

## Syndromic Colon Cancer: Lynch Syndrome and Familial Adenomatous Polyposis

Tusar K. Desai, MD<sup>a,b,\*</sup>, Donald Barkel, MD<sup>a,b</sup>

<sup>a</sup>Division of Gastroenterology, Department of Medicine, William Beaumont Hospital, 3601 West Thirteen Mile Road, Royal Oak, MI 48073, USA

<sup>b</sup>Department of Surgery, William Beaumont Hospital, 3601 West Thirteen Mile Road, Royal Oak, MI 48073, USA

Colon cancer, the third leading cause of mortality from cancer in the United States, afflicts about 150,000 patients annually. More than 10% of these patients exhibit familial clustering [1]. The most common and well characterized of these familial colon cancer syndromes is hereditary nonpolyposis colon cancer syndrome (HNPCC or Lynch syndrome), which accounts for about 2% to 3% of all cases of colon cancer in the United States [1].

Lynch syndrome, an autosomal dominant condition with incomplete penetrance, was initially defined by clinical and family history criteria, known as the Amsterdam criteria (Box 1). Subsequently, genetic mutations in six distinct DNA mismatch repair genes have been identified, and testing for three of these genes (MLH1, MSH2, MSH6) has become widely available to clinicians. Lynch syndrome now refers to patients who have mutations in one of four DNA mismatch repair (MMR) genes—MLH1, MSH2, MSH6, and PMS2—regardless of whether the Amsterdam criteria for family history are met [2,3]. About 1 in 1000 to 1 in 3000 Americans are carriers for MMR gene mutations [4,5], and 100,000 to 300,000 Americans have Lynch syndrome. Genetic testing for these mutations is now used for the diagnosis, although genetic testing is limited by its cost of more than \$2000 and concerns regarding privacy. Patients may be reluctant to be identified as a carrier of a cancer-causing genetic mutation that may limit their ability to obtain insurance, home mortgage loans, or employment. This aversion to being identified, potentially publicly, as a cancer gene carrier, has impeded the diagnosis of Lynch syndrome even in European countries where nationalized health care renders concerns about insurability irrelevant [6].

\*Corresponding author. Department of Medicine, William Beaumont Hospital, 3601 West Thirteen Mile Road, Royal Oak, MI 48073. E-mail address: tusardesai@aol.com (T.K. Desai).

### **Box 1: Amsterdam criteria: family risk for hereditary nonpolyposis colorectal cancer<sup>a</sup>**

At least three relatives have a cancer associated with hereditary nonpolyposis colorectal cancer<sup>b</sup>

One should be first-degree relative of the other two relatives.

At least two successive generations should be affected.

At least one relative should be diagnosed before age 50 years.

Familial adenomatous polyposis should be excluded.

Tumors should be verified by pathologic examination.

<sup>a</sup>About half of the families meeting Amsterdam I criteria have Lynch syndrome (hereditary DNA mismatch repair gene mutation); conversely, many families that have Lynch syndrome do not meet these criteria.

<sup>b</sup>Colorectal cancer, cancer of the endometrium, small bowel, or renal pelvis. Amsterdam I criteria included only colorectal cancer. Amsterdam II criteria included all cancers listed.

We review the current knowledge of familial cancer syndromes, with an emphasis on Lynch syndrome and familial adenomatous polyposis (FAP).

## **MISMATCH MUTATION REPAIR GENE FUNCTION**

Six MMR genes have been identified (Box 2). The two major genes are MLH1 and MSH2. The four minor MMR genes are MSH6, MSH3, PMS2, and MLH3. Mutations in MLH3 and MSH3 are not believed to cause malignancy [3]. MMR genes work as dimers or in pairs: MLH1 can pair with PMS2 or MLH3, whereas MSH2 can pair with MSH3 or MSH6. A mutation in MSH3 can therefore be overcome as MSH2 pairs with MSH6, and MSH6 mutations can be overcome by MSH2 pairing with MSH3 [3]. Similarly, a mutation in PMS2 can be overcome as MLH1 pairs with MLH3. A mutation in MLH1, however, leads to loss of MLH1 function and also PMS2 and MLH3 function because these two latter genes cannot function without MLH1. A mutation in MSH2 leads to loss of function for MSH3 and MSH6 because the protein products of these genes require the MSH2 protein for stabilization [3]. Mutations in MSH6 and PMS2 therefore lead to an attenuated form of familial cancer and Lynch syndrome, although there is one case report of a family with individuals who had colon cancer, uterine cancer, and three other cancers all occurring before age 25 associated with homozygous PMS2 mutations [7]. Gene sequencing for PMS2 is not commercially available.

Mutations in these genes can be truncating, leading to highly abbreviated mRNA transcription and complete lack of normal protein function, resulting in complete absence of immunohistochemical staining. MMR gene mutations often are missense mutations, however, which lead to single amino acid substitutions in MMR proteins. Such mutations may or may not express the cancer phenotype [3]. Missense mutations in MSH2 are almost always pathogenic [8],

**Box 2: DNA mismatch repair genes**

MLH1: Mutations lead to classic form of Lynch syndrome; 30% of mutations are missense mutations

PMS2: Usually leads to attenuated form of Lynch syndrome  
MSI-H cancers  
Onset cancer 7 to 8 years later than classic Lynch

MLH3: Not pathogenic

MSH2: Classic form of Lynch syndrome

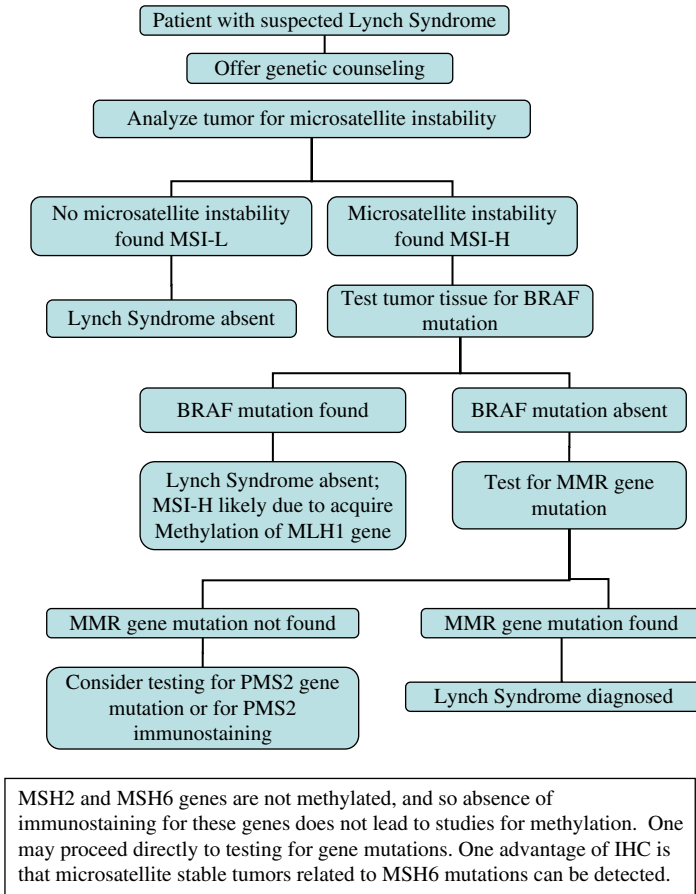
MSH6: Usually leads to attenuated form of Lynch syndrome  
Onset cancer 7 to 8 years later  
Cancers often MSI-L or stable

MSH3: Not pathogenic

whereas nontruncating missense mutations in MSH6 are usually not associated with MMR dysfunction and a high cancer risk [9]. MLH1 mutations are the most common MMR gene mutation found; 30% of these mutations are missense mutations. Some pathologic missense MLH1 gene mutations result in a minimally functional protein, which leads to falsely positive immunohistochemical staining [3].

**LYNCH SYNDROME: CLINICAL PRESENTATION AND DIAGNOSIS**

Inssofar as every patient who has colon cancer should undergo a detailed family history, the Amsterdam criteria, listed in Box 1, represents the starting point for evaluating the genetic basis of colon cancer. Genetic testing, however, reveals MMR gene mutations in only half of patients who meet the Amsterdam criteria [10]. Conversely, at least half of patients who have genetic mutations that define Lynch syndrome do not meet the Amsterdam criteria [11–14]. The Amsterdam criteria are, therefore, obsolete. They are clinically useful only when a patient and family meet the Amsterdam criteria; in this case one may proceed directly to genetic testing for MMR mutations (Fig. 1). In the dominant familial colon cancer pedigree, a family meets the Amsterdam criteria in number of colon cancers, but all family members developed the cancer after age 50. If, however, a patient does not meet the Amsterdam criteria, then the Bethesda guidelines should be followed. The revised Bethesda guidelines (Box 3) were established to identify patients who had colon cancer who should undergo testing for either microsatellite instability or immunohistochemistry for MMR proteins as a prelude to genetic testing [15]. Testing for microsatellite instability is beyond the capability of most community hospitals, but immunohistochemical testing for MMR proteins is technically easier and can be



**Fig. 1.** Diagnostic algorithm for Lynch syndrome using microsatellite instability.

performed in most pathology laboratories [4]. Missense mutations, however, can lead to weakly false-positive immunostaining for the MMR protein.

Each of the above genes except MLH1 has in its coding sequence a nucleotide repeat of seven or more elements, so they are particularly susceptible to mutation in the event of MMR gene dysfunction.

The finding of microsatellite instability should lead to genetic testing because it is found in more than 90% of patients who have Lynch syndrome, but in only 15% to 20% of patients who have sporadic colon cancer. Sporadic colon cancer that has high microsatellite instability (MSI-H) is believed to arise from serrated adenomas due to hypermethylation of the MLH1 gene promoter [4,16]. MLH1 gene hypermethylation is an age-related process [17]. Sporadic colon cancer that is MSI-H thus usually occurs after age 60.

**Box 3: Revised Bethesda guidelines<sup>a</sup>**

Tumors should be tested for microsatellite instability when one or more of the following exist:

Colorectal cancer diagnosed in a patient who is younger than 50 years

Presence of colorectal cancers that are synchronous (simultaneous) or metachronous (diagnosed at different times) or other tumors associated with hereditary nonpolyposis colorectal cancer,<sup>b</sup> regardless of age

Colorectal cancer with a high amount of microsatellite instability<sup>c</sup> or histology<sup>d</sup> diagnosed in a patient who is younger than 60 years<sup>e</sup>

Colorectal cancer or tumor associated with hereditary nonpolyposis colorectal cancer<sup>b</sup> diagnosed before age 50 years in at least one first-degree relative<sup>f</sup>

Colorectal cancer or tumor associated with hereditary nonpolyposis colorectal cancer<sup>b</sup> diagnosed at any age in two first- or second-degree relatives<sup>f</sup>

<sup>a</sup>These guidelines are intended for colorectal cancer patients to identify those who may benefit from tumor microsatellite instability testing. The guidelines are not diagnostic criteria for hereditary nonpolyposis colorectal cancer or Lynch syndrome. When a tumor is not available for testing, germline DNA testing can be offered if clinical presentation is strongly suggestive of Lynch syndrome.

<sup>b</sup>Includes colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas, and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.

<sup>c</sup>Refers to changes in two or more of the five panels of microsatellite markers recommended by the National Cancer Institute.

<sup>d</sup>Presence of tumor infiltrating lymphocytes, Crohn disease–like lymphocytic reaction, mucinous or signet-ring differentiation, or medullary growth pattern.

<sup>e</sup>There was no consensus among the Bethesda workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep age younger than 60 years in the guidelines.

<sup>f</sup>Criteria 4 and 5 have been reworded to clarify the revised Bethesda guidelines.

Up to 50% of MSI-H colon cancers diagnosed before age 60 are related to Lynch syndrome [17,18]. Only 20% to 25% of patients who have MSI-H colon cancer have an MMR gene mutation and Lynch syndrome [11,13]. Patients who have microsatellite instability and do not have MMR gene mutation are believed to have hypermethylation and inactivation of the MLH1 gene [4,16]. This situation frequently correlates with BRAF proto-oncogene mutations and may represent the pathway from serrated adenomas to sporadic colon cancer [19,20].

Microsatellites refer to long segments of nontranscribed DNA that are composed of a repeating mononucleotide (eg, AAAAAAAAAAAAAAAAAA) or dinucleotide sequences (eg, GTGTGTGTGTGTGT). These long DNA sequences provide a simple indicator of genetic mutation rate and risk because mutations are readily apparent in these long repeating sequences. For example, in the

sequence AAAAAAAAAAAAAACAAAAA, the C is an obvious mutation. Microsatellite instability is tested at five loci, the best known of which are BAT 26 and BAT 25, which consist of 25 and 26 adenine nucleotides in a row, respectively. BAT 25 or 26 is short for Big A Nucleotide Tract 25 or 26, respectively. Short nucleotide repeats of 7 to 8 elements are commonly present in the expressed portion of various genes. These genes are susceptible to mutation when MMR dysfunction exists.

The test sites for MSI are not involved in the carcinogenic process. Thirty-two genes in the human genome have mononucleotide repeats of more than 7 elements [21]. These long repetitive sequences are believed to be more prone to mutation than nonrepetitive sequences. Common target genes for mutation attributable to mismatch repair deficiency are the *TGFB1R2* gene, the neurofibroma 1 gene, and the four minor mismatch repair genes themselves (*MSH6*, *MSH3*, *MLH3*, *PMS2*) [22]. Each of these six genes has a repetitive sequence of eight or more nucleotides in its coding sequence, whereas *MSH2*, a major MMR gene, has an A7 repeat sequence in its coding region. The presence of microsatellites within the coding region of *MSH2*, *MSH6*, and *MSH3* and their susceptibility to MMR deficiency leads to a vicious cycle whereby a mutation in one MMR gene leads to mutations in other MMR genes. This phenomenon may account for the rapid development of adenomas and their rapid transition to cancer in Lynch syndrome [23].

*TGFB2* mutations are found in more than 90% of Lynch colorectal cancers, but are not found in nonmalignant tissues of Lynch patients, nor are they found in sporadic colorectal cancer without MMR deficiency [24,25].

Once microsatellite instability is found in a resected cancer, genetic testing may be offered to the patient or family members. Some 70% to 75% of patients whose cancer demonstrates microsatellite instability do not demonstrate mutations in the MMR genes. The high cost of genetic testing, coupled with the observation that genetic testing is negative in more than 70% of patients who have MSI, has led to a search for other cancer markers. Mutations in the *BRAF* proto-oncogene are nearly always absent in patients who have Lynch syndrome [19,20]. Testing for *BRAF* gene mutations in the cancer tissue may be performed for approximately \$200.

Some authorities have suggested the protocol outlined in Fig. 1. Candidates for MSI testing are identified according to the Bethesda guidelines. If MSI is detected, then *BRAF* gene mutation should be tested. If the *BRAF* gene has a mutation, genetic testing is unnecessary because the presence of this mutation suggests hypermethylation. If the *BRAF* gene does not have a mutation, genetic testing should be performed. If a patient who has MSI-H lacks all of the above (ie, has no mutations in MMR genes, *BRAF* genes, or abnormal methylation) then it is currently speculated that this patient has an undetectable MMR gene mutation. One possibility is mutation of the *PMS2* gene, as discussed below. The cost of genetic testing to identify mutations in the MMR genes is approximately \$2200. If a proband is diagnosed with a specific MMR gene mutation,

however, family members can be checked for mutations within this specific gene at a cost of approximately \$300 per patient. The cost effectiveness of these various approaches has not been evaluated. It seems that for families, screening the proband for MMR gene mutations is cost effective. Genetic diagnosis is important because of the high lifetime risk of 70% for cancer in these patients [26]. Moreover, the average age of the initial cancer diagnosis is only 44 years of age. Once an index patient is identified, therefore, the \$300 per person cost to identify family members who need intensive screening is cost effective. The lifetime risk for cancers associated with Lynch syndrome is provided in [Box 4](#).

Immunohistochemistry to stain a resected cancer specimen for functional MLH1 and its heterodimer partner PMS2, MSH2, or MSH6 protein has been used to screen for Lynch syndrome ([Fig. 2](#)). Staining for MLH1 or PMS2 is often absent in sporadic colon cancer because of hypermethylation of these genes. If immunohistochemistry shows the absence of either of these two proteins, testing for hypermethylation or the BRAF mutation should be performed, and if hypermethylation or the BRAF mutation are found, genetic testing is not indicated. If hypermethylation or the BRAF mutation are not found, genetic testing is indicated. If immunohistochemistry reveals an absence of MSH2 or MSH6, one may proceed directly to genetic testing without testing for hypermethylation [12]. Missense mutations in MLH1 or MSH6 may lead to defective function with a weakly staining protein on immunohistochemistry.

Immunohistochemistry for PMS2 is usually not performed for PMS2 but should be included in any investigation of familial cancer or MSI-H cancer. Among 12 patients who had MSI-H cancer and staining for MLH1, MSH2, and MSH6, 8 patients showed a loss of PMS2 staining. Five of these patients had an MMR gene mutation in either MLH1 or PMS2 [27]. Of 775 patients who had familial colon cancer undergoing immunohistochemical stains for MLH1, MSH2, MSH6, and PMS2, 8 patients had loss of only PMS2, of whom 7 had PMS2 gene mutations associated with MSI-H [28]. Finally, a kindred with PMS2 mutations has recently been reported that

#### **Box 4: Lifetime risk for cancer in Lynch syndrome**

Colon cancer 70%

Endometrial cancer 60% to 70%

Ovarian cancer 7% to 10%

Gastric cancer 13% (higher in East Asia)

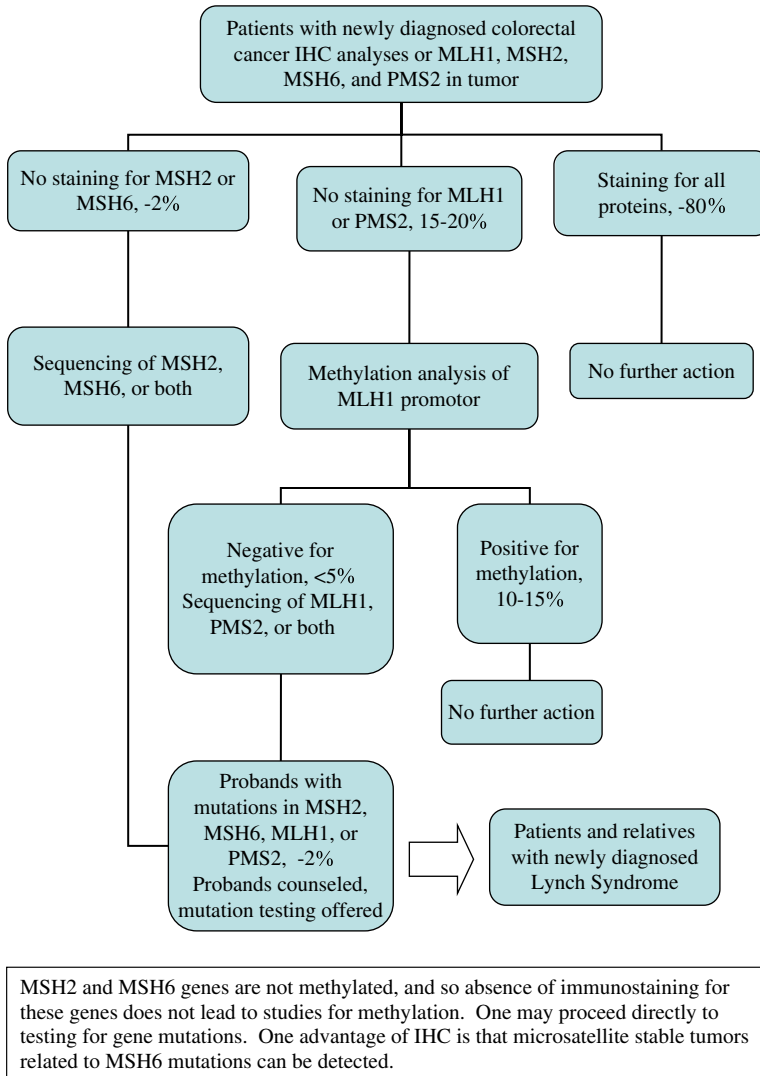
Small bowel cancer 4% (age at onset 40; 50% within range of upper endoscope)

Brain cancer 3.5%

Pancreas cancer

Renal ureteral

Sebaceous adenoma



**Fig. 2.** Diagnostic algorithm for Lynch syndrome using immunohistochemistry.

met the Amsterdam criteria; all the children had homozygous biallelic mutations for PMS2 [7].

Colon cancer related to Lynch syndrome tends to be proximal and have a specific histologic appearance characterized by a heavy infiltration of lymphocytes, medullary growth pattern, and a mucinous or signet ring differentiation [2]. Colon cancers with these histologic characteristics in a patient who is less

than 60 years of age, or has had other HNPCC-related cancers, should undergo testing for MSI. The clinicopathologic features most predictive of MSI-H cancers are lymphocytic infiltration and proximal cancer location [29]. There is considerable heterogeneity in the pathologic appearance of colon cancers from family members who have the same MMR gene mutation, and even among metachronous colorectal cancers from the same individual, suggesting that factors other than MMR gene mutations influence carcinogenesis [30]. Distal cancers are less likely than proximal cancers to demonstrate the characteristic pathologic features of Lynch colon cancer [30].

The initial Bethesda guidelines suggested that patients who have adenomas diagnosed before age 40 undergo evaluation for HNPCC. A small study of such patients, however, found that none of the adenomas demonstrated MSI or lacked immunohistochemical markers for MSH1 or MLH2 [31]. Microsatellite instability occurs in 40% to 80% of benign adenomas from patients who have documented colon cancer and MMR gene mutations [32,33]. The revised Bethesda guidelines no longer suggest detailed evaluation of patients who have adenoma before age 40 unless the adenoma demonstrates high-grade dysplasia or multiple adenomas are found.

Cancers associated with HNPCC are listed in **Box 4**. Gynecologic cancers may be more common than colon cancer in patients who have HNPCC, and some gynecologists have proposed changing the name to not refer to colon cancers or to also mention endometrial cancer [34]. These gynecologists argue that it is difficult to raise awareness of this syndrome when the name of the syndrome includes colon cancer but not the frequently associated gynecologic malignancies [34]. It has been proposed that all patients younger than age 50 years who have endometrial cancer be tested for Lynch syndrome mutations. In one study, 34% of women who had endometrial cancer younger than age 50 had HNPCC mutations [35].

The phenotypic expression of Lynch syndrome varies according to ethnicity. In Korea and China, gastric cancer is far more common among patients who have Lynch syndrome than in Holland [36,37]. The high risk for endometrial cancer seems to affect all ethnic groups, however. A study of 385 Japanese women who had endometrial cancer reported that 0.5% satisfied the Amsterdam criteria for family history, 30% demonstrated MSI-H in the cancer tissue, and 8.3% demonstrated MMR gene mutations [38].

Other cancers associated with Lynch syndrome include stomach, pancreas, biliary tract, small bowel, renal pelvis and ureter, and brain (see **Box 3**) [2]. The cumulative lifetime risk for gastric cancer in patients who have Lynch syndrome is estimated at 13% [39]. It is much higher in East Asia and lower in the West [36,37]. In China and Korea, the risk for gastric cancer exceeds the risk for endometrial cancer among MMR gene mutation carriers [36,37]. The relative risk for brain tumors among patients who have MMR gene mutations is six times the baseline in the general population [40]. Despite this high relative risk, the lifetime risk for brain tumor among patients who have Lynch syndrome is only 3.5%, so screening for this tumor is not recommended [40].

The lifetime risk for small bowel cancer among MMR gene mutation carriers is estimated at 4%, which is too low to justify screening [41]. Up to 50% of small bowel cancers are located in the duodenum or proximal jejunum within reach of the upper endoscope [42]. The average age of onset of small bowel cancer is 39 years. In about 50% of patients who have small bowel cancer, this cancer was the first indicator of Lynch syndrome [42]. This cancer exhibits intense lymphocytic infiltration similar to that for colon cancer [42]. There have been reports of cancer of the prostate and thyroid in association with HNPCC, with microsatellite instability in these cancers [43].

### Confounding Features of Microsatellite Instability Testing

Cancers from about 6% of patients who have Lynch syndrome do not manifest microsatellite instability; these patients usually have MSH6 mutation [44,45]. MSH6 mutations account for approximately 8% of all Lynch syndrome cancers.

There are data suggesting that cigarette smoking increases the risk for colonic adenomas [46,47], but the link to colon cancer has been harder to establish. It has been recently found, however, that cigarette smoking selectively increases the risk for colon cancer that is MSI-H [48,49]. Similarly, the incidence of colon cancer is 1.2-fold higher in African Americans than Caucasians. In two small series from Washington DC a high level of microsatellite instability was found in more than 40% of cancers in African Americans, representing a doubling of the rate found in cancers in Caucasians [50]. The colon cancers found with MSI-H were proximal, well differentiated, and mucinous, a pattern suggestive of colon cancer in Lynch syndrome [50]. The proportion of patients who have MSI who have defective expression of MMR gene function is similar in African Americans and in Caucasians. In African Americans, females accounted for 68% of the cancers with MSI-H, whereas in Caucasians, most patients who had MSI-H colon cancer were male [11,12]. Alpha-1-antitrypsin deficiency is also associated with MSI-H colon cancer [51].

### Management of Patients Who Have Amsterdam Criteria

Approximately half of the families who meet the Amsterdam criteria do not demonstrate microsatellite instability or MMR gene mutation. They are classified as familial colon cancer X. They seem to have a colon cancer risk intermediate between that of Lynch syndrome and the general population [10]. The standardized incidence ratio of colon cancer in familial colon cancer X is 2.3, compared with 6 for Lynch syndrome. These patients do not seem to be at risk for other cancers. Patients who have familial colon cancer X syndrome develop colon cancer at an older age, with a mean age of about 60 years [10].

Colon cancer screening of these families should probably be more aggressive than the recommendations of the American Cancer Society for patients who have one family member who has colon cancer or polyps before age 60 of undergoing colonoscopy every 5 to 10 years starting at age 40 or 10 years before the age of the youngest immediate family member who developed cancer. It therefore seems reasonable to advise these patients to undergo colonoscopy

every 5 years beginning 5 years earlier than the age of the youngest family member to develop cancer. Colon cancer in these patients tends to be proximal and associated with multiple adenomas, whereas more than three adenomas is rare in Lynch syndrome [52].

### Mutational Heterogeneity

Among patients who have documented MMR gene mutations, 90% have mutations in MLH1 and MSH2. The risk for cancer presumably varies according to the specific mutation, but guidelines are presented for mutations in aggregate. The risk for various cancers in patients who have Lynch mutations is presented in **Box 3**. It varies according to ethnicity. A study from the German HNPCC registry reported that families that have the MSH6 mutation have a lower risk for colon cancer and a later age of cancer onset [53]. A large series from the Dutch HNPCC database reported that the risk for cancer in MSH6 carriers is related to sex and the type of cancer. Among women who have the MSH6 mutation the risk for colon cancer was significantly less than the risk for colon cancer among women who have MLH1 or MSH2 mutations, whereas the risk for endometrial cancer in women who have MSH6 mutation was significantly higher than that for women who have MLH1 or MSH2 mutation [54]. About 70% of women who have MSH6 mutation develop endometrial cancer by age 70 [54]. Among men who have MSH6 mutations, the risk for colon cancer was less, but the difference was not statistically significant. MSH6-related cancers are usually microsatellite stable [44,45]. Missense mutations of MSH6 lead to weakly (false) positive immunostaining for the MSH6 protein, but deficient MMR function [3].

Mutations of the PMS2 gene lead to an attenuated form of Lynch syndrome with onset of cancer at a later age, like mutations in the MSH6 gene. Cancers in patients who have PMS2 mutations are MSI-H, however [27,28]. Interestingly, families who have mutations in either PMS2 or MSH6 tend to be small and not meet the Amsterdam criteria [28]. The largest series of families that had PMS2 mutations reported breast cancer to be the second most common cancer after colon cancer [28].

### Screening for Patients/Families Who Have Lynch Syndrome

A proband identified as having Lynch syndrome should undergo subtotal colectomy for colon cancer and screening for the other associated cancers. The evaluation of immediate family members depends on their willingness to undergo genetic testing. If family members who have a documented MMR gene mutation refuse genetic testing, colonoscopy should be offered every 1 to 2 years starting at age 20 to 25 years. If genetic testing is negative, screening colonoscopy should still be offered in a program similar to that for the family colon cancer X syndrome, at every 5 years starting at age 10 years before the youngest family member to develop cancer. If genetic testing is positive, screening is intensive and prophylactic surgery may be considered. The adenoma-to-carcinoma progression in Lynch syndrome is believed to occur more rapidly than in sporadic colon cancer, and may occur in flat adenomas that are easily

missed by colonoscopy. Colonoscopic screening was shown to be effective in reducing the rate of colon cancer and overall mortality among 133 patients who had Lynch syndrome, as compared with 119 patients who had Lynch syndrome who refused screening colonoscopy [55]. Colon cancer was found in 8 screened subjects and in all cases it was local and potentially curable, with no deaths of colon cancer. Colon cancer occurred in 19 controls who refused screening, of whom 9 died of colon cancer [55]. Despite intensive counseling and education, almost 50% of patients told that they had a 70% lifetime risk for colon cancer from Lynch syndrome refused screening [55]. This finding has been confirmed. The Netherlands launched a large-scale surveillance program in the late 1980s, and a retrospective analysis of the national cancer registry revealed that mortality from colon cancer decreased among patients who had Lynch syndrome after the introduction of this surveillance program [56]. In this study, there was no absolute excess mortality for any of Lynch syndrome-related cancers, except for colon and brain cancers [56].

Among 394 primary relatives of patients who had Lynch syndrome undergoing surveillance colonoscopy, 5 patients developed an interval colon cancer within 3.5 years of a reportedly normal colonoscopy [57]. This presumed high miss rate has led to the performance of frequent colonoscopies on these patients, and the use of chromoendoscopy to increase mucosal contrast and improve detection of flat adenomas. Back-to-back colonoscopy using indigo carmine dye during the second colonoscopy was performed in 25 patients who met the Amsterdam criteria, of whom 84% had MSH2 or MLH1 germline mutations. The initial colonoscopy revealed 24 lesions in 10 patients, but the segmental pancolonial chromogen dye contrast colonoscopy revealed 52 lesions in 15 patients. One cancer in this series was detected by conventional colonoscopy, but 7 conventional adenomas with high-grade dysplasia and 4 serrated adenomas were found by chromogen dye colonoscopy that were missed on conventional colonoscopy [58]. This intriguing study should stimulate further study of chromocolonoscopy in this high-risk population.

Lecomte and colleagues [59], after performing a conventional colonoscopy, sprayed indigocarmine dye into only the proximal colon and then re-evaluated the proximal colon with chromocolonoscopy in 36 patients from HNPCC families. Conventional colonoscopy revealed 25 lesions, whereas chromocolonoscopy of the proximal colon revealed an additional 45 lesions. Most missed lesions detected by chromocolonoscopy were less than 5 mm, flat, and hyperplastic [59]. East and colleagues [60] compared use versus nonuse of narrow band imaging (NBI) with high-definition colonoscopes in 62 patients from HNPCC families who already underwent a prior clearing colonoscopy. Conventional colonoscopy detected 25 adenomas in 17 patients, whereas NBI colonoscopy detected an additional 21 adenomas. Because the progression from adenoma to cancer can occur within 2 years in patients who have Lynch syndrome, the enhanced detection rate of these newer colonoscopic techniques seems to be clinically significant. The above data suggest that chromocolonoscopy or high-definition colonoscopy with NBI may be highly useful in this high-risk population.

Virtual CT colonography has been proposed for screening of sporadic colon cancers, but this would likely be inappropriate for patients who have proven or suspected Lynch syndrome who are at high risk for flat adenomas. In a study from Finland both CT colonography and optical colonoscopy were performed in 78 patients who had proven MMR gene mutations [61]. Two colon cancers were detected by both modalities. A total of 26 polyps were found (13 adenomatous and 13 hyperplastic). The sensitivity of CT colonography for detecting polyps was only 27%, and for polyps greater than 1 cm the sensitivity was only 80% [61]. Given the rapid progression of adenomas to carcinomas in Lynch syndrome, CT colonography seems inadequate for screening these patients. Among patients who have documented Lynch syndrome who have not had colon cancer, colonoscopy should be performed every 2 years starting at age 25 to 30, depending on the age of onset of the colon cancer in the family.

### Natural History of Lynch Syndrome Colon Cancer

Large population-based studies have reported that survival of patients who have colon cancer related to MMR gene mutations is similar to survival of patients who have sporadic colon cancer [11,62,63]. Patients who have Lynch syndrome who have distal colon cancer may develop metachronous colon cancer more quickly than those who have proximal colon cancer.

MSI-H cancers, considered as a group, including Lynch syndrome and acquired methylation of MLH1, have different clinical characteristics. Patients who have MSI-H colon cancer exhibit improved survival for all cancer stages, so that the improved survival is not because of earlier detection [64]. Patients who have stage I or II colorectal cancer have 5-year survivals of 90% or more [64]. Among patients who have stage II and III colon cancer, microsatellite stability may affect the response to chemotherapy. Patients who have MSI-H cancers are less likely to respond to alkylating agents or 5-FU [65,66] but are more likely to respond to irinotecan than patients who have MSI-L colon cancers [67]. In vitro studies of MSI-H cell lines suggest that demethylating agents can restore sensitivity to alkylating agents and 5-FU [68].

### Postoperative Surveillance for Rectal Cancer

Patients who have colon cancer related to Lynch syndrome should undergo subtotal colectomy and then undergo aggressive endoscopic surveillance of the rectum. A retrospective study estimated the risk for rectal cancer to be 12% during 12 years after abdominal colectomy [69].

### Gynecologic Management

Gynecologic malignancies may be as common in Lynch syndrome as colon cancer. The lifetime risk in Lynch syndrome for colon cancer is 70%, for endometrial cancer is 50%, and for ovarian cancer is 10% to 20%. These risks may be overestimated because of ascertainment bias and may actually be lower [70].

Among 543 unselected patients who had endometrial cancer, 22% were MSI-H or had abnormal immunohistochemistry. Ten patients demonstrated MMR gene mutations. Remarkably, 7 of these 10 patients would not have

met any published criteria for HNPCC, and 6 of these patients were more than 50 years old [35].

About 2% to 4% of women who have ovarian cancer carry MMR gene mutations, compared with 11% of women who carry BRCA gene mutations [71]. If the BRCA mutation has been excluded in patients who have familial ovarian cancer, genetic testing for Lynch syndrome should be considered [71]. The lifetime risk for endometrial cancer may be underestimated because so many women have a hysterectomy for benign causes. Prophylactic hysterectomy and bilateral salpingo-oophorectomy have been proposed as an alternative to intensive annual screening. Prophylactic surgery is generally accepted as a cost-effective measure for women between 30 and 35 years old [72]. A retrospective study comparing 245 women who had an MMR gene mutation who did not undergo hysterectomy and salpingo-oophorectomy to 61 women who did undergo surgery, for either prophylaxis or a benign indication, reported that endometrial cancer occurred in 33% of women who did not have surgery, whereas no woman undergoing preventive surgery developed gynecologic cancers [72]. Patients who have ovarian cancer related to Lynch syndrome have a survival similar to that of patients who have sporadic ovarian cancer [73].

### Homozygous Mutations

Lynch III syndrome refers to patients who are either homozygous or compound heterozygotes for MMR gene mutations, rather than simple heterozygotes. These patients develop malignancies of the colon, endometrium, brain, and hematopoietic system at a very young age in the first through third decades of life (Box 5) [74]. Café-au-lait spots and neurofibromas frequently occur [7]. The neurofibroma gene may undergo mutation because of MMR gene mutation [75]. MLH1 deficiency accelerates the development of leukemia in mice heterozygous for the neurofibromatosis gene [76]. Among children who have neurofibromatosis, MLH1 mutations lead to a higher likelihood of hematologic malignancy [77] and an earlier onset of cancer [78].

A literature review revealed 59 individuals among 24 families with members who had café-au-lait spots and biallelic gene mutations [7]. Among these 59

#### **Box 5: Lynch syndrome homozygotes or compound heterozygotes**

Cancer onset in first 3 decades of life

Pediatric malignancies

Leukemia lymphoma

Brain cancer

Café-au-lait spots

If suspected check normal tissue for microsatellite instability. If normal tissue MSI-H suspect biallelic mutations.

individuals 42 (71%) developed colon cancer at a mean age of 32, and 28 individuals developed a brain tumor at a mean age of 16. Remarkably, 2 of these families had only PMS2 mutation, but in 1 of these families it was a homozygous PMS2 mutation [7]. In a study of consecutive children who developed cancer before age 15 years, 6% to 10% of children were homozygous for MMR gene mutations [79]. Among children who had 6 or more café-au-lait spots, 80% had a serious disease [80].

Patients who have complete absence of MMR function present with hematologic and brain malignancies in the first 2 decades of life, whereas those who have biallelic mutations and minimal residual MMR function present with gastrointestinal and endometrial cancer in the third and fourth decades of life [74]. In carriers of biallelic mutations, MSI-H can be found in normal tissue [81]. When a Lynch syndrome-associated cancer is found in a very young patient (<30 years old), normal tissue should be checked for microsatellite instability, and if detected, biallelic mutations should be searched for.

### Muir-Torre Syndrome

Muir-Torre syndrome refers to the occurrence of sebaceous adenomas on the skin of patients who have cancers related to Lynch syndrome [82]. These adenomas demonstrate microsatellite instability and MMR gene mutations. The presence of sebaceous adenomas should trigger screening for colonic and gynecologic malignancy and testing for MSI.

### Colon Cancer Prevention

Observational studies have shown that aspirin reduces the risk for sporadic colon cancer, but prospective randomized controlled trials have shown no benefit [83], possibly because of the low dose of aspirin used in prospective trials [83]. There are no data among patients who have Lynch syndrome. Nonsteroidal anti-inflammatory drugs, particularly sulindac, have been studied to prevent colon cancer in high-risk groups, such as FAP, but a recent study of sulindac in patients who had Lynch syndrome was disappointing [84]. The investigators analyzed epithelial proliferation as a surrogate marker for carcinogenesis before and after a 1-month course of orally administered sulindac. Such studies have shown reduced colonic epithelial cell proliferation in patients who had sporadic adenomas and familial adenomatous polyposis [85]. In patients who had Lynch syndrome, however, sulindac did not suppress colonic epithelial proliferation, and it stimulated epithelial cell proliferation in the proximal colon [84].

Folic acid has been suggested to reduce the risk for sporadic colon cancer [86] and for colon cancer among patients who have ulcerative colitis [87], but there are no data among patients who have Lynch syndrome. Calcium supplementation has been shown to reduce the risk for benign adenomas and the rate of epithelial proliferation in patients who have adenomas [88], but calcium supplementation did not reduce the rate of epithelial proliferation among patients who had Lynch syndrome [89].

Cigarette smoking was shown to increase the risk for cancer in patients who had Lynch syndrome, whereas alcohol use did not alter the colon cancer risk

[90]. This study reported that a mean cigarette consumption of 24 pack-years increased the hazard ratio for colon cancer to 1.43. This study was limited by the lack of a dose–response relationship and lack of a tobacco history in 236 of the 596 study patients [90]. Patients who have familial colon cancer should be advised to follow a healthy lifestyle that incorporates exercise and a diet low in fat and red meat and high in whole grains, legumes, fruits, and vegetables.

### Barriers to Screening

Few physicians follow a systematic protocol to evaluate for Lynch syndrome, even among patients who present with colon or uterine cancer before age 50. Clinicians typically do not obtain a family history for the Amsterdam criteria. Screening for gynecologic cancer is particularly inadequate because of provider- and patient-related factors. In a highly educated population in Northern California, only 69% of women who had HNPCC had undergone screening for endometrial and ovarian cancer, and only 12% underwent this screening as a result of the advice of their gynecologist [91]. In a survey of 2845 women in California's Silicon Valley, 313 were identified as being at risk for familial cancer syndromes, and yet when contacted, less than 25% were interested in follow-up, cancer screening, or genetic testing [92].

A survey in 2002 reported that only 51% of 815 gastroenterologists who were members of the American Gastroenterological Association referred patients for genetic counseling before cancer predisposition testing. When presented with a family history consistent with Lynch syndrome, only 26% advised genetic testing and only 16% advised appropriate screening [93]. Pathologists may not screen for microsatellite instability or immunohistochemistry even when pathologic criteria are present in a patient who is younger than 50 years of age. The pathologist has the additional burden of the cost of the MSI or immunohistochemical testing, which may not be reimbursed by insurance. Even if insurance paid for these tests, the insurance company might inquire about the test results or infer positive results from a subsequent request for genetic testing. Once MMR mutations have been identified, the insurance company might decline further coverage for the patient or primary relatives. In the Netherlands, where there is universal health coverage, 40% of healthy carriers of MMR gene mutations experienced problems obtaining disability or life insurance or a home mortgage [6].

### Patient-Related Lapses in Screening

After an index patient is diagnosed with Lynch syndrome, there remain barriers to identify family members who are carriers and to ensure proper screening of affected relatives. Patients may not inform their primary relatives, partly because of their natural reluctance to reveal that they have a life-threatening genetic disorder. If the health care providers take the initiative by contacting family members, this poses a time burden on a busy physician, infringes on patient privacy, and potentially violates doctor–patient

confidentiality. Both studies that proposed direct physician communication with relatives of Lynch probands were conducted outside the United States [94,95]. Canadian patients felt that a physician did not have the right to inform relatives without the patient's permission [94]. There may be legal risks to not informing the relatives of a Lynch proband, however. Three lawsuits have been filed in the United States against physicians who failed to warn family members about the risk for a hereditary disease [96]. Even after relatives have been informed of the risk for carrying an MMR gene and the potential of a 70% lifetime risk for cancer, 28% to 40% of at-risk relatives decline genetic testing [97,98].

Among those who accept genetic testing and are proved to be carriers, only about 75% comply with colonoscopy; most of these studies have been performed in Australia or Europe where cost is not an issue because of universal health care coverage [55,99,100]. Finally, less than 65% of female carriers of the MMR gene comply with gynecologic screening in Australia and the United States [99,100]. Relatives may decline genetic testing or subsequent screening because of a lack of interest in prevention or a fatalistic attitude toward the disease.

The current cost of genetic testing for MMR gene mutations is estimated at about \$2700, but this cost will certainly decline as technological advances in gene sequencing revolutionize medicine. Complete human genome sequencing currently costs \$100,000 but should decline to \$10,000 within 5 years.

### CLASSICAL FAMILIAL ADENOMATOUS POLYPOSIS

FAP is a dominantly inherited syndrome in which affected people develop hundreds, if not thousands, of colonic polyps that inevitably lead to colon cancer at a relatively young age. It is caused by mutations of the APC gene, a tumor-suppressor gene located on chromosome 5q21-q22 [101]. The APC gene normally blocks DNA transcription that would otherwise lead to uncontrolled cellular growth, helps control cell adhesion, and helps regulate migration of enterocytes [102]. APC gene mutation and protein inactivation trigger the growth of the numerous polyps found in this syndrome. The specific location of the mutation on the APC gene predicts the specific phenotype.

Classical FAP occurs in about 1 in 20,000 live births. It affects both sexes equally. It represents less than 1% of the total colon cancer risk in the United States. It is caused by mutations in exon 15 of the APC gene [103]. Variants of FAP include Turcot syndrome (FAP associated with brain tumors), Gardner syndrome (FAP associated with extraintestinal manifestations), and attenuated FAP (aFAP). Genetic evaluation has shown that 10% to 30% of patients who have classical FAP do not have an APC mutation and up to 90% who have aFAP do not have a detectable APC mutation. One third of patients who have FAP have no family history of the syndrome and represent *de novo* germline APC mutations [104].

Up to 10% of patients who have FAP eventually develop periampullary cancer. Other neoplasms triggered by FAP include gastric polyps, small intestinal

adenomas, osteomas, and adrenal, liver, and thyroid tumors. These associations historically led to the use of eponyms, such as Turcot syndrome and Gardner syndrome, but these associations represent variant presentations of FAP. Other features occurring in FAP include abdominal desmoid tumors and congenital hypertrophic retinal pigment epithelium.

### Attenuated Familial Adenomatous Polyposis

Attenuated FAP is caused by mutations in the 5' or 3' end of the APC gene. Attenuated expression occurs because these mutations can be bypassed by an internal translation initiation site in the APC gene [105]. Patients who have attenuated FAP have fewer polyps, a later age of diagnosis, and more frequent involvement of the proximal colon [106]. These patients can develop the extracolonic features of classical FAP.

### MUTYH-Associated Polyposis

MUTYH-associated polyposis is an autosomal recessive form of FAP. The MUTYH gene is located on the p-arm of chromosome 1. This gene results in the production of an enzyme involved in DNA repair. Mutations allow for an accumulation of mutations in the APC gene that can lead to a form of FAP. MUTYH mutation most frequently results in the development of numerous polyps, typically fewer than 100 polyps, but up to 1000 polyps, and can result in cancer even without polyps [107]. These patients can also manifest the extracolonic features of FAP. Most cancers in MUTHY-associated polyposis are right-sided.

### I1307K

This APC mutation, which affects 6% of the Ashkenazi Jewish population, leads to an increased risk for colon polyps and cancer. Ashkenazi Jews are descended from Jewish communities from middle or eastern Europe. Ashkenazi Jews constitute approximately 80% of Jews worldwide [108]. The I1307K mutation is so named because it involves codon 1,307 in exon 15 of the APC gene. This mutation is autosomal dominant, is of low penetrance, and has no detectable effect on APC function, but seems to render the gene more susceptible to additional mutations that can then lead to the development of colon cancer. An individual who has the I1307K mutation has a 10% to 20% lifetime risk for colorectal cancer.

### Screening

Recognition of these and other inherited colorectal cancer syndromes is essential to reduce colorectal cancer risks and to advise at-risk family members regarding screening. The diagnosis of FAP is straightforward in a patient presenting with hundreds to thousands of colonic polyps. Suspicion should also be raised when patients present with colon cancer when they are younger than age 45, adenomatous polyps younger than age 40, or multiple colonic malignancies; when patients develop more than 10 polyps in their lifetime together with a positive family history; or when a family has multiple generations with colon cancer and clustering of extracolonic cancers [109].

*Screening: familial adenomatous polyposis*

Patients affected with FAP and their first-degree relatives should be offered genetic counseling. An affected patient should undergo APC gene testing and if positive, at-risk family members should be tested. If no APC mutation is found in the affected member, additional gene testing is not indicated. If the family mutation is known, genetic testing of at-risk family members can determine if they have FAP. If the family mutation is unknown or the affected family member is unavailable, at-risk family members can still be tested but a negative result in this setting is inconclusive. It is inadvisable to genetically test children before the age of 10 years because it does not change treatment strategy and can lead to emotional and family conflicts [110]. Gene carriers or at-risk family members who have inconclusive results should begin annual endoscopic surveillance starting at age 10 to 12.

*Screening: attenuated familial adenomatous polyposis*

Genetic screening of patients and families that have suspected aFAP is similar to that for classical FAP. Fewer of these individuals have a detectable mutation, however. As right-sided tumors predominate in aFAP, it is mandatory to use colonoscopy as the screening tool in at-risk individuals.

*Screening: MUTYH-associated polyposis*

Patients who have FAP or aFAP phenotypes who have negative genetic tests for APC mutations should undergo MUTYH genetic testing. About 10% to 20% of such patients test positive for MYH-associated polyposis (MAP) [106]. Siblings of patients who have MAP should be considered for this genetic test. Children of MAP patients are carriers (MAP is autosomal recessive), and therefore screening of their partners to determine risk for MAP in any future children should be considered.

*Screening: duodenal adenomas*

Patients should undergo screening esophagogastroduodenoscopy beginning at age 20 because of their high risk for developing duodenal adenomas [111]. The rate of conversion of a duodenal adenoma to invasive cancer is low and therefore the aim of esophagogastroduodenoscopy in these patients is to detect severe dysplasia rather than to eradicate all neoplasia. Adenomas greater than 1 cm or with known dysplasia should be removed [112]. Endoscopic removal of these lesions is often incomplete and duodenectomy must be considered for lesions with high-grade dysplasia.

*Screening: I1307K*

A person of Ashkenazi heritage who has a personal or family history of colonic neoplasia may consider gene testing. Because these patients are already considered at increased risk, however, knowledge of their I1307 status is unlikely to change the screening guidelines, which call for colonoscopic surveillance at 3- to 5-year intervals [113].

## Treatment

Treatment of FAP is surgical removal of the colon and rectum. The timing and type of surgery depend on the severity of the polyposis phenotype and to a lesser extent on the genotype, age, and clinical and social circumstances of the patient. Cancer is rare under the age of 20 [114]. If the syndrome is severe or symptomatic, surgery is done as soon as convenient. If the disease is mild, surgery can be delayed to the mid-teen years.

The three main surgical options are: colectomy and ileorectal anastomosis (IRA), total proctocolectomy and ileostomy (TPC), and proctocolectomy with ileal pouch–anal anastomosis (IPAA). TPC is almost never done as the first operation, except when IPAA is contraindicated. Contraindications include mesenteric desmoids, advanced low rectal cancer, or poor anal sphincter function. IRA has the advantage of better functional outcome, but has the disadvantage of a continuing risk for rectal cancer. Because this cancer risk is related to the severity of polyposis, IRA is a reasonable option in patients who have mild disease (<20 rectal adenomas, <1000 colonic adenomas) [115]. Proponents of IPAA for FAP cite rectal cancer risk after IPA and studies showing equivalent quality of life after the two operations [116]. Technical factors, including the type of pouch constructed and whether it is hand-sewn, stapled, or with or without mucosectomy, have little functional impact [117].

## Follow-up After Surgery

Patients must undergo yearly endoscopic surveillance after IRA or IPAA surgery for FAP. Rectal polyps occurring after IRA should be removed if greater than 5 mm in diameter. Random mucosal biopsies should be performed to exclude severe dysplasia even in patients who do not have polyps. Sulindac and celecoxib reduce the polyp load, but do not completely prevent cancers. Long-term chemoprevention for rectal polyposis is therefore of doubtful benefit [118]. Annual endoscopic evaluation of the pouch is necessary after IPAA because polyposis can occur in ileal pouches. The impact of pouch polyposis will not be known until a large number of FAP patients who have IPAA reach 20 years of follow-up, because 20 years is the time for development of most ileostomy cancers. It is disturbing that cancer in patients who have IPAA is beginning to be reported [119].

## References

- [1] Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;348:919–32.
- [2] Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome. *JAMA* 2006;296:1507–17.
- [3] Boland CR, Koi M, Chang D, et al. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. *Fam Cancer* 2007 July.
- [4] Boland CR. Decoding hereditary colorectal cancer. *N Engl J Med* 2006;354:2815–7.
- [5] Dunlop MG, Farrington SM, Nicholl I, et al. Population carrier frequency of hMSH2 and hMLH1 mutations. *Br J Cancer* 2000;83:1643–5.

- [6] Wagner A, Van Kessel I, Kriege MG, et al. Long term follow-up of HNPCC gene mutation carriers: compliance and satisfaction with counseling and screening procedures. *Fam Cancer* 2005;4(4):295–300.
- [7] Trimbath JD, Petersen GM, Erdman SH, et al. Café-au-lait spots and early onset colorectal neoplasia: a variant of HNPCC? *Fam Cancer* 2001;1(2):1010–5.
- [8] Ollila S, Sarantaus L, Kariola R, et al. Pathogenicity of MSH2 missense mutations is typically associated with impaired repair capability of the mutated protein. *Gastroenterology* 2006;131:1408–17.
- [9] Kariola R, Hamel H, Frankel WL, et al. MSH6 missense mutations are often associated with no or low cancer susceptibility. *Br J Cancer* 2004;91:1287–92.
- [10] Lindor NM, Rabe K, Petersen GM, et al. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency. *Familial colorectal cancer type X. JAMA* 2005;293:1979–85.
- [11] Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N Engl J Med* 2006;356(26):2751–63.
- [12] Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary non-polyposis colorectal cancer). *N Engl J Med* 2005;352:1851–60.
- [13] Aaltonen LA, Saloraara R, Kristip P, et al. Incidence of hereditary non-polyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;338:1481–7.
- [14] Casey G, Lindor NM, Papadopoulos N, et al. Conversion analysis for mutation detection in MLH1 and MLH2 patients with colorectal cancer. *JAMA* 2005;293:799–809.
- [15] Umar A, Boland CR, Syngal S, et al. Revised Bethesda guidelines for hereditary non-polyposis colorectal cancer (Lynch Syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004;96:261–8.
- [16] Herman JG, Umar A, Plyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci* 1998;95:6870–5.
- [17] Kakar S, Burgart LJ, Thibodeau SN, et al. Frequency of loss of MLH1 expression in colorectal cancer increases with advancing age. *Cancer* 2003;97(6):1421–7.
- [18] Farrington SM, Lin-Goerke J, Ling J, et al. Systematic analysis of MSH2 and MLH1 in young colon cancer patients and controls. *Am J Hum Genet* 1998;63:749–59.
- [19] Deng G, Bell J, Crawley S, et al. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1 but not in hereditary non-polyposis colorectal cancer. *Clin Cancer Res* 2004;10:191–5.
- [20] Kambara T, Simms LA, Whitehall VLJ, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004;53:1137–44.
- [21] Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. *Cancer Res* 2002;62(9):2447–54.
- [22] Chang DK, Metzgar D, Wills C, et al. Microsatellites in the eukaryotic DNA mismatch repair genes as modulators of evolutionary mutation rate. *Genome Res* 2001;11(7):1145–6.
- [23] Perucho M. Microsatellite instability: the mutator that mutates the other mutator. *Nat Med* 1996;2(6):630–1.
- [24] Markowitz S, Wang J, Myeroff L, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 1995;268(5215):1336–8.
- [25] Parsons R, Myeroff LL, Liu B, et al. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res* 1995;55(23):5548–50.
- [26] Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA-mismatch-repair gene mutation. *Hum Mol Genet* 1997;6:105–10.

- [27] Halvarsson B, Lindblom A, Rambach E, et al. The added value of PMS2 immunostaining in the diagnosis of hereditary non-polyposis colorectal cancer. *Fam Cancer* 2006;5(4): 353–8, Epub 2006 July 12.
- [28] Hendriks Y, Jagmohan-Changur S, Van Der Klift H, et al. Heterozygous mutations in PMS2 cause hereditary non-polyposis colorectal carcinoma (Lynch syndrome). *Gastroenterology* 2006;130:312–22.
- [29] Jenkins MA, Hayashi S, O'Shea M, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability; a population-based study. *Gastroenterology* 2007;133:48–56.
- [30] Halvarsson B, Muller W, Planck M, et al. Phenotypic heterogeneity in hereditary non-polyposis colorectal cancer: identical germline mutations associated with variable tumor morphology and immunohistochemical expression. *J Clin Pathol* 2007;60:781–6.
- [31] Velayos FS, Allen BA, Conrad PG, et al. Low rate of microsatellite instability in young patients with adenomas: reassessing the Bethesda guidelines. *Am J Gastroenterol* 2005;10:1143–9.
- [32] DeJong AE, Morreau H, Van Puijenbroek M, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. *Gastroenterology* 2004;126: 42–8.
- [33] German HNPCC Consortium, Muller A, Beckman C, et al. Prevalence of mismatch-repair-deficient phenotype in colonic adenomas arising in HNPCC patients: results of a 5-year follow-up study. *Int J Colorectal Dis* 2006;21(7):642–4.
- [34] Lu HK, Broaddus RR. Gynecologic cancers in Lynch syndrome. *Fam Cancer* 2005;4(3): 249–54.
- [35] Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome among endometrial cancer patients. *Cancer Res* 2006;66(15):7810–7.
- [36] Cai SJ, Xu Y, Cai GX, et al. Clinical characteristics and diagnosis of patients with hereditary non-polyposis colorectal cancer. *World J Gastroenterol* 2003;9:284–7.
- [37] Park YJ, Shin KH, Park JG. Risk of gastric cancer in hereditary non-polyposis colorectal cancer in Korea. *Clin Cancer Res* 2000;6:2994–8.
- [38] Banno K, Susumu N, Yanokura M, et al. Association of HNPCC and endometrial cancers. *Int J Clin Oncol* 2004;9(4):262–9.
- [39] Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch repair genes. *Int J Cancer* 1999;81:214–8.
- [40] Vasen HF, Sanders EA, Taal BG, et al. The risk of brain tumors in hereditary non-polyposis colorectal cancer. *Int J Cancer* 1996;65(4):422–5.
- [41] Ten Kate GL, Kleibeuker JR, Nagengast FM, et al. Is surveillance of the small bowel indicated for Lynch syndrome families? *Gut* 2007;56:1198–201.
- [42] Schulmann K, Brasch FE, Kunstanann E, et al. HNPCC associated small bowel cancer: clinical and molecular characteristics. *Gastroenterology* 2005;128(3):590–9.
- [43] Maul JS, Warner NR, Kuwanda SK, et al. Extra-colonic cancers associated with hereditary non-polyposis colorectal cancer in the Utah population database. *Am J Gastroenterology* 2006;101(7):1591–6.
- [44] Wu Y, Berends MJ, Mensink RG, et al. Association of hereditary non-polyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. *Am J Hum Genet* 1999;65:1291–8.
- [45] Wijnen J, de Leeuw W, Vasen H, et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. *Nat Genet* 1999;23:142–4.
- [46] Martinez ME, McPherson RS, Annegers JF, et al. Cigarette smoking and alcohol consumption as risk factors for colorectal adenomatous polyps. *J Natl Cancer Inst* 1995;87:274–9.
- [47] Kikendall JW, Bowen PE, Burgess MB, et al. Cigarettes and alcohol as independent risk factors for colonic adenomas. *Gastroenterology* 1989;97:660–4.
- [48] Neugut AI, Terry MB. Cigarette smoking and microsatellite instability: casual pathway or marker-defined subset of colon tumors? *J Natl Cancer Inst* 2000;92:1791–3.

- [49] Samovitz WS, Albertsen H, Sweeney C, et al. Association of smoking CpG Island methylator phenotype, and V600E BRAF mutations in colon cancer. *J Natl Cancer Institute* 2006;98:1731–8.
- [50] Ashktorab H, Smoot DT, Carethers JM, et al. High incidence of microsatellite instability in colorectal cancer from African-Americans. *Clin Cancer Res* 2003;9(3):1112–7.
- [51] Yang P, Cunningham JM, Halling KC, et al. Higher risk of mismatch-repair deficient colorectal cancer in alpha-antitrypsin deficiency carriers and cigarette smokers. *Mol Genet Metab* 2000;71:639–45.
- [52] Dove-Edwin J, Dejong AE, Adams T, et al. Prospective results of surveillance colonoscopy in dominant familial colorectal cancer with and without Lynch syndrome. *Gastroenterology* 2006;130:1995–2000.
- [53] Plaschke J, Engel C, Kruger S, et al. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations; the German HNPCC consortium. *J Clin Oncol* 2004;22(22):4486–94.
- [54] Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. *Gastroenterology* 2004;127(1):17–25.
- [55] Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary non-polyposis colorectal cancer. *Gastroenterology* 2000;118:829–34.
- [56] DeJong AE, Hendriks YM, Kleibeuker JF, et al. Decrease in mortality in Lynch syndrome families because of surveillance. *Gastroenterology* 2006;130(3):655–71.
- [57] Vasen H, Nagenast F, Meera Khan P. Interval cancer in hereditary non-polyposis colorectal cancer (Lynch syndrome). *Lancet* 1995;345:1183–4.
- [58] Hurlstone DP, Karajeh M, Cross SC, et al. The role of high-magnification chromoscopic colonoscopy in hereditary non-polyposis colorectal cancer screening: a prospective “back to back” endoscopic study. *Am J Gastroenterol* 2005;100:2167–73.
- [59] Lecomte T, Cellier C, Meatchi T, et al. Chromoendoscopic colonoscopy for detecting preneoplastic lesions in hereditary nonpolyposis colorectal cancer. *Clin Gastroenterol Hepatol* 2005;3:897–902.
- [60] East JE, Suzuki N, Starrinidis M, et al. Narrow band imaging for colonoscopic surveillance in hereditary nonpolyposis colorectal cancer. *Gut* 2008;57:65–70.
- [61] Renkonen-Sinisablo L, Kivisaari A, Kivisaari L, et al. Utility of computed tomographic colonography in surveillance for hereditary nonpolyposis colorectal cancer syndrome. *Fam Cancer*; 2007 [Epub ahead of print].
- [62] Bertario L, Russo A, Sala P, et al. Survival of patients with hereditary colorectal cancer in comparison to sporadic colorectal cancer. *Int J Cancer* 1999;80:183–7.
- [63] Goecke T, Schulmann K, Engel C, et al. Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome. *J Clin Oncol* 2006;24(26):4285–92.
- [64] Gryfe R, Kim H, Hsieh ETK, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 2000;342:69–77.
- [65] Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;349(3):247–57.
- [66] Jover R, Zapater P, Castells A, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. *Gut* 2006;55(6):848–55.
- [67] Fallik D, Borroni F, Boige V, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. *Cancer Res* 2003;63(18):5738–44.
- [68] Arnold CN, Goel A, Castells CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. *Int J Cancer* 2003;106(1):66–73.

- [69] Rodriguez-Bigas MA, Vasen HF, Pekka-Mecklin J, et al. Rectal cancer risk in hereditary non-polyposis colorectal cancer after abdominal colectomy. *Ann Surg* 1997;225:202-7.
- [70] Risk of colorectal and endometrial cancers for carriers of mutations of hMLH1 and hMSH2 gene: correction for ascertainment. *J Med Genetics* 2005;42(6):491-6.
- [71] Farrell C, Lyman M, Freitag k, et al. The role of hereditary non-polyposis colorectal cancer in the management of familial ovarian cancer. *Genet Med* 2006;8(10):653-7.
- [72] Scmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. *N Engl J Med* 2006;354:261-9.
- [73] Crijnen TE, Janssen-Heijnen ML, Gelderblom H, et al. Survival of patients with ovarian cancer due to mismatch repair defect. *Fam Cancer* 2005;4(4):301-5.
- [74] Bandipalliam P. Syndrome of early onset colon cancers, hematologic malignancies and features of neurofibromatosis in HNPCC families with homozygous mismatch repair gene mutations. *Fam Cancer* 2005;4(4):323-33.
- [75] Wang Q, Montmain G, Ruano E, et al. Neurofibromatosis type 1 gene as a mutational target in a mismatch repair-deficient cell type. *Hum Genet* 2003;112(2):117-23.
- [76] Kort BR. Diagnostic outcome in children with multiple café-au-lait spots. *Pediatrics* 1992;90:924-7.
- [77] Gutmann DH, Winkler E, Kabbarah O, et al. M1H1 deficiency accelerates myeloid leukemogenesis in neurofibromatosis 1 (Nf1) heterozygous mice. *Oncogene* 2003;22(29):4581-5.
- [78] Wang Q, Lasset C, Desseigne F, et al. Neurofibromatosis and early onset of cancers in hMLH1-deficient children. *Cancer Res* 1999;59:294-7.
- [79] Ricciardone MD, Ozcelik T, Cevher B, et al. Human MLH1 deficiency predisposes to hematological malignancy and neuro-fibromatosis type 1. *Cancer Res* 1999;59:290-3.
- [80] Poley JW, Wagner A, Hoogmans MM, et al. Biallelic germline mutations of mismatch-repair genes: a possible cause for multiple pediatric malignancies. *Cancer* 2007;109:2349-56.
- [81] DeRosa M, Fasano C, Panariello L, et al. Evidence for recessive inheritance of Turcot's syndrome caused by a compound heterozygous mutations in the PMS2 gene. *Oncogene* 2000;19:1719-23.
- [82] Jones B, Oh C, Mangold E, et al. Muir Torre syndrome: diagnostic and screening guidelines. *Australas J Dermatol* 2006;47(4):266-9.
- [83] Dubé C, Rostom A, Lewin G, et al. The use of aspirin for primary prevention of colorectal cancer; a systematic review prepared for the U.S. Preventive service task force. *Ann Intern Med* 2007;146:365-75.
- [84] Rijcken FE, Hollema H, van der Zee AG, et al. Sulindac treatment in hereditary non-polyposis colorectal cancer. *Eur J Cancer* 2007;43:1251-6.
- [85] Giardello FM, Yang VW, Hyland LM, et al. Primary chemoprevention of familial adenomatous polyposis with sulindac. *N Engl J Med* 2002;346:1054-9.
- [86] Pufulete M, Al-Ghnam R, Leather AJ, et al. Folate status, genomic DNA hypomethylation, and the risk of colorectal adenoma and cancer: a case control study. *Gastroenterology* 2003;124:1240-8.
- [87] Chan EP, Lichtenstein GR. Chemoprevention: risk reduction with medical therapy of inflammatory bowel disease. *Gastroenterol Clin North Am* 2006;35:675-712.
- [88] Baron JA, Beach M, Mandel JS, et al. Calcium supplement for the prevention of colorectal adenomas. Calcium polyp prevention study group. *N Engl J Med* 1999;340(2):101-7.
- [89] Cats A, Kleibeuker JH, van der Meer R, et al. Randomized double blinded, placebo-controlled intervention study with supplemental calcium in families with hereditary non-polyposis colorectal cancer. *J Natl Cancer Inst* 1995;87(8):598-603.
- [90] Watson P, Ashwathnarayan R, Lynch HT, et al. Tobacco use and increased colorectal cancer risk in patients with hereditary non-polyposis colorectal cancer. *Arch Intern Med* 2004;164:2429-31.

- [91] Yang K, Allen B, Conrad P, et al. Awareness of gynecologic surveillance in women from hereditary non-polyposis colorectal cancer families. *Fam Cancer* 2006;5(4):405–9, Epub 2006 Aug 26.
- [92] Manuel MR, Lilja J, Kieran S, et al. Cancer risk assessment in a community setting: prevalence of patients with high risk family histories (abstract). *Gynecol Oncol* 2007;104(535):15.
- [93] Batra S, Valdimarsdóttir H, McGovern M, et al. Awareness of genetic testing for colorectal cancer predisposition among specialists in gastroenterology. *Am J Gastroenterol* 2002;97(3):729–33.
- [94] Kohut K, Manno M, Gallinger S, et al. Should healthcare providers have a duty to warn family members of individual with an HNPCC-causing mutation? A survey of patients from the Ontario Familial Colon Cancer Registry. *J Med Genet* 2007;44(6):404–7.
- [95] Aktan-Collan K, Haukka A, Pylvänäinen K, et al. Direct contact in inviting high-risk members of hereditary colon cancer families to genetic counseling and DNA-testing. *J Med Genet* 2007;44:732–8.
- [96] Offit K, Groeger E, Turner S, et al. The “duty to warn” a patient’s family members about hereditary disease risks. *JAMA* 2004;292(12):1469–73.
- [97] Halbert CH, Lynch H, Lynch J, et al. Colon cancer screening practices following genetic testing for hereditary non-polyposis colon cancer (HNPCC) mutations. *Arch Intern Med* 2004;164(17):1881–7.
- [98] Ponz de Leon M, Benatti P, Di Gregorio C, et al. Genetic testing among high-risk individuals in families with hereditary non-polyposis colorectal cancer. *Br J Cancer* 2004;90(4):882–7.
- [99] Collins V, Meiser B, Gaff C, et al. Screening and preventive behaviors one year after predictive genetic testing for hereditary non-polyposis colorectal carcinoma. *Cancer* 2005;104(2):273–81.
- [100] Hadley DW, Jenkins JF, Dimond E, et al. Colon cancer screening practices after genetic counseling and testing for hereditary non-polyposis colorectal cancer. *J Clin Oncol* 2004;22(1):39–44.
- [101] Burt RW, DiSario JA, Cannon-Albright L. Genetics of colon cancer: impact of inheritance on colon cancer risk. *Annu Rev* 1995;46:371–9.
- [102] Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. *Annu Rev Genomics Hum Genet* 2002;3:101–28.
- [103] Russell AM, Zhang J, Luz J, et al. Prevalence of MYH germline mutations in Swiss APC mutation-negative polyposis patients. *Int J Cancer* 2006;118:1937–40.
- [104] Hernegger GS, Moore HG, Guillem JG. Attenuated familial polyposis: an evolving and poorly understood entity. *Dis Colon Rectum* 2002;45:827–34.
- [105] Heppner GK, Trzepak C, Tuohy TM, et al. Attenuated APC alleles produce functional protein from internal translation initiation. *Proc Natl Acad Sci U S A* 2002;99:8161–6.
- [106] Galiatsatos P, Foulkes WD. Familial adenomatous polyposis. *Am J Gastroenterol* 2006;101:385–98.
- [107] Wang L, Baudhuin I, Boardman L, et al. MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. *Gastroenterology* 2004;127:9–16.
- [108] Elazar DJ. Can Sephardic Judaism be reconstructed? Jerusalem center for public affairs. Available at: ([www.jcpa.org/dje/articles3/sephardic.htm](http://www.jcpa.org/dje/articles3/sephardic.htm)). Accessed August 31, 2007.
- [109] Kaz AM, Brentnall TA. Genetic testing for colon cancer. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:670–9.
- [110] American Society of Clinical Oncology Policy Statement Update: genetic testing for cancer susceptibility. *J Clin Oncol* 2003;21:2397.
- [111] Wallace MH, Phillips RK. Upper gastrointestinal disease in patients with familial adenomatous polyposis. *Br J Surg* 1998;85:742–50.

- [112] Church J, Simmang C. Practice parameters for the treatment of patients with dominantly inherited colorectal cancer (familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer). *Dis Colon Rectum* 2003;46:1001–12.
- [113] Strul H, Barenboim E, Lehno M, et al. The 11307K adenomatous polyposis coli gene variant does not contribute in the assessment of the risk for colorectal cancer in Ashkenazi Jews. *Cancer Epidemiol Biomarkers Prev* 2003;12:1012–5.
- [114] Church JM, McGannon E, Burke C, et al. Teenagers with familial adenomatous polyposis: what is their risk for colorectal cancer? *Dis Colon Rectum* 2002;45:887–9.
- [115] Church J, Burke C, McGannon E, et al. Predicting polyposis severity by proctoscopy: how reliable is it? *Dis Colon Rectum* 2001;44:1249–54.
- [116] Ambroze WL Jr, Dozois RR, Pemberton JH, et al. Familial adenomatous polyposis: results following ileal pouch-anal anastomosis and ileorectostomy. *Dis Colon Rectum* 1992;35:12–5.
- [117] von Roon AC, Tekkis PP, Clark SK, et al. The impact of technical factors on outcome of restorative proctocolectomy for familial adenomatous polyposis. *Dis Colon Rectum* 2007;50:952–61.
- [118] Lynch HT, Thorson AG, Smyrk T. Rectal cancer after prolonged sulindac chemoprevention. A case report. *Cancer* 1995;75:936–8.
- [119] Church J. Ileoanal pouch neoplasia in familial adenomatous polyposis: an underestimated threat. *Dis Colon Rectum* 2005;48:1708–13.