

Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability

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Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is a common autosomal dominant syndrome characterized by early age at onset, neoplastic lesions, and microsatellite instability (MSI). Because cancers with MSI account for approximately 15% of all colorectal cancers and because of the need for a better understanding of the clinical and histologic manifestations of HNPCC, the National Cancer Institute hosted an international workshop on HNPCC in 1996, which led to the development of the Bethesda Guidelines for the identification of individuals with HNPCC who should be tested for MSI. To consider revision and improvement of the Bethesda Guidelines, another HNPCC workshop was held at the National Cancer Institute in Bethesda, MD, in 2002. In this commentary, we summarize the Workshop presentations on HNPCC and MSI testing; present the issues relating to the performance, sensitivity, and specificity of the Bethesda Guidelines; outline the revised Bethesda Guidelines for identifying individuals at risk for HNPCC; and recommend criteria for MSI testing. [J Natl Cancer Inst 2004;96:261–8]

INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC) is a common, autosomal dominant syndrome characterized by early onset (average age at onset <45 years), the development of neoplastic lesions in a variety of tissues (e.g., endometrial, gastric, renal, ovarian, and skin), and microsatellite instability (MSI) (1–3). Cancers with MSI account for approximately 15% of all colorectal cancers (usually MLH1 methylation), and for HNPCC germline mutations, there are three key DNA mismatch repair (MMR) genes (i.e., MSH2, MLH1 and, in attenuated cases, MSH6) that are responsible for these cancers. A few candidate genes (e.g., PMS2 and MLH3) are still awaiting additional validation regarding their role in the etiology of colorectal cancers with MSI (1–3).

In 1991, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC), in an attempt to standardize diagnostic criteria for multicenter studies, developed the original Amsterdam Criteria for recruiting HNPCC patients for collaborative studies. A better understanding of the

clinical and histologic manifestations of HNPCC led to the National Cancer Institute (NCI) International Workshop on HNPCC in 1996 and to the development of the Bethesda Guidelines, in which criteria for the identification of colorectal tumors that should be tested for MSI were present (4). In this commentary, we outline the revised Bethesda Guidelines recommendations for identifying individuals with HNPCC and recommend criteria for MSI testing that were outlined following a recent (2002) HNPCC workshop conducted by the NCI in Bethesda, MD.

WORKSHOP SUMMARY

HNPCC and MSI Testing

Dr. Henry Lynch (Creighton University, Omaha, NE) briefly described the history of HNPCC starting with Dr. Albert

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Warthin, who first suspected and documented the disorder in an affected woman in 1895 (5). A detailed discussion of the historical perspective of HNPCC is provided elsewhere (6).

Dr. C. Richard Boland (University of California, San Diego School of Medicine, San Diego, CA) explained that, if two or more of the five microsatellite sequences [NCI-recommended panel of microsatellites; see Table 1 (7)] in the tumor DNA have been mutated, then the tumor is termed MSI-high (MSI-H). If only one of the five microsatellite sequences in the tumor DNA have been mutated, then the tumor is termed MSI-low (MSI-L). If none of the five microsatellite sequences in the tumor DNA have been mutated, then the tumor is termed microsatellite stable (MSS) (7,8). When tumors are classified as MSI-L, an additional panel of microsatellite sequences is recommended for testing to accurately characterize the tumor.

Genetic and Epigenetic Mechanisms Leading to MSI-H

Dr. Annika Lindblom (Karolinska Institute, Stockholm, Sweden) led a discussion in which she explained that germline mutations are generally the cause of MSI-H tumors in HNPCC and that somatic mutations occur in only a small fraction of both MLH1 and MSH2 genes in sporadic cases (9,10). The explanation for sporadic MSI-H tumors is generally a silencing of the MLH1 gene (11), which is also seen in HNPCC (12). There is also a strong association between MSI-H tumors and loss of expression of the Mlh1 protein in sporadic tumors and the Mlh1 and Msh2 proteins in familial tumors (12,13). Interestingly, only 2% of MSI-H tumors express both proteins (14); the clinical significance of this observation has not yet been determined. Patients with MSI-H sporadic tumors generally show good prognosis (15), and these tumors have been shown to be associated with proximal (or ascending) colon distribution (16). In very young MSI-H patients (i.e., age at onset <30 years), patients have an even distribution (48% proximal and 52% distal colon) and a poor prognosis (17). In contrast, there was a marked predominance (75%) of proximal colon tumors among the MSI tumors arising in the colon of older patients.

Mutations Associated With HNPCC Predisposition

Dr. Paivi Peltomaki (University of Helsinki, Helsinki, Finland) explained that the MMR genes, MLH3 and PMS1, in

addition to MSH2, MLH1, and MSH6, may also play a role in HNPCC. The ICG-HNPCC mutation database focuses on the primary causes of HNPCC susceptibility, which combine familial features with defined DNA MMR defects (18).

Defective DNA Mismatch Repair in Sporadic and Inherited Colon Cancer

Dr. Lawrence J. Burgart (Mayo Clinic, Rochester, MN) presented clinical data on MSI and how that data relate to HNPCC. A total of 257 consecutive colorectal cancer case patients from a prospective Mayo Clinic cohort were studied using immunohistochemistry (IHC), four mononucleotide markers, and six dinucleotide markers (14,19–22). Approximately 20% of the cases were MSI-H, mostly because of MLH1 loss (due to methylation). In addition, approximately 2% of the cases were due to germline mutations in MLH1 (1%) and MSH2 (1%). It was noted that younger patients with MLH1 protein loss often also had a germline mutation. IHC plays a strong role in segregating these types of MSI patients into their respective risk level and in determining cases in which DNA MMR genes should be analyzed.

Has Anything Really Changed Since the Findings of Dietmaier et al. in 1997?

Dr. Richard Fishel (Kimmel Cancer Center, Philadelphia, PA) noted that the article by Dietmaier et al. (8) is the foundation for much of the current research on diagnostic guidelines for MSI and HNPCC. In that study, 32 markers were used to scan a well-defined cohort of 58 tumors, some of which were known to be positive for HNPCC. Of those 58 tumors, 29 displayed some degree of instability. The Dietmaier et al. article also presented a series of recommendations and statements regarding MSI and HNPCC. First, it suggested that there should be only one nomenclature to describe MSI. Second, it suggested that not all microsatellite sequences display the MSI phenotype. In fact, most tri-, tetra-, and pentanucleotide microsatellites were stable in MSI tumors, and there was a large variation in the degree of stability of microsatellites. Third, there are two classes of MSI: 1) MSI-H, in which 40% or more of the microsatellite markers demonstrate instability and 2) MSI-L, in which less than 40% of the microsatellite markers demonstrate instability. Fourth, a five-panel microsatellite marker with 100% sensitivity and specificity for MSI-H was identified, and a panel with an additional five microsatellite markers was identified with a fairly high sensitivity for the MSI-L phenotype. Fifth, IHC is associated with MSI, but this association is not without exceptions; for example, an MSI phenotype is possible while still being positive for one of the MMR proteins via IHC.

German Collaborative Study Group on HNPCC

Dr. Josef Ruschoff (Klinikum Kassel, Institute of Pathology, Kassel, Germany) described a German multicenter prospective study that started in 1998 and included a collaborative/hereditary cohort of 718 patients and a mixed hereditary/sporadic cohort of 580 patients (23). The MSI-H rate in the collaborative/hereditary cohort was 52%, whereas the MSI-H rate in the mixed hereditary/sporadic cohort was only 21%. The MSI-L rate was 4% in both cohorts. Approximately 81% of the collaborative/hereditary cohort and 90% of the mixed hereditary/sporadic cohort showed loss of repair gene expression by IHC.

Table 1. Recommendations for the evaluation of MSI-H and MSI-L*

The original National Cancer Institute (NCI) microsatellite panel included BAT25, BAT26, D2S123, D5S346 and D17S250 (7); however, the following caveats may apply:

1. If only dinucleotide repeats are mutated, test a secondary panel of microsatellite markers with mononucleotide repeats (e.g., BAT40 and/or MYCL) to exclude MSI-L.
2. Dinucleotide repeats are less sensitive than mononucleotide repeats for MSI-H; however, they provide an internal control for the prevention of sample mix-up.
3. A pentaplex panel of five quasimonomorphic mononucleotide repeats may be more sensitive for MSI-H tumors than other microsatellite markers and may obviate the need for normal tissue for comparison; this approach requires three or more mutant alleles to indicate MSI-H (25).

*MSI-H = microsatellite instability–high in tumors refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers in tumors. MSI-L = microsatellite instability–low in tumors refers to changes in only one of the five NCI-recommended panels of microsatellite markers in tumors.

On the basis of these observations, the following recommendations were put forward for the evaluation of MSI: 1) MSI markers should remain the same as those recommended by the NCI in 1997; 2) IHC is not a substitute for MSI testing in general, and the criteria for positive and negative immunostaining needs to be defined more accurately; 3) the most advanced lesions within the patient or family should be tested because the value of MSI and IHC assays in identifying MSI in early adenomatous lesions is not firmly established; and 4) in unclear and/or equivocal colorectal cancer cases, other, possibly more advanced, HNPCC-related carcinomas in the same family should be tested.

Quasimonomorphic Mononucleotide Repeats and Pentaplex Polymerase Chain Reaction

Dr. Richard Hamelin (Institut National de la Santé et de la Recherche Médicale, Unit 434 [INSERM U434], Centre d'Etude du Polymorphisme Humain [CEPH], Paris, France) reminded the Workshop participants that, in early 1997, BAT-25 and BAT-26 mononucleotide repeats were shown to be quasimonomorphic in normal DNA and to be effective markers for determining the MSI status of human tumors (24). Recently, a single pentaplex polymerase chain reaction (PCR) with five quasimonomorphic mononucleotide repeats was also described (25). This approach is sensitive and specific enough to detect MSI-H tumors in gastric and colorectal cancers and may obviate the need for normal matching DNA for the tumors being tested.

MSI-H Versus MSI-L

Dr. Miguel A. Rodriguez-Bigas (The University of Texas M. D. Anderson Cancer Center, Houston) stated that the mechanisms for colorectal carcinogenesis are far from clear. MSI-L colorectal cancers do not appear to differ clinically or pathologically from MSS cancers in terms of quality (i.e., gross abnormalities), but they do differ in quantity (i.e., the level of MSI). Hence, this phenomenon needs to be clearly defined. More confusion surrounds MSI-L colorectal cancers, and the Workshop participants felt that, for clinical purposes, the MSI-L classification for colorectal cancers should be revised, and MSI-L tumors should be included with MSS tumors. Research is needed to identify microsatellite sequences that are indicative of a rapid or elevated mutation rate within the tumor and to identify a set of markers that are highly reliable for detecting MSI-L tumors or the instability phenotype that is found in developing tumors. It is believed that MSI-L tumors exist as a distinct group separate from MSS tumors. However, there is evidence (26) to suggest that all colorectal cancers have inherent instability and, if enough markers are tested, almost all colorectal cancers will have some degree of MSI.

Value of MSI and IHC for Identification of MMR Mutation Carriers

More than 10 years ago, the ICG-HNPCC proposed the Amsterdam Criteria to enable collaborative studies to identify HNPCC patients and to provide uniformity in the literature. Since that time, many clinicians have used the Amsterdam Criteria to make a clinical diagnosis of HNPCC and to select families for intensive surveillance and mutation analysis. Dr. Hans F. A. Vasen (The Netherlands Foundation for the Detection of Hereditary Tumors, Leiden University, Leiden, The

Netherlands), on behalf of Dr. Hans Morreau (Leiden University), described the Dutch experience with MSI and immunostaining in the detection of MMR mutation carriers.

Performance Characteristics of the Bethesda Guidelines

Dr. Jonathan P. Terdiman (University of California, San Francisco [UCSF], CA) conducted a set of studies to determine 1) the sensitivity and specificity of the modified Bethesda Guidelines (age cutoff of 50 years) (27) for the identification of HNPCC among high-risk patient populations and 2) whether the Bethesda Guidelines perform differently when applied to patients in a high-risk registry population, a referral population, or the general population.

The investigators interviewed 127 colorectal cancer patients from the UCSF high-risk cancer registry (28) and performed a medical record review and an MSI analysis of tumors for each patient. MSI-H tumors were found in 53 (42%) of the 127 patients, with mutations identified in 22 (61%) of the 36 patients tested. Statistically significant predictors of MSI were early age at colorectal cancer diagnosis, number of colorectal cancers per family, presence of other HNPCC cancers in the family, and presence of multiple cancers in a single family member. Interestingly, tumor location and histology were not independent predictors of MSI status. However, multiple colorectal tumors in an individual, regardless of age, were a specific predictor of MSI status. The likelihood of detecting an MSH2 or MLH1 germline mutation among high-risk patients with an MSI-H tumor was greater than 60%.

The study by Terdiman et al. (28) showed that, overall, the Bethesda Guidelines have good sensitivity (96%) but only modest specificity (27%) for identifying MSI-H tumors in high-risk populations. Individual Bethesda Guidelines had a wide range of performance characteristics, with guideline 2 (i.e., individuals with two or more HNPCC-related cancers) having the lowest sensitivity (42%) and highest specificity (96%) and guideline 4 (i.e., colorectal or endometrial cancer under age 50 years) having the highest sensitivity (85%) and the lowest specificity (32%). Terdiman et al. (29) also compared very early onset of colorectal cancer in patients diagnosed under the age of 36 years in the UCSF high-risk patient registry and the Northern California Kaiser Permanente Cancer Registry. A total of 54 probands were identified from the Kaiser Permanente registry population. Despite the clinical similarities between the patients in the two registries, statistically significant differences were found in terms of molecular test results between the two registry populations. Seventy percent of the UCSF patients had MSI-H tumors, whereas only 33% of the Kaiser Permanente patients had MSI-H tumors. Of those patients tested, 62% the UCSF patients had an MMR mutation, and none of the Kaiser Permanente patients had an MMR mutation. The extent of a family history of cancer and the institution from which a patient came were strong predictors of HNPCC. The investigators concluded that family history of cancer is an important determinant of HNPCC, even with early-onset colorectal cancer, and that caution must be exercised when applying clinical data regarding HNPCC in high-risk patients to the general population (29).

Sensitivity and Specificity of the Bethesda Guidelines

Dr. Sapna Syngal (Brigham and Women's Hospital and Dana-Farber Cancer Institute, Boston, MA) noted that some of

the difficulties associated with MSI and IHC testing may play an important role in patient care.

The goal of the Bethesda Guidelines is to identify HNPCC patients, not to identify MSI-H tumors from patients in sporadic populations that may have better prognoses or different therapeutic implications. Therefore, how well do the Bethesda Guidelines identify MSH2 and MLH1 mutation carriers? In a study (30) of 70 families collected from a family registry and referred to the Dana-Farber Cancer Institute, Dr. Syngal et al. performed a full-gene sequence analysis for MSH2 and MLH1 mutations on affected individuals without prescreening for MSI. The performance of the Bethesda Guidelines was compared with other existing HNPCC clinical criteria for predicting germline mutations in MSH2 and MLH1. The Bethesda Guidelines were found to be the most sensitive of the existing criteria for the identification of mutation carriers but were also found to be the least specific (30). The NCI panel of five markers (Table 1) identified every patient with a germline mutation of MSH2 or MLH1, and all germline mutations were found to be associated with MSI-H tumors (31).

Syngal et al. (30) also enrolled 433 colorectal cancer patients who presented at a gastrointestinal oncology clinic. Although patients who fulfilled the Bethesda Guidelines were more likely to be advised to undergo genetic assessment than those who did not fulfill the criteria, the majority of patients remained untested. The fact that there is this untested patient cohort indicates that the importance of family history is still being somewhat ignored in clinical practice.

Best Strategies for Identifying Individuals With MLH1 or MSH2 Gene Mutations

Dr. Andrew N. Freedman (Division of Cancer Control and Population Sciences, NCI, Bethesda, MD) conducted a literature search and found 10 population-based colorectal cancer studies (32–42) that examined clinical and family history and prevalence of HNPCC. Despite the fact that these studies were conducted in different populations and with different sample sizes, age structures, and ascertainment methods, similar prevalence estimates were found for HNPCC across all studies. The prevalence of HNPCC in population-based studies that used clinical and family history was 1.5% (95% confidence interval [CI] = 1.2% to 1.8%) for Amsterdam Criteria I or II and 2.6% (95% CI = 2.2% to 3.0%) when revised Amsterdam Criteria were used. When combining results from the five large population-based colorectal cancer studies (42–46) that examined both MSI status and mutations in the MLH1 and MSH2 genes, 15.2% (500/3300) of tumors were positive for MSI-H. The prevalence of MLH1 or MSH2 mutations in these population-based studies of colorectal cancer patients (using MSI as a screener) ranged from 0.9% to 2.7%. However, it should be noted that test performance characteristics of family history and clinical criteria and MSI in the general colorectal cancer patient population may differ from high-risk patient populations.

Dr. Freedman, together with Dr. Ann Chao (American Cancer Society, Atlanta, GA) and Dr. Anna Wu (University of Southern California [USC], Los Angeles), examined 199 colorectal cancer patients from the New Mexico Surveillance, Epidemiology, and End Results (SEER)¹ Program site and 239 colorectal cancer patients from the USC SEER site (36,47). Consistent with the population-based studies (42–46), 1.8% of

patients across the two SEER sites fulfilled the Amsterdam Criteria (26% fulfilled the Bethesda Guidelines criteria). Using MSI as a screen, the researchers identified 438 colon cancer tumors, 16% (70/438) of which were found to be MSI-H. Full MMR gene sequencing was performed on 65 of the MSI-H tumors, and an MLH1 or MSH2 mutation prevalence rate of 2.7% was found. The sensitivity, specificity, and positive predictive value of the Bethesda Guidelines criteria at identifying an HNPCC carrier was similar to that of the MSI-H criteria in this preliminary analysis.

An Economic Viewpoint on Alternative Strategies for Identifying Individuals With HNPCC

Dr. Scott Ramsey (Fred Hutchinson Cancer Research Center, Seattle, WA) presented data that showed that the Bethesda Guidelines are highly cost-effective for identifying HNPCC patients when first-degree relatives are included in the analysis. Out of a total of 148 300 newly diagnosed colorectal cancer patients, 23 417 patients would have their tumors tested for MSI status based on the Bethesda Guidelines; 2810 patients would be offered counseling and testing to determine their MMR gene mutation status (3612 probands and first-degree relatives combined); there would be 833 years of life gained in the probands (7615 years with the probands and first-degree relatives combined); the cost per MMR gene mutation carrier detected would be \$20 313 (\$15 787 with the probands and first-degree relatives combined); and the cost per life-year gained would be \$73 711 (\$11 865 with probands and first-degree relatives combined).

Dr. Ramsey emphasized that, although the Bethesda Guidelines are, in theory, the most efficient strategy for identifying HNPCC patients, the cost-effectiveness of this method depends on locating, testing, and screening first-degree relatives of patients identified with MMR gene mutations.

Pathologic Manifestations of MSI-H in Clinical Disease

Dr. Stanley R. Hamilton (The University of Texas M. D. Anderson Cancer Center, Houston) discussed MSI from a reverse-engineering standpoint and reminded the Workshop participants that MSI-H occurs in tumors other than those in HNPCC, such as sporadic neoplasms, hyperplastic polyposis syndrome, and serrated adenomas. One interesting finding from studies on colorectal cancer rates in MMR mutation carriers (48–50) is that males have a higher reported rate of MMR mutations than females; the explanation for this reported sex difference among MMR mutation carriers is unclear. MSI-H plays an important role in the morphogenesis of neoplasms in numerous organ sites and tissue types. Heterogeneity of pathologic and histopathologic manifestations of MSI-H in clinical disease is the rule rather than the exception. Pathologic and histologic findings can provide clues to the presence of MSI-H but tend to have poor sensitivity, specificity, and positive predictive values.

In a recent study (47), MSI analysis of 323 sporadic colon cancer cases using 10 microsatellite markers showed a very high specificity for identifying MSI-H tumors for a number of histopathologic characteristics (e.g., mucinous and signet-ring cell component) but had the tradeoff of poor sensitivity. There was a high level of agreement in identifying signet-ring cell carcinoma and poor differentiation among pathologists involved in the study, but there was a low level of agreement in identifying other

tumor characteristics associated with MSI-H, such as tumor-infiltrating lymphocytes.

Clinical Manifestations of HNPCC

Dr. Noralane M. Lindor (Mayo Clinic) described preliminary evidence from an ongoing study (Lindor NM: unpublished data) in which the rates of colorectal and other typical HNPCC-associated cancers are lower in Amsterdam Criteria families without MMR gene mutations than in families with MMR gene mutations. The risk of cancer was calculated for first- and second-degree relatives. From the 53 families analyzed, 1738 relatives were analyzed (693 were from the Amsterdam Criteria families with MMR gene mutations). The percentage of first- and second-degree relatives with colorectal cancer in families with an MMR gene mutation was higher than that in families without an MMR gene mutation (8.23% versus 2.58%). Similar results were found for endometrial cancer (4.18% versus 0.98% for families with and without an MMR gene mutation, respectively). The goal of this study is to enroll more than 100 families and to compare cancer risks in the clinic- and population-based families with cancer risks in the general population.

Similarly, previous studies give us a good comparison of IHC and MSI testing. Compared with MSI testing, IHC can provide unique as well as overlapping information about MMR defects. Loss of expression of the MLH1 or MSH2 genes is associated approximately 100% with an MSI-H phenotype; however, normal expression of these genes has been shown to predict an MSS phenotype 93% of the time (14). In addition, loss of expression can identify the specific MMR gene defects. However, the loss of expression of the MLH1 gene, mostly in sporadic MSI cases, can be genetic or epigenetic. The final decision to conduct MSI and IHC testing together or in some specific order may be patient- and/or center-specific and is based on prior probabilities of an abnormal test, local expertise in these techniques, rigorous cost analyses, and whether the test is being used as a screening or a diagnostic test (51). Current data (6) suggest that in HNPCC approximately 95% of MSI-H cases can be accounted for by a loss of expression of MLH1 (~40%), MSH2 (~40%), MSH6 (~10%), or PMS2 (~5%). For a small fraction of MSI-H cases (~5%), the etiology of the MSI remains unknown.

WORKSHOP DISCUSSION

After a detailed discussion, it was recommended that the Bethesda Guidelines be revised to clarify the issues mentioned above and to further aid in the identification of HNPCC kindreds for genetic testing. Explanations for the absence of a strong family history of cancer may include non-paternity, adoption, new mutation, lack of disease penetrance, denial of a family history of cancer, and small families. These ambiguous cases could be identified by screening all cancers for a DNA MMR defect. However, this approach would be costly, and it would be necessary to determine whether the presence of an MMR gene defect was due to HNPCC or whether it represented a genuinely sporadic occurrence. The rationale for developing testing criteria that are specific and sensitive for HNPCC as well as for defining the role of the pathologist in the diagnosis of HNPCC was discussed by Dr. Jeremy Jass (McGill University, Montreal, Quebec, Canada).

The quality assurance and quality control aspects of MSI and IHC analyses—from the time of patient identification to the time

of delivery of results and treatment—require careful attention and additional research, especially with respect to IHC analysis (e.g., Which fixatives work best for tissue processing?). Although most laboratories and/or research facilities use buffered formalin as the tissue fixative, it is not clear which fixative is most effective in facilitating MSI detection. Processing and tissue-handling protocols also vary widely across the country and around the world.

If MSI and/or IHC testing are not available, the pathologist may still raise the possibility of a diagnosis of HNPCC on the basis of histologic findings. When IHC and/or MSI testing are available, the clinician may still prefer to instigate MSI and/or IHC testing after discussing the issue with the patient, because MSI and IHC testing for DNA MMR proteins could be construed as ‘genetic tests,’ even though these tests are assessing phenotype rather than genotype. The most sensitive pathologic feature of MSI-H status, which can be assessed and quantified with hematoxylin–eosin-stained sections, is the presence of intraepithelial lymphocytes [i.e., tumor infiltrating lymphocytes (51,52)].

The role of clinicians and geneticists in the HNPCC diagnosis was discussed by Dr. Albert de la Chapelle (Ohio State University, Comprehensive Cancer Center, Columbus, OH). It was noted that 90% or more of the literature in this field is from the study of high-risk families or high-risk individuals. Hence, the HNPCC research community should consider studying HNPCC from the perspective of the general population or the general cancer population, in addition to focusing on the high-risk patient population groups. de la Chapelle et al. (53–55) have studied the general cancer population to extract more information about HNPCC by not excluding, *a priori*, any cancer patient from scrutiny. From this perspective, the natural history of HNPCC changes to reflect a later age onset and less familial influence (43,44).

The two central problems in the diagnosis of HNPCC are 1) the detection of large deletions and some splicing errors (56–58), mostly in the MSH2 gene, and 2) the interpretation of missense mutations, mostly in the MLH1 and MSH6 genes. These problems are a much greater source of HNPCC misdiagnosis than the lack of sensitivity of MSI or IHC testing. Researchers and clinicians generally cannot determine whether missense mutations are pathogenic when these mutations are first encountered. Thus, efforts must be undertaken to develop better methods to evaluate the clinical significance of missense mutations in these genes.

Workshop participants noted that the target audience for the revised Bethesda Guidelines (Tables 2 and 3) needs to be defined (e.g., primary care physicians, specialty physicians who interact with cancer patients, pathologists, surgeons, or a combination of them) and that the composition of the target audience would probably have an impact on the wording of the Guidelines and the venue for their publication. The Workshop participants recognized that clinicians who see and treat many cancer patients (e.g., oncologists, gynecologists, and gastroenterologists) are a critical audience. However, the important role that pathologists can play in helping to make the diagnosis of HNPCC was also noted. It was also recognized that dissemination of the Guidelines to primary care providers and to the general public is crucial if most cases of HNPCC are to be diagnosed. Another issue to consider when defining the target audience is the fact that the Bethesda Guidelines are directed at the evaluation of the

Table 2. The Revised Bethesda Guidelines for testing colorectal tumors for microsatellite instability (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,* regardless of age.
3. Colorectal cancer with the MSI-H[†] histology[‡] diagnosed in a patient who is less than 60 years of age.§
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.

*Hereditary nonpolyposis colorectal cancer (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 (48).

[†]MSI-H = microsatellite instability-high in tumors refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.

[‡]Presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

[§]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.

proband, but much of the benefit of these Guidelines and HNPCC testing would be directed at the relatives. Moreover, many health care providers fail to understand that the benefit of HNPCC testing extends to an entire family.

Another important issue is the choice of microsatellite markers and their sensitivity. The original NCI five-marker microsatellite panel for the evaluation of MSI may underestimate the number of MSI-H tumors (because of the use of three dinucleotide repeats) and, instead, overestimate the number of MSI-L tumors (because of the use of two mononucleotide repeats). The addition of mononucleotide markers to the analysis improves the sensitivity of the panel; hence, it has been suggested by the Workshop participants that more mononucleotide markers should be included in the evaluation of MSI (Table 1). Further modification of the original five-marker microsatellite panel might select for a different pool of MSI-L tumors. For example, use of the MYCL marker has been shown to be sensitive for MSI-L tumors (59). The clinical, pathologic, and biologic significance of MSI-L tumors is still not fully determined; thus, these areas of research represent legitimate opportunities for further investigation.

Dinucleotide repeats present interpretive challenges, and monomorphic markers may obviate the need for normal tissue in MSI testing. MSI testing of tumor types other than colorectal and endometrial requires further validation. MSI-H tumors are readily detected in the presence of germline mutations in the MSH2 and MLH1 genes. In addition, germline mutations in the MSH6 gene may also result in MSI-H, MSI-L, or MSS tumors. Thus, additional mononucleotide markers for the MSH6 gene need to be validated. Another area of research involves the detection of HNPCC cases due to MSH6 mutations and other modifier genes that may make classical HNPCC less penetrant and less obvious. Furthermore, it is not known how many MSI-L tumors represent occult MSH6 germline mutations. Additional research is also needed for the identification and validation of

Table 3. Recommendations for the process of molecular evaluation of patients identified as being at risk, based on meeting the Bethesda Guidelines*

Process of molecular evaluation:

1. The optimal approach to evaluation is microsatellite instability (MSI) or immunohistochemical (IHC) analysis[†] of tumors, followed by germline MSH2/MLH1 testing in patients with MSI-H tumors or tumors with a loss of expression of one of the mismatch repair genes.[‡]
 2. After the mutation is identified, at-risk relatives should be referred for genetic counseling and tested if they wish.
 3. An alternative approach, if tissue testing is not feasible, is to proceed directly to germline analysis of the MSH2/MLH1 genes.
 4. If no mismatch repair gene mutation is found in a proband with an MSI-H tumor and/or a clinical history of hereditary nonpolyposis colorectal cancer (HNPCC), the genetic test result is non-informative. The patients and the at-risk individuals (i.e., relatives) should be counseled as if HNPCC was confirmed and high-risk surveillance should be undertaken.
 5. There is a need to assure patients of confidentiality to allay fears related to discrimination based on genetic status.
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*MSI-H = microsatellite instability-high in tumors refers to changes in two or more of the five National Cancer Institute (NCI)-recommended panels of microsatellite markers. MSI-L = microsatellite instability-low in tumors refers to changes in only one of the five NCI-recommended panels of microsatellite markers.

[†]Standard mutation detection techniques include single-strand conformational polymorphism, denaturing gradient gel-electrophoresis analysis, and DNA sequencing. Alternative mutation detection techniques include monoallelic expression analysis, Southern analysis, and quantitative polymerase chain reaction (60); these methods may detect certain mutations such as large genomic rearrangements that may be missed by more conventional mutation detection methods.

[‡]For families with a strong suspicion of HNPCC, germline testing should be considered, even when the MSI/IHC results indicate MSI-L, microsatellite stable, or normal expression. The likelihood of finding a germline mutation in the MLH1/MSH2 genes of patients with colorectal cancer tumors that are not MSI-H is expected to be low; however, this likelihood has not been thoroughly studied.

microsatellite markers that can identify defects in modifier genes such as EXO1 and MSH3.

The Workshop participants discussed the notion of adapting the name of "Lynch syndrome (HNPCC)," because they realized that HNPCC is a misnomer that describes a syndrome that, in women, can lead to a predisposition for endometrial cancer. The participants also recommended future research priorities for the clinical and molecular evaluation of MSI. Recommendations for future clinical research priorities included 1) assessing the clinical significance of early-onset adenomas as a determinant of genetically defined HNPCC, 2) evaluating the performance characteristics of individual and combinations of pathologic features in predicting HNPCC, 3) examining the performance of the revised Bethesda Guidelines in the general population, and 4) ascertaining cancer risks in high-risk families who are mutation- and/or MSI-negative. The recommendations for future molecular research priorities included 1) determining the molecular mechanisms for MSI-L versus MSI-H tumors, 2) determining additional genes involved in the development of MSI-positive and -negative colorectal cancer, 3) defining dietary and chemopreventive approaches that might help the cancer disposition of HNPCC kindred, and 4) investigating genomic and proteomic approaches for the early detection and risk assessment of HNPCC cancer development.

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NOTE

¹*Editor's note:* SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

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