Molecular Classification of Patients With Unexplained Hamartomatous and Hyperplastic Polyposis

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Context Significant proportions of patients with hamartomatous polyposis or with hyperplastic/mixed polyposis remain without specific clinical and molecular diagnosis or present atypically. Assigning a syndromic diagnosis is important because it guides management, especially surveillance and prophylactic surgery.

Objective To systematically classify patients with unexplained hamartomatous or hyperplastic/mixed polyposis by extensive molecular analysis in the context of central rereview of histopathology results.

Design, Setting, and Patients Prospective, referral-based study of 49 unrelated patients from outside institutions (n=28) and at a comprehensive cancer center (n=21), conducted from May 2, 2002, until December 15, 2004. Germline analysis of PTEN, BMPR1A, STK11 (sequence, deletion), SMAD4, and ENG (sequence), specific exon screening of BRAF, MYH, and BHD, and rereview of polyp histology results were performed.

Main Outcome Measures Molecular, clinical, and histopathological findings in patients with unexplained polyposis.

Results Of the 49 patients, 11 (22%) had germline mutations. Of 14 patients with juvenile polyposis, 2 with early-onset disease had mutations in ENG, encoding endoglin, previously only associated with hereditary hemorrhagic telangiectasia; 1 had hemizygous deletion encompassing PTEN and BMPR1A; and 1 had an SMAD4 mutation. One individual previously classified with Peutz-Jeghers syndrome had a PTEN deletion. Among 9 individuals with an unknown hamartomatous polyposis, 4 had mutations in STK11 (1), BMPR1A (2), and SMAD4 (1). Of the 23 patients with hyperplastic/mixed polyposis, 2 had PTEN mutations. Substantial discrepancies in histopathology results were seen.

Conclusions Systematic molecular classification of 49 patients with unexplained hamartomatous or hyperplastic polyposis uncovered a potential novel susceptibility gene, ENG, for juvenile polyposis. Importantly, given the substantial proportion of patients found to have germline mutations, more extensive analysis of the known susceptibility genes is indicated. Rereview of histology results by a dedicated gastrointestinal pathologist should be considered routinely, as organ-specific surveillance rests on defining syndromic diagnosis.
However, there is increasing evidence for several important categories of polyposis and colorectal carcinoma that may develop from alternative routes. This includes the hamartomatous polyposis syndromes and the serrated neoplasia pathway, whose morphologic spectrum includes the hyperplastic polyp and sessile serrated adenoma. Given the attendant cancer risks and medical management issues inherent in these forms of polyposis (Table 1), it is imperative that physicians recognize that variability in histopathology and molecular etiology can hinder appropriate diagnosis.

The known forms of inherited hamartomatous polyposis include Peutz-Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), and Cowden syndrome. Although collectively accounting for less than 1% of colorectal cancer in North America, proper identification of these clinically confusing syndromes remains of critical importance, because each syndrome carries significant risks for extraintestinal malignancy and other component features that must be managed (Table 1). Peutz-Jeghers syndrome (Online Mendelian Inheritance in Man [MIM] 175200) causes gastrointestinal polyposis, especially of the upper jejunum (78%), and mucocutaneous pigmentation. Approximately 50% of all PJS cases are due to germline mutation in the nuclear seirene threonine kinase gene STK11. Germline mutations in the genes of the signaling pathway of the transforming growth factor β (TGF-β) superfamily (Figure) can lead to a range of common heritable disorders, including JPS (MIM 174900), and vascular disorders, such as hereditary hemorrhagic telangiectasia (HHT) (MIM 187300) and primary pulmonary hypertension (MIM 178600). For example, a combined syndrome consisting of both juvenile polyposis and hereditary HHT type 2 (MIM 175050) is known to be due to germline mutations in the SMAD4 gene. Mutations in another member of this pathway, BMPRIA, account for 20% to 30% of additional JPS cases, although apparently without stigmata of HHT. While other members of the TGF-β family would be ideal candidates for JPS, no germline mutations in the genes BMPR2, ACRV1, SMAD1, SMAD2, SMAD3, SMAD5, and SMAD7 have been identified to date, leaving approximately 50% of cases unexplained at the molecular level. A third hamartoma syndrome, Cowden syndrome (MIM 158350), differs from both PJS and JPS in that polyposis is not the defining feature. Rather, most cases are ascertained because of distinctive mucocutaneous lesions, benign and malignant thyroid and breast disease, and macrocephaly. Approximately 85% of patients with Cowden syndrome who meet established diagnostic criteria have mutations of the PTEN gene (Table 1).

It is widely believed that hyperplastic polyps have no malignant potential. Commonly seen on colonoscopic examination (10% of patients younger than 50 years, 50% of those younger than 70 years), they seldom exceed 0.5 cm and are often localized to the distal colon and rectum. However, there is evolving evidence linking the sessile serrated adenoma (SSA), a lesion related to hyperplastic polyp, with neoplasia. SSAs tend to be multiple and large (>1 cm), have a greater propensity for the proximal colon, and may comprise 15% to 20% of traditional hyperplastic polyps. The initiating event in the progression from hyperplastic polyp to SSA may be the activating somatic BRAF (hotspot V600E) mutation. Importantly, this process can be clinically investigated in hyperplastic polyposis syndrome (HPS), as reappraisal of histological subtype supports the development of SSA. Interestingly, it was recently shown in the serrated adenoma mouse model that SMAD4

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Table 1. Incidence and Cancer Risks for Known Polyposis Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>MIM No.</th>
<th>Gene(s)</th>
<th>Patients Meeting Clinical Criteria With Mutation, %</th>
<th>Population Incidence</th>
<th>Cancer Risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial adenomatous polyposis</td>
<td>175000</td>
<td>APC</td>
<td>90</td>
<td>1/5000</td>
<td>Colorectal, duodenal, papillary thyroid, pancreatic, hepatoblastoma, CNS tumors</td>
</tr>
<tr>
<td>MYH-adenomatous polyposis</td>
<td>608456</td>
<td>MYH</td>
<td>Unknown</td>
<td>1/5000</td>
<td>Colorectal tumors, other?</td>
</tr>
<tr>
<td>Hyperplastic polyposis syndrome</td>
<td>601228</td>
<td>CRAC1</td>
<td>NA</td>
<td>1/100,000</td>
<td>Colorectal tumors, other?</td>
</tr>
<tr>
<td>Hereditary mixed polyposis syndrome</td>
<td>175050</td>
<td>SMAD4</td>
<td>20-40</td>
<td>1/100,000</td>
<td>Colorectal, gastric, duodenal, pancreatic tumors</td>
</tr>
<tr>
<td>Juvenile polyposis/hemorrhagic telangiectasia syndrome</td>
<td>174900</td>
<td>BMPRIA</td>
<td>20-40</td>
<td>1/100,000</td>
<td>Colorectal, gastric, duodenal, pancreatic tumors</td>
</tr>
<tr>
<td>Cowden syndrome</td>
<td>158350</td>
<td>PTEN</td>
<td>80-85</td>
<td>1/200,000</td>
<td>Breast, thyroid, uterine, melanoma, renal cell tumors</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>175200</td>
<td>STK11</td>
<td>50</td>
<td>1/30,000-1/100,000</td>
<td>Colorectal, small intestine, stomach, breast, pancreatic, sex-cord tumors</td>
</tr>
<tr>
<td>Birt-Hogg Dube syndrome</td>
<td>135150</td>
<td>BHD</td>
<td>50-70</td>
<td>1/200,000</td>
<td>Renal tumors, other?</td>
</tr>
</tbody>
</table>

Abbreviations: CNS, central nervous system; MIM, Mendelian Inheritance in Man; NA, not available.

*From Online Mendelian Inheritance in Man.*
mutation results in serrated adenomas and mixed polyposis, suggesting the potential for involvement of the TGF-β pathway in this alternative route to colorectal cancer.27

Although DNA analysis often makes it possible to differentiate polyposis syndromes at the gene level, a significant percentage of patients remain without molecular diagnosis or with atypical presentation (Table 1). We therefore sought to molecularly classify 49 patients with unexplained hamartomatous polyps or with hyperplastic/mixed polyps in the context of central review of all histopathology results. Seventeen patients (34%) met clinical criteria for diagnosis of a known hamartomatous polyposis syndrome and were sequence-negative for their respective genes prior to study entry. For the entire cohort, extensive comprehensive germline sequencing and deletion analysis of PTEN, BMPR1A, and STK11, as well as sequence analysis of SMAD4 and ENG, the latter a known cause of HHT type 1, were performed. Specific exon screening was performed for BRAF, MYH, and BHD. Moreover, this is the first study to evaluate germline mutation status of known polyposis genes in a cohort of patients with hyperplastic/mixed polyposis to determine which, if any, have hereditary disease.

**METHODS**

**Patients**

This was a prospective, referral-based study in which 49 unrelated patients were recruited from May 2, 2002, until December 15, 2004, from genetics clinics at outside institutions (n=28) and at a comprehensive cancer center (n=21). All patients provided written informed consent as part of a protocol approved by the respective human subjects protection committees. To be included in the study, patients had to have a minimum of 5 gastrointestinal polyps on consecutive colonoscopic procedures, at least 1 of which was either a hamartomatous or hyperplastic polyp. Patients were classified into 3 groups based on the pathological diagnosis from standard clinical pathology review from their respective hospitals of referral. Only patients in group 1 (n=17) had previous analysis of known hamartomatous polyposis genes.

Group 1 (Table 2) consisted of 17 patients with a clinical diagnosis of either JPS (n=14) or PJS (n=3) based on established clinical criteria.28,29 Each individual also had to have tested negative for mutations in SMAD4, BMPR1A, or STK11, respectively. Mean age at diagnosis of JPS was 9 years; the mean number of juvenile polyps was 17. Mean age at diagnosis of PJS was 33 years; the mean number of PJS polyps was 7.

Group 2 (Table 3) comprised 9 patients with an undefined hamartoma-
tous polyposis, due either to discrepancies in the pathology report(s) or to lack of supportive clinical features to establish a diagnosis. Mean age at diagnosis of first polyp was 36 years.

Group 3 (Table 4) consisted of 23 patients with a combination of hyperplastic polyps and adenomas. Eleven patients met criteria for the diagnosis of hyperplastic polyposis syndrome. Mean age at diagnosis was 47 years. An additional 5 patients had a single large hyperplastic polyp (>1 cm) among other polyps and a mean age at diagnosis of 52 years. Initial pathology reports identified SSA in only 3 members of group 3.

Medical records were requested and hematoxylin and eosin–stained slides obtained. History of colorectal or other cancer was noted. Size and site of polyps were recorded from the accompanying colonoscopic requisition sheet or from the gross description on the pathology report. The histopathology slides were centrally reviewed by a single gastrointestinal pathologist (J.W.) without knowledge of the molecular findings.

Molecular Genetic Analyses

Mutation analysis was carried out on coded samples in a blinded fashion. Ge-
RESULTS

Group 1

In group 1, comprising previously mutation-negative individuals with a clinical diagnosis of JPS or PJS, 5 of 17 (29%) were found to have germline mutations (Table 2 and Table 5). Central pathology review confirmed the diagnosis of multiple juvenile polyps in all 14 patients with JPS. Among the 14 patients with JPS who tested mutation-negative in the 2 known genes, SMAD4 and BMPRIA, 4 occult germline mutations were found. Specifically, 2 patients were found to have germline mutations in ENG, encoding endoglin, previously associated only with HHT (Table 5). Patient 1N had a 1711C→T mutation of exon 12, causing the amino acid change R571C. A second patient (1D) had the 1538 A→G (K513R) mutation in exon 11 of ENG. The K513R mutation involves a residue within the zona pellucida domain that is conserved from Gal-lus gallus to Mus musculus. Neither mutation was found in 105 North American controls. Both patients presented with juvenile polyposis of unusually early onset (age 3 and 5 years, respectively), with no abnormalities noted in skin pigmentation or features consistent with a diagnosis of HHT (Table 5). A third patient with JPS (1B) was found to have a 1.2-Mb deletion encompassing BMPRIA and PTEN. This patient presented at an early age (18 months) with multiple juvenile polyps (Table 5). This early polyp presentation clearly distinguished these 3 patients (mean age, 3 years; range, 18 months to 5 years) from the remaining patients with JPS in group 1 (mean age, 11 years; range, 2-31 years).

Table 4. Group 3: Patient Demographics, Characteristics, and Initial and Final Histopathologic Classification

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at First Polyp, y</th>
<th>No. of Polyps by Histologic Examination</th>
<th>Cancer Diagnosis (Age, y)</th>
<th>Family History of HPS</th>
<th>Family History of CRC</th>
<th>Central Pathology Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>41</td>
<td>HP 31, TA 3, SSA 41</td>
<td>Sigmoid (41)</td>
<td>–</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>B</td>
<td>57</td>
<td>HP 74, TA 8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>C</td>
<td>19</td>
<td>HP 52, TA 3</td>
<td>Cecum (19)</td>
<td>+</td>
<td>–</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>D</td>
<td>68</td>
<td>HP &gt;30, TA 5, SSA 4</td>
<td>Sigmoid (68) Ascending colon (69) Transverse colon (70)</td>
<td>–</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>E</td>
<td>62</td>
<td>HP 3 (2&gt;1.0 cm), TA 2</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>F</td>
<td>50</td>
<td>HP 9 (2&gt;1.0 cm), TA 1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>G</td>
<td>45</td>
<td>HP &gt;30, TA 2</td>
<td>Splenic flexure (45)</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>H*</td>
<td>16</td>
<td>HP &gt;10, TA &gt;10</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>I</td>
<td>54</td>
<td>HP &gt;30, TA 2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>J</td>
<td>62</td>
<td>HP 20 (2&gt;1.0 cm), TA 9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>K</td>
<td>40</td>
<td>HP 22, TA 2</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>L</td>
<td>23</td>
<td>HP 10, TA 2</td>
<td>Cecum (26)</td>
<td>–</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>HP 12, TA 3</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>N</td>
<td>58</td>
<td>HP 14, TA 3</td>
<td>Cecum (60)</td>
<td>–</td>
<td>+</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>O</td>
<td>43</td>
<td>HP 12, TA 1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>P</td>
<td>53</td>
<td>HP 13, TA 34</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>Q</td>
<td>52</td>
<td>HP 10, TA 17</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>R</td>
<td>58</td>
<td>HP 8, TA 8</td>
<td>CLL (54)</td>
<td>–</td>
<td>–</td>
<td>HP, TA, and SSA</td>
</tr>
<tr>
<td>S</td>
<td>47</td>
<td>HP 3, TA 5</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>T</td>
<td>59</td>
<td>HP 6, TA 11</td>
<td>GIST (60)</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>U*</td>
<td>63</td>
<td>HP 5, TA 9</td>
<td>NSCL (63), breast (64)</td>
<td>–</td>
<td>–</td>
<td>HP and TA</td>
</tr>
<tr>
<td>V</td>
<td>37</td>
<td>HP 2, TA 3</td>
<td>Seminoma (35)</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
<tr>
<td>W</td>
<td>45</td>
<td>HP 2, TA 6</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>HP and TA</td>
</tr>
</tbody>
</table>

Abbreviations: CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; GIST, gastrointestinal stromal tumor; HP, hyperplastic polyps; HPS, hyperplastic polyposis syndrome; NSCL, non–small cell lung cancer; SSA, sessile serrated adenoma; TA, tubular adenomas.

*Found to have germline mutation (see Table 5).
A fourth patient with JPS (1H) was found to have a 1524T→A (W508R) mutation in exon 11 of SMAD4. This mutation was not found in 100 normal controls. This mutation had been missed on previous analysis by an outside research laboratory but was confirmed on reanalysis.

Among the 3 individuals presenting with the diagnosis of PJS, patient 1O was found to have a hemizygous deletion involving the PTEN promoter region and exon 1. Interestingly, all 3 individuals lacked characteristic mucocutaneous pigmentation and had been diagnosed solely on polyp histology results. Indeed, central reevaluation of the polyp results did not confirm initial clinical pathology findings for any of the patients with PJS (Table 2).

**Group 2**

Of the 9 individuals with undefined hamartomatous polyps, 4 (44%) were found to have germline mutations (Tables 3 and 5). Patient 2C had a 29-bp deletion of exon 1 of STK11 and an IVS7-32 (A→T) polymorphism of SMAD4. Central pathology review based on 5 lesions was consistent with the diagnosis of juvenile polyps. Thus, unexpectedly, a germline STK11 mutation, previously associated only with PJS, has been found in an individual with juvenile polyps.

Patient 2F was found to carry a germline IVS3 + 5G→C mutation of BMPR1A. RNA extraction and reverse transcriptase polymerase chain reaction revealed that this mutation led to a splicing alteration, resulting in a 97-bp intronic insertion between the coding sequences of exons 3 and 4. Central pathology review noted juvenile polyps, some with adenomatous change, from each colonoscopic procedure.

A second splice site mutation, IVS1 + 1 (G→T) of BMPR1A, was found in patient 2G. A fourth patient in this group (2H) harbored a 4-bp insertion (c.1409_10insCCCT) in exon 10 of SMAD4. Notably, these 2 individuals (2G and 2H) had previous negative molecular workup for the attenuated form of familial adenomatous polyposis prior to study entry. Central pathology review noted a higher degree of juvenile polyposis with marked predisposition to adenomatous transformation compared with the other study cases.

**Group 3**

Of 23 patients with a combination of hyperplastic and adenomatous polyps, 2 (9%) were found to have germline PTEN mutation (Tables 4 and 5). Review of the clinical phenotype revealed features reminiscent of, but not diagnostic for, Cowden syndrome (Table 5). Patient 3H, with a 612insC mutation in exon 6 of the PTEN gene, presented with a 20-year history of polyposis and a significant family history. Patient 3U had the IVS3-3~7delCTTTT mutation of PTEN, a

### Table 5. Mutation Results and Clinical Features

<table>
<thead>
<tr>
<th>Group/Patient</th>
<th>Mutation</th>
<th>Age at Diagnosis, y</th>
<th>Final Pathology Result</th>
<th>Polyp Location</th>
<th>Extracolonic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 A</td>
<td>Hemizygous deletion of BMPR1A and PTEN</td>
<td>1.5</td>
<td>JP</td>
<td>Duodenum and colon</td>
<td>Macrocephaly (OFC, 53.5 cm); frontalbossing; depressed nasal bridge; higharched palate; broad thumbs andtoes; ASD with VSD withabsence of portal vein; cytogeneticstudies of 46,XX,t(2;10)(q31;p15).</td>
</tr>
<tr>
<td>D</td>
<td>ENG, K513R (1538 A→G)</td>
<td>3</td>
<td>JP</td>
<td>Pancolon</td>
<td>None reported</td>
</tr>
<tr>
<td>H</td>
<td>SMAD4, W508R (1524T→A)</td>
<td>31</td>
<td>JP and TA</td>
<td>Pancolon</td>
<td>None reported</td>
</tr>
<tr>
<td>N</td>
<td>ENG, R571C (1711C→T)</td>
<td>5</td>
<td>JP</td>
<td>Distal descending and cecum</td>
<td>Macrocephaly (OFC, 54.5 cm); frontalbossing; down-squinting, palpebralfissures; elongated philtrum; higharched palate</td>
</tr>
<tr>
<td>O</td>
<td>Hemizygous deletion of PTEN promoter and exon 1</td>
<td>59</td>
<td>JP, HP, and TA</td>
<td>Gastric and pancolon</td>
<td>Mucinous ovarian adenocarcinoma (64 y); deep vein thrombosis*</td>
</tr>
<tr>
<td>Group 2 C</td>
<td>STK11, 191-219 del 29 of exon 1; SMAD4, IVS7-32 (A→T)</td>
<td>33</td>
<td>JP</td>
<td>Pancolon</td>
<td>None reported</td>
</tr>
<tr>
<td>F</td>
<td>BMPR1A, IVS3 + 5 (G→C)</td>
<td>17</td>
<td>JP and TA</td>
<td>Pancolon</td>
<td>None reported</td>
</tr>
<tr>
<td>G</td>
<td>BMPR1A, IVS1 + 1 (G→T)</td>
<td>36</td>
<td>JP and TA</td>
<td>Pancolon</td>
<td>Hepatic flexure CRC (36 y)*</td>
</tr>
<tr>
<td>H</td>
<td>SMAD4 c.1409_10insCCCT</td>
<td>8</td>
<td>JP and TA</td>
<td>Pancolon</td>
<td>None reported</td>
</tr>
<tr>
<td>Group 3 H</td>
<td>PTEN, 612insC</td>
<td>16</td>
<td>HP and TA</td>
<td>Rectosigmoid</td>
<td>Macrocephaly (OFC 64.4 cm); singlelipoma; keratoderma palmarepunctata</td>
</tr>
<tr>
<td>U</td>
<td>PTEN, IVS3-3 - 7delCTTTT</td>
<td>63</td>
<td>HP and TA</td>
<td>Pancolon</td>
<td>Macrocephaly (OFC 58.0 cm); non-small cell lung cancer (62 y); invasive ductal carcinoma (63 y)*</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, atrial septal defect; CRC, colorectal cancer; HP, hyperplastic polyps; JP, juvenile polyps; OFC, occipital-frontal circumference; PJ, Peutz-Jeghers polyps; SSA, sessile serrated adenomas; TA, tubular adenomas; VSD, ventricular septal defect.

*Numbers in parentheses indicate age at diagnosis.
history of non–small cell lung cancer and invasive ductal carcinoma of the breast, and 5 hyperplastic polyps and 9 adenomas identified since age 63 years. Neither PTEN mutation–positive patient had SSA on central pathology review.

Central review of pathology reports revealed the highest frequency of discrepancy in group 3. Initial pathology reports identified only 3 of 23 patients (13%) with SSA. Central pathology review noted that 13 of 23 patients (57%) had at least 1 SSA. Of the 11 patients with HPS, 9 had at least 1 SSA, with the majority (78%) found in the proximal colon. Seven colon carcinomas occurred in 4 patients with HPS (mean age at diagnosis, 54 years; range, 19–70 years). In the remaining cohort of patients in group 3, 4 had at least 1 SSA. Of these, 2 (aged 26 and 60 years, respectively) were diagnosed with cecal carcinoma.

COMMENT

Our systematic histopathologic and molecular evaluation of 49 unrelated patients with unexplained hamartomatous polyposis or mixed polyps revealed that 11 (22%) had germline mutations. Of these, we characterize a new genetically defined form of juvenile polyposis in 2 patients with germline ENG mutations previously found only in individuals with HHT. Seven additional patients had germline mutation of known polyposis genes, and 2 others were found to have a PTEN mutation, although their clinical history was not diagnostic for Cowden syndrome (Table 5). Colorectal polyps do occur at increased frequency in patients with Cowden syndrome, sometimes as early as age 5 years, and are typically found distal to the hepatic flexure.38,39 Usually these are hamartomatous, although adenomas and hyperplastic and inflammatory polyps have been reported.38,39 It remains to be seen what role the PTEN gene plays in the development of colorectal polyps. Overall, we reclassified 6 patients (1B, 1C, 2C, 2F, 2G, 2H), not only on the basis of this more extensive molecular analysis but also by reevaluation of polyp histology results.

It has been predicted that a proportion of patients with polyposis would share clinical and molecular features, given the crosstalk and interaction that occurs between many of the known genes. As an example, the TGF-β signaling pathway is an important mechanism for the pathogenesis of many heritable diseases, including juvenile polyposis, HHT, and primary pulmonary hypertension (Figure). Patients with mutations in ALK1 need careful assessment and management for complications not only of HHT but also of primary pulmonary hypertension. Similarly, mutations in the SMAD4 gene can cause a combined syndrome of juvenile polyposis and HHT.8 Other candidate genes of the TGF-β family have been studied in patients with juvenile polyposis, but no mutations have been identified.10 Endoglin, a protein product of the ENG gene previously associated with only HHT, acts as a coreceptor and accessory protein in this signaling pathway. Previously, 1 small series of patients (n=7) with features of both HHT and JPS were studied for either ENG, ACVRL1, or SMAD4 mutations; each was found to have a germline SMAD4 mutation.4 Therefore, this represents the first report of germline ENG mutation as a cause of juvenile polyposis. Interestingly, the 2 patients with ENG mutation in our series presented with juvenile polyposis of unusually early onset (Table 5). Neither patient has stigmata of HHT, although both are young. This suggests either that a subset of patients with JPS and ENG mutations will have polyposis without HHT, as may be the case with BMPRIA mutation–positive families, or that HHT may have age-related penetrance. Penetration of HHT symptoms by age 8 years is approximately 25% (D.A.M., unpublished data). The advent of clinical disease might also be dependent on the site and type of mutation within a particular gene. Interestingly, the vast majority of HHT type 1–specific missense mutations occur between exons 1 and 9, compared with the novel JPS-related ENG mutations that occur in exons 11 and 12.40 It is tempting to speculate that the missense mutations in this region of the protein may be gain-of-function mutations. This has been seen in achondroplasia41 and in the NEU/erbB2 receptor,42 in which mutations in the transmembrane domains are activated due to altered dimerization properties of the proteins.

Currently there is great interest focused on angiogenesis and its potential role in solid-tumor development, as seen with colorectal carcinoma. It is known that angiogenesis promotes the development of small adenomatous polyps, with recent evidence showing alterations in the microvasculature at even earlier stages of colon carcinogenesis.43–45 Endoglin is a homodimeric transmembrane glycoprotein predominantly expressed in vascular endothelial cells but also present in several nonendothelial tissues.46,47 Notably, elevated levels of endoglin expression have been detected on human microvascular endothelium and on vascular endothelial cells in tissues and tumors undergoing active angiogenesis.46–48 In fact, endoglin serves as a useful prognostic marker, as the microvessel density increases during the progressive stages of colorectal carcinogenesis.49,50 Gain-of-function mutations might serve to further up-regulate endoglin expression in the vasculature network, which, along with modifier genes and environment, contribute to polyp development and carcinogenesis. Recently, Lebrin et al51 implicated endoglin as a modulator of the balance between the TGF-β/ACVRL1 and TGF-B/ALK-5 signaling pathways in endothelial cells (Figure). Thus, imbalance between signaling pathways might be a clue to understanding the biological role of endoglin in the context of JPS and HHT.

Hyperplastic polyposis syndrome is not a single entity, as histological and molecular analysis has shown a mixed picture. This condition is characterized by multiple or large hyperplastic polyps. Recent studies suggest that the SSA may be a component of HPS and, as such, may be associated with the in-
reased risk for neoplasia.13-16,24-26 It remains unknown whether SSAs arise from traditional hyperplastic polyps or are a unique polyp of themselves, as well as which of a number of molecular pathways may be involved. In our series of patients with HPS, most polyps were hyperplastic polyps, indistinguishable from common sporadic hyperplastic polyps apart from the number and, in some cases, the large size and proximal location. The finding that most (81%) of our cases, the large size and proximal location of a number of molecular pathologists, suggests that the finding of histopathology results. Because mechanistic studies illustrate the importance of defining molecular diagnosis to influence surveillance and medical intervention strategies for the patient and to allow for determination of disease status for at-risk family members. This is because each specific syndrome carries different organ-specific risks of neoplasia. Many of our study patients, previously diagnosed and managed as having a particular form of polyposis, were reclassified based on extended molecular analyses and review of histopathology results. Because phenotypic features can be shared by a number of hamartoma syndromes, a molecular analysis of a battery of the known susceptibility genes (PTEN, BMPRIA, SMAD4, and STK11) should be considered for patients who have suggestive clinical and histopathologic features but who are negative for mutations in the most obvious genes. Lastly, given the significant discrepancies seen in histology reports, review by a dedicated gastrointestinal pathologist should be considered, since that may guide accurate selection of which gene(s) to begin testing.

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