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Genetic Testing for Hereditary Colorectal Cancer

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Genetic testing for hereditary colorectal cancer syndromes is more complex than genetic testing for hereditary breast-ovarian cancer syndrome. This is due to the following: 1) there are many more syndromes to consider in the differential diagnosis, 2) some of the syndromes have considerable phenotypic overlap, 3) many of the syndromes can be caused by mutations in any one of a number of causative genes, and 4) testing for Lynch syndrome (the most common hereditary cause of colorectal cancer) includes a combination of tumor testing and germline genetic testing. As a result, many clinicians find genetic testing for hereditary colorectal cancer to be a challenge. The clinical features of all the various hereditary colorectal cancer syndromes have been reviewed in other chapters in this issue. In addition, genetic counseling, which is a prerequisite for genetic testing to ensure appropriate informed consent, has been reviewed by Aronson. This chapter will focus on genetic testing for hereditary colorectal cancer syndromes.

The hereditary colorectal cancer syndromes can be divided into the polyposis and nonpolyposis syndromes keeping in mind that there are polyps in the nonpolyposis syndromes, however, they are usually less numerous. The presence of ten colonic polyps is sometimes used as a rough threshold for when to consider genetic testing for a polyposis syndrome. The polyposis syndromes can be further subdivided by the histology of the polyps; namely the hamartomatous and adenomatous polyposis syndromes. Hamartomatous polyposis syndromes include Juvenile Polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), Hyperplastic Polyposis syndrome (HPS), and the *PTEN* Hamartomatous Tumor syndrome (comprised of Cowden syndrome and Bannayan Ruvalcaba-Riley syndrome). Adenomatous polyposis syndromes include Familial Adenomatous Polyposis (FAP), Attenuated FAP (AFAP), and *MUTYH*-associated Polyposis (MAP). A diagnosis of Mixed Polyposis syndrome should be considered in individuals with both hamartomatous and adenomatous colon polyps and with no features of the other polyposis syndromes. The nonpolyposis syndromes include Lynch syndrome (LS), Familial Colorectal Cancer syndrome Type X, and occasionally can include individuals with MAP and a low polyp count. See Figure 1 for a flowchart demonstrating this differential diagnosis.

It is important to note that genetic testing is now available on a clinical basis for every gene discussed in this chapter. To find a list of laboratories performing genetic testing for any of

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these genes visit the GeneTests website at www.genetests.org. When selecting a laboratory for clinical testing, there are many considerations. While price and billing considerations may be most important to the patient, it is also very important to consider the testing modality used (sequencing vs. other typically less sensitive mutation screening modalities and whether testing for large rearrangements is included) as this can significantly affect the sensitivity of the test. The importance of including large rearrangement testing varies from gene to gene and is discussed throughout this chapter.

Genetic testing for the polyposis syndromes

Genetic testing for the polyposis syndromes is generally more straightforward than testing for the nonpolyposis syndromes, however, several of the conditions are polygenic and genetic testing does not have 100% sensitivity for any of the syndromes at this time. As such, the most important prerequisite for genetic testing in the polyposis syndromes is making certain of the differential diagnosis in order to select the correct gene test. To that end, it is often recommended that a dedicated gastrointestinal pathologist review the pathology of the polyps to confirm the type of polyp when there is any doubt. Once the syndrome has been correctly identified, testing should proceed as described below.

Hamartomatous polyposis syndromes

Juvenile Polyposis syndrome (JPS)

JPS (OMIM 174900) can be due to mutations in either the *BMPRIA* gene or the *SMAD4* gene. *SMAD4* is located on chromosome 18q21.1 and was identified as a susceptibility gene for JPS in 1998 (1). *BMPRIA* is located on chromosome 10q22.3 and was identified as the second gene responsible for JPS in 2001 (2). Data from all studies involving sequencing and large rearrangement testing (using multiplex ligation-dependent probe assay, or MLPA) of these genes in JPS patients were reviewed recently (3). Point mutations were found in 40.1% of JPS patients. This included 21.6% (77/357) with *SMAD4* mutations and 18.5% (62/336) with *BMPRIA* mutations. Large rearrangements were found in 8.7% of JPS patients. This included 4.6% (9/194) with *SMAD4* deletions and 4.1% (8/194) with *BMPRIA* deletions. The likelihood of finding a *SMAD4* mutation (26.8%) was approximately the same as the likelihood of finding a *BMPRIA* mutation (23.7%) when the analysis was restricted to only the three studies that included both sequencing and MLPA. Although the likelihood of finding a mutation in either gene is about equal, testing could still be ordered one gene at a time to potentially save the patient money (because the second gene test would not be necessary if a mutation was found in the first gene). Due to the association of *SMAD4* mutations with Hereditary Hemorrhagic Telangiectasia, mutations in this gene are more likely if a patient with JPS reports a history of recurrent nosebleeds, arteriovenous malformations, or telangiectasias (4). Unfortunately, the causative mutation will only be identified in around 50% of patients with a clinical diagnosis of JPS when genetic testing includes both sequencing and MLPA of the *SMAD4* and *BMPRIA* genes. This suggests that there are possibly additional genes that cause JPS or other mutations that inactivate *SMAD4* and *BMPRIA* that cannot be detected currently. This also means that a negative genetic test result for *SMAD4* and *BMPRIA* will not rule out the diagnosis of JPS and cannot be used to exclude the diagnosis in a patient who does not meet the clinical diagnostic criteria for JPS.

Peutz-Jeghers syndrome (PJS)

PJS (OMIM 175200) is caused by mutations in the *STK11* gene (also known as *LKB1*). *STK11* is located on chromosome 19p13.3 and mutations in this gene were found to cause PJS in 1998 (5). Combining data from the two largest series of PJS patients tested for mutations in *STK11* by full sequencing and MLPA yields the following results (5,6). Out of 132 patients

meeting the clinical diagnostic criteria for PJS, mutations were identified in 85% (112/132). This includes 84 (63.6%) point mutations identified by sequencing and 28 (21.2%) large deletions identified by MLPA. These results make it less likely that there is another as yet unidentified gene for PJS, however, there are some linkage studies in PJS families that have reported exclusion of the *STK11* locus (7–9). At this point, a negative test result cannot rule out a diagnosis of PJS but mutations will be identified in the majority of patients with a clinical diagnosis of PJS if testing includes both sequencing and large rearrangement analysis of the *STK11* gene.

Hyperplastic Polyposis syndrome (HPS)

The gene(s) responsible for HPS (not included in OMIM) have not been identified. Clinicians should try to enroll patients meeting a clinical diagnosis of HPS into a research study attempting to identify the responsible gene. In the meantime, the diagnosis can only be made clinically based on the WHO diagnostic criteria; ≥ 5 hyperplastic polyps proximal to the sigmoid colon with two measuring > 1 cm in diameter or > 30 hyperplastic polyps anywhere in the colon (10).

***PTEN* Hamartoma Tumor syndrome (PHTS)**

PHTS includes syndromes caused by germline mutations in the *PTEN* gene including Cowden syndrome (CS; OMIM 158350) and Bannayan Ruvalcaba Riley syndrome (BRR; OMIM 153480). The *PTEN* gene is located on chromosome 10q23.3 and mutations in this gene were associated with CS and BRR in 1997 (11,12). A recent review (13) found that there is not strong evidence of an increased risk for colorectal cancer among patients with PHTS, however, it is likely that 70–80% of patients with PHTS have colon polyps when they are evaluated with colonoscopy (14–16). Although it is generally stated that 80% of CS patients will have *PTEN* mutations, by combining the published findings of five studies that included sequencing of the *PTEN* gene in patients meeting the diagnostic criteria for CS, it appears that a mutation will be identified only 53% (54/101) of the time (11,17–19). Large deletions seem to be rare in this gene with two studies reporting a combined prevalence of 2.3% (4/175) among CS or CS-like patients without identifiable point mutations in the *PTEN* gene (20,21). Variations in the promoter region have been reported in 10% (9/95) of patients who are negative for a *PTEN* mutation on sequencing (around 2% of all CS patients); however, it is not clear yet whether these mutations are deleterious (20). *PTEN* gene sequencing seems to be sufficient at present for *PTEN* testing in a patient suspected of having PHTS since the addition of large deletion or promoter mutation analysis will only add 2–3% to the overall mutation detection rate. This may indicate that there are other genes responsible for some cases of CS, other changes that inactivate the *PTEN* gene that cannot be detected at present, or possibly that the diagnostic criteria are not stringent enough given that many of the features of this syndrome (fibrocystic breasts, thyroid nodules, endometrial fibroids) are quite common in the general population. A negative result cannot rule out the diagnosis given the low detection rate at this time.

Adenomatous Polyposis syndromes

Since there is considerable overlap among the adenomatous polyposis syndromes, the approach to genetic testing for all three syndromes will be described at the end of this section.

Familial Adenomatous Polyposis (FAP)

FAP (OMIM 175100) is caused by mutations in the *APC* gene located on chromosome 5q21-22. Mutations in the *APC* gene were determined to cause FAP in 1991 (22,23). Genetic testing for *APC* mutations has changed significantly over the past ten years. In the late 1990's the only *APC* testing available clinically in the United States was protein truncation testing. This was

followed by full sequencing of the *APC* gene and then large rearrangement analysis (using an approach like MLPA). Depending on the clinical features present in the patient and the testing modality utilized [single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), or protein truncation testing (PTT) were used in most studies], mutations in the *APC* gene are found in 48–80% of patients with polyposis (24–26). In the largest study to date involving 680 polyposis families, *APC* mutations were found in 31.7% of patients with <100 adenomas and 58.2% of patients with >100 adenomas (24). The addition of large rearrangement testing of the *APC* gene has been shown to detect mutations in an additional 4–33% of polyposis patients who had previously tested negative (27–31). Therefore, *APC* gene testing should always include both testing for point mutations (using sequencing or another technique) and large rearrangement analysis. In addition, since 2002 it has been shown that 7.5–17% of classic polyposis patients have biallelic *MUTYH* mutations. As a result, for patients who previously tested negative for *APC* mutations, there may be additional testing available that could help identify the cause of their polyposis.

Attenuated FAP (AFAP)

AFAP (OMIM 175100) is described in patients with 10–99 adenomas. It can be caused either by germline mutations in the *APC* gene (discussed above) or by biallelic mutations in the *MUTYH* gene (described below). In a series of 25 Dutch families who met AFAP criteria (32), *APC* mutations were found in nine families (36%) and biallelic *MUTYH* mutations were found in nine families (36%). There was not a significant difference in the clinical features of the families with *APC* or *MUTYH* mutations. Despite the fact that the *APC* mutations responsible for AFAP tend to occur in either the 5' end of the gene (the first five exons), in exon 9, or in the distal 3' end of the gene (33), full gene sequencing is necessary since exceptions have been reported and there is no targeted *APC* test available for AFAP.

MUTYH-Associated Polyposis (MAP)

MAP (OMIM 608456) is caused by biallelic mutations in the *MUTYH* gene. This is the only known example of an autosomal recessive colon cancer susceptibility syndrome. The *MUTYH* gene is located on chromosome 1p32.1 and was found to cause polyposis and colon cancer in 2002 (34). The presentation is quite variable; most patients have attenuated polyposis but some have classic polyposis indistinguishable from FAP and others have colon cancer without polyposis (leading to clinical overlap with Lynch syndrome). A recent review (35) found that 5–22% of patients with 3–100 adenomas and 7.5–17% of patients with more than 100 adenomas have biallelic *MUTYH* mutations (36–40). Most of the mutations found in the *MUTYH* gene have been missense mutations. The two most common *MUTYH* mutations are the Y179C (previously known as Y165C before a nomenclature change) and G396D (previously known as G382D before a nomenclature change) mutations which account for 73% of *MUTYH* mutations found in Caucasian populations with Northern European ancestry (41). Other *MUTYH* mutations seen commonly include the R245C and IVS10-2a>g mutations in Japanese MAP patients, the c.1145delC mutation in Italian MAP patients, A473D in Finnish MAP patients, and E383fxX451 found in Portuguese MAP patients (35).

Given the recessive inheritance pattern, siblings of an affected individual are at the highest risk for also having biallelic *MUTYH* mutations (25% risk) and should be offered genetic testing. In addition, testing should be offered to the spouse of a biallelic mutation carrier if there are at-risk children in the family because of the relatively high carrier frequency (2% in North America) (42). If the spouse does not have a *MUTYH* mutation, then the children will all be carriers and should be aware of the future risk to their offspring (should they have children with a carrier) but none of them will have MAP due to biallelic mutations. If the spouse does have a *MUTYH* mutation, then the children will each have a 50% chance of being a biallelic

mutation carrier and having MAP. This can create a “pseudodominant” inheritance pattern since the parent and 50% of the children will be affected with MAP.

Genetic testing for FAP, AFAP, and MAP

It is often not easy to distinguish FAP patients (due to *APC* mutations) from *MUTYH*-associated polyposis (MAP) patients given 1) the high *de novo* mutation rate of *APC* mutations (leading to single affected individuals), 2) the high carrier rate of *MUTYH* mutations (leading to pseudodominant inheritance), and 3) that FAP-associated extracolonic features (congenital hypertrophy of the retinal pigment epithelium, upper GI polyps, and desmoids tumors) have occasionally been reported in MAP patients (35). Genetic testing for patients with more than 10 adenomatous polyps should include testing for both the *APC* gene and the *MUTYH* gene. This is particularly important to the family members of polyposis patients since the inheritance pattern of *APC* (autosomal dominant) and *MUTYH* (autosomal recessive) differ and this significantly affects the risks to the children and siblings of affected individuals.

If a family appears to have a vertical transmission pattern of polyposis (i.e. affected individuals in more than one generation), genetic testing should begin with *APC*. If negative, testing should proceed to *MUTYH* despite the vertical transmission pattern given the possibility of pseudodominant inheritance. Practically speaking, a combination test including *APC* testing and screening for the two most common *MUTYH* mutations is available and is often a good approach for patients with adenomatous polyps. *APC* testing should always include testing for point mutations and large rearrangements to maximize mutation detection. In North America, *MUTYH* genetic testing often begins with the Y179C and G396D mutations and proceeds to full sequencing if the patient is found to be heterozygous for one of these two mutations. The concern with this approach is that it will miss those patients who carry two *MUTYH* mutations other than Y179C or G396D. Studies have found 8.3 – 20% of polyposis patients with biallelic *MUTYH* mutations identified through full sequencing did not have either of the two common mutations (36,42,43). As a result, it is appropriate and cost-effective to begin testing with the *APC* gene and the two common *MUTYH* mutations in Caucasian polyposis patients with Northern European ancestry. However, full sequencing of the *MUTYH* gene is indicated if no mutations are found in *APC* or in the initial *MUTYH* two mutation screening test. Full sequencing of the *MUTYH* gene (instead of the two mutation screen) would be more appropriate for anyone who is not Caucasian with Northern European ancestry.

Hereditary Mixed Polyposis syndrome (HMPS)

The gene(s) responsible for HMPS (OMIM 601228 & 610069) have not been identified at this time although there is linkage to the CRAC1 locus on chromosome 15q13-14 among Ashkenazi Jewish patients (44–46). Linkage to chromosome 10q23 was found in two Singapore Chinese families and one was found to have a *BMPRIA* gene mutation (47). As a result, clinicians should try to enroll patients meeting a clinical diagnosis of Mixed Polyposis syndrome into a research study attempting to identify the responsible gene(s). Patients with HMPS have a combination of juvenile, hyperplastic and adenomatous polyps causing this to often be a diagnosis of exclusion after the known genes for other polyposis syndromes have been ruled out.

Nonpolyposis syndromes

The most common hereditary colorectal cancer syndrome is Lynch syndrome and this accounts for most cases of the hereditary nonpolyposis colorectal cancers. As a result, this syndrome needs to be ruled out by tumor testing prior to consideration of the other possible syndromes within this category.

Lynch syndrome (LS)

LS (OMIM 120435) is the most common hereditary cause of colorectal cancer accounting for around 3% of all cases (48,49). Lynch syndrome can be caused by mutations in any one of at least four mismatch repair genes; *MLH1* (chromosome 3p21.3), *MSH2* (chromosome 2p22-21), *MSH6* (chromosome 2p16), and *PMS2* (chromosome 7p22.2). Testing for Lynch syndrome is more complicated than testing for most other cancer genetic syndromes because of the genetic heterogeneity and because there are two screening tests that can be performed on tumor material from the patients prior to gene testing. The tumor screening tests include microsatellite instability testing and immunohistochemistry staining for the four mismatch repair proteins.

Microsatellite Instability (MSI)

Microsatellites are repetitive DNA sequences (usually a lengthy repeat of 1–2 nucleotides) that occur throughout the genome. These areas are prone to an increase or decrease in the number of repeats when the mismatch repair genes are not functioning properly. This occurs in the tumor DNA from LS patients and is known as microsatellite instability (50–52). MSI testing assesses the length of ≥ 5 microsatellites using tumor DNA and for comparison, normal DNA (52). MSI-high tumors are defined as tumors having changes in the length of at least two of the five ($>20\%$) microsatellites in tumor DNA versus normal DNA. MSI-low tumors are those tumors having changes in the length of one of the five ($<20\%$) microsatellites. Microsatellite stable (MSS) tumors have no changes in the length of the microsatellites being studied (53).

Immunohistochemical staining (IHC)

IHC for the four mismatch repair (MMR) proteins determines whether these proteins are present in the tumor. When one or more of the MMR proteins is absent in the tumor tissue, IHC is considered abnormal (54,55). This is an indication that the protein is not being expressed in the tumor either as a result of germline mutation or epigenetic silencing. Since genetic testing is expensive, IHC is quite helpful since the results can narrow down the number of MMR genes which need to be tested from all four to only one or two. Understanding the underlying functions of the MMR proteins helps in the interpretation of IHC results. The MMR proteins are only stable when they are in heterodimer pairs (56–59). MSH2 can pair with either MSH6 or MSH3 while MSH6 can only pair with MSH2 (56). As a result, if there is a germline mutation in *MSH2*, MSH6 has no partner with whom to make a heterodimer pair and the tumor generally is missing both the MSH2 and MSH6 proteins. However, if there is a germline mutation in *MSH6*, MSH2 is typically stable since it can partner with MSH3 and the tumor will generally exhibit the absence of MSH6 only and presence of MSH2 on IHC. Similarly, MLH1 can pair with PMS1, PMS2 or MLH3 while PMS2 can only pair with MLH1 (59,60). This means that if there is a germline mutation (or acquired promoter methylation) of the *MLH1* gene, PMS2 is unstable because it has no partner protein with whom to pair and both MLH1 and PMS2 will be absent in the tumor. If there is a germline mutation in *PMS2* on the other hand, MLH1 will generally be present in the tumor since it can partner with other mismatch repair proteins.

Sensitivity and Specificity of MSI and IHC

It is important to note that the majority of patients whose colorectal tumors are MSI-high (75%) or have abnormal IHC (88%) do not have Lynch syndrome (49,61). In these cases, the cause of the deficient mismatch repair is usually acquired hypermethylation of the *MLH1* gene promoter. As a result, while MSI and IHC can identify colorectal cancer patients who are more likely to have Lynch syndrome, these tests are not diagnostic. Some argue that proceeding directly to gene testing is an acceptable approach in patients with a high likelihood of having LS (eg. families meeting the Amsterdam criteria (62,63), or families with ≥ 5 –10% likelihood of having a MMR gene mutation based on current risk assessment models, MMRpredict (64),

MMRpro (65), or PREMM1,2 (66)). However, around 50% of families that meet Amsterdam I criteria do not have an identifiable MMR gene mutation and would undergo expensive and unnecessary genetic testing with this approach (67). In addition, ordering IHC will save money overall by reducing the number of genes which need to be tested. As a result, many argue that when possible, LS testing should always begin with MSI and IHC on a LS-associated tumor.

A recent review (68) found that the sensitivity of MSI was 89% for patients with *MLH1* and *MSH2* mutations and 77% for patients with *MSH6* mutations as long as at least 3 mononucleotide repeats were included in the panel of microsatellites tested. The sensitivity dropped to 80–84% for *MLH1* and *MSH2* mutation carriers and 55% for *MSH6* mutation carriers if only two mononucleotide repeats were included in the test. The specificity was 90.2%. The sensitivity of IHC was 83% regardless of which MMR gene was involved and specificity was 88.8%. This review did not assess the sensitivity of using both MSI and IHC on the same tumor to screen for LS since its aim was to select one of the two screening tests for use among all newly diagnosed colorectal cancer patients. However, assuming that some of the tumors from LS patients which are missed by MSI are detected by IHC and vice versa, which has been reported (48,49), a gain in sensitivity would be expected by the use of both tests. Therefore, when screening a patient in a high risk clinic setting, it is advisable to order both MSI and IHC on the LS-associated tumor.

How to follow-up MSI and IHC results

If the tumor is MSS and IHC indicates that all four MMR proteins are present, then the patient is unlikely to have LS. Genetic testing for the MMR genes would not be indicated in this case. If the family history is strong, it may be worthwhile to test a tumor from another affected family member in case the first person tested was a phenocopy (someone who happened to have a LS-associated cancer in a family with LS who did not inherit the germline MMR gene mutation segregating in the family).

If IHC indicates that one or more MMR proteins are absent (regardless of the MSI results), then the patient is more likely to have LS and genetic testing should be offered to the patient using the IHC results for guidance (See Figure 2). When the *MSH2* and *MSH6* proteins are absent, then the patient probably has LS given the lack of evidence of other epigenetic causes for this staining pattern. *MSH2* genetic testing should be ordered first since mutations in this gene are more common among colorectal cancer patients with this IHC result. If no mutation is found in *MSH2*, *MSH6* genetic testing should be ordered. If the *MSH6* protein is the only protein absent in the tumor, then *MSH6* genetic testing should be ordered. If only the *PMS2* protein is absent, then *PMS2* genetic testing is indicated.

Follow-up is more complicated when the *MLH1* and *PMS2* proteins are absent in a tumor. If the patient has a strong family history, then they may have LS due to a germline mutation in *MLH1* and genetic testing for *MLH1* is appropriate. If the patient does not have a strong family history (perhaps they received IHC as part of routine screening at the hospital where they had surgery for their colorectal cancer), then the patient probably has somatic (acquired) methylation of the *MLH1* gene promoter but it is still possible that they may have LS. There are two tumor tests available that can help distinguish the patients with LS from the patients with *MLH1* promoter methylation.

MLH1 promoter methylation and BRAF testing on tumor DNA

Methylation of the *MLH1* gene promoter can be assessed directly in tumor DNA. In addition, tumors can be studied for somatic *BRAF* gene mutations (most commonly the V600E mutation). *BRAF* mutations are identified in 69% of colorectal tumors from individuals with *MLH1* promoter methylation and thus far, have not been reported in patients with germline

MLH1 mutations (68). It is important to note that *BRAF* testing is not informative for endometrial tumors. Tumors found to have both *MLH1* promoter methylation and a *BRAF* mutation are most likely due to acquired *MLH1* promoter methylation and genetic testing for LS is not necessary. However, tumors found to have *MLH1* promoter methylation and *BRAF* results which are not concordant could still be the result of a germline *MLH1* mutation and genetic testing for *MLH1* is appropriate. *MLH1* promoter methylation without a *BRAF* mutation might occur if the *MLH1* methylation was the “second hit” in a patient with a germline *MLH1* mutation. A *BRAF* mutation might be found in a tumor from a patient with a germline *MLH1* mutation (without *MLH1* promoter methylation in the tumor) since it is unlikely that *BRAF* testing will be 100% specific. Colorectal cancer patients with *MLH1* promoter methylation are generally diagnosed at later ages and are less likely to have a family history of colon cancer. As such, it may be prudent to order *MLH1* promoter methylation and *BRAF* testing for colorectal cancer patients with *MLH1* and *PMS2* absence on IHC if they are diagnosed after age 60 and have no first degree relatives with colorectal or endometrial cancer. If they are diagnosed ≤ 60 or have a first degree relative with colorectal or endometrial cancer, testing could begin with the *MLH1* gene.

Unexpected IHC results

Occasionally, the IHC results will be confusing based on the known heterodimer pairs that occur with MMR proteins. For example, Baudhuin et al. found that *MLH1* and *MSH6* were absent in <1–3% of colon tumors studied and *MSH6* and *PMS2* were absent in <1% of colon tumors (69). There are mononucleotide repeats in the coding region of the *MSH2*, *MSH6* and *PMS2* genes which can become unstable in an MSI-high tumor (70). When this occurs, it can create a frameshift resulting in premature truncation of one allele of the gene. Since something would have to destroy the function of the other allele of the same gene to result in the absence of the protein in the tumor, this only occurs rarely. It has been noted that this probably occurs in subclones of an MSI-high tumor which could also explain partial loss of IHC staining in some tumors (i.e. focal staining) (71).

MSI-high tumors with normal IHC results

Occasionally, a tumor will be found to be MSI-high but the presence of all four MMR proteins will be demonstrated by IHC. This is more likely in the case of a germline missense mutation which might lead to a full length protein which is nonfunctional but present in the tumor and stable in its heterodimer pair. These results can be followed up in two ways depending on the *a priori* likelihood that the patient has LS. One option is to order *MLH1* methylation and/or *BRAF* mutation testing on the colorectal tumor. Results of the *MLH1* methylation and *BRAF* mutation testing should be interpreted as described previously. This is a good option if there is not a strong family history. The second option is to proceed directly to genetic testing using a blood sample from the patient. In this case, genetic testing should start with the *MLH1* and *MSH2* genes since they are the most common causes of LS accounting for 70% of cases (68). If negative, proceed to *MSH6* testing and *PMS2* testing in any sequence since these genes each account for around 15% of LS (68).

LS gene testing

Gene testing for all four mismatch repair genes should include both full sequencing of the gene and large rearrangement testing because large deletions account for around 22% of *MLH1* and *PMS2* mutations, 26% of *MSH2* mutations and ~7% of *MSH6* mutations (48,49). Deleterious mutations in any of the four mismatch repair genes are diagnostic for LS. Variants of uncertain significance are found fairly frequently (~7%) in the mismatch repair genes. (48,49) For more information about a variant, one can contact the testing laboratory, conduct additional segregation testing in the family, and for variants in *MLH1* and *MSH2* use the MAPP-MMR

program (<http://mappmmr.blueankh.com/>) to find out if the mutation is likely to be a deleterious or a benign polymorphism (74). Until the pathogenicity of the mutation has been established, predictive testing should not be offered to at-risk relatives.

Management recommendations are complicated if IHC shows absence of MSH2 and MSH6, or absence of MSH6 or PMS2 alone and genetic testing is negative. Similarly, if MLH1 and PMS2 are absent on IHC, genetic testing is negative and *MLH1* promoter methylation has been ruled out, surveillance recommendations are complicated. A conservative approach would be to recommend that these families follow LS cancer surveillance guidelines but this has to be interpreted in the context of the family history and clinical judgment.

Familial Colorectal Cancer syndrome Type X

For patients with a strong family history of colorectal cancer and MSS tumors, one should consider a diagnosis of Familial Colorectal Cancer Type X (75). The strict definition includes only families that meet the Amsterdam I criteria with no evidence of deficient mismatch repair (62). These families do not have an increased risk for extracolonic cancers as seen in Lynch syndrome. In addition, the colorectal cancer risk appears lower than in families with Lynch syndrome (75). The gene(s) responsible for the colorectal cancer risk in these families have yet to be discovered which is the reason they are referred to as “type X” at this time. This syndrome appears to also be inherited in an autosomal dominant manner so the children of an affected individual are at 50% risk for also having inherited the colorectal cancer susceptibility. Since clinical testing is not available, it may be useful to try to enroll these families in research studies looking for new colorectal cancer susceptibility genes. All at-risk individuals should receive increased colorectal cancer surveillance.

MUTYH-Associated Polyposis (MAP) in the differential diagnosis for nonpolyposis colorectal cancer

It is clear that patients with MAP do not always have significant numbers of colon polyps leading to some overlap with the nonpolyposis syndromes. In addition, there are case reports of MAP patients with sebaceous adenomas or carcinomas which would be typical in the Muir-Torre variant of Lynch syndrome. Further underscoring the clinical overlap with Lynch syndrome, a commercial laboratory found that 1% of 306 patients who had been negative for LS genetic testing had biallelic *MUTYH* mutations (42). Biallelic MYH mutations have been identified in around 0.8 – 6% of colorectal cancer patients diagnosed under age 50 sometimes without associated adenomatous polyps (40,76,77). A large population-based series of 9268 colorectal cancer patients was recently tested for the common Y179C and G396D *MUTYH* mutations (77). It was determined that biallelic mutations account for 0.3% (27/9268) of all CRCs, these cancers are MSS, and biallelic mutations infer a 28-fold increased risk for colorectal cancer. Four of the 27 (14.8%) patients with biallelic *MUTYH* mutations did not have any concurrent adenomas. A review of the clinical findings of 257 patients with biallelic *MUTYH* mutations found that 20% of G396D homozygotes had fewer than 10 colon polyps compared to only 2% of the Y179C homozygotes (78). In general the Y179C mutation and truncating mutations in *MUTYH* were associated with higher numbers of colon polyps. Nine percent of patients with non-truncating mutations in *MUTYH* had fewer than 10 polyps compared to none of the patients with two truncating mutations. At this time, it is not clear when to perform *MUTYH* gene testing in cases with less than 10 adenomatous polyps, however, it could be considered in patients with MSS colorectal cancers diagnosed under age 50.

Genetic testing in family members

Clinicians must take reasonable steps to guarantee that immediate family members are warned about their risk for inherited cancer susceptibility syndromes. There is legal precedent for this in the United States (*Safer v Estate of Pack*, 1996) where the daughter of a man with FAP won

a lawsuit against her father's physician for not having warned her about her risk for inheriting FAP. We frequently encounter FAP patients who have never undergone genetic testing because it was felt that this was not important given the obvious clinical diagnosis. However, this is important to the at-risk relatives of these patients who may or may not have inherited the causative gene mutation which would significantly affect their medical management. In general, all patients with a known or suspected hereditary colorectal cancer syndrome need to be referred for genetic counseling and possibly genetic testing because of the implications to their relatives and the possible implications to their own management.

Once a deleterious mutation has been identified in any of the colorectal cancer susceptibility genes in an affected family member, at-risk relatives can undergo single mutation testing for the known mutation (or mutations in the case of *MUTYH*) in the family which is less costly and very reliable. Genetic testing can be offered to minors for conditions that become require medical management in childhood (which is the case for all the polyposis syndromes). Any relative who has the mutation(s) or who defers genetic testing should be managed as if they have the condition. Any relative who does not have the known familial mutation can follow the American Cancer Society guidelines for cancer screening in the general population as long as they do not have any other cancer risk factors.

If a deleterious mutation cannot be identified in the family, then at-risk relatives either need to screen as if they have the syndrome for which they are at risk or follow a modified screening program which may relax over time if they do not begin to exhibit signs of the condition. For example, if a person at risk for FAP does not have adenomas by a certain age, it becomes rather unlikely that they inherited FAP and they may be able to undergo colonoscopy less frequently. It is important to consider when the original genetic testing occurred in the family and what testing modality was utilized since advances in technology have led to increased mutation detection rates. For example, a patient who was tested for *APC* mutations in 1999 may have only had protein truncation testing. If no protein truncation was detected at that time, it would be important to offer *APC* testing using DNA sequencing and large rearrangement analysis along with *MYH* gene testing since the underlying genetic cause of their polyposis may be identified with these new tests.

Summary

With the recent availability of PMS2 genetic testing on a clinical basis, genetic testing is now available in North America for all of the known hereditary colorectal cancer genes. In addition, most of these tests have improved significantly in the past few years with the inclusion of techniques to detect large rearrangements. As a result, we are in a better position than ever to help families with these syndromes to identify the underlying genetic cause. This will ensure that they receive appropriate management and will enable their relatives to determine their precise risks and to tailor their cancer surveillance. Since colorectal cancer can often be prevented if surveillance is initiated at the correct age and frequency, this is important for the relatives of patients with hereditary colorectal cancer syndromes.

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Figure 1.
Flowchart for Hereditary Colorectal Cancer Differential Diagnosis

Figure 2.
Flowchart for Lynch syndrome testing depending on IHC result