Cancer Predisposition Syndromes of the Gastrointestinal Tract

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Introduction

Cancer predisposition syndromes affecting the gastrointestinal (GI) tract represent a small proportion of GI cancers and may arise in the background of a polyposis syndrome (Table 7.1). The molecular mechanisms underlying these syndromes have been instrumental in our understanding of the molecular basis of development and progression of the more frequent counterpart sporadic neoplasms, sharing many common molecular features. Syndromic hereditary cancers can involve any segment of the GI tract but predominantly involve the colon, and the most common cancers are colorectal adenocarcinomas (CRC). The most frequent inheritable GI cancer syndromes are those associated with germline mutations in the DNA mismatch repair (MMR) genes, in which case cancers do not arise in a polyposis background, and those attributed to underlying germline mutations in the *APC* or *MYH* genes in patients who manifest an adenomatous polyposis phenotype in the intestine. In addition to the well-characterized cancer syndromes, there are families with clustering of colon cancer, including patients with colon cancers before age 50, for whom the susceptibility gene loci have not been identified.[1–3]

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Hereditary Non-Polyposis Colorectal Cancer–Lynch Syndrome

Clinical Features

Hereditary Non-Polyposis Colorectal Cancer, also known as Lynch syndrome (HNPCC/LS) is diagnosed on the basis of a germline mutation in one of the DNA MMR genes that results in deficient DNA mismatch repair (dMMR).[4–6] The deficiency of DNA mismatch repair results in increased mutation accumulation in the genome and higher risk of neoplastic transformation. HNPCC/LS represents an estimated 3–6% of all colorectal cancer cases.[4,7] HNPCC/LS is inherited as an autosomal dominant cancer predisposition syndrome, and is characterized by early onset of colorectal cancers, and increased frequency of cancers in the small bowel, stomach, biliary tract, pancreas, endometrium, ovary, urothelium (urinary bladder, ureter, and renal pelvis), brain, sebaceous gland adenomas, and keratoacanthomas.[5–9] The extra-gastrointestinal neoplasms associated with HNPCC/LS will be described in further detail later in this chapter. Sebaceous gland adenomas and keratoacanthomas are part of the Muir-Torre syndrome, whereas tumors of the brain, usually glioblastomas, occur in the Turcot syndrome.[5,9]

The average age of presentation of colorectal cancer in HNPCC patients is 45 years of age, but the disease can be discovered in older patients.[10,11] Tumors are located in the proximal colon in two-thirds of cases, but can occur in any segment of the gastrointestinal tract.[10,11] Tumors are often multiple or associated with other synchronous or metachronous neoplasms of the HNPCC-cancer spectrum.[5,7,9,10] HNPCC/LS patients are at higher lifetime risk for colorectal cancer than the general population, developing CRC in up to 80% and endometrial carcinoma in up to 60% of mutation carriers.[5]

Table 7.1 Polyposis Syndromes of the Gastrointestinal Tract[172,173,180,181,187]

Syndrome	Major clinical features	Risk of CRC cancer	Gene	Gene product function
Familial adenomatous polyposis	>100 colonic adenomas; duodenal and periampullary adenomas; gastric fundic gland polyps	100% lifetime risk for colorectal carcinoma (later age in AFAP)	APC	Growth inhibitory: targets beta-catenin for destruction
–Gardner syndrome	FAP and CHRPE, osteomas, desmoid tumors			
–Turcot syndrome	FAP and medulloblastoma			
–Attenuated FAP (AFAP)	>15 but <100 colonic adenomas; other FAP manifestations are less frequent			
MYH-associated polyposis	FAP-like presentation: colonic adenomas; but no APC mutation identifiable	Slight increase in risk of CRC	MYH	DNA damage repair
Peutz-Jeghers syndrome	Hamartomatous gastrointestinal polyps; predisposition for multiple extracolonic malignancies	GI and extra-GI malignancies (>90% risk of cancer by 65 years of age)	STK11	Serine/threonine kinase
Cowden syndrome	Hamartomatous gastrointestinal polyps: juvenile type polyps, ganglioneuromas, lymphoid hyperplasia, lipomas	Rare GI malignancy	PTEN	Protein phosphatase
BRR syndrome	Hamartomatous gastrointestinal polyps: juvenile-type polyps	Rare GI malignancy	PTEN	Protein phosphatase
Juvenile polyposis	Hamartomatous gastrointestinal polyposis	Increased risk for colorectal carcinoma	SMAD4 BMPR1A, ENG	TGF-beta signaling
Neurofibromatosis type I	Neurofibromas; ganglioneuromas; malignant peripheral nerve sheath tumors	N/A	NF1	Negative regulator of Ras oncogene
Multiple endocrine neoplasia type II	Neural polyps: ganglioneuromas, most common in colon and rectum	N/A	RET	Proto-oncogene

The polyposis syndromes listed in the table are inherited as Mendelian autosomal dominant disorders, except for MYH-associated polyposis, which is inherited as an autosomal recessive disease. Two-thirds of Turcot syndrome occur in patients with APC gene mutation and one-third in DNA mismatch repair gene mutation (LS). *CHRPE* Congenital hypertrophy of the retinal pigment epithelium, *BRR* Bannayan–Ruvalcaba–Riley syndrome

Pathologic Features

Unique histopathologic features may be seen in colorectal adenocarcinomas with underlying dMMR that suggest the possibility of HNPCC/LS (reviewed in Gologan et al[11,12]), (Fig. 7.1). These features are not specific for HNPCC/LS cancers, and are also seen frequently in sporadic CRC with dMMR, as well as in some tumors that are proficient in DNA MMR.[11,13] Three major histopathologic groups of dMMR colorectal cancers can be recognized (reviewed in Gologan
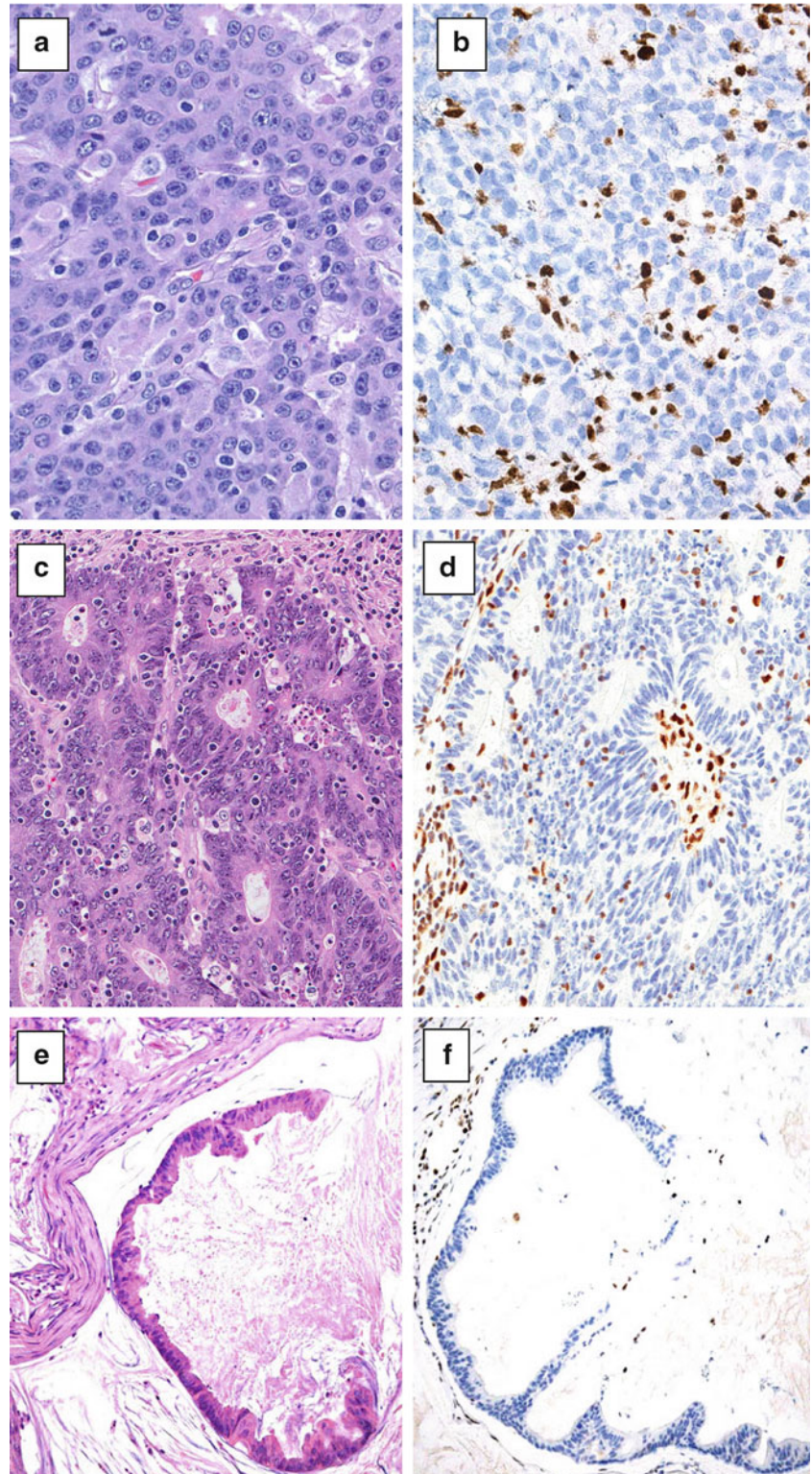

Fig. 7.1 (**a**) Poorly differentiated adenocarcinoma with medullary features, showing prominent TILs; (**b**) Immunohistochemistry for MLH1 shows loss of expression of the protein in tumor cell nuclei, whereas MLH1 remains positive in the nucleus of infiltrating lymphocytes and surrounding stromal cells. (**c**) Moderately differentiated CRC with prominent TILs. (**d**) Immunohistochemistry for MSH2 shows loss of expression in tumor cell nuclei, whereas MSH2 remains positive in the nucleus of infiltrating lymphocytes and surrounding stromal cells. (**e**) Mucinous adenocarcinoma (H&E). (**f**) Immunohistochemistry for MLH1 shows loss of expression in tumor cell nuclei. Original magnifications ×200

et al[12] (Fig. 7.1): (1) poorly differentiated adenocarcinomas, including medullary-type carcinomas, in which the neoplastic epithelium is infiltrated with high numbers of lymphocytes (tumor-infiltrating lymphocytes/TILs), and carcinomas with signet ring cell features; (2) mucinous adenocarcinomas, and (3) well to moderately differentiated adenocarcinomas. The presence of prominent intratumoral lymphocytes, with at least four lymphocytes infiltrating the tumor epithelium in a single 400× microscopic field,[11] is the most predictive histologic finding of dMMR in CRC (Fig. 7.1). TILs may be particularly numerous not only in poorly differentiated cancers but also occur in the other morphologic types of HNPCC/LS-associated cancers. The intratumoral lymphocytes are CD3-positive T cells and most are CD8-positive cytotoxic T lymphocytes. Peritumoral lymphocytic inflammation and lymphoid aggregates forming a Crohn-like reaction are also frequent in carcinomas with dMMR.[11,12,14–18]

Patients with HNPCC/LS typically develop a small number of colonic adenomas of traditional type (tubular or tubulovillous adenomas) at earlier age (mean 42–43, range 24–62 years) as compared to noncarriers of DNA mismatch repair gene mutations.[11,14–16,19–21] Progression from adenoma to invasive adenocarcinoma occurs rapidly, in many patients within less than 3 years, in contrast to an average of 15 years in patients without HNPCC/LS.[19,22] HNPCC/LS adenomas arise most frequently in the proximal colon and often contain high-grade dysplasia.[19,23]

Molecular Mechanisms of Cancer Development and Progression in HNPCC/LS

As introduced in Chap. 1 and further discussed in Chap. 8, deficiencies of DNA mismatch repair have been implicated in the development and progression of CRC in two contexts: (1) in HNPCC/LS patients with inhered DNA MMR gene mutations and (2) in patients with sporadic colorectal cancers, where deficient MMR is nearly always attributed to epigenetic silencing of the DNA MMR MLH1 gene.[24]

The two most frequent genes underlying MMR deficiency in HNPCC/LS are *MLH1* and *MSH2*. Although the reported proportions vary in different studies, HNPPC/LS patients inherit germline mutations affecting the coding regions of *MLH1* (approximately 40%), *MSH2* (approximately 40%), *MSH6* (approximately 10%), and *PMS2* (approximately 5%).[12,25–35] In addition to inherited germline mutations, germline epimutation of the promoter regions of *MLH1* or *MSH2* have been reported in rare cases of HNPCC/LS[35–37] (Table 7.1).

Deficient DNA MMR occurs when both alleles of one of the MMR genes are inactivated. During normal DNA replication, errors such as base mismatches and insertion or deletion loops, especially in repetitive regions, are corrected by the DNA mismatch repair proteins. MMR deficiency allows for replication-associated errors to be propagated in newly synthesized DNA strands (Fig. 7.2). In HNPCC/LS, pathogenic mutations of MMR genes are inherited and are present in constitutional DNA such as is found in peripheral blood; however, dMMR only develops in somatic tissues, especially in colonic epithelium and a few other cellular targets, when a second hit affects the MMR gene and leads to its loss of function. It has been shown that the second hits in HNPCC/LS can be caused by large or small chromosomal deletions or by mitotic recombination-mediated gene conversion, occurring in up to 46% of tumors.[3] Somatic methylation of the promoter of the wild-type allele occurs in some cases, affecting *MLH1* more often than *MSH2*.[3] Somatic point mutations are thought to be the least common second-hit mechanism.[3] The double allelic dMMR leads to a mutational phenotype that affects primarily repetitive nucleotides, known as microsatellite regions, with resulting microsatellite instability (MSI) seen in more than 90% of CRC cases in HNPCC/LS patients[10] (Fig. 7.3), as well as mutations in target genes that may contribute to neoplastic development.

The DNA MMR genes encode the MutS proteins MSH2, MSH3, and MSH6, and the MutL proteins MLH1, PMS1, PMS2, and MLH3[38,39] (Fig. 7.2). In the process of DNA MMR, the MMR proteins form heterodimers (MutS and MutL)[38,40–43] (Fig. 7.2). The MSH2 protein can interact with MSH6, forming the MutS-alpha complex, or with MSH3, forming the MutS-beta complex. MutS-alpha and MutS-beta heterodimers recognize and bind to post-DNA replication mismatched sequences. The MutS-alpha heterodimers are involved in the repair of single base mispairs and small insertion or deletion mispairs, whereas MutS-beta heterodimers primarily are involved in the correction of insertion or deletion mispairs.[44–46] MutL heterodimers bind MutS-alpha or MutS-beta.[38] Of the two possible MutL heterodimers, only MutL-alpha (MLH1 and PMS2) heterodimers are involved in DNA MMR, whereas the MutL-beta heterodimers (MLH1 and PMS1) do not appear to be significantly involved in DNA mismatch repair functions.[47,48] The MMR proteins are stabilized by heterodimerization, and loss of the heterodimer protein(s) may result in their degradation. Since MSH2 and MLH1 have two possible proteins to heterodimerize with (Fig. 7.2), their expression is only lost when their respective genes are primarily inactivated by a mutation or epigenetic silencing. However, MSH6 only heterodimerizes with MSH2, and PMS2 only heterodimerizes with MLH1; thus, loss of MSH6 expression can be secondary to primary loss of MSH2 and loss of PMS2 expression can be secondary to primary loss of MLH1 (Figs. 7.2, 7.4, and 7.5).

The molecular mechanisms that underlie the neoplastic development and progression in HNPCC/LS characterize the so-called microsatellite instability (MSI pathway).[32,49,51]

Fig. 7.2 DNA mismatch repair proteins and mechanisms of repair. MutS heterodimers (MSH2/MSH6 or MSH2/MSH3) recognize a DNA strand loop or base mismatch and recruit MutL (MLH1/PMS2) heterodimers to the site. Other molecules required for strand discrimination, helicase, endonuclease activity, resynthesis, and ligation are recruited in the complex with correction and maintenance of the native DNA sequence
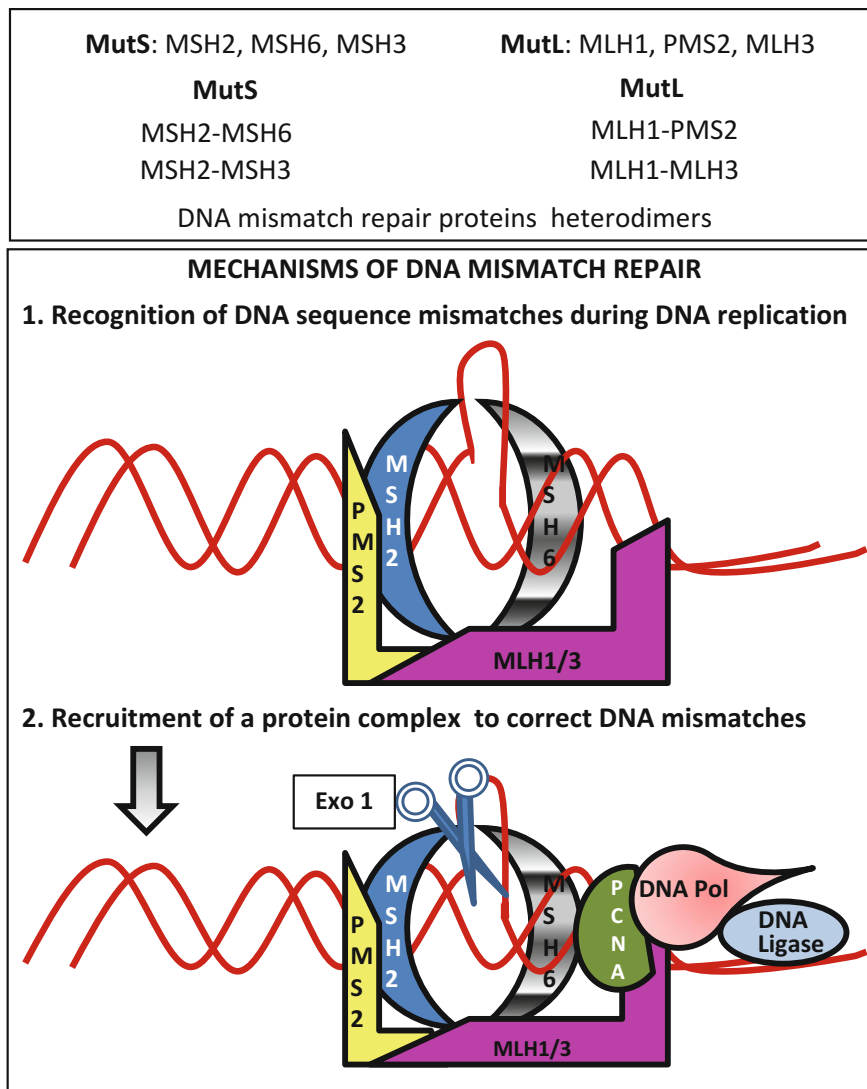
As in sporadic MSI-pathway carcinomas, dMMR may lead to accumulation of mutations in cancer-related genes, such as the TGF-beta receptor II, *BAX*, and *MSH6* genes among others, in early pre-neoplastic cell populations as well as during the steps of neoplastic progression.[52] In contrast to sporadic CRC, patients with germline mutations in DNA mismatch repair genes do not carry *BRAF* activating mutations in their tumors.[53] *BRAF* V600E activating mutations have been reported in about 5% of all CRCs, 4–12% of CRC without microsatellite instability (MSS tumors), 40–70% of sporadic CRC with microsatellite instability (MSI-H), but are not seen in HNPCC/LS CRCs which nearly always show MSI-H.[54–56] HNPCC/LS tumors, similar to sporadic CRCs with dMMR, show frequent aberrant nuclear beta-catenin, but aberrant p53 expression, 5q loss of heterozygosity, and *KRAS* mutations are uncommon.[11]

Criteria for Identification of HNPCC/ LS Patients and Molecular Testing Approaches

Historically, the first criteria to identify patients with HNPCC/LS, known as the Amsterdam criteria, were established in the early 1990s, with subsequent revisions.[10,57] Later, more inclusive criteria, known as the Bethesda guidelines were established by a consensus group.[5,35,58–60] According to Amsterdam II Criteria, patients are diagnosed with HNPCC when[10]:

1. The family includes three or more relatives with an HNPCC-associated cancer, verified by pathological examination and:
   (a) One affected patient is a first-degree relative of the other two
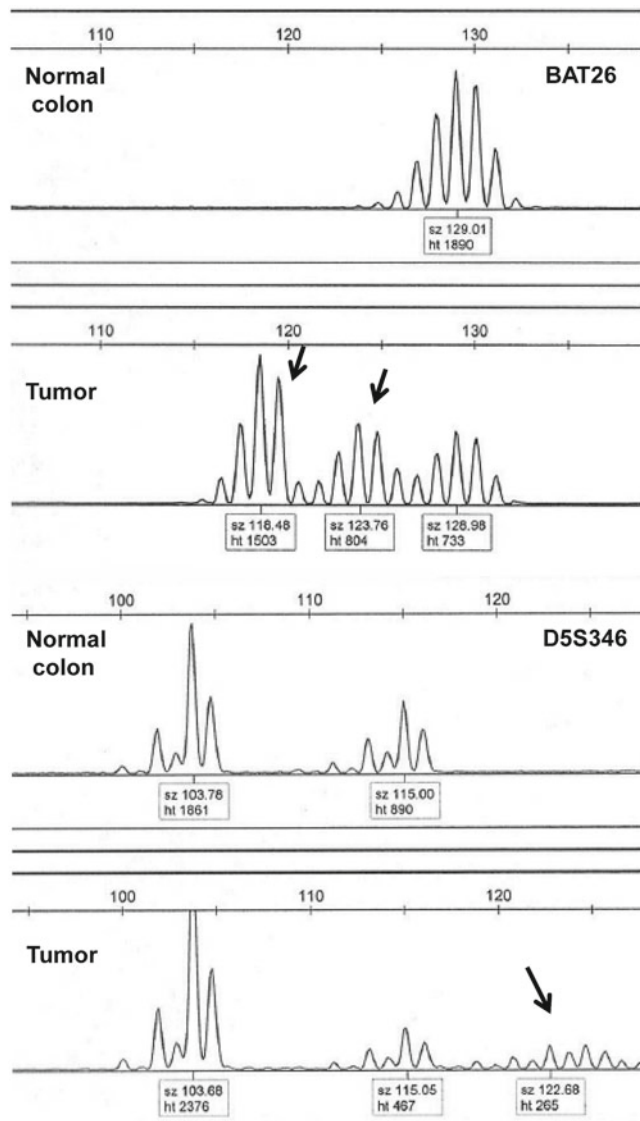   (b) two or more successive generations are affected

Fig. 7.3 Microsatellite instability detected by amplification of markers of the NCI panel. PCR amplification of the BAT26 locus reveals the appearance of novel alleles (*arrows*), of smaller size, in the tumor DNA as compared to non-neoplastic colonic DNA. The dinucleotide repeat marker D5S346 shows microsatellite instability characterized by a novel allele (*arrow*), of larger size, in the tumor DNA

(c) cancer in one or more affected relatives is diagnosed before the age of 50 years

(d) familial adenomatous polyposis is excluded.

2. Alternatively, patients meet one of the following modified Amsterdam criteria:

(a) Very small families can be considered to have HNPCC with only two colorectal cancers in first-degree relatives if at least 2 generations have cancer and at least one case of CRC was diagnosed by the age of 55 years

(b) In families with two first-degree relatives affected by colorectal cancer, the presence of a third relative with

an unusual early onset neoplasm or endometrial cancer is sufficient

(c) If an individual is diagnosed before the age of 40 years and does not have a family history that fulfills Amsterdam II or modified Amsterdam criteria, they are still considered as having HNPCC/LS.

The most recently revised Bethesda criteria recommend testing patients to rule out HNPCC/LS if there is one of the following criteria[5,35,59,60]:

1. Patient is diagnosed with colorectal cancer before the age of 50 years.

2. Patient has synchronous or metachronous colorectal cancer and another HNPCC-related tumor [stomach, urinary bladder, ureter and renal pelvis, biliary tract, brain (glioblastoma), sebaceous gland adenomas, keratoacanthomas, and small bowel], regardless of age.

3. Colorectal cancers with histopathologic features suggestive of dMMR–microsatellite instability: abundant tumor infiltrating lymphocytes, Crohn-like lymphocytic reaction, mucinous or signet ring cell differentiation, or medullary growth pattern of poorly differentiated adenocarcinoma, diagnosed before the age of 60 years.

4. Colorectal cancer patient with one or more first-degree relatives with CRC or other HNPCC-related tumors. One of the cancers must have been diagnosed before the age of 50 years.

5. Colorectal cancer patient with two or more relatives with colorectal cancer or other HNPCC-related tumors, regardless of age.

The revised Bethesda guidelines resulted in an increased identification of patients with dMMR as compared to previous guidelines, identifying not only more HNPCC/LS cancers but also more sporadic-type cancers with dMMR.[18] Given recent knowledge of the molecular changes underlying sporadic as compared to HNPCC-associated dMMR cancers, algorithms have been proposed to determine whether a patient has sporadic or HNPCC/LS cancers.[61] Alternatively, as discussed later, universal testing for dMMR of all CRC has been proposed.[3,62–64]

Molecular Testing for HNPCC/LS

The diagnostic workup for dMMR either due to HNPCC/LS or due to sporadic CRC is initiated by testing cancer tissue for DNA for microsatellite instability (MSI) (Fig. 7.3), the functional end point of dMMR, and/or by performing immunohistochemistry (IHC) of tumor tissues for MSH2, MLH1, MSH6, and PMS2 DNA mismatch repair proteins, to evaluate for their preserved (in DNA MMR-proficient tumors) or loss of expression (in deficient DNA MMR) tumor cell nuclei. Usually, CRC tissue is used for testing but other tumors of the HNPCC/LS spectrum, such as endometrial carcinoma, can be used.
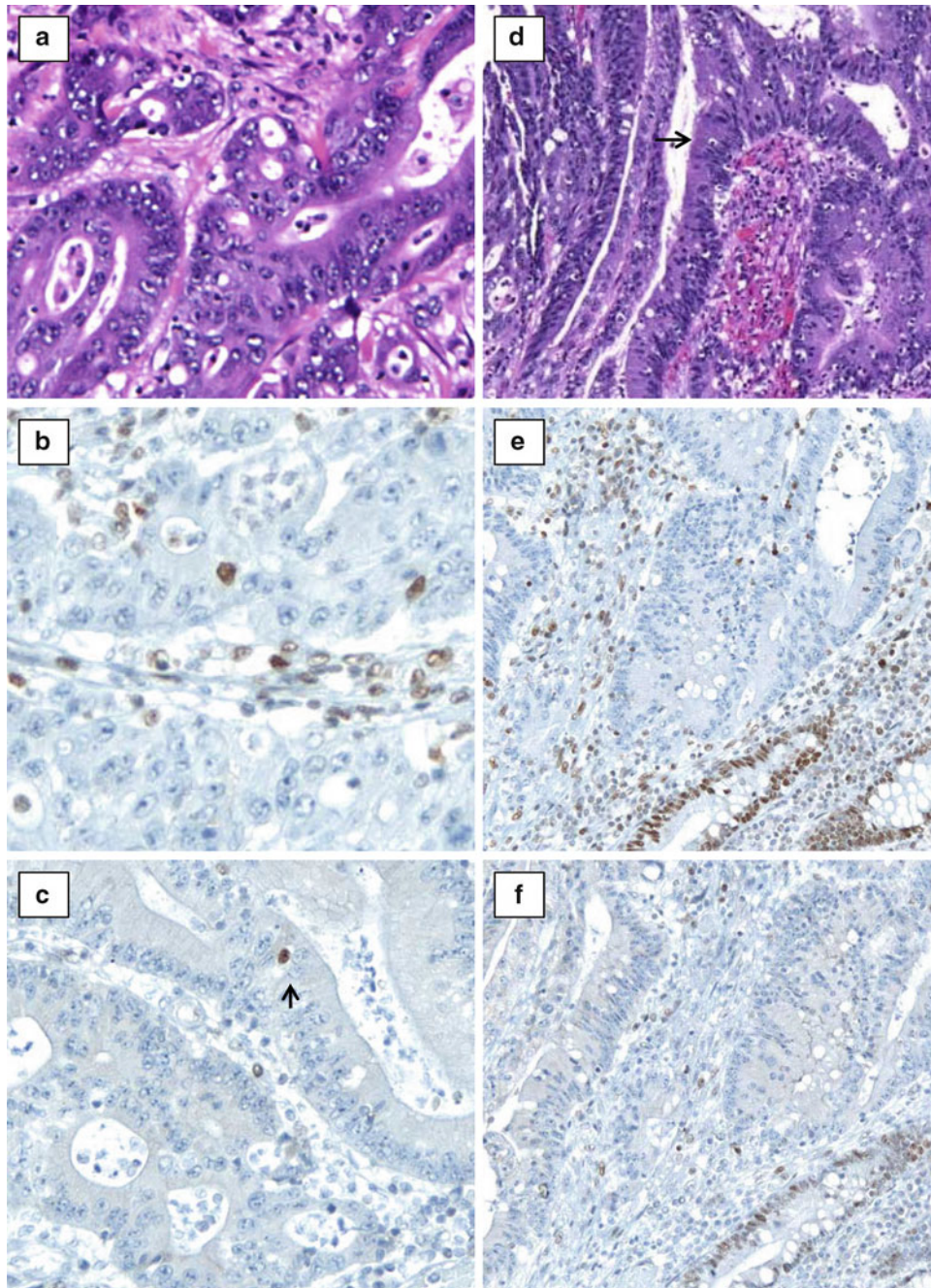
Fig. 7.4 Loss of expression of MLH1 (**b**) or MSH2 (**e**) is associated with loss of their MMR heterodimer proteins PMS2 (**c**) and MSH6 (**f**), respectively, exemplified in these two cases of moderately differentiated colonic adenocarcinoma. Note the prominent TILs in both cases, indicated by the *arrows* (**c** and **d**). Hematoxylin and Eosin stain (**a**) and (**b**).

MSI DNA Test

The DNA-based test to assess for microsatellite instability (MSI) in tumor cells is based on the evaluation of instability in small 100–200 base pair DNA segments that consist of repetitive nucleotides, called microsatellite regions or short tandem repeats (STRs). The nucleotide sequences within the repetitive elements of marker loci used in the MSI test are mononucleotide repeats of adenine $(A)_n$ or cytosine–adenine $(CA)_n$ dinucleotide repeats. During DNA replication these repetitive sequences may undergo variations in length due to DNA strand slippage, leading to increased or reduced length of STRs. In normal cells, proficient DNA MMR proteins are able to correct these replication-associated DNA sequence errors; however, in dMMR cells with loss of function of both
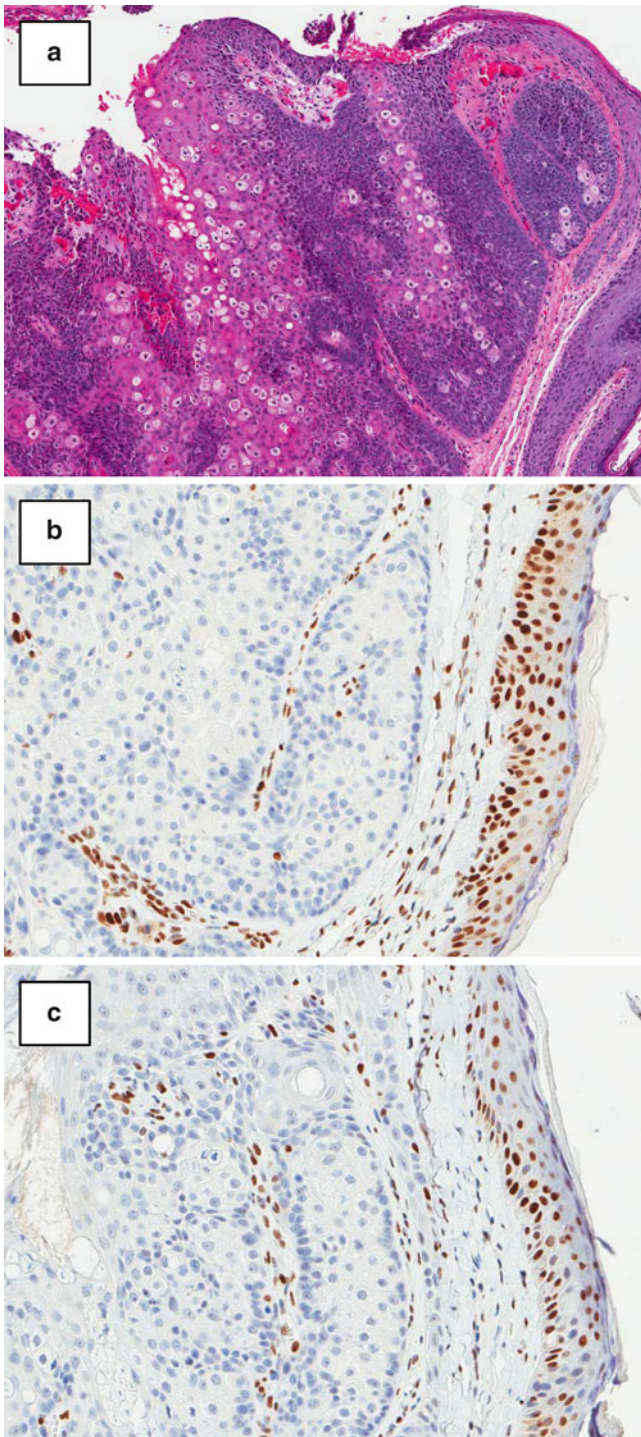
Fig. 7.5 Sebaceous adenoma in a patient with Muir-Torre syndrome (**a**) shows loss of expression of MSH2 (**b**) and MSH6 (**c**), suggestive of germline mutation in *MSH2* and secondary loss of MSH6 due to inability to form stabilizing heterodimers. (**a**) H&E stain, (**b** and **c**) immunohistochemistry

alleles, these mutations persist in the genome of daughter cells and in future cell generations in the tumor. The resulting changes in length of nucleotide repeats are known as microsatellite instability. Therefore, MSI can be detected in

cells with dMMR, as occurs in patients with HNPCC/LS and sporadic dMMR cancers, as these mutations are not repaired and persist in the DNA of tumor cells. Tissue sections from formalin-fixed tumor tissue used for routine pathologic diagnosis and embedded in paraffin (FFPE) are adequate for the MSI test. Tumor enrichment by micro or macro-dissection is recommended. One of the most used sets of microsatellite markers was recommended by a NCI consensus group and consists of 5 loci: 2 poly(A) mononucleotide repeat markers (BAT25 and BAT26) and 3 poly(CA) dinucleotide repeat markers (D2S123, D5S346, and D17S250).[35,58] The NCI panel of five microsatellite markers requires comparison of the tumor DNA profile with that of non-neoplastic/normal tissue, which can be obtained from non-neoplastic colonic mucosa and adjacent wall layers, away from the tumor. The results of the MSI test using the NCI panel are reported as MSI-high (MSI-H), MSI-low (MSI-L), or microsatellite stable (MSS). MSI-H tumors have MSI in at least two of the five markers, MSI-L tumors have MSI in only one marker, and MSS tumors do not have instability at any of the five markers. Figure 7.3 illustrates amplification of the microsatellite loci in a colorectal cancer with MSI-H. Alternative panels consisting of mononucleotide repeat markers that are highly monomorphic in the germline DNA of a wide spectrum of populations have been used, with the advantage that they perform well without the use of normal control DNA.[65,66] One such panel includes five microsatellite loci (BAT-25, BAT-26, NR-21, NR-24, and NR-27) providing a pentaplex assay.[66–68] The pentaplex assay data correlate well with MMR protein expression for MLH1, MSH2, and PMS2, but less robustly with MSH6 expression, similar to the NCI panel.

Testing for MSI-H with the DNA MSI test is highly sensitive for HNPCC/LS cancers; however, the test may yield about 5% false negative cases among overall CRC cases from patients with MMR gene mutations.[4] Additionally, the MSI DNA test may detect MSI-H status in only 86% of the CRC cases from patients with germline mutations in MSH6.[4]

Immunohistochemistry of cancer tissues for DNA MMR proteins is a surrogate marker for the MSI status and when informative has the advantage of identifying the underlying deficient DNA mismatch repair gene based on the finding of which MMR protein is primarily lost in tumor cells. Overall, the sensitivity of immunohistochemistry to detect dMMR is about 95%.[4] Limitations of immunohistochemistry result in part from occasional problems with tissue immunoreactivity and interpretation pitfalls.[69] In MLH1-deficient tumors of sporadic type, where the *MLH1* gene is silenced by promoter methylation, IHC shows complete loss of MLH1 expression in tumor cells, and this is accompanied by parallel loss of PMS2, since the latter proteins are unstable in the absence of MLH1.[70] However, IHC may be difficult to interpret in cancers of HNPCC/LS patients with *MLH1* missense mutations that result in nonfunctional protein and MSI-H phenotypes,

where the mutant protein may be expressed and retain its immunoreactivity, at least partially, with variable levels of expression of PMS2 in parallel.[70–72] In addition, MSI-H CRCs with preserved or variable expression of MLH1 protein, with associated loss or variably reduced PMS2 in tumor tissue, may represent a germline mutation in *PMS2*. IHC for MSH2 in HNPCC/LS patients with germline mutations in *MSH2* usually cause a complete loss of gene expression in tumor tissues, accompanied by loss of MSH6 protein expression, but IHC heterogeneity has been reported in a rare case.[70] Since both the MSI DNA test and IHC for DNA mismatch repair proteins will miss a small number of tumors in patients with underlying HNPCC/LS, it has been proposed to perform both tests upfront in the evaluation of CRC for potential HNPCC/LS.[73]

Genetic Testing for Constitutional Germline Mutations in DNA MMR Genes

If the tumor tissue reveals MSI-H and/or there is loss of expression of one of the DNA repair proteins by immunohistochemistry, constitutional germline testing should be performed for the gene encoding the deficient protein, after appropriate genetic counseling of the patient. If tissue testing is not feasible, or if there is sufficient clinical evidence of HNPCC/LS, germline analysis of the *MSH2* and/or *MLH1* genes can be done as first line testing.[35,61,74] Mutation analysis can be performed by using a number of approaches, including single-strand conformational polymorphism analysis, denaturing gradient gel electrophoresis analysis, DNA sequencing, monoallelic expression analysis, Southern analysis, and quantitative polymerase chain reaction.[35] Overall, the likelihood of finding a germline mutation in the *MLH1* or *MSH2* genes of patients with colorectal cancers that are not MSI-H is low.[35] The germline mutations that occur in *MSH2* and *MLH1* are widely distributed throughout the two genes. More than 200 pathogenic mutations have been reported in *MLH1* and in *MSH2*.[75]

Two additional tests, *MLH1* methylation and *BRAF* V600E activating mutation assays, may help discriminate between a sporadic MSI-tumor and HNPCC/LS tumor with loss of expression of *MLH1* by immunohistochemistry but an undetected *MLH1* mutation.[61] Using quantitative methylation analysis, HNPCC/LS patients showed no or low level of *MLH1* promoter methylation, in contrast to high levels of methylation (greater than a cutoff value of 18% methylation) in sporadic MSI-cancers.[24] In addition, none of the patients with an unambiguous germline mutation in DNA mismatch repair genes demonstrated *BRAF* activating mutation.[53] If no loss of expression of *MSH2, MLH1, MSH6,* or *PMS2* is seen in MSI-H tumors or if the tumor is MSI-L or MSS but there is clinical suspicion of HNPCC/LS testing for germline constitutional mutations in the DNA MMR genes should be performed.[35,76] Identification of a germline mutation in index

cancer patients is important because it confirms the diagnosis of HNPCC/LS and the identified mutation may then be used to screen at risk relatives who may be mutation carriers and benefit from increased colonoscopic surveillance.

After a germline mutation is identified or the patient is diagnosed with HNPCC/LS, relatives should be referred for genetic counseling and testing should be offered. If no mismatch repair gene mutation is found in a proband with an MSI-H tumor and/or a clinical history of HNPCC/LS, the genetic test result is noninformative. The patients and the relatives at-risk should be counseled as if HNPCC/LS was confirmed and high-risk surveillance should be performed.[5,35,61]

Testing dMMR in Adenomas

In HNPCC/LS a significant association was found between MSI-H and high-grade dysplasia in adenomas, with loss of either MLH1 or MSH2 expression.[77] Based on these findings it was recommended that immunohistochemical staining/ MSI testing of large adenomas with high-grade dysplasia in young patients (younger than 50 years) may be performed to help identify patients with suspected HNPCC/LS.[19,77]

Universal Testing of CRC for dMMR

During the past two decades, patients who presented with one of the tumors of the HNPCC/LS syndrome, in particular colon cancer, were offered testing for HNPCC/LS following guidelines based on family history (as described by the Amsterdam criteria) or based on a combination of family history, age of presentation, and histopathologic features of the tumor (as described by the Bethesda guidelines). However, more recently, studies have suggested a universal screening approach entailing the evaluation of all diagnosed colorectal cancers for markers of HNPCC/LS regardless of the family history.[3,62,64] One of these studies evaluated more than 1,000 patients with colorectal cancer whose tumors were tested for MSI.[78] Patients with MSI-positive tumors were tested for expression of MMR genes and germline mutations in *MSH2, MLH1, MSH6*, and *PMS2* by genomic sequencing and deletion studies. A mutation causing HNPCC/LS was detected in 23 patients (2.2%), of whom as many as 22% would have been missed if Amsterdam or Bethesda criteria had been used alone. In families that meet strict clinical criteria for HNPCC/LS, germline mutations in *MSH2* or *MLH1* have been found in 45–70% of the families, and overall, germline mutations in these two genes account for 95% of HNPCC/LS cases with an identified mutation.[31,74] These data show that despite extensive testing there is still a significant number of families without an identified germline mutation accounting for HNPCC/LS. It has been proposed that if an individual has a family history that is suggestive of HNPCC/LS, but does not fulfill the Amsterdam criteria, they are considered to represent an HNPCC variant, or familial

colorectal cancer type X.[79,80] Further, it was observed that nearly 40–60% of all cases who meet Amsterdam criteria for HNPCC/LS do not have characteristic MMR deficiency or germline mutation in a DNA MMR gene. The age at diagnosis of these MSS familial CRC patients is 6 years older on average and most tumors occur on the left side of the colon.[2] The underlying genetic defect for these tumors is not yet known. Conversely there are also individuals who have tumors with MSI and germline mutation in a DNA MMR gene, but whose family history does not meet the Amsterdam criteria.[3]

In 2010, a consensus recommendation was published, stating that all CRCs should be screened using IHC for the four DNA MMR gene products, or alternatively by MSI, in order to evaluate for HNPCC/LS.[3] Another point raised by this consensus group was that MSI and IHC testing should not be considered genetic tests and should be ordered by appropriate medical personnel as needed for medical care.[3] Further, other investigators in the USA have advocated that all newly diagnosed colorectal and endometrial cancer patients should be screened for HNPCC/LS.[62,81–83] Review of this topic by the Evaluation of Genomic Applications in Prevention and Practice (EGAPP) working group[56] led to the recommendation that all newly diagnosed colorectal cancer patients should be screened for HNPCC/LS to reduce the morbidity and mortality from colorectal cancer in their at-risk unaffected relatives. A cost analysis of several possible screening methods indicated that the most cost-effective approach for screening all newly diagnosed colorectal cancer patients for HNPCC/LS would be to test tumor tissue with IHC followed by genetic testing in patients in whom any MMR protein was absent, after ruling out epigenetic (*MLH1* CpG methylation) causes of protein absence to exclude sporadic dMMR cases.[84] However, as reviewed earlier in this chapter, it should be cautioned that IHC alone will miss a small proportion of MSI-H cancers.[73] A report from the Association for Molecular Pathology proposed a testing strategy combining IHC for the four DNA MMR proteins and MSI DNA test upfront, followed by *BRAF* and *KRAS* mutation testing, serving both the purposes of screening for HNPCC/LS as well as for evaluation for targeted anti-EGFR therapies.[73]

Extra-colonic Neoplasia in Lynch Syndrome

Early-onset colorectal cancer (CRC) is the hallmark of HNPCC/LS, but it has been estimated that extracolonic manifestations may be as common as CRC, particularly in women. MMR germline mutations collectively confer an 80% risk of CRC before age 70 in men and 30–50% in women.[85,86] In comparison, the best characterized of the extracolonic manifestations is early-onset endometrial cancer, which occurs in an estimated 40–60% of at-risk patients.[4,85,87]

Weaker but definite associations[10] have been made between LS and cancers of the ovary, stomach, hepatobiliary tract, and small bowel, transitional cell carcinoma of the ureter and renal pelvis, glioblastoma (Turcot variant of Lynch syndrome), and sebaceous adenoma/carcinoma (Muir-Torre variant of Lynch syndrome).[88]

Breast cancer has often been detected in patients with LS.[89–95] The lifetime (to age 70) risk of breast cancer in LS patient series has been 2.2–5.4%.[87,96] This risk is not convincingly higher than the 7.6% rate seen in the general population,[97] in contrast to the dramatic enrichment seen for most LS-associated tumors.[96] It remains unsettled whether breast cancer is part of the LS spectrum.

While there are no conclusive studies linking LS with other cancers, case reports have suggested potential associations of LS with cancer of the pancreas, adrenal cortical carcinoma,[98] sarcomas (malignant fibrous histiocytoma,[99] rhabdomyosarcoma,[100] liposarcoma[101,102]), prostate cancer,[103] and neurofibromatosis-like features including café-au-lait macules and plexiform neurofibromas.[104]

The prevalence of extracolonic tumors in HNPCC/LS is variable and reflects the incomplete penetrance of the underlying mutation. CRC is relatively more common in *MLH1* germline mutants, while other cancers are more common in *MSH2* mutation.[88] *MSH6* mutation also appears to favor endometrial cancer.[88] *PMS2* mutants tend to present with atypical tumors in childhood.[88]

Endometrial Cancer

Endometrial cancer (EC) is the most common extracolonic manifestation of HNPCC/LS and has been extensively studied and reviewed.[105–110] The cumulative incidence of EC was 60% to age 70 in a study of 183 Finnish women with confirmed MMR mutations,[87] compared with 54% for colorectal cancer in the same population. Other series of women with MMR mutations showed a 42% lifetime risk of EC and 30–54% lifetime risk of CRC.[85,111]

EC becomes all the more salient as a feature of Lynch syndrome when one notes that CRC is less common in women than in men with HNPCC/LS, for whom the lifetime cumulative incidence of CRC is 74–83%.[85,111]

Given that its cumulative incidence approaches or exceeds that of CRC, it is clear that EC may be the sentinel cancer in some Lynch syndrome patients and kindreds. In a series of 111 women meeting Amsterdam criteria and having both CRC and EC, the CRC presented first in 49 (median age of 40), EC presented first in 46 (median age of 45), and the tumors presented simultaneously in 12.[112] When EC presented

first in this series, the mean lead time before subsequent development of CRC was 11 years, allowing for follow-up and prevention if the Lynch phenotype is successfully identified.[112] The converse situation, of course, also provides an opportunity for cancer prevention, since Lynch-associated CRC patients have a markedly elevated risk of subsequently developing endometrial cancer (10-year cumulative risk of 23.4% in LS patients, versus 1.6% in patients with sporadic CRC).[113] The reported average lead time from CRC to the second malignancy (usually EC or ovarian carcinoma) was 8 years.[112]

LS-associated EC occurs at a younger age than sporadic cancer, and Lynch syndrome is more common in women presenting with EC at a young age. Functionally significant MMR gene mutations are present in 1.4–2.6% of EC overall,[114,115] but 9% of EC occurring before age 50.[116] Young age at presentation of EC has therefore been proposed as a trigger for further testing, usually either IHC testing for loss of MMR proteins or molecular testing for the MSI phenotype. Importantly, LS patients with EC are less likely than average women to fit the typical clinical profile of EC (obese, nulliparous), given that their tumors arise due to a genetic lesion. Thus, low BMI, family history suggestive of LS, and LS-associated histology (see below) have all been suggested as additional features to prompt further testing.[117] Using age below 50 as the main trigger for further testing would, however, result in low sensitivity for detecting Lynch syndrome. In one study of 562 unselected EC patients, the 13 women with eventual discovery of LS had a mean age of 54.1 years at diagnosis.[82] In another group of 7 women with Lynch syndrome related to inactivating *MSH6* mutations, the mean age at diagnosis was 54.8 years, compared to 64.6 years in the overall population.[118]

There are conflicting data on the prognosis of LS-associated EC, with some studies showing no difference in 5-year survival between LS patients and matched controls.[119–121] Other studies have variously shown either worse[122] or better survival[123] in MSI-H tumors. Taking these data together, it appears to be difficult to demonstrate a significant prognostic difference in LS-associated EC. Its prognostic importance lies, rather, in the likelihood of observing other associated malignancies in the patient and family members.

Pathologic Features

HNPCC/LS-associated endometrial carcinoma has a characteristic morphology.[124–127] Typical features include numerous peritumoral and tumor-infiltrating lymphocytes (TILs). These features related to the inflammatory response are reminiscent of the Crohn-like reaction and dense TILs that are common in MSI-H CRC cases. Both features show a statistically significant association with MSI-H status, but neither of them is sensitive or specific enough to serve as a diagnostic marker.[124] Although there appears to be no specific TIL

density that sensitively and specifically identifies MSI-H cases,[125] 40 TILs/10 HPF (using a 400× high-power field) was suggested in one study as an analytically useful cutoff by which to define this feature.

The majority of LS-associated EC is of endometrioid histology, but these endometrioid cases show some propensity to demonstrate a biphasic morphology in which a typical, rather well-differentiated endometrioid adenocarcinoma (FIGO grade 1 or 2) is admixed with an undifferentiated component.[124,125] The resulting "dedifferentiated" carcinoma must, by definition, contain at least 10% of each component. 21% of MSI-H cases showed this phenotype versus 6% of non-MSI-H cases ($p = 0.06$).[124]

LS-associated EC also shows a propensity toward nonendometrioid histology. One series of 23 patients with germline MMR mutations consisted of 57% endometrioid tumors and 43% nonendometrioid ones, while sporadic controls were 96% endometrioid.[128] Two other reports have shown endometrioid histology in 86–87% of LS-associated cases, as compared to >96% in both sporadic controls[129] and *MLH1* hypermethylated cases.[118,129] The nonendometrioid tumors were predominantly serous, clear cell, and carcinosarcoma in these reports.

It has been reported that the prevalence of LS is higher in patients with carcinoma of the lower uterine segment (29%) as compared to the general population with endometrial cancer.[130]

Molecular Features

EC can be associated with mutation in any of the MMR genes (*MSH2*, *MLH1*, *MSH6*, *PMS2*). However, the lifetime risk of EC appears to be highest in *MSH6* mutation, followed by *MSH2* and *MLH1*.[131] This is the inverse of the behavior seen for CRC in women, for which the lifetime risk is highest for *MLH1* mutants and lowest for *MSH6*. Indeed, while *MSH6* mutation has been considered something of a hypomorph from the viewpoint of CRC, it is potentially the most significant of the MMR proteins from the viewpoint of EC. Data on EC risk in *PMS2* mutants are relatively scarce, but in one series of 61 confirmed mutants, the cumulative risk of EC was 15% to age 70 years, showing the penetrance of *PMS2* mutation to probably be lower than the other three MMR genes.[132]

Ovarian Cancer

An estimated 5–10% of epithelial ovarian cancers (EOC) are hereditary, consisting mainly of those associated with *BRCA1* and *BRCA2* mutations.[110,133] LS patients make up most of the remaining 10–15% of hereditary epithelial ovarian cancer cases.[110,134] Although these figures suggest that approximately

1% of ovarian cancer is associated with LS, the MSI-H phenotype is present in approximately 12% of all EOC cases,[135] indicating that this defect probably arises de novo and plays a pathogenetic role in many sporadic tumors.

LS-associated ovarian cancers present at an earlier age than sporadic tumors (mean 49.5 versus 60.9 years).[134,136] These cases are also identified at earlier stages than sporadic tumors. In one study, the stage at diagnosis was FIGO I for LS-associated tumors, but FIGO III for sporadic ones.[136] Both age and stage differences, when they occur, probably reflect more active screening in the Lynch patients. However, a stage difference was not seen in a series of 37 hereditary ovarian cancers that included predominantly *BRCA* mutants, although these would presumably also have been heavily screened (in an era, however, predating the appreciation of the role of the fallopian tube in EOC carcinogenesis).[134]

Pathologic Features

EOCs in general exhibit several histologic patterns. In sporadic cases, the most common histology is serous (accounting for as many as 78% of all EOCs), followed by endometrioid, mucinous, clear cell, and malignant Brenner tumor in decreasing order of frequency.[137,138] Carcinomas of mixed or indeterminate (i.e., poorly differentiated) histology also occur. LS-associated and MMR-deficient EOCs appear to be enriched in nonserous histologies, which in one meta-analysis constituted 57% of LS-associated ovarian cancers (128 patients in 6 studies).[139] In this meta-analysis, 25% of LS-associated EOCs were endometrioid, 17% were clear cell, and 16% were mucinous. Similar proportions were found in MSI-H tumors occurring outside the context of Lynch syndrome, suggesting that the relative scarcity of serous tumors somehow reflects the biology of the MMR-deficient state.[86]

The prognosis of EOC in Lynch patients appears to be similar to that of non-Lynch controls. In one report, there was a small and statistically insignificant decrement in overall survival for Lynch patients,[136] conceivably attributable to these patients' risk of other malignancies. This situation contrasts with that for CRC, where survival is better in Lynch-associated tumors versus sporadic ones.[4]

Gastric Cancer

Gastric cancer was present, along with CRC and EC, in the original "family G of Warthin" in whom LS was initially described. A study of 60 families from a Brazilian registry found a history of gastric cancer in 12/1,040 = 1.1% of men and 12/1,055 = 1.1% of women meeting Amsterdam I or II criteria.[89] The prevalence of gastric cancer in a Dutch LS registry population was similar (21/948 = 2.2% of men,

11/1,066 = 1.0% of women); Kaplan-Meier analysis in this population gave an estimated incidence of 6.2% in men and 2.0% in women to age 70.[140] In the Dutch series, gastric cancer occurred only in *MSH2* and *MLH1* mutant kindreds. *MSH6* mutation was well represented in the sample, but was not associated with any gastric cancers. *PMS2* mutants had no gastric cancers, but represented only two of the 236 families.

The histology of Lynch-associated gastric cancers is predominantly intestinal type in the Lauren classification (at least two-thirds of patients), the remainder being of diffuse type.[140,141] This finding is somewhat unexpected, given that the diffuse type is statistically more likely to have a primary genetic etiology (e.g., in hereditary diffuse gastric carcinoma).[142,143] Intestinal-type tumors are numerically more common than diffuse type in the genetically normal background population (51% intestinal type, 37% diffuse type).[144] *H. pylori* was noted in only 20% of LS-associated gastric cancers,[141] a fraction that is lower relative to the general gastric cancer population and reflects the presence of an inherited cancer predisposition. The histologic pattern of LS-associated gastric cancer is not distinctive enough to be used to triage patients for further testing in the absence of contributory personal or family history.

Pancreatobiliary Carcinoma

The cumulative lifetime risk of pancreatobiliary carcinoma in confirmed and presumed MMR gene mutation carriers has been estimated at 2–4% to age 70,[87,96] compared with 0.2% in the general population. These tumors appear to be almost entirely cholangiocarcinomas arising at various sites along the biliary tract, including common bile duct (7/18 in a series of 315 Finnish subjects), ampulla of Vater (4/18), intrahepatic biliary tree (4/18), and pancreas (3/18).[145] A mucinous cholangiocarcinoma has been reported in a patient with Muir-Torre syndrome related to *MSH2* mutation.[146] LS does not seem to have an association with gallbladder cancer.

Small Intestinal Carcinoma

Carcinomas of the small bowel are approximately 100 times more common in LS than in the general population, with a cumulative lifetime risk of 4%.[147] This is roughly similar to the relative risk in Crohn disease or familial adenomatous polyposis.[148] The cancer risk is higher in *MSH2* and *MLH1* mutants, and lower in *MSH6* and *PMS2*.[149] Small bowel tumors in LS are adenocarcinomas, have up to 3:1 male:female bias,[147] have no particular site of predilection within the small bowel, and may occur synchronously at several sites.[148] The median age at diagnosis is 40–50, at

least a decade younger than the general population with small bowel cancer.[147,148] As in the colon, the prognosis for patients with small bowel carcinoma in the setting of Lynch syndrome may be slightly better than for the general population.[148]

Transitional Cell Carcinoma

Lynch syndrome carries a 22-fold increased risk of transitional cell carcinoma (TCC) of the upper urinary tract (renal pelvis and ureter),[150] occurring in approximately 4% of the Lynch syndrome population.[87,111,151] There is no increase in TCC of the bladder in LS.[150,152] The tumors present around age 56, a decade earlier than in the background population.[150] They have an MSI phenotype,[153] confirming that they are part of the Lynch syndrome spectrum. Special histologic features of these tumors have not been described.

CNS Tumors in Turcot Syndrome

The eponym "Turcot syndrome" has been used to describe the association of colorectal and CNS tumors. It is now apparent that this association occurs in two distinct sets of patients with fundamentally different genetic lesions.[154] The larger group has APC mutations, with the characteristic florid pancolonic polyposis, and with medulloblastoma as the CNS manifestation. A smaller group of patients has MMR mutations, typical Lynch-type CRC, and glioblastoma as the CNS manifestation.[155] Intriguingly, much as CRC associated with Lynch syndrome has a favorable prognosis, the survival of Turcot patients with glioblastoma appears to be better than that of patients with sporadic glioblastoma, although the number of patients reported is small.[155]

Sebaceous Adenoma/Carcinoma in Muir-Torre Syndrome

The Torre syndrome,[156] simultaneously reported by Muir,[157] was initially believed to represent the co-occurrence of cutaneous lesions with visceral malignancies, without mention of any familial association. It was subsequently noted that several of these patients belonged to families with what was then known as the cancer family syndrome, now HNPCC/LS.[158] The cutaneous lesions are frequently sebaceous adenomas, sebaceous carcinomas, or keratoacanthomas, i.e., low-grade squamous cell carcinomas arising from the pilosebaceous unit.

Further study has confirmed Muir-Torre syndrome (MTS) to be a variant of Lynch syndrome[159] defined by the presence of sebaceous skin neoplasms. The skin lesions of MTS have MMR mutations, usually in *MSH2*,[160] and a MSI-H phenotype.[161,162] The presence of sebaceous neoplasms is variably penetrant, having been reported in 28% of families and 9.2% of individuals meeting criteria for LS. There were 42% of kindreds with *MSH2* mutations, and 44% of kindreds with *MLH1* mutations, that had at least one individual with MTS, while MTS was not found in kindreds with *MSH6* or *PMS2* mutation.

HNPCC/LS: Clinical Management

Colorectal Cancer

Carriers of DNA mismatch repair gene mutations seen in HNPCC/LS are recommended to have colonoscopic surveillance staring at early age.[61] Colonoscopy is recommended every 1–2 years starting at age 20–25 years (age 30 years for those with *MSH6* mutations). For individuals who will undergo surgical resection of colon cancer, subtotal colectomy may be favored.

Endometrial and Ovarian Cancer

Patients Without Known Lynch Syndrome

Although identification of a CRC meeting Amsterdam or Bethesda criteria has been suggested as a cue to screen for EC, it appears that EC is more common than CRC in these patients, indicating that EC with appropriate features may equally well be taken as a sentinel event to identify Lynch syndrome patients and families.[127] Examining the converse situation, in a prospective study of 100 women with endometrial cancer diagnosed before age 50, nine carried germline MMR mutations (seven *MSH2* mutants, one *MLH1* mutant, and one *MSH6* mutant).[116]

MMR testing by immunohistochemistry, MSI testing, or gene sequencing is commonly recommended when faced with a new diagnosis of colon cancer in an appropriate clinicopathologic setting. Similar reasoning dictates that EC patients of sufficiently low age be tested for MMR mutations (with age <50 years often suggested as a cutoff). As has been mentioned, the prevalence of such mutations is in the neighborhood of 9%.[116] As age rises, the relative number of Lynch-associated mutations would be expected to fall, as sporadic tumors begin to predominate, but in absolute terms, Lynch cases continue to accumulate. Furthermore, with rising age, sporadic hypermethylation begins to predominate as a cause of *MLH1* silencing, causing an apparent dMMR/MSI phenotype in a patient with no germline mutation and no increased tumor risk at other sites. Thus the relative diagnostic utility of immunohistochemistry as compared to other methods can be expected to change with age.

A consensus statement of the Society of Gynecologic Oncologists instructs practitioners to identify women who have a 20–25% chance of having an inherited predisposition to endometrial, colorectal, and related cancers, for whom

genetic risk assessment is "recommended," and those with a 5–10% chance of the same, for whom genetic risk assessment "may be helpful".[163] The SGO statement does not specify the form that the risk assessment should take, but underlines the need for it to be integrated with genetic counseling and education. Since LS-associated malignancies are uncommon before age 21, it is suggested that the benefits of testing do not outweigh its potential adverse effects in this age group.

Strategies for risk assessment in the patient with EC and suspected Lynch syndrome resemble those available in CRC, with immunohistochemistry, MSI testing, and gene sequencing representing the state of the art in approximately increasing order of cost.[117] Application of the Amsterdam criteria to triage patients for testing increases specificity at the cost of decreased sensitivity. As in CRC, immunohistochemical staining of the endometrial tumor for MSH2, MLH1, MSH6, and PMS2 is a simple gatekeeper strategy that can serve as a useful initial screen. When sequencing is used, nonsense mutations are relatively simple to interpret, but missense mutations are heterogeneous and may or may not be of clinical significance.[82]

Several heuristics[116,163] or specific algorithms[4,105,117,124] have been proposed to combine clinicopathologic features with stepwise diagnostic testing in order to achieve sensitivity and specificity while minimizing cost.[117] While the relative merits of each specific testing strategy will depend on the cost–benefit tradeoffs one chooses to make, the lowest cost-effectiveness ratio appears to be achieved with four-protein immunohistochemistry, followed by confirmatory sequencing of any putatively affected gene.[117] When cost represents a major consideration, an initial panel consisting only of MSH6 and PMS2 IHC can provide clues regarding dMMR status at a reduced cost.[164]

Patients with Suspected Lynch Syndrome

Once a presumptive diagnosis of Lynch syndrome has been established, further management can involve a combination of surveillance and, potentially, prophylactic surgery. For women, in whom EC and EOC are the main malignancies to exclude, proposed surveillance models usually involve imaging, usually by ultrasound, and periodic endometrial sampling.[105,165–169]

Other HNPCC/LS Associated Carcinomas

Patients Without Known Lynch Syndrome

Endometrial and ovarian cancers are markedly more common than the other extracolonic Lynch-associated tumors and have been the focus of most of the research on screening. However, when other tumors associated with the syndrome present at a young age or with suspicious family history,

tumor testing for features of Lynch syndrome is broadly recommended. As for endometrial cancer, IHC testing for loss of MMR proteins or molecular testing for MSI are both appropriate initial modalities, without clear consensus to favor one over the other.[152] DNA sequencing should be reserved for later steps of the investigation due to its expense and complexity.[170] For clinicians, it is important to emphasize the details of family history and to inform the pathologist of these details so that testing can be initiated or justified.

Patients with Suspected Lynch Syndrome

The heterogeneity in extracolonic tumors between Lynch kindreds and between patients within a given kindred,[111] combined with the relatively low frequency of any given extracolonic tumor, makes it difficult to recommend any specific screening protocol in patients with suspected or confirmed Lynch syndrome. For some tumors, such as glioblastoma, no specific screening method seems practical. In the case of the small bowel, screening by capsule endoscopy or double-balloon enteroscopy has been suggested; rough calculations with rather favorable assumptions suggest a reasonable cost per QALY if screening is performed once above age 40.[147] Urine cytology is a straightforward screening method for transitional cell carcinoma. None of these methods have been explicitly adopted or endorsed, and screening of the Lynch patient therefore seems to currently require an individualized approach.

Polyposis Syndromes of the Gastrointestinal Tract

Polyposis syndromes are characterized by the development of variable numbers of polypoid lesions throughout the colon, small intestine, and stomach and include developmental polypoid abnormalities or hamartomatous polyps, inflammatory-type polyps, and adenomas. The risk of adenocarcinoma is highest in the syndromes that develop adenomas, but increased risk of malignancy also characterizes the other polyposis syndromes. A summary of the gastrointestinal polyposis syndromes is depicted in Table 7.1.

Familial Adenomatous Polyposis

Clinical Features, Pathology, and Natural History

Familial adenomatous polyposis (FAP) is the most frequent gastrointestinal polyposis syndrome. Patients develop numerous adenomas preferentially involving the colorectum (Fig. 7.6), but also affecting the small intestine, in particular the duodenum and periampullary region.[171–173] The stomach typically develops numerous fundic gland polyps, although
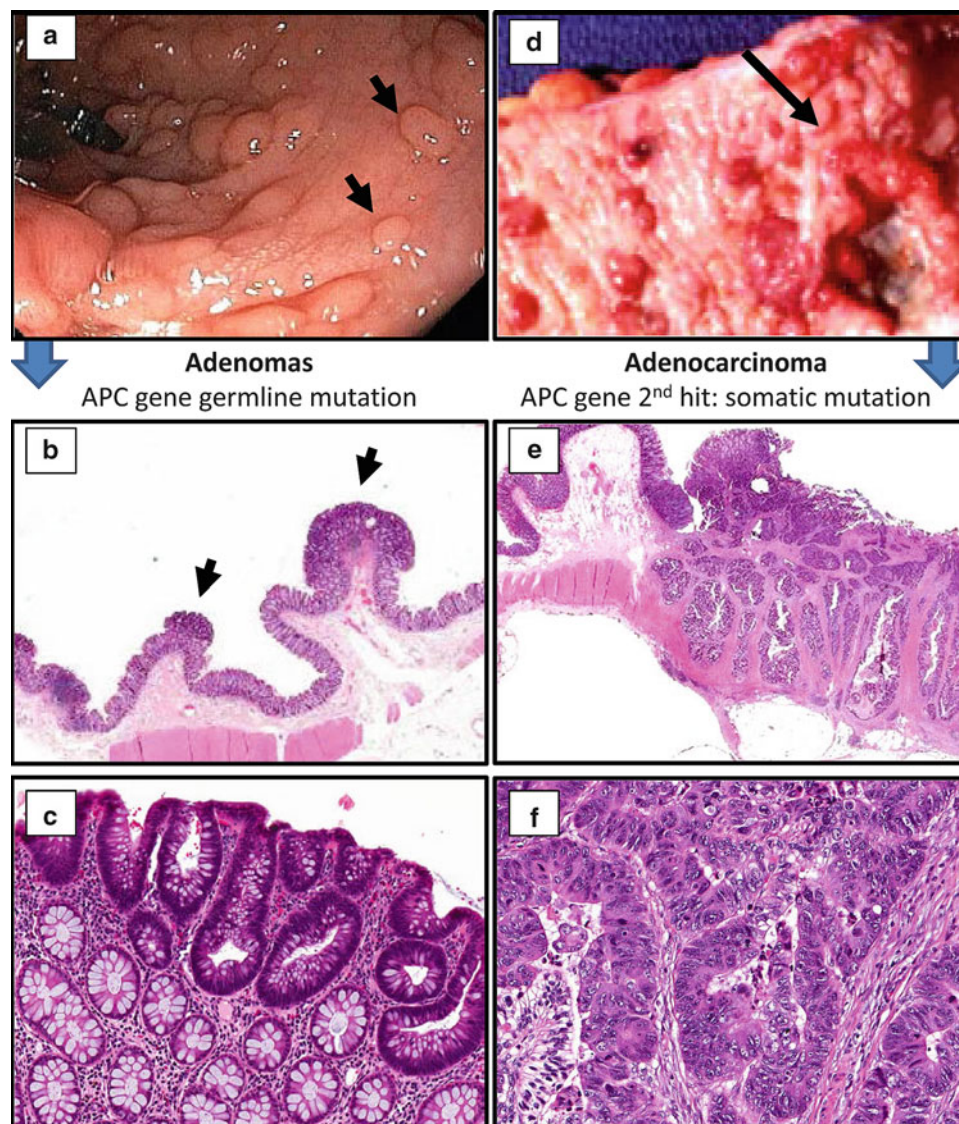
Fig. 7.6 Colonic adenomas and progression to adenocarcinoma in a patient with FAP. (**a**) Numerous polyps are identified at colonoscopy (image provided by courtesy of Dr. David Metz, University of Pennsylvania); (**b** and **c**) Histologic features of representative tubular adenomas. (**d**) Adenocarcinoma (*arrow*) arising in the background of numerous colonic polyps. (**e** and **f**) Histologically, the tumor is a moderately differentiated adenocarcinoma extending into the subserosal tissue

adenomas may also occur.[173–178] If the disease progresses through its natural history, 100% of affected individuals eventually develop colorectal cancer at early age[179] (Fig. 7.6). Overall, FAP patients represent less than 1% of all colorectal carcinoma cases in the United States, affecting 1 in 8,000–10,000 individuals.[180] FAP patients are also at higher risk of developing duodenal and ampullary carcinomas and have a slightly increased risk of gastric adenocarcinoma.[181]

The majority of FAP patients inherit germline mutations in the adenomatous polyposis coli (*APC*) gene on 5q21–22 chromosome locus,[182–184] but up to a third of FAP cases present as de novo germline mutations.[180,185]

In addition to the characteristic gastrointestinal polyposis, FAP patients manifest other lesions, including congenital hypertrophy of retinal pigmented epithelium (CHRPE) in 70–80% of FAP patients, desmoid tumors in 15% of cases, thyroid carcinoma (papillary and follicular types, including the characteristic cribriform-morular variant) in 1–2% of FAP patients,[174] and hepatoblastoma in young children.[174]

In addition to the classic FAP syndrome, inherited mutations in the *APC* gene are associated with several variants of FAP that include Gardner Syndrome, Turcot Syndrome, and Attenuated FAP (AFAP), summarized in Table 7.1.[173,180,181,186,187]

Gardner syndrome: These patients develop a number of extra-colonic manifestations including osteomas, desmoid tumors, dental abnormalities, ophthalmologic abnormalities including congenital hypertrophy of retinal pigment epithelium (CHRPE) and cutaneous cysts.

Turcot syndrome: These patients develop colorectal polyposis and brain tumors, most commonly medulloblastoma (see also above).

Attenuated FAP: These patients develop a smaller number of colonic polyps as compared to classic FAP, but with sufficient colonic polyposis (frequently over 15), suggesting an underlying polyposis syndrome.[181]

Genetic and Molecular Features

FAP provides the basis to understand the molecular pathways underlying the most common mechanisms of stepwise progression in colorectal cancer.[188] *APC* codes for a 312 kDa protein that is expressed in many tissues and is thought to participate in several cellular functions including Wnt-mediated signaling and transcriptional regulation, cell adhesion, cell migration, and chromosomal segregation. The *APC* gene product is a key mediator in the Wnt signaling pathway for cellular growth and proliferation. In the absence of a Wnt-mediated growth signal, APC occurs in a complex with beta-catenin, leading to beta-catenin phosphorylation and targeting it for destruction by the proteasome. When the Wnt pathway is activated by ligand binding, the APC–beta-catenin protein complex is disrupted, beta-catenin phosphorylation cannot occur, and this results in stability and nuclear localization of beta-catenin. Pathogenic mutations in the *APC* gene result in a disrupted APC–beta-catenin protein complex, leading to constitutive activation of the Wnt pathway.[188–190]

The APC protein contains multiple functional domains, including an oligomerization domain, an "armadillo" domain region which is thought to be involved in binding of APC to proteins related to cell morphology and motility, a beta-catenin-binding domain, an axin-binding domain, and a microtubule-binding domain.[174,191]

The majority of germline mutations associated with FAP are either frameshift or nonsense mutations that result in a truncated protein product, leading to disruption of interaction with beta-catenin, stabilization of beta-catenin with nuclear accumulation of the protein, and a phenotype of constitutive activation of the Wnt pathway.[191,192] The most common germline mutations occur at codons 1061 and 1309 and account for 17% and 11% of all germline APC mutations, respectively. The region between codons 1286 and 1513 is known as the "mutation cluster region" (MCR), and many of the identified *APC* mutations occur in this segment of the gene.[193] Some associations between the location of the germline APC mutation and the clinical phenotype have been

found.[194] Mutations within the MCR are associated with extensive polyposis of typical FAP patients, whereas mutations in the 5′ end of the gene (exons 4 and 5), and mutations in the alternatively spliced form of exon 9 or the 3′ distal end of the gene, are seen in attenuated polyposis. An intermediate phenotype is observed in patients with mutations between codon 157 and 1249 and between 1465 and 1595. The association of FAP with desmoid tumor formation has been correlated to mutations downstream of codon 1400.[181,194,195] Mutations beyond codons 934, 1395, and within codons 564–1465 may be associated with upper gastrointestinal tumors. CHRPE is associated with mutations that occur between codons 311 and 1444.

Molecular Diagnosis

Molecular testing for germline mutations in the *APC* gene is recommended in specific settings as summarized by the American Gastroenterological Association.[171] The primary indications include high clinical suspicion for FAP (>100 colorectal adenomas), first-degree relatives of FAP patients, >20 cumulative colorectal adenomas (suspected AFAP), and first-degree relatives of patients with AFAP.[171,196]

Different testing approaches are used in various laboratories, dictated by factors such as the volume of testing, platforms, and experience available in the laboratory. Sequencing of the entire coding region is the gold standard for diagnosis. Other methods include protein truncation tests and mutation scanning.

As numerous pathogenic mutations have been described in *APC*, including large exonic or whole gene deletions, a comprehensive approach to molecular diagnosis is required.[197] If the patient has a phenotype suggestive of FAP but no mutations are found in the APC gene, underlying mutations in the *MUTYH* gene should be considered (see also below).[198] When a mutation is identified, targeted genetic testing can be offered to family members.

When germline mutations are not detected in patients clinically suspicious for FAP and related syndromes, other mechanisms of gene deficiency may be interrogated. These alternatives include alterations in epigenetic regulation of the *APC* gene, contribution of genes encoding other proteins involved in the beta-catenin pathway such as axin, alterations of allelic mRNA ratios, and somatic APC mosaicism.[199] Germline hypermethylation of the *APC* gene has been shown not to be a significant cause of FAP in *APC* mutation-negative cases.[200] In cases in which no other discrete mutation exists, unbalanced *APC* allelic mRNA expression, resulting in reduced "dosage" of *APC* and functional haploinsufficiency has been reported.[201]

Individuals who are carriers of an *APC* gene germline mutation are recommended to have regular surveillance

starting early in life with annual sigmoidoscopy or colonoscopy, beginning at 10–12 years of age. Prophylactic colectomy is recommended, usually by the time the patient is in his or her early 20s. Regular esophagogastroduodenoscopy (EGD) is also recommended for identification of gastric, duodenal, and periampullary lesions. Additional recommendations are directed at extra-colonic manifestations and include annual palpation of the thyroid, serum alpha-fetoprotein (AFP) levels, and abdominal palpation every 6 months in young children of FAP families to detect hepatoblastoma.

MUTYH-Associated Polyposis (MAP)

MUTYH-associated polyposis is transmitted as an autosomal recessive colorectal cancer syndrome, occurring in patients with colorectal polyposis and underlying germline mutations in the DNA repair gene *MYH*[202–204] (Table 7.1). Patients with MAP usually present with fewer polyps, but they may show no polyps or even numerous polyps as seen in FAP.[204] Colorectal cancer arising in the background of MAP represents an estimated 0.5–1% of all colorectal cancers, with a lifetime risk of CRC reaching 80%, and as in FAP the risk of duodenal cancer is also increased.[204]

The *MUTYH* gene encodes a protein with glycosylase functions, involved in the base excision repair (BER) system critical to the repair of DNA damage caused by oxidative stress. The oxidized base 7,8-dihydroxy-8-oxoguanine (8-oxo-G) is often mistakenly paired with adenine (A), resulting in the appearance of guanine:cytosine>thymine:adenine (G:C>T:A) transversions at the next round of DNA replication, as the detection of stable 8-oxo-G:A base-pairs is missed by the replicative DNA polymerases.[205,206] The DNA damage-specific glycosylases OGG1, MUTYH, and MTH1 function by recognizing and facilitating the removal of 8-oxo-G adducts.[206] Consequently, deficient MUTYH function is associated with an increased frequency of G:C>T:A transversions,[207] which occur in regions of cancer-related genes such as *APC, KRAS,* and *BRCA1/2*. Mutations in two hotspots, Y165C and G382D, account for 70% of all *MUTYH* mutations in Caucasian patients. Germline mutational testing is indicated in individuals with greater than 10 adenomatous polyps (particularly with family history of colon cancer consistent with recessive inheritance) and significant polyposis similar to AFAP/FAP who test negative for mutations in *APC*.[204] Screening is recommended to begin at age 18–20 years with colonoscopies every 1–2 years. Colectomy can be considered in cases with larger numbers of polyps mimicking FAP. Guidelines for the screening of duodenal cancers in FAP/AFAP should be applied to MAP patients as well.[204]

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