigmentation and generalized intestinal polyposis; (Peutz-Jeghers syndrome). Mayo Clin Proc 1954; 29(6):168–171.

8. Hemminki A, Tomlinson I, Markie D, et al. Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nat Genet 1997; 15(1):87–90.
9. Hemminki A, Markie D, Tomlinson I, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998; 391(6663):184–187.
10. Boudeau J, Sapkota G, Alessi DR. LKB1, a protein kinase regulating cell proliferation and polarity. FEBS Lett 2003; 546(1):159–165.
11. Gruber SB, Entius MM, Petersen GM, et al. Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome. Cancer Res 1998; 58(23):5267–5270.
12. Lim W, Hearle N, Shah B, et al. Further observations on LKB1/STK11 status and cancer risk in Peutz-Jeghers syndrome. Br J Cancer 2003; 89(2):308–313.
13. Ballhausen WG, Gunther K. Genetic screening for Peutz-Jeghers syndrome. Expert Rev Mol Diagn 2003; 3(4):471–479.
14. Utsunomiya J, Gocho H, Miyanaga T, et al. Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med J 1975; 136(2):71–82.
15. Boardman LA, Couch FJ, Burgart LJ, et al. Genetic heterogeneity in Peutz-Jeghers syndrome. Hum Mutat 2000; 16(1):23–30.
16. Bartholomew LG, Moore CE, Dahlin DC, et al. Intestinal polyposis associated with mucocutaneous pigmentation. Surg Gynecol Obstet 1962; 115:1–11.
17. Bartholomew LG, Dahlin DC, Waugh JM. Intestinal polyposis associated with mucocutaneous melanin pigmentation Peutz-Jeghers syndrome; review of literature and report of six cases with special reference to pathologic findings. Gastroenterology 1957; 32(3):434–451.
18. Westerman AM, Wilson JH. Peutz-Jeghers syndrome: risks of a hereditary condition. Scand J Gastroenterol Suppl 1999; 230:64–70.
19. Foley TR, McGarrity TJ, Abt AB. Peutz-Jeghers syndrome: a clinicopathologic survey of the "Harrisburg family" with a 49-year follow-up. Gastroenterology 1988; 95(6):1535–1540.
20. Kato N, Romero M, Catasus L, et al. The STK11/LKB1 Peutz-Jegher gene is not involved in the pathogenesis of sporadic sex cord-stromal tumors, although loss of heterozygosity at 19p13.3 indicates other gene alteration in these tumors. Hum Pathol 2004; 35(9):1101–1104.
21. Young S, Gooneratne S, Straus FH II, et al. Feminizing Sertoli cell tumors in boys with Peutz-Jeghers syndrome. Am J Surg Pathol 1995; 19(1):50–58.
22. Young RH, Welch WR, Dickersin GR, et al. Ovarian sex cord tumor with annular tubules: review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. Cancer 1982; 50(7):1384–1402.
23. Wilson DM, Pitts WC, Hintz RL, et al. Testicular tumors with Peutz-Jeghers syndrome. Cancer 1986; 57(11):2238–2240.
24. Coen P, Kulin H, Ballantine T, et al. An aromatase-producing sex-cord tumor resulting in prepubertal gynecomastia. N Engl J Med 1991; 324(5):317–322.
25. Alikasifoglu A, Gonc EN, Akcoren Z, et al. Feminizing Sertoli cell tumor associated with Peutz-Jeghers syndrome. J Pediatr Endocrinol Metab 2002; 15(4):449–452.
26. Solh HM, Azoury RS, Najjar SS. Peutz-Jeghers syndrome associated with precocious puberty. J Pediatr 1983; 103(4):593–595.
27. Zung A, Shoham Z, Open M, et al. Sertoli cell tumor causing precocious puberty in a girl with Peutz-Jeghers syndrome. Gynecol Oncol 1998; 70(3):421–424.

28. Scully RE. Sex cord tumor with annular tubules a distinctive ovarian tumor of the Peutz-Jeghers syndrome. Cancer 1970; 25(5):1107–1121.
29. Lele SM, Sawh RN, Zaharopoulos P, et al. Malignant ovarian sex cord tumor with annular tubules in a patient with Peutz-Jeghers syndrome: a case report. Mod Pathol 2000; 13(4):466–470.
30. Clement S, Efrusy ME, Dobbins WO III, et al. Pelvic neoplasia in Peutz-Jeghers syndrome. J Clin Gastroenterol 1979; 1(4):341–343.
31. Christian CD, McLoughlin TG, Cathcart ER, et al. Peutz-Jeghers syndrome associated with functioning ovarian tumor. JAMA 1964; 190:935–938.
32. Hales SA, Cree IA, Pinion S. A poorly differentiated Sertoli-Leydig cell tumour associated with an ovarian sex cord tumour with annular tubules in a woman with Peutz-Jeghers syndrome. Histopathology 1994; 25(4):391–393.
33. Mangili G, Taccagni G, Garavaglia E, et al. An unusual admixture of neoplastic and metaplastic lesions of the female genital tract in the Peutz-Jeghers syndrome. Gynecol Oncol 2004; 92(1):337–342.
34. Chen KT. Female genital tract tumors in Peutz-Jeghers syndrome. Hum Pathol 1986; 17(8):858–861.
35. Song SH, Lee JK, Saw HS, et al. Peutz-Jeghers syndrome with multiple genital tract tumors and breast cancer: a case report with a review of literatures. J Korean Med Sci 2006; 21(4):752–757.
36. Gilks CB, Young RH, Aguirre P, et al. Adenoma malignum (minimal deviation adenocarcinoma) of the uterine cervix. A clinicopathological and immunohistochemical analysis of 26 cases. Am J Surg Pathol 1989; 13(9):717–729.
37. Kaku T, Enjoji M. Extremely well-differentiated adenocarcinoma (“adenoma malignum”) of the cervix. Int J Gynecol Pathol 1983; 2(1):28–41.
38. Srivatsa PJ, Keeney GL, Podratz KC. Disseminated cervical adenoma malignum and bilateral ovarian sex cord tumors with annular tubules associated with Peutz-Jeghers syndrome. Gynecol Oncol 1994; 53(2):256–264.
39. Tsuruchi N, Tsukamoto N, Kaku T, et al. Adenoma malignum of the uterine cervix detected by imaging methods in a patient with Peutz-Jeghers syndrome. Gynecol Oncol 1994; 54(2):232–236.
40. Choi CG, Kim SH, Kim JS, et al. Adenoma malignum of uterine cervix in Peutz-Jeghers syndrome: CT and US features. J Comput Assist Tomogr 1993; 17(5):819–821.
41. Boardman LA, Pittelkow MR, Couch FJ, et al. Association of Peutz-Jeghers-like mucocutaneous pigmentation with breast and gynecologic carcinomas in women. Medicine (Baltimore) 2000; 79(5):293–298.
42. Spigelman AD, Murday V, Phillips RK. Cancer and the Peutz-Jeghers syndrome. Gut 1989; 30(11):1588–1590.
43. Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000; 119(6):1447–1453.
44. Westerman AM, Entius MM, de Baar E, et al. Peutz-Jeghers syndrome: 78-year follow-up of the original family. Lancet 1999; 353(9160):1211–1215.
45. Dodds WJ, Schulte WJ, Hensley GT, et al. Peutz-Jeghers syndrome and gastrointestinal malignancy. Am J Roentgenol Radium Ther Nucl Med 1972; 115(2):374–377.
46. Burdick D, Prior JT. Peutz-Jeghers syndrome. A clinicopathologic study of a large family with a 27-year follow-up. Cancer 1982; 50(10):2139–2146.
47. Nakamura T, Suzuki S, Yokoi Y, et al. Duodenal cancer in a patient with Peutz-Jeghers syndrome: molecular analysis. J Gastroenterol 2002; 37(5):376–380.

48. Hizawa K, Iida M, Matsumoto T, et al. Neoplastic transformation arising in Peutz-Jeghers polyposis. Dis Colon Rectum 1993; 36(10):953–957.
49. Bowlby LS. Pancreatic adenocarcinoma in an adolescent male with Peutz-Jeghers syndrome. Hum Pathol 1986; 17(1):97–99.
50. Giardiello FM, Welsh SB, Hamilton SR, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med 1987; 316(24):1511–1514.
51. Lim W, Olschwang S, Keller JJ, et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004; 126(7):1788–1794.
52. Jarvinen HJ. Genetic testing for polyposis: practical and ethical aspects. Gut 2003; 52(suppl 2):ii19–ii22.
53. Leggett BA, Young JP, Barker M. Peutz-Jeghers syndrome: genetic screening. Expert Rev Anticancer Ther 2003; 3(4):518–524.
54. Giardiello FM, Trimbath JD. Peutz-Jeghers syndrome and management recommendations. Clin Gastroenterol Hepatol 2006; 4(4):408–415.
55. Oncel M, Remzi FH, Church JM, et al. Benefits of 'clean sweep' in Peutz-Jeghers patients. Colorectal Dis 2004; 6(5):332–335.
56. Spigelman AD, Thomson JP, Phillips RK. Towards decreasing the relaparotomy rate in the Peutz-Jeghers syndrome: the role of peroperative small bowel endoscopy. Br J Surg 1990; 77(3):301–302.
57. Paterlini A, Huscher C, Salmi A. Jejunal endoscopic polypectomy in the Peutz-Jeghers syndrome. Endoscopy 1983; 15(4):270–271.
58. Boardman LA, Thibodeau SN, Schaid DJ, et al. Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann Intern Med 1998; 128(11):896–899.
59. Lloyd KM II, Dennis M. Cowden's disease. A possible new symptom complex with multiple system involvement. Ann Intern Med 1963; 58:136–142.
60. Starink TM, van der Veen JP, Arwert F, et al. The Cowden syndrome: a clinical and genetic study in 21 patients. Clin Genet 1986; 29(3):222–233.
61. Hanssen AM, Fryns JP. Cowden syndrome. J Med Genet 1995; 32(2):117–119.
62. Nelen MR, Kremer H, Konings IB, et al. Novel PTEN mutations in patients with Cowden disease: absence of clear genotype-phenotype correlations. Eur J Hum Genet 1999; 7(3):267–273.
63. Eng C. Will the real Cowden syndrome please stand up: revised diagnostic criteria. J Med Genet 2000; 37(11):828–830.
64. Longy M, Lacombe D. Cowden disease. Report of a family and review. Ann Genet 1996; 39(1):35–42.
65. Mallory SB. Cowden syndrome (multiple hamartoma syndrome). Dermatol Clin 1995; 13(1):27–31.
66. Marsh DJ, Dahia PL, Caron S, et al. Germline PTEN mutations in Cowden syndrome-like families. J Med Genet 1998; 35(11):881–885.
67. De Vivo I, Gertig DM, Nagase S, et al. Novel germline mutations in the PTEN tumour suppressor gene found in women with multiple cancers. J Med Genet 2000; 37(5):336–341.
68. Nelen MR, Padberg GW, Peeters EA, et al. Localization of the gene for Cowden disease to chromosome 10q22-23. Nat Genet 1996; 13(1):114–116.
69. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997; 275(5308): 1943–1947.

70. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997; 15(4):356–362.
71. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997; 16(1): 64–67.
72. Nelen MR, van Staveren WC, Peeters EA, et al. Germline mutations in the PTEN/MMAC1 gene in patients with Cowden disease. Hum Mol Genet 1997; 6(8): 1383–1387.
73. Zhou XP, Waite KA, Pilarski R, et al. Germline PTEN promoter mutations and deletions in Cowden/Bannayan-Riley-Ruvalcaba syndrome result in aberrant PTEN protein and dysregulation of the phosphoinositol-3-kinase/Akt pathway. Am J Hum Genet 2003; 73(2):404–411.
74. Waite KA, Eng C. Protean PTEN: form and function. Am J Hum Genet 2002; 70(4): 829–844.
75. Stambolic V, Suzuki A, de la Pompa JL, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 1998; 95(1):29–39.
76. Dahia PL, Aguiar RC, Alberta J, et al. PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanisms in haematological malignancies. Hum Mol Genet 1999; 8(2):185–193.
77. Shin I, Yakes FM, Rojo F, et al. PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. Nat Med 2002; 8(10):1145–1152.
78. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev 1999; 13(22):2905–2927.
79. Risinger JI, Hayes AK, Berchuck A, et al. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res 1997; 57(21):4736–4738.
80. Dahia PL, Marsh DJ, Zheng Z, et al. Somatic deletions and mutations in the Cowden disease gene, PTEN, in sporadic thyroid tumors. Cancer Res 1997; 57(21): 4710–4713.
81. Ali IU, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. J Natl Cancer Inst 1999; 91(22):1922–1932.
82. Stambolic V, Tsao MS, Macpherson D, et al. High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/− mice. Cancer Res 2000; 60(13):3605–3611.
83. NCCN practice guidelines: genetics/familial high risk cancer. Oncology (Williston Park) 1999; 13:161–186.
84. www.nccn.org/Professionals/Physician_gls/PDF/genetics_screening.pdf. 2007. Accessed in April 13, 2007.
85. Hildenbrand C, Burgdorf WH, Lautenschlager S. Cowden syndrome-diagnostic skin signs. Dermatology 2001; 202(4):362–366.
86. Brownstein MH, Mehregan AH, Bilowski JB. Trichilemmomas in Cowden's disease. JAMA 1977; 238(1):26.
87. Weary PE, Gorlin RJ, Gentry WC Jr., et al. Multiple hamartoma syndrome (Cowden's disease). Arch Dermatol 1972; 106(5):682–690.
88. Bosserhoff AK, Grussendorf-Conen EI, Rubben A, et al. Multiple colon carcinomas in a patient with Cowden syndrome. Int J Mol Med 2006; 18(4):643–647.

89. Omote K, Kawamata T, Imaizumi H, et al. Case of Cowden's disease that caused airway obstruction during induction of anesthesia. Anesthesiology 1999; 91(5): 1537–1540.
90. Schrager CA, Schneider D, Gruener AC, et al. Clinical and pathological features of breast disease in Cowden's syndrome: an underrecognized syndrome with an increased risk of breast cancer. Hum Pathol 1998; 29(1):47–53.
91. Fackenthal JD, Marsh DJ, Richardson AL, et al. Male breast cancer in Cowden syndrome patients with germline PTEN mutations. J Med Genet 2001; 38(3):159–164.
92. Lyons CJ, Wilson CB, Horton JC. Association between meningioma and Cowden's disease. Neurology 1993; 43(7):1436–1437.
93. Haibach H, Burns TW, Carlson HE, et al. Multiple hamartoma syndrome (Cowden's disease) associated with renal cell carcinoma and primary neuroendocrine carcinoma of the skin (Merkel cell carcinoma). Am J Clin Pathol 1992; 97(5): 705–712.
94. Yen BC, Kahn H, Schiller AL, et al. Multiple hamartoma syndrome with osteosarcoma. Arch Pathol Lab Med 1993; 117(12):1252–1254.
95. Li FP, Fraumeni JF Jr. Prospective study of a family cancer syndrome. JAMA 1982; 247(19):2692–2694.
96. Birch JM, Hartley AL, Marsden HB, et al. Excess risk of breast cancer in the mothers of children with soft tissue sarcomas. Br J Cancer 1984; 49(3):325–331.
97. Strong LC, Stine M, Norsted TL. Cancer in survivors of childhood soft tissue sarcoma and their relatives. J Natl Cancer Inst 1987; 79(6):1213–1220.
98. Williams WR, Strong LC. Genetic epidemiology of soft tissue sarcomas in children. In: Weber M, ed. Familial Cancer. 1st International Research Conference. Basel, Switzerland: Karger, 1985:151–153.
99. Li FP, Fraumeni JF Jr., Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988; 48(18):5358–5362.
100. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990; 250(4985):1233–1238.
101. Srivastava S, Zou ZQ, Pirollo K, et al. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature 1990; 348(6303): 747–749.
102. Law JC, Strong LC, Chidambaram A, et al. A germ line mutation in exon 5 of the p53 gene in an extended cancer family. Cancer Res 1991; 51(23 pt 1):6385–6387.
103. Hollstein M, Sidransky D, Vogelstein B, et al. p53 mutations in human cancers. Science 1991; 253(5015):49–53.
104. Lalloo F, Varley J, Moran A, et al. BRCA1, BRCA2 and TP53 mutations in very early-onset breast cancer with associated risks to relatives. Eur J Cancer 2006; 42(8): 1143–1150.
105. Lalloo F, Varley J, Ellis D, et al. Prediction of pathogenic mutations in patients with early-onset breast cancer by family history. Lancet 2003; 361(9363):1101–1102.
106. Chompret A, Brugieres L, Ronsin M, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer 2000; 82(12):1932–1937.
107. Hwang SJ, Lozano G, Amos CI, et al. Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk. Am J Hum Genet 2003; 72(4): 975–983.
108. Hisada M, Garber JE, Fung CY, et al. Multiple primary cancers in families with Li-Fraumeni syndrome. J Natl Cancer Inst 1998; 90(8):606–611.

109. Birch JM, Hartley AL, Tricker KJ, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. Cancer Res 1994; 54(5): 1298–1304.
110. Chompret A, Abel A, Stoppa-Lyonnet D, et al. Sensitivity and predictive value of criteria for p53 germline mutation screening. J Med Genet 2001; 38(1):43–47.
111. Wagner J, Portwine C, Rabin K, et al. High frequency of germline p53 mutations in childhood adrenocortical cancer. J Natl Cancer Inst 1994; 86(22):1707–1710.
112. Varley JM, McGown G, Thorncroft M, et al. Are there low-penetrance TP53 Alleles? Evidence from childhood adrenocortical tumors. Am J Hum Genet 1999; 65(4):995–1006.
113. Krutilkova V, Trkova M, Fleitz J, et al. Identification of five new families strengthens the link between childhood choroid plexus carcinoma and germline TP53 mutations. Eur J Cancer 2005; 41(11):1597–1603.
114. Diller L, Sexsmith E, Gottlieb A, et al. Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma. J Clin Invest 1995; 95(4): 1606–1611.
115. Strong L. Li-Fraumeni syndrome. In: Isaacs C RT, ed. Hereditary Breast Cancer: Risk, Prevention and Management. New York, NY: Isaacs and Rebbeck; 2007:95–109.
116. Kupryjanczyk J, Thor AD, Beauchamp R, et al. p53 gene mutations and protein accumulation in human ovarian cancer. Proc Natl Acad Sci U S A 1993; 90(11): 4961–4965.
117. Berchuck A, Kohler MF, Marks JR, et al. The p53 tumor suppressor gene frequently is altered in gynecologic cancers. Am J Obstet Gynecol 1994; 170(1 pt 1):246–252.
118. Malkin D, Jolly KW, Barbier N, et al. Germline mutations of the p53 tumor-suppressor gene in children and young adults with second malignant neoplasms. N Engl J Med 1992; 326(20):1309–1315.
119. Jolly KW, Malkin D, Douglass EC, et al. Splice-site mutation of the p53 gene in a family with hereditary breast-ovarian cancer. Oncogene 1994; 9(1):97–102.
120. Nichols KE, Malkin D, Garber JE, et al. Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomarkers Prev 2001; 10(2): 83–87.
121. Birch JM, Alston RD, McNally RJ, et al. Relative frequency and morphology of cancers in carriers of germline TP53 mutations. Oncogene 2001; 20(34):4621–4628.
122. Olivier M, Goldgar DE, Sodha N, et al. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. Cancer Res 2003; 63(20):6643–6650.
123. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA 2006; 295(12): 1379–1388.
124. Ruijs MW, Schmidt MK, Nevanlinna H, et al. The single-nucleotide polymorphism 309 in the MDM2 gene contributes to the Li-Fraumeni syndrome and related phenotypes. Eur J Hum Genet 2007; 15(1):110–114.
125. Senzer N, Nemunaitis J, Nemunaitis M, et al. p53 therapy in a patient with Li-Fraumeni syndrome. Mol Cancer Ther 2007; 6(5):1478–1482.
126. Ries LAG, Melbert D, Krapcho M, et al. (eds). SEER Cancer Statistics Review, 1975–2005. Bethesda, MD: National Cancer Institute. Available at: http://seer.cancer.gov/csr/1975_2005/, based on November 2007 SEER data submission, posted to the SEER Web site, 2008. Accessed April 24, 2008.

127. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat 2007; 28(6):622–629. IARC TP53 Mutation Database R12. Available at: http://www-p53.iarc.fr/Whatsnew.html. Release Date November 2007. Accessed December 15, 2007.
128. Schneider KA, DiGianni LM, Patenaude AF, et al. Accuracy of cancer family histories: comparison of two breast cancer syndromes. Genet Test 2004; 8(3): 222–228.
129. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007; 57(2): 75–89.
130. Martins CP, Brown-Swigart L, Evan GI. Modeling the therapeutic efficacy of p53 restoration in tumors. Cell 2006; 127(7):1323–1334.
131. Ventura A, Kirsch DG, McLaughlin ME, et al. Restoration of p53 function leads to tumour regression in vivo. Nature 2007; 445(7128):661–665.
132. Xue W, Zender L, Miething C, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007; 445(7128):656–660.
133. Vassilev LT. MDM2 inhibitors for cancer therapy. Trends Mol Med 2007; 13(1): 23–31.
134. Sarek G, Kurki S, Enback J, et al. Reactivation of the p53 pathway as a treatment modality for KSHV-induced lymphomas. J Clin Invest 2007; 117(4):1019–1028.
135. Bell HS, Dufes C, O'Prey J, et al. A p53-derived apoptotic peptide derepresses p73 to cause tumor regression in vivo. J Clin Invest 2007; 117(4):1008–1018.
136. Kurzrock R. Studies in target-based treatment. Mol Cancer Ther 2007; 6(5):1477.

16

Genetic Risk Assessment for Hereditary Ovarian Cancer: BRCA1 and BRCA2

Sheri A. Babb

Siteman Cancer Center at Barnes-Jewish Hospital and Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

KEY POINTS

- When obtaining a family history, efforts should be made to confirm cancer diagnoses through medical records.
- Criteria have been established by National Comprehensive Cancer Network (NCCN), American Society of Clinical Oncology (ASCO), and Society of Gynecologic Oncologists (SGO) for referring individuals for risk assessment for hereditary breast-ovarian cancer syndrome.
- Risk assessment models, such as BRCAPRO, are available to guide referrals.
- Discussion of the psychologic impact of genetic testing and the implication of results should be included in pretest counseling.
- BRCA test results may be reported as positive, negative, or variant of uncertain significance. Therefore, pre- and posttest counseling are a significant part of assessment and testing.

INTRODUCTION

Ovarian cancer is relatively rare in the general population; the average woman has a lifetime risk to develop ovarian cancer of approximately 1.4% (1). However, women who have a hereditary predisposition to ovarian cancer are at

significantly elevated risk to develop ovarian and other cancers. Studies of lifetime risks for ovarian cancer range from 39% to 54% in a woman identified as having a BRCA1/BRCA2 mutation, with a lower risk associated with BRCA2 mutations as compared with BRCA1 (2–5). The ability to identify women at high lifetime risk for ovarian cancer offers "the opportunity to provide tailored screening and prevention strategies such as surveillance, chemoprevention, and prophylactic surgery that may reduce the morbidity and mortality associated with these syndromes" (6).

Population-based studies of the prevalence of hereditary cancer predisposition genes in invasive epithelial ovarian cancer identified a germline BRCA mutation frequency of 11% to 15% (7–9). Germline mismatch repair (MMR) mutations [responsible for Lynch syndrome, also known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC)] account for approximately 2% of invasive epithelial ovarian cancer (10) and possibly a higher percentage in individuals diagnosed with invasive epithelial cancer before age 40 (11).

REFERRAL FOR GENETIC RISK ASSESSMENT

Referral of the Ovarian Cancer Patient

Referral for genetic risk assessment and consideration of genetic testing may be warranted for all invasive epithelial ovarian cancer patients. Histopathologic data may be useful in risk assessment and determining appropriateness of genetic testing. Several population-based studies of ovarian cancer series report BRCA mutations predominantly in invasive epithelial (nonmucinous) ovarian cancers, which include the histopathologic subtypes papillary serous, endometrioid, and, less commonly, Malignant Mixed Mullerian Tumor (MMMT) and clear cell. Primary peritoneal and fallopian tube malignancies are also part of spectrum of epithelial tumors associated with BRCA mutations. BRCA mutations are rarely reported in borderline ovarian tumors or mucinous ovarian tumors (7,12,13).

The recommendation for referral of the ovarian cancer patient for cancer genetic risk assessment is even more compelling if there is a family history of cancer. Recommendations for referral criteria for genetic risk assessment for Hereditary Breast and Ovarian Cancer (HBOC) incorporate age of onset of breast cancer diagnosis, ovarian cancer diagnoses (not considering age of onset), and number of close relatives (first, second, or third degree) with breast and/or ovarian cancer (6,14,15). The Society of Gynecologic Oncologists recommends cancer genetic risk assessment for the following high-risk categories (6):

- Women with personal history of breast cancer and ovarian cancer at any age
- Women with personal history of ovarian cancer and close relative (first, second, or third degree) with breast cancer $\leq$ age 50 or ovarian cancer at any age

- Women with ovarian cancer and Ashkenazi Jewish ancestry
- Women with breast cancer $\leq$ age 50 and a close relative with ovarian cancer or male breast cancer at any age
- Women of Ashkenazi Jewish ancestry and breast cancer $\leq$ 40 years
- Women with first- or second-degree relatives with a known BRCA1 or BRCA2 mutation

Referral for genetic risk assessment should not be strictly limited to those individuals who meet high-risk criteria. Additional family medical information is sometimes revealed during the process of genetic risk assessment that significantly changes the recommendation for genetic testing, resulting in either testing for different genes than for what the patient was initially referred, additional genetic testing, or no genetic testing at all. Certain features can mask the dominant expression of the BRCA1 and BRCA2 genes, including incomplete or inaccurate reporting of family medical history information, family members undergoing surgical removal of target organs (i.e., ovaries) for benign conditions, and family structure such as small family size or few at-risk females (15,16). In addition, BRCA1/BRCA2 mutations can be inherited from either the mother or the father, and paternal transmission of a hereditary predisposition to breast and ovarian cancer may be masked by the relatively low cancer risk for males. Therefore, referral for genetic risk assessment may also be helpful for the following: (6)

- Women with breast cancer $\leq$40 years
- Women with bilateral breast cancer (particularly if the first cancer is $\leq$50 years)
- Women with breast cancer $\leq$50 years and a close relative (first, second, or third degree) with breast cancer $\leq$50 years
- Women of Ashkenazi Jewish ancestry and breast cancer $\leq$50 years
- Women with breast or ovarian cancer at any age and two or more close relatives with breast cancer at any age (particularly if one is $\leq$50 years)
- Unaffected women with a first- or second-degree relative who meets one of the above criteria.

Timing of Referral for the Ovarian Cancer Patient

Referral of the ovarian cancer patient for genetic risk assessment should be initiated during the time of diagnosis/treatment for several practical reasons. While this is not always easy, since women with ovarian cancer can be overwhelmed by their diagnosis and treatment, it is ultimately of benefit both to the patient and her family.

First, given the high risk of breast cancer associated with BRCA1/BRCA2 mutations, women with ovarian cancer who test positive would be offered the high-risk breast cancer screening and prevention options discussed elsewhere in this book. While genetic test results do not currently alter treatment strategies for

the ovarian cancer patient, ongoing research may provide data to allow for individualized therapy related to mutation status in the future.

In addition, women with ovarian cancer often express concern to their treating physician/surgeon regarding their relatives' risk for ovarian cancer, early in their own diagnosis and treatment process. Referral for genetic counseling is an appropriate way to address this concern in a constructive manner. Genetic testing for hereditary cancer predisposition genes is most informative when first performed on an individual with a cancer diagnosis associated with the hereditary cancer syndrome (in this case, the woman with ovarian cancer). It is important to first determine if there is an identifiable mutation in the family that will allow for mutation-specific testing for "at-risk" family members. Therefore, testing should be initiated in the individual most likely to have a mutation when an individual/family is referred for suspected hereditary cancer.

Other practical reasons for referral at the time of diagnosis and treatment include the fact that the overall five-year survival for ovarian cancer is only 44.7% (1), and the potential impact of genetic information for family members is significant. Referral for genetic risk assessment at the time of diagnosis and treatment will provide the patient and family the option of seeking this information in a timely manner. Unfortunately, many individuals are not referred or do not seek genetic risk assessment until after the relative with ovarian cancer has died. It is not uncommon for genetic counseling clients to mention with regret that the option of cancer genetic counseling and genetic testing was brought up to the deceased relative with ovarian cancer, but the family chose not to pursue the testing at that time; or to have an ovarian cancer patient come in for genetic risk assessment and postpone genetic testing, only to have the family call when the patient is terminally ill, requesting an immediate blood draw for genetic testing.

Initiating genetic risk assessment and, possibly, genetic testing in an unaffected individual with a family history suspicious for hereditary disease is not recommended but is sometimes unavoidable if the relative(s) with cancer is deceased, estranged, or unwilling to seek genetic risk assessment. Negative genetic testing in the unaffected individual in the absence of a known familial mutation does not rule out increased hereditary risk for disease. In these cases, cancer risk assessment and recommendations for prevention and early detection of cancer will be based primarily on the family history of cancer.

GENETIC COUNSELING AND GENETIC TESTING FOR BRCA1 AND BRCA2

Genetic counseling is the process of helping people understand and adapt to medical, psychologic, and familial implications of genetic contributions to disease. This process integrates the following: interpretation of family and medical histories to assess the chance of disease occurrence or recurrence; education about inheritance, testing, management, prevention, resources, and

research; and counseling to promote informed choices and adaptation to the risk or condition (17).

Clients presenting for genetic counseling may be cancer patients who are newly diagnosed, in the process of treatment, experiencing recurrence, or in the end stages of disease; or they may be unaffected by cancer themselves but have a family history of cancer. Each of these life stages presents with different issues and challenges. Schneider (18) provides an excellent resource and discussion of the motivations, risk perceptions, coping strategies, and family issues for clients at various life stages. It is essential to provide the genetic counseling within the context of these motivations, risk perceptions, coping strategies, and family issues.

OBTAINING AN ACCURATE CANCER FAMILY HISTORY

The cancer family history is the fundamental tool for identifying individuals at hereditary risk for cancer. The collection and interpretation of family history information has been identified as a core skill necessary for health care providers (19). In 2005, the U.S. surgeon general, in cooperation with other agencies within the U.S. Department of Health and Human Services (HHS), launched a national public health campaign called the U.S. Surgeon General's Family History Initiative (http://www.familyhistory.hhs.gov) to encourage all American families to learn more about their family health history (20). The National Society of Genetic Counselors also has an online tool to assist individuals in beginning to gather their medical family history (http://www.nsgc.org/consumer/familytree/index.cfm).

Physicians should document cancer history for first- and second-degree relatives and then use this information to determine the necessity of referral to an appropriate cancer genetic professional and/or expansion of the family history. Targeted questions regarding first- and second-degree relatives' health history may be necessary to elicit the relevant information.

Full genetic risk assessment usually calls for a minimum of a three-generation pedigree including first-, second-, and third-degree relatives from both maternal and paternal families (15,16,21). Table 1 provides a list of questions to be asked about all relatives with and without cancer. This level of detail may not be available for all relatives, but should be requested. The patient needs to understand that the risk assessment is contingent on the accuracy and detail of the family history provided.

Patients' ancestry should also be ascertained, as this information contributes significantly to the risk assessment. Approximately 2% of individuals of Ashkenazi (Eastern European) Jewish ancestry have a BRCA mutation, typically one of three founder mutations: 187delAG (BRCA1), 5385insC (BRCA1), and 6174delT (BRCA2) (22,23). Targeted testing for these three mutations is available and is a recommended starting point for individuals of Ashkenazi Jewish ancestry for whom BRCA testing is appropriate. Proceeding with sequence analysis if the targeted testing is negative is recommended depending

Table 1 Questions to Ask When Collecting Personal and Family History of Cancer

For all patients and relatives with and without cancer	For all patients and relatives with cancer
• Age • Personal history of benign or malignant tumors • Major illnesses • Hospitalizations • Surgeries • Biopsy history • Reproductive history[b] • Cancer surveillance • Environmental exposures	• Organ in which tumor developed • Age at time of diagnosis • Number of tumors[a] • Pathology, stage, and grade of malignant tumor • Pathology of benign tumors • Treatment regimen (surgery, chemotherapy, radiation)

[a]For patients who have developed more than one tumor, it is important to discriminate whether the additional tumor(s) was a separate primary, recurrence, or the result of metastatic disease.
[b]Especially important for women at increased risk of breast, ovarian, or endometrial cancer. Inquire about age at menarche, age at first live birth, and history of oral contraceptive use, infertility medications, or hormone replacement therapy, including dosage and duration and age at menopause.
Source: Adapted from Ref. 16.

on the cancer family history and the a priori risk for a mutation. Many insurance companies require a stepwise approach to BRCA testing in individuals of Ashkenazi Jewish ancestry.

Importance of Medical Record Confirmation

Medical record verification of multiple cancer diagnoses in the family contributes significantly to accurate risk assessment. Inaccurate reporting can lead to an incorrect assessment of the cancer family history, which directly impacts clinical recommendations for genetic testing, cancer surveillance, and surgical or chemoprevention options. Multiple studies have documented the inaccuracy of a verbal cancer family history both in the clinical and the research setting (24–30). Gynecologic cancers are reported less accurately than other cancer sites such as colon and breast, and cancers are reported with decreasing accuracy the more distant the familial relationship (31).

The pathology report is the most reliable source for verification of both the age of diagnosis and histology and should be obtained whenever possible. However, it must also be acknowledged that it is not always feasible to obtain relevant family member medical records; patients should be informed that risk assessment will be based on the available information and will be only as accurate as that information.

Cancer patients should be encouraged to gather the medical family history even if they decline referral for genetic risk assessment. This gift of family medical history is important for the next generation, since this information may significantly impact future medical recommendations for that family.

The psychosocial impact of genetic testing is covered in detail in a subsequent chapter. However, it is important to recognize and openly discuss the potential for a significant emotional response while gathering and discussing the medical family history. We have found this to be particularly true for women seeking genetic risk assessment whose mothers died of cancer when they themselves were young.

Risk Estimation Models

The probability that a client will have a BRCA1 or BRCA2 mutation can be determined by several models that rely on a complete and accurate family history. This estimation should be shared with clients so that they may incorporate this information into their decision-making process regarding genetic testing. Clients should also be informed that all models used to determine the likelihood of finding a mutation have limitations. Research continues to evaluate validity of the variety of probability models available.

Multiple methods are available to aid clinicians in determining appropriateness of genetic testing for BRCA1 and BRCA2. These include published clinical criteria (15,16,21,32) and models, which can determine the probability that an individual has a germline BRCA1 or BRCA2 mutation, based on the personal and family history (33–37). Several sources provide a comparison of these methods (16,38–40).

The model BRCAPRO (41–44) is commonly utilized in the clinical setting and is a standard with which the performance of other methods has been compared (45–48). BRCAPRO is based on the known autosomal dominant inheritance of BRCA1 and BRCA2, Surveillance Epidemiology and End Results (SEER) estimates of risk of breast and ovarian cancer in the general population, and published estimates of the prevalence of BRCA1 and BRCA2 mutations, as well as the breast and ovarian cancer risks for mutation carriers. Using Bayesian analysis of the input data (gender, Ashkenazi Jewish ancestry, current age or age at death, age at cancer diagnosis, and additional variables including breast tumor pathology and oophorectomy status) on the proband and all first- and second-degree relatives (including both those with cancer and those unaffected), BRCAPRO calculates the probability that the proband has a BRCA1 or BRCA2 mutation.

Validation studies have shown that BRCAPRO generally performs quite well, with a high concordance between the model prediction and the results of genetic testing (39,42,47). Calculation of BRCAPRO does require computer input of a complete family history of first- and second-degree relatives, which does make it somewhat time consuming. The authors' acknowledged limitations

of BRCAPRO (42) include that it cannot consider family history beyond second-degree relatives, it relies on published penetrance and prevalence estimates and is therefore subject to any inaccuracies in these estimates, it does not consider cancers other than breast or ovarian cancer (such as pancreatic cancer) that do have known associations with BRCA1/BRCA2, and it assumes that BRCA1 and BRCA2 are the only relevant susceptibility genes.

Another commonly used risk assessment tool is the BRCA1/BRCA2 mutation prevalence table compiled by Myriad Genetic Laboratories (33) and available for viewing and download at www.myriadtests.com. This table reports the observations of deleterious BRCA1/BRCA2 mutations by Myriad Genetic Laboratories through its clinical testing service. These observations are reported as the percentage testing positive per category, with the categories delineated by gender, personal and family history of breast and ovarian cancer, and Ashkenazi Jewish status. The user then identifies the category that corresponds to their patient's personal and family history. The reported percentage testing positive in that category can then be used as an estimate of the likelihood that the patient will test positive for a BRCA mutation. The strengths of this risk assessment tool include that it is very quick and easy to use, the data are periodically updated on the website, and the data represent a large number of observations (as of the spring 2006 update, $N > 60{,}000$). Limitations of this tool include its reliance on the personal and family history information reported on test requisition forms by the health care providers ordering clinical BRCA1/BRCA2 genetic testing, without any independent verification of this information; its lack of consideration of the number and age of unaffected relatives; and the inherent ascertainment bias in the study population, which consists entirely of individuals for whom clinical BRCA1/BRCA2 genetic testing was ordered, and therefore is not representative of the general population.

CancerGene (49) is a user-friendly PC-based program that includes BRCAPRO, the Myriad prevalence table, and several other published models for calculating BRCA mutation probability. CancerGene also includes models for calculating breast cancer risk for individuals who do not have an identified hereditary predisposition (e.g., Gail and Claus models), as well as risk for Lynch syndrome–associated mutations and risk for hereditary pancreatic cancer. CancerGene is available for download at http://www4.utsouthwestern.edu/breasthealth/cagene/.

Risk assessment tools aid the clinician in determining which patients are appropriate candidates for genetic testing and can also aid patients in making informed decisions regarding whether to pursue genetic testing. However, these tools should be regarded as a supplement to, rather than a replacement for, good clinical judgment. Consistent with this notion, the current American Society of Clinical Oncology (ASCO) policy statement does not include a specified a priori risk required to offer genetic testing, leaving the decision to the discretion of the clinician and the client (21).

Informed Consent

Published guidelines for clinical genetic testing clearly recommend that testing only be provided in the setting of pre- and posttesting counseling by appropriately trained health care professionals (15,16,21,32). Health professionals who specialize in cancer genetic services can be found through the National Cancer Institute's Cancer Genetic Services Directory (http://www.cancer.gov/search/genetics_services or 1-800-4-CANCER) and the National Society of Genetic Counselors (http://www.nsgc.org/resourcelink.cfm or 1-312-321-6834).

Informed consent is a necessary component of genetic testing and should include the following (16,21):

- Psychosocial assessment and support
- Purpose of the test and who to test
- General information about the genes
- Possible test results and the implication of each of those results
- Likelihood of finding a mutation
- Technical aspects and accuracy of the test including possibility that the test will not be informative
- Fees involved in testing and counseling
- Risks of genetic discrimination
- Psychosocial aspects including anticipated reaction to results, timing and readiness for testing, family issues, and preparing for results
- Confidentiality issues
- Utilization of test results including options and limitations of medical surveillance and strategies for prevention
- Options for risk estimation without genetic testing
- Importance of sharing genetic test results with at-risk relatives
- Storage and potential reuse of genetic material sent to the laboratory

Ordering Clinical BRCA Testing

Clinical genetic testing is available for BRCA1 and BRCA2 and is typically performed on a blood sample. In the United States, full sequence analysis of BRCA1 and BRCA2 is currently available only through Myriad Genetic Laboratories. The cost of full sequence analysis is currently $3120. Testing for the three Ashkenazi founder mutations is available for $415. Site-specific testing is available for $385 for at-risk relatives of an individual with a previously identified deleterious mutation. There are several other clinical laboratories in the United States that offer site-specific and founder mutation genetic testing; a listing of these laboratories can be found on GeneTests (www.genetests.org). GeneTests, funded by the National Institutes of Health, provides an updated listing of laboratories offering clinical and research genetic testing and information on genetic testing and its use in diagnosis, management, and genetic counseling.

A component of the informed consent process is discussion of the fees and insurance benefits for genetic testing. ASCO supports efforts to ensure that all individuals at significantly increased risk of hereditary cancer have access to appropriate genetic counseling, testing, screening, surveillance, and all related medical and surgical interventions, which should be covered without penalty by public and private third-party payers (21). Most health insurance plans provide coverage for the majority of the cost of genetic testing. Many companies use specific personal and family cancer history guidelines/criteria to determine coverage of genetic testing, and coverage is also often based on medical necessity (i.e., how the genetic test results will impact medical management). Medicare provides coverage for individuals meeting specified criteria, which are available online through the Centers for Medicare and Medicaid Services (http://www.cms.hhs.gov/).

Genetic Test Results and Interpretation

Possible test results from genetic testing of BRCA1 and BRCA2 include (15,16)

1. Positive. Deleterious mutation identified. This is a true positive, meaning that the mutation is known to be functionally significant with subsequent increased risks of developing cancer.
2. Negative upon site-specific genetic testing for a known familial mutation. The familial mutation is not found in an individual from a family with a known deleterious BRCA mutation. This is an informative, reassuring negative result that typically indicates that this individual is at general population risk to develop breast and ovarian cancer. However, it is still important to assess any cancer family history not attributable to the BRCA mutation (e.g., on the other side of the family).
3. Negative upon comprehensive BRCA1/BRCA2 genetic testing. No mutation identified in an individual for whom no mutation has been identified in other family members. The possibility of a hereditary predisposition to cancer due either to a BRCA1/BRCA2 mutation that could not be detected by the genetic testing method employed, or due to other genes, known or unknown, should also be considered. Surveillance and prevention recommendations should be based on the individual's personal and family medical history. Testing another relative diagnosed with breast or ovarian cancer may be recommended; if a BRCA1/BRCA2 mutation is identified in a relative, then this result can often be reinterpreted as a "true negative."

   Comprehensive BRCA testing includes testing for five specific BRCA1 rearrangements (50,51). An additional full rearrangement panel [BRACAnalysis® Rearrangement Test (BART)] is currently automatically run in high-risk families (generally includes proband with breast

cancer diagnosed before age 50 or ovarian cancer, and two or more close relatives with breast cancer diagnosed before age 50 or ovarian cancer). The full rearrangement panel can be requested, for an additional charge, if high-risk criteria are not met. This rearrangement panel detects deletions or duplications not detected by the technology used for sequence analysis (52). Even with the addition of the large rearrangement panel, sensitivity of the clinically available BRCA1/BRCA2 genetic testing is not 100%.

4. Variant of uncertain significance (VUS). This is an inconclusive result. An alteration in BRCA1/BRCA2 has been identified, but it is unknown whether the alteration will affect gene function. This does not rule out a hereditary cancer syndrome in the family. Testing unaffected relatives for the VUS is not appropriate, because medical recommendations cannot be made on the basis of presence or absence of a VUS. Appropriate medical management should be based on family history. Research testing is often offered to the parents and to close relatives who have had cancer, as this data can be helpful in attempting to establish the clinical significance of a variant. Of note, the majority of BRCA mutation studies have been performed on white, European ancestry families. A higher frequency of BRCA VUS has been seen in individuals of African-American ancestry (48,53,54).

Results Disclosure and Follow-Up

Posttest counseling is an essential component of the genetic testing process. This includes results disclosure, discussion of the medical significance of the test results and recommended medical management options, assessment and counseling regarding the emotional reaction to results for client, discussion of medical and psychosocial significance of genetic testing results for family, offer of assistance in facilitation of communication with family regarding results, recommendations regarding future contact, and provision of resources (16).

The goal of results disclosure is to share the genetic testing results and provide immediate information and emotional support as needed (18). This includes determining the proper setting for disclosure, either in person or over the telephone. Telephone disclosure should be a scheduled telephone appointment, particularly if clients provide a cellular number, to ensure that the client is in an appropriate place to receive genetic testing results and has a support person present, if desired. Telephone disclosure should include referral for a follow-up appointment in the clinic for further discussion of the results.

Schneider (18) provides useful strategies for disclosing the results in a professional yet empathetic manner. The method of presenting cancer risks associated with a mutation in a hereditary cancer predisposition gene is an

important consideration and should include a discussion of the clients' perceived risk of developing cancer as well as the current information for cancer risks associated with mutations. Presenting cancer risks as absolute risks (the probability an event will occur over a defined time period) is generally the easiest way for clients to process this information. Cancer risks associated with hereditary cancer predisposition genes are often reported as lifetime risks. Age-specific cumulative risks, provided in intervals of time, are often easier for the clients to process relative to their current life stage, which will then allow them to incorporate that risk into their medical decision making. Chen et al. (3) provides age-specific cumulative risk for breast and ovarian cancer and predicted risk for breast and ovarian cancer in 10-year intervals.

Genetic information has an impact on extended family members that is quite unlike any other medical information. Patenaude et al. (55) succinctly state that the telling or not telling of others at risk has enormous overall impact, as it can initiate or impede a cascade of testing over many generations, with potential gains or losses of many quality years of life. Health care professionals have an ethical responsibility to encourage the sharing of genetic information within families and to assist clients in communicating this complex medical information with relatives while also protecting the client's privacy and confidentiality (16,18,21,56,57). This can include discussion of the family dynamics and current communication patterns, provision of letters and educational materials for the client to distribute to family members, and/or referral to cancer genetic health professionals located geographically near the at-risk family members.

When genetic testing for BRCA is initiated in an older patient with ovarian cancer, the focus of discussion of the impact of the genetic information for extended family members is often centered on the risk for sisters, daughters, and nieces. However, these patients may also have at-risk women in the preceding generation, and it is important to include discussion of potential risk for ovarian cancer and breast cancer in these women and to offer genetic testing, if appropriate.

CLIENT RESOURCES FOR THE FUTURE

Importance of Continued Contact

Another important component of the process of cancer genetic risk assessment is to provide the client with resources for the future. Genetic risk assessment is a dynamic process, both in technologic advances and the cancer family history information. Continued research on hereditary cancer families and advances in human genetics and technology may result in new management guidelines and/or the availability of new or improved genetic testing. Additionally, the risk assessment and subsequent recommendations are based on the cancer family history information provided at the time of the consultation, and new information

may significantly impact the risk assessment and subsequent recommendations. Therefore, the recommendation should be made for the clients to recontact their genetics provider for new advances and also with relevant changes to their personal or family medical history (58). Clients who utilize the Internet can be directed to websites that contain updated information regarding genetics of hereditary cancer, such as the National Cancer Institute (www.cancer.gov) or Facing Our Risk of Cancer Empowered (FORCE) (www.facingourrisk.org). FORCE is a nonprofit organization with an advisory board of cancer genetic specialists that provides updated information and support for individuals concerned with hereditary breast and ovarian cancer.

The popular media is increasingly covering the human perspective, ethical dilemmas, and psychosocial impact of cancer genetic testing with dramatic titles such as "A threat of cancer, a drastic decision" (59), "Facing life with a lethal gene" (60), and "Previvor: A personal voyage into the strange new world of genetic testing" (61). Clients should be encouraged to contact the genetics clinic for follow-up appointments to be able to put the new technology, updated family history, and popular media reports into perspective with their personal and family medical history.

DNA Banking

DNA banking should be offered to individuals who are suspected to have a hereditary predisposition to cancer, in cases where either genetic testing was unable to identify a causative mutation, or who decline or postpone genetic testing. DNA banking is an important alternative for terminally ill cancer patients, when the patient and/or family does not have the time for traditional genetic risk assessment or are simply overwhelmed by planning for the end of life and cannot process additional medical information. The cost ranges from $100 to more than $300 with storage times from 5 years to 25+ years. The National Society of Genetic Counselors produced a patient-oriented brochure *DNA BANKING: Saving for the Future* (62) to promote the awareness, understanding, and utilization of DNA banking by both health care practitioners and the general public.

CONCLUSION

Genetic information has a tremendous impact, unlike that of any other medical information, for the woman with ovarian cancer and her extended family members. Referral for genetic risk assessment and consideration of genetic testing may be warranted for all invasive epithelial ovarian cancer patients and should be initiated during the time of treatment and diagnosis. Referral to a health care professional with expertise in genetics and cancer family history evaluation is recommended.

CASE REPORT

L.S., a woman of Ashkenazi Jewish descent, decided to pursue hereditary cancer risk assessment and genetic testing at the age of 52, never having been diagnosed with any type of cancer. Her mother, three maternal aunts, and two cousins had been diagnosed with breast cancer at the ages of 83, 68, 60, 52, 50, and 37. Her sister was diagnosed with ovarian cancer at the age of 52 and passed 3 years later. Her father and paternal uncle had both succumbed to prostate cancer. L.S. referred herself for genetic counseling out of concern for her own risk and risk to her two daughters. Her mother was not interested in genetic testing and other affected family members were deceased. BRCA testing revealed that L.S. had one of three Ashkenazi Jewish founder mutations. Since that time she has undergone a prophylactic bilateral salpingo-oophorectomy and is seen every six months for breast cancer screening.

LEARNING POINTS

- Family history may dictate the appropriateness of hereditary cancer risk assessment in unaffected individuals.
- For individuals of Ashkenazi Jewish heritage, limited testing for one of three Ashkenazi Jewish founder BRCA1 and BRCA2 mutations [187delAG (BRCA1), 5385insC (BRCA1), and 6174delT (BRCA2)] may be a reasonable initial step.
- Intensive breast screening protocols, chemoprevention, and prophylactic surgical options should be addressed in posttest counseling.

REFERENCES

1. SEER. Cancer Stat Fact Sheets: cancer of the ovary, National Cancer Institute, 2007.
2. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003; 72(5): 1117–1130.
3. Chen S, Iversen ES, Friebel T, et al. Characterization of BRCA1 and BRCA2 mutations in a large United States sample. J Clin Oncol 2006; 24(6):863–871.
4. Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA 1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994; 343(8899):692–695.
5. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302(5645):643–646.
6. Lancaster JM, Bethan Powell C, Kauff ND, et al. Society of gynecologic oncologists education committee statement on risk assessment for inherited gynecologic cancer predispositions. Gynecol Oncol 2007; 107(2):159–162.
7. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005; 104(12):2807–2816.

8. Risch HA, McLaughlin JR, Cole DE, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl Cancer Inst 2006; 98(23):1694–1706.
9. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001; 68(3):700–710.
10. Malander S, Rambech E, Kristoffersson U, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol 2006; 101(2):238–243.
11. Domanska K, Malander S, Masback A, et al. Ovarian cancer at young age: the contribution of mismatch-repair defects in a population-based series of epithelial ovarian cancer before age 40. Int J Gynecol Cancer 2007; 17(4):789–793.
12. Lakhani SR, Manek S, Penault-Llorca F, et al. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. Clin Cancer Res 2004; 10(7):2473–2481.
13. Werness BA, Ramus SJ, DiCioccio RA, et al. Histopathology, FIGO stage, and BRCA mutation status of ovarian cancers from the Gilda Radner Familial Ovarian Cancer Registry. Int J Gynecol Pathol 2004; 23(l):29–34.
14. Hampel H, Sweet K, Westman JA, et al. Referral for cancer genetics consultation: a review and compilation of risk assessment criteria. J Med Genet 2004; 41(2):81–91.
15. NCCN. Clinical Practice Guidelines in Oncology-v.1.2007: Genetic/familial high-risk assessment: breast and ovarian. Available at: http://www.nccn.org.
16. Trepanier A, Ahrens M, McKinnon W, et al. Genetic cancer risk assessment and counseling: recommendations of the national society of genetic counselors. J Genet Couns 2004; 13(2):83–114.
17. Resta R, Biesecker BB, Bennett RL, et al. A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. J Genet Couns 2006; 15(2): 77–83.
18. Schneider K. Counseling About Cancer: Strategies for Genetic Counselors. New York: Wiley-Liss, Inc., 2002.
19. NCHPEG. Recommendations of core competencies in genetics essential for all health professionals. Genet Med 2001; 3(2):155–159.
20. HHS, U.S.D. U.S. Surgeon General's Family History Initiative, 2005.
21. ASCO. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003; 21(12):2397–2406.
22. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997; 336(20):1401–1408.
23. Tonin P, Weber B, Offit K, et al. Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. Nat Med 1996; 2(11):1179–1183.
24. Aitken J, Bain C, Ward M, et al. How accurate is self-reported family history of colorectal cancer? Am J Epidemiol 1995; 141(9):863–871.
25. Bondy ML, Strom SS, Colopy MW, et al. Accuracy of family history of cancer obtained through interviews with relatives of patients with childhood sarcoma. J Clin Epidemiol 1994; 47(1):89–96.
26. Husson G, Herrinton LJ. How accurately does the medical record capture maternal history of cancer? Cancer Epidemiol Biomarkers Prev 2000; 9(7):765–768.

27. Ivanovich J, Babb S, Goodfellow P, et al. Evaluation of the family history collection process and the accuracy of cancer reporting among a series of women with endometrial cancer. Clin Cancer Res 2002; 8(6):1849–1856.
28. Love RR, Evans AM, Josten DM. The accuracy of patient reports of a family history of cancer. J Chronic Dis 1985; 38(4):289–293.
29. Ziogas A, Anton-Culver H. Validation of family history data in cancer family registries. Am J Prev Med 2003; 24(2):190–198.
30. Schneider KA, DiGianni LM, Patenaude AF, et al. Accuracy of cancer family histories: comparison of two breast cancer syndromes. Genet Test 2004; 8(3):222–228.
31. Murff HJ, Spigel DR, Syngal S. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. JAMA 2004; 292(12):1480–1489.
32. USPSTF. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: recommendation statement. Ann Intern Med 2005; 143(5):355–361.
33. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol 2002; 20(6):1480–1490.
34. Couch FJ, DeShano ML, Blackwood MA, et al. BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. N Engl J Med 1997; 336(20): 1409–1415.
35. Shattuck-Eidens D, Oliphant A, McClure M, et al. BRCA1 sequence analysis in women at high risk for susceptibility mutations. Risk factor analysis and implications for genetic testing. JAMA 1997; 278(15):1242–1250.
36. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med 2004; 23(7):1111–1130.
37. Antoniou AC, Pharoah PP, Smith P, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancer. Br J Cancer 2004; 91(8):1580–1590.
38. Nelson HD, Huffman LH, Fu R, et al. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med 2005; 143(5):362–379.
39. Parmigiani G, Chen S, Iversen ES Jr., et al. Validity of models for predicting BRCA1 and BRCA2 mutations. Ann Intern Med 2007; 147(7):441–450.
40. Petrucelli N, Daly M, Culver JO, et al. BRCA1 and BRCA2 Hereditary Breast/ Ovarian Cancer. GeneReviews 2007 June 19, 2007. Available at http://www.genetests.com. Accessed on November 1, 2007.
41. Berry DA, Iversen ES Jr., Gudbjartsson DF, et al. BRCAPRO validation, sensitivity of genetic testing of BRCA1/BRCA2, and prevalence of other breast cancer susceptibility genes. J Clin Oncol 2002; 20(11):2701–2712.
42. Berry DA, Parmigiani G, Sanchez J, et al. Probability of carrying a mutation of breast-ovarian cancer gene BRCA1 based on family history. J Natl Cancer Inst 1997; 89(3):227–238.
43. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet 1998; 62(1): 145–158.
44. BRCAPRO, 2006. Available at http://astor.som.jhmi.edu/BayesMendel/brcapro.html. Accessed on April 10, 2007.

45. Barcenas CH, Hosain GM, Arun B, et al. Assessing BRCA carrier probabilities in extended families. J Clin Oncol 2006; 24(3):354–360.
46. Euhus DM, Smith KC, Robinson L, et al. Pretest prediction of BRCA1 or BRCA2 mutation by risk counselors and the computer model BRCAPRO. J Natl Cancer Inst 2002; 94(11):844–851.
47. James PA, Doherty R, Harris M, et al. Optimal selection of individuals for BRCA mutation testing: a comparison of available methods. J Clin Oncol 2006; 24(4):707–715.
48. Nanda R, Schumm LP, Cummings S, et al. Genetic testing in an ethnically diverse cohort of high-risk women: a comparative analysis of BRCA1 and BRCA2 mutations in American families of European and African ancestry. JAMA 2005; 294(15):1925–1933.
49. Euhus DM. CancerGene. 2006, UT Southwestern Medical Center at Dallas: http://www4.utsouthwestern.edu/breasthealth/cagene/
50. Myriad. BRACAnalysis Technical Specifications, 2006. Available at: http://www.myriadtests.com/provider/doc/BRACAnalysis-Technical-Specifications.pdf. Accessed in November, 2007.
51. Hendrickson BC, Judkins T, Ward BD, et al. Prevalence of five previously reported and recurrent BRCA1 genetic rearrangement mutations in 20,000 patients from hereditary breast/ovarian cancer families. Genes Chromosomes Cancer 2005; 43(3): 309–313.
52. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA 2006; 295(12): 1379–1388.
53. Haffty BG, Silber A, Matloff E, et al. Racial differences in the incidence of BRCA1 and BRCA2 mutations in a cohort of early onset breast cancer patients: African American compared to white women. J Med Genet 2006; 43(2):133–137.
54. Fackenthal JD, Sveen L, Gao Q, et al. Complete allelic analysis of BRCA1 and BRCA2 variants in young Nigerian breast cancer patients. J Med Genet 2005; 42(3): 276–281.
55. Patenaude AF, Dorval M, DiGianni LS, et al. Correspondance: in reply. J Clin Oncol 2006; 24(18):2971–2971.
56. Offit K, Dorval M, DiGianni LS, et al. The "duty to warn" a patient's family members about hereditary disease risks. JAMA 2004; 292(12):1469–1473.
57. AMA. E-2.131 Disclosure of familial risk in genetic testing. American Medical Association Code of Medical Ethics 2003. Available at http://www.amaassn.org/apps/pf_new/pf_online?f_n=browse&doc=policyfiles/HnE/E-2.131.HTM&&s_t=&st_p=&nth=1&prev_pol=policyfiles/HnE/E-1.02.HTM&nxt_pol=policyfiles/HnE/E-2.01.HTM&. Accessed in November, 2007.
58. Hirschhorn K, Fleisher LD, Godmilow L, et al. Duty to re-contact. Genet Med 1999; 1(4):171–172.
59. Gorman A. A Threat of Cancer, A Drastic Decision. Los Angeles, CA: Los Angeles Times, 2007.
60. Harmon A. Facing Life with A Lethal Gene. New York: New York Times, 2007.
61. DeBare I. Previvor: A personal voyage into the strange new world of genetic testing. San Francisco Chron. November 13, 2005:CM20.
62. NSGC. DNA BANKING: Saving for the Future, 2007. Available at http://www.nsgc.org/dnabanking.cfm.

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Genetic Risk Assessment for Hereditary Endometrial Cancer: Lynch Syndrome

Molly S. Daniels

Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- Women with young age at onset of endometrial carcinoma should be considered for evaluation of Lynch syndrome.
- Personal history of endometrial, colorectal, small bowel, ureter, renal pelvis cancer, and/or a family history of these cancers can be important indicators of Lynch syndrome.
- In addition to family history, studies on tumor tissue, such as immunohistochemistry and microsatellite instability testing, are excellent methods of screening for Lynch syndrome.
- Germline genetic testing of mismatch repair genes is the criterion standard for diagnosis of Lynch syndrome and allows for informative predictive genetic testing of at-risk family members.

INTRODUCTION

Endometrial cancer is the most common gynecologic cancer in U.S. women and accounts for 6% of all cancers in women (1). The lifetime risk of endometrial cancer for the average U.S. woman is 2% to 3% (2), with a median age at diagnosis of 63 years (3). As is the case with breast, colorectal, and most other

Table 1 Selected Risk Factors and Relative Risks for Endometrial Cancer

Risk factor	Relative risk (Ref.)
Obesity (200+ lbs vs. <125 lbs)	7.2 (4)
Nulliparity	2.8 (4)
Early age at menarche (<12 vs. > = 15)	2.4 (4)
Diabetes mellitus	2.1 (5)
Tamoxifen use	2.2–2.3 (6,7)
Combination oral contraceptive use for at least 12 mo	0.6 (10)

cancers, the majority of endometrial cancer is not hereditary. Risk factors for endometrial cancer have been identified, many of which are thought to affect the level of estrogen to which the endometrium is exposed. The strongest risk factor for sporadic endometrial cancer is obesity, with a relative risk of 7.2 for women 200 lbs or greater versus 125 lbs or less (4). Other risk factors include nulliparity (4), early age at menarche (4), diabetes mellitus (5), and the use of tamoxifen (6,7). Unopposed estrogen therapy was found to significantly increase the risk of endometrial cancer (8,9) and is therefore no longer recommended for women who have an intact uterus. Combination estrogen/progesterone oral contraceptives have been shown to decrease the risk of endometrial cancer (10). See Table 1 for a summary of endometrial cancer risk factors and relative risks.

Endometrial cancer risk is also significantly elevated in women with Lynch syndrome. Lynch syndrome is a hereditary cancer predisposition syndrome that causes an increased risk of colorectal cancer, endometrial cancer, and other cancers including ovarian cancer. Lynch syndrome is caused by germline mutations in any one of the mismatch repair genes (MLH1, MSH2, MSH6, PMS2, and possibly others). Loss of function of any of these mismatch repair genes leads to genomic instability, which can eventually lead to cancer. Lynch syndrome is inherited in an autosomal dominant manner; each child of a person with Lynch syndrome has a 50% chance to have inherited the causative mutation, irrespective of gender.

Women with Lynch syndrome have up to a 60% lifetime risk to develop endometrial cancer (11,12), which is comparable to their lifetime risk to develop colorectal cancer. Endometrial cancer is also associated with other hereditary cancer syndromes, including PTEN hamartomatous tumor syndrome (also known as Cowden syndrome) (13) and Peutz–Jeghers syndrome (14). However, as these syndromes are both much rarer than Lynch syndrome and also comprise a lower risk of endometrial cancer, Lynch syndrome is the focus of hereditary cancer risk assessment for women with endometrial cancer.

The mean age of onset of endometrial cancer in women with Lynch syndrome is 47 to 55 years (15–17), which is younger than in the general population. While the term "Lynch syndrome" is used to describe individuals with germline mutations in any of the aforementioned genes, there do appear to be significant genotype/phenotype correlations within the syndrome. For example,

families with MSH2 mutations may have a higher risk of cancer than those with MLH1 mutations (18). In addition, MSH6 mutations, currently thought to be less common than MLH1 or MSH2 mutations, are associated with a preponderance of endometrial cancer and an older age of onset of colorectal cancer (19). The phenotype of Lynch syndrome families with MSH6 or PMS2 mutations is less well understood overall, and further studies are needed.

Individuals with Lynch syndrome are at high risk for second primary cancers. Women with Lynch syndrome who have had both endometrial and colorectal cancer are equally likely to have been diagnosed with colorectal or endometrial cancer as their first or "sentinel" cancer (20). Therefore, it is imperative that women with endometrial cancer be evaluated for the possibility of Lynch syndrome, so that the prevention of colorectal cancer can be integrated into the medical management of those women who do have Lynch syndrome. In addition, the identification of Lynch syndrome in women with endometrial cancer allows for identification of other at-risk family members who would then have the option of undertaking early detection and preventive strategies before any cancer develops. Therefore, this chapter will focus on strategies to identify which women with endometrial cancer are most likely to have Lynch syndrome.

AGE OF ONSET OF ENDOMETRIAL CANCER AS A RISK FACTOR FOR LYNCH SYNDROME

As noted above, the age of onset of endometrial cancer is lower in Lynch syndrome than in the general population. It therefore stands to reason that an earlier age of onset of endometrial cancer could be considered as an indicator of Lynch syndrome. Two studies have examined the prevalence of Lynch syndrome in women diagnosed with endometrial cancer before 50 years of age (21,22). Both studies found that 9% of women diagnosed with endometrial cancer before age 50 years had germline mutations in MLH1, MSH2, or MSH6 and that germline mutations are more likely in women who have a positive family history for Lynch syndrome–associated cancers.

Two important caveats to the correlation of early age of onset of endometrial cancer with Lynch syndrome must be noted. First, Lynch syndrome–associated endometrial cancer does not occur solely in women diagnosed before age 50 years. In a population-based study of women diagnosed with endometrial cancer at any age, 1.8% (10 of 543) were found to have Lynch syndrome mutations (16). Six out of ten of these women were diagnosed after age 50 years. Therefore, Lynch syndrome must still be considered in women diagnosed with endometrial cancer after age 50 years.

The second caveat is that risk factors for sporadic endometrial cancer, such as obesity, can also result in an earlier age of onset. Lu et al. (22) found that among women diagnosed with endometrial cancer before age 50 years who did not have identifiable Lynch syndrome mutations, the median body mass index

(BMI) was 37.5, indicating that these women are obese. Median BMI in women with Lynch syndrome mutations was significantly lower at 27.6. When considering the risk of Lynch syndrome in a young woman with endometrial cancer, sporadic risk factors such as obesity should also be considered.

Overall, the population of women diagnosed with endometrial cancer before age 50 years is enriched for Lynch syndrome; whereas 1.8% of women with endometrial cancer unselected for age have Lynch syndrome (16), 9% of women diagnosed before age 50 years have Lynch syndrome (21,22). Therefore, young age of onset of endometrial cancer can be used as a "red flag" for consideration of Lynch syndrome. However, young age of onset lacks both sensitivity and specificity as a screening criterion for Lynch syndrome, for the reasons noted above.

PERSONAL HISTORY OF OTHER CANCERS AS A RISK FACTOR FOR LYNCH SYNDROME

One of the hallmark features of hereditary cancer predisposition is the presence of multiple primary tumors in one individual. This criterion may also be used to identify women at risk for Lynch syndrome. Millar et al. (23) found that 7 of 40 women (18%) who had both endometrial and colorectal cancer had a germline mutation in MLH1 or MSH2. When both cancers were diagnosed before age 50 years, the mutation detection rate was higher (6 of 14, 43%). Any woman who has had both endometrial and colorectal cancer should undergo further evaluation for the possibility of Lynch syndrome.

The question has been raised about whether the same holds true for women who have had synchronous primary endometrial and ovarian cancers. The risk of ovarian cancer is elevated in women who have Lynch syndrome and has been estimated to be 12% (11). Therefore, a woman who has had synchronous primary ovarian and endometrial cancers meets the criterion of having multiple primary Lynch syndrome–related tumors. However, synchronous primary endometrial and ovarian cancer is a distinct clinical phenomenon that is thought to be linked to an underlying hormonal field effect and is characterized by early age of onset and the presence of sporadic endometrial cancer risk factors such as obesity and nulliparity (24). Soliman et al. (25) found evidence of Lynch syndrome in 7 of 102 (7%) women with synchronous primary endometrial and ovarian cancers. While this is higher than the 1.8% risk for Lynch syndrome in a general population sample of women with endometrial cancer (16), this difference could simply be due to the skewing toward younger age in the population of women with synchronous primary endometrial and ovarian cancers. (In this study, half of the sample were younger than 50 years old at diagnosis.). In addition, all seven women who met criteria for Lynch syndrome had a prior history or a first-degree relative with a Lynch syndrome–associated cancer. In summary, synchronous primary endometrial and ovarian cancers do occur in women with Lynch syndrome. Women with synchronous primary endometrial and ovarian

Table 2 Risk of Lynch Syndrome for Women with Endometrial Cancer, Based on Their Personal Cancer History

Personal history of cancer	Percentage likelihood of Lynch syndrome (Ref.)
Endometrial cancer at any age	1.8% (16)
Endometrial cancer diagnosed under age 50 yr	9% (21,22)
Endometrial and colorectal cancer at any age	18% (23)
Endometrial and colorectal cancers diagnosed under age 50 yr	43% (23)
Synchronous primary endometrial and ovarian cancers	7% (25)

cancers who have additional personal or family history suggestive of Lynch syndrome should be further evaluated.

Several other cancer types have been found in association with Lynch syndrome, including gastric cancer, small bowel cancer, transitional cell carcinomas of the ureter and renal pelvis, hepatobiliary tract cancer, sebaceous skin cancers, and brain tumors. The risk of Lynch syndrome in a woman who has had both endometrial cancer and one of these other cancers has not been quantified to our knowledge. It would seem prudent, however, to consider further evaluation for Lynch syndrome for any woman who has had both endometrial cancer and any of the other Lynch syndrome–associated cancers. Table 2 summarizes risk of Lynch syndrome for women with endometrial cancer, based on their personal cancer history.

FAMILY HISTORY OF CANCER AS A RISK FACTOR FOR LYNCH SYNDROME

Cancer family history is a hallmark feature of hereditary cancer predisposition syndromes. Criteria, including the Amsterdam criteria (26) and the Amsterdam II criteria (27), have been developed to identify Lynch syndrome on the basis of family history. While the original Amsterdam criteria addressed family history of colorectal cancer only, the Amsterdam II criteria incorporate extracolonic cancers, including endometrial cancer. The Amsterdam II criteria are outlined in Table 3. It is widely acknowledged that not all families that have Lynch syndrome will meet these criteria. In a small family, for example, there may simply not be enough at-risk family members to express the phenotype this clearly. In addition, the Amsterdam criteria were developed prior to the identification of MSH6 families' distinct phenotype of lower penetrance and later onset colorectal cancer (19) and therefore may not be sensitive in identifying families with MSH6 mutations.

Risk assessment models have been developed to predict the chance of Lynch syndrome given a certain family history and, in some cases, the results of

Table 3 Amsterdam II Criteria

Three relatives with a Lynch syndrome-associated cancer[a], one of which is a first-degree relative (i.e., parent, child, or sibling) of the other two;
At least two successive generations affected;
At least one diagnosis under age 50 yr;
Exclusion of familial adenomatous polyposis in any cases of colorectal cancer;
Tumor(s) should be verified by pathologic examination.

[a]Lynch syndrome–associated cancers were defined as colorectal, endometrial, small bowel, ureter, and renal pelvis.
Source: From Ref. 27.

tumor microsatellite instability (MSI) analysis and immunohistochemical (IHC) analysis (28–32). Two of these models in particular, MMRpro (30) and $PREMM_{1,2}$ (28), have been developed from and/or validated with relatively large, clinic-based sample sizes.

The $PREMM_{1,2}$ model was constructed and validated from 1914 unrelated probands from whom blood samples were submitted to Myriad Genetic Laboratories, Inc. for clinical MLH1 and MSH2 genetic testing. Personal and family history information for these probands was collected from test requisition forms filled out by the ordering physician. MSI and IHC data were not routinely collected on the whole sample and were not included in this model. The authors found that MLH1 or MSH2 mutations were present in 15% of the probands and that the strongest predictors of the presence of a mutation were the proband having had colorectal or endometrial cancer, particularly two or more colorectal cancer diagnoses; age at colorectal cancer diagnosis; and the number of first-degree relatives with colorectal or endometrial cancer. $PREMM_{1,2}$ calculates the risk of a given proband having a MLH1 or MSH2 mutation based on personal history of colorectal cancer, endometrial cancer, other Lynch-associated cancer, and colon adenomas; family history of colorectal cancer, endometrial cancer, and other Lynch-associated cancer in first- or second-degree relatives; and age at diagnosis of colorectal and endometrial cancers. This model is available for use at http://www.dfci.org/premm.

$PREMM_{1,2}$ is easy to use and can provide clinicians with an assessment of the likelihood of detecting an MLH1 or MSH2 mutation on the basis of personal and family history factors noted above. Accuracy of the model was demonstrated with the validation sample, which showed an area under the receiver operating characteristic curve of 0.80 (95% CI, 0.76–0.84). There are several acknowledged limitations to this model. The model does not incorporate MSI and IHC data, which (as discussed in the next section) can be very helpful in distinguishing colorectal and endometrial cancers that are associated with Lynch syndrome. The model is currently not able to estimate risk of an MSH6 mutation. The personal and family history data used in the model were neither centrally collected nor verified, but instead were provided by the many different health

care professionals who ordered clinical genetic testing on these patients. Only those cancer diagnoses that the health care professional chose to report are represented, and there was no data collected on unaffected relatives.

The MMRpro model was developed from published estimates of mutation prevalence and penetrance of the mismatch repair genes and of the sensitivity and specificity of MSI, IHC, and germline genetic testing. This model was validated on 279 individuals from 226 clinic-based families. These individuals were all seen in cancer genetics clinics and/or enrolled in familial colorectal cancer registries, and extensive family history information was therefore available to the investigators. The model incorporates personal and family history (in first- and second-degree relatives) of colorectal and endometrial cancer, age at diagnosis of colorectal and endometrial cancer, any available results of MSI/IHC and germline genetic testing, and the current age of all first- and second-degree relatives (whether or not they had cancer).

MMRpro uses the input data and Bayesian analysis to calculate the chance that a given individual has a germline MLH1, MSH2, or MSH6 mutation, including calculating the residual risk for an individual to have Lynch syndrome after receiving inconclusive negative genetic test results. It can also be used to calculate the probability that an asymptomatic proband will develop colorectal and/or endometrial cancer. The authors argue that this model is particularly helpful in determining when to offer germline genetic testing without prescreening by MSI/IHC or when MSI/IHC analysis cannot be performed (e.g., when tumor blocks are not available or when the proband is unaffected). In addition, it could aid clinicians in determining when to pursue further genetic testing when the initial genetic testing is inconclusive and/or negative. MMRpro is available for download at http://astor.som.jhmi.edu/BayesMendel/ and at http://www4.utsouthwestern.edu/breasthealth/cagene/.

MMRpro will be most accurate when a complete pedigree of all first- and second- degree relatives and their cancer status is available for input; however, the model can accommodate missing data. The authors report high accuracy, with their validation sample demonstrating a concordance index of 0.83 (95% CI, 0.78–0.88) and a ratio of observed to predicted cases of 0.94 (95% CI, 0.84–1.05). One limitation of this model is the lack of incorporation of data regarding the methylation status of the MLH1 promoter in cases of high MSI and loss of staining for MLH1; incorporation of this information would improve the specificity of these tumor study results for Lynch syndrome. In addition, the model relies heavily on the currently available literature regarding prevalence, penetrance, sensitivity, and specificity. As the authors acknowledge, these estimates may change over time as further research is performed, particularly in the case of the estimates of mismatch repair gene mutation carriers' cancer risks.

Both MMRpro and PREMM$_{1,2}$ were validated using high-risk populations (patients seen in cancer genetics clinics and/or enrolled in hereditary colorectal cancer registries and patients for whom clinical MLH1 and MSH2 genetic testing were ordered, respectively). These populations may well be representative of

patients presenting for hereditary cancer evaluation in high-risk clinics. However, further validation studies would be needed before these models could be applied to a general population sample, such as using them to screen all colorectal cancer or endometrial cancer patients for Lynch syndrome.

Clearly, family history is a valuable tool in identifying women with endometrial cancer who are at risk for Lynch syndrome and for quantifying how likely it is that a Lynch syndrome mutation would be identified through clinical genetic testing. However, family history information can be limited by a variety of circumstances, including small family size, limited family communication about cancer family history, misattributed paternity, adoption, etc. Family history also does not identify which of the mismatch repair genes is most likely to contain the pathogenic mutation. Therefore, if Lynch syndrome clinical genetic testing is undertaken based solely on family history, then full sequence and large rearrangement analysis of all mismatch repair genes must be considered, which is time consuming and expensive and can lead to inconclusive negative results.

THE ROLE OF MSI AND IHC ANALYSIS OF TUMORS TO IDENTIFY LYNCH SYNDROME

Lynch syndrome is unique among hereditary cancer predisposition syndromes in that tumors caused by an underlying mismatch repair gene defect demonstrate distinct molecular features. Loss of function of the mismatch repair pathway can be directly visualized through MSI analysis. Microsatellites are short tandem DNA repeat sequences, generally mono- or dinucleotide repeats. These repeat sequences can expand and/or contract significantly in a tumor where mismatch repair is impaired, and this variation in microsatellite repeat length between normal and tumor tissue can then be detected using a polymerase chain reaction (PCR)-based assay. The National Cancer Institute (NCI) has developed a consensus panel of five microsatellites that should be included in MSI analysis (33). The presence of MSI in two or more of the five microsatellites classifies the tumor as MSI-high (MSI-H). Tumors lacking MSI at any of the five markers are classified as microsatellite stable (MS-stable). MSI-low (MSI-L) is used to indicate a tumor where only one of the five microsatellites demonstrates MSI; the clinical significance of this finding is not known. It should be noted that this consensus panel was developed for use in colorectal cancers; however, the same panel does seem to be effective in endometrial cancers as well.

However, not all tumors that demonstrate MSI are caused by Lynch syndrome. MSI can also occur sporadically, primarily through epigenetic hypermethylation of the MLH1 promoter, leading to silencing of the gene. Up to 20% of all colorectal tumors demonstrate MSI, most often due to hypermethylation of the MLH1 promoter (34). Similarly, up to 29% of endometrial cancers demonstrate MSI, again most often due to hypermethylation of the MLH1 promoter

Table 4 Expected Immunohistochemistry Results Given Inactivation of a Mismatch Repair Gene

Inactivation of this gene:	Leads to this IHC result (staining present = "+"; staining absent = "−"):			
	MLH1	MSH2	MSH6	PMS2
MLH1	−	+	+	−
MSH2	+	−	−	+
MSH6	+	+	−	+
PMS2	+	+	+	−

Abbreviation: IHC, immunohistochemical.

(35). Assays have been developed to detect the presence of hypermethylation of the MLH1 promoter, and these assays can be useful in determining whether MSI is sporadic or due to Lynch syndrome.

IHC analysis of tumors is useful in both identifying whether a particular tumor is due to an underlying mismatch repair gene defect as well as identifying the specific mismatch repair gene involved. Mismatch repair proteins function in heterodimers, therefore loss of expression of one mismatch repair gene can lead to loss of staining for more than one mismatch repair protein. Table 4 lists the expected staining pattern for inactivation of the MLH1, MSH2, MSH6, or PMS2 genes. These staining patterns are certainly not absolute, and exceptions will occur. Promoter hypermethylation should be considered whenever IHC analysis demonstrates loss of expression of MLH1.

MSI/IHC/MLH1 promoter hypermethylation studies have been shown to be effective in identifying women with endometrial cancer who have Lynch syndrome. Goodfellow et al. (35) screened 441 consecutive endometrial cancer cases by MSI. Of these cases, 127 samples demonstrated high MSI, of which 92 (72.4%) also demonstrated hypermethylation of the MLH1 promoter and are therefore unlikely to be attributable to Lynch syndrome. Of the 35 endometrial cancers that were MSI-H and did not show MLH1 promoter hypermethylation, five were found to have germline MSH2 mutations and seven were found to have germline MSH6 mutations (MLH1 mutations were not ascertained). No germline MSH2 or MSH6 mutations were identified in a sampling of the endometrial cancers that were MSI-H and methylated, MSI-L, or MS-stable.

Hampel et al. (16) screened an unselected, population-based sample of 543 endometrial cancers by MSI and performed germline genetic testing of MLH1, MSH2, and MSH6 on all women who had MSI-H tumors (118 patients). Of 118 patients, nine had confirmed deleterious mismatch repair gene mutations. In addition, one MS-stable tumor demonstrated abnormal MSH6 IHC analysis, and a germline MSH6 mutation was subsequently found. This study demonstrates the

feasibility of screening endometrial cancers by MSI and IHC analysis. It also demonstrates that MSI analysis can miss cases of Lynch syndrome. IHC analysis can also miss cases of Lynch syndrome. As an example, a missense mutation could lead to formation of a nonfunctional mismatch repair protein that would yet still be detectable by IHC analysis. For this reason, when the index of suspicion for Lynch syndrome is high for a particular patient, it is preferable to perform both MSI and IHC analysis.

In conclusion, MSI/IHC/MLH1 promoter hypermethylation analysis is an effective tool to identify women with endometrial cancer who have Lynch syndrome. The use of IHC prior to initiating germline genetic testing allows for the genetic testing to be targeted to the gene(s) most likely to be involved. MSI, IHC, and MLH1 promoter hypermethylation analysis can either be performed "in-house" by a qualified molecular pathology laboratory, or can be sent out to commercial labs that offer this service (see www.genetests.org). In the case of inconclusive negative or variant of uncertain significance genetic test results, the tumor study results are crucial in aiding in the interpretation of that genetic test result. For example, if an individual's endometrial tumor is MSI-H with demonstrated absence of staining for MSH2 and MSH6 and genetic testing of MSH2 and MSH6 yielded negative or variant results, that individual still most likely has Lynch syndrome and should be counseled accordingly regarding cancer risk. Conversely, Lindor et al. (36) studied families who met Amsterdam I criteria but for whom tumor studies showed no evidence of mismatch repair defect. These families had a lower incidence of colorectal cancer than families with tumor evidence of mismatch repair defect. The authors therefore conclude that these "familial colorectal cancer type X" families should not be described or counseled as having Lynch syndrome.

At a recent consensus conference on Lynch syndrome and endometrial cancer (personal communication), clinical guidelines for identifying endometrial cancer patients at risk for Lynch syndrome were proposed. The guidelines proposed are that IHC analysis be routinely performed on endometrial cancers meeting any of the following criteria: diagnosed before age 50 years, synchronous or metachronous colorectal cancer or other Lynch-associated cancer, or first-degree relative with colorectal cancer or other Lynch-associated cancer. Women with abnormal IHC analysis results, or whose personal and/or family history is otherwise thought to be suggestive of Lynch syndrome, would then be referred for further evaluation. Guidelines have already been proposed for MSI analysis of colorectal cancers (33,37).

MOLECULAR GENETIC TESTING FOR LYNCH SYNDROME

The gold standard for the diagnosis of Lynch syndrome is molecular genetic testing. When a clearly deleterious mutation in MLH1, MSH2, or MSH6 is detected, this confers an unequivocal diagnosis of Lynch syndrome.

Identification of a deleterious mutation in a proband also allows for site-specific predictive genetic testing of his or her relatives. While initial genetic testing of a proband is expensive (~$1000 per gene analyzed), follow-up site-specific genetic testing of relatives is less expensive (~$300–$400). Clinical genetic testing for Lynch syndrome mutations is available through several commercial laboratories in the United States (see www.genetests.org).

As mentioned in the previous sections on family history and MSI/IHC analysis, the currently clinically available genetic testing does have limitations. Sensitivity is not 100%; it is possible for an individual who clearly has Lynch syndrome to receive negative genetic test results. Sensitivity of clinically available genetic testing has improved over time, and this trend will almost certainly continue. The most important innovation within the last few years has been the addition of specific analyses for large gene rearrangements; this is now generally performed in tandem with direct sequencing of exons and immediately adjacent intronic regions. Large gene rearrangements are not detected by direct sequencing in diploid organisms (including humans), unless a technique such as conversion analysis is employed (38). However, they can be detected without conversion analysis through techniques such as Southern blotting or multiplex ligation-dependent probe amplification (MLPA) analysis. Large gene rearrangements are currently thought to account for approximately 5% of MLH1 mutations and approximately 20% of MSH2 mutations (39,40). In the United States, a specific MSH2 founder deletion of exons one to six has been identified (41). Therefore, clinical genetic testing should routinely include both sequence analysis and large gene rearrangement analysis.

Even when both techniques are used, however, mutations can still be missed. Mutations in promoters or within introns could be missed, as these regions are not routinely sequenced in their entirety. While PMS2 mutations probably account for a minority of cases of Lynch syndrome, the current lack of clinically available PMS2 genetic testing does limit sensitivity of mismatch repair gene testing overall. A negative result from genetic testing is therefore only clearly interpretable in the case of predictive genetic testing for a known familial mutation. Genetic variants of uncertain significance, such as a missense mutation whose functional significance is not known, can also be detected. Any given variant could be a harmless polymorphism versus a true deleterious mutation. At this time, there are no universally accepted criteria for what standard of evidence is necessary to interpret the significance of a variant, and therefore this genetic test result is of limited or no clinical utility.

When the initial proband in a family receives negative or variant genetic test results, that result should be considered inconclusive. Interpretation of the inconclusive negative or variant genetic test result should be specific to the personal and family history of the proband. As discussed above, the results of MSI/IHC/promoter hypermethylation studies can be especially helpful in this case.

GENETIC COUNSELING FOR LYNCH SYNDROME

Genetic counseling should be provided both pre- and post genetic testing. The importance of pre- and posttest counseling and informed consent has been affirmed by the American Society of Clinical Oncology (42). Genetic test results can be a powerful tool in cancer prevention and early detection, but the results will only accomplish these goals to the extent that the patient is willing, able, and ready to use the information provided.

The focus of pretest genetic counseling is on risk assessment, anticipatory guidance regarding the possible results, and informed consent. The construction of a complete pedigree, encompassing at least three generations, will allow the clinician to provide the patient with an accurate risk assessment regarding the likelihood of Lynch syndrome as well as providing a context within which to interpret the results of genetic testing. Risk assessment would also incorporate any additional relevant information, such as results of tumor studies. Prior to the initiation of genetic testing, patients should be aware of the three possible results (in the case of initial genetic testing of a proband): positive, uninformative negative, and variant of uncertain significance as well as what the medical management recommendations for the patient and family would be in each circumstance. Pretest counseling should also address ethical and psychosocial issues, which are covered in other chapters; some common areas of concern include emotional sequelae of the genetic test results, fears regarding genetic discrimination, and strategies for informing at-risk family members of genetic test results. This comprehensive discussion will provide patients with the information necessary to make an informed decision regarding hereditary cancer genetic testing.

Posttest counseling focuses on interpretation of the genetic test results in the context of personal and family history. This discussion should include information on the screening and risk reduction options available to the patient based on the interpretation of the genetic test results. Patients should be reminded of the importance of sharing their results with at-risk relatives and provided with tools such as family letters to aid them in doing so.

CONCLUSION

The identification of women with endometrial cancer who have Lynch syndrome is crucial to their future medical management as well as to their at-risk relatives. Age of onset, personal history of other cancers, and family history of cancer are all important indicators or red flags of Lynch syndrome. Tumor screening studies (MSI, IHC, MLH1 promoter hypermethylation) are an appropriate first step in the evaluation of women with endometrial cancer who may have Lynch syndrome; the results of these tumor studies will aid in the decision on whether germline genetic testing, and of which gene(s), is warranted. Routine IHC screening of all endometrial cancers meeting certain criteria should be

considered (37). Germline genetic testing in the absence of tumor studies may also be considered, but the limited ability to interpret a negative result in this case must be fully appreciated.

CASE REPORT

S.W. is a 57-year-old woman who initially presented to her primary physician with heavy vaginal bleeding. Her workup revealed a high-grade papillary serous endometrial carcinoma. Hysterectomy and staging procedures revealed a stage IIb endometrial carcinoma. She was subsequently treated with chemotherapy and radiation.

A detailed family history was suggestive of Lynch syndrome. Her brother was diagnosed with right-sided colon cancer at age 39 years. In addition, her father was diagnosed with "kidney cancer"; medical records review revealed this to be a transitional cell carcinoma of the renal pelvis. S.W. was referred for genetic counseling; tumor studies were subsequently ordered, which showed that her endometrial tumor exhibited high microsatellite instability and immunohistochemical loss of staining for MSH2 and MSH6. Genetic testing of the MSH2 gene revealed a germline MSH2 mutation, confirming the diagnosis of Lynch syndrome. S.W. has chosen to pursue annual colonoscopy regarding her risk of colorectal carcinoma. She informed her two children of their 50% risk to have inherited Lynch syndrome, and they are considering predictive genetic testing.

LEARNING POINTS

- Endometrial cancer can be the presenting cancer in women with Lynch syndrome.
- Endometrial cancer patients who have a family history of colorectal and/or other Lynch syndrome-associated cancers should be assessed for Lynch syndrome.
- Immunohistochemical analysis of endometrial tumors for mismatch repair proteins contributes to the Lynch syndrome risk assessment and allows for gene-specific genetic testing.
- Both the endometrial cancer patient with Lynch syndrome and her at-risk relatives can benefit from cancer risk reduction strategies, including earlier and more frequent colonoscopy.

REFERENCES

1. National Cancer Institute. Endometrial cancer treatment (PDQ®). Available at: http://www.cancer.gov/cancertopics/pdq/treatment/endometrial/healthprofessional. Accessed May 2007.
2. Ries LAG, Melbert D, Krapcho M, et al., eds. SEER Cancer Statistics Review, 1975–2004. Bethesda, MD: National Cancer Institute, 2007. Available at: http://

seer.cancer.gov/csr/1975_2004/, based on November 2006 SEER data submission, posted to the SEER Web site, 2007.
3. Ries LAG, Eisner MP, Kosary CL, et al., eds. SEER Cancer Statistics Review, 1975–2002. Bethesda, MD: National Cancer Institute, 2005.
4. Brinton LA, Berman ML, Mortel R, et al. Reproductive, menstrual, and medical risk factors for endometrial cancer: results from a case-control study. Am J Obstet Gynecol 1992; 167(5):1317–1325.
5. Friberg E, Orsini N, Mantzoros CS, et al. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. Diabetologia 2007; 50(7):1365–1374. [Epub 2007, May 3].
6. van Leeuwen FE, Benraadt J, Coebergh JW, et al. Risk of endometrial cancer after tamoxifen treatment of breast cancer. Lancet 1994; 343(8895):448–452.
7. Fisher B, Costantino JP, Redmond CK, et al. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994; 86(7):527–537.
8. Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. N Engl J Med 1975; 293(23):1167–1170.
9. Jick SS, Walker AM, Jick H. Estrogens, progesterone, and endometrial cancer. Epidemiology 1993; 4(1):20–24.
10. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. Combination oral contraceptive use and the risk of endometrial cancer. JAMA 1987; 257(6):796–800.
11. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214–218.
12. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997; 6(1):105–110.
13. GeneReviews, NIH. PTEN Hamartoma Tumor Syndrome (PHTS). Available at: http://www.genetests.org/query?dz=phts. Accessed May 2007.
14. GeneReviews, NIH. Peutz-Jeghers Syndrome. Available at: http://www.genetests.org/query?dz=pjs. Accessed May 2007.
15. Hakala T, Mecklin JP, Forss M, et al. Endometrial carcinoma in the cancer family syndrome. Cancer 1991; 68(7):1656–1659.
16. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer research 2006; 66(15):7810–7817.
17. Watson P, Vasen HF, Mecklin JP, et al. The risk of endometrial cancer in hereditary nonpolyposis colorectal cancer. Am J Med 1994; 96(6):516–520.
18. Vasen HF, Stormorken A, Menko FH, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001; 19(20):4074–4080.
19. Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 2004; 127(1):17–25.
20. Lu K, et al. Gynecological malignancy as a "Sentinel Cancer" for women with HNPCC. Gynecol Oncol 2004; 92:421.
21. Berends MJ, Wu Y, Sijmons RH, et al. Toward new strategies to select young endometrial cancer patients for mismatch repair gene mutation analysis. J Clin Oncol 2003; 21(23):4364–4370.

22. Lu KH, Schorge JO, Rodabagh KJ, et al. Prospective determination of prevalence of Lynch syndrome in young women with endometrial cancer. J Clin Oncol 2007; 25(33): 5158–5164. [Epub 2007 Oct 9].
23. Millar AL, Pal T, Madlensky L, et al. Mismatch repair gene defects contribute to the genetic basis of double primary cancers of the colorectum and endometrium. Hum Mol Genet 1999; 8(5):823–829.
24. Soliman PT, Slomovitz BM, Broaddus RR, et al. Synchronous primary cancers of the endometrium and ovary: a single institution review of 84 cases. Gynecol Oncol 2004; 94(2):456–462.
25. Soliman PT, Broaddus RR, Schmeler KM, et al. Women with synchronous primary cancers of the endometrium and ovary: do they have Lynch syndrome? J Clin Oncol 2005; 23(36):9344–9350.
26. Vasen HF, Mecklin JP, Khan PM, et al. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991; 34(5):424–425.
27. Vasen HF, Watson P, Mecklin JP, et al. New clinical criteria for hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999; 116(6):1453–1456.
28. Balmana J, Stockwell DH, Steyerberg EW, et al. Prediction of MLH1 and MSH2 mutations in Lynch syndrome. JAMA 2006; 296(12):1469–1478.
29. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006; 354(26):2751–2763.
30. Chen S, Wang W, Lee S, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA 2006; 296(12):1479–1487.
31. Lipton LR, Johnson V, Cummings C, et al. Refining the Amsterdam criteria and Bethesda guidelines: testing algorithms for the prediction of mismatch repair mutation status in the familial cancer clinic. J Clin Oncol 2004; 22(24):4934–4943.
32. Marroni F, Pastrello C, Benatti P, et al. A genetic model for determining MSH2 and MLH1 carrier probabilities based on family history and tumor microsatellite instability. Clin Genet 2006; 69(3):254–262.
33. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96(4):261–268.
34. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Gen 2001; 69(4):780–790.
35. Goodfellow PJ, Buttin BM, Herzog TJ, et al. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. Proc Natl Acad Sci U S A 2003; 100(10):5908–5913.
36. Lindor NM, Rabe K, Petersen GM, et al. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. JAMA 2005; 293(16):1979–1985.
37. Rodriguez-Bigas MA, Boland CR, Hamilton SR, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997; 89(23):1758–1762.

38. Casey G, Lindor NM, Papadopoulos N, et al. Conversion analysis for mutation detection in MLH1 and MSH2 in patients with colorectal cancer. JAMA 2005; 293(7): 799–809.
39. Charbonnier F, Olschwang S, Wang Q, et al. MSH2 in contrast to MLH1 and MSH6 is frequently inactivated by exonic and promoter rearrangements in hereditary nonpolyposis colorectal cancer. Cancer Res 2002; 62(3):848–853.
40. Wagner A, Barrows A, Wijnen JT, et al. Molecular analysis of hereditary non-polyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene. Am J Hum Gen 2003; 72(5):1088–1100.
41. Lynch HT, de la Chapelle A, Hampel H, et al. American founder mutation for Lynch syndrome. Prevalence estimates and implications. Cancer 2006; 106(2):448–452.
42. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003; 21(12):2397–2406.

18

Legal Aspects of Genetic Testings

Patrick M. Lynch

Department of GI Medicine, Nutrition, and Hepatology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Kate M. Kraycirik

Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- Federal and state laws offer protection against insurance and employment discrimination on the basis of genetic information.
- The American Medical Association and the American Society of Clinical Oncology suggest that physicians encourage their patients to share genetic information from test results with family members.
- Clinical Practice Guidelines regarding hereditary cancer testing and management are evolving. Physicians need to stay abreast of current practice recommendations.

INTRODUCTION

This chapter will highlight legal issues involving genetic testing for gynecologic hereditary cancer syndromes. One of the most significant concerns that patients and health care providers express regarding genetic testing is the possibility of genetic discrimination. We will explore current legislation regarding health and life insurance. We will discuss the duties of the physician with respect to the

"duty to warn" other family members when a patient has a positive test for a hereditary cancer predisposition. Finally, the duty to provide a standard of care regarding referral to genetic testing and management of at-risk individuals will be discussed in relation to medical liability.

LEGISLATION ON GENETIC DISCRIMINATION

Fear of genetic discrimination is a commonly cited reason for not undergoing genetic testing for a hereditary cancer syndrome. This fear includes fears of discrimination in life and health insurance as well as fear of workplace discrimination. Likewise, the fears of discrimination extend beyond the individual to include fears of discrimination for family members. Multiple population-based studies have shown that patients are extremely concerned about genetic discrimination, and this fear plays a significant role in the decision whether or not to pursue genetic testing (1–3).

Many states have adopted legislation prohibiting access to existing genetic information or using knowledge of test information as a basis for denying insurance or "rating" individuals (4). Thus far in 2007, 41 states have passed legislation that protects the public from genetic discrimination by insurance companies, and 32 states have passed laws protecting against discrimination in the workplace (5). However, the state laws vary greatly in terms of defining genetic information and providing privacy and confidentiality guidelines. Some states consider family history genetic information, while other states narrow the definition to only genetic test results (4,6). The lack of definition of what constitutes genetic information leaves vast room for interpretation. The extent to which these new laws can be defined and enforced is still undetermined, as very few issues have been brought to court.

The Health Insurance Portability and Accountability Act (HIPAA) offers some protection against genetic discrimination to people who are members of group health insurance plans. Employment-based insurance, a type of group health insurance, which is the most common form of health insurance coverage in the United States, is based on membership in a large pool of individuals of widely varying personal health risks and costs. Insurance premiums are calculated for the pool, and individual rates do not vary based on the risk profile of each member (6). Under HIPAA, protection is offered to individuals who are members of a group plan, including provisions from being denied insurance, having insurance canceled, and having rates raised due to an individual's pre-existing condition (4,9). However, HIPAA would allow insurance companies to raise premiums or deny coverage to an entire group based on the medical records of one member of that group. Thus, this federal law offers only limited protection and has not yet been adequately defined by the court. Private health

insurance more closely resembles life insurance, with individual risk assignment the norm. Typically, genetic carrier status *can* be considered as a preexisting condition that constitutes a basis for either denying coverage or charging more for it (6).

For the last few years, there has been an attempt to pass national legislation that would broaden protection from genetic discrimination protection to national legislation. Opponents have argued that the national legislation is unnecessary as there is no current evidence that discrimination is currently a threat (7). However, a new act, the Genetic Information Nondiscrimination Act (GINA) of 2008, offers protection to individuals from health insurance and employment discrimination on the basis of genetic information. While some protection is offered by HIPAA and various state laws, GINA strengths and broadens the existing safeguards by limiting an insurers' ability to raise rates for an entire group, and prohibits individual health insurers for determining eligibility or premiums on the basis of genetic information (8). GINA offers protection from employment discrimination by prohibiting employers from requesting or requiring a person to undergo a genetic test, or using a person's genetic information in making employment decisions. Although opposing voices still exist, this federal legislation reflects the rise of public awareness and societal concerns about the issue of genetic discrimination.

Life insurance companies argue that they would be put at an unfair advantage if individuals were able to obtain life insurance based on their own knowledge of carrier status, without the would-be insurer having access to the same information. The argument has to do with "adverse selection" whereby a woman with, for example, a BRCA mutation obtains a million dollar term life insurance policy (10,11). She knows her risk of early cancer death is increased, but if the insurer does not, the policy cannot be priced according to standard actuarial principles. The insurance company loses money and/or must charge the rest of the pool of insured more to compensate for the distorted pricing for the individual in question. These same authors argue that they should have access to specific test information just as they have access to family history information (10). Therefore, patients should be counseled that while some laws exist to protect against health insurance discrimination, no laws exist to protect against life insurance discrimination.

Employment discrimination based on genetic information is also a concern. The introduction of state legislation and HIPAA offers some protection against employment discrimination. However, as with insurance discrimination, it is difficult to predict exactly what protection these laws will offer as they have not yet been tested in the courts (12). In 1995, the Equal Employment Opportunity Commission (EEOC) stated that the American Disabilities Act would offer protection to individuals who were discriminated on the basis of "genetic information relating to illness, disease, or other disorders" (12). As with state

legislation, this statement offers ambiguous protection due to the lack of court cases.

The possibility of genetic discrimination should be discussed with the patient during the informed consent process. Interestingly, in the 15 years since the discovery of the BRCA1 gene, thousands of hereditary cancer genetic tests have been performed, and to our knowledge, there have been no documented cases of an individual losing health insurance based on hereditary cancer genetic test information. Rather, clinicians have noted that the fear of genetic discrimination prevents patients from undergoing genetic testing. Therefore, clinicians and genetic counselors have a responsibility to adequately address the concerns of the patient, to help them weigh the potential risks of genetic testing against the potential benefits, and even in the absence of concrete answers, provide direction to other sources of helpful information.

DUTIES AND OBLIGATIONS OF THE PHYSICIAN

Duty to Warn

Both ethics and the law contribute to the discussion surrounding the physician's duty to warn other family members when a patient has a positive test for a mutation for a hereditary cancer susceptibility gene. When a patient with a positive genetic test result indicating a hereditary predisposition to a cancer syndrome does not wish to share this information with family members, the clinician is left in both a legal and ethical dilemma. Offit et al. discuss how the "beneficence" principle of informing a relative directly conflicts with the "autonomy" of the tested individual to decide when and how to disclose personal health information. They conclude that although well intentioned, overriding the autonomy of a patient is paternalistic and recommend that physicians do not override the right of the patient to confidentiality (12).

Case law has helped to define the obligations of a physician regarding the duty to warn family members about hereditary cancer risk. In the Florida case *Pate v. Threkel*, the court ruled that warning and educating the affected patient, in this case with medullary thyroid cancer, would allow the doctor's duty to be met, and there was no need for other familial notification (13,14). However, in the case *Safer v. Estate of Pack*, litigated in 1996, the court expanded the definition of the duties of physicians to warn. In this case, the daughter of a patient with familial adenomatous polyposis (FAP) who died from colon cancer later developed colon cancer herself at the age of 36 years (15). She sued the estate of her father's surgeon claiming: (*i*) that he had a duty to warn those known to be at risk of avoidable harm from a genetically transmissible condition, (*ii*) that the physician's duty did extend to members of the immediate family of his patient, and (*iii*) that he had breached these duties. Although the initial case was dismissed, the decision was reversed by a New Jersey Appeals Court that ruled "a physician's interest and duties may extend beyond the interests of the immediate

family" and "the attending physician had the distinct and definite duty to warn the parents to monitor their children's health conditions" (15).

Genetic testing results are unique among medical tests in that a positive germline mutation in an individual has direct medical implications on family members who may harbor the same deleterious mutation. While the clinician may feel obligations to alert family members of the test results when a patient does not wish to disclose the results, patient confidentiality must be maintained. Strict laws, such as the Standards for Privacy of Individually Identifiable Health Information (Privacy Rule) passed as part of HIPAA in 1996, protect patient's private health information (12). The guidelines of the American Medical Association (AMA) and the American Society of Clinical Oncology (ASCO) suggest that physicians should encourage their patients to share family genetic information as a means of meeting a responsibility to family members (16,17).

Duty to Provide a Standard of Care

Clinical practice guidelines (CPGs), which have become more prevalent over the last decade, can help inform the standard of care. However, in the United States, standards of care are determined on a specialty-by-specialty basis (18). CPGs exist that help define patients who may benefit from genetic counseling and testing. After identifying those with an inherited susceptibility to cancer, CPGs can also be useful in outlining who may benefit from more intensive screening or risk reduction procedures to decrease the risk for the development of primary or secondary cancers.

In the United States, the issue of professional liability for negligence based on failure to adhere to standards is a realistic concern (18). Four basic elements must be established to find a physician liable for medical malpractice. These are injury, duty, negligence, and proximate causation. These have all been elaborated elsewhere (19). In most instances, the injury has clearly occurred, and the physician-patient relationship established (source of duty). Most cases thus hinge on whether the provider has in fact performed negligently and if so, whether the negligence was the main basis for the injury that occurred (proximate cause).

Unlike the situation in most other areas of tort law, negligence in medical malpractice cases has traditionally been defined in terms of the "standard of care." This last issue seems to be pivotal in most cases, with experts providing opinions as to whether the alleged acts or omissions were in keeping with the usual or customary practices. It is often contended that the bar is set rather low, with "usual," "customary," or "average" being good enough to protect the provider. Many plaintiff advocates complain that physicians are relatively unique in being able to set their own standards, with little or no external oversight (20). Guided by the differences of opinion and CPGs in the details of the prevailing practice and whether it was adhered to in the case in point, the judge and jury need only arrive at a judgment as to the physician's actions in relation to the expert-defined standard.

For hereditary cancer syndromes, case law has established that the duty to provide a standard of care encompasses not just appropriate and timely referral to genetic counseling and testing but also offering appropriate screening and risk reduction strategies for cancer prevention to those with strong family history or known deleterious germline mutations. In 2006, the Seattle Times reported a malpractice suit against a medical center in Seattle, Washington, that settled for 1.6 million dollars for failure to diagnose a patient with hereditary breast and ovarian cancer syndrome and for not offering risk-reductive salpingo-oophorectomy (21). In this article, a young woman was diagnosed and survived bilateral breast cancer at the ages of 28 and 37 years but ultimately passed away from ovarian cancer at the age of 43 years. The lawsuit settled in 2001, and the file was sealed on a motion by the plaintiffs. Thus the lawsuit's outcome was never reported in an electronic database of jury verdicts or settlements.

CONCLUSION

Genetic testing, a relatively new technological advance in medicine, leads to new challenges for patients and clinicians alike. From the patient's perspective, the theoretical possibility of genetic discrimination in the form of loss of health insurance, inability to obtain life insurance, and employment discrimination remains a palpable concern even in the absence of documented cases of discrimination from hereditary cancer test results. For physicians and other health care providers, the importance of adequately counseling patients to weigh the potential risks and benefits of genetic testing should be emphasized. In addition, for physicians, the potential struggle between ethical and legal concerns of trying to ensure that the larger, extended family of at-risk individuals are informed while respecting patient confidentiality remains a challenge. Finally, CPGs regarding hereditary cancer genetic testing and management are evolving. From both a medical-legal standpoint and for optimal patient care, clinicians must stay abreast of the current practice recommendations.

Resources for Health Care Providers and Patients

- The National Conference of State Legislatures. This site includes information on State genetic privacy laws, employment, and health insurance.
  Available at: http://www.ncsl.org/programs/health/genetics/charts.htm.
- State Genetic Privacy Laws. Last updated January 2008.
  Available at: http://www.ncsl.org/programs/health/genetics/prt.htm.
- National Human Genome Research Institute, National Institutes of Health. Genome.gov. Genetic Discrimination. This Web site has links to multiple topics related to genetic discrimination, previous reports of genetic discrimination, and current legislation.
  Available at: http://www.genome.gov/pfv.cfm?pageID=10002077.

CASE REPORT

J.K. is a 41-year-old woman with endometrial cancer. Her family history was limited but significant for a mother diagnosed with colon cancer at the age of 53 years. J.K. was tested and found to have a deleterious mutation in MSH2. J.K. has been estranged from all of her biologic family and was reticent to contact them to communicate the results from her genetic testing. After much discussion with the genetic counselor, the decision was made to provide an anonymous letter to family members.

LEARNING POINTS

- There are many different ways to communicate genetic test result information to family members.
- Through encouragement and counseling, health care providers can help patients through potential barriers to communicate this important information.

REFERENCES

1. Lehmann LS Weeks JC, Klar N, et al. A population-based study of Ashkenazi Jewish women's attitudes toward genetic discrimination and BRCA 1/2 testing. Genet Med 2002; 4(5):346–352.
2. Sankar P. Genetic privacy. Annu Rev Med 2003; 54:393–407.
3. Peterson EA, Milliron KJ, Lewis KE, et al. Health insurance and discrimination concerns and BRCA1/2 testing in a clinic population. Cancer Epidemiol Biomarkers Prev 2002; 11(1):79–87.
4. Hall MA Rich SS. Laws restriciting health insurers' use of genetic information: impact on genetic discrimination. Am J Hum Geneti 2000; 66:293–307.
5. genome.gov National Human Genome Research Institute NIoH. Genetic Discrimination. http://www.genome.gov/pfv.cfm?pageID=10002077. Accessed March 6, 2008.
6. Nowlan WJ. Ethics and genetics. N Engl J Med 2003; 349(19):1870–1872; author reply 1870–1872 (comment).
7. Code of Federal Regulations Title 45 Section 160.5105.
8. Hudson KL, Holohan MK, Collins FS. Keeping Pace with the Times-The Genetic Information Nondiscrimination Act of 2008. N Engl J Med 2008; 385(25):2661–2663.
9. Subramanian K, Lemaire J, Hershey JC, et al. Estimating adverse selection costs from genetic testing for breast and ovarian cancer: the case of life insurance. J Risk and Insurance 1999; 66:531–550.
10. MacDonald A. How will improved forecasts of individual lifetimes affect underwriting. Brit Actuarial J 1997; 3:1009–1025.
11. Rothenberg K, Fuller B, Rothstein M, et al. Genetic information and the workplace: legislative approaches and policy changes. Science 1997; 275(5307):1755–1757.

12. Offit K, Groeger E, Turner S, et al. The "duty to warn" a patient's family members about hereditary disease risks. JAMA 2004; 292(12):1469–1473.
13. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006; 296(12):1507–1517.
14. Trepanier A, Ahrens M, McKinnon W, et al. Genetic cancer risk assessment and counseling: recommendations of the national society of genetic counselors. J Genet Couns 2004; 13(2):83–114.
15. Lynch HT, Paulson J, Severin M, et al. Failure to diagnose hereditary colorectal cancer and its medicolegal implications: a hereditary nonpolyposis colorectal cancer case. Dis Colon Rectum 1999; 42(1):31–35.
16. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003; 21(12):2397–2406.
17. Burke W, Petersen G, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. JAMA 1997; 277(11):915–919.
18. Lynch PM. Standards of care in diagnosis and testing for hereditary colon cancer. Fam Cancer 2008; 7:65–72.
19. Mazur D. The shifting role of the jury in malpractice law and the diffusion of medical knowledge in the biomedical community. Med Decis Making 2002; 22:57–61.
20. Pelly JE, Newby L, Tito F et al Clinical practice guidelines before the law: sword or shield?. Med J Aust. 1998; 169:330–333.
21. Steve Miletich KA, Justin Mayo. Life or death question, but debate was hidden for years. Your Courts, Their Secrets. Available at: http://seattletimes.nwsource.com/html/yourcourtstheirsecrets/2003311951_virginiamason19m.html. Accessed January 3, 2008.

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Psychological Impact of Genetic Counseling and Testing for Hereditary Gynecologic Cancers

Susan K. Peterson

Department of Behavioral Science, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

KEY POINTS

- Studies have found that in general most mutation carriers do not report clinically significant levels of psychological distress in the first year after testing. However, long-term effects have not been adequately studied.
- Some subsets of patients, such as those with preexisting anxiety or depression, may experience higher levels of distress during and after genetic counseling and testing. Adequate counseling and support throughout the process may be beneficial.
- Communication aids such as a genetic counseling summary letter or informational booklet may be helpful to mutation carriers trying to communicate the test results to family members.

INTRODUCTION

The primary hereditary cancer syndromes that confer an increased risk for gynecologic cancers are hereditary breast-ovarian cancer syndrome (HBOC) and Lynch syndrome/hereditary nonpolyposis colorectal cancer (HNPCC).

Approximately 5% of uterine cancers and 10% of ovarian cancers are attributed to a hereditary cause. Deleterious germ line mutations associated with these syndromes have been identified in the BRCA1 and BRCA2 genes for HBOC and in mismatch repair genes (i.e., *hMLH1*, *hMSH2*, *hMLH6*, *PMS1*) for Lynch syndrome. Through genetic testing, health care providers can identify women who carry such mutations and subsequently have a risk for developing uterine or ovarian cancer that substantially exceeds the general population risk for these diseases. A primary benefit of genetic testing is the ability to offer targeted options for cancer risk reduction and risk management to those high-risk persons.

Since genetic testing for HBOC and Lynch syndrome became clinically available over a decade ago, psychosocial research has focused on understanding individuals' motivations and decisions regarding genetic testing, the psychological impact of genetic risk notification, effects on family and interpersonal relationships, and factors that influence the uptake of risk reduction options (e.g., screening, risk-reducing surgery, or chemoprevention). This chapter will highlight the relevant literature on these topics for HBOC and Lynch syndrome and the implications for clinical practice. It is important to note that most studies do not report outcomes specifically in terms of gynecologic cancers associated with these two syndromes, with the exception of studies that evaluate decisions regarding screening or risk-reducing surgery. The majority of participants in psychosocial research on *BRCA1*/*BRCA2* testing are women; however, most psychosocial research on Lynch syndrome includes both men and women. Nonetheless, findings from these studies can guide clinicians toward understanding why people seek genetic counseling and testing, what they hope to gain from it, and how they cope with the results of testing and subsequently integrate that information into cancer prevention and treatment decisions.

UPTAKE OF GENETIC COUNSELING AND TESTING FOR HEREDITARY BREAST AND OVARIAN CANCER AND LYNCH SYNDROME

Decision-making about genetic testing for inherited cancer susceptibility is complex and may be influenced by medical, psychological, and social factors (1). Genetic counseling and testing is a multistep process that involves several decision points, such as decisions about whether to seek counseling, undergo mutation testing, and receive test results. Following the disclosure of genetic test results, individuals also face decisions about whether and when to share results with family members, health care providers or others, and about risk management choices regarding screening, risk-reducing surgery, or chemoprevention.

An increasing number of studies have examined acceptance rates for genetic counseling and testing for *BRCA1*/*BRCA2* and Lynch syndrome–associated mutations and have identified demographic, clinical, and psychosocial predictors of testing participation. Most studies recruited participants from familial cancer registries or clinical settings, such as cancer genetics or oncology clinics, and

many studies offered free genetic counseling and testing as part of research protocols (2–6). However, comparison of uptake rates across studies is challenging because of differences in methodological characteristics, including the sampling strategy used and the recruitment setting (2). There are many points in the genetic counseling and testing process at which an individual may decline, and a standard methodology for reporting uptake rates is lacking (7).

Genetic Testing for *BRCA1*/*BRCA2* Mutations

Uptake rates for *BRCA1*/*BRCA2* mutation testing have varied widely. A systematic review of studies that reported the proportions of persons who underwent genetic testing showed that uptake rates ranged from 20% to 96%, with an average uptake rate of 59% across all studies (2). Results of multivariate analysis indicated that testing uptake was associated with having a personal or family history of breast or ovarian cancer (2), which was supported by later research (8). Methodological features of the studies, including the use of convenience sampling strategies and recruitment from clinical settings, also were associated with greater testing uptake (2). Several psychosocial factors have been positively correlated with *BRCA1*/*BRCA2* testing uptake, including the presence of cancer-specific distress (8,9) and perceived risk of developing breast or ovarian cancer. Having children or having a greater number of cancer-affected relatives also has been correlated with greater testing uptake; however, no clear pattern has emerged regarding the relationship between testing uptake and other demographic factors, such as age and educational level (1,9–13).

Relatively little is known about the characteristics of persons who decline genetic testing. This may be partly because persons who have declined testing also may have been reluctant to take part in research studies, making access to these samples difficult. The limited data on decliners of *BRCA1*/*BRCA2* mutation testing suggest that those persons are more likely to be male, unmarried, childless, and to be younger and have fewer cancer-affected relatives compared with testing acceptors (11,14,15). Compared with those who pursued *BRCA1*/*BRCA2* testing, decliners reported lower levels of cancer worry (11) and were more likely to report positive changes in family relationships (16). Nonetheless, the decision to decline genetic testing may be influenced by apprehension about the potential negative impact of receiving test results, particularly with regard to worries about one's own health or children's health, and possible effects on job or life insurance discrimination (11). Few data exist on longer-term psychological effects of declining *BRCA1*/*BRCA2* genetic testing. One prospective study of 327 persons identified as mutation carriers, noncarriers, and testing decliners suggested that decliners might be at greater risk of experiencing distress. In this study, depression rates in decliners increased significantly from 26% at the baseline (pretest) assessment to 47% at one- and six-month follow-up assessments, while rates among carriers and noncarriers remained unchanged or decreased, respectively (14).

Genetic Testing for Lynch Syndrome–Associated Mutations

Genetic testing uptake rates for Lynch syndrome–associated mutations have ranged from 14% to 59% (4,5,17,18). The wide range of uptake rates suggests that factors such as cost, test characteristics, and the context in which counseling and testing were offered may have influenced participants' decisions. For example, uptake rates tend to be highest (i.e., 36–59%) among studies that offered free genetic counseling and testing as part of a research protocol (3–5,18,19).

The uptake of genetic testing for Lynch syndrome susceptibility has been associated with having a personal history of cancer, a greater number of affected relatives, a greater perceived risk of developing colorectal cancer, and more frequent thoughts about colorectal cancer (3–5,18). Test acceptors also were more likely to be employed and have higher educational levels compared with decliners, and there appeared to be no differences in testing uptake for men and women (3,5,18). Participation in genetic counseling to learn about Lynch syndrome–associated cancer risk also has been correlated with having greater perceived social support (20), and the desire to learn about one's mutation status may be motivated by the belief that testing will help family members (6).

While less is known about the characteristics of persons who decline genetic testing for Lynch syndrome, decliners may be more likely to report depressive symptoms, a lack of prior colorectal cancer screening, and a lower perceived ability to cope with mutation-positive test results (3,5). Other reasons cited for not seeking genetic counseling or testing have included concerns about potential insurance discrimination, how genetic testing would affect one's family, and emotional reactions to genetic test results (4).

In summary, studies have revealed that clinical factors (having a personal history of cancer or having a greater number of cancer-affected relatives) as well as psychological factors (greater perceived risk of developing cancer, greater distress or worries related to cancer) are consistently and positively associated with the decision to undergo testing for *BRCA1*/*BRCA2* and Lynch syndrome–associated mutations. Individuals' decisions to undergo genetic counseling and testing may reflect a strong motivation to gain knowledge about why they were diagnosed with cancer and/or about their family members' cancer risk (1). These findings suggest that persons may undergo genetic testing to reduce cancer-related distress and to feel reassured. Additionally, the decision to decline testing may be approached with some apprehension, although relatively less is known about the long-term consequences of these decisions, since decliners may not maintain contact with genetic counselors or other providers.

Given the complexity of the decision to undergo genetic testing, researchers have begun to test innovative strategies to facilitate education and decision making about inherited cancer risk and genetic testing. Decision aids have been developed using diverse formats from booklets to personalized, interactive computer technology (21–26). Randomized controlled trials have shown that the use of decision aids during the counseling and testing process can

improve knowledge and accuracy of risk perception, facilitate clarification of values, and reduce decisional conflict and that their use does not cause anxiety or distress (21,23,26). A computer-based decision aid also was shown to enhance the quality and efficiency of genetic counseling sessions by enabling counselors or other providers to spend less time on the delivery of factual information and to reallocate more time to addressing individual risk and psychosocial concerns (27). Results from these studies indicate that decision aids can be useful adjuncts to standard counseling and education, particularly as genetic testing increasingly moves into the realm of primary care where access to genetic counselors or other genetics specialists may be limited.

THE PSYCHOLOGICAL IMPACT OF UNDERGOING GENETIC COUNSELING AND TESTING

When clinical genetic testing for hereditary cancer risk first became available, a primary concern was whether, or to what degree, persons would experience adverse psychological consequences as a result of undergoing counseling and testing. Clinicians and researchers also sought to characterize those persons most vulnerable to experiencing negative effects to identify specific needs for psychological support during the counseling and testing process. Studies have examined psychological distress outcomes (most commonly, depression, anxiety, and cancer-specific worries or distress) in persons before genetic counseling, after counseling, and for various lengths of time after disclosure of mutation status and have delineated responses in terms of mutation-positive, mutation-negative, and inconclusive/uninformative results. Much of the research to date has focused on the psychological impact of genetic testing in cancer-unaffected persons; however, a smaller number of studies also have examined effects on persons diagnosed with cancer.

BRCA1/BRCA2 Testing

A recent review of studies examining psychological outcomes (including anxiety, depression, general distress, and cancer-specific distress) following *BRCA1/BRCA2* genetic testing among cancer-unaffected women concluded that, in general, mutation carriers experience no adverse effects up to one year after disclosure of results and noncarriers may gain psychological benefits from testing (1). Studies have shown that mean scores on psychological outcome measures either improved or did not change for unaffected noncarriers (12,28–30). For unaffected carriers, most studies have shown that unaffected carriers' distress following disclosure of their mutation status did not change relative to baseline (12,28,30,31) or increases over the short term (29,32,33). It is important to note that psychological distress measured in these studies generally did not reach levels of clinical significance and usually remained within normal ranges. There are limited data regarding the long-term psychological impact of *BRCA1/*

BRCA2 mutation testing. A study that examined anxiety and distress up to five years after results disclosure found that distress levels did not differ between mutation carriers and noncarriers up to one year postdisclosure (34). However, anxiety and depression increased from one to five years' follow-up, and long-term distress was associated with the presence of cancer-specific distress at the time of testing, having young children, and having lost a family member to breast or ovarian cancer (34). Also, a majority of carriers had undergone risk-reducing surgery during the follow-up time period, which could possibly confound the distress outcomes (1). Taken together, these findings suggest that notification of positive mutation carrier status does not appear to significantly impact psychological distress; however, psychosocial research is needed to further explore the long-term impact of genetic testing.

Most studies have focused on psychological outcomes of unaffected women who underwent *BRCA1/BRCA2* genetic testing, and the limited focus on cancer-affected women's experience with testing may have stemmed from an early assumption that the impact of genetic risk notification is attenuated by their prior experience with a cancer diagnosis. In contrast, findings from some studies of affected carriers tell a somewhat different story. Often, cancer-affected mutation carriers experienced no change in distress levels over time after disclosure of results (12,30,35), although strong declines in well-being were reported by affected carriers in one study, particularly among those who had been diagnosed with cancer within the previous year (32). In fact, affected women may underestimate their own emotional response to receiving a mutation-positive test result, which in turn can exacerbate distress. A study by Dorval et al. (36) showed that affected *BRCA1* carriers experienced higher levels of anger and worry after disclosure than they had anticipated, and their underestimation of postdisclosure distress was associated with higher levels of general distress at six-month follow-up. Because testing protocols advise beginning mutation testing with affected individuals, being the first person identified as a mutation carrier in one's family may pose an additional psychological burden (37).

The relatively limited data that exist on the impact of receiving uninformative genetic test results suggest that a person receiving such a result may not experience the same decrease in distress as a person receiving a true negative result (30). Understanding the meaning of inconclusive results may be difficult, and accurately communicating their meaning to family members may be challenging (38–40).

Lynch Syndrome

Longitudinal studies of psychological outcomes after genetic testing for Lynch syndrome–related mutations indicated that carriers may experience increased general distress (41,42), cancer-specific distress (43), or cancer worries (42), relative to their pretest assessments immediately following disclosure of their mutation status (e.g., two weeks to one month). Carriers' distress often was

significantly higher postdisclosure compared with noncarriers' distress (41–44). However, in most cases, distress responses were short term, and carriers' distress levels subsided during the course of the year after disclosure (41,42) and did not differ from pretest distress levels at one year postdisclosure (43,44). Findings from these studies also indicated that noncarriers may derive psychological benefit from testing, as they experienced a reduction or no change in distress up to a year following results disclosure (41–44). Less is known about the long-term psychological impact of HNPCC genetic counseling and testing beyond one year following notification of mutation carrier status. One study evaluated psychological outcomes up to three years after disclosure of mutation status (44). Carriers' and noncarriers' three-year mean scores on measures of depression, state anxiety, and cancer-specific distress were similar to scores obtained prior to genetic testing, with one exception: noncarriers' cancer-specific distress scores showed sustained decreases posttesting and were significantly lower compared with their baseline scores and with carriers' scores at one year posttesting, with a similar trend observed at three years posttesting.

Subgroups of individuals may be at higher risk of psychological distress following disclosure of test results, including those who present with relatively higher scores on measures of general or cancer-specific distress before undergoing testing (6,42,45–47). In a sample of colorectal cancer patients who had donated blood for genetic testing, higher levels of depressive symptoms and/or anxiety were found among women, younger persons, and nonwhites, as well as those with less formal education and fewer and less satisfactory sources of social support (47). A subgroup of individuals who showed higher levels of psychological distress and lower quality of life and social support were identified from the same population; in addition, this subgroup was more likely to worry about finding out that they were HNPCC mutation carriers and being able to cope with learning their test results (20). In a follow-up report that evaluated psychological outcomes following disclosure of test results among both colorectal cancer patients as well as relatives at risk of having a HNPCC mutation, a subgroup with the same psychosocial characteristics experienced higher levels of general distress and distress specific to the experience of having genetic testing within the year after disclosure, regardless of mutation status. Nonwhites and those with lower education had higher levels of depression and anxiety scores at all time compared with whites and those with higher education, respectively (42). Other studies have also found that a prior history of major or minor depression, higher pretest levels of cancer-specific distress, having a greater number of cancer-affected first-degree relatives, greater grief reactions, and greater emotional illness–related representations predicted higher levels of distress from one to six months after disclosure of test results (46,48). While further research is needed in this area, case studies indicate that it is important to identify persons who may be at risk of experiencing psychiatric distress and to provide psychological support and follow-up throughout the genetic counseling and genetic testing process (49).

FAMILY COMMUNICATION ABOUT GENETIC TESTING AND INHERITED CANCER RISK

Cancer genetic test results provide information about the individual tested as well as his or her biological relatives, and individuals who undergo testing (particularly index cases, or the first person tested in the family) are the gatekeepers for this information in their families (50). It is generally accepted that communication about genetic risk information within families is largely the responsibility of family members, rather than health care providers. The American Society for Clinical Oncology (ASCO) has advised that health care providers educate persons who undergo genetic testing for inherited cancer susceptibility about the importance of communicating test results to family members (ASCO 2003). It is encouraging to note that studies have consistently shown that persons generally are willing to share their genetic test results with at least some of their relatives, often within a few weeks after disclosure (51–53). Typically, communication is more likely to occur with first-degree relatives (e.g., siblings, children) rather than with more distant relatives (51–53). Motivations for sharing genetic risk information include a desire to increase family awareness about health care options and predictive genetic testing as well as a perceived moral obligation and responsibility to help others in the family (51,52).

While communication about genetic risk is generally perceived by most study participants as an open process, some barriers to doing so were reported across studies. Reasons for not informing a relative included lack of a close relationship and lack of contact with the individual; in fact, emotional rather than relational closeness seemed to be a more important determinant of the degree of risk communication. Disclosure seemed less likely if at-risk individuals were considered too young to receive the information (i.e., children), or if information about the hereditary cancer risk had previously created conflict in the family (52), or if it was assumed that relatives would be uninterested in information about testing (51). Prior existence of conflict seemed to inhibit discussions about hereditary cancer risk, particularly if such discussions involved disclosure of bad news (52).

In some cases, probands reported feeling particularly obliged to inform family members about a hereditary cancer risk (52) and were often the strongest advocates for encouraging their family members to undergo genetic counseling and testing for the family mutation (53). Some gender and family role differences also emerged regarding the dissemination of hereditary cancer risk information. One study reported that female probands were more comfortable discussing genetic information than were male probands and that male probands showed a greater need for professional support during the family communication process (51). Female *BRCA1/BRCA2* mutation carriers were more likely to inform their fathers or brothers about genetic test results if the inheritance of mutations occurred through a paternal line or if there was the presence of

paternal family cancer history (54). Mothers may be particularly influential members of the family network regarding communicating health risk information (55) and were more likely to be involved in communication about *BRCA1/BRCA2* or Lynch syndrome mutation results (54). Parents from high-risk families often communicated with their minor children about their genetic test results, and this communication was more likely to occur with older rather than younger children and in families that favored a more open communication style (56,57). Mutation-negative individuals, persons who chose not to be tested, and spouses of at-risk persons reported not feeling as personally involved with the risk communication process compared with probands and other at-risk persons who had undergone genetic testing (53). It was suggested that families who are more comfortable and open with cancer-related discussions might be more receptive and accepting of news about genetic risk (52).

Various modes of communication (e.g., in-person, telephone, or written contact) may typically be used to disclose genetic risk information within families (51–53). In one study, communication aids such as a genetic counseling summary letter or HNPCC booklet were viewed as helpful adjuncts to the communication process but were not considered central or necessary to its success (51). Studies have suggested that recommendations by health care providers to inform relatives about hereditary cancer risk may encourage communication about HNPCC (52) and that support by health care professionals may be helpful in overcoming barriers to communicating such information to family members (58).

RISK MANAGEMENT RECOMMENDATIONS FOR CARRIERS OF *BRCA1/BRCA2* AND LYNCH SYNDROME–ASSOCIATED MUTATIONS: DECISION MAKING AND PSYCHOLOGICAL CONSEQUENCES

Carriers of *BRCA1/BRCA2* or Lynch syndrome–associated mutations are advised to follow recommendations for reducing their gynecologic cancer risk, which include options for screening and risk-reducing oophorectomy and/or hysterectomy (59,60). A primary goal of genetic testing is ultimately to reduce cancer morbidity and mortality in families with HBOC and Lynch syndrome; thus it is important for clinicians to understand factors that influence women's decisions regarding risk reduction options, barriers to adoption of the recommendations, and the effects on quality of life and psychological adjustment.

Screening For Gynecologic Cancers

Ovarian Cancer Screening in *BRCA1/BRCA2* Mutation Carriers

Risk management recommendations for *BRCA1/BRCA2* mutation carriers include the option of screening for ovarian cancer risk by transvaginal ultrasound (TVU) and serum CA-125 testing every six months, although efficacy data for

these strategies are lacking (59,61). Studies have evaluated the adoption of recommended screening within the year following disclosure of mutation status and have found wide variation in uptake rates. Within the year following disclosure of *BRCA1/BRCA2* mutation status, uptake of TVU among carriers ranged from 15% to 100%, and uptake of CA-125 testing ranged from 21% to 68% (62–68). Positive *BRCA1/BRCA2* mutation status was the most consistent predictor of ovarian cancer screening use after testing (33,62,64–66,69). Studies also reported that greater perceived risk of developing ovarian cancer, having a greater number of ovarian cancer–affected relatives, and physician recommendation were positively associated with adherence to ovarian cancer screening following mutation testing (65,70,71).

Endometrial Cancer Screening in Lynch Syndrome

Gynecologic cancer risk management recommendations for Lynch syndrome include the option of annual endometrial biopsy with TVU for women with a suspected or documented mismatch repair mutation beginning at age 30 to 35 years (60); again, these strategies have no proven efficacy in the early detection of endometrial cancer. Few studies have examined adherence to endometrial screening in Lynch syndrome and have comprised small numbers of women at risk. Available data suggest that mutation carriers do not universally adopt intensive gynecologic cancer screening; however, use of screening appears to increase following genetic counseling and testing, in response to notification that one is at increased risk for endometrial cancer. A cross-sectional study of persons surveyed six months to nine years after genetic testing for Lynch syndrome found that 69% of mutation-positive women reported following gynecologic screening advice, significantly more than had done so prior to testing (10%); however, the screening interval and specific gynecologic tests were not described (72). Among women enrolled in a Lynch syndrome registry who had received genetic counseling and risk assessment with or without genetic testing, 69% had undergone at least one endometrial biopsy (73). Other studies have reported that within one to three years after disclosure of test results, 53% to 54% of carriers underwent endometrial biopsy and 47% to 86% underwent TVU (44,74,75).

Risk-Reducing Surgery

Risk-Reducing Salpingo-Oophorectomy in *BRCA1/BRCA2* Mutation Carriers

The benefits of risk-reducing salpingo-oophorectomy (RRSO) for high-risk women include a reduction in both breast and ovarian cancer risk, with an 85% to 90% reduction in lifetime ovarian cancer risk for *BRCA1/BRCA2* carriers (76,77). There is a wide variation in uptake rates for RRSO among *BRCA1/BRCA2* carriers following genetic testing, ranging from 5% to 75% across studies (33,63,65,68,69,76,78,79). Clinical factors associated with uptake of RRSO

include positive *BRCA1*/*BRCA2* mutation status, prior to breast cancer diagnosis or risk-reducing mastectomy, and having a family history of ovarian cancer (62,69,79,80). Psychosocial and other factors also associated with uptake include greater perceived benefits of surgery, higher perceived cancer risk, older age, and having children (33,66,80–82). Limitations of current ovarian cancer screening options and the perceived severity of ovarian cancer also may influence decisions to undergo RRSO (66,80).

Available data suggest that there is a psychological benefit to undergoing RRSO, specifically regarding reductions in cancer worry and in perceived risk of developing cancer (80,83,84). However, the effect of RRSO on long-term psychological adjustment and quality of life warrants further study. Bresser et al. (85) found that about one-fourth of women who had undergone RRSO reported clinically significant levels of cancer-specific distress at one-year follow-up (86–88). Other studies have reported long-term dissatisfaction with body image (87) and reduced quality of sexual functioning in 42% to 54% of women who had undergone RRSO (86,88).

Despite the obvious risk reduction benefits of undergoing RRSO, the decision to do so carries considerable consequences, particularly for premenopausal women (40,66,78). Reasons for not undergoing or delaying the decision to have RRSO include the desire for childbearing, worries about feeling a loss of femininity, and concerns about long-term use of hormone replacement therapy (HRT) (78,89). To facilitate decision making about RRSO, women reported a need for information about the possible physical and emotional effects to expect after surgery, including the resulting premature menopause and about benefits and risks of HRT (83,87). To help assure successful outcomes after RRSO, including optimizing quality of life, these findings suggest the need for presurgical patient education and communication about what to expect after surgery, as well as careful follow-up to address post-RRSO physical symptoms and emotional outcomes (Patenaude et al., personal communication).

Risk-Reducing Hysterectomy and Oophorectomy in Lynch Syndrome

Few data are available regarding the use of risk-reducing hysterectomy (RRH) or RRSO among women with Lynch syndrome. One study of individuals who had undergone genetic testing for Lynch syndrome suggested that consideration of risk-reducing surgery may have motivated interest in testing (90). Before receiving results, 69% of women reported considering RRH and RRSO; however, this study did not assess whether persons actually followed through with risk-reducing surgery after they received their test results (90). In a longitudinal study of cancer-unaffected persons who underwent genetic testing for Lynch syndrome, 5% of women indicated that they would have an RRH and an RRSO, if they were found to be mutation positive (44,74). At three years following disclosure of results, two women (of 13 female mutation carriers) who had undergone an RRH

before genetic testing underwent RRSO within one year after testing, but risk-reducing surgery was not elected by any other female mutation carriers (44).

The relatively low uptake of RRH and RRO among women with Lynch syndrome may reflect individual preferences, such as delayed decision making about surgery until childbearing has been completed. In a study by Sun et al. (2005, personal communication), patient preferences were elicited for colorectal and endometrial risk management strategies among women with Lynch syndrome. Women strongly preferred screening tests for Lynch syndrome risk management, and the least attractive strategies were surgical interventions as a means of cancer prevention, with the exception of postmenopausal total abdominal hysterectomy (TAH)/bilateral salpingo-oophorectomy (BSO) (91). Evidence showing the efficacy of hysterectomy and oophorectomy in reducing the occurrence of endometrial and ovarian cancers for Lynch syndrome was published relatively recently (79), and it is possible that the dissemination of these efficacy data over time may increase provider recommendations about risk-reducing surgery and influence patients to choose this option more frequently.

IMPLICATIONS FOR FUTURE RESEARCH AND CLINICAL PRACTICE

The availability of clinical genetic testing for hereditary cancer syndromes has brought about rapid changes in the care of patients and their families who face inherited cancer risk. The progress in clinical cancer genetics has yielded both medical as well as psychological benefits for families with hereditary cancers. Genetic testing presents these individuals with an opportunity to resolve uncertainty about their personal and familial risk and to obtain information to guide future health care decisions. Many persons have adopted recommended strategies to reduce or manage their cancer risk, which is critical in translating genetic information into reductions in cancer morbidity and mortality. Future research should continue to explore the long-term psychosocial impact of genetic testing, genetic risk notification, and adoption of risk reduction recommendations at both the individual and family level, to address current gaps in knowledge as well as to inform the delivery of optimal clinical services for high-risk populations.

CASE REPORT

F.R. is a 38-year-old woman with a strong family history of breast and ovarian cancer. Her aunt with ovarian cancer was recently diagnosed with a *BRCA1* mutation. F.R. presented to a genetic counselor to discuss her personal cancer risk. During the risk assessment, the genetic counselor discovered a past history of a generalized anxiety disorder. Special attention was given to the implications to the patient's life of both a positive or negative result. A referral to a psychologist was made prior to further action. F.R. decided to proceed with testing

and was diagnosed with the same *BRCA* mutation. She reported feeling increased levels of worry about a future cancer but did well with continued therapy and with discussions regarding risk reduction options with an oncologist.

LEARNING POINTS

- Women with preexisting anxiety or depression may need extra support during the testing process.
- Counseling prior to genetic testing should encourage patients to contemplate the impact a positive, negative, or uninformative result may have on their daily life.

REFERENCES

1. Meiser B. Psychological impact of genetic testing for cancer susceptibility: an update of the literature. Psychooncology 2005; 14(12):1060–1074.
2. Ropka ME, Wenzel J, Phillips EK, et al. Uptake rates for breast cancer genetic testing: a systematic review. Cancer Epidemiol Biomarkers Prev 2006; 15(5):840–855.
3. Codori AM, Petersen GM, Miglioretti DL, et al. Attitudes toward colon cancer gene testing: factors predicting test uptake. Cancer Epidemiol Biomarkers Prev 1999; 8(4 pt 2):345–351.
4. Hadley DW, Jenkins J, Dimond E, et al. Genetic counseling and testing in families with hereditary nonpolyposis colorectal cancer. Arch Intern Med 2003; 163:573–582.
5. Lerman C, Hughes C, Trock BJ, et al. Genetic testing in families with hereditary nonpolyposis colon cancer. J Am Med Assoc 1999; 281(17):1618–1622.
6. Vernon SW, Gritz ER, Peterson SK, et al. Intention to learn results of genetic testing for hereditary colon cancer. Cancer Epidemiol Biomarkers Prev 1999; 8(4 pt 2):353–360.
7. Bowen DJ, Patenaude AF, Vernon SW. Psychosocial issues in cancer genetics: from the laboratory to the public. Cancer Epidemiol Biomarkers Prev 1999; 8(4 pt 2):326–328.
8. Andrews L, Meiser B, Apicella C, et al. Psychological impact of genetic testing for breast cancer susceptibility in women of Ashkenazi Jewish background: a prospective study. Genet Test 2004; 8(3):240–247.
9. Lerman C, Schwartz MD, Lin TH, et al. The influence of psychological distress on use of genetic testing for cancer risk. J Consult Clin Psychol 1997; 65(3):414–420.
10. Biesecker BB, Ishibe N, Hadley DW, et al. Psychosocial factors predicting BRCA1/BRCA2 testing decisions in members of hereditary breast and ovarian cancer families. Am J Med Genet 2000; 93(4):257–263.
11. Foster C, Evans DG, Eeles R, et al. Non-uptake of predictive genetic testing for BRCA1/2 among relatives of known carriers: attributes, cancer worry, and barriers to testing in a multicenter clinical cohort. Genet Test 2004; 8(1):23–29.
12. Lerman C, Narod S, Schulman K, et al. BRCA1 testing in families with hereditary breast-ovarian cancer. A prospective study of patient decision making and outcomes. JAMA 1996; 275(24):1885–1892.

13. Meijers-Heijboer EJ, Verhoog LC, Brekelmans CT, et al. Presymptomatic DNA testing and prophylactic surgery in families with a BRCA1 or BRCA2 mutation. Lancet 2000; 355(9220):2015–2020.
14. Lerman C, Hughes C, Lemon SJ, et al. What you don't know can hurt you: adverse psychologic effects in members of BRCA1-linked and BRCA2-linked families who decline genetic testing. J Clin Oncol 1998; 16(5):1650–1654.
15. Lodder L, Frets PG, Trijsburg RW, et al. Attitudes and distress levels in women at risk to carry a BRCA1/BRCA2 gene mutation who decline genetic testing. Am J Med Genet A 2003; 119(3):266–272.
16. McInerney-Leo A, Biesecker BB, Hadley DW, et al. BRCA1/2 testing in hereditary breast and ovarian cancer families II: impact on relationships. Am J Med Genet A 2005; 133(2):165–169.
17. Codori AM. Psychological opportunities and hazards in predictive genetic testing for cancer risk. Gastroenterol Clin North Am 1997; 26(1):19–39.
18. Aktan-Collan K, Mecklin JP, Jarvinen H, et al. Predictive genetic testing for hereditary non-polyposis colorectal cancer: uptake and long-term satisfaction. Int J Cancer 2000; 89(1):44–50.
19. Keller M, Jost R, Kadmon M, et al. Acceptance of and attitude toward genetic testing for hereditary nonpolyposis colorectal cancer: a comparison of participants and nonparticipants in genetic counseling. Dis Colon Rectum 2004; 47(2):153–162.
20. Gritz ER, Vernon SW, Peterson SK, et al. Distress in the cancer patient and its association with genetic testing and counseling for hereditary non-polyposis colon cancer. Cancer Res Ther Control 1999; 8:35–49.
21. Lobb EA, Butow PN, Moore A, et al. Development of a communication aid to facilitate risk communication in consultations with unaffected women from high risk breast cancer families: a pilot study. J Genet Couns 2006; 15(5):393–405.
22. Wakefield CE, Meiser B, Homewood J, et al. A randomized controlled trial of a decision aid for women considering genetic testing for breast and ovarian cancer risk. Breast Cancer Res Treat 2008; 107(2):289–301.
23. Tiller K, Meiser B, Gaff C, et al. A randomized controlled trial of a decision aid for women at increased risk of ovarian cancer. Med Decis Making 2006; 26(4):360–372.
24. Wang C, Gonzalez R, Milliron KJ, et al. Genetic counseling for BRCA1/2: a randomized controlled trial of two strategies to facilitate the education and counseling process. Am J Med Genet A 2005; 134(1):66–73.
25. Green MJ, Biesecker BB, McInerney AM, et al. An interactive computer program can effectively educate patients about genetic testing for breast cancer susceptibility. Am J Med Genet 2001; 103(1):16–23.
26. Green MJ, Peterson SK, Baker MW, et al. Effect of a computer-based decision aid on knowledge, perceptions, and intentions about genetic testing for breast cancer susceptibility: a randomized controlled trial. JAMA 2004; 292(4):442–452.
27. Green MJ, Peterson SK, Baker MW, et al. Use of an educational computer program before genetic counseling for breast cancer susceptibility: effects on duration and content of counseling sessions. Genet Med 2005; 7(4):221–229.
28. Lodder L, Frets PG, Trijsburg RW, et al. Psychological impact of receiving a BRCA1/BRCA2 test result. Am J Med Genet 2001; 98(1):15–24.
29. Meiser B, Butow P, Friedlander M, et al. Psychological impact of genetic testing in women from high-risk breast cancer families. Eur J Cancer 2002; 38(15): 2025–2031.

30. Schwartz MD, Peshkin BN, Hughes C, et al. Impact of BRCA1/BRCA2 mutation testing on psychologic distress in a clinic-based sample. J Clin Oncol 2002; 20(2): 514–520.
31. Croyle RT, Smith KR, Botkin JR, et al. Psychological responses to BRCA1 mutation testing: preliminary findings. Health Psychol 1997; 16(1):63–72.
32. van Roosmalen MS, Stalmeier PF, Verhoef LC, et al. Impact of BRCA1/2 testing and disclosure of a positive test result on women affected and unaffected with breast or ovarian cancer. Am J Med Genet A 2004; 124(4):346–355.
33. Watson M, Foster C, Eeles R, et al. Psychosocial impact of breast/ovarian (BRCA1/2) cancer-predictive genetic testing in a UK multi-centre clinical cohort. Br J Cancer 2004; 91(10):1787–1794.
34. van Oostrom I, Meijers-Heijboer H, Lodder LN, et al. Long-term psychological impact of carrying a BRCA1/2 mutation and prophylactic surgery: a 5-year follow-up study. J Clin Oncol 2003; 21(20):3867–3874.
35. Reichelt JG, Heimdal K, Moller P, et al. BRCA1 testing with definitive results: a prospective study of psychological distress in a large clinic-based sample. Fam Cancer 2004; 3(1):21–28.
36. Dorval M, Patenaude AF, Schneider KA, et al. Anticipated versus actual emotional reactions to disclosure of results of genetic tests for cancer susceptibility: findings from p53 and BRCA1 testing programs. J Clin Oncol 2000; 18(10):2135–2142.
37. Bonadona V, Saltel P, Desseigne F, et al. Cancer patients who experienced diagnostic genetic testing for cancer susceptibility: reactions and behavior after the disclosure of a positive test result. Cancer Epidemiol Biomarkers Prev 2002; 11(1):97–104.
38. Hallowell N, Foster C, Ardern-Jones A, et al. Genetic testing for women previously diagnosed with breast/ovarian cancer: examining the impact of BRCA1 and BRCA2 mutation searching. Genet Test 2002; 6(2):79–87.
39. Hughes C, Lerman C, Schwartz M, et al. All in the family: evaluation of the process and content of sisters' communication about BRCA1 and BRCA2 genetic test results. Am J Med Genet 2002; 107(2):143–150.
40. Tiller K, Meiser B, Butow P, et al. Psychological impact of prophylactic oophorectomy in women at increased risk of developing ovarian cancer: a prospective study. Gynecol Oncol 2002; 86(2):212–219.
41. Aktan-Collan K, Haukkala A, Mecklin JP, et al. Psychological consequences of predictive genetic testing for hereditary non-polyposis colorectal cancer (HNPCC): a prospective follow-up study. Int J Cancer 2001; 93(4):608–611.
42. Gritz ER, Peterson SK, Vernon SW, et al. Psychological impact of genetic testing for hereditary nonpolyposis colorectal cancer. J Clin Oncol 2005; 23(9):1902–1910.
43. Meiser B, Collins V, Warren R, et al. Psychological impact of genetic testing for hereditary non-polyposis colorectal cancer. Clin Genet 2004; 66(6):502–511.
44. Collins VR, Meiser B, Ukoumunne OC, et al. The impact of predictive genetic testing for hereditary nonpolyposis colorectal cancer: three years after testing. Genet Med 2007; 9(5):290–297.
45. Esplen MJ, Urquhart C, Butler K, et al. The experience of loss and anticipation of distress in colorectal cancer patients undergoing genetic testing. J Psychosom Res 2003; 55(5):427–435.
46. van Oostrom I, Meijers-Heijboer H, Duivenvoorden HJ, et al. Experience of parental cancer in childhood is a risk factor for psychological distress during genetic cancer susceptibility testing. Ann Oncol 2006; 17(7):1090–1095.

47. Vernon SW, Gritz ER, Peterson SK, et al. Correlates of psychologic distress in colorectal cancer patients undergoing genetic testing for hereditary colon cancer. Health Psychol 1997; 16(1):73–86.
48. Murakami Y, Okamura H, Sugano K, et al. Psychologic distress after disclosure of genetic test results regarding hereditary nonpolyposis colorectal carcinoma. Cancer 2004; 101(2):395–403.
49. Patenaude AF. Genetic Testing for Cancer: Psychological Approaches for Helping Patients and Families. Washington, DC: American Psychological Association, 2005.
50. Peterson SK. The role of the family in genetic testing: theoretical perspectives, current knowledge, and future directions. Health Educ Behav 2005; 32(5):627–639.
51. Gaff CL, Collins V, Symes T, et al. Facilitating family communication about predictive genetic testing: Probands' perceptions. J Genet Couns 2005; 14(2):133–140.
52. Mesters I, Ausems M, Eichhorn S, et al. Informing one's family about genetic testing for hereditary non-polyposis colorectal cancer (HNPCC): a retrospective exploratory study. Fam Cancer 2005; 4(2):163–167.
53. Peterson SK, Watts BG, Koehly LM, et al. How families communicate about HNPCC genetic testing: findings from a qualitative study. Am J Med Genet 2003; 119C(1):78–86.
54. Patenaude AF, Dorval M, DiGianni LS, et al. Sharing BRCA1/2 test results with first-degree relatives: factors predicting who women tell. J Clin Oncol 2006; 24(4): 700–706.
55. Koehly LM, Peterson SK, Watts BG, et al. A social network analysis of communication about hereditary nonpolyposis colorectal cancer genetic testing and family functioning. Cancer Epidemiol Biomarkers Prev 2003; 12(4):304–313.
56. Tercyak KP, Hughes C, Main D, et al. Parental communication of BRCA1/2 genetic test results to children. Patient Educ Couns 2001; 42(3):213–224.
57. Tercyak KP, Peshkin BN, DeMarco TA, et al. Parent-child factors and their effect on communicating BRCA1/2 test results to children. Patient Educ Couns 2002; 47(2): 145–153.
58. Pentz RD, Peterson SK, Watts B, et al. Hereditary nonpolyposis colorectal cancer family members' perceptions about the duty to inform and health professionals' role in disseminating genetic information. Genet Test 2005; 9(3):261–268.
59. Daly MB, Axilbund JE, Bryant E, et al. Genetic/familial high-risk assessment: breast and ovarian. J Natl Compr Canc Netw 2006; 4(2):156–176.
60. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006; 296(12):1507–1517.
61. Lu KH. Hereditary gynecologic cancers: differential diagnosis, surveillance, management and surgical prophylaxis. Fam Cancer 2008; 7(1):53–58.
62. Scheuer L, Kauff N, Robson M, et al. Outcome of preventive surgery and screening for breast and ovarian cancer in BRCA mutation carriers. J Clin Oncol 2002; 20(5): 1260–1268.
63. Lerman C, Hughes C, Croyle RT, et al. Prophylactic surgery decisions and surveillance practices one year following BRCA1/2 testing. Prev Med 2000; 31(1):75–80.
64. Botkin JR, Smith KR, Croyle RT, et al. Genetic testing for a BRCA1 mutation: prophylactic surgery and screening behavior in women 2 years post testing. Am J Med Genet A 2003; 118(3):201–209.

65. Schwartz MD, Kaufman E, Peshkin BN, et al. Bilateral prophylactic oophorectomy and ovarian cancer screening following BRCA1/BRCA2 mutation testing. J Clin Oncol 2003; 21(21):4034–4041.
66. Claes E, Evers-Kiebooms G, Decruyenaere M, et al. Surveillance behavior and prophylactic surgery after predictive testing for hereditary breast/ovarian cancer. Behav Med 2005; 31(3):93–105.
67. Diefenbach MA, Miller SM, Daly MB. Specific worry about breast cancer predicts mammography use in women at risk for breast and ovarian cancer. Health Psychol 1999; 18(5):532–536.
68. Watson M, Kash KM, Homewood J, et al. Does genetic counseling have any impact on management of breast cancer risk? Genet Test 2005; 9(2):167–174.
69. Phillips KA, Jenkins MA, Lindeman GJ, et al. Risk-reducing surgery, screening and chemoprevention practices of BRCA1 and BRCA2 mutation carriers: a prospective cohort study. Clin Genet 2006; 70(3):198–206.
70. Isaacs C, Peshkin BN, Schwartz M, et al. Breast and ovarian cancer screening practices in healthy women with a strong family history of breast or ovarian cancer. Breast Cancer Res Treat 2002; 71(2):103–112.
71. Tinley ST, Houfek J, Watson P, et al. Screening adherence in BRCA1/2 families is associated with primary physicians' behavior. Am J Med Genet A 2004; 125(1):5–11.
72. Wagner A, van Kessel I, Kriege MG, et al. Long term follow-up of HNPCC gene mutation carriers: compliance with screening and satisfaction with counseling and screening procedures. Fam Cancer 2005; 4(4):295–300.
73. Yang K, Allen B, Conrad P, et al. Awareness of gynecologic surveillance in women from hereditary non-polyposis colorectal cancer families. Fam Cancer 2006; 5(4): 405–409.
74. Collins V, Meiser B, Gaff C, et al. Screening and preventive behaviors one year after predictive genetic testing for hereditary nonpolyposis colorectal carcinoma. Cancer 2005; 104(2):273–281.
75. Claes E, Denayer L, Evers-Kiebooms G, et al. Predictive testing for hereditary nonpolyposis colorectal cancer: subjective perception regarding colorectal and endometrial cancer, distress, and health-related behavior at one year post-test. Genet Test 2005; 9(1):54–65.
76. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002; 346(21):1609–1615.
77. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002; 346(21):1616–1622.
78. Madalinska JB, Hollenstein J, Bleiker E, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. J Clin Oncol 2005; 23(28):6890–6898.
79. Schmeler KM, Sun CC, Bodurka DC, et al. Prophylactic bilateral salpingo-oophorectomy compared with surveillance in women with BRCA mutations. Obstet Gynecol 2006; 108(3 pt 1):515–520.
80. Madalinska JB, van Beurden M, Bleiker EM, et al. Predictors of prophylactic bilateral salpingo-oophorectomy compared with gynecologic screening use in BRCA1/2 mutation carriers. J Clin Oncol 2007; 25(3):301–307.
81. Meiser B, Butow P, Barratt A, et al. Attitudes toward prophylactic oophorectomy and screening utilization in women at increased risk of developing hereditary breast/ovarian cancer. Gynecol Oncol 1999; 75(1):122–129.

82. Fang CY, Miller SM, Malick J, et al. Psychosocial correlates of intention to undergo prophylactic oophorectomy among women with a family history of ovarian cancer. Prev Med 2003; 37(5):424–431.
83. Meiser B, Dunn S. Psychological impact of genetic testing for Huntington's disease: an update of the literature. J Neurol Neurosurg Psychiatry 2000; 69(5):574–578.
84. Antill YC, Reynolds J, Young MA, et al. Screening behavior in women at increased familial risk for breast cancer. Fam Cancer 2006; 5(4):359–368.
85. Bresser PJ, Seynaeve C, Van Gool AR, et al. Satisfaction with prophylactic mastectomy and breast reconstruction in genetically predisposed women. Plastic and reconstructive surgery 2006; 117(6):1675–1682; discussion 83-4.
86. Elit L, Esplen MJ, Butler K, et al. Quality of life and psychosexual adjustment after prophylactic oophorectomy for a family history of ovarian cancer. Fam Cancer 2001; 1(3-4):149–156.
87. Hallowell N. A qualitative study of the information needs of high-risk women undergoing prophylactic oophorectomy. Psychooncology 2000; 9(6):486–495.
88. Robson M, Hensley M, Barakat R, et al. Quality of life in women at risk for ovarian cancer who have undergone risk-reducing oophorectomy. Gynecol Oncol 2003; 89 (2):281–287.
89. Hallowell N, Foster C, Eeles R, et al. Accommodating risk: responses to BRCA1/2 genetic testing of women who have had cancer. Soc Sci Med 2004; 59(3):553–565.
90. Lynch HT, Lemon SJ, Karr B, et al. Etiology, natural history, management, and molecular genetics of hereditary nonpolyposis colorectal cancer (Lynch Syndromes): genetic counseling implications. Cancer Epidemiol Biomarkers Prev 1997; 6:987–991.
91. Sun CC, Peterson SK, White KG, et al. Preferences for cancer prevention strategies in women with hereditary nonpolyposis colorectal cancer. J Clin Oncol 2006; 24(18S): 53s.

Index

Oncology and Gynecology

about the book...

Hereditary Gynecologic Cancer: Risk, Prevention and Management fills the need that exists for a book addressing highly relevant clinical issues associated with the new field of hereditary gynecologic cancers. Written with the clinician in mind, the authors will cover a broad range of topics, beginning with an overview discussing clinical relevance of hereditary ovarian and hereditary endometrial cancers. Succeeding sections will provide in-depth analyses of Hereditary Breast Ovarian Cancer Syndrome, Lynch Syndrome, and other syndromes with gynecologic cancer components, and genetic risk assessment.

Hereditary Gynecologic Cancer: Risk, Prevention and Management:

- is the first clinically focused reference detailing gynecologic patient management issues of BRCA1 and BRCA2 mutation carriers
- includes a practical section on genetic risk assessment and genetic testing
- examines case studies to demonstrate management techniques and decision-making
- provides detailed discussion of ovarian cancer screening, prevention and risk reducing surgery

about the editor...

Karen H. Lu is Professor of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA. Dr. Lu received her M.D. from Yale University School of Medicine, New Haven, Connecticut, USA and her medical training from Brigham and Women's Hospital and Massachusetts General Hospital, Boston, Massachusetts, USA. She is the Co-Director of the Clinical Cancer Genetics Program and Director of the Ovarian Cancer Screening Clinic and Uterine Cancer Research Program at MD Anderson Cancer Center, Houston, Texas, USA. Dr. Lu is a recognized scholar of hereditary gynecologic cancers and has published extensively on BRCA-associated ovarian cancer and Lynch syndrome-associated endometrial cancer.

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