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Feature

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Hereditary Colorectal Cancer: Hereditary Nonpolyposis Colon Cancer

Introduction

The most prevalent hereditary colorectal cancers are hereditary nonpolyposis colon cancer (HNPCC), familial adenomatous polyposis (FAP), and MYH-associated polyposis (MAP). The September 2004 *Communiqué* feature discussed the familial adenomatous polyposis (FAP) and MYH-associated polyposis (MAP) hereditary colorectal cancers. This month we will focus on hereditary nonpolyposis colon cancer (HNPCC).

HNPCC is the third most commonly diagnosed cancer and the third leading cause of cancer deaths in the United States. It is estimated that there were over 147,000 newly diagnosed cases and 57,000 deaths due to colorectal cancer in 2003. Roughly 10-30% of cases arise within familial aggregates, with known genetic conditions such as HNPCC accounting for a small fraction of these cases. To date, some of the genes responsible for the hereditary CRC syndromes have been identified (Table 1), and routine genetic testing is available for many.

Recognition of a hereditary cancer syndrome within families and individuals is critical to optimal patient screening, surveillance, and management. Molecular genetic testing is now commercially available for HNPCC. However, such testing can be involved, costly, and time-consuming. Because there are numerous genes and gene defects responsible for HNPCC, strategies should be employed to minimize unnecessary testing while maximizing the informativeness of test results.

Clinical Hallmarks

The first description of a cancer-prone family with HNPCC dates back to the late-1800s. However, it was not until the work of Lynch in

Table 1. Classification of Hereditary Colorectal Cancer (CRC) Syndromes

- | |
|--|
| I. Nonpolyposis syndromes (2-3% of all CRCs) (MMR gene mutations) |
| A. Hereditary nonpolyposis colorectal cancer (HNPCC; Lynch syndrome) |
| B. Muir-Torre syndrome |
| C. Turcot syndrome (associated with glioblastoma) |
| II. Polyposis syndromes (~1% of all CRCs) |
| A. Adenomatous polyposis syndromes |
| 1. Familial adenomatous polyposis (FAP) (APC gene mutations) |
| a. Gardner syndrome |
| b. Turcot syndrome (associated with medulloblastoma) |
| c. Attenuated familial adenomatous polyposis (AFAP) |
| 2. MYH-associated polyposis (MAP) (MYH gene mutations) |
| B. Hamartomatous polyposis syndromes |
| 1. Peutz-Jeghers syndrome (LKB/STK11 gene mutations) |
| 2. Juvenile polyposis (SMAD4 gene mutations) |
| 3. Cowden syndrome (PTEN gene mutations) |

the 1970s that a more complete clinical picture of this disorder began to emerge. HNPCC is an autosomal dominant disorder characterized by the early onset of tumors in the setting of few polyps. The average age of colon cancer diagnosis in individuals with HNPCC is in the early- to mid-40s, although many tumors may occur in the 20s or even in teenage years. Colon cancer in HNPCC occurs more frequently in the proximal colon and has common histopathological features. Interestingly, although many of the histologic features of HNPCC are considered aggressive, HNPCC is paradoxically associated with a more favorable prognosis than sporadic colorectal cancer. In addition to colorectal cancer, several other tumor types are observed at an increased frequency in families with HNPCC. These include endometrial, gastric, ovarian, small intestine, brain, and upper urinary tract, and biliary tract tumors.

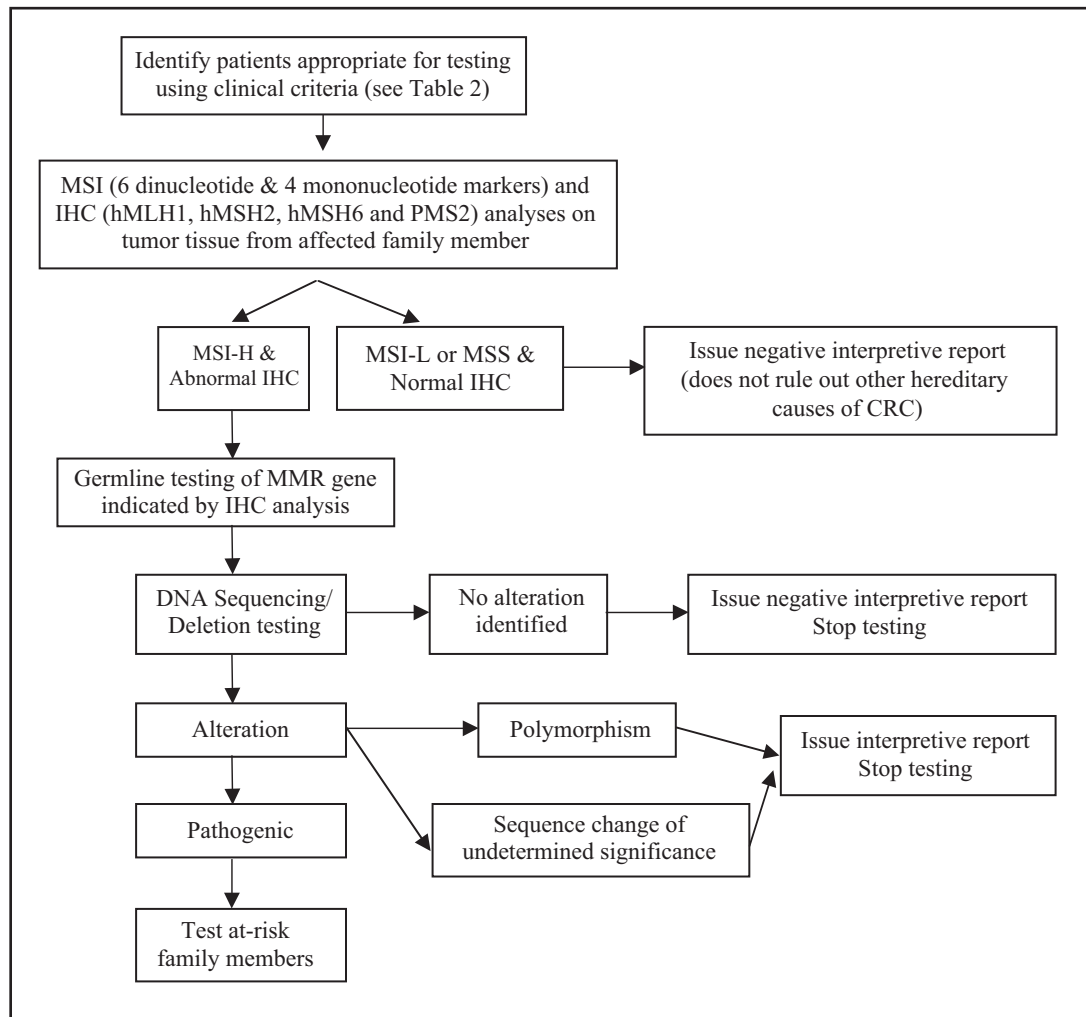


Figure 1. Flowchart for recommended HNPCC diagnostic testing.

Molecular Basis

Current data suggests that molecular defect for clinically defined HNPCC is likely to be very heterogeneous. Of these hereditary cases, approximately two-thirds have germline mutations in any 1 of several genes involved in DNA mismatch repair (MMR). Based on the recommendations of an NCI-sponsored meeting, this specific group of patients is now referred to as Lynch syndrome. Although still heterogeneous with respect to the DNA mismatch repair genes involved, this designation does represent a more homogeneous clinical patient population. DNA MMR is 1 of several mechanisms involved in the correction of mutations that occur because of exogenous or endogenous mutagens or misincorporation during DNA replication. This DNA repair process involves a complex set of proteins that include hMLH1, hMSH2, hMSH6, PMS2, and hMSH3.

Germline mutations in the MMR genes hMSH2 and hMLH1 account for approximately 80% of the reported mutations in families with defective MMR-associated HNPCC (Lynch syndrome). Nearly 10% of germline mutations occur in the hMSH6 gene, while a smaller number occur in PMS2. Of interest, MMR gene defects have also been demonstrated in patients with Muir-Torre syndrome and Turcot syndrome (Table 1). Muir-Torre is characterized by the presence of sebaceous skin lesions (adenomas and adenocarcinomas) in addition to colorectal cancer. Turcot syndrome is clinically distinguished by the presence of brain tumors (medulloblastoma and glioblastoma). Glioblastoma-associated Turcot syndrome is more commonly caused by defective DNA MMR, whereas medulloblastoma-associated Turcot is caused by mutations in the APC gene that are generally linked to FAP.

A hallmark of Lynch syndrome patients with defective MMR is the presence of microsatellite instability (MSI) in the patient's tumor. Tumor MSI is characterized by expansions or contractions in the number of tandem repeats at numerous microsatellite loci in the DNA. This characteristic feature in tumors from patients with defective MMR has been employed as part of a testing strategy for the identification of this group of patients. It is important to note that approximately 20% of sporadic colorectal cancers also exhibit defective MMR and MSI. However, unlike Lynch syndrome, the majority of these sporadic cases are due to hypermethylation of the hMLH1 promoter, a somatic alteration that results in a reduction of hMLH1 transcription, but does not infer an inherited predisposition.

Proband Identification

Because there are no characteristic phenotypic features of Lynch syndrome, its diagnosis has historically been established based on family history. In 1991, the International Collaborative Group on HNPCC (ICG-HNPCC) established the Amsterdam criteria to aid in the identification of families with HNPCC and to make the process of defining families for research purposes more uniform (Table 2). These criteria were based primarily on an extended family history of colorectal cancer and were instrumental in helping to identify the genes responsible for a majority of families with HNPCC. However, due to the strict nature of the Amsterdam I criteria, the more liberal Amsterdam II criteria, Bethesda guidelines, and revised Bethesda guidelines were subsequently proposed (Table 2) to aid in the identification of individuals at high risk for having a germline alteration in 1 of the MMR genes (Lynch syndrome). These criteria cast a wider net and include individuals with Lynch syndrome-associated tumors as well as patients with early onset and/or multiple primary tumors whose family history is either incomplete or the pedigree is small. These guidelines also include criteria based on the common histopathological presentations of the tumors and make recommendations for germline DNA testing.

Molecular Testing of Proband

As mentioned earlier, because there are no characteristic phenotypic features (such as polyposis for FAP), establishing the diagnosis of HNPCC/Lynch syndrome has been difficult. Family history, by itself, may not be sufficient to make a diagnosis. Furthermore, current data suggest that only 70% or so of HNPCC

families involve the MMR pathway (Lynch syndrome) and 30% likely involve other genes not yet identified. Finally, of those families that do involve the MMR genes, abnormalities may be present in a minimum of 4 different MMR genes. Thus, to most efficiently identify germline mutations in patients identified through family history and/or clinical criteria to be at risk for Lynch syndrome, a stepwise diagnostic procedure is recommended (Fig. 1).

Tumor Screening

Utilizing this approach, MSI and immunohistochemical analyses are first performed on tumor tissue as an initial screen for individuals at increased risk to have germline MMR defects ([#17073 Hereditary Nonpolyposis Colorectal Cancer \[HNPCC\] Screen](#)). MSI analysis begins with DNA extracted from paraffin-embedded tissue sections ideally containing >50% tumor cells. The corresponding normal control DNA is derived from adjacent normal mucosa. Isolated DNA is analyzed by polymerase chain reaction (PCR) and then subjected to size-based electrophoretic separation. MSI can be observed by comparing the electrophoretic patterns of amplified DNA from both tumor and normal tissue, and is scored as the presence of novel fragments in tumor DNA compared to normal DNA (Fig. 2). Tumors analyzed for MSI can be divided into 3 discrete groups: MSI-H (high frequency MSI with $\geq 30\%$ marker instability), MSI-L (low frequency MSI with $< 30\%$ marker instability), and MSS (microsatellite stable). Current data indicate that the MSI-H phenotype is due almost exclusively to defective MMR. MSI-L and MSS tumors appear to share the same phenotypic characteristics with little or no involvement of defective MMR.

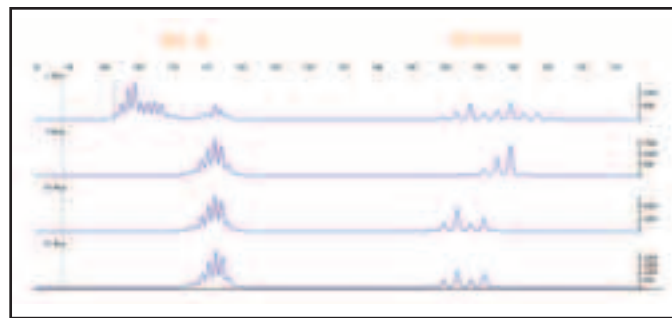


Figure 2. Microsatellite instability analysis. Example of microsatellite instability (MSI) testing of 2 patients with the mononucleotide marker BAT 26 and the dinucleotide marker D17S250 (A-D). Comparisons are made between tumor and normal tissue DNA. Patient 1: A. Tumor tissue, demonstrating MSI for both markers; B. Normal tissue. Patient 2: C. Tumor tissue, demonstrating MSS for both markers; D. Normal tissue.

Immunohistochemistry (IHC) provides additional important information about the specific MMR gene that is most likely to be mutated. IHC analysis of tumors is now routinely available for hMSH2, hMLH1, hMSH6, and PMS2 (Fig. 3). Tumor cells that demonstrate an absence of nuclear staining in the presence of positively stained normal cells are interpreted as having an absence of protein expression and defective MMR. Interestingly, loss of expression of hMSH2 is accompanied by loss of hMSH6, and similarly, loss of expression of hMLH1 is accompanied by loss of PMS2. However, loss of hMSH6 and PMS2 can occur alone. In practice, the loss of expression of an MMR protein in the appropriate clinical setting (Table 2) is strong evidence for the presence of a germline mutation in that respective gene and efforts to determine the precise germline mutation can take place.

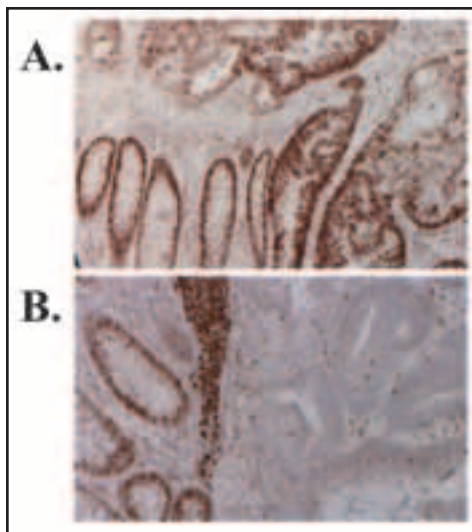


Figure 3. Immunohistochemical analysis with MSH6 antibody. A. Tumor and surrounding normal tissue showing nuclear staining for MSH6 expression. B. Normal tissue with positive stain, but tumor tissue shows an absence of staining, indicating a loss of MSH6 protein expression in the tumor.

The HNPCC screen ([#17073 Hereditary Nonpolyposis Colorectal Cancer \(HNPCC\) Screen](#)) is a comprehensive tumor analysis that includes MSI for 10 markers and IHC for 4 proteins (hMLH1, hMSH2, hMSH6 and PMS2). This assay allows us to determine if the tumor is caused by defective MMR and, if so, which gene should be targeted for germline testing. Tumors that do not exhibit defective MMR and do not show absence of protein expression are highly unlikely to be caused by germline mutations within any of the MMR genes.

These patients likely fall into the 30% of HNPCC-related clinical diagnoses that are not caused by defects of MMR. The benefit of performing MSI and IHC in tandem is to provide the most information to the patient and allow for more cost-effective testing. By identifying the gene of interest (via IHC) the cost of germline testing is reduced as it is generally only necessary to test for the gene that lacks expression. Additionally, a small proportion of patients will have tumors that are MSI-H with normal protein expression. Although very rarely observed, absence of protein expression along with an MSS or MSI-L tumor phenotype may occur. When taken together, a more comprehensive interpretation and recommendations for further analysis can be provided to the clinician and patient in an interpretive report.

For diagnostic cases, it is generally more informative to perform MSI and IHC concurrently. However, certain circumstances may result in the need to perform 1 or the other alone. For example, a number of institutions are considering and/or have already implemented screening protocols for the detection of patients at high risk for having Lynch syndrome, as recommended in the revised Bethesda guidelines (Table 2). Such a program was recently implemented at Mayo Clinic. For all patients having colon cancer surgery and who are less than 50 years old, reflex testing on tumor tissue is performed for MSI, but not IHC. IHC testing for MSI-H cases is deferred until the patient has been notified of results because IHC results for some of the proteins are highly predictive of a germline mutation (eg, hMSH2). This strategy allows patients to decide if they wish to have further testing for Lynch syndrome. Tumor MSI testing is currently only available as part of [#17073 Hereditary Nonpolyposis Colorectal Cancer \(HNPCC\) Screen](#). Individual MSI and IHC tests are currently under development and will be offered to our clients in the near future.

Tumor Sample Submission

The submission of tumor specimens for MSI/IHC analysis can be complicated. Some individuals have multiple tumor specimens, while in other families it is nearly impossible to track down the most appropriate specimen for testing. The accuracy of Lynch syndrome screening can vary significantly depending upon the type of tumor being analyzed. Colon, endometrial, gastric (small bowel and upper GI), transitional cell of the renal pelvis/ureter, and sebaceous adenocarcinomas are the most informative tumor types for Lynch syndrome screening. Secondly, analysis can be performed on neoplasms from the uterus or brain

(ie, glioblastoma). Those tumors that are weakly associated with Lynch syndrome (ie, ovarian, hepatobiliary, pancreatic, renal, and bile duct) have very limited results as this population is not enriched for Lynch syndrome. Breast, lung, prostate, and bladder cancers are very poor candidates for MSI/IHC. Additionally, colon polyps can be problematic for MSI/IHC testing. In general, it is often difficult to obtain enough DNA from a polyp to perform MSI

Table 2. Criteria for Clinical Diagnosis of HNPCC

The following minimum criteria should be met:

Amsterdam criteria I

1. At least 3 relatives should have histologically verified colorectal cancer; 1 of them should be a first-degree relative to the other 2.
2. At least 2 successive generations should be affected.
3. In 1 of the relatives, colorectal cancer should be diagnosed under 50 years of age.
4. Familial adenomatous polyposis should be excluded.

Amsterdam criteria II

1. There should be at least 3 relatives with an HNPCC-associated cancer (CRC, cancer of the endometrium, small bowel, ureter, or renal pelvis)
2. One should be a first-degree relative of the other 2.
3. At least 2 successive generations should be affected.
4. At least 1 should be diagnosed before age 50.
5. Familial adenomatous polyposis should be excluded in the CRC case(s), if any.
6. Tumors should be verified by pathological examination.

Bethesda guidelines

1. Individuals with cancer in families that meet the Amsterdam criteria.
2. Individuals with 2 HNPCC-related cancers including synchronous and metachronous CRC, or associated extracolonic cancers*.
3. Individuals with CRC and a first-degree relative with CRC and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; 1 of the cancers diagnosed at age less than 45, the adenoma at age less than 40.
4. Individuals with CRC or endometrial cancer diagnosed at age less than 45.
5. Individuals with right-sided CRC showing undifferentiated pattern on histopathology**.
6. Individuals with signet ring cell type CRC***.
7. Individuals with adenomas diagnosed at age less than 40.

Revised Bethesda guidelines

1. CRC diagnosed in individual <50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors*, regardless of age.
3. CRC with the MSI-H histology, in patient <60 years of age.
4. CRC in 1 or more first-degree relatives with an HNPCC-related tumor, with 1 of the cancers being diagnosed under age 50 years.
5. CRC diagnosed in 2 or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

* endometrial, ovarian, gastric, hepatobiliary, small bowel, transitional cell carcinoma of the renal pelvis or ureter

** solid cribriform defined as a poorly undifferentiated carcinoma composed of irregular solid sheets of large eosinophilic cells and containing small glandlike spaces

*** composed of more than 50% signet ring cells

and, due to the type of surgical removal performed, there is rarely enough normal tissue present for comparison in MSI analysis. Finally, because polyps are relatively common under the age of 50, the population is not well selected for Lynch syndrome. Presence of a few polyps in context of a family history of Lynch syndrome-related cancers does not ensure that the polyps in that patient are caused by the same genetic mechanism as the other cancers in the family. Therefore, a negative result from MSI/IHC on a polyp can provide false reassurance. As a result, analysis of polyps is not generally recommended. It is strongly recommended that Lynch syndrome screening be performed on the most appropriate neoplasm in the family. A pathology consultation on the submitted tumor specimen is included with MSI/IHC analysis and will determine if Lynch syndrome screening is appropriate based on the sample provided.

Germline Testing

Once the gene of interest has been implicated using MSI/IHC, DNA sequencing (Fig. 4) can be performed to identify a specific genetic alteration and genetic predisposition. Direct sequencing is limited by the fact that it does not detect large deletions, duplications, or

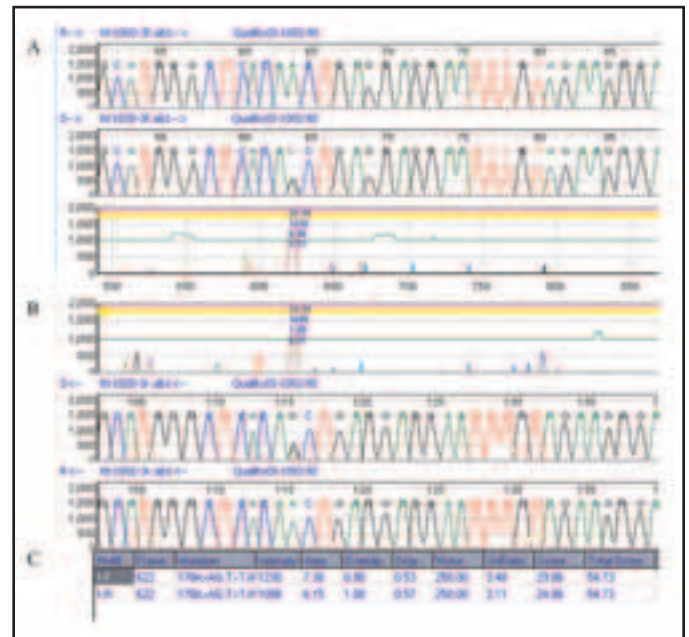


Figure 4. Automated fluorescent sequence of hMLH1 demonstrating a heterozygous A>G mutation. A. Forward sequence (mutation at nt 64). B. Reverse sequence (mutation at nt 116). C. Summary of mutation in both forward and reverse. R = reference (normal) sequence, S = patient sample.

other genomic rearrangements. Our studies indicate that these mutations make up roughly 30% of mutations observed in hMSH2 and 5-10% of hMLH1 mutations. Recently, Lynch et al described the identification of a founder mutation in hMSH2, specifically a deletion of exons 1-6, in a large outbred US population with a wide geographic distribution. Due to the presence of deletions and duplications, analysis by multiplex ligation-dependent probe amplification (MLPA), or Southern blot, is a part of our routine mutation detection protocol for Lynch syndrome (Fig. 5). Comprehensive mutation screening is currently available for hMLH1 (#83015 MLH1 Mutation Screen), hMSH2 (#83016 MSH2 Mutation Screen). Testing for hMSH6 will be available in the near future. Analysis of each of these genes includes DNA sequencing and MLPA for large rearrangements. The vast majority of germline mutations in these genes will be detected using this analytical approach. Mutations that are not detected by this approach, or other standard sequencing techniques, are those that occur in the promoter or in noncoding areas of the gene.

If a tumor specimen cannot be located within the family for Lynch syndrome screening, direct germline testing can be performed on a symptomatic individual;

however, due to the number of genes involved and the limitations of testing, it is not recommended that gene testing be performed on an asymptomatic individual until a mutation has been identified in an affected family member.

Molecular Testing of At-Risk Family Members

Once a mutation has been identified in an affected family member, site-specific analysis can be performed on blood specimens from at-risk family members using our known mutation assays for hMLH1 (#83002 MLH1 Known Mutation), hMSH2 (#83082 MSH2 Known Mutation), and hMSH6 (which also will be introduced in the near future). Demonstrating the presence of a familial mutation in an at-risk family member can assist asymptomatic individuals in making decisions regarding surveillance, management, and prophylactic surgery. Similarly, absence of a familial mutation in an at-risk family member can eliminate the need for costly surveillance and the patient can be put on general population screening protocols.

Conclusions

Molecular genetic testing in an individual with a suspected diagnosis of hereditary colorectal cancer has important implications for both the affected individual and at-risk family members. The identification of a deleterious germline mutation constitutes a genetically defined diagnosis of hereditary colorectal cancer and is important for the treatment and subsequent surveillance of the disease. In addition, the identification of a germline mutation allows predictive testing to be performed on unaffected relatives (Fig. 1) at a relatively small cost and with essentially 100% accuracy. Thus, the identification of a germline mutation in families with suspected hereditary colorectal cancer is of paramount importance and optimized screening strategies should be considered by clinicians.

Adapted with permission from the American Association for Clinical Chemistry, Washington, DC, from Clinical Laboratory News, July 2004.

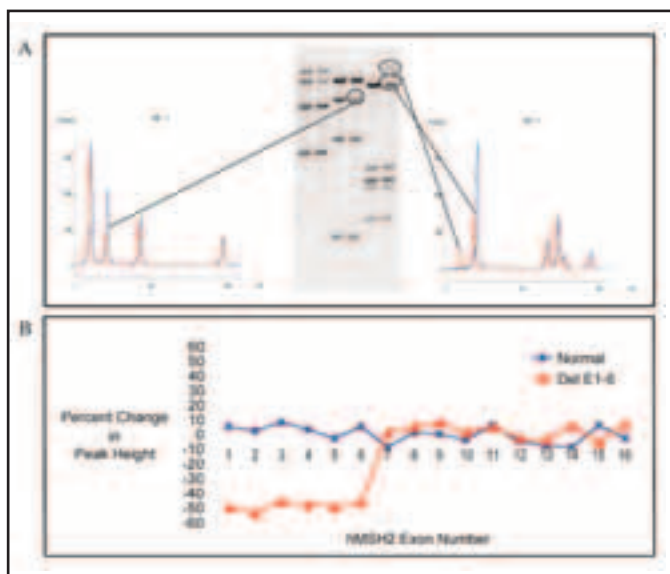


Figure 5. Deletion of hMSH2 exons 1-6.
A. Southern blot analysis using the G3 probe (middle panel) and corresponding densitometry (red peaks indicate patient sample). B. Multiplex Ligation-Dependent Probe Amplification (MLPA) demonstrating same deletion.

Test Updates

Immunoglobulin Gene Rearrangement Method Change

The polymerase chain reaction (PCR) method for **#83123 Immunoglobulin Gene Rearrangement** was changed from an in-house assay to a commercial kit. The new method uses a total of 34 upstream and 5 downstream primers in 7 separate PCR reactions (InVivo Scribe IGH and IGK Gene Clonality Assay kits). The primers are designed to amplify fragments from all theoretical rearrangements of the IG heavy and IG kappa light chain genes. Each unique rearrangement should produce PCR fragment(s) of unique size(s). The primers cannot amplify anything if the IG genes are not rearranged because the distance is too great. The primers are labeled with a fluorescent tag so that the PCR product can be detected. PCR fragments are analyzed by capillary gel electrophoresis using the Applied Biosystems (ABI) 3100. Please note that this kit is labeled For Research Use Only, and is used per the manufacturer's instructions.

The test classification will now include the comment "For Research Use Only" when PCR is performed.

Surgical Pathology Consultation Expanded to Include Ewing Sarcoma Algorithm FISH

MML's **#5439 Surgical Pathology Consultation** currently utilizes molecular testing to differentiate small round cell tumors. The algorithm for Ewing sarcoma has been expanded to include a fluorescence in situ hybridization (FISH) step when there is insufficient RNA or questionable results by the reverse transcriptase-polymerase chain reaction (RT-PCR) assay. **#83656 Ewing Sarcoma (EWS) Anomalies at 22q12, Fluorescence In Situ Hybridization (FISH)** is available only as part of the **#5439 Surgical Pathology Consultation**, and will be performed under the direction of the reviewing Mayo pathologist. Specific questions can be directed to Mayo Laboratory Inquiry at 800-533-1710.

Hepatitis C Genotyping Test Changes

To facilitate ordering, the unit code and test name have been changed for **#15158 Hepatitis C Genotype, Serum**. No other aspect of the test was impacted by these changes.

New Unit Code

#84434

New Test Name

Hepatitis C Virus (HCV) Genotype After Amplification, Serum

Syphilis Testing Improvements

Syphilis (*Treponema palladium*) test offerings have now been expanded to include 2 new tests:

#84419 Syphilis Antibody IgM, Serum

#84425 Syphilis Antibody IgG & IgM, Serum

(See New Test Announcements in this issue for detailed information.)

Additionally, **#81814 Syphilis Antibody, Serum** has been converted from the CAPTIA method to the Trep-Chek Anti-Treponema EIA. In response to this change the test name has been changed to more appropriately reflect test performance: **#81814 Syphilis Antibody, IgG, Serum**.

Toxoplasma Antibody Testing Changes

Previously providing only a qualitative result, the following *Toxoplasma gondii* antibody tests will now provide quantitative results.

#8267 Toxoplasma Antibody, IgG, Serum
New Reference Values

IgG
Negative: <4 IU/mL
Equivocal: 5-7 IU/mL
Positive: >8 IU/mL

#8865 Toxoplasma Antibody, IgM, Serum
New Reference Values

IgM
Negative: <0.55
Equivocal: >0.55 to <0.65
Positive: >0.65

2004 Education Calendar

Interactive Satellite Programs . . .

Thyroid Disease – Laboratory Support For Diagnosis and Management

December 7, 2004

Presenter: George Klee MD, PhD

Moderator: Robert M. Kisabeth MD

Upcoming Education Conferences . . .

Introductory Clinical Mycology

November 18-19, 2004

Mayo Clinic, Siebens Building

Rochester, Minnesota

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