The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations


Background: Juvenile polyposis (JP) is an autosomal dominant syndrome predisposing to colorectal and gastric cancer. We have identified mutations in two genes causing JP, MADH4 and bone morphogenetic protein receptor 1A (BMPR1A); both are involved in bone morphogenetic protein (BMP) mediated signalling and are members of the TGF-β superfamily. This study determined the prevalence of mutations in MADH4 and BMPR1A, as well as three other BMP/activin pathway candidate genes in a large number of JP patients.

Methods: DNA was extracted from the blood of JP patients and used for PCR amplification of each exon of these five genes, using primers flanking each intron-exon boundary. Mutations were determined by comparison to wild type sequences using sequence analysis software. A total of 77 JP cases were sequenced for mutations in the MADH4, BMPR1A, BMPR1B, BMPR2, and/or ACVR1 (activin A receptor) genes. The latter three genes were analysed when MADH4 and BMPR1A sequencing found no mutations.

Results: Germline MADH4 mutations were found in 14 cases (18.2%) and BMPR1A mutations in 16 cases (20.8%). No mutations were found in BMPR1B, BMPR2, or ACVR1 in 32 MADH4 and BMPR1A mutation negative cases.

Discussion: In the largest series of JP patients reported to date, the prevalence of germline MADH4 and BMPR1A mutations is approximately 20% for each gene. Since mutations were not found in more than half the JP patients, either additional genes predisposing to JP remain to be discovered, or alternate means of inactivation of the two known genes are responsible for these JP cases.

Juvenile polyposis (JP) is an autosomal dominant, hamartomatous polyposis syndrome which predisposes to gastrointestinal cancer. The prevalence is approximately one in 100 000 persons1 and affected individuals develop primarily colorectal and sometimes upper GI juvenile polyps. Juvenile polyps are characterised by normal epithelium and a lamina propria markedly expanded by dilated glands, abundant stroma, and an inflammatory infiltrate. The GI cancers which develop in JP patients are epithelial and the mechanism by which polyps transform into cancers remains unknown.

Significant progress has been made in our knowledge of the genes predisposing to JP. In 1998, a locus for JP was mapped to chromosome 18q21 by genetic linkage in a large JP family from Iowa. Subsequently, MADH4 (SMAD4, DPC4) germline mutations were identified in all affected members of this kindred as well as four other pedigrees.2 This finding was later confirmed by several investigators,3–5 but MADH4 mutations have only accounted for a fraction of JP cases.6 In 2001, a second locus for JP was localised to chromosome 10q22-23 by genetic linkage in four North American JP kindreds, and germline mutations were found in the bone morphogenetic protein receptor 1A (BMPR1A) gene in all affected members of these families.7 This finding was subsequently confirmed in other JP cases.8–10 However, the relative contribution of each of these genes in a large series of JP patients remains to be determined.

Since both genes known to predispose to JP are members of the TGF-β superfamily, another unanswered question is whether mutations of other genes in these pathways also cause JP. To date, studies examining JP cases for mutations of MADH1, MADH2, MADH3, MADH5, and MADH7 have been negative.11–12 JP may possibly result from alteration of BMP-mediated signalling pathways, since these both require BMPR1A and MADH4, and, therefore, alterations of other genes in the BMP pathway might also play a role in JP predisposition. It is of interest that bone morphogenetic protein receptor type II (BMPR2) is mutated in the germline of patients with familial pulmonary hypertension13 and mutations in the type I receptor homologue in the activin pathway (ACVR1) predispose to hereditary hemorrhagic telangectasia (HHT).14–17 JP patients sharing features with these disorders have been described18–20 and, therefore, BMPR2, ACVR1, and the other BMP type I receptor, BMPR1B, might also be plausible candidate genes for JP. In this study, we set out to determine the prevalence of MADH4 and BMPR1A mutations in JP, and whether JP patients without germline mutations in these two genes have mutations in BMPR1B, BMPR2, or ACVR1.

METHODS

Patients

The project was approved by the University of Iowa Institutional Review Board. After informed consent was obtained from each patient or guardian, blood was drawn and patients completed medical history questionnaires. Medical records were obtained and reviewed, and the standard criteria for the diagnosis of JP were used, which included: 1) more than five juvenile polyps of the colorectum;

Abbreviations: BMP, bone morphogenetic protein; BMPR1A, bone morphogenetic protein receptor 1A gene; CS, Cowden syndrome; HHT, hereditary hemorrhagic telangectasia; JP, juvenile polyposis; MH1, Mad Homology 1
2) juvenile polyps throughout the GI tract; or 3) any number of juvenile polyps with a family history of JP. Specific features suggestive of Peutz-Jeghers syndrome, Cowden syndrome (CS), Bannayan-Ruvalcaba-Riley syndrome, and Gorlin syndrome were sought out from medical records and questionnaires and patients with these characteristics were excluded from this study. DNA was extracted from whole blood using a salting-out method and then quantitated by spectrophotometry.

**Defining intron-exon boundaries**

The cDNA sequences of **BMPR1B** (Alk-6), **BMPR2**, and **ACVR1** (Alk-1) were retrieved from GenBank and segments of each were then used using the BLAST algorithm against the draft version of the human genome sequence and Celera genome sequences. The intron-exon boundaries of each gene were defined by comparison of these sequences and then primers were designed from intronic sequence flanking each exon using the Primer3 program.

**BMPR1B (Alk-6) primers**

Exons 1–3 were identified using the BAC clone 2025F7 (acc. no. AP001950), exon 4 on BAC RP11-59I16 (acc. no. AC011818), and exons 6–10 from BAC 2212D22 (acc. no. AP001969). The primers selected using the Primer3 program which were found to work well for amplification and sequencing were (capital letters represent nucleotides within exons, non-capsitals within introns): exon 1-Alk-6a: cccca cagatgctcaacct, Alk-6b: gctcaacctgcagatggttaa (269 bp product); exon 2-Alk-6a2a: acaactcgctcttcagttgc, Alk-6b2h: tttactgaaactgtggtaa (204 bp); exon 3-Alk-6a3a: gacaa gcttcgctgaggtaa (292 bp); exon 4-Alk-6a4a: ttctccatctggttaaagtga, Alk-6b4b: gacaa gcttcgctgaggtaa (292 bp); exon 5-Alk-6a5a: tttctccatgctgaggtaa (292 bp); exon 6-Alk-6a6a: aacaactctgttcaggttaa, Alk-6b6b: gaccagctgaaacacttaag (302 bp); exon 7-Alk-6a7a: tggagaacacctggttaa, Alk-6b7b: ggcctcagctcttcagta (416 bp); exon 8-Alk-6a8a: tcgttttattgtctcagttgc, Alk-6b8b: ggcctcagctcttcagta (416 bp); exon 9-Alk-6a9a: tcgtattgtaaactct, Alk-6b9b: ggcctcagctcttcagta (416 bp); exon 10-Alk-6a10a: ggcctcagctcttcagta (416 bp); exon 10-Alk-6a10b: cccca cagatgctcaacct, Alk-6b10b: gctcaacctgcagatggttaa (269 bp product).

**BMPR2 primers**

Exons 1 and 3–7 were identified on BAC clone RP11-68606 (acc. no. AC066836) and exons 8–13 on BAC clone RP11-345N12 (acc. no. AC075930). Exon 2 was not found in the human genome sequence and, therefore, primers could only be chosen from within this exon. Exon 12 was very large (1280 bp) and sequence and, therefore primers could only be chosen from within this exon. DNA from each exon was amplified by PCR using intronic primers as previously described for **BMPR1B**, **BMPR2**, and **ACVR1**. The primers developed for **BMPR1B**, **BMPR2**, and **ACVR1** are described above. PCR products were purified from 2% agarose gels using Qiaquick columns (Qiagen), quantitated, then cycle-sequenced using ABI-Prism dye terminators (Applied Biosystems) and one of the PCR primers. Sequences were determined using a Model 377 ABI Sequencer. Patient sequences were compared to wild type sequences using the Sequencher software (GeneCodes) and alterations from the wild type sequence were confirmed by sequencing in the opposite direction. When available, other family members were sequenced for the mutated exon to determine whether the same changes were present. Point mutations which did not change an amino acid were assumed to be silent polymorphisms.

**RESULTS**

**Patients**

DNA from a total of 77 different JP families and sporadic cases was sequenced. The majority of cases were referred from outside physicians and genetic counsellors within the United States, with additional cases coming from Canada, South America, and Europe. Seven Finnish JP cases in which we previously reported **MAHD4** sequencing results are not counted in this total, but are considered in later discussions of combined studies. Other patients previously reported from our laboratory were included. The **MAHD4** and **BMPRIA** genes were sequenced first in all cases, although **BMPRIA** was not sequenced in cases in which **MAHD4** mutations had already been established. The **PTEN** gene was also sequenced in the first 40 cases (except for four in which **MAHD4** mutations had been found), but this was discontinued thereafter because these mutations were considered to represent misdiagnoses of JP for CS. Other patients previously reported from our laboratory were included. The **MAHD4** and **BMPRIA** genes were sequenced first in all cases, although **BMPRIA** was not sequenced in cases in which **MAHD4** mutations had already been established. The **PTEN** gene was also sequenced in the first 40 cases (except for four in which **MAHD4** mutations had been found), but this was discontinued thereafter because these mutations were considered to represent misdiagnoses of JP for CS. Other patients previously reported from our laboratory were included. The **MAHD4** and **BMPRIA** genes were sequenced first in all cases, although **BMPRIA** was not sequenced in cases in which **MAHD4** mutations had already been established. The **PTEN** gene was also sequenced in the first 40 cases (except for four in which **MAHD4** mutations had been found), but this was discontinued thereafter because these mutations were considered to represent misdiagnoses of JP for CS.
premature stop codons, and of the six substitutions, one was a nonsense mutation and five were missense mutations. These mutations were distributed across six of the 11 MADH4 exons. In six cases, additional affected family members were available for sequencing and the proband mutation was found in the affected kindred members.

BMPR1A mutations were identified in 16 of 77 (20.8%) JP cases (table 2). These included six deletions and 10 substitutions. All deletions predicted for premature stop codons, and four substitutions were nonsense and six were missense mutations. These mutations were found in eight of 11 BMPR1A exons. Additional affected family members were available in six cases and the proband mutation was found in the affected kindred members.

**PTEN** mutations were found in only one patient, who, in retrospect, had features most consistent with Cowden syndrome. This mutation was an insertion of an additional adenine in a stretch of four adenines in exon 8 (871insA), which would lead to a premature stop at codon 297. No mutations were found upon sequencing BMPR1B, BMPR2, or ACVR1 genes in 52 patients (table 3).

**DISCUSSION**

Mutations in members of the TGF-β pathway have been implicated in the development of colon cancer, including those affecting the type I receptor, type II receptor, and MADH4. The finding that germline mutations in MADH4 were responsible for a proportion of JP cases raised the possibility that mutations of other members of the TGF-β pathway could be responsible for JP. The discovery that germline mutations in BMPR1A also caused JP suggested that disruption of BMP-mediated signalling rather than TGF-β could be responsible for this unusual phenotype predisposing to colon cancer. The BMP pathway is within the TGF-β superfamily, which envelopes the TGF-β, BMP, activin, and inhibin pathways. All share MADH4 as the common intracellular mediator of signal transduction. In these pathways, the respective ligands bind to specific type II receptors, which then activate specific type I receptors. The type I receptors phosphorylate MAD proteins (MADH2 and MADH3 for TGF-β; MADH1, MADH5, and MADH8 for BMP), which then form oligomers with MADH4, migrate to the nucleus, and regulate the transcription of specific genes by binding to particular sequences in conjunction with DNA binding proteins.

This study represents the largest single series of JP patients examined for MADH4 or BMPR1A mutations, and, as such, gives an accurate estimate of the prevalence of these alterations. We found an overall mutation rate of 18.2% for MADH4 and 20.8% for BMPR1A, suggesting that either additional JP genes remain to be discovered, or alternate methods of inactivation of these genes (which are not detected by sequencing) may be responsible for JP. With respect to correlation of mutations with phenotype, we have previously reported that 89% (17/19) of MADH4 and BMPRIA mutation positive (MUT+) cases and 63% (17/27) of mutation negative (MUT-) cases were familial (p = 0.09), and 89% (17/19) of MUT+ and 52% (13/25) of MUT- cases had a family history of GI cancer (p = 0.01). Interestingly, the incidence of a family history of upper GI juvenile polyps was 86% (6/7) in MADH4 mutation positive cases, 10% in BMPRIA mutation positive cases, and 23% (5/22) in mutation negative cases. Friedl et al also found that four of seven MADH4 mutation positive cases, only one of five cases with BMPR1A mutations and two of 17 MUT- cases had gastric polyposis. It therefore appears that MADH4 mutations predispose to generalised JP, while those cases which are MADH4 mutation negative are more likely to represent juvenile polyposis coli, a JP subtype with polyps involving only the colorectum.

Combining the present data with our previous reports and those of others, a total of 26 different MADH4 mutations have been found in JP patients (fig 1). Friedl et al described mutations in seven of 29 (24%) JP cases, Kim et al in three of five (60%) Korean JP cases, Woodford-Richens in five of 24 (21%) cases, and Howe et al in five of seven Finnish kindreds. If these studies are combined with the current series, taking into account three kindreds in common, then the total prevalence of MADH4 mutations is 22.7% (32 of 141 cases). These mutations consisted of 15 deletions, two insertions, and 15 substitutions (five nonsense, 10 missense). No splice site mutations have been described to date. Three mutations have been found in multiple patients. The 1244delACAG mutation has been reported in a total of six familial cases (four included in the present series). In depth haplotype analysis in four of these kindreds revealed no evidence for a common ancestor, suggesting this region of the gene is a mutational hotspot. Another common mutation was 1162C>T (E388X), described in one of our families and also in a sporadic Korean case with generalised JP. Lastly, there were four different substitutions found in codon 361, three changing a positively-charged arginine amino acid to a neutral one (glycine, serine, cysteine) and the other to a positively-charged histidine. The R361C mutation has also been described in the colorectal cancer cell line Mx5. A substitution similar to the E330G in this study (changing a neutral, polar amino acid to a neutral non-polar one) has also been described in colorectal cancer (E330A).

Of the 26 MADH4 unique mutations, only one (a deletion of nine nucleotides, amino acids 64–66) mapped to the Mad Homology 1 (MH1) domain, in a patient with extensive colonic polyposis. The MH1 domain can directly bind to DNA

<table>
<thead>
<tr>
<th>Table 1 MADH4 mutations in JP cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>JP23</td>
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</tr>
<tr>
<td>JP69</td>
</tr>
<tr>
<td>JP68</td>
</tr>
<tr>
<td>JP22</td>
</tr>
</tbody>
</table>

Fam, familial; Unk, unknown.
SMAD4 and BMPR1A mutations in JP

in target genes, such as the plasminogen activator inhibitor-1. Moren et al examined a G65V missense MADH4 mutation and found that it could translocate to the nucleus, activate a MAD-dependent promoter, and complex with Madh3, but had impaired DNA binding properties and decreased protein stability. Mutations between amino acids 43 and 135 in the MH1 domain markedly reduce the DNA binding activity of MADH4, and this may be a mechanism by which tumours evade the growth suppressive effects of the TGF-β superfamily. Five of the 26 JP mutations mapped to the linker and extensive colonic polyposis. SAD is a 48 amino acid MADH4 activating domain (SAD) in a patient with a VSD phenotype or minimal diagnostic criteria for CS9. The other negative JP cases and one patient with either CS-like vascularisation. Interestingly, there are case reports of JP patients also having pulmonary osteoarthropy, making BMPR2 a plausible candidate gene to examine in JP patients without MADH4 or BMPR1A mutations. Another type I

![Table 2](#)

**Table 2** BMPR1A mutations in JP cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Type*</th>
<th>Affected sequenced (n)</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP17</td>
<td>Fam</td>
<td>9</td>
<td>1</td>
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<td>2</td>
<td>170C&gt;G</td>
<td>P57R</td>
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<tr>
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<td>Fam</td>
<td>2</td>
<td>2</td>
<td>184T&gt;G</td>
<td>Y62D</td>
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<tr>
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<td>Fam</td>
<td>1</td>
<td>3</td>
<td>233G&gt;T</td>
<td>T78I</td>
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<tr>
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<td>Spor</td>
<td>1</td>
<td>3</td>
<td>245G&gt;A</td>
<td>C82Y</td>
</tr>
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<td>3</td>
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<td>6</td>
<td>673del</td>
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<td>7</td>
<td>715C&gt;T</td>
<td>Q239X</td>
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<td>Fam</td>
<td>6</td>
<td>7</td>
<td>812G&gt;A</td>
<td>W271X</td>
</tr>
<tr>
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<td>Spor</td>
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<td>7</td>
<td>864-868delACTTGIVS7+1-2delgt</td>
<td>Loss of exon 7 splice site; stop at new codon 292</td>
</tr>
<tr>
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<td>Spor</td>
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<td>8</td>
<td>961delC</td>
<td>Stop 321-2</td>
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<td>8</td>
<td>1013C&gt;A</td>
<td>A338D</td>
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<td>Fam</td>
<td>1</td>
<td>8</td>
<td>1061delG</td>
<td>Stop 363-4</td>
</tr>
<tr>
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<td>Spor</td>
<td>1</td>
<td>9</td>
<td>1327C&gt;T</td>
<td>R443C</td>
</tr>
</tbody>
</table>

*Fam, familial; Spor, sporadic; Unk, unknown.

Since the original description of BMPR1A mutations in JP patients, there have been two additional reports. One described mutations in 10 of 25 (40%) MADH4 mutation negative JP cases and one patient with either CS-like phenotype or minimal diagnostic criteria for CS. The other found five BMPR1A mutations in 29 JP cases (17.2%). In the current study, we report that 16 of 77 (20.8%) JP cases had BMPR1A mutations. In all, 31 different BMPR1A mutations have been described, two cases with a common mutation (fig 2). The best estimate of the overall prevalence of BMPR1A mutations in JP would combine the current series with that of Friedel et al (Zhou et al examined at only MADH4 negative cases and included those with CS/BRRS and CS-like features). This gives an overall BMPR1A mutation rate of 19.8% (21 of 106 cases). Of 31 unique mutations described, nine were deletions, one was an insertion, 19 were substitutions (10 missense, nine nonsense), and two were splice site mutations (not including a 7 bp deletion at the end of exon 7, which included the 2 bp splice acceptor site). The most commonly affected region was the protein kinase domain of the gene, where six of seven mutations in exon 7 and all eight mutations in exon 8 involved this area of the intracellular domain. There were eight mutations involving the cytoenrich region of the extracellular domain, including two non-sense mutations, five missense mutations, and one deletion. Of these missense mutations, three changed a cysteine to another amino acid (C82Y, C124R, C130R) and the other two changed a neutral amino acid with polar side chains to a negatively charged amino acid (Y62D) or to a neutral, non-polar amino acid (T78I). There have been no mutations described in the transmembrane domain of the gene.

Several investigators have examined other MAD genes for germline mutations in JP patients without MADH4 mutations. Thirty such cases were negative for mutations in MADH1, MADH2, MADH3, and MADH5, and four were negative for mutations in MADH2, MADH3, and MADH7. However, the involvement of other BMP receptor genes in JP, which include BMPR1B and BMPR2, has not been studied. Mutations in BMPR2 have been demonstrated to cause familial primary pulmonary hypertension, a condition characterised by endothelial proliferation in the pulmonary vasculature. Interestingly, there are case reports of JP patients also having pulmonary osteoarthropy, making BMPR2 a plausible candidate gene to examine in JP patients without MADH4 or BMPR1A mutations. Another type I
A receptor gene within the TGF-β superfamily has also been implicated in human disease. Germline mutations in ACVR1, a type I activin receptor, have been described in hereditary hemorrhagic telangiectasia, and JP patients with pulmonary osteoarthropathy have also had features reminiscent of HHT. In this study, 32 MADH4 or BMPR1A mutation negative JP cases were negative for mutations in BMPR1B, BMPR2, or ACVR1 by sequencing.

Only one familial JP patient in this series clearly had a history of HHT, with the proband having AV malformations of the lung requiring pulmonary resection and later embolisation. This patient had telangiectasias noted from her nose to throughout the gastrointestinal tract. Her daughter had pulmonary arteriovenous malformations of the left lower lobe of the lung treated by embolisation at age 18. She also had a mucinous adenocarcinoma of the rectum resected at age 22 and has had gastric, duodenal, and jejunal juvenile polyps. This patient’s daughter (the grand-daughter of the proband) also had diffuse colonic polyposis, hypoalbuminemia, ascites, and digital clubbing. These individuals were found to have a substitution in exon 8 of MADH4, nucleotide 1054G>A (G352R). Sequencing of ACVR1, BMPR1A, BMPR1B, and BMPR2 were all negative for mutations in the proband. Another patient and her father have been described with cavernous transformation of the portal vein and JP, but no mutations of MADH4, BMPR1A, BMPR1B, BMPR2, or ACVR1 were found in this patient. No clear history of PPH or HHT was found in the other 31 cases sequenced for BMPR1B, BMPR2, or ACVR1. However, since these patients were collected from throughout North America and the evaluation of each was variable, a small number of other cases could also have had PPH or HHT. Gallione et al recently reported a series of eight patients with both JP and HHT who had germline MADH4 mutations, but no mutations of ACVR1 or endoglin, confirming MADH4 as a third predisposing gene for HHT.

A controversial issue in JP genetics is whether PTEN mutations cause JP. Two reports have described four patients with JP who had PTEN mutations, but some of these patients could have represented cases of undiagnosed Cowden syndrome. Furthermore, no mutations were found by two groups who sequenced PTEN in 36 JP cases. We found only one case of PTEN mutation in 32 MADH4 and BMPR1A mutation negative JP cases and in retrospect this patient had a family history of goitre more suggestive of Cowden syndrome than JP. In CS, the incidence has been estimated to be 35–40% for GI polyps, 70% for fibroadenomas of the breast, 40–60% for thyroid adenomas and goitre and 99% for trichilemmomas and mucocutaneous papules. The lifetime risk is 25–50% for breast cancer and 3–10% for thyroid cancer. We believe that JP is not caused by PTEN mutation, but instead, some patients with CS may have only hamartomatous polyps of the GI tract identified, especially if they present with GI bleeding at a young age. Therefore, we recommend that initial genetic screening in JP patients include MADH4 and BMPR1A sequencing, but if negative, PTEN sequencing may be of value to detect undiagnosed
cases of CS. These patients would then potentially benefit from close screening for breast and thyroid malignancies.

In conclusion, multiple studies support a prevalence of approximately 20% each for \textit{MADH4} and \textit{BMPR1A} germline mutations in JP. These data suggest there are other genes which predispose to JP and we have excluded \textit{BMPR1B}, \textit{BMPR2}, and \textit{ACVR1} in this study by sequencing. It is also possible that alternate methods of inactivation of \textit{MADH4} and \textit{BMPR1A} account for some cases. In a subset of hereditary non-polyposis colorectal cancer cases, the effects of subtle mutations affecting splicing or exonic duplication were not realised until separation of alleles was accomplished in somatic cell hybrid lines.53 It has also been shown that single nucleotide polymorphisms within a gene may cause increased or reduced expression of that gene and be inherited in a Mendelian fashion.54 At present, it is unclear whether the JP phenotype results specifically from altered BMP signal transduction or disruption of other TGF-\(\beta\) superfamily mediated pathways. The discovery of new JP genes, careful analysis of JP cases for alternate methods of \textit{MADH4} and \textit{BMPR1A} inactivation, and gene expression studies of juvenile polyps will be important in order to define the underlying genetic basis of JP.

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**ELECTRONIC-DATABASE INFORMATION**


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REFERENCES


