

Lynch syndrome (HNPCC) and microsatellite instability analysis guidelines

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The “International Workshop on Diagnostic Guidelines for Hereditary Non-Polyposis Colorectal Cancer and Microsatellite Instability” represents the third workshop in this series. In November 1996, NCI’s DCP convened a workshop entitled “Intersection of Pathology and Genetics in HNPCC Syndrome.” In December 1997, another workshop, “International Workshop on Microsatellite Instability and Replication Error Phenotypes in Cancer Detection and Familial Predisposition,” was sponsored by the NCI. Recommendations developed at these two workshops included the Bethesda Guidelines and panel of five specific microsatellite markers that have broad utility in several experimental and diagnostic settings. However, recent findings warranted a re-examination of the current guidelines for HNPCC diagnosis. MSI and immunohistochemistry (IHC) detection are other molecular tests that have been developed for detecting DNA mismatch repair (MMR) defects and were not included in previous HNPCC diagnostic guidelines.

The “International Workshop on Diagnostic Guidelines for Hereditary Non-Polyposis Colorectal Cancer and Microsatellite Instability,” held in December 2002, brought together experts in the areas of hereditary non-polyposis colorectal cancer (HNPCC) and microsatellite instability (MSI). Participants were charged with reviewing, evaluating, and updating existing criteria for HNPCC and MSI, as well as with providing recommendations to the NCI based on new insights into the disease and its manifestations. The workshop’s primary goal was to generate recommendations on appropriate strategies for: (1) evaluating MSI, (2) diagnosing HNPCC, and (3) identifying HNPCC mutation carriers.

In applying the results of new research on HNPCC, workshop participants were asked to consider the previous recommendations for MSI testing, refined these recommendations, and identify the most effective screening approaches.

First case of Lynch syndrome (HNPCC) may have been reported by Albert Warthin, who first suspected and documented the disorder in his affected seamstress (she died of endometrial cancer), in 1895. Dr. Warthin published the woman’s family history, characterized by a pattern of gynecologic cancer – specifically endometrial cancer – and many other gastrointestinal cancers. In 1961, Dr. Lynch documented a similar experience, with a patient whose family was riddled with colon and endometrial cancers. Dr. Lynch and colleagues subsequently identified other families with the same pattern of cancers, noting that in addition to colon and endometrial cancers, gastric, small bowel, and other cancers occurred significantly more often in these families [1–4].

It was observed that there was a significantly marked 70–80% excess of proximal colon cancers in these patients. Cutaneous manifestations such as Muir Torre features [5,6], sebaceous adenomas, and sebaceous carcinomas also were found to be associated with the disorder. Aside from colorectal cancers (CRC), endometrial cancers were identified as the second-leading cancer associated with the syndrome. With current detection and treatment options, it is felt that no one with HNPCC should die from colorectal cancer, assuming that the patient has been identified, has a dedicated physician, and has been referred to a gastroenterologist

who prescribed frequent screening colonoscopies (initiated at age 25). HNPCC patients who develop cancer should have a subtotal colectomy, given the excess of synchronous and metachronous cancers associated with HNPCC.

HNPCC is caused by an inherited mutation in the DNA mismatch repair (MMR) genes (*hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2*) and has the following cardinal features (reviewed in [7–10]):

- (1) early age of onset
- (2) proximal colon involvement
- (3) increased incidence of synchronous and metachronous colon cancers
- (4) an autosomal dominant inheritance pattern
- (5) extracolonic adenocarcinomas
- (6) distinctive pathologic features
- (7) increased survival from colorectal cancers
- (8) accelerated carcinogenesis and interval colorectal cancer.

To date, extracolonic cancers that are known to be associated with HNPCC include endometrial, ovary, breast, stomach, small bowel, pancreas, hepatobiliary, upper urologic, and brain. It is believed that the complete tumor complement for HNPCC has not yet been identified, and other tumors may eventually be associated with HNPCC as new data are collected. Current hypotheses as to the disorder's mechanism of action include: (1) mutator genes (e.g., *hMLH1*, *hMSH2*) causing genomic instability, leading to an enormous burden of microsatellite disturbance that overwhelms CRC, sending cells to apoptosis; and (2) immune response (peritumoral, lymphocytic infiltration, Crohn's-like reaction).

Lynch syndrome (HNPCC) as well as a number of sporadic cancers of multiple organs manifest repetitive DNA sequence instability termed as microsatellite instability (MSI). MSI is representation of the cells internal state of lack of DNA proofreading at the spellcheck or DNA mismatch repair correction steps and almost always manifested by genome wide hypermutability, particularly at single base pair mismatches and insertion/deletion frameshift mutations. Hence it is not surprising that MSI tumors are targets of multiple mutations that continue to accumulate and represent a true mutator phenotype. MSI occurs due to loss of DNA MMR activity, MSI is normally measured by PCR of microsatellite DNA. A typical panel involves testing five microsatellites; 40 or greater percent (two or more out of five) of microsatellites mutated is termed MSI-H, and 20 percent or less (one out of five) is termed MSI-L.

When no microsatellites are mutated tumors are considered MSS or microsatellite stable. Although MSI pathway is not one of the most common pathway in colorectal carcinogenesis. The most common form of genomic instability that leads to tumor development is chromosomal instability, for which familial adenomatous polyposis is the model disease and we lose tumor suppressor genes by loss of heterozygosity. It seems that in sporadic cancers genetic defects are not as common reasons for tumors to achieve MSI, rather most tumors start straight away by losing tumor suppressor genes through promoter methylation and become cancerous. It is a subset of these that happen to silence *MLH1* and that makes the microsatellite unstable.

Mismatch repair system in which we have protein information so far include MSH-2 and MLH-1 as the major mismatch repair proteins, MSH-2 can partner at least with MSH-6 and MSH-3, giving it somewhat different specificity in terms of recognizing mismatches. That triggers a process whereby the mismatch repair system recognizes and eventually chews up the mismatch in the newly synthesized strand. This serves to repair single base pair mismatches and insertion/deletion errors as they occur. The major mismatch repair proteins have some options with their protein pairs, and at least in the case of the Mut S homologs (MSH2, MSH3 and MSH6) which are recognition proteins; it confers some specificity for what is going to be repaired [9,11]. Lynch syndrome (HNPCC) is usually characterized by germline mutations in MSH2 or MLH1 and "attenuated" HNPCC by germline mutation in the MSH6 gene. Currently, there are no answers in the area of variant phenotypes. Muir-Torre Syndrome is mostly but not always caused by germline mutations in MSH2, but some people get unusual skin tumors (e.g., sebaceous adenomas, sebaceous carcinomas, carotoid acanthomas), and it is not known what does that, may be some modifier gene that slips into the family along with the MSH 2 germline mutation. For example, a cyclin D polymorphism is present in half of the population if you have one of the polymorphism, you get a more severe phenotype with earlier onset cancers than the other one. MSH6 germline mutations are called "attenuated" HNPCC, with a later onset, weaker penetrance and more endometrial tumors.

There are many diagnostic challenges even though it is known that there are two genes that are the main culprits. About one-half of the time, the mutation cannot be found, even though you know that that is what the disease is. It is not clear whether there is modifier genes involved, and whether there are more "HNPCC

Table 1
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 tumors¹, regardless of age.
3. Colorectal cancer with the MSI-H² histology³ diagnosed in a patient who is less than 60 years of age.⁴
4. Colorectal cancer or HNPCC-associated tumor diagnosed under age 50 years in at least one first-degree relative.
5. Colorectal cancer or HNPCC-associated tumor diagnosed at any age in two first or second-degree relatives.

¹Hereditary nonpolyposis colorectal cancer (HNPCC)-associated 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.

²MSI-H microsatellite instability-high in tumors refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.

³Presence of tumor infiltrating lymphocytes.

Crohn disease-like lymphocytic reaction, mucinous/ signet-ring differentiation, or medullary growth pattern.

⁴There 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.

genes” or hidden mutations in MSH2 and MLH1. Genetic testing for HNPCC is negative about one-half of the time, even when Amsterdam Criteria are met. Direct sequencing of MSH2 and MLH 1, as you broaden criteria to get more families in, the number of people for whom you can find mutations decreases.

The Amsterdam Criteria have been relaxed to accommodate new information about reduced penetrance and phenotypic variation in Lynch (HNPCC) syndrome. Many families with Lynch (HNPCC) syndrome may have negative genetic tests because of MSH2 deletions, which are non-detectable by routine measures; the presence of Alus indicates the mechanism, and where to look for more of these. One may use MSI testing of tumors to screen for these difficult cases.

The original Amsterdam criteria were developed in 1991 and were:

- (1) three or more family members with CRC, and all of the following features
- (2) one is a first degree relative of two others, and at least two successive generations are affected
- (3) pathological confirmation, FAP is excluded
- (4) one affected person has developed a tumor by 50 years of age or younger.

These criteria were modified in 1999 (Amsterdam II) and are:

- (1) at least three relatives with HNPCC-associated cancers (CRCA, endometrium, si, ureter, kidney)
- (2) one must be a first degree relative of two others
- (3) at least 2 successive generations involved
- (4) at least one younger than age 50 with cancer
- (5) pathological verification, exclude FAP.

Simplified original Bethesda Guidelines were released in 1996 and were:

- (1) Amsterdam I criteria met
- (2) individuals with more than one HNPCC cancer
- (3) CRCA and FDR with CRC/HNPCC cancer, one cancer younger the 45 years or adenoma younger than 40
- (4) CRC/endometrial cancer younger than age 45
- (5) right-sided CRCA, undifferentiated, younger than 45
- (6) signet ring CRCA younger than 45
- (7) adenomas younger than 40 years.

As a primary outcome of the 2002 meeting it was decided that the Bethesda criteria should be further simplified (Table 1) and the expert panel agreed on the revision of the original Bethesda guidelines that are outlined in Table 1 [12].

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