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## Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications

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### Abstract

More than one million patients will manifest colorectal cancer (CRC) this year of which, conservatively, approximately 3% (~30,700 cases) will have Lynch syndrome (LS), the most common hereditary CRC predisposing syndrome. Each case belongs to a family with clinical needs that require genetic counseling, DNA testing for mismatch repair genes (most frequently *MLH1* or *MSH2*) and screening for CRC. Colonoscopy is mandated, given CRC's proximal occurrence (70–80% proximal to the splenic flexure). Due to its early age of onset (average 45 years of age), colonoscopy needs to start by age 25, and because of its accelerated carcinogenesis, it should be repeated every 1 to 2 years through age 40 and then annually thereafter. Should CRC occur, subtotal colectomy may be necessary, given the marked frequency of synchronous and metachronous CRC. Because 40–60% of female patients will manifest endometrial cancer, tailored management is essential. Additional extracolonic cancers include ovary, stomach, small bowel, pancreas, hepatobiliary tract, upper uroepithelial tract, brain (Turcot variant) and sebaceous adenomas/carcinomas (Muir-Torre variant). LS explains only 10–25% of familial CRC.

### Keywords

colorectal cancer; endometrial cancer; hereditary cancer; hereditary nonpolyposis colorectal cancer; immunohistochemistry; Lynch syndrome; microsatellite instability; mismatch repair; mismatch repair genes

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The estimated annual worldwide incidence of colorectal cancer (CRC) is 1,023,152 (1). Lynch syndrome (LS), previously called hereditary non-polyposis colorectal cancer or HNPCC, accounts, conservatively, for approximately 3% (2) of this incidence (~30,700 cases), compared with familial adenomatous polyposis (FAP) syndrome which is about one-tenth as common, occurring in only about 1 in 10,000 of the population (3,4). Hampel et al. studied 500 tumors from unselected CRC affected individuals. Among these 500 CRC patients, 18 (3.6%) had LS. When these results were added to data on 1066 previously studied patients, the entire study cohort (N = 1566) showed 44 patients (2.8; 95% confidence interval (CI), 2.1–3.8) manifesting LS. These authors concluded that approximately 1 in every 35 patients who

manifested CRC had LS (2). Given the mentioned worldwide incidence figures of CRC, and extrapolating from the findings of Hampel et al. that 2.8% of the CRC cases they investigated were confirmed to be LS, we arrive at a figure of approximately 28,600 cases of LS to be newly diagnosed this year, worldwide. We have only crude estimates of familial CRC, as defined by the presence of two or more first-degree relatives with CRC, but it is thought by some to involve approximately 20% of all cases of CRC (5,6). These statistics are important given that each hereditary case comes from a family that could benefit from genetic counseling, DNA testing, surveillance, and targeted management (7–9). Yet, when a patient's family risk is overlooked, so too are opportunities for early screening (10). For example, the United States Surgeon General was concerned enough to launch a campaign promoting awareness of the medical value of family history and encouraging data collection and analysis (10,11). A series of papers dealing with LS diagnosis, molecular genetics, screening and management, has recently been published (12–15), prompting us to review LS and to summarize the recent advances in knowledge of hereditary CRC, highlighting LS (7,8).

## History of hereditary CRC

### Familial adenomatous polyposis

The first clinical report of hereditary CRC was in 1861 (16), of a patient who likely had FAP. Lockhart-Mummary (17) was the first to report that the relevant hereditary factor in this disease is not the cancer *per se*, but rather the presence of multiple colonic adenomas, which have a tendency to undergo malignant transformation. Herrera et al. reported a patient in 1986 with developmental abnormalities as well as multiple colonic polyps, and interstitial deletion of chromosome 5 (18). In 1987, Bodmer et al. provided evidence that the FAP gene, now known as *APC*, was on chromosome 5q (19). Since then, there have been rapid advances in the understanding of the genotypic–phenotypic correlations related to different mutations in the *APC* gene (Table 1) (20). For a more detailed history of FAP, see Bussey (21).

### Lynch syndrome

In 1913, Warthin published a large pedigree including numerous cases of CRC in the absence of polyposis, along with cases of gastric and uterine cancer (22), designating it 'Family G.' In 1966, Lynch et al. reported two large Midwestern families (Families N and M), whose constellation of tumors was strikingly similar to Warthin's Family G (23). The study of Families G, N, and M, and hundreds of similar families helped to define the cardinal features of LS as described in Table 2.

In 1991 an international collaborative group of researchers agreed upon criteria, which became known as the Amsterdam Criteria (AC), for diagnosing HNPCC (24). These were broadened to recognize a diagnostic role for extracolonic tumors; hence, the Amsterdam Criteria II (AC-II) (25). Subsequently, the broad Bethesda Guidelines were developed to select CRC patients to receive preliminary testing (Table 3) (26).

The molecular genetics era for LS began when Peltomäki et al., through a genome-wide search and linkage analysis in large informative families, identified a cancer susceptibility locus on chromosome 2p (27). Shortly thereafter, a second locus for LS was identified on chromosome 3p by Lindblom in Sweden (28).

During this same time period, it was demonstrated that tumors occurring in LS patients had a characteristic molecular change, initially called 'ubiquitous somatic mutations in simple repeated sequences' (29), or a 'replication error' phenotype (RER) (30). These characteristic molecular changes are now called microsatellite instability (MSI) (31,32). Recognition that MSI is a consequence of defective DNA replication error repair (33) or post-synthetic DNA

proofreading, contributed to the identification of the first two LS genes on 2p and 3p, namely *MSH2* and *MLH1*. These genes encode proteins involved in the identification and repair of DNA mismatch errors (34–37). The identification of germline mutations in *MLH1* and *MSH2* was quickly followed by the discovery of other human genes that encode proteins involved in the mismatch repair (MMR) complex. The genes identified to date include *MLH1* (27,36,37), *MSH2* (28,34,35), *MSH6* (38), *PMS2* (39), and possibly *MLH3* (40,41).

## The diagnosis of LS

LS is defined in terms of having a germline mutation in a DNA MMR gene (42). However, it is not the standard of care to test every CRC patient for germline mutations in the DNA MMR genes. LS can be difficult to diagnose, due to a lack of specific phenotypic features. Figure 1 and Table 1 show the CRC-prone disorders which are part of the differential diagnosis of LS.

The diagnosis of LS is facilitated by the acquisition of a comprehensive family history of cancer of *all* anatomic sites, with attention paid to the cardinal features of LS as shown in Table 2. Importantly, Table 2 includes the extra-colonic cancers that have been found to be integral to the syndrome. Figure 2 depicts a classical LS family with a known *MSH2* mutation; notable are individuals with diagnoses of extracolonic cancers (III-7, III-8, III-12, III-14), and numerous individuals with CRC.

An algorithmic approach to LS has been adopted by professional organizations with clinical practice guidelines developed for risk assessment, genetic testing, and clinical management for patients and families. Among these guidelines are those offered by the American Cancer Society (43), American Society of Colorectal Surgeons (44), National Comprehensive Cancer Network (NCCN) (45), and a United States gastrointestinal consortium (American Gastroenterology Association, among others) (46). These groups have developed a general consensus for surveillance and management. Other groups such as the American Society of Clinical Oncology and the National Society of Genetic Counselors have focused heavily on genetic testing. Lynch and Lynch have critiqued the various guidelines (47).

## MSI testing

A suspicion of LS is raised when a CRC or LS-related cancer occurs in a young person, and is increased when a positive family history is present, or when a person develops multiple primary LS-related cancers. Identification of patients in whom the tumor should undergo MSI testing has been facilitated by the development of the Bethesda Guidelines (see Table 3). However, only a fraction of people with CRC before age 50 have LS. In one series, 58% of CRC patients < 35 years old had MSI in their tumors, but only 17% of those between 35 and 55 years of age had MSI (48). Fully 40% of families that fulfill AC-I do not have MSI, which has led to the designation of a ‘familial colorectal cancer type X’ (49). Germline testing of all young patients with CRC or testing of cases solely on family history grounds carries a diagnostic yield for deleterious mutations of < 50% in most series. Tumors without MSI predict a much lower yield on mutational testing.

Thus, MSI testing enhances the predictive power of clinical selection features. For this reason, a step-wise approach is widely used: (i) clinical suspicion (such as early onset or family history), followed by (ii) MSI testing (see below). When a tumor is found to be microsatellite instability-high (MSI-H), germline mutation testing is generally recommended. A thorny exception, discussed below, involves sporadic, nonfamilial tumors that manifest MSI. These have a later age at onset, with loss of *MLH1* protein expression and evidence of hypermethylation of the *MLH1* promoter and frequent presence of a specific *BRAF* mutation (V600E). If tumor is unavailable or MSI cannot be done for whatever reason, it may be

appropriate to proceed directly to germline testing when clinical features are compelling. Clinical practice guidelines have embraced this thinking.

Jass et al. have shown how clinical, molecular, and pathology features may prove effective in LS diagnosis. When a CRC is found with tumor infiltrating lymphocytes, a Crohn's-like lymphocytic response, an excess of mucin, or signet-ring cell pathology with poor differentiation, the informed pathologist should consider testing for MSI (50,51).

Historically, MSI testing was the first test to be used for the purpose of identifying tumor characteristics predictive of an underlying MMR mutation. The assays required standardization and selection of the optimal microsatellite sequences for routine testing (52). According to the Bethesda Guidelines (Table 3), five microsatellite markers are used, and three dinucleotide repeats and two mononucleotide repeats comprised the initial panel of markers. When tumor DNA shows a new allele compared with normal tissues in two or more of these five microsatellites, this is classified as MSI-high (MSI-H). Although MSI is characteristic of LS tumors, it may be found in about 15% of unselected groups of CRC. Of this subset of MSI tumors, 20–25% represent LS, and the other 75–80% are sporadic MSI, caused by methylation-induced silencing of *MLH1* (53–55). Another 20% of all CRCs have a mutation at only one of the five microsatellite sequences, and this is termed MSI-low (MSI-L). MSI-L tumors do not share the clinical or pathological features seen in MSI-H cancers, and are not associated with germline mutations in DNA MMR genes. Finding MSI-L in a CRC does not suggest LS, and this abnormality appears to be due to a non-mutational down-regulation of the *MSH3* gene (56). A novel panel of five microsatellite markers has been identified that can be run in a single pentaplex polymerase chain reaction (PCR) that is more sensitive and specific than the original markers, and does not require normal tissue to be analyzed (57). In addition to its use in screening for LS, MSI testing is important because MSI-H is associated with a better clinical prognosis overall and stage-for-stage (58), and it predicts a relatively worse response to adjuvant chemotherapy for both stage II and III CRCs (59).

### Immunohistochemistry

MSI is sensitive but not specific for LS, as only 20–25% of all MSI-H tumors are associated with germline mutations in a DNA MMR gene. Though testing for MSI-H is quite sensitive for LS, it will not be detected in tumors of about 5% of all LS mutation carriers and is only 86% sensitive in CRCs from families with germline mutations in *MSH6* (38). For this reason, immunohistochemistry (IHC) is of value to supplement MSI testing. Antibodies are commercially available for MSH2, MSH6, MLH1 and PMS2 proteins, and IHC is being performed in many academic and community hospitals either to complement MSI testing, or as a substitute. IHC is probably ~95% sensitive for DNA MMR deficiency, but it requires experience on the part of the pathologist to interpret the slides, as there can be ambiguous staining of the tissues (60). The DNA MMR genes are subject to physiological regulation, and may not be robustly expressed in every cell. The normal cells in the slide (e.g., non-neoplastic epithelium, lymphocytes, stromal tissues, etc.) serve as positive controls for the technical issues in the staining. The pertinent finding is the absence of staining of the gene product in the tumor cell nuclei compared to normal cells.

Also, there are two 'major' DNA MMR genes: *MSH2* and *MLH1*, which are stabilized by interactions between any of several 'minor' DNA MMR genes, including *MSH6* and *PMS2* (61,62). The 'minor' DNA MMR genes are dependent upon their binding partners for expression at the protein level. Thus, when a tumor loses expression of MSH2, there is concomitant loss of MSH6 expression. Similarly, loss of MLH1 leads to loss of PMS2 expression. However, when there are germline mutations in *MSH6* or *PMS2*, the tissue shows isolated loss of these proteins (61).

The majority of the germline mutations in *MSH2* lead to complete loss of gene expression, and CRC tissues from patients with LS-*MSH2* type usually show complete loss of both *MSH2* and *MSH6* protein expression. However, LS-*MLH1* type is frequently caused by missense mutations (63). Some missense mutations result in an altered, nonfunctional protein, but the mutant protein may be expressed and retain its immunoreactivity. Thus, the finding of MSI-H in a CRC, with normal or weak expression of *MLH1* protein, in association with loss of *PMS2* protein in the tumor tissue could either be due to an inactivating germline mutation in *PMS2*, or to a missense germline mutation in *MLH1*. This conundrum has led to the development of functional assays for the *MLH1* protein (64–66). On the other hand, complete loss of *MLH1* and *PMS2* proteins is the universal observation with sporadic MSI-H CRCs caused by epigenetic silencing of *MLH1*, as this process completely silences both *MLH1* alleles (67).

This discrepancy between MSI-H status and ‘uninformative’ *MLH1* staining has been validated in findings from several clinical series. Salahshor et al. described intact staining for *MLH1* in 2 of 15 MSI-H cancers from patients with known mutations of *MLH1*. In a large series of unselected cases, Lindor et al. found that 27 of 818 tumors with intact staining of *MLH1* (68) and *MSH2* were MSI-H, concluding that IHC is a highly specific (100%) and reasonably sensitive (92.3%) screening tool, but that some MSI-H tumors will be missed if only IHC is performed (69).

In a study that involved MSI and IHC testing on 500 tumors from unselected patients with CRC, Hampel et al. concluded that, for screening purposes, IHC was found to be almost equally as sensitive as MSI, but advantages of IHC are that it is more readily available and often enables identification of the gene that should be tested (2).

### Conversion analysis

Certain MMR mutations are not detected when conventional DNA sequencing is used, as the wild-type allele can ‘mask’ the mutant one. Consequently, the sensitivity of genetic testing, even in AC positive cases with MSI, is no greater than about 70%. To deal with this, a diploid to haploid conversion analysis was developed to separate the paternal and maternal alleles, unmasking certain cryptic mutations (see below). This technique was evaluated in comparison with DNA sequencing alone for its potential to detect heterogeneous germline mutations in *MLH1*, *MSH2*, and *MSH6* in CRC patients suspected of having LS. Conversion analysis provided an increase of 56% (35/63) in the diagnostic yield of genetic testing compared with genomic DNA sequencing alone. Conversion analysis can substantially increase the diagnostic yield of genetic testing for MMR mutations in patients diagnosed with CRC (70). In fact, the germline mutation in Family G was initially not appreciated by direct sequencing, but was found to be a T → G transversion mutation at the splice acceptor site of exon 4 of *MSH2* using conversion (71). However, this technique is cumbersome to perform and has not come into routine clinical use.

### *PMS2* mutations

Although *MSH2* and *MLH1* account for most cases of LS, one must be prepared to evaluate a suspected LS patient for evidence of mutation in one of the ‘minor’ MMR genes. Senter et al. (72) investigated 99 probands diagnosed with LS-associated tumors that had isolated loss of *PMS2* by IHC. Employing the long-range PCR and multiplex ligation-dependent probe amplification (MLPA), they detected germline *PMS2* mutations in 62% of probands ( $n = 55$  monoallelic and 6 with biallelic mutations). In describing the clinical phenotype of LS in the presence of *PMS2* mutations in this large series of *PMS2* mutation carriers, they found that 65.5% of families with monoallelic *PMS2* mutations met the Revised Bethesda Guidelines. The incidence of CRC was 5.2-fold higher and the incidence of endometrial cancer was 7.5-fold higher than expected in the general population. These findings translate to a cumulative

cancer risk in North America, to age 70 years, of 15–20% for CRC, 15% for endometrial cancer, and 25–32% for any LS-associated cancer. Thus, the penetrance of *PMS2* with monoallelic mutation carriers was lower than that for other MMR genes. Although these findings potentially merit modified counseling and cancer surveillance in *PMS2* families, no recommendations for liberalized management have been offered to date.

### Homozygous biallelic MMR mutations

To date, virtually all attention has been devoted to the typical case of dominant mutations involving one MMR gene allele. However, there are rare cases of biallelic mutations. Ricciardone et al. (73) first reported three siblings that appeared to have neurofibromatosis (NF1) accompanied by leukemia or lymphoma in infancy, associated with biallelic germline mutations in *MLH1*. Their parents were first cousins. Gallinger et al. (74) described a similar family from another geographical area with a different *MLH1* mutation. An 11-year-old boy had *café-au-lait* macules and developed metastatic duodenal adenocarcinoma (complicating a tubulovillous adenoma). His 9-year-old sister, also with *café-au-lait* spots and axillary freckling, presented with malignant colonic polyps. Their 6-year-old sister manifested *café-au-lait* macules, hairy nevi, and a plexiform neurofibroma of the tongue, but had not yet developed internal malignancies. While AC-I criteria were not met, two relatives in their 60s had gastric cancer and CRC. The parents of these children are first cousins, but remain cancer free. The duodenal cancer and malignant colon polyps were MSI-H but retained *MLH1*, *MSH2*, and *MSH6* proteins by IHC. (*PMS2* IHC was not performed.) Such cases, while rare, pose one situation in which reliance upon IHC as a surrogate for MSI may be unwarranted.

Several groups have attempted to put a novel name on the disease caused by biallelic mutations in DNA MMR genes whose deficiency targets the NF genes. Bandipalliam (75) summarized this clinical entity, emphasized the features of colon tumors, leukemia/lymphoma, and features of NF, and coined the term ‘CoLoN.’ Felton et al. (76) suggested the term ‘Lynch syndrome III,’ recalling the terms Lynch syndrome I and II that were proposed in 1984 (77). Wimmer and Etzler (78) recently reviewed 35 reports of phenotypes in patients with biallelic mutations in one of the MMR genes, noting cancer in childhood, hematological malignancies, and *café au lait* spots, and suggested the term ‘constitutional mismatch repair-deficiency (CMMR-D) syndrome.’ None of these terms has come into general usage.

### Recurrent and founder mutations

Mutations that are widespread can be recurrent, i.e. occurring repeatedly *de novo*, or ancestral (founder mutations), having occurred once and passed on to succeeding generations. An important aspect of recurrent and founder mutations is that testing for them as a first step may lower the cost of molecular diagnosis in appropriate populations.

The best-known recurrent mutation causing LS is an A → T transversion in the donor splice site of intron 5 of *MSH2* (designation c.942 + 3A → T) (79,80). It occurs world-wide (81) and may account for as much as 5–10% of all LS (82). In Newfoundland it also occurs as a founder mutation, accounting for 20–25% of all LS, having been introduced by a settler more than 300 years ago (83).

In Finns, a genomic deletion of exon 16 of *MLH1* probably dates back 1000 years or more and in parts of Finland it accounts for > 50% of all LS (84,85). Another highly enriched founder mutation has been documented in Ashkenazi Jews (*MSH2* 1906G → C; accounting for ~20% of LS (86)) (for review see de la Chapelle (87)).

A founder mutation in the heterogeneous general United States population was not expected. In fact, one such mutation has been uncovered (88,89), a genomic deletion of exons 1–6 of

*MSH2*. It had remained undiscovered because initially it could not be detected by standard sequencing methods. The sequence of the breakpoints of this exon 1-6 deletion and the haplotypes surrounding the mutation were identical in kindreds spread over 14 states (88,90, 91), suggesting a common origin of this mutation, termed the American Founder Mutation (AFM). Clendenning et al. (92) subsequently estimated that large deletions, including the AFM, account for up to 20% of all deleterious deletions in *MSH2*. A robust multiplex PCR aided in identifying 32 families carrying the AFM. Testing of family members with a site-specific diagnostic PCR identified 126 carriers of the AFM. Genealogic studies then connected 27 of 41 AFM families into seven extended pedigrees, which were then traced back to the 18th century. (92)

### Risk modifiers: an example

Wijnen et al. (93) have shown that genome-wide association studies have identified common low-risk variants impacting CRC. These investigators genotyped these variants in 675 individuals from the Dutch Lynch Syndrome Registry who were known carriers of LS-associated mutations. They genotyped 8q24.21, 8q23.3, 10p14, 11q23.1, 15q13.3, and 18q21.1. Results disclosed a significant association between CRC risk in these LS mutation carriers and the single nucleotide polymorphisms (SNPs) rs16892766 on chromosome 8q23.3 and rs3802842 on chromosome 11q23.1. They concluded that the two loci which they identified may be helpful in identifying LS family members who require more intensive surveillance, specifically for CRC. The possibility exists that the presence of one of these identified low-risk variants may slightly increase the risk of CRC for members of LS families who are negative for an MMR mutation.

### Clinical issues

If detection of LS is possible clinically and amenable to confirmation by genetic testing, what is the physician's responsibility for translating this molecular genetic knowledge into patient care? The answer is it may be quite difficult, given the rapid pace of molecular genetic discoveries during the past two decades. Reliance upon genetic counselors may help bridge the knowledge gap (8) and provide patients the in-depth counseling and educational services the busy clinician may have difficulty providing. Some of the issues that follow fall more strictly within the clinician's purview.

### CRC survival and LS

Improved survival with CRC has long been considered a feature of LS, though methodologic problems left the actuality of a survival advantage in question for some time. Watson et al. (94) compared CRC stage and survival in a retrospective cohort of LS cases with an unselected hospital series of patients with sporadic CRC. Compared with the unselected series, the LS family members had lower stage disease and less distant metastases at diagnosis. After adjusting for their younger age at diagnosis, the LS patients had a significant survival advantage over the unselected series, with a hazard ratio of 0.67 ( $p < 0.0012$ ). The basis for such a survival advantage remains elusive; this advantage is also seen in CRC patients with MSI that is not due to LS (29,31,58). Investigation continues regarding survival characteristics of extracolonic LS cancers. Contrary to the improved survival in CRC, Crijnen et al. (95) showed no 5-year survival rate difference between ovarian cancer patients with LS and those with sporadic ovarian cancer.

### Defective DNA MMR and adjuvant chemotherapy for CRC

The DNA MMR system was initially discovered in microorganisms, and the genes were given the designations 'Mut S' and 'Mut H' etc., because when inactivated, they conferred a 'mutator' ('mut') phenotype to the bacteria. More importantly, loss of DNA MMR activity

rendered the organisms relatively resistant to the effect of toxic compounds that damaged DNA, such as the alkylating agents. Thus, when the DNA MMR genes were found to be involved in the pathogenesis of CRCs with the MSI-H phenotype, it was speculated that these tumors might also be relatively resistant to the effects of chemotherapeutic agents that act by damaging DNA (96).

The first test of this hypothesis in human CRC occurred when the missing DNA MMR gene was restored to a DNA MMR-deficient cell line (HCT116) by stable chromosome transfer; the resulting cell line was resistant to the cytotoxic effects of the alkylating agent N-methyl-N-nitro-N-nitrosoguanidine (MNNG) (97). Subsequent experiments demonstrated that DNA MMR deficiency abrogated the G2/M cell cycle checkpoint, and that DNA MMR activity was involved in the cytotoxic response to DNA damage, either through DNA alkylation, or incorporation of 6-thioguanine (98,99). Moreover, resistance was found to a number of commonly used chemotherapeutic agents, including cisplatin (100) and (unexpectedly) 5-fluorouracil (5-FU) (101).

This finding raised the question of whether patients with DNA MMR-deficient tumors might not have the expected response to adjuvant chemotherapy. It was known that only a minority of CRCs actually respond to 5-FU-based regimens, and it was speculated that DNA MMR-deficiency might be a marker of a poor response. The first paper on the subject did not use prospectively randomized patients; rather, the patients were selected for adjuvant chemotherapy on the basis of younger age and better clinical status. Consequently, the least ill patients were given treatment, and the older, sicker ones were used as controls, which was an inappropriate study design. In this first retrospective study, the patients with MSI had better outcomes, and it appeared that they were preferred candidates for 5-FU-based chemotherapy (102). However, this erroneous conclusion reflected patient selection, rather than the utility of chemotherapy for CRCs with MSI. When confirmation of this observation was sought using patients who had been prospectively randomized into adjuvant chemotherapy or observation (or placebo) groups, most studies have demonstrated either no benefit from the treatment (103–106), or a two- to threefold increase in mortality, for stage III and stage II cancers, respectively (59). Thus, both the *in vitro* predictions and the empirical observations support the conclusion that patients with LS, or with the acquired form of MSI due to methylation-induced silencing of *MLH1*, should not be offered adjuvant chemotherapy with a 5-FU based regimen. Whether other drug modifications in adjuvant chemotherapeutic regimens might result in a beneficial outcome remains to be demonstrated. There is *in vitro* evidence that some drugs may be cytotoxic to CRC cells with DNA MMR defects (107), and preliminary evidence in patients with advanced CRC suggests that this may be of benefit (108).

### Pathology of LS CRCs

CRCs which arise in the MSI pathway, regardless of whether they are hereditary or sporadic, show characteristic light-microscopic findings. CRCs in LS frequently show mucinous or signet ring cell morphology (109). Medullary carcinoma has been described as a subtype by the World Health Organization classification, and appears almost exclusively in those tumors with MSI-H (110). It is common in MSI-H tumors to find a host lymphoid response characterized by lymphoid aggregates at the periphery of the tumor and/or lymphocytes infiltrating the tumor (111). When pathologic suspicion is high for the possibility of an MSI-H tumor, immunohistochemical stains for the protein products of the DNA MMR genes can be informative (112), as discussed above.

Available data can be summarized by stating that a characteristic pathology exists for LS CRC. This appearance, alone or in combination with easily identified clinical considerations such as age, tumor multiplicity, and family history, make a case for pathologist-initiated MSI and/or IHC testing. A plan for further evaluation of MSI/IHC informative cases for evidence of



hypermethylation and *BRAF* mutations appears to be a requirement for developing a comprehensive clinical strategy.

### Colon surveillance

Järvinen et al. (113) first suggested a benefit for colonoscopic screening in LS through an observational trial extending over 15 years. The incidence of CRC was compared in two cohorts of at-risk members of 22 families. CRC developed in 8 screened subjects (6%), compared with 19 nonscreened relatives (16%;  $p = 0.014$ ), a 62% reduction. All CRCs in the screened group were localized, causing no deaths, compared with 9 CRC-related deaths in those who declined colonoscopy (this was a nonrandomized, noncontrolled study). Vasen et al. (114) found five interval cancers in LS patients within 3.5 years following a negative colonoscopy. The relatively high incidence of CRC even in the screened subjects argued for shorter screening intervals, e.g. 1–2 years.

In a follow-up to the early Finnish screening experience, Mecklin et al. (115) assessed the cumulative risk for development of colorectal adenoma or carcinoma in prospective colonoscopic surveillance of 420 LS mutation carriers without previous CRC. Cumulative risk of adenoma by age 60 was approximately 68% in males and 48% in females. Cumulative risk of CRC by age 60 at surveillance intervals of 2–3 years was 35% in males and 22% in females. Approximately half of the adenomas were located proximal to the splenic flexure. In relation to historical cancer rates, removal of adenomas clearly appears to decrease risk of CRC. Notably, although surveillance colonoscopy at intervals of 2–3 years was still associated with a fairly high rate of interval CRCs, there was no CRC-associated mortality when surveillance recommendations were followed.

In another series, Hurlstone et al. (116) note the typicality of flat and diminutive adenomas in the proximal colon, with a high risk of malignant transformation. Various techniques have been employed to improve the yield of such small, flat lesions. Chromoendoscopy with indigo carmine or methylene blue has been employed and narrow band imaging (NBI) has emerged, but have yet to gain truly widespread use. Hurlstone et al. (116) studied 25 asymptomatic AC-I positive patients who underwent a pair of tandem colonoscopies. This included conventional colonoscopy using targeted chromoscopy followed by pan-colonic chromoscopic colonoscopy. Compared with conventional colonoscopy, pan-chromoscopy identified significantly more pedunculated adenomas ( $p = 0.001$ ) and flat adenomas ( $p = 0.004$ ), supporting the notion that pan-colonic chromoscopy improves detection of significant neoplasia.

In order to test the efficacy of a screening program in the Netherlands, de Jong et al. (117) compared CRC mortality rates before and after 1990. Their study showed a 70% decrease in CRC mortality following the advent of more widespread colonoscopy screening after this arbitrarily chosen date.

Rijcken et al. (118,119) studied 100 HNPCC adenomas (25 from individuals with LS-associated MMR mutations, and 75 from individuals chosen from AC-I families) compared with 152 sporadic adenomas. Fifty percent of colonic adenomas in the HNPCC cases were found in the proximal colon, compared with 26% in sporadic cases. In addition, among familial cases, proximal adenomas progressed to high-grade dysplasia more commonly than distal adenomas, and were also more often highly dysplastic than larger distal adenomas ( $p < 0.001$ ). Adenomas in familial cases were smaller when compared with sporadic adenomas, and proximal familial adenomas  $\geq 5$  mm showed more severe dysplasia than larger proximal sporadic polyps.

Lecomte et al. (120) compared conventional colonoscopy with chromoendoscopy using indigo carmine dye sprayed onto the proximal colon. Chromoendoscopy significantly increased detection of adenomas in the proximal colon from 3/33 patients to 10/33 patients ( $p = 0.005$ ).

East et al. (121) performed conventional white-light colonoscopy followed by NBI in 62 LS surveillance patients. More flat adenomas vs pedunculated adenomas were found during the NBI colonoscopy (45% vs 12%).

Stoffel et al. (122) evaluated the colonic adenoma miss rate among LS patients undergoing colonoscopy. They compared the sensitivity of chromoendoscopy versus intensive inspection for identifying polyps missed by conventional colonoscopy. This included 54 subjects with LS who underwent tandem colonoscopies initially. All had a conventional colonoscopy with removal of all visualized polyps. The second colonoscopy randomly assigned subjects to pan-colonic indigo carmine chromoendoscopy or white light but 'intensive' (20 minute pull-back from cecum). The mean interval since last colonoscopy was 17.5 months. A total of 17 polyps (10 adenomas and 7 hyperplastic polyps) were identified during the standard colonoscopies, while 23 additional polyps (12 adenomas and 11 hyperplastic polyps) were identified in the second exams, yielding an adenoma miss rate of 55%. Furthermore, 15 polyps (5 adenomas and 10 hyperplastic polyps) were identified in those cases that had chromoendoscopy, while 8 polyps (7 adenomas and 1 hyperplastic polyp) were identified in those who had intensive white light inspection. After various adjustments, chromoendoscopy detected more polyps ( $p = 0.04$ ), but adenoma detection was not significantly different.

One can conclude from these studies that colonoscopy, early and at frequent intervals, is of value in detecting flat and rapidly progressive adenomas. Chromoendoscopy and related techniques such as NBI appear to improve the detection yield, but have not yet come to be regarded as the standard of care. It is not known whether the use of chromoendoscopy will eventually allow longer surveillance intervals.

### Extracolonic screening and management

Women who carry a germline mutation for LS have a 20–60% risk for endometrial cancer (123,124), and should have annual endometrial screening beginning at age 30 to 35 years, which should comprise endometrial aspiration and transvaginal ultrasound; however, we lack evidence-based data showing survival benefit from such screening. Prophylactic hysterectomy and oophorectomy can be considered when childbearing is completed. Schmeler et al. (125) have shown a significant reduction in endometrial and ovarian cancer among those patients with LS who underwent prophylactic surgery vs those who did not have surgical prophylaxis.

It is recommended that LS mutation carriers with a family history of ureter/renal pelvis cancer and/or hematuria should have annual ultrasound and urinalysis with cytologic examination, beginning at age 30 or at first evidence of hematuria. Periodic upper endoscopy is recommended for mutation carriers in families with gastric or small bowel cancer, as well as those of oriental origin in which gastric cancer is more common. Evidence-based data showing survival advantage for urologic, gastric, and small bowel screening are not available, but we consider these recommendations prudent.

### Genetic counseling

Genetic counseling is a process that provides the patient and extended family important details about genetic risk for cancer of specific anatomic sites (126,127). Education may be efficiently provided to multiple family members in a family information session (126), but counseling itself is ideally accomplished by a personal one-on-one discussion. Informed consent to undergo genetic testing implies the communication of a number of specific elements, and Offit

has nicely summarized these (7). Certainly, the well-informed clinician could take the time to counsel the patient on each of these elements. However, an appropriately detailed approach typically requires at least 60–90 minutes. We have found that the use of genetic counselors experienced in the counseling of LS family members enables the clinician to use his or her time more effectively, and to focus on clinical management issues.

## Non-LS familial CRC

### ***CHEK2* 1100delC mutation and HNPCC-related families**

The *CHEK2* gene 1100delC pathogenic variant has a role as a susceptibility allele in predisposing to breast cancer. Wasielewski et al. (128) reported Dutch *CHEK2* 1100delC families that include CRC cases. FAP cases did not show evidence of *CHEK2* 1100delC; but it was identified in 10 of 237 (4.2%) HNPCC/HNPCC-related cases, significantly higher than the 1.0% population frequency. Out of 10 *CHEK2* 1100delC CRC cases, 9 fulfilled the AC-II or the Bethesda Guidelines.

A *CHEK2* I157T variant has also been associated with CRC risk in the Finnish and Polish populations (129–131), indicating that the CRC risk of *CHEK2* is not limited to the 1100delC variant. *CHEK2* variants may account for a fraction of familial CRC fulfilling AC-I in the absence of LS-associated MMR gene mutations, i.e. familial CRC type X. A variety of low-penetrance alleles have been associated with increased risk of CRC, but to date none have really been found to account for familial or ‘syndromic’ CRC. Thus, although there is an increase in CRC among some families with the *CHEK2* 1100delC mutation (129), there is insufficient evidence at this time that this mutation accounts for obvious syndromic CRC (132). This is an area that requires additional study and clarification.

### **BRAF V600E mutation and Lynch syndrome**

Although MSI characterizes LS, the earliest studies of CRC in unselected populations found evidence of MSI in 15% of the cases, and these often involved older, family history negative patients (133). Such cases always involved inactivation of *MLH1* and did not have evidence of germline mutations in this gene. It was subsequently shown that most of the MSI CRCs had an epigenetic basis (53). Bessa et al. (134) and others have confirmed that apparently sporadic CRCs with MSI frequently have acquired hypermethylation of the promoter region of *MLH1*. Cases with evidence of *MLH1* promoter hypermethylation have also been found to commonly carry a V600E mutation in the *BRAF* gene. MSI (111 cases) and/or loss of protein expression (81 cases) was identified in 119 of 1222 CRCs. *BRAF* mutation was identified in 22 (18.5%), but was not identified in any patients who had an unambiguous MMR germline mutation (i.e. LS). It was concluded that exclusion of an LS diagnosis by detection of the *BRAF* V600E mutation simplified and improved the cost effectiveness of genetic testing, particularly in cases where the family history was either incomplete or unknown.

*BRAF* and *KRAS* participate in the same proliferation-supporting signaling pathway, and one finds either *KRAS* or *BRAF* mutations – but not both – in most CRCs. *BRAF* mutations are closely linked with non-LS, sporadic MSI CRCs, whereas *KRAS* mutations are seen in LS CRCs. Sporadic MSI CRCs are a consequence of the CpG island methylator phenotype, with consequent methylation of the *MLH1* gene. LS CRCs have a methylator phenotype as well, but it is defined by methylation of a different set of targets, and activating mutations in *KRAS*. These two observations illustrate how different, and perhaps parallel, mechanisms are operating to stimulate rapid growth in CRCs with MSI (135).

A key question, therefore, surrounds the role for methylation testing and *BRAF* V600E mutation testing in cases of MSI. The lower the clinical suspicion for LS, the more likely a given case will have sporadic, methylation/*BRAF*-related MSI. This underscores the

importance of carefully thinking about the prior probability of LS when selecting cases for MSI testing in the first place. As Hampel's study (82) points out, a population-based assessment of MSI that is intended to find cases of LS needs to include a process for unmasking cases of sporadic MSI, a process that may include methylation assays.

### Familial CRC type X

As previously mentioned, one occasionally encounters families that fulfill the AC-I, but in which there is neither evidence of MSI nor detection of MMR germline mutations. This phenomenon has been referred to by Lindor et al. (49) as 'familial colorectal cancer type X,' and makes up 40% of the AC-I positive kindreds. Such families have also been confirmed by Llor et al. (136) Perhaps not surprisingly, these families manifest later age of cancer onset and fewer CRCs when compared with LS, and no excess of extracolonic cancers. Along with the features described by Lindor et al. (49) others (136–138) have reported that these patients presented with mostly left-sided CRCs lacking a lymphocytic infiltrate.

### Privacy, informed consent, and protection

Among the barriers to delivery of effective genetic counseling and testing is the fear of discrimination at the level of life and health insurance, as well as employment. Although no objective basis for this has ever really emerged, fear is not usually an emotion based on rationality and may serve as a bar to action. For this reason, state and Federal efforts to provide legislative reassurance have been undertaken.

Hudson et al. (139) discuss the federal Genetic Information Nondiscrimination Act (GINA) signed into U.S. law in 2008. It is important to note that GINA addresses only employment concerns and health insurance, excluding life insurance, disability insurance, and long-term-care insurance. Importantly, it prohibits health insurers from using an individual's genetic information for determining eligibility or premiums; in addition, an insurer is prohibited from requesting or requiring that a person undergo a genetic test.

GINA is timely, given the fact that genomic information has grown exponentially. It is hoped that GINA and the Health Insurance Portability and Accountability Act (HIPAA) will relieve fears and anxieties of patients contemplating DNA mutation testing. We remain skeptical that the more distrustful patients will be reassured, even by Federal legislation, at least in the short term, and have already experienced patients refusing testing due to discrimination fears.

Offit and Thom (140) and Offit et al. (141) address 'The Duty to Warn' relatives of mutation carriers regarding their risk. Cases from New Jersey and Florida have arrived at opposing views on this duty to warn vs a duty to maintain confidentiality, and the issue remains quite unsettled in other states.

It has been suggested that direct marketing of genetic testing to patients and providers may be justified on the grounds of inadequate access to genetic counselors, with a number of reasons that may be advanced: (i) lack of cancer-oriented counselors within a reasonable geographic distance of the provider and patient, (ii) inability of counselors to effectively bill for their time, and (iii) fear of loss of patients through referral to potentially competing academic centers. 'Direct-to-consumer' advertising has already been conducted for *BRCA* assessment and can be anticipated for MMR mutations as well. Without commenting on whether this is a good or bad practice, the reality is that providers will need to find some way to accomplish the basic elements of the counseling process, whether or not a genetic counselor is formally involved in the process.

## Conclusion

It is now possible, in the clinical setting, to identify CRC patients likely to have LS, and to substantially confirm the diagnosis by clinically available testing of the tumor for MSI and/or IHC abnormalities. Genetic testing, while still suboptimally sensitive for detecting mutations in all likely LS cases, carries a ~70% likelihood of detecting deleterious mutations in one of the four implicated MMR genes. This enables predictive testing of at-risk relatives and targeted surveillance in those found to be carriers.

Realistically, most patients with LS currently are not evaluated at all for the available diagnostic endpoints and many continue to receive incomplete genetic counseling and treatment. Limited colon resection followed by second and third primary CRCs due to follow-up that is not sufficiently aggressive, and/or the development of extracolonic tumors, remain significant problems. Their at-risk relatives continue to receive haphazard screening which is empirically based on 'positive family history' instead of being based on sound diagnostic grounds or genetic testing. Primary care providers and many surgical and endoscopic specialists seem to still be intimidated by the process of working up patients with early-onset CRC and/or a family history of cancer. While this seems to be gradually changing, perhaps fostered by very pointed clinical practice guidelines as well as by wider availability and marketing of both tumor and germline testing, there is still a place for expert consultation that includes genetic counseling. Our hope is that some of the technical advances outlined in this review will actually make it easier to diagnose and treat LS patients and their families.

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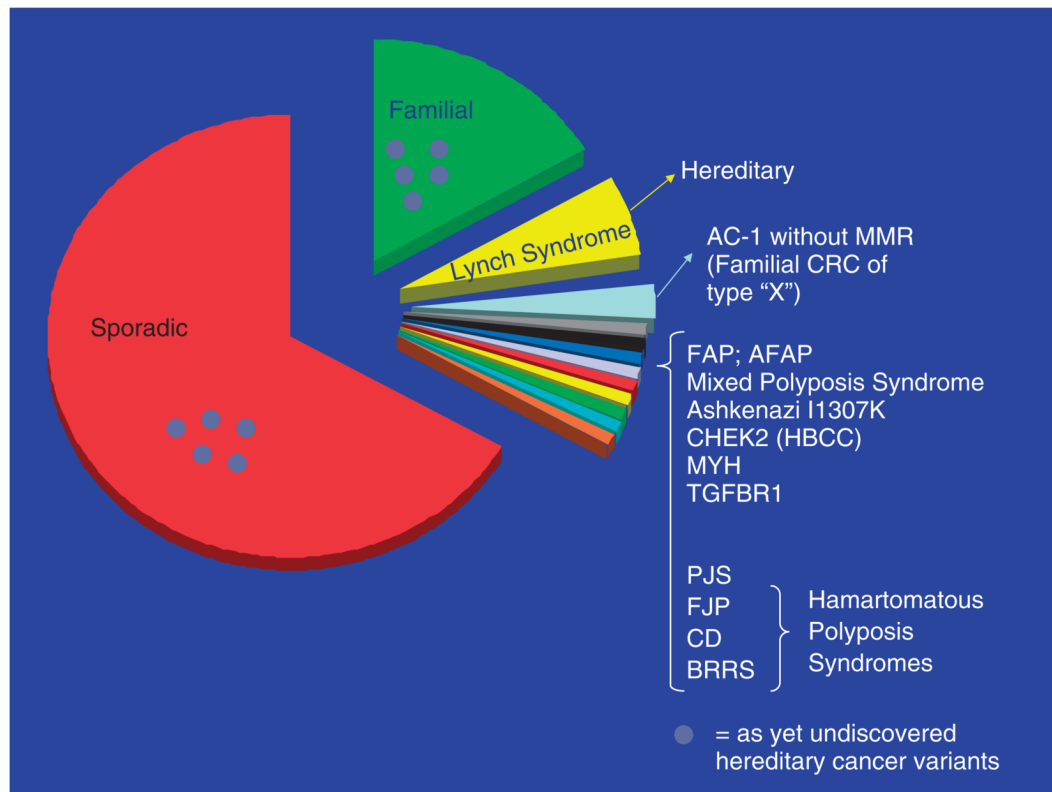


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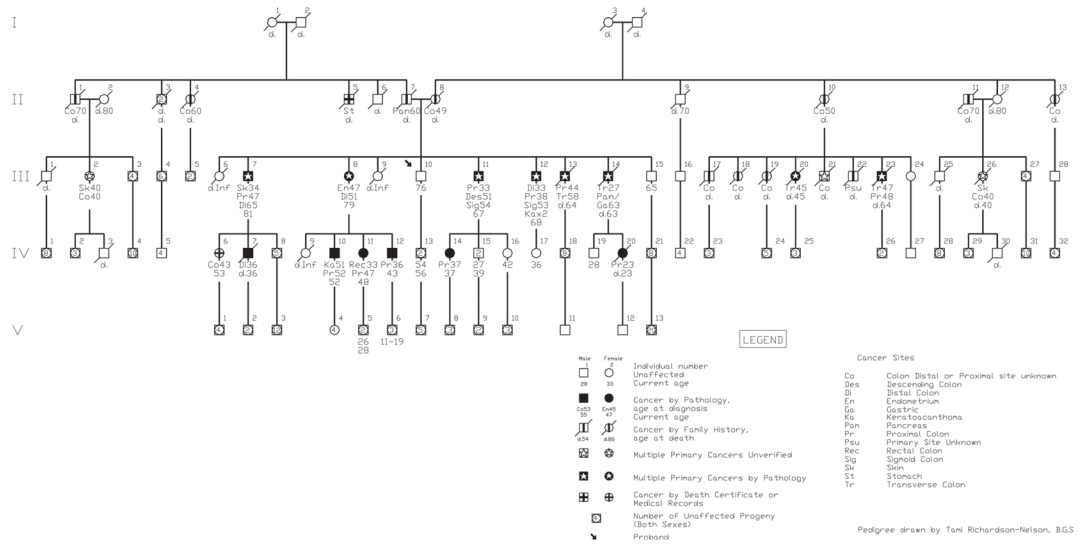
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**Fig. 1.** Circle graph depicting the marked genotypic and phenotypic heterogeneity in hereditary colorectal cancer syndromes. AC-I, Amsterdam Criteria I; MMR, mismatch repair; FAP, familial adenomatous polyposis; AFAP, attenuated familial adenomatous polyposis; HBCC, hereditary breast and colorectal cancer; PJS, Peutz–Jeghers syndrome; FJP, familial juvenile polyposis; CD, Cowden’s disease; BRRS, Bannayan–Ruvalcaba–Riley syndrome. (Revised with permission from Lynch et al. *Cancer* 2004;100:53–64).



**Fig. 2.** Pedigree of a classical Lynch syndrome (LS) family showing colorectal cancer (CRC) as well as extracolonic cancers integral to the syndrome.

Table 1

Incidence and cancer risks for known polyposis syndromes

Syndrome	MIM No.*	Gene(s)	Patients meeting clinical criteria with mutation, %	Population incidence	Cancer risks
Familial adenomatous polyposis (FAP)	175000	<i>APC</i>	90	1/5000	Colorectal, duodenal, papillary thyroid, pancreatic, hepatoblastoma, CNS tumors
MYH-adenomatous polyposis	608456	<i>MYH</i>	Unknown	1/5000	Colorectal tumors, other?
Hyperplastic polyposis syndrome		NA	NA	1/100,000	Colorectal tumors, other?
Hereditary mixed polyposis syndrome	601228	<i>CRAC1</i>	NA	NA	Colorectal tumors, other?
Juvenile polyposis/hemorrhagic telangiectasia syndrome	175050	<i>SMAD4</i>	20–40	1/100,000	Colorectal, gastric, duodenal, pancreatic tumors
Juvenile polyposis syndrome	174900	<i>BMPRIA</i>	20–40	1/100,000	Colorectal, gastric, duodenal, pancreatic tumors
Cowden syndrome	158350	<i>PTEN</i>	80–85	1/200,000	Breast, thyroid, uterine, melanoma, renal cell tumors
Peutz-Jeghers syndrome	175200	<i>STK11</i>	50	1/30,000–1/100,000	Colorectal, small intestine, stomach, breast, pancreatic, sex-cord tumors
Birt-Hogg-Dube syndrome	135150	<i>BHD</i>	50–70	1/200,000	Renal tumors, other?

CNS, central nervous system; MIM, *Mendelian Inheritance in Man*; NA, not available.\* From *Online Mendelian Inheritance in Man* (142).Republished by permission from Sweet et al. Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. *JAMA* 294(19):2465–2473, November 16, 2005. Copyright © 2005 by the American Medical Association. All rights reserved.

**Table 2**

## Cardinal features of Lynch syndrome (LS)

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<ul style="list-style-type: none"> <li>• Autosomal dominant inheritance pattern seen for syndrome cancers in the family pedigree</li> <li>• Earlier average age of colorectal cancer (CRC) onset than in the general population Average age of 45 years in LS vs 69 years in the general population</li> <li>• Proximal (right-sided) colonic cancer predilection ~70% of LS CRCs are proximal to the splenic flexure</li> <li>• Accelerated carcinogenesis (tiny adenomas can develop into carcinomas more quickly) Within 2–3 years in LS vs 8–10 years in the general population</li> <li>• High risk of additional CRCs 25–30% of patients having surgery for a LS-associated CRC will have a second primary CRC within 10 years of surgical resection if the surgery was less than a subtotal colectomy</li> <li>• Increased risk for malignancy at certain extracolonic sites (143,144) <ul style="list-style-type: none"> <li>– Endometrium (40–60% lifetime risk for female mutation carriers)</li> <li>– Ovary (12–15% lifetime risk for female mutation carriers)</li> <li>– Stomach (higher risk in families indigenous to the Orient, reason unknown at this time)</li> <li>– Small bowel</li> <li>– Hepatobiliary tract</li> <li>– Pancreas</li> <li>– Upper uro-epithelial tract (transitional cell carcinoma of the ureter and renal pelvis), especially in males with LS-<i>MSH2</i> type (143)</li> <li>– Brain (in the Turcot's syndrome variant of the LS)</li> <li>– Multiple sebaceous adenomas, sebaceous carcinomas, and keratoacanthomas in the Muir-Torre syndrome variant of LS</li> </ul> </li> <li>• Pathology of CRCs is more often poorly differentiated, with an excess of mucoid and signet-cell features, a Crohn's-like reaction, and a significant excess of tumor-infiltrating lymphocytes within the tumor</li> <li>• Increased survival from CRC</li> <li>• The <i>sine qua non</i> for diagnosis is the identification of a germline mutation in a mismatch repair gene (<i>MLH1</i>, <i>MSH2</i>, <i>MSH6</i>, or <i>PMS2</i>) that segregates in the family: i.e., members who carry the mutation show a much higher rate of syndrome-related cancers than those who do not carry the mutation</li> </ul>
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**Table 3**

## Amsterdam Criteria I and II (AC-I and II) and Bethesda Guidelines

**AC-I (24)**

- At least three relatives with histologically verified colorectal cancer:
  1. One is a first-degree relative of the other two;
  2. At least two successive generations affected;
  3. At least one of the relatives with colorectal cancer diagnosed at <50 years of age;
  4. Familial adenomatous polyposis (FAP) has been excluded.

**AC-II (25)**

- At least three relatives with an hereditary nonpolyposis colorectal cancer (HNPCC)-associated cancer [colorectal cancer, endometrial, stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, and skin (sebaceous tumors)]:
  1. One is a first-degree relative of the other two;
  2. At least two successive generations affected;
  3. At least one of the syndrome-associated cancers should be diagnosed at <50 years of age;
  4. FAP should be excluded in any colorectal cancer cases;
  5. Tumors should be verified whenever possible.

## Bethesda Guidelines for testing of colorectal tumors for microsatellite instability (MSI) (145)

- 1 Colorectal cancer diagnosed in a patient who is <50 years of age.
- 2 Presence of synchronous or metachronous colorectal, or other syndrome-associated tumors<sup>a</sup> regardless of age.
- 3 Colorectal cancer with microsatellite instability-high (MSI-H)<sup>b</sup> histology<sup>c</sup> diagnosed in a patient who is <60 years of age<sup>d</sup>.
- 4 Colorectal cancer or syndrome-associated tumor<sup>a</sup> diagnosed under age 50 years in at least one first-degree relative<sup>e</sup>.
- 5 Colorectal cancer or syndrome-associated tumor<sup>a</sup> diagnosed at any age in two first- or second-degree relatives<sup>e</sup>.

<sup>a</sup> Syndrome-associated tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter or 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>b</sup> MSI-H = microsatellite instability-high in tumors refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.

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

<sup>d</sup> There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep <60 years of age in the guidelines.

<sup>e</sup> Criteria 4 and 5 have been reworded to clarify the Revised Bethesda Guidelines.