Diagnostic Approach and Management of Lynch Syndrome (Hereditary Nonpolyposis Colorectal Carcinoma): A Guide for Clinicians[1]

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ABSTRACT The patient with a family history for colorectal carcinoma constitutes a complicated diagnostic challenge involving many clinicians. The diagnostic workup of familial colorectal cancer is an elaborate and time consuming process in which the family and several medical specialists closely collaborate. However, establishing a diagnosis can be very rewarding. If a mutation is detected in the family, a satisfactory explanation can be provided for an accumulation of tumors at young age, and often of untimely death. Appropriate presymptomatic testing can be offered to reduce mortality among at-risk family members, and relatives not at risk can avoid uncertainty and needlessly intensive surveillance.

We show the differential diagnostic considerations when an individual with a family history of colorectal carcinoma is encountered, with emphasis on Lynch syndrome (Hereditary Nonpolyposis Colorectal Carcinoma [HNPCC]). Practical recommendations for laboratory workup of suspected Lynch syndrome, including analysis of tumor tissue by microsatellite instability analysis and immunohistochemistry, and germline DNA analysis are given. Furthermore, the clinical management after a molecular diagnosis has been made is described. The diagnostic scheme presented here allows efficient and effective analysis of colorectal carcinoma cases with (suspected) Lynch syndrome, making optimal use of currently available technology. (*CA Cancer J Clin 2006;56:213–225.)* *© American Cancer Society, Inc., 2006.*

INTRODUCTION

The cumulative lifetime risk for colorectal cancer in the United States is approximately 6%.[1] Up to 15% of cases are attributable to an inherited or familial predisposition.[2] Three relatively common and two rare hereditary conditions with an increased risk for colon carcinoma are known. Lynch syndrome (Hereditary Nonpolyposis Colorectal Carcinoma [HNPCC])[3–4] is the most common hereditary colorectal carcinoma syndrome. Germline mutations resulting in Lynch syndrome have been found in four mismatch repair (MMR) genes, *MSH2*,[5] *MLH1*,[6] *PMS2*,[7] and *MSH6*.[8–9] Familial Adenomatous Polyposis (FAP) is characterized by the development of a hundred to thousands of adenomatous polyps[10] and is caused by mutations in the *APC* gene located on chromosome 5q21-q22.[11–12]

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Recently mutations have been found in the *MutYH* gene.[13] Contrary to the above mentioned conditions, the colorectal cancer syndrome resulting from *MutYH* mutations is inherited in an autosomal recessive fashion.[13–15] The phenotypes associated with biallelic *MutYH* mutations are multiple adenomatous colorectal polyps (similar to attenuated FAP, 10 to 100 adenomas), or to a lesser extent, similar to classical FAP.[15–16]

Two rare syndromes with an increased risk for colorectal carcinoma, Peutz-Jeghers syndrome and Juvenile Polyposis, are both characterized by hamartomatous polyps.[17–20] In these conditions, germline mutations have been identified in the *LKB1*-gene in Peutz-Jeghers syndrome and in *SMAD4, PMPR1A,* and rarely *ENG* in Juvenile Polyposis.[20–24] This review focuses on Lynch syndrome.

It is essential to identify individuals at increased risk to offer adequate surveillance programs to prevent the development of tumors or recognize them at an early stage.[25] However, the patient with a family history for colorectal carcinoma poses several challenging questions for the clinician. How can one discriminate between coincidence of several sporadic tumors and hereditary predisposition? How should patients be selected for genetic counseling and testing? What is the optimal management of patients carrying a mutation conferring a high colorectal cancer risk? In this paper, we provide recommendations for clinicians encountering these questions. Our review emphasizes Lynch syndrome.

FAMILY HISTORY

Although a considerable number of patients diagnosed with colorectal carcinoma have a family history for this disease, most do not have any of the known colorectal cancer syndromes. A pedigree should be drawn of each patient; this permits a rapid assessment of whether or not a hereditary form of colorectal carcinoma should be suspected. It is essential to ask not only about polyps and colorectal carcinoma in family members, but also about other associated neoplasms. Carriers of Lynch syndrome have an increased risk of colorectal carcinoma (60%-70% at age 70), endometrial carcinoma (30%-40% at age 70), and to a lesser extent, carcinoma of the small bowel, transitional cell carcinoma of the upper urinary tract, stomach cancer, ovarian cancer, brain tumors (Turcot syndrome), and sebaceous gland tumors (Muir-Torre syndrome).[26]

Mutations in the *APC* gene are also associated with an increased risk of carcinoma of the biliary tract, the duodenum, stomach, the Ampulla of Vater, and the thyroid, as well as desmoid tumors, congenital hypertrophy of the retinal pigment epithelium (CHRPE), mandibular osteomas, dental abnormalities, epidermal cysts, lipomas, and fibromas.[27]

One of the following situations—multiple cases of colorectal carcinoma in different generations diagnosed at a relatively young age (<50 years) or (numerous) adenomatous polyps at a relatively young age, the combination of syndrome-related tumors in other organs or synchronous or metachronous tumors in one person—are indicators of hereditary colorectal carcinoma and should prompt further analysis (of the family) of the patient. Patients for whom we recommend referral to a department of clinical genetics are listed in Table 1.

When analyzing a pedigree, it is important to consider the size of the family. A small family with two cases of colorectal carcinoma among first-degree relatives is more suspicious than a large family with two cases of this diagnosis. In cases where *MutYH* associated polyposis is considered, one should ask for consanguinity because of this condition's recessive transmission. It is also important to realize that the accuracy and completeness of a family history depends on the patient's contact with his/her family and the knowledge of relatives' medical histories. In a study comparing the patient-reported family history with data from a cancer registry, Mitchell, et al. found that one-third of colorectal carcinoma cases among first-degree relatives and two-thirds of colorectal cases among second-degree relatives were not reported by the family member, especially if the tumor was diagnosed at an older age (mean age of 63.3 years in cases that were reported cor-

TABLE 1 Criteria for Referral for Genetic Counseling

Classical FAP [>100 adenomatous polyps]
More than 10 adenomatous polyps
Adenomatous polyp before age 40 years
Multiple colorectal carcinomas or other Hereditary Nonpolyposis Colorectal Carcinoma-related tumors in one individual
Colorectal carcinoma or endometrial carcinoma before age 50 years
Two first-degree relatives with colorectal carcinoma/or Hereditary Nonpolyposis Colorectal Carcinoma-related tumors independent of age of diagnosis

rectly and mean age of 70.2 years in cases that were reported incorrectly).[28]

FURTHER ANALYSIS OF THE FAMILY HISTORY AND DIFFERENTIAL DIAGNOSIS

In our practice we always check medical records after consent has been obtained. If a family member is deceased, a first-degree family member can provide consent. Confirmation of the diagnoses is crucial since sometimes a tumor appears not to exist or to be benign, or the site of the tumor is incorrectly reported. Stomach cancer is most often incorrectly reported in that the correct diagnosis is often a carcinoma of some other abdominal organ.[29] Also gynecological tumors are often incorrectly reported. Second primary tumors appeared to be underreported.[29] For further diagnostic workup, it is essential to obtain information on the localization of a colorectal tumor and the type, number, and localization of adenomatous polyps, as well.

After having completed the family history, a differential diagnosis is made (Figure 1). If medical records show that classical FAP has been diagnosed in a sporadic patient or in several individuals following an autosomal dominant pattern of inheritance, DNA analysis of the *APC* gene by protein truncation test (PTT), denaturating gradient gel electrophoresis (DGGE), Multiplex Ligation-dependent Probe Amplification (MLPA), and sequence analysis is indicated, preferably on an affected individual. If no mutation is found, DNA analysis of *MutYH* by sequence analysis is the next step.

If medical records confirm the presence of 10 to 100 adenomatous polyps in a sporadic patient or in several individuals in one generation, analysis of the *MutYH*-gene analysis should be done first. If DNA analysis of the *MutYH* gene does not reveal a mutation, analysis of the *APC* gene is the second step.

If the family history including first-, second-, and third-degree relatives and medical records do not identify individuals with more than five polyps, if the inheritance pattern is autosomal dominant, and if Lynch syndrome-related tumors occur in the family, the question is addressed of whether the family diagnosis is suggestive of this syndrome. The clinical diagnosis of Lynch syndrome is made by applying the Amsterdam Criteria (Table 2).[30–31] These criteria are too stringent to identify all Lynch families and are specially developed for scientific purposes to identify families eligible for the identification of the genes causing Lynch syndrome in the period when these genes were not known yet. If a family does not fulfill these criteria, a mutation in one of the mismatch repair genes could still segregate in the family.[32] Therefore, the (revised) Bethesda Criteria have been formulated (Table 3)[33–34] to identify families in which further analysis for Lynch syndrome is indicated.

If a family does not fulfill the Bethesda Criteria, no specific analysis for Lynch syndrome is indicated. This does not, however, exclude a hereditary factor in the development of colorectal carcinoma in a family. For that reason the referral criteria for genetic counseling (Table 1) are broader than the Bethesda Criteria (Table 3). Also, it is not always clear, before a family is referred and before the family history and medical records are analyzed, if a family truly fulfills these criteria. Individuals with a first-degree relative with colorectal carcinoma have an increased relative risk of developing colorectal carcinoma compared with the population risk, but the cumulative risk is not higher than 10%. If a first-degree relative was diagnosed before the age of 45 years or if an individual has 2 first-degree relatives with colorectal carcinoma, the risk is increased four to sixfold (cumulative risk higher than 10%).[35] For these indications, a colonoscopic examination every 5 years from the age of 45 to 50 years has been recommended.[36] However, the American Gastroenterological Association, U.S. Multi-Society Task Force on Colorectal Cancer, and the American

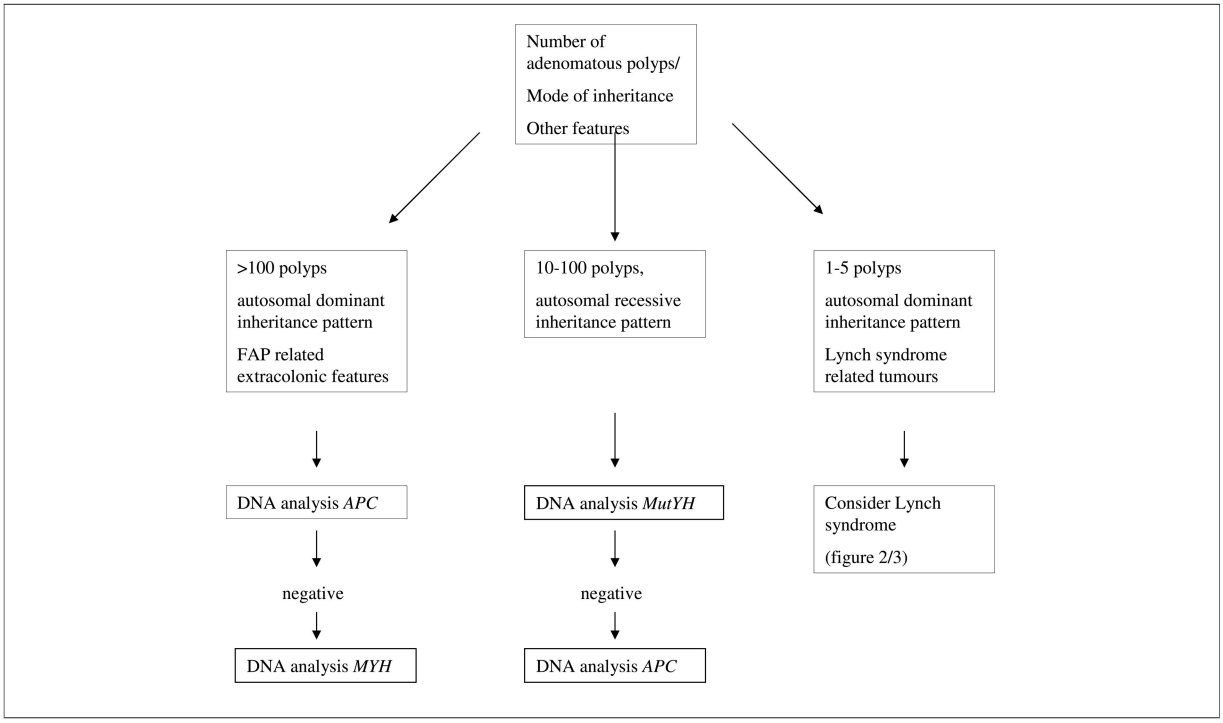

FIGURE **1** Differential Diagnosis

TABLE **2** Amsterdam Criteria[30–31]

Colorectal carcinoma and/or endometrial carcinoma or transitional cell carcinoma of the ureter or pyelum or carcinoma of the small bowel in at least three individuals in the family
One of the patients is a first-degree family member of two other patients
Patients occur in at least two successive generations
At least one of the diagnoses was made before age 50 years
The diagnoses are histologically confirmed
Familial adenomatous polyposis is excluded

TABLE **3** Bethesda Criteria (Revised)[33,34]

Colorectal carcinoma <age 50 years
Presence of synchronous or metachronous HNPCC-related carcinomas, regardless of age*
Colorectal carcinoma with specific pathological features <60 years†
Colorectal carcinoma diagnosed in one or more first-degree relatives with an Hereditary Nonpolyposis Colorectal Carcinoma-related tumor, with one of the diagnoses under age 50 years
Colorectal carcinoma in two or more first- or second-degree relatives with an Hereditary Nonpolyposis Colorectal Carcinoma-related tumor, regardless of age

*Colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain, sebaceous gland and small bowel carcinoma.
†Tumor infiltrating lymphocytes, Crohn's-like lymphocyte reaction, mucinous/signet ring differentiation, or medullary growth pattern.

Cancer Society (ACS) recommended a colonoscopy every 5 years from age 40 or 10 years before the earliest diagnosis if an individual has 2 or more first-degree relatives with colon cancer, or a single first-degree relative with colon cancer or adenomatous polyp diagnosed at an age <60 years.[37–38]

ANALYSIS OF TUMOR TISSUE; MICROSATELLITE INSTABILITY, AND IMMUNOHISTOCHEMISTRY

When a family fulfills the Bethesda Criteria (Table 3), examination of tumor tissue is indicated, where microsatellite instability (MSI) analysis, and immunohistochemistry (IHC) are used as prescreening tests in tumor tissue to select individuals eligible for DNA mutation analysis in blood with DGGE, MLPA, and sequence analysis, which can avoid unnecessary, expensive, and time-consuming DNA-analyses.

Immunohistochemistry

Using IHC in the presence or absence of the MLH1, MSH2, MSH6, and PMS2 proteins (Table 4) can be visualized using specific antibodies.[39–42] IHC is sensitive in predicting a truncating MMR defect in one of the genes where antibodies are available against the respective proteins.[41–42] IHC has the additional advantage, when compared with MSI analysis, that it indicates the MMR gene most eligible for DNA analysis. Since the mismatch repair proteins form heterodimeric complexes, distinct IHC patterns can be expected (Table 4). The MSH2, MSH3, MSH6, MLH1, and PMS2 proteins mediate mismatch repair in humans.[42] Recognition of single nucleotide mismatches and insertion and deletion loops (IDLs) is carried out by a heterodimer of MSH2 and MSH6, whereas a heterodimer of MSH2 and MSH3 recognizes IDLs in absence of MSH6.[43] The heterodimer of MLH1 and PMS2 mediates cross talk between mismatch recognition and the actual repair complex.[44] In absence of PMS2, the MLH3 protein is a candidate protein for forming a heterodimer with MLH1.[45] Thus individuals can be selected for DNA mutation analysis, and the assessment of which gene to test first can be made. Previous research by our group and other groups has shown a high sensitivity in predicting mutations in *MSH2* (92%) and *MSH6* (90%) by applying IHC in colorectal tumors. IHC has a lower sensitivity in *MLH1* (48%), when only the MLH1 specific antiserum is used. However, when PMS2 staining is applied in addition, the yield increases by 23% to 71%.[41–42,46]

IHC is especially indicative for MMR mutations that result in truncation of the protein, such as nonsense, frameshift, splicesite mutations, and large genomic rearrangements. In case of missense mutations, IHC is not always diagnostic. In these cases the protein can be functionally abnormal, but still be detected by IHC.

Microsatellite Instability Analysis

MSI analysis was first described in 1993.[47–49] This method gives an indication of abnormal mismatch repair in general, irrespective of the MMR gene and type of mutation that is involved. MSI analysis is a test that detects failure of the DNA Mismatch Repair (MMR) machinery to repair errors occurring during DNA replication. Such failure leads to increased length variation of simple, repetitive sequences distributed throughout the genome. An international set of markers is developed to test for MSI, consisting of the markers D2S123, D5S346, D17S250, BAT25, and BAT26.[50] In addition, we recommend testing for the BAT40 marker[41] because it increases the sensitivity of the test.[41] Tumors are scored MSI-high if at least 30% of the markers show instability, MSI-low if less than 30% show instability, or MS-stable if none of the markers show instability. An MSI-H phenotype is reported in 85% to 92% of Colorectal Carcinoma (CRC) associated with HNPCC and in 10% to 15% of sporadic CRC.[43,47,51–55] MSI analysis has a sensitivity of 93% in detecting MMR deficiency in carriers of a pathogenic MMR mutation.[41,46,56] MSI analysis is not suitable to predict which of the MMR genes is harboring a mutation.

TABLE **4** IHC Findings Associated with MLH1, MSH2, MSH6, and PMS2 Mutations

MMR mutation	IHC staining			
	MLH1	MSH2	MSH6	PMS2
MLH1	−	+	+	−
MSH2	+	−	−	+
MSH6	+	+	−	+
PMS2	+	+	+	−

AMSTERDAM CRITERIA POSITIVE FAMILIES

In families that do fulfill the Amsterdam Criteria, the chance of identifying a mutation in one of the MMR genes, *MLH1, MSH2,* and *MSH6*, is high, at 50% to 92%.[57–59] For this reason we have chosen to perform IHC as a first diagnostic step in these families. This technique has a high sensitivity and has the additional advantage that it indicates directly which gene to test first.[41,42] Especially concerning immunohistochemistry, it is impor-

tant that the technique is performed in an experienced laboratory. The decision tree for the group that fulfills the Amsterdam Criteria is shown in Figure 2. It is essential to carefully select the index patient in whom tissue analysis and DNA analysis will be performed. In our experience, the yield of DNA analysis is 3 times higher when an index with any Lynch syndrome-related tumor below the age of 50 is tested than when the index patient developed a Lynch syndrome related-tumor above this age (Wijnen, et al, unpublished data). Adenomas are also suitable for IHC if they are large, if they have high-grade dysplasia, and if they occur in a patient younger than 50 years.[42]

MLH1

An IHC pattern with absent staining for MLH1 and PMS2 and positive staining for MSH2 and MSH6 is indicative for a mutation in *MLH1* (first column of Figure 2); in absence of MLH1, the heterodimer of this protein with PMS2 will not be formed, the PMS2 protein will quickly degrade, and both proteins will not stain in IHC. Absent staining for MLH1 can also be caused by hypermethylation of the promoter region of this gene, a somatic event restricted to the tumor and irrespective of a germline *MLH1* mutation. This phenomenon has been shown to be related with specific mutations in tumor tissue in the *BRAF* gene. *BRAF* analysis in tumor is a low-cost and effective strategy to distinguish between a somatic event/hypermethylation and a possible germline mutation in the *MLH1* gene. If a specific mutation in *BRAF*, V600E, is found in tumor DNA, mutation analysis of the *MLH1* gene is thus not indicated.[60,61] If both IHC and analysis of *BRAF* do not indicate somatic abrogation, *MLH1* DNA analysis of this gene is the next diagnostic step. However, in the case of a relatively young patient, *MLH1* mutation analysis should always be performed. Both point mutations, and more recently, large genomic deletions have been identified in this gene.[62,63] DNA analysis should include techniques to identify both. If mutation screening remains negative, analysis of a second tumor can be considered. The pattern found in the first examined tumor may be confirmed, indicating an as yet undetected *MLH1* mutation, or a different IHC pattern may be found, directing DNA mutation analysis of another MMR gene. If no tumor tissue is available in an Amsterdam Criteria positive family, mutation analysis of the mismatch repair genes, starting with *MLH1* and *MSH2,* should be performed.

MSH2

An IHC pattern with no staining for MSH2 and MSH6 and positive staining for MLH1 and PMS2 indicates a mutation in *MSH2* (second column in Figure 2). Both point mutations and genomic deletions are frequently identified in *MSH2*.[63,64] The sensitivity and specificity for IHC in predicting a mutation in *MSH2* is high.[41,56] However, in 5% (2/40) of the tumors from carriers of a germline mutation in *MSH6,* we have detected an IHC pattern compatible with a mutation in *MSH2*,[26] with absent staining for both MSH2 and MSH6. Thus, if *MSH2* mutation screening turns out negative, we recommend DNA analysis of *MSH6.*

MSH6

In the third column of Figure 2, the IHC pattern matching a mutation in *MSH6* is shown as absent staining for MSH6 with positive staining for the remaining three MMR proteins. Also in this gene, both point mutations, and recently, large genomic rearrangements have been described.[26,65,66] We found in 1/12 (8%) of the tumors from carriers of a pathogenic mutation in *MSH2* an IHC pattern indicative of a mutation in *MSH6*.[41] Therefore, if no mutation is identified, DNA analysis of *MSH2* can be considered.

PMS2

The fourth column of Figure 2 shows the IHC pattern expected in tumors from carriers of a *PMS2* mutation. Only seven heterogygous truncating mutations have been described in

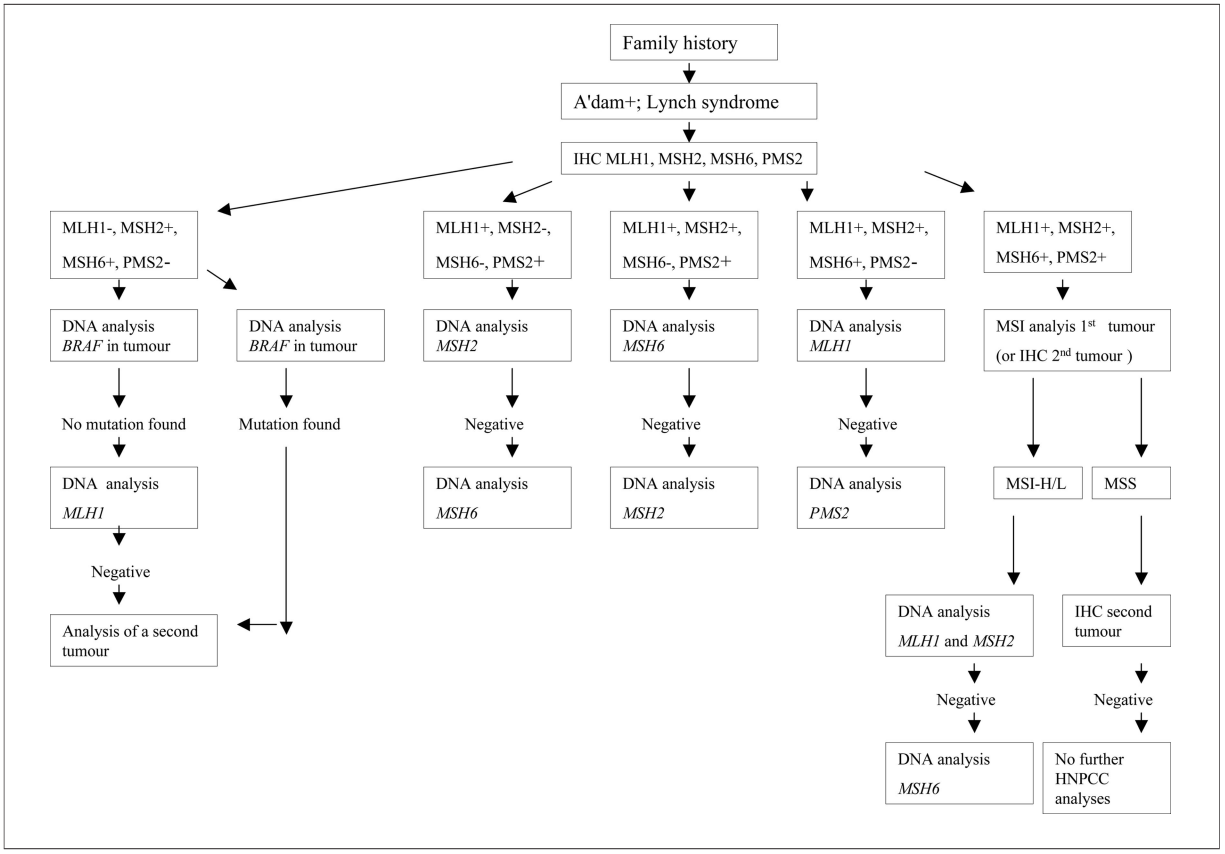

FIGURE **2** Amsterdam Positive Families. A'dam+/− = Amsterdam criteria positive/negative; IHC = Immunohistochemistry; MSI-(H/L/S) = Microsatellite instability (High/Low/Stable); MLH1− = Negative staining for the MLH1 protein in IHC

individuals with suspected Lynch syndrome.[7,67–69] Other studies in large series of Lynch syndrome suspected families did not reveal any pathogenic mutations in *PMS2*.[70–72] However, a number of mutations have been described in patients with Turcot's syndrome, with possible recessive inheritance.[73–75] Recently, we identified 4 genomic rearrangements in a group of 112 HNPCC suspected patients negative for a mutation in *MLH1, MSH2,* and *MSH6.* Furthermore in a group of eight individuals with negative staining for PMS2 only in a Lynch syndrome-related tumor, three different pathogenic point mutations were identified in the *PMS2* gene.[76] We have thus demonstrated that *PMS2* plays a role in Lynch syndrome. The exact cancer risks in these families have yet to be established. However, when an IHC pattern with no staining of the PMS2 protein in combination with present MLH1 staining is encountered, frequently *MLH1* unclassified variants (see paragraph 'unclassified variants' below) are found.[42] Also, mutation analysis of *PMS2* is complicated by the fact that this gene is part of a family of highly homologous genes clustered on chromosome 7. If no mutation is identified in *MLH1,* mutation analysis of *PMS2* is the next step.

MSI-high/IHC Staining Pattern Indicative of a Mutation in One of the MMR Genes, No Mutation Detected in DNA Analysis

Possibly in these cases, the current DNA-analysis techniques are not yet adequate to detect all mutations in the MMR genes. If new techniques will become available, additional tests might reveal a mutation.

MSI-high/MSI-low

If IHC for all four proteins is positive in an Amsterdam positive family, MSI analysis is the next diagnostic step. Using this method a

MMR defect in general may be confirmed. If either an MSI-high or MSI-low phenotype is found (fifth column of Figure 2), the latter especially when instability of one of the mononucleotide markers is established, we recommend to start mutation analysis with the *MLH1* and *MSH2* genes since the likelihood of finding a mutation in one of these genes is the highest in families fulfilling the Amsterdam Criteria. Analysis of the other MMR genes can be considered. If another tumor is easily obtainable, IHC of a second tumor could be considered in addition. This is also recommended when MSI analysis of the first tumor shows a MS-stable phenotype.

MS-stable

If an MS-stable phenotype is found, analysis of a second tumor should be performed to rule out the possibility that the individual tested first is a phenocopy (sixth column of Figure 2). In proven Lynch syndrome families, frequently tumors are encountered with no indications of MMR involvement in MSI analysis and IHC. These individuals could have developed, for example, a colorectal carcinoma or endometrial carcinoma simply because these tumors have a relatively high prevalence in the population.[26,76]

AMSTERDAM CRITERIA NEGATIVE FAMILIES

In families with multiple colon cancers not fulfilling the Amsterdam Criteria, but fulfilling the less stringent Bethesda Criteria, a mutation is detected in approximately 30% of the families,[63,64,77–80] mostly in the[66,78–80] *MSH6* gene. The majority of families with a mutation in *MSH6* do not fulfill the Amsterdam Criteria.[26,58,81] Since the likelihood of finding a truncating mutation in one of the MMR genes is considerably lower in the Amsterdam negative group than in the Amsterdam Criteria positive group, we recommend to start with MSI analysis as the first prescreening method in the former group[41] (Figure 3). MSI analysis gives general information on loss of MMR function, including alterations in MMR genes other than the known genes, with probably a lower penetrance and therefore a lesser extent of fulfillment of the Amsterdam Criteria.[82]

MSI-high/MSI-low

If an MSI-high or MSI-low phenotype is encountered, IHC of the mismatch repair proteins should follow (Figure 3). If negative staining for one or two of the proteins is seen, DNA analysis of the respective gene is the obvious next step (Figure 2). If all proteins show normal staining, no direction can be given toward DNA analysis. In this situation we recommend DNA analysis of *MLH1* and *MSH2*, and if no mutation is found, analysis of *MSH6*. Also, MSI analysis and subsequent IHC in a second tumor can be considered (fifth column, Figure 2).

MS-stable

Carcinomas in *MSH6* carriers, particularly endometrial carcinomas, have been shown to present with a MS-Stable phenotype in a minority that cannot be neglected.[26,83] Therefore, if an MS-stable phenotype is found, IHC of MSH6 is the next step (Figure 3). If IHC is negative for MSH6, mutation analysis follows. If staining is present, MSI analysis of a second tumor can be considered.

UNCLASSIFIED VARIANTS

Both in the group of Amsterdam positive and the group of Amsterdam negative families, missense mutations can be encountered in DNA analysis. A missense mutation changes the composition of the protein. The pathogenic significance of such mutations is often unclear. A functional test to reliably assess the competence of the mismatch repair proteins in vitro is currently not available. Therefore, most missense mutations are designated as 'unclassified variants.' These variants cannot be used for diagnostic purposes.

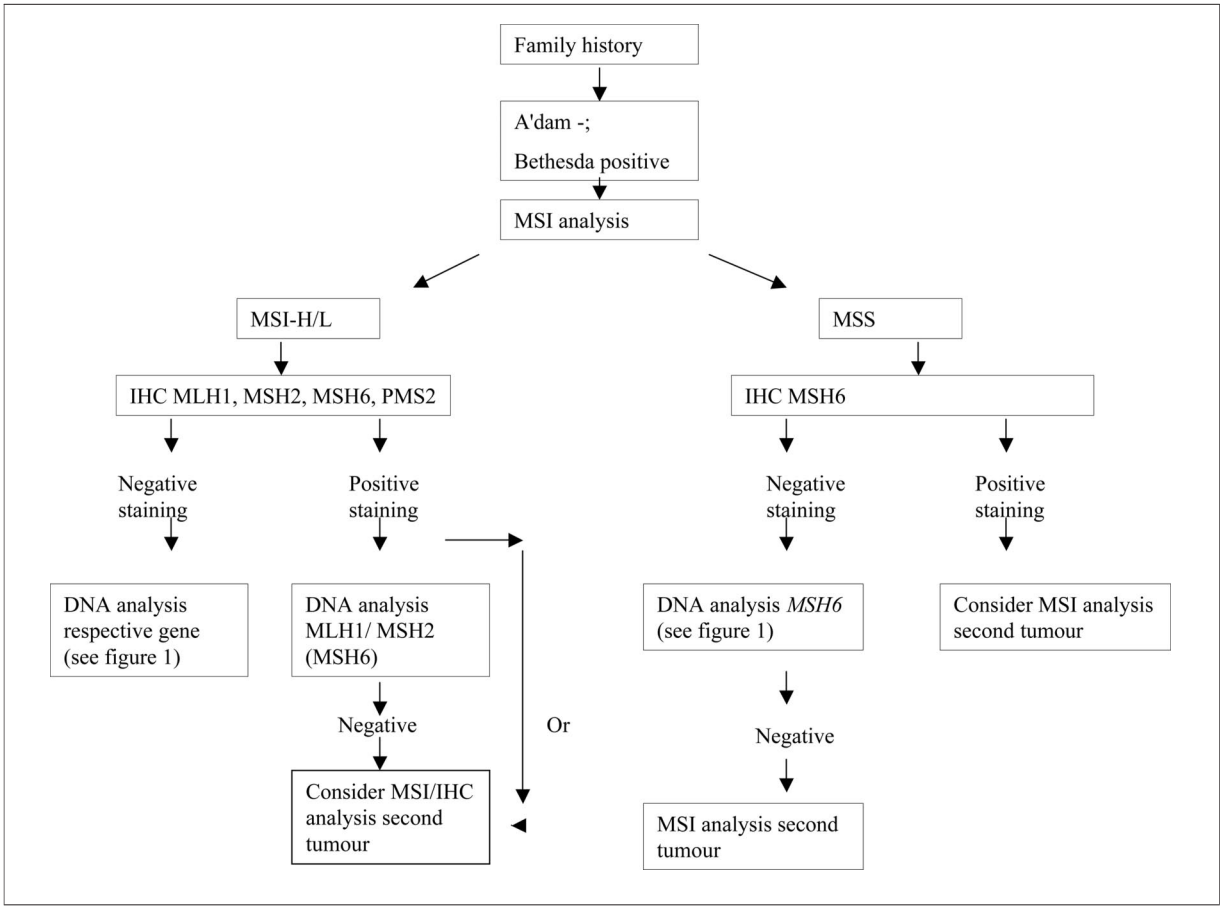

FIGURE 3 Amsterdam Negative Families. A'dam+/− = Amsterdam criteria positive/negative; IHC = Immunohistochemistry; MSI-(H/L/S) = Microsatellite instability (High/Low/Stable); MLH1− = Negative staining for the MLH1 protein in IHC

Management

When the diagnostic process has been completed, colon cancer risk can be assessed for the family (Table 5) and recommendations for periodic surveillance can be formulated. When a mutation in one of the MMR genes has been identified, presymptomatic testing (mutation specific testing) is available for all family members. Such presymptomatic testing is initiated after having discussed all pros and cons in individual genetic counseling sessions. A consultation with a psychologist is offered for guidance in decision making. Carriers of a mutation are offered periodic surveillance. Noncarriers (true negatives) can be discharged from follow up and are guarded against intensive surveillance protocols.

When no mutation was identified, but when the family fulfills the Amsterdam Criteria, the surveillance protocol for Lynch syndrome is recommended. The surveillance protocol, used in the Netherlands for carriers of a mutation in one of the MMR genes, is shown in Table 6.[84] For colorectal carcinoma it has been shown that mortality rates decrease substantially with periodic surveillance.[85,86] It is, however, still questionable whether surveillance of the endometrium will lead to the early detection of cancer and improvement of the prognosis.[87] It is therefore disputable whether surveillance of the endometrium is opportune. Also screening for other organs is currently not evidence-based. The ACS guideline recommends that annual endometrial cancer screening be offered by age 35 for women with or at risk for hereditary nonpolyposis colorectal cancer (HNPCC), and that these women should be informed about the potential benefits, risks,

TABLE 5 Cancer Risk Assessment[26]

MMR Gene	Cumulative Colorectal Carcinoma Risk (at age 70)	Mean Age of Diagnosis of Colorectal Carcinoma	Cumulative Endometrial Carcinoma Risk (at Age 70)	Mean Age of Diagnosis of Endometrial Carcinoma
MLH1, males	65%	43 years		
MLH1, females	53%	43 years	27%	48 years
MSH2, males	63%	44 years		
MSH2, females	68%	44 years	40%	49 years
MSH6, males	69%	55 years*		
MSH6, females	30%	57 years	71%	54 years

*Difference between males with a mutation in *MSH6* and a mutation in *MLH1* and *MSH2* is not significant (P=0.0845).

TABLE 6 Netherlands Surveillance Protocol for Carriers of an MMR-Gene Mutation[26,84]

Surveillance	MLH1, MSH2, MSH6 (males)	MSH6 (females)
Colon	Colonoscopy, every 1 to 2 years, starting at age 20-25 years	Colonoscopy, every 1 to 2 years, starting at age 30 years
Endometrium	Ultrasound and CA-125, every 1 to 2 years, starting at age 30-35 years	Ultrasound and CA-125, every 1 to 2 years, starting at age 30–35 years. Consider hysterectomy above age 50 years
Upper urinary tract	Urine cytology analysis, every 1 to 2 years, starting at age 30-35 (MSH6 from age 50) years, if it occurs 2 or more times in family	Urine cytology analysis, every 1 to 2 years, starting at age 50 years, if it occurs 2 or more times in family
Stomach	Gastroscopy every 1 to 2 years, starting at age 30-35 years, if it occurs 2 or more times in family	Gastroscopy every 1 to 2 years, starting at age 30-35 years, if it occurs 2 or more times in family

and limitations of testing for early endometrial cancer detection.[38] Lynch, et al. mentioned this in their most recent review examination of the colon and endometrium as part of the Lynch syndrome surveillance protocol. The age of onset of surveillance and intervals are the same as recommended in the Netherlands.[4]

In families with an *MSH6* mutation, the age of onset of colorectal carcinoma in females is significantly higher than in carriers of a mutation in *MLH1* or *MSH2*, and the cumulative risk is significantly lower. The age of onset of colorectal carcinoma in male carriers of an *MSH6* mutation is also increased, but the difference was not statistically significant. The cumulative risk for endometrial carcinoma is significantly higher in female carriers of a mutation in *MSH6*.[26] Therefore, we propose to consider to start colonoscopies from age 30 years and to consider hysterectomy after the age of 50 years in female *MSH6* mutation carriers (Table 6).

If a family does fulfill the Amsterdam Criteria, but two tumors with a microsatellite stable phenotype are encountered, the current recommendation is the Lynch syndrome surveillance protocol. However, this recommendation is currently under discussion because the high frequency of colonoscopies in carriers of a mutation in one of the MMR genes is based on the defect in mismatch repair indicated by microsatellite instability (Vasen, personal communication). Recently Lindor, et al. compared cancer risks in Amsterdam I Criteria positive families with a mutation in one of the mismatch repair genes with an Amsterdam I Criteria positive group without a mutation in one of these genes. Cancer risks in the latter group were lower, and the incidence for other cancers than colorectal cancer might not be increased. They proposed the designation "familial colorectal cancer type X," and thus not Lynch syndrome to describe this type of familial aggregation of colorectal cancer.[88] Furthermore, Boland suggested using the term Lynch syndrome instead of HNPCC for the autosomal disease caused by a mutation in one of the mismatch repair genes since the name HNPCC only refers to colorectal cancer.[89]

CONCLUSIONS

The diagnostic workup of familial colorectal cancer is elaborate and time consuming and involves the cooperation of several medical specialists. However, establishing a diagnosis provides many advantages. If a mutation is detected in the family, an explanation can be provided for the accumulation of colorectal carcinoma and potential other tumors at young age. Also, presymptomatic testing can be offered to at-risk family members, thus providing certainty and a tailor-made protocol for periodic surveillance.

If however, no mutation is detected, which is quite often the case, risk calculations can be performed using empirical data. Family members whose cancer risk is elevated can be offered periodic screening. Clearly, the rapid detection of new genes involved in cancer, as well as the development of new diagnostic techniques and tools, may necessitate modification of our approach to familial colon cancer. However, the diagnostic scheme presented here allows efficient and effective analysis of families making optimal use of currently available technology.

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