Hereditary Colorectal Cancer-Part II

Lynch Syndrome

There are 2 broad classes of hereditary forms of colorectal cancer (CRC), based on the predominant anatomic location of the CRC: distal versus proximal. CRCs involving the distal colon are more likely to show aneuploid DNA content, harbor mutations in APC, p53, and K-ras genes, and behave more aggressively\textsuperscript{303,304}; proximal CRCs are more likely to show diploid DNA, possess microsatellite instability, harbor mutations in the mismatch repair (MMR) genes, and behave less aggressively, as in hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome.\textsuperscript{303,304} Familial adenomatous polyposis (FAP) and most sporadic cases may be considered a paradigm for the first, or distal, CRC class, whereas Lynch syndrome more clearly represents the second, or proximal, CRC class.\textsuperscript{303,304}

The term Lynch syndrome is used in preference to HNPCC, given the fact that the syndrome’s phenotype is not completely without polyps, and in addition to CRC it involves a litany of extracolonic cancer types that are integral to the syndrome. It is the most commonly occurring hereditary syndrome that predisposes to CRC\textsuperscript{304} (Fig 2; Part I). Its diagnosis requires a sufficiently detailed family history of colorectal carcinoma as well as cancer of all anatomic sites, particularly those that are integral to this disorder (e.g., carcinoma of the endometrium [the second most common cancer], ovary, stomach [particularly in families from Japan and Korea],\textsuperscript{305} hepatobiliary tract, small bowel, pancreas, upper uroepithelial tract, and brain).\textsuperscript{303,304,306,307}

History of Lynch Syndrome

The history of what is known as Lynch syndrome dates to an observation of Aldred Warthin, pathologist at the University of Michigan School of Medicine.\textsuperscript{308} He became deeply moved when his seamstress, in 1895, told him that she would likely die of cancer of the colon, stomach, or her female organs, because of the enormous proclivity to these cancers in her family (unfortunately, just as she had told Warthin, she died at a
young age of metastatic endometrial carcinoma). Warthin listened intently, developed her pedigree, and along with other similar cancer prone families published this work in 1913.\(^{309}\) Warthin updated the family’s history in 1925.\(^{310}\) The seamstress’s family has since been known as Family G.

Lynch and colleagues\(^{311}\) described the natural history and genetics of 2 large Midwestern kindreds (Families N and M) in 1966. Dr. A. James French, Warthin’s successor as chairman of pathology at the University of Michigan, heard about Lynch’s research on Families N and M, and recalled that Warthin, his predecessor, had discovered a similar family (Family G) in 1895. Lynch was then invited by French to take custody of all the detailed documents and pathology specimens that the meticulous Warthin had investigated, catalogued, and published over a span of more than 30 years.\(^{309,310}\) The history of Family G was then updated and published in 1971.\(^{312}\) This material is discussed in a more detailed review of the history of HNPCC.\(^{313}\) Through the use of conversion technology, an \(MSH2\) mutation was identified in Family G in the year 2000.\(^{314}\) Table 7 summarizes the important points in the history of HNPCC.

**Clinical Features and Diagnostic Criteria**

The cardinal clinical features of the Lynch syndrome are listed in Table 8 and are discussed in more detail in other review articles.\(^{303,304,315}\) Several international diagnostic criteria for the Lynch syndrome have been developed, the foremost being the Amsterdam I,\(^{316}\) Amsterdam II,\(^{317}\) and the Bethesda Guidelines.\(^{318}\) The variety of criteria that have been developed for the syndrome testifies to its complexity. The original Amsterdam I criteria were developed for the purpose of consistent classification of subjects in research programs and focused on CRC (Table 9). The subsequent Amsterdam II criteria added the syndrome’s integral extracolonic cancers to the focus to be more useful for clinical diagnosis purposes. The Bethesda Guidelines introduced molecular genetic considerations, linking the diagnostic criteria of the Lynch syndrome to the high percentage of Lynch syndrome tumors that are positive for microsatellite instability (MSI), to provide guidelines for MSI testing. Other criteria, such as those developed in Korea and Japan, make use of emerging knowledge regarding the syndrome’s phenotypic variability in different geographic regions. These criteria are based heavily on the family history, which we consider to be potentially the most cost-beneficial component of a patient’s medical evaluation; however, we are also cognizant of the fact that it remains 1 of the most neglected portions
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<td>RER+ (MSI) phenotype described</td>
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<td>MSH2 mutation identified</td>
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<td>MSH2; MLH1 mutations</td>
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<td>Amsterdam II criteria</td>
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of a cancer patient’s medical evaluation in the average clinical practice setting.\textsuperscript{319,320}

Differing stages in the development of a Lynch syndrome pedigree

### TABLE 7. Continued

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<th>Feature</th>
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<tr>
<td>A complex mutation of $MLH1$ at codon 222 is associated with adolescent onset of CRC (more early-onset CRC families needed for study)</td>
<td>2001</td>
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<td>Fluorouracil-based adjuvant chemotherapy benefits patients with stage II or stage III CRC with MSS or MSI-L tumors but not those with MSI-H tumors</td>
<td>2003</td>
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<tr>
<td>$H_2O_2$ effect improves survival in DNA MMR-deficient cell line</td>
<td>2003</td>
<td>611</td>
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<tr>
<td>$MSH2$ del1-6 founder mutation in the United States</td>
<td>2004</td>
<td>315</td>
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Abbreviations: CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer; TAH-BSO, total abdominal hysterectomy and bilateral salpingo-oophorectomy; ICG-HNPCC, International Collaborative Group on HNPCC; FAP, familial adenomatous polyposis; MMR, mismatch repair; NIH, National Institutes of Health; NCI, National Cancer Institute; MSI, Microsatellite instability; MSS, Microsatellite stable.

### TABLE 8. Cardinal features of Lynch syndrome

- Earlier average age of CRC onset than in the general population; the average age of CRC onset in HNPCC is $\sim$45 years, whereas the average age of onset in sporadic CRC is $\sim$63 years
- Proximal colon involvement (70% of CRCs arise proximal to the splenic flexure)
- A significant excess of synchronous and metachronous CRCs ($\sim$25% to 30% among patients having a second primary CRC within 10 years of surgical resection for initial CRC, if the operation was anything less than a subtotal colectomy)
- Autosomal dominant inheritance pattern
- Increased risk for malignancy at certain extracolonic sites, foremost of which is endometrial carcinoma, followed by carcinoma of the ovary, stomach, small bowel, hepatobiliary tract, pancreas, upper uroepithelial tract, and brain
- CRC tumors in HNPCC are more often poorly differentiated, with an excess of mucoid and signet-cell features, show a Crohn’s-like reaction, and contain a significant excess of infiltrating lymphocytes within the tumor. Microsatellite instability (MSI) is found in most CRC tumors in the Lynch syndrome
- Increased survival from CRC
- Accelerated carcinogenesis and interval CRC; a tiny adenoma may emerge into a carcinoma within 2–3 years, as opposed to 8–10 years in the general population
- Sebaceous adenomas, sebaceous carcinomas, and multiple keratoacanthomas in the Muir-Torre syndrome (MTS) variant of Lynch syndrome
- The sine qua non, the identification of a germline MMR mutation segregating with syndrome-affected individuals in the family

Abbreviations: HNPCC, hereditary nonpolyposis colorectal cancer; CRC, colorectal cancer; MMR, mismatch repair.
wherein each component provides a pictorial review of its genesis are shown in Figure 16. For example, note that the proband had multiple primary cancers at an early age (Panel A), which should provide the clinician with vital clues of the possibility of the Lynch syndrome. This much information does not fulfill any of the criteria in Table 9, yet we believe the information is sufficient to test for a mismatch repair gene (MMR) germline mutation. However, when the pedigree is further extended, it essentially epitomizes an HNPCC diagnosis (Panels B and C), and when extended even further, as shown in Panel D, it essentially

<table>
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<th>TABLE 9. Amsterdam I and Amsterdam II criteria and Bethesda Guidelines</th>
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<td>Amsterdam I criteria(^{316})</td>
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<td>At least 3 relatives with histologically verified colorectal cancer:</td>
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<td>1. One is a first degree relative of the other 2</td>
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<td>2. At least 2 successive generations affected</td>
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<td>3. At least 1 of the relatives with colorectal cancer diagnosed at less than 50 years of age</td>
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<td>4. Familial adenomatous polyposis has been excluded</td>
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Bethesda Guidelines for testing of colorectal tumors for microsatellite instability\(^{318}\) |

1. Individuals with cancer in families that meet the Amsterdam criteria 
2. Individuals with 2 HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers* 
3. Individuals with colorectal cancer and a first degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; 1 of the cancers diagnosed at age less than 45 years and the adenoma diagnosed at age less than 40 years 
4. Individuals with colorectal cancer or endometrial cancer diagnosed at age less than 45 years 
5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribiform) on histopathology diagnosed at age less than 45 years† 
6. Individuals with signet-ring-cell-type colorectal cancer diagnosed at age less than 45 years‡ 
7. Individuals with adenomas diagnosed at age less than 40 years

*Endometrial, ovarian, gastric, hepatobiliary, or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter. 
†Solid/cribiform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces. 
‡Composed of more than 50% signet ring cells.
evolves into highly detailed information that is used for research purposes. The extended pedigree is also valuable for identifying other family members at risk for developing HNPCC and its related extracolonic cancers.
From a practical standpoint, the clinician does not need to go into this much depth to diagnose the Lynch syndrome. We believe that the modified nuclear pedigree (Fig 17), wherein attention is given to a detailed description of the proband, with pathologic documentation of cancer, and is extended to that individual’s primary relatives (parents, siblings, and progeny), and then to secondary relatives (maternal and paternal aunts and uncles, and both sets of grandparents), who will be highly genetically informative because they have passed through the cancer risk age, will, in most cases, provide enough information to diagnose the Lynch syndrome, should it be present in the family. The sine qua non for this diagnosis will be the presence of a germline mutation in 1 of the mismatch repair genes.

**Genetics of Lynch Syndrome**

*Microsatellite Instability*. Microsatellite instability (MSI) is a phenomenon observed in some colorectal tumors, especially in patients with HNPCC. It is present in approximately 90% to 95% of cancers in Lynch syndrome, and in approximately 15% of sporadic CRCs. When DNA is amplified using primers derived from highly repetitive DNA sequences, new alleles frequently appear in tumor DNA relative to normal DNA (Fig 18). These new alleles represent small insertions or deletions as a consequence of uncorrected errors in DNA replication. In 1 of the first studies, Thibodeau and colleagues found MSI in 25 of 90 (28%) colorectal cancers, which was more common in proximal versus distal cancers ($P = 0.003$), and these tumors were associated with a better

![PATIENT'S MODIFIED NUCLEAR PEDIGREE](image)

**FIG 17.** The necessary information on first and second degree relatives, which will aid significantly in hereditary cancer syndrome diagnosis.
prognosis than those without microsatellite instability ($P = 0.02$). Aaltonen and colleagues$^{322,323}$ found 86% (25 of 29) of HNPCC colorectal tumors to be replication error positive (RER+, synonymous with MSI) but only 16% (8 of 49) of sporadic colorectal cancers were RER+. This group found that 3% (1 of 33) of sporadic adenomas and 16% (8 of 49) of sporadic carcinomas were RER+, whereas 57% (8 of 14) of adenomas from HNPCC patients were RER+. This suggested that adenomatous polyps were precursor lesions to colorectal cancer in HNPCC.$^{323}$

MSI in colorectal cancers reminded investigators of abnormalities seen in DNA mismatch repair genes from yeast cells, where increases or decreases in the number of repeat units within repetitive DNA sequences

*FIG 18. Microsatellite instability, as demonstrated in 3 normal [N] and colon tumor [T] tissue pairs using the dinucleotide repeat marker D3S1029. Note an extra group of bands in each tumor, representing a new allele (reproduced with permission from Lindblom A, Tannergard P, Werelius B, Nordenskjold M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. Nat Genet 1993;5:279-82).*
result.\textsuperscript{324} DNA mismatch repair genes in bacteria encode for the enzymes mutS (which binds to base mismatches), mutH (which binds to methylated CpG nucleotides and cuts the opposite strand), mutL (a facilitator of mutS and mutH), UvrD (a helicase that excises mismatched strands), DNA polymerase III holoenzyme (which adds new bases), and DNA ligase.\textsuperscript{325} Since yeast lack methylated DNA, they do not have mutH. The homologous 2 mutL proteins in yeast are PMS1 and MLH1, and the homologous protein to mutS is MSH2 (Fig 19). When these yeast genes are mutated, there is a corresponding 100- to 700-fold increase in MSI. It is believed that these genetic changes lead to errors in the number of repetitive sequences replicated, due to lack of repair and DNA polymer-
ase slippage. The observation that tumors in patients with Lynch syndrome had similar changes to those seen in bacterial and yeast cells with DNA mismatch repair gene mutations led investigators to examine the human homologs of these genes in HNPCC.

**Identification of Lynch Syndrome Genes.** In 1993, Peltomäki and colleagues from Finland and Lindblom and colleagues from Sweden discovered sites on chromosomes 2p and 3p by linkage analysis, respectively, as being etiologic for cancer susceptibility in the Lynch syndrome. Aaltonen and colleagues also demonstrated that tumors in Lynch syndrome were RER+.

**hMSH2 Gene Mutations.** Fischel and colleagues identified the mutS homolog MSH2 in humans (hMSH2) and mapped it to chromosome 2. They found that 2 of 7 MSI+ tumors had point mutations affecting a splice acceptor site in hMSH2 and that this same mutation was present in affected members of 2 Lynch syndrome families. The same gene was identified by Leach and colleagues by using a positional cloning strategy, and a gene homologous to mutS was found to map within an interval defined by critical recombinants in Lynch syndrome families linked to a 2p locus. The gene was 2802 bp in length, and a missense mutation was found in 11 affected members of 1 kindred, a splice site mutation in another, and a nonsense mutation in another. Both germline and somatic mutations were found in 1 of 4 RER+ sporadic CRC, suggesting that this gene was a tumor suppressor gene. The important role of hMSH2 in Lynch syndrome was subsequently confirmed by several investigators. Nystrom-Lahti and colleagues found that 4 of 6 Lynch syndrome families linked to chromosome 2p had hMSH2 mutations. Liu and colleagues reported that 10 of 25 Lynch syndrome kindreds had germline hMSH2 mutations, with 9 mutations leading to a truncated protein. Wijnen and colleagues described 7 hMSH2 mutations in 34 Lynch syndrome kindreds leading to protein truncation, and found no evidence of correlation of genotype with phenotype in Lynch syndrome. Maliaka and colleagues found that 4 of 11 Russian and Moldavian Lynch syndrome kindreds had hMSH2 mutations.

**hMLH1 Gene Mutations.** Papadopoulos and colleagues screened human gene databases for sequences with similarities to the yeast mutL genes and found 3 genes, called hMLH1, hPMS1, and hPMS2. These genes were localized to chromosomes 3, 2, and 7, respectively, by somatic cell hybrids. hMLH1 held immediate interest due to the previous finding of genetic linkage in some Lynch syndrome families to chromosome 3p21. This gene was 2268 bp in length and expressed in a wide variety of tissues. They found that 9 of the 10 Lynch syndrome families
demonstrating linkage to markers on 3p had germline hMLH1 mutations whereas only 1 of 18 Lynch syndrome kindreds that were too small to be studied by linkage had mutations, thereby establishing hMLH1 as another important predisposition gene for Lynch syndrome.\textsuperscript{334}

Bronner and colleagues\textsuperscript{335} independently identified hMLH1 by searching a human cDNA library for genes homologous to mutL. They found a gene encoding for a 756-amino acid protein that localized to chromosome 3p21.3-23. They also found that 4 affected members of a Lynch syndrome family had a germline substitution in a highly conserved exon of hMLH1.\textsuperscript{335} Han and colleagues\textsuperscript{336} reported that 8 of 34 (24\%) unrelated Lynch syndrome patients had hMLH1 mutations, and Nystrom-Lahti and colleagues\textsuperscript{330} demonstrated that 3 of 4 Lynch syndrome kindreds linked to 3p had hMLH1 mutations. Kolodner and colleagues\textsuperscript{337} defined the genomic structure of hMLH1 and found a frameshift mutation in 9 of 19 members of a Lynch syndrome kindred linked to markers on 3p. Wijnen and colleagues\textsuperscript{332} found 12 of 34 (35\%) kindreds with hMLH1 mutations, and exons 15 and 16 were hot spots for mutation, accounting for 50\% of reported mutations. No genotype-phenotype correlations were observed for hMLH1 either, as suggested by 3 Lynch syndrome families with a common exon 16 deletion, where 1 had Turcot’s syndrome and the other 2 did not have family members with CNS tumors.\textsuperscript{338}

In cell lines, Parsons and colleagues\textsuperscript{339} demonstrated that mutation of 1 allele in a DNA mismatch repair gene alone did not result in the RER+ phenotype. Hemminki and colleagues\textsuperscript{340} found that one third of patients with germline mutation in hMLH1 also had somatic loss of wild-type hMLH1 in RER+ tumors, consistent with a tumor-suppressor gene function.

Herman and colleagues\textsuperscript{341} described methylation of the hMLH1 promoter in sporadic CRC demonstrating MSI, with subsequent loss of hMLH1 expression. No methylation of hMSH2 was found. Methylation of the hMLH1 promoter is also common in MSI-positive sporadic endometrial cancers, and Simpkins and colleagues\textsuperscript{342} reported this in 41 of 53 such cases.

**hPMS1 and hPMS2 Mutations.** Papadopoulos and colleagues\textsuperscript{334} discovered the human mutL homologs hPMS1 and hPMS2 in 1994 and mapped them to chromosomes 2 and 7. Nicolaides and colleagues\textsuperscript{343} mapped hPMS1 to chromosome to 2q31-33 by FISH and demonstrated the gene encoded for a 932-amino acid protein; hPMS2 mapped to 7p22 and encoded for a 862-amino acid protein. They examined 22 hMSH2 and hMLH1 mutation-negative Lynch syndrome patients and found 1 with a hPMS1 missense mutation and another with a hPMS2 deletion. A tumor
from the latter patient demonstrated deletion of the other hPMS2 allele, supporting a tumor suppressor role. A family with Turcot’s syndrome has also been reported with a missense mutation of hPMS2.

**hMSH6 Gene Mutations.** Another mutS gene homolog was identified in 1995, by virtue of it forming heterodimers with hMSH2, called GTBP (G/T binding protein) or hMSH6. It was found to map in close proximity to hMSH2 on chromosome 2p16, and mutations were rare in Lynch syndrome patients. Cells lacking hMSH6 generally have changes in mononucleotide tracts rather than other simple tandem repeat sequences more commonly present with other MMR genes. Miyaki and colleagues found that 1 of 5 HNPCC families lacking hMSH2 or hMLH1 mutations had an hMSH6 mutation. Wijnen and colleagues reported 10 hMSH6 mutations in 214 hMLH1 and hMSH2 mutation negative families, 71 meeting the Amsterdam criteria (3 mutations) and 143 suspected of having Lynch syndrome but not meeting the Amsterdam criteria (7 mutations). The age at which colon cancer developed was later in these patients, and there was a higher incidence of atypical endometrial hyperplasia and cancer (73%, versus 29% in hMSH2 and 31% in hMLH1 mutation carriers). Wu and colleagues demonstrated that 4 of 18 individuals with Lynch syndrome-associated tumors that were MSI-low had hMSH6 mutations. Berends and colleagues found hMSH6 mutations in 25 of 316 suspected Lynch syndrome cases, and Huang and colleagues detected only 1 of 90 cases with hMSH6 mutations in which hMSH2 or hMLH1 mutations were not found.

**Endometrial Cancer and MMR Germline Mutations.** Berends and colleagues examined the frequency of MMR germline mutations in 58 patients with endometrial carcinoma who were diagnosed at less than 50 years of age. The objective was to relate the occurrence of MMR mutations to family history, as well as to histopathologic data in the presence of MSI positivity and immunostaining to develop criteria for genetic testing. The results showed that 5 of 22 patients who had a positive first degree family history for Lynch syndrome-related cancers had pathogenic germline mutations (1 MLH1, 3 MSH2, and 1 MSH6). Four of the mutation carriers were members of families that fulfilled the revised Amsterdam criteria, whereas no mutations were identified in the 35 patients who lacked such family history ($P = 0.006$). In turn, MSI was identified in 20 of 57 cancers in which 1 or more MMR proteins were absent. Among all 5 MMR mutation carriers, immunostaining identified the involved MMR gene. These findings support the conclusion that an HNPCC-related cancer in a first degree relative is an important selection.
criterion for mutation analysis. Immunostaining of MMR proteins will then aid in targeting the gene(s) that should be analyzed.

**Overview of Genetic Changes.** Lynch syndrome is an autosomal dominant condition, caused by germline changes in DNA mismatch repair genes, which behave as tumor suppressor genes. Individuals born with these germline alterations have 1 normal copy (wild-type) of the gene in each cell and 1 mutant copy. When the wild-type (functional) copy is lost as a somatic event (in a nongerm cell), that cell develops the characteristic of microsatellite instability. The inevitable mistakes that occur during DNA replication are not efficiently repaired, and these changes may lead to unrestrained growth that leads to adenoma, then carcinoma. The numbers of the mutations of various MMR genes in 49 Lynch syndrome families fulfilling the Amsterdam criteria were 21 $hMLH1$ (43%; 19 point mutations, 2 deletions), 22 $hMSH2$ (45%; 10 point mutations, 12 deletions), 2 $hMSH6$ (4%), and 4 (8%) were negative for point mutations or genomic rearrangements. The frequency of $hMSH2$ mutations may have been overestimated in this study, however, because 7 families had the same 20 kb deletion encompassing exons 1-6 and appeared potentially to share a common ancestor. A compilation of published and unpublished mutations have been established by the International Collaborative Group on HNPCC (http://www.nfdht.nl), in which the numbers of $hMLH1$ mutations reported were 323 (63% of total), $hMSH2$ 156 (25%), $hMSH6$ 30 (6%), $hPMS2$ 2 (0.4%), and $hPMS1$ 1 (0.2%). The mutations found in the $hMLH1$ and $hMSH2$ genes are distributed throughout the exons, and no clear correlations between genotype and phenotype are known, with the exception of the higher incidence of MSI-low and endometrial cancers in those patients with $hMSH6$ mutations.

One might appropriately probe more deeply into the question about why only approximately one half of Lynch syndrome families will have an MMR mutation identified, or why some classical FAP families may not have an $APC$ germline mutation identified. This vexing problem has been addressed by Renkonen and colleagues in both HNPCC and FAP families that lack any sequence changes for their respective known susceptibility genes. These authors suggest that these genes either have alterations that have escaped detection by conventional techniques, or alternatively, other, as yet unknown, susceptibility genes are involved. Furthermore, they suggest that significant proportions of families that are presumed to be mutation negative (11 of 26 families in their study, or 42% for Lynch syndrome; 4 of 16 families, or 25% for FAP) harbor “hidden” alterations in known predisposition genes, leading to their
conclusion that such changes may be detected by expression-based methods.\textsuperscript{354,355}

**Founder Mutations in Lynch Syndrome.** De la Chapelle and Wright\textsuperscript{356} evaluated 2 founder mutations in the \textit{hMLH1} gene in Finland, which accounted for approximately one half of all Lynch syndrome germline mutations in that country.\textsuperscript{357,358} They identified haplotype sharing “. . . over a genomic region as large as 18 cM indicated a relatively recent founding of the more prevalent mutation. . . wherein the ‘age’ of this mutation in most of the 19 kindreds studied could be estimated at 16-43 generations in keeping with historical records and compatible with a founding in a regional subsisolate in new Finland in the early 1500s.”\textsuperscript{357,359} This research, once the mutations became more fully understood and characterized, indicated that their presence in a patient or family could identify individuals who could benefit significantly from highly targeted cancer control programs.\textsuperscript{360}

A founder mutation consisting of a 4-bp deletion beginning at the first nucleotide of codon 727 in \textit{hMLH1} has been identified in Navajo families from Arizona. A deletion encompassing exons 1-6 of the \textit{MSH2} gene has been identified in an extended American kindred of German origin.\textsuperscript{315}

Foulkes and colleagues\textsuperscript{361} described a founder mutation of \textit{hMSH2}(1906G\textless{}C) in the Ashkenazi Jewish population. This mutation results in a substitution of proline for alanine at codon 636 in the MSH2 protein and was identified in 15 unrelated Ashkenazi Jewish families with Lynch syndrome who met the Amsterdam criteria. These families shared the same alleles with 18 polymorphic loci within and flanking the \textit{MSH2} region, which was consistent with a single origin for this mutation. Through immunohistochemical analysis, all CRCs that were tested were found to harbor MSI and absence of the MSH2 protein. These authors then provided an analysis of a population-based incidence series of 686 Ashkenazi Jews from Israel who manifested CRC. Their results showed that 3 (0.44\%) were mutation carriers. Furthermore, those patients with a family history of CRC or endometrial cancer were more likely to harbor the mutations than those without such a family history (\textit{P} = 0.042). Individuals with CRC who were mutation carriers were, on average, younger than CRC-affected individuals who were noncarriers (\textit{P} = 0.033). Five hundred sixty-six unaffected Ashkenazi Jews from Israel and 1022 controls from New York were all found to be noncarriers. In hospital-based series, the 1906 allele was identified in 5 of 463 Ashkenazi Jews with CRC, 2 of 197 with endometrial cancer, and 0 of 83 with ovarian cancer.

Guillem and colleagues\textsuperscript{362} identified this rare founder mutation, \textit{hMSH2}
1906C→G, in Ashkenazi Jews in 8 of 1345 individuals (0.6%) with CRC. They then investigated the proportion of individuals of Ashkenazi heritage who manifested early-onset CRC (age ≤ 40 years) that could be explained by hMSH2 1906C→G. They detected this mutation in 3 of the 41 samples (7.14%) among Ashkenazi patients with CRC diagnosed before age 40, an incidence found to be significantly greater than the 8 in 1345 (0.6%) that was observed in CRC among Ashkenazi Jews who were not selected for age (P = 0.004). They concluded that their results merit testing for the hMSH2 1906C→G mutation among Ashkenazi Jews manifesting early-onset CRC.362

Strategy for Genetic Testing. As in other autosomal dominant conditions, the affected proband should be studied thoroughly for mutations to allow for directed testing later in other members at risk. This should be performed in patients meeting the Amsterdam criteria, or in those who are suspected of having Lynch syndrome but do not meet the criteria (as in the Bethesda guidelines). The Lynch syndrome patient’s tumor should first be tested for MSI, and testing for MSH2 or MLH1 expression by immunohistochemistry can also be quite helpful in determining which gene is most likely affected in the family. If MSI is not found, then sequencing of MMR genes is not warranted. If the tumor is MSI-positive or loss of protein expression is found, then sequencing of hMLH1 and/or hMSH2 is indicated. The frequency of PMS gene mutations is so low that their sequencing is not generally performed; hMSH6 sequencing may be indicated in cases with a family history suggestive of Lynch syndrome where tumors are MSI-low or there is a high incidence of endometrial cancer. In rare cases, there may be mutations in PMS2, MLH3, and EXO1.363

Lynch Syndrome Versus Early Onset Sporadic Colon Cancer. Even in those cases in which the Amsterdam criteria have not been met, Jass has suggested that the profile of early-onset MSI-high (MSI-H) CRCs resembles that of Lynch syndrome cancer, with emphasis on the histologic features and site of tumors. Furthermore, Jass provides 5 cogent and interrelated reasons for questioning the presumption that early-onset “sporadic” MSI-H CRCs are truly sporadic. Specifically, he notes that “... first is the fact that the incidence of HNPCC peaks at around 45 years. Second is the finding of germline mutations in DNA mismatch repair genes in subjects presenting with early-onset “sporadic” MSI-H colorectal cancer. Third is the evidence that methylation of hMLH1 in sporadic MSI-H cancer is strongly age-related. Fourth is the fact that methylation of hMLH1 may occur selectively in HNPCC cancers in subjects who carry a germline mutation in hMLH1. Fifth is the finding of
HNPCC-type molecular features among early-onset “sporadic” MSI-H colorectal cancers.”

**Pathologic Features in Lynch Syndrome.** The pathologic features of Lynch syndrome CRCs include a solid growth pattern, which appears to be responsible for the high frequency of poorly differentiated carcinomas. Surprisingly, however, is the finding that CRCs in the Lynch syndrome are not as aggressive as their failure to form tubules might suggest. Lynch and colleagues have called attention to the “undifferentiated carcinoma” described by Gibbs and the “medullary carcinoma” described by Jessurun and Manivel, which were published in small case series and shown to have a more favorable prognosis when compared with typical CRC. It is also noteworthy that similar histologic features characterize the 15% of sporadic CRCs that harbor microsatellite instability (MSI+), which have been found to constitute a molecular aberration in CRCs that lack MMR activity. This special histologic feature has been referred to as “solid cribiform growth” wherein there is a positive predictive value of 53% for MSI+ status.

Smyrk and colleagues described a postlymphoid response referred to as “Crohn’s-like reaction” to be more common in Lynch syndromes as opposed to sporadic cancers, a finding that has been shown to be consistent in all series, but which is similar to the tendency to form lymphoid aggregates around the tumor, and which is a frequent feature of sporadic MSI+ CRCs. Importantly, the Crohn’s-like reaction is often associated with improved prognosis in the general population, suggesting that this phenomenon may account for the more favorable prognosis of CRC that occurs in HNPCC. De Jong and colleagues studied the role of MMR defects and the manner in which they promote development of adenomas in Lynch syndrome families compared with controls from the Dutch HNPCC Registry. They identified 249 MMR mutation carriers and 247 controls. They found that the proportion of patients lacking an adenoma at the age of 60 years was 29.7% for carriers and 70.8% for controls ($P < 0.05$). They verified previous studies showing that the adenomas in carriers were larger and showed a higher proportion of villous components and/or high-grade dysplasia ($P < 0.05$, all analyses). Furthermore, they found the adenomas and carcinomas of the carriers to be located predominantly in the proximal colon, and most of the adenomas showed absent staining of the MMR proteins. They concluded that their study “... indicates that the MMR defect is involved in the early stages of development of adenomas.” They recommended immunohistochemical staining of large adenomas showing high-grade dysplasia in
patients younger than 50 years old, in the interest of identifying patients with suspected Lynch syndrome.  

**Pattern Recognition.** An alternative to the Amsterdam I, Amsterdam II, Bethesda, and other criteria is what we have described as “pattern recognition.” This involves careful scrutiny of the phenotypic cancer features expressed in the family in accord with their mode of distribution and transmission throughout the family. This is particularly important when evaluating small families, or for problems of reduced penetrance of the deleterious MMR mutation, should this be present in the family. It is valuable then to follow what have been considered to be the cardinal features of hereditary forms of cancer, including the Lynch syndrome, when present even in a single patient. These phenotypic features have often been sufficient to raise the confidence level of a possible Lynch syndrome diagnosis and include the following: 1) autosomal dominant inheritance pattern; 2) gene penetrance for CRC of ~85% to 90%; 3) gene carriers develop CRC at an early age (~45 years); 4) most (~70%) of the CRCs are proximal to the splenic flexure; 5) multiple CRCs, both synchronous and metachronous, are common; 6) the prognosis is better than that for sporadic CRC; 7) the pathologic features of CRC are often distinguishable (but not pathognomic) and include poor differentiation, mucoid excess with increased signet cells, medullary features, peritumoral lymphocytic infiltration, Crohn’s-like reaction, and tumor infiltrating lymphocytes (TILs) admixed with tumor cells; and 8) there is an increased risk for malignancy at several extracolonic sites, particularly the endometrium, ovary, stomach, small bowel, hepatobiliary tract, pancreas, ureter, renal pelvis, and brain. In addition, breast cancer excess may be present in some HNPCC families. Although gastric cancer has declined in Lynch syndrome families since 1900, paralleling its decline in the general population, it is still an important feature of the syndrome in geographic areas in which gastric cancer is endemic, most notably Japan and Korea.  

**Muir-Torre Syndrome (MTS).** In 1981, Lynch and colleagues reported the first observation of MTS in the Lynch syndrome. MTS is characterized by multiple cutaneous sebaceous adenomas, sebaceous carcinomas, multiple keratoacanthomas, and multiple visceral cancers, in Lynch syndrome families. Several publications have elucidated the clinical and molecular genetic features of MTS in the Lynch syndrome. Kruse and colleagues examined 13 patients with CRC and sebaceous lesions of the skin and found that 8 had hMSH2 mutations and 1 had an hMLH1 mutation. Data suggest that the identification of these MTS cutaneous features in a patient merit a detailed family history.

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in the search for evidence of the Lynch syndrome. Indeed, patients with these stigmata merit MMR germline testing, particularly for evidence of \textit{hMSH2} germline mutation. The pedigree of an extended Lynch syndrome family with MTS that is known to carry an \textit{hMSH2} mutation is shown in Fig 20.

**Surgical Management.** In Lynch syndrome patients diagnosed with colorectal cancer, the preferred treatment is subtotal colectomy. Fitzgibbons and colleagues\textsuperscript{388} studied 116 patients with Lynch syndrome and demonstrated several significant differences in this patient population relative to patients with sporadic CRC. They found that 69\% of Lynch syndrome CRC were proximal to the splenic flexure, whereas 23\% were in the rectosigmoid. Eighteen percent had synchronous cancers, and 40\% developed metachronous lesions over 10 years of follow-up.\textsuperscript{388} Because of the predominance of proximal lesions and the high risk of developing new cancers in the future, subtotal colectomy with IRA and lifetime surveillance of the rectosigmoid is recommended. For women beyond childbearing age, serious consideration should also be given to performing prophylactic hysterectomy and bilateral salpingo-oophorectomy.\textsuperscript{388}

In reviewing this subject, Church\textsuperscript{389} suggests that interval CRCs develop from normal epithelium within 3 years and/or from adenomas that were missed. It is also important to realize that colonoscopy “miss” rates are as high as 29\% for polyps less than 5 mm in diameter.\textsuperscript{390} Therefore, patients should be advised that colonoscopy, although not a perfect screening procedure, is nevertheless highly effective.\textsuperscript{360} The option of prophylactic colectomy should be discussed, weighing it against lifetime colonoscopy.\textsuperscript{391-394} The problem in effective cancer control through the use of prophylactic colectomy is a decision based heavily on the attitudes, feelings, and concerns of the patient and the manner in which these could impact on his/her compliance with annual colonoscopy. For example, the presence of fear and anxiety about what might be found at colonoscopy, in our experience, has deterred patients in showing compliance with this screening procedure for CRC. Criteria for prophylactic surgery are the following: 1) lack of compliance with surveillance; 2) morbid fear and desire to have these high risk organs surgically excised; 3) presence of colonic adenomas, particularly at a young age and/or with features of moderate to severe dysplasia and carcinoma in situ in polyps.

Women at risk for the Lynch syndrome should have annual screening for endometrial and ovarian cancer beginning at age 30 to 35 years. Endometrial aspiration coupled with transvaginal ultrasound is advised. CA-125 testing, in addition to transvaginal ultrasound and Doppler color
FIG 20. Pedigree shows the Muir-Torre syndrome.
blood flow imagery, should be performed semiannually for ovarian cancer. Prophylactic hysterectomy and oophorectomy can be considered when childbearing is completed.

**Screening in Lynch Syndrome.** Jass\(^{395}\) has elucidated the pathologic nature of precursor lesions in HNPCC. He has postulated the “aggressive adenoma” theory (i.e., adenomas form earlier but about as often in HNPCC patients as in the general population).\(^{395}\) However, once formed, these colonic adenomas progress to carcinoma more quickly and/or more often than their sporadic counterparts. A tiny colonic adenoma in the Lynch syndrome may evolve into a carcinoma in as short a time frame as 2 to 3 years, compared with approximately 8 to 10 years in the general population.\(^{303,365,396}\) Strong clinical evidence in support of a more rapid evolution of adenoma to carcinoma comes from a Finnish study showing a marked decrease in colon cancer incidence for HNPCC patients given regular colonoscopic surveillance with removal of adenomas.\(^{397}\) Because of accelerated carcinogenesis, proximal colonic predilection, and early age of CRC onset in the Lynch syndrome, we strongly recommend that annual full colonoscopy be initiated at age 25 (Fig 21). A good clean-out is necessary and excellent visualization of the cecum is mandatory, because approximately one third of CRCs occur at that particular site.

Järvinen and colleagues\(^{360}\) demonstrated the benefit of colonoscopic screening in HNPCC through a controlled clinical trial extending over 15 years. The incidence of CRC was compared in 2 cohorts of at-risk members of 22 HNPCC families. CRC developed in 8 screened subjects (6%), compared with 19 controls (16%; \(P = 0.014\)). The CRC rate was reduced by 62% in those who were screened. All CRCs in the screened group were local, causing no deaths, compared with 9 deaths caused by CRC in the controls. It was concluded that CRC screening at 3-year intervals more than cuts in half the risk of CRC, prevents CRC deaths, and decreases the overall mortality rate by approximately 65% in HNPCC families.\(^{360}\) The relatively high incidence of CRC even in the screened subjects (albeit without deaths) in our opinion argues for shorter screening intervals (i.e., 1 year). For example, Vasen and colleagues\(^{398}\) discovered 5 interval cancers in HNPCC patients within 3.5 years following a normal colonoscopy. Prophylactic colectomy, as discussed, is an alternative to intensive colonoscopic surveillance in highly selected members of Lynch syndrome families. An algorithm showing the appropriate sequence for diagnosis and management of Lynch syndrome is shown in Figure 21.
Juvenile Polyposis

The colorectal polyps most commonly found in children are different from those found in adults and are called juvenile polyps. Polyps in adults are generally adenomatous, whereas polyps in children tend to be hamartomatous (juvenile polyps being a type of hamartomatous polyp). In most children, the juvenile polyps are few in number or solitary, will slough off and not require treatment, and are not associated with a heritable predisposition. Juvenile polyposis (JP; OMIM 174900) is a heritable syndrome in which there are multiple juvenile polyps (Fig 22). Patients with juvenile polyposis, Cowden syndrome (CS; OMIM 158350), and Bannayan-Riley-Ruvalcaba syndrome (BRRS; OMIM 153480) may all develop juvenile polyps of the GI tract, which are indistinguishable microscopically. Patients with Peutz-Jeghers syndrome (PJS; OMIM 175200) also have hamartomatous polyps, but these can be differentiated histologically from juvenile polyps.
Part B

Screening and management melded to cardinal features of Lynch Syndrome

<table>
<thead>
<tr>
<th>Cardinal Features of Lynch Syndrome</th>
<th>Screening/Management</th>
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<tr>
<td>Proximal colonic predilection</td>
<td>Colonoscopy</td>
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<td>Early age of onset</td>
<td>Initiate at age 25</td>
</tr>
<tr>
<td>Accelerated carcinogenesis</td>
<td>Repeat colonoscopy annually</td>
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<tr>
<td>Predisposition to synchronous and metachronous CRCs</td>
<td>If CRC, subtotal colectomy</td>
</tr>
<tr>
<td>Extracolonic cancers:</td>
<td></td>
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<tr>
<td>Most Common:</td>
<td></td>
</tr>
<tr>
<td>Endometrial</td>
<td>Endometrial aspiration semi-annually, Transvaginal US</td>
</tr>
<tr>
<td>Ovary</td>
<td>Transvaginal US, Doppler color blood flow imagery, CA-125 and repeat annually</td>
</tr>
<tr>
<td>Stomach</td>
<td>Upper endoscopy, particularly in Orient (Japan, Korea) or families with gastric cancer, repeat annually</td>
</tr>
<tr>
<td>Hepatobiliary, small bowel, pancreas</td>
<td>No practical screening with acceptable sensitivity/specificity</td>
</tr>
<tr>
<td>Upper uroepithelial tract (ureter, and/or renal pelvis)</td>
<td>Urine cytology, US, positive FH of lesions, families with MTS</td>
</tr>
<tr>
<td>Brain</td>
<td>Positive FH, but no known screening efficacy</td>
</tr>
<tr>
<td>Sebaceous adenomas, sebaceous carcinomas, multiple keratoacanthomas (MTS)</td>
<td>Cutaneous beacon to screen family for Lynch syndrome tumors, coupled with meticulous cutaneous examinations</td>
</tr>
<tr>
<td>Distinguishing pathology features: Poorly differentiated, mucinous features with signet cell excess, increased diploidy, tumor infiltrating lymphocytes, lymphocytic infiltration at periphery, Crohn’s-like reaction, increased diploidy</td>
<td>Useful for diagnosis</td>
</tr>
<tr>
<td>MMR mutations</td>
<td>Enable certainty in diagnosis</td>
</tr>
<tr>
<td>Most common:</td>
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<td>MLH1, MSH2, MSH6</td>
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<tr>
<td>Survival advantage</td>
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**Abbreviations:**
- CRC: colorectal cancer
- FH: family history
- MMR: mismatch repair
- MSI: microsatellite instability
- MTS: Muir-Torre syndrome
- US: ultrasound

FIG 21. Continued
History. The first description of a juvenile polyp was by Diamond\textsuperscript{399} in 1939, who originally called this a rectal adenoma. Diamond\textsuperscript{399} described it grossly as a 2.5 by 2.0 cm pedunculated tumor that was injected and friable. One small area on the polyp surface was covered with a fibrinous exudate. Histologically, the polyp was lined with high columnar epithelial cells with an occasional bare area. The glands were distended and filled with a fibrinous cellular material. The stroma was diffusely infiltrated with lymphocytes, plasma cells, eosinophils, and neutrophils.\textsuperscript{399} In 1957, Mauro and Prior\textsuperscript{400} clearly distinguished between juvenile polyps and adenomatous polyps. Contrasting the 2, they found juvenile polyps to be globular in shape, gray to slightly red, with a granular external surface. Villous processes were not present. It was common to have areas of denudation of the columnar epithelium replaced by granulation tissue. There was cystic gland dilation, containing mucous and nuclear debris. The abundant stroma contained an inflammatory cell infiltrate with a predominance of eosinophils.\textsuperscript{400} Later in the same year, Horrilleno and colleagues\textsuperscript{401} coined the term juvenile polyp for these histologically similar polyps described previously by Diamond and by Mauro and Prior.
In 1962, Morson\textsuperscript{402} supported the view that juvenile polyps were different from adenomatous polyps and suggested they were hamartomatous polyps. Hamartomas are an overgrowth of mature cells and tissues that normally occur in the affected part, but often with 1 element predominating. Juvenile polyps satisfy this definition with their excessive proliferation of the lamina propria and attenuation of the muscularis mucosa (Fig 23).

**Definition and Manifestations.** The term juvenile polyposis (JP) was defined by McColl and colleagues\textsuperscript{403} in 1964. This distinguished juvenile polyps from JP, a heritable syndrome in which there are multiple juvenile polyps involving the GI tract. In 1974, following the definition of JP by McColl, Sachatello and colleagues\textsuperscript{404} proposed a working definition, later modified by Jass and colleagues,\textsuperscript{405} with the following diagnostic criteria: 1) more than 5 juvenile polyps involving the colon and rectum, 2) juvenile polyps throughout the GI tract, and 3) any number of juvenile polyps involving the GI tract with a family history of JP. JP has been further divided based on the clinical presentation and disease course into the following three classifications:\textsuperscript{404,405} 1) juvenile polyposis of infancy, 2)
juvenile polyposis coli, and 3) generalized juvenile polyposis. Sachatello and colleagues defined juvenile polyposis of infancy in a case report and reviewed 6 similar cases in the literature. In all 7 cases, the infants died before 1 year of age. Characteristic symptoms included diarrhea (usually bloody), protein losing enteropathy, hypoproteinemia, anemia, anasarca, and eventually failure to thrive. It was common to find rectal polyp prolapse and intussusception. Congenital anomalies ranged in these series from none to clubbing, motor retardation, macrocephaly, alopecia, double renal pelvis, and bifid uterus/vagina.

Juvenile polyposis coli and generalized juvenile polyposis are defined by the sites of GI involvement with juvenile polyps. As the name implies, juvenile polyposis coli occurs when juvenile polyps are limited to the colon and rectum, whereas in generalized juvenile polyposis, the polyps are present in both the upper and lower GI tract (Fig 24). Both subtypes will usually manifest in the first and second decades with rectal bleeding, a prolapsed rectal polyp, abdominal cramps or pain, diarrhea, or ane-

FIG 24. Juvenile polyposis affecting the stomach, resulting in a dense carpet of polyps.
In addition, generalized GI juvenile polyposis may include protein-losing enteropathy, intussusception, and severe malnutrition. However, these children, unlike those with juvenile polyposis of infancy, eventually recover. Coburn and colleagues reviewed the English literature and identified 218 patients meeting the criteria for JP. These patients with JP were stratified into 2 groups: juvenile polyposis coli and generalized juvenile polyposis. Patients with juvenile polyposis coli usually presented during childhood between the ages of 5 and 15 years, whereas patients diagnosed with generalized juvenile polyposis present at an earlier age. Otherwise, there did not appear to be any difference in the family history of juvenile polyposis in either subtype.413 It should be mentioned that the distinction between juvenile polyposis of infancy, juvenile polyposis coli, and generalized juvenile polyposis may be somewhat arbitrary. Stemper and colleagues reported a kindred with some members having generalized GI juvenile polyposis whereas others had juvenile polyposis coli, suggesting the phenotype of juvenile polyposis is of variable penetrance. Similarly, Grotsky and colleagues described an extended family with both juvenile polyposis coli and juvenile polyposis of infancy.

**Associated Anomalies.** In their 1964 series, McColl and colleagues described 4 of 11 patients with associated congenital anomalies. Coburn and colleagues reported nearly a 15% prevalence of congenital malformations with JP. Interestingly, Desai and colleagues, who routinely obtained radiographs and an echocardiogram if clinically indicated, reported 18 of 23 (78%) patients with JP having extracolonic manifestations. This number is clearly skewed since after the extensive re-evaluation 5 patients had alternative syndromes diagnosed, and an unspecified number were described as having similarities to known syndromes. Removing these cases leaves 8 of 13 (62%) patients with presumed JP associated with extracolonic manifestations. Throughout the JP literature, some of the recurrent anomalies include the following: macrocephaly, mental retardation, atrial and ventricular septal defects, pulmonary arteriovenous malformations, pulmonary stenosis, Meckel’s diverticulum, malrotation, cryptorchidism, hypertelorism, and telangiectasias.

**Malignant Potential.** An extensive portion of the literature on JP has been dedicated to the discussion of its malignant potential. It was initially thought that JP did not predispose to GI malignancy, since juvenile polyps are hamartomatous. As it became apparent that adenomatous polyps had malignant potential, an increased prevalence of colorectal cancer was recognized with JP patients. During this time adenomatous changes in
juvenile polyps were recognized\textsuperscript{414,415,417-434} and hypothesized occasionally to undergo dysplastic change and eventually progress to adenocarcinoma. In 1979, Goodman and colleagues\textsuperscript{422} reported a 23-year-old woman undergoing a colectomy with numerous juvenile polyps mainly localized to the cecum, ascending colon, and rectum. Several of the juvenile polyps showed adenomatous changes, and an adenocarcinoma had also developed in the rectum.

After adenomatous changes, the next step in the progression to malignancy would be dysplasia/carcinoma in situ changes in the juvenile polyps of patients with JP. Jass and colleagues\textsuperscript{405} reviewed the pathologic features of 1038 polyps collected from 80 patients in the St. Mark’s Polyposis Registry with JP. They divided the polyps into 2 groups: 1) multilobulated or having a villous configuration, and 2) typical juvenile polyps. Seventy-nine of 169 (47\%) atypical juvenile polyps had epithelial dysplasia, whereas 76 of the 840 (9\%) typical juvenile polyps had similar dysplastic changes.\textsuperscript{405} Several other investigators have reported the finding of dysplastic changes in juvenile polyps in JP patients.\textsuperscript{428,435,436} There have been many cases of documented colorectal adenocarcinoma reported in patients with JP.\textsuperscript{414,422,427,428,430-432,437,438} These cancers are often closely associated with juvenile polyps and juvenile polyps with adenomatous changes, supporting a juvenile polyp-adenomatous changes-dysplasia-carcinoma sequence of events.

The establishment of JP registries has allowed estimates of the cumulative risk for developing GI malignancies to be made. Howe and colleagues\textsuperscript{439} examined a 117-member Iowa JP family and found that 11 of the 59 (38\%) affected kindred members developed colon cancer, whereas 6 of 59 (21\%) developed upper GI cancers. The cumulative risk for upper and lower GI cancers was 55\%. The cumulative risk for colorectal cancer in JP patients has ranged from 17\% to 68\% in other reports.\textsuperscript{413,440}

The distinction between JP and patients with juvenile polyps is important when estimating the risk of GI malignancy. Many authorities have suggested that there is no inherent increased risk of GI malignancy for patients with solitary juvenile polyps not meeting the definition of JP. Giardello and colleagues\textsuperscript{432} found that only 1 of 26 patients with only 1 or 2 juvenile polyps and a negative family history had adenomatous changes in a polyp, whereas 9 of 31 (29\%) patients with 3 or more juvenile polyps or a positive family history of JP had colonic adenomas or adenocarcinoma. There is only 1 reported case of an adenocarcinoma arising from a presumed solitary juvenile polyp in a 16-year-old boy.\textsuperscript{441} Nugent and colleagues\textsuperscript{442} reported that the relative risk of dying of
colorectal malignancy in a patient with a solitary juvenile polyp was 0.66, and therefore these patients are not at an increased risk of death from colorectal carcinoma.

**Genetics.** The autosomal dominant nature of JP was first suggested by Smilow and colleagues and Veale and colleagues in 1966. Further descriptions of kindreds with juvenile polyposis confirmed an autosomal dominant inheritance. More recent investigation has shifted from the clinical to the molecular basis of JP. Jacoby and colleagues described a patient in 1997 with JP having a deletion at chromosome 10q22. Soon thereafter, Olschwang and colleagues reported 3 cases of JP with germline PTEN mutations, which maps to 10q22. However, on closer evaluation by Eng and Ji, these patients either had a phenotype suggestive of CS or, due to their young age, CS could not be ruled out. Howe and colleagues examined a family of 43 individuals of which 13 had the clinical diagnosis of JP and found no linkage to chromosome 10q markers. Marsh and colleagues were unable to identify germline mutations in PTEN in 14 familial and 11 sporadic JP cases. Riggins and colleagues also found no PTEN mutations in 11 JP patients. In 1998, Howe and colleagues demonstrated genetic linkage to markers on chromosome 18q21, and subsequently reported germline mutations of MADH4 in 5 of 9 JP cases. This was independently confirmed in multiple small series. Woodford-Richens and colleagues found 7 of 44 (16%) cases of JP with MADH4 mutations, and Howe and colleagues reported mutations in 14 of 77 JP cases (18.2%). In the latter report, 8 mutations were deletions and 6 were substitutions (1 nonsense, 5 missense). Reviewing all published mutations, Howe and colleagues described a total prevalence of 23% (32 of 141 cases with MADH4 mutations). One mutation had been described in 6 cases, a 4-bp deletion in exon 9. Of 32 total mutations, 1 mapped to the MH1 domain, 5 to the linker region and the remainder to the MH2 domain (Fig 25).

The MADH4 protein is the common mediator involved in the transforming growth factor-β (TGF-β) superfamily, which includes TGF-β, activin, and bone morphogenetic protein (BMP) signal transduction pathways. Functionally, MADH4 acts as a tumor suppressor gene, as demonstrated by Hahn and colleagues, who found loss of heterozygosity (LOH) of MADH4 in 25 of 84 (30%) pancreatic xenografts. Members of the TGF-β superfamily initiate a wide spectrum of effects on a variety of cell types, including control of differentiation, proliferation, and apoptosis. TGF-β binds to plasma membrane serine/threonine kinases, specifically the type II TGF-β receptor. TGF-β and the type II
TGF-β receptor complex then binds with the type I receptor, causing phosphorylation in a glycine-serine-rich domain of the receptor (Fig 26). In the TGF-β pathway, these activated type I receptors then phosphorylate cytoplasmic MADH2 or MADH3. Phosphorylation allows these proteins to oligomerize and then to associate with MADH4. The complex then migrates to the nucleus, where it recruits a DNA binding protein, and the complex binds to specific DNA sequences, regulating the transcription of various genes, many of which remain to be identified. The MH2 domain of MADH4, which is frequently altered in JP patients and sporadic pancreatic cancers, is important in nuclear localization, MADH4 binding, and activation of transcription. There are 8 known MADHs in...
vertebrates, with MADH2 and MADH3 functioning as the cytoplasmic effectors in the TGF-β and activin pathways; MADH1, MADH5, and MADH8 are those involved in the BMP pathway. MADH4 is the common mediator for these pathways, whereas MADH6 and MADH7 function as inhibitors of all these pathways by binding to the type I receptors and by interfering with phosphorylation. To date, no other MADH genes have been implicated in the development of JP. Roth and colleagues found no mutations in MADH2, MADH3, or MADH7 in 4 unrelated patients without MADH4 mutations, and Bevan and colleagues found no mutations in MADH1, MADH2, MADH3, or MADH5 in 30 JP patients without MADH4 mutations.

Since MADH4 mutations only accounted for one fifth of JP cases, Howe and colleagues searched for another JP gene through a linkage-based approach.

genome screen in 4 additional JP families. They found linkage to markers on chromosome 10q22-23, and subsequently each family was found to have a truncating mutation in the BMPR1A gene.462 Since the initial report of BMPR1A mutations in JP patients, there have been 2 confirmatory studies published.463,464 In a total of 77 JP cases studied by Howe and colleagues, there have been 16 (21%) with BMPR1A mutations, consisting of 6 deletions and 10 substitutions (4 nonsense, 6 missense).457 Pooling all 3 reports, 31 unique mutations have been described, distributed in 8 of 11 exons with nearly one half affecting the intracellular protein kinase region in exons 7 and 8. One quarter of mutations affect the extracellular cysteine-rich domain (Fig 27).457 BMPR1A is another member of the TGF-β superfamily involved in a pathway that also depends on MADH4 as the intracellular mediator of signal transduction (Fig 26). Like TGF-β, the various BMPs bind to specific type II receptors or to the type II and type I receptors together, then the type II receptor phosphorylates a type I receptor, such as BMPR1A or BMPR1B.465 The type I/type II receptor complex then phosphorylates MADH1, MADH5, and/or MADH8, which then form oligomers with MADH4, and these proteins migrate to the nucleus to regulate transcription of specific genes.460 BMPs exert many effects on a variety of tissues, including control of osteogenesis, chondrogenesis, mesoderm development, synthesis of extracellular matrix, and epithelial/mesenchymal cell relationships contributing to morphogenesis.466

A recent study examining MADH4 and BMPR1A mutation-positive (MUT+?) and negative (MUT−?) cases found that that 17 of 19 (89%) MUT+ patients and 13 of 25 (52%) MUT− patients cases had a family history of GI cancer (P = 0.01), and that 89% of MUT+ and 63% of MUT− cases were familial (P = 0.09).467 The familial prevalence of upper GI juvenile polyps was 86% in MADH4 mutation-positive JP families, 10% in BMPR1A families, and 23% in MUT− cases. Friedl and colleagues464 reported gastric polyposis in 4 of 7 MADH4 mutation-positive cases, in 1 of 5 cases with BMPR1A mutations, and 2 of 17 mutation-negative cases. It therefore appears that MADH4 mutations predispose to generalized juvenile polyposis, whereas MADH4 mutation-negative cases more commonly cause juvenile polyposis coli. Since MADH4 and BMPR1A mutations only account for 40% of JP cases, there are likely other genes responsible for JP that await discovery.

**Treatment.** Because of the malignant potential of juvenile polyps in JP, endoscopic screening and polypectomy, and prophylactic surgery in some cases, are very important means of cancer control. Screening should begin at age 15, and include esophagogastroduodenoscopy, colonoscopy, and a
hemogram in those at risk for JP. If a mutation of MADH4 or BMPR1A is found in a family, then the process is simplified by testing individuals at risk for the same mutation. If no mutation is found, then that individual can be followed by using the guidelines for screening the general population for sporadic colorectal cancer. If either a mutation is found in an at-risk patient, or a mutation has not been found in the family, then colonoscopy and upper endoscopy should be performed every 3 years. When juvenile polyps are found in the colon, they should be removed endoscopically, which may require multiple sessions if the number is greater than 20 to 50 polyps.

**FIG 27.** BMPR1A mutations reported in juvenile polyposis (JP). The upper rectangle represents the BMPR1A gene, with the exons shown within the rectangle and nucleotides above and below. Lines designate mutations identified in JP cases, with those shown above each exon corresponding to those identified by, while those below are derived from the reports of. The lower rectangle represents the different known domains of the BMPR1A gene (reproduced with permission from Howe JR, Sayed MG, Ahmed AF, Ringold J, Larsen-Haidle J, Merg A, et al. The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. J Med Genet 2004;41:484-91).
Subsequently, annual endoscopy should be performed until the all polyps are removed, and then every 3 years after this if no dysplastic polyps are found. If the colon cannot be rendered polyp free using the endoscope, then surgical management may be needed. The range of surgical options include segmental colectomy, subtotal colectomy, and total proctocolectomy with ileoanal pull-through. Since the malignant potential of juvenile polyps in JP is low relative to more aggressive polyposis syndromes, one must consider carefully the impact of the chosen procedure on quality of life versus the need for future endoscopic surveillance. Subtotal colectomy is preferred over segmental colectomy since it limits the amount of colon at risk, and patients can be screened easily by flexible sigmoidoscopy. Total proctocolectomy should be

<table>
<thead>
<tr>
<th>TABLE 10. International Cowden Consortium diagnostic criteria*</th>
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<tr>
<td>Pathognomonic criteria</td>
</tr>
<tr>
<td>Facial trichilemmomas</td>
</tr>
<tr>
<td>Acral keratoses</td>
</tr>
<tr>
<td>Papillomatous papules</td>
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<tr>
<td>Mucosal lesions</td>
</tr>
<tr>
<td>Major criteria</td>
</tr>
<tr>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Thyroid carcinoma, nonmedullary</td>
</tr>
<tr>
<td>Macrocephaly</td>
</tr>
<tr>
<td>Lhermitte-Duclos disease (LDD)</td>
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<tr>
<td>Endometrial carcinoma</td>
</tr>
<tr>
<td>Minor criteria</td>
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<tr>
<td>Thyroid lesions, adenoma or multinodular goiter</td>
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<tr>
<td>Mental retardation</td>
</tr>
<tr>
<td>Gastrointestinal hamartomas</td>
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<tr>
<td>Fibrocystic disease of the breast</td>
</tr>
<tr>
<td>Lipomas</td>
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<tr>
<td>Fibromas</td>
</tr>
<tr>
<td>Genito-urinary tumors (renal cell carcinoma or uterine fibroids) or malformations</td>
</tr>
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Operational diagnosis in an individual
1) Mucocutaneous lesions alone if there are
   i) Six or more facial papules, of which 3 or more must be trichilemmomas
   ii) Cutaneous facial papules and oral mucosal papillomatosis
   iii) Oral mucosal papillomatosis and acral keratoses
   iv) Six or more palmar or plantar keratoses
2) Two major criteria, 1 of which must be macrocephaly or LDD
3) One major and 3 minor criteria
4) Four minor criteria

Operational diagnosis in a family in which 1 individual has had a diagnosis of Cowden syndrome
1) One or more of pathognomonic criteria
2) Any 1 major criterion with or without minor criteria
3) Two minor criteria

*Reproduced with permission from Waite KA, Eng C. Protean PTEN: form and function. Am J Hum Genet 2002;70:829–44.612
reserved for those with significant involvement of both the colon and the rectum, or those with dysplastic rectal polyps. Polyps of the stomach are usually diffuse and cannot be effectively removed endoscopically. Significant anemia is often present, and these patients may require subtotal or total gastrectomy.\textsuperscript{468} Patients with JP or at risk for JP should receive genetic counseling. Here, recommendations for endoscopic screening, the risk of upper and lower GI symptoms and cancer, and the ramifications of genetic testing should be discussed.

**Cowden Syndrome**

Cowden Syndrome (CS) was first described by Lloyd and Dennis\textsuperscript{469} in the propositus Rachel Cowden, and Weary and colleagues\textsuperscript{470} later
designated CS as a multiple hamartoma syndrome after observing its autosomal dominant inheritance in her family. CS involves hamartomatous changes in all 3 germ layers, including predominantly the skin, mucous membranes, breast, thyroid, GI tract, and CNS. The International Cowden Consortium has established a group of features that establish the diagnosis of CS (Table 10). Lhermitte and Duclos disease (LDD) is included in these criteria, which causes a variety of CNS symptoms. In the 1990s, it was realized that LDD and CS were variants of the same genetic condition, which manifest as neurologic and cutaneous hamartomas, respectively.

**Phenotype.** Lesions of the skin and mucous membranes are pathognomonic for CS, and are present with nearly 100% prevalence. Trichilemmoma, 1 of the characteristic lesions in CS, is a hamartoma of the hair follicle’s root sheath. Typically trichilemmomas are found around the eyes, nose, and mouth (Fig 28). Oral fibromas with a papillomatosis morphology can cause a cobblestoning appearance of the gingiva and/or tongue, also referred to as a scrotal tongue (Fig 29). Acral keratosis is also prominent in CS (Fig 30). Three quarters of patients with CS have thyroid abnormalities, most commonly goiters or adenomas. Non-
medullary thyroid malignancies are more common than in the general population and range in prevalence from 3% to 7%.478,479,482-486

Two large series of CS patients have demonstrated breast involvement in approximately 70%. Fifty percent to 60% have benign lesions, and breast cancer is found in approximately 20% to 30% of cases.478,479 Owing to the lack of prospective, long-term follow-up studies, the lifetime risk of breast cancer in CS patients may be underestimated.

Approximately 30% of patients with breast cancer have bilateral involvement, and the mean age at presentation is younger than for the general population, ranging from 38 to 46 years. Breast cancer is not limited to women with CS; 2 cases of breast cancer have been reported in men with CS.

The GI tract manifestations in CS are adenomas, hamartomas, ganglioneuromas, leiomyomas, lipomas, lymphoid, neuromas, as well as hyperplastic, inflammatory, and occasionally juvenile polyps. The incidence of GI involvement may range from 35% to 85%, depending on whether patients have contrast studies and endoscopy performed. In contrast to JP, there are only 4 reported cases of colon carcinoma.

Menstrual irregularities and ovarian cysts are found in approximately 20% of patients with CS, and leiomyomas of the uterus may also be present. Other cancers associated with CS are uterine adenocarcinoma (6%), transitional cell carcinoma (5%), and cervical carcinoma (3%). Eng recently suggested a possible association between CS and both renal cell carcinoma as well as melanoma, based on anecdotal evidence.
**Lhermitte-Duclos Disease.** Lhermitte and Duclos first described this syndrome in the French literature in 1920. This was later referred to as dysplastic gangliocytoma of the cerebellum, due to the histologic features of this peculiar hamartoma. This cerebellar hamartoma usually manifests with symptoms of increased pressure or cerebellar ataxia, which include headaches (75%), papilledema (70%), cerebellar syndrome (50%), megalencephaly (40%), cranial nerve deficit (30%), ataxia (20%), and pyramidal syndrome (20%). Associated anomalies found in patients with Lhermitte-Duclos disease (LDD) have also included macrocephaly, megalencephaly, hydrocephalus, heterotopia, and hydromyelia. 

Ambler and colleagues found LDD to be autosomal dominant in nature. By the 1980s, the diagnosis could be established by MRI findings, manifesting as a nonenhancing gyriform pattern with enlargement of the cerebellar folia. In 1921, Padberg and colleagues reported 2 patients with biopsy-proven LDD and CS. This was followed by similar reports, leading to the realization that these were the same hamartomatous syndrome involving cutaneous and neurologic ectoderm. This was supported in 1996, when Nelen and colleagues reported the localization of the predisposing gene for CS to chromosome 10q22-23. Among the 12 families studied for linkage, 4 families had clear evidence of LDD that showed the same linkage to 10q22-33. Hence, LDD was incorporated into the diagnostic criteria for CS (Table 10).

**Genetics.** CS was demonstrated to have an autosomal dominant inheritance by Gentry and colleagues in 1974 and Nuss and colleagues in 1978. Although within families there were generally equal numbers of affected men and women, between families, interfamilial series may show a female predisposition ranging from 1:1 to 1.6:1. This could also reflect a bias for presentation in females due to the breast disease predisposition in CS.

Excellent progress has been made in understanding the genetic basis of CS in the last decade. In 1996, Nelen and colleagues found linkage for CS to markers on chromosome 10q22-23, with a lod score of 8.92 (theta = 0.02) in 12 families. This was closely followed by independent identification of the **PTEN** gene on chromosome 10 by 3 groups: Li and Sun, Li and colleagues, and Steck and colleagues. Li and colleagues found **PTEN** to have a sequence consistent with a protein tyrosine phosphatase domain, and homology to the chicken protein tensin and bovine protein auxilin. In the name **PTEN**, the phosphatase function is represented by P; TEN refers to the homology to tensin and its location on chromosome 10. They also found mutations of the **PTEN** gene in breast cancer, glioblastoma, and prostate cancer cell lines.
colleagues found that 4 of 5 CS families had PTEN germline mutations in 1997 and Nelen and colleagues found that 9 of 19 CS cases had PTEN mutations.

PTEN is a dual specificity phosphatase that causes dephosphorylation of serine/threonine and tyrosine residues and of protein and lipid substrates. PTEN is involved in embryonic development, the cell cycle, apoptosis, and tumor formation. It plays an important role in the phosphatidylinositol 3-kinase (PI3K) protein kinase B (PKB)/antiapoptotic serine threonine kinase (AKT) pathway. Upregulation of PI3K-PKB/AKT promotes cell growth and survival, which is regulated by PTEN-mediated dephosphorylation of the phosphatidylinositol triphosphate (PIP3) substrate. Reduced PIP3 levels leads to lower PKB/AKT in the cell membrane, causing cell cycle arrest in G1 and apoptosis. Other pathways regulated by PTEN may include the mitogen-activated kinase (MAPK) pathway, which is involved in cell proliferation and differentiation and the insulin signaling pathway. Li and Sun, Li and colleagues, and Steck and colleagues all recognized that the mutation of PTEN leads to inactivation of its phosphatase activity and inactivation of its role in growth inhibition, implying a tumor suppressor role.

Marsh and colleagues examined a series of 37 families with CS and identified 30 families with germline PTEN mutations, for an incidence of 81%. Zhou and colleagues re-examined 97 patients with CS that were negative for PTEN mutations. They found mutations in the promoter region of PTEN in 9 (9.3%) patients. Combining both mutations in the PTEN promoter and coding sequence therefore accounts for approximately 90% of germline mutations seen in patients with CS.

Treatment. The first step in appropriate screening for CS is recognition of its predisposition to malignancy. Once CS is diagnosed, patients should be considered for genetic counseling. This provides an opportunity for support and education, including its social, familial, and personal ramifications. In a family in which a PTEN mutation has been detected, if the individuals at risk are negative for the same mutation, they may then undergo routine health and cancer screening. At-risk CS patients or patients in families without identified PTEN mutations should undergo a general comprehensive physical examination starting at 18 years of age or 5 years before the earliest cancer was diagnosed in another family member.

The most common cancers encountered in CS patients are of the breast and thyroid gland. Starink and colleagues reported 3 patients younger than the age of 30 years in whom breast cancer developed.
at-risk patients should perform monthly breast self-examinations starting at age 18, with annual physical examinations beginning at age 25, or 5 to 10 years earlier than the youngest case of breast cancer in the family. Mammography should be performed beginning at age 30 to 35 years, or at least 5 years before the youngest age of onset of breast cancer in the family.

Thyroid cancer in CS patients may develop by 10 to 30 years of age. Screening should begin with a thyroid ultrasound at age 18, with annual physical examinations thereafter and repeat ultrasounds as determined by the level of clinical suspicion.

To screen for endometrial cancer, annual endometrial suction biopsies should be performed beginning at the age of 35 to 40 years, or 5 years younger than the earliest premenopausal case of endometrial cancer in the family. Postmenopausal women should have an annual endometrial ultrasound. Families with a history of renal cancer should have annual urine cytology, and renal ultrasound as indicated. Dermatologic examination is also suggested annually to screen for melanoma.

**Bannayan-Riley-Ruvalcaba Syndrome**

The name Bannayan-Riley-Ruvalcaba syndrome (BRRS) was proposed in 1990 to encompass 3 phenotypically similar syndromes (Ruvalcaba-Myhre-Smith syndrome, Bannayan-Zonana syndrome, and the Riley-Smith syndrome). The syndromes are defined by macrocephaly, and variable penetrance of developmental delay, hemangiomas, lipomas, genital pigmentation, intestinal polyps, and lipid myopathy. The predominant features of BRRS as reported by Gorlin and colleagues are listed in Table 11.

The GI polyps associated with BRRS were initially described as hamartomatous. Further review by Haggitt and Reid of the histologic features of polyps reported by Ruvalcaba found that they were juvenile polyps, except for 1 ganglioneuroma. Recent reports have confirmed that most polyps are juvenile polyps, which occasionally will display adenomatous changes.

**Genetics.** It may be difficult to distinguish between BRRS and CS. Fargnoli and colleagues described a patient with findings consistent with both BRRS and meeting the diagnostic criteria for CS. This patient had macrocephaly, 2 lipoma resections, a thyroid follicular adenoma (that had been removed), scoliosis, colonic polyps, and hyperpigmented macules on the glans penis (Fig 31). Both BRRS and CS have an autosomal dominant inheritance.

Marsh and colleagues found a *PTEN* germline mutation in 4 of 7 unrelated BRRS
families in 1998. One of the these $PTEN$ mutations had previously been found in 2 CS families, suggesting that the 2 syndromes were allelic. The largest series to date has been that of Marsh and colleagues, who evaluated 43 individuals with BRRS. Of these 43 patients with BRRS, 16 were sporadic and 27 were familial. Eleven of the 27 familial cases were mixed, having individuals of both CS and BRRS phenotypes. $PTEN$ mutations were found in 26 of 43 individuals, for a prevalence of 60%. Additional reports of $PTEN$ mutations associated with patients having BRRS have been published.

In families with BRRS, the recommendations for follow-up are less clear, because the risk for cancers has not been established. The findings from genetic studies that BRRS and CS are allelic syndromes suggests that the clinician maintain an elevated level of suspicion and examine the patient annually for the same tumors seen in patients with CS.

**Peutz-Jeghers Syndrome**

Peutz-Jeghers syndrome (PJS) is characterized by hamartomatous polyps of the upper and lower GI tract, and lentigines (pigmented lesions) of the buccal mucosa, perioral region, palms, feet, or anus (Fig 32). The association of melanosis and polyposis was first recognized by Peutz in 1921, and Jeghers and colleagues described an autosomal dominant inheritance in 1949. The prevalence of PJS has been estimated to be between 1 in 8300 and 1 in 29,000. The mean age of diagnosis of PJS ranges between 22 and 26 years.
The intestinal polyps are most commonly found in the jejunum, and large series have found the incidence of polyps in the small intestine to be 78%, colon 42%, stomach 38%, and rectum 28%. These polyps resemble the hamartomatous polyps in patients with juvenile polyposis and Cowden syndrome, but differ in the fact that there is prominent smooth muscle tissue found within the lamina propria (Fig 33). Adenomatous and hyperplastic polyps may also be encountered. Gastric polyps tend to be more sessile, whereas small and large intestinal polyps are generally pedunculated. The most common problems arising from these GI polyps are intussusception and GI bleeding, as highlighted by the report of 1 family with 12 affected members that had 32 abdominal operations and more than 70 endoscopic procedures performed for removal of polyps.

The skin lesions in PJS patients are pigmented macules, caused by increased numbers of melanocytes. These lesions are not premalignant. They are most commonly seen around the lips, mouth, hands, feet, buccal mucosa, eyes, nose, and perianal areas. The degree of pigmentation in cutaneous regions may decrease with age, but it may persist in the buccal mucosa.
In a study of 72 PJS patients, Spigelman and colleagues\textsuperscript{553} found that 16 (22\%) had developed cancer, with 10 GI cancers (4 small bowel, 3 stomach, 2 colon, 1 pancreas) and 7 non-GI cancers (2 unknown primary, 1 ovary, 1 lung, 1 thyroid, 1 fallopian tube, 1 basal cell carcinoma). The relative risk of GI cancer death was 13, and 48\% of those with cancer had died by age 57.\textsuperscript{553} Giardiello and colleagues\textsuperscript{554} found that 15 of 31 (48\%) PJS patients developed cancers over a 12-year follow-up interval. Four had GI cancers (2 stomach, 2 colon), 4 had pancreatic cancers, and 7 had other cancers (2 breast, 2 lung, 1 ovary, 1 uterus, 1 myeloma). The relative risk of cancer was calculated to be 18 times higher than the normal population, and the average age at diagnosis was 25 years (ranging from 1 to 64 years) after the diagnosis of PJS (at mean age of 17 years).\textsuperscript{554} Lim and colleagues\textsuperscript{555} found that 8 of 70 affected PJS patients developed cancers (2 stomach, 2 breast, 2 colorectal, 1 pancreas, 1 cervical). They calculated the risk of developing cancer by age 65 in mutation carriers to be 47\%, with a standardized mortality ratio of 13.2 for all cancers, 32.0 for GI cancer, and 13.9 for breast cancer. Boardman and colleagues\textsuperscript{556} found that 18 of 34 PJS patients developed cancer, in

which there were 10 GI (7 colon, 1 duodenum, 1 esophagus, 1 stomach) and 16 extraintestinal cancers (6 breast, 3 lung, 2 cervix, 1 ovary, 1 uterus, 1 thyroid, 1 prostate, 1 unknown primary). Six patients had multiple cancers. Women were at an 18.5-fold increased risk for cancer, 20.3 times higher for breast and gynecologic malignancies. Men were at a 6.2-fold increased risk for cancer, and the mean age of cancer diagnosis was 39.4 years in all patients. In a review of PJS, Hemminki found 39 cases of small bowel, 31 colorectal, 17 gastric, 8 pancreatic, and 1 esophageal cancer. There were 22 cases of breast cancer, 13 cervical, 9 lung, 8 ovarian, 3 hepatobiliary, 2 thyroid, 2 myelomas, 2 leiomyosarcomas, 2 endometrial, 1 testicular, 1 fallopian tube cancer, and 1 osteosarcoma. Benign tumors of the breast, ovary, thyroid, and of sex cord origin have also been reported. Giardiello and colleagues performed a meta-analysis and showed that the relative risk of small bowel cancer in PJS was 520, stomach 213, pancreas 132, colon 84, esophagus 57, ovary 27, lung 17, breast 15, and uterus 16. The risk of cervical and testicular cancer was not increased. The mechanism of GI cancer development is likely through hamartoma to adenoma to carcinoma, although this has not been completely elucidated. Spigelman and colleagues did find that 4 to 5 of their 10 GI cancers appeared to have developed within a hamartomatous polyp.

Genetics of PJS. In 1997, evidence for a PJS locus on chromosome 19p was discovered by Hemminki and colleagues, who performed comparative genomic hybridization and detected a region that appeared to be lost in PJS tumors relative to normal tissues. Loss of heterozygosity studies found consistent losses of markers from 19p in tumors. Then by genetic linkage, they found a multipoint lod score of 7.00 at theta = 0 with the marker D19S886 in 12 PJS families. Amos and colleagues performed genetic linkage studies in 5 PJS families and confirmed linkage to the same marker (multipoint lod score of 7.52 at theta = 0), placing the PJS susceptibility gene within the telomeric 3 megabases of chromosome 19p. Mehenni and colleagues also found linkage to D19S886 with a 2-point lod score of 4.74 at theta = 0.045. Interestingly, they found

linkage to the marker \textit{D19S891} from the long arm of chromosome 19 (19q) in 1 Indian PJS family (lod of 3.52 at theta = 0), suggesting a possible second PJS locus.

Hemminki and colleagues\textsuperscript{562} mapped cDNA clones to a cosmid contig created from the human chromosome 19 mapping project, spanning an 800-kb region near \textit{D19S886} known to contain the PJS gene by critical recombinants in PJS families. Twenty-seven genes were mapped to this region, and mutations were found in 1 called \textit{LKB1}, a previously unmapped serine threonine protein kinase gene. Of 12 unrelated PJS patients sequenced, 11 were found to have \textit{LKB1} mutations (5 deletions, 1 insertion, 5 substitutions; Fig 34). This gene was expressed in all tissues examined, and the mutations found were predicted to diminish the kinase activity of the protein, in contrast to the activation of kinases seen in other heritable cancer syndromes (such as \textit{RET} in MEN2, and \textit{CDKN2} in familial melanoma).\textsuperscript{562} A similar effort from Jenne and colleagues\textsuperscript{563} mapped 21 genes from overlapping cosmid clones spanning 400 kb in the region of \textit{D18S886}, and 1 of these genes was found to have a rearrangement in a PJS patient. This was a serine threonine protein kinase gene consisting of 9 exons over 23 kb of genomic DNA, which they called \textit{STK11} (although it had previously been given the name \textit{LKB1}). The PJS proband had a deletion of exons 4 and 5, and inversion of exons 6 and 7, and all 3 affected members of her family also had this rearrangement. Analysis of 4 other unrelated PJS patients revealed 3 nonsense and 1 splice site mutation of the gene.\textsuperscript{563}

Gruber and colleagues\textsuperscript{564} found an \textit{LKB1} mutation in the original family reported by Jeghers,\textsuperscript{548} as well as 5 others (2 deletions, 2 insertions, 2 nonsense mutations). They confirmed that \textit{LKB1} was a tumor suppressor gene by demonstrating that 11 of 12 hamartomas or adenocarcinomas from 4 PJS patients had loss of the wild-type allele with retention of the mutated allele. The losses seen in hamartomas implied that this gene is involved at an early stage of tumor progression.\textsuperscript{564} Jiang and colleagues\textsuperscript{565} found only 1 mutation of \textit{LKB1} in 10 unrelated PJS patients, and suggested that there may be other genes responsible for JPS. Nakagawa and colleagues\textsuperscript{566} analyzed 15 unrelated PJS patients for mutations, and found them in 10. Four were deletions causing frameshifts, 3 were insertions, 2 were splice site mutations, and 1 was a nonsense mutation.

Miyaki and colleagues\textsuperscript{567} found \textit{LKB1} mutations in 6 of 9 PJS families studied, which consisted of 3 deletions, 2 insertions, and 1 splice site mutation, all predicted to cause protein truncation. They also found somatic \textit{LKB1} mutations in 5 of 27 (19%) polyps, 4 of which shared a
4-bp deletion in exon 6. Fourteen other polyps had LOH of 19p markers, for a total of 70% of polyps showing potential somatic inactivation of \( LKB1 \). Six larger polyps also had \( \beta \)-catenin mutations, suggesting these changes may be important in polyp progression. One carcinomatous polyp examined had 19p LOH, \( \beta \)-catenin mutation, and p53 mutation, suggesting a hamartoma-adenoma-carcinoma sequence in PJS polyps.\textsuperscript{567} Gruber and colleagues\textsuperscript{564} found that 11 of 12 hamartomas and adenocarcinomas had 19p LOH, 1 of 18 had 18q LOH, and 0 of 18 had \( K-ras \) mutations.

Wang and colleagues\textsuperscript{568} found \( LKB1 \) germline mutations in 7 of 12 (58%) PJS patients, which included 4 deletions, 2 nonsense mutations, and 1 missense mutation. Four families without mutations had findings compatible with \( LKB1 \) linkage, suggesting that alterations might be present but were not detected by their sequencing or single strand conformational polymorphism (SSCP) analysis.\textsuperscript{568} Westerman and colleagues\textsuperscript{569} found \( LKB1 \) mutations in 12 of 19 PJS families (63%), which consisted of 3 deletions, 3 insertions, 3 missense mutations, 2 splice site mutations, and 1 nonsense mutation.

Boardman and colleagues\textsuperscript{570} screened 10 familial and 23 sporadic cases of PJS for \( LKB1 \) changes by conformation-sensitive gel electrophoresis (CSGE), and only found mutations in 2 and 4 cases, respectively. The low mutation rate found in this study may have been due to the lower sensitivity rate of CSGE for identifying base-pair changes (which is approximately 90%), other mechanisms of \( LKB1 \) alteration, or the possibility that other genetic loci for PJS may exist (genetic heterogeneity).\textsuperscript{570} Lim and colleagues\textsuperscript{555} also used CSGE to screen 33 JPS families for \( LKB1 \) mutations, and found mutations in 17 (52%; 5 deletions, 3 splice site mutations, 2 insertions, 3 nonsense, and 4 missense mutations).

Boudeau and colleagues\textsuperscript{571} studied the functional consequences of 6 germline \( LKB1 \) mutations (4 deletions and 2 missense mutations) in PJS patients by cloning the cDNA into a CMV expression vector. They found that each of these \( LKB1 \) isoforms was unable to autophosphorylate at Thr336, and to suppress the growth of a melanoma cell line, in contrast to wild-type \( LKB1 \). They concluded that these were loss-of-function mutations, and that loss of kinase activity was responsible for PJS.\textsuperscript{571}

Esteller and colleagues\textsuperscript{572} studied 51 cancer cell lines (15 colon, 11 lung, 7 ovary, 5 breast, 3 thyroid, 3 brain, 3 prostate, 3 leukemia, 1 cervix) for methylation of a CpG island just upstream from \( LKB1 \), and found this in 3 colorectal and 1 cervical cancer cell lines with coincident loss of \( LKB1 \) expression. They also found methylation in 4 of 22 (18%)
hamartomatous polyps in PJS patients, suggesting that this mechanism of gene inactivation may be involved in a subset of PJS cases. Entius and colleagues examined 39 polyps and 5 carcinomas from 17 PJS patients for LOH for markers at the LKB1 locus on 19p13, 5q21 (APC), 17p13 (p53), and 18q21 to 22 (MADH2, MADH4). Fifteen of 39 (38%) polyps had 19p13 LOH, as did 5 of 5 carcinomas. Six of 7 polyps from the 5 patients with carcinoma demonstrated 19p LOH. No LOH was seen at 5q21, but 1 cancer had an APC mutation. LOH was not found at 17p13 in any of these samples, but was found in 1 cancer at 18q; however, all polyps and cancers had p53 and MADH4 expression by immunohistochemistry. The authors concluded that LKB1 loss was important in tumorigenesis in PJS patients, and the genetic mechanisms responsible differed from those in sporadic colorectal carcinogenesis.

**Surveillance and Follow-Up.** Recommendations for surveillance of patients with PJS or at risk for PJS are not clear, but must address the significantly elevated risk of a variety of cancers, such as the aforementioned increased relative risk of small bowel cancer (520-fold increased risk), stomach, pancreas, colon, esophagus, ovary, lung, uterus, and breast. Dunlop recommended that colonoscopy or barium enema be performed every 3 years beginning at age 18, and that upper endoscopy be performed every 3 years beginning at age 25. Hemminki recommends biannual upper and lower endoscopy beginning in the mid teen years, and others suggest these be performed every 2 to 5 years. How to follow the small bowel in PJS patients remains a challenge. These polyps can lead to intussusception or anemia and develop into cancers. Small bowel follow-through and enteroclysis will show larger polyps, but the relatively new technique of capsule endoscopy may be the best available method for visualization of small bowel polyps. It would seem prudent to perform capsule endoscopy at least every 3 years in asymptomatic PJS gene carriers, which might preclude the need for screening colonoscopy and upper endoscopy, which could be reserved for therapeutic interventions. If multiple polyps are found, then screening should be performed at least annually until the polyps are removed.

Gastric and colonic polyps may be removed endoscopically, with

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surgery being reserved for lesions with dysplasia or cancer. Less invasive therapeutic interventions for small bowel polyps are more limited, since most enteroscopes will only reach the proximal jejunum. Surgery is therefore the required treatment for symptomatic polyps (causing intussusception, anemia, or pain) or those suspicious for cancer. At operation, intraoperative endoscopy through an enterotomy can be performed to examine the entire small bowel, and polyps may be removed through the endoscope by using a snare cautery. Larger or suspicious polyps will require enterotomy or small bowel resection for their removal.

Surveillance recommendations for extraintestinal cancers in PJS patients are not well established. Screening for breast cancers should include annual breast examination and mammography (or ultrasound in patients younger than age 35), with the latter beginning between the ages of 25 and 35. Gynecologic malignancy should be ruled out by annual transvaginal ultrasound, pap smear, and pelvic examination. Annual testicular examination and ultrasound should be initiated in males by age 10. Screening for pancreatic cancer by abdominal ultrasound, endoscopic ultrasound, or computed tomography should be performed every 1 to 2 years starting at age 30.\textsuperscript{552,557,575}

The diagnosis of PJS gene carriers may be evident from within the first year of life based on the characteristic mucocutaneous pigmentation. These patients will clearly need surveillance. Gene testing for PJS involves direct sequencing of the entire gene for probands and directed mutation screening of other at-risk individuals once a mutation is found in the family. Genetic testing of at-risk individuals from families with known \textit{LKB1} mutations will benefit those found not to harbor the disease-causing mutation because they may be excused from the screening regimens. Those found to be gene carriers will need to be educated regarding their risk for developing cancer and be given these surveillance recommendations.

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