

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

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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,^{3–6} but *MADH4* mutations have only accounted for a fraction of JP cases.^{3–7} 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.⁸ This finding was subsequently confirmed in other JP cases.^{9–10} 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 telangiectasia (HHT).^{14–15} JP patients sharing features with these disorders have been described^{16–18} 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 telangiectasia; 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.¹⁹ 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 method²⁰ 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 searched using the BLAST algorithm against the draft version of the human genome sequence²¹ and Celera genome sequences.²² 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 upon 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-capitals within introns): exon 1-Alk6-1a: ccca cagatgcctaactct, Alk6-1b: gctcacagttgcatagttgaaaa (269 bp product); exon 2-Alk6-2a: acacattctgtttctcactttgc, Alk6-2b: ttgcttgaactgtttggaaa (204 bp); exon 3-Alk6-3a: gactttgaaa ttggaagaaactca, Alk6-3b: ctccagcagtggaagtgtt (266 bp); exon 4-Alk6-4a: ttttcacattttgtgctgta, Alk6-4b: gaaactaa caggcaaaagca (294 bp); exon 5-Alk6-5a: tttactcatcattgca ttca, Alk6-5b: gctttctctgtccagtg (302 bp); exon 6-Alk6-6a: acaaatctgtagcgtgtgaa, Alk6-6b: ggaaagctagaaacctgaa (292 bp); exon 7-Alk6-7a: ttggaaactaaaccttttca, Alk6-7b: cctagaagcactgcctaca (416 bp); exon 8-Alk6-8a: tcgtttttgt gcctcttc, Alk6-8b: gcaatgactgcatcaacaga (370 bp); exon 9-Alk6-9a: gcaattctctttgagaactgtg, Alk6-9b: ccatcaccatcaccat caaa (339 bp); exon 10-Alk6-10a: ccagccttcagatgataca, Alk6-10b: tctgcccaacaacagaagag (234 bp).

BMPR2 primers

Exons 1 and 3–7 were found on BAC clone RP11-68606 (acc. no. AC064836) and exons 8–13 on BAC RP11-345N12 (acc. no. AC009960). 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 required four primer pairs to span this sequence. The primers chosen were: exon 1-BMPR2-1c: tctttctttgcctctctga, BMPR2-1d: ggcgatttccctggaagg (288 bp); exon 2-BMPR2-2a: tagCTTCGCAGAAATCAAGAAC, BMPR2-2b: tgcCTTGTTTTAC AAGATTTATGTC (177 bp); exon 3-BMPR2-3a: tctgttgatttg caaaactgt, BMPR2-3b: tgcaaatctttggagaaagga (358 bp); exon 4-BMPR2-4a: cagcctttctaaaggcagctc, BMPR2-4b: aggctgggtg tattttgcat (320 bp); exon 5-BMPR2-5a: gctgctaattcttctg cagctc, BMPR2-5b: gaatgaagtcactgttccaggtc (273 bp); exon 6-BMPR2-6a: gcaacagagagctgtagcatt, BMPR2-6b: tacagcca taagccaccaca (391 bp); exon 7-BMPR2-7a: catggaatcctagcc tatttgc, BMPR2-7b: cccatgatgagtcaatttca (264 bp); exon 8-BMPR2-8a: tcatttcattgtaaatgctccttt, BMPR2-8b: cacactgg ccagtagatgtt (298 bp); exon 9-BMPR2-9a: tgttctcagaatagc tacgttctc, BMPR2-9b: gaagatataactgcttcaactcaaaaa (258 bp); exon 10-BMPR2-10a: gctgaaggggatgaaaaa, BMPR2-10b: ggcattagcgaactcaaaaa (388 bp); exon 11-BMPR2-11a: catgtgg taaactgaaaagctca, BMPR2-11b: tctttgtgggtctcattttct (310 bp); exon 12-BMPR2-12a: aaatttggagagacagttttgca, BMPR2-12b: TTCATCTGGGTATGGCATCTC (391 bp); BMPR2-12c: CACA

GGACTCACGCCAAGTA, BMPR2-12d: ATCAAACATGCTTGG CCATT (351 bp); BMPR2-12e: CTGGACAGCAGGACTT CACA, BMPR2-12f: CTGGGCCCTATGTGTCACT (381 bp); BMPR2-12g: CCACAACCCAATATGCCAAT, BMPR2-12h: ccccaaaagacacacaggag (449 bp); exon 13-BMPR2-13a: caccctctgagacattggt, BMPR2-13b: ggggtgctgacaggagataa (458 bp).

ACVR (Alk-1) primers

All exons were found on the Celera chromosome 12 scaffold segment GA_x2HTBL3Q5WG.²² The nine exons corresponded to 1512 bp in cDNA, which were distributed over 8428 bp in genomic DNA. The primers used were: exon 1-Alk1-1a: gctgtcacacttcatggtct, Alk1-1b: cccagcttcaagtgcagc (216 bp); exon 2-Alk1-2a: tagcacagaaatgggtgtgc, Alk1-2b: ccagagcat gagaggaaagg (407 bp); exon 3-Alk1-3a: gggagctgacctagtg aag, Alk1-3b: tcctatctgccctctctaca (308 bp); exon 4-Alk1-4a: tcccagctcagagatagaga, Alk1-4b: gagagccttgctctctac (216 bp); exon 5-Alk1-5a: tgtgtgccagtggtgtaacc, Alk1-5b: aacttgagcctt gactgagc (255 bp); exon 6-Alk1-6a: acgactccagctcccttag, Alk1-6b: ctccgccacctgtgaag (383 bp); exon 7-Alk1-7a: tttctcagtcaccaacttg, Alk1-7b: gctgattcccccttccctac (320 bp); exon 8-Alk1-8a: tggattgggctcctctaga, Alk1-8b: ggctcagaca caatttct (272 bp); exon 9-Alk1-9-5': tctcctctgacctctctcccaa, Alk1-9-3': ctgcaggcagaaggaatcaggtgct (197 bp).

Sequencing

DNA from each exon was amplified by PCR using intronic primers as previously described for *MADH4*² and *BMPR1A*.⁸ 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 *MADH4* sequencing results are not counted in this total, but are considered in later discussions of combined studies.² Other patients previously reported from our laboratory were included.^{2, 8, 23} The *MADH4* and *BMPR1A* genes were sequenced first in all cases, although *BMPR1A* was not sequenced in six cases in which *MADH4* mutations had already been established. The *PTEN* gene was also sequenced in the first 40 cases (except for four in which *MADH4* mutations had been found), but this was discontinued thereafter because these mutations were considered to represent misdiagnoses of JP for CS.⁷ *BMPR1B*, *BMPR2*, and *ACVR1* were sequenced in a total of 32 *MADH4*, *BMPR1A*, and *PTEN* mutation negative cases.

Results of sequencing

Germline *MADH4* mutations were found in 14 of 77 (18.2%) JP cases (table 1). These consisted of eight deletions and six substitutions. All deletions were predicted to result in

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.

BMPRIA 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 *BMPRIA* 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.⁷ 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 *BMPRI1B*, *BMPRI2*, or *ACVRI* genes in 32 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,²⁴ type II receptor,²⁵ and *MADH4*.²⁶ The finding that germline mutations in *MADH4* were responsible for a proportion of JP cases² raised the possibility that mutations of other members of the TGF- β pathway could be responsible for JP. The discovery that germline mutations in *BMPRIA* 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 encompasses the TGF- β , BMP, activin, and inhibin pathways.²⁷ 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*,²⁸ migrate to the nucleus, and regulate the transcription of specific genes by binding to particular sequences in conjunction with DNA binding proteins.²⁹

This study represents the largest single series of JP patients examined for *MADH4* or *BMPRIA* 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 *BMPRIA*, 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 ($p = 0.01$).²³ 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 *BMPRIA* mutations and two of 17 MUT- cases had gastric polyposis.¹⁰ 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.³⁰

Combining the present data with our previous reports^{2, 7} and those of others,^{5, 6, 10, 12} 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,¹⁰ Kim *et al* in three of five (60%) Korean JP cases,⁵ Woodford-Richens in five of 24 (21%) cases,⁶ and Howe *et al* and Roth *et al* in five of seven Finnish kindreds.^{2, 12} If these studies are combined with the current series, taking into account three kindreds in common,^{2, 12} 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).^{2, 4} 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.³¹ 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 Mx5.²⁶ 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).³²

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.⁶ The MH1 domain can directly bind to DNA

Table 1 *MADH4* mutations in JP cases

Case	Type*	Affected sequenced (n)	Exon	Nucleotide change	Result
JP23	Fam	2	4	608delC	Stop 240-1
JP11	Fam	1	8	989A>G	E330G
JP8	Unk	1	8	1037delC	Stop 383-4
JP47	Fam	2	8	1054G>A	G352R
JP64	Fam	1	8	1081C>G	R361G
JP66	Fam	1	8	1081C>A	R361S
JP21	Fam	6	9	1162C>T	E388X
JP1	Fam	17	9	1244delACAG	Stop 435-6
JP2	Fam	1	9	1244delACAG	Stop 435-6
JP3	Fam	6	9	1244delACAG	Stop 435-6
JP4	Fam	3	9	1244delACAG	Stop 435-6
JP69	Unk	1	10	del1343-1365	Stop
JP68	Unk	1	11	1529G>T	G510V
JP22	Fam	1	11	1588delC	Stop 536-7

*Fam, familial; Unk, unknown.

Table 2 *BMPRIA* mutations in JP cases

Case	Type*	Affected sequenced (n)	Exon	Nucleotide change	Result
JP17	Fam	9	1	44-47delTGTT	Stop 35-6
JP74	Unk	1	2	170C>G	P57R
JP5	Fam	2	2	184T>G	Y62D
JP72	Unk	1	3	233C>T	T78I
JP57	Spor	1	3	245G>A	C82Y
JP20	Fam	2	3	262G>T	E84X
JP46	Fam	1	4	349C>T	Q117X
JP14	Fam	1	4	353delT	Stop 122-3
JP53	Fam	1	6	673delT	Stop 259-60
JP16	Fam	8	7	715C>T	Q239X
JP18	Fam	6	7	812G>A	W271X
JP25	Spor	1	7	864-868delACTTGIVS7+1-2delgt	Loss of exon 7 splice site; stop at new codon 292
JP19	Fam	5	8	961delC	Stop 321-2
JP43	Spor	1	8	1013C>A	A338D
JP9	Fam	1	8	1061delG	stop 363-4
JP58	Spor	1	9	1327C>T	R443C

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

in target genes, such as the plasminogen activator inhibitor-1.³³ Moren *et al* examined a G65V missense *MADH4* mutant 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.³⁴ 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.^{35, 36} Five of the 26 JP mutations mapped to the linker region of *MADH4*. One of these (831-2delAC) was within the *MADH4* activating domain (SAD) in a patient with a VSD and extensive colonic polyposis.⁴ SAD is a 48 amino acid regulatory unit, that when mutated, still allows for other MAD protein and transcription factor binding, but not activation of transcription.³⁷ The remaining mutations all map to the MH2 domain of *MADH4*, which is important in directing transcriptional responses.³⁸ The majority of *MADH4* mutations thus far described in diverse human tumours are found in the MH2 domain, such as eight of 10 mutations described in pancreatic cancers^{39, 40} and nine of 11 in colorectal cancers.^{26, 32, 41} The MH2 region appears to play an important role for nuclear localisation, interaction with other MADs, and transcriptional activation.^{28, 42} Of the different missense mutations found in the MH2 domain, five of the seven occurred at highly conserved amino acids shared in the C-terminal regions of human *MADH1-5*, *Drosophila* Mad, and *C. elegans* Sma2-4 proteins, and, therefore, these specific amino acids may have important structural roles.⁴³

Since the original description of *BMPRIA* mutations in JP patients,⁸ 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.⁹ The other found five *BMPRIA* mutations in 29 JP cases (17.2%).¹⁰ In the current study, we report that 16 of 77 (20.8%) JP cases had *BMPRIA* mutations. In all, 31 different *BMPRIA* mutations

have been described, two cases with a common mutation (fig 2). The best estimate of the overall prevalence of *BMPRIA* mutations in JP would combine the current series with that of Friedl *et al* (Zhou *et al* looked at only *MADH4* negative cases and included those with CS/BRRS and CS-like features). This gives an overall *BMPRIA* 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 cysteine-rich region of the extracellular domain, including two nonsense 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*,¹¹ and four were negative for mutations in *MADH2*, *MADH3*, and *MADH7*.¹² 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.¹³ Interestingly, there are case reports of JP patients also having pulmonary osteoarthropathy,^{16, 17} making *BMPR2* a plausible candidate gene to examine in JP patients without *MADH4* or *BMPRIA* mutations. Another type I

Table 3 Summary of mutations in JP cases

	<i>MADH4</i>	<i>BMPRIA</i>	<i>PTEN</i>	<i>BMPR1B</i>	<i>BMPR2</i>	<i>ACVR1</i>
Chromosomal locus	18q21	10q22-23	10q22-23	4q22-24	2q33-34	12q13
Mutations (n)	14	16	1	0	0	0
Cases (n)	77	77	36	32	32	32
Rate of mutation	18.2%	20.8%	2.8%	0%	0%	0%

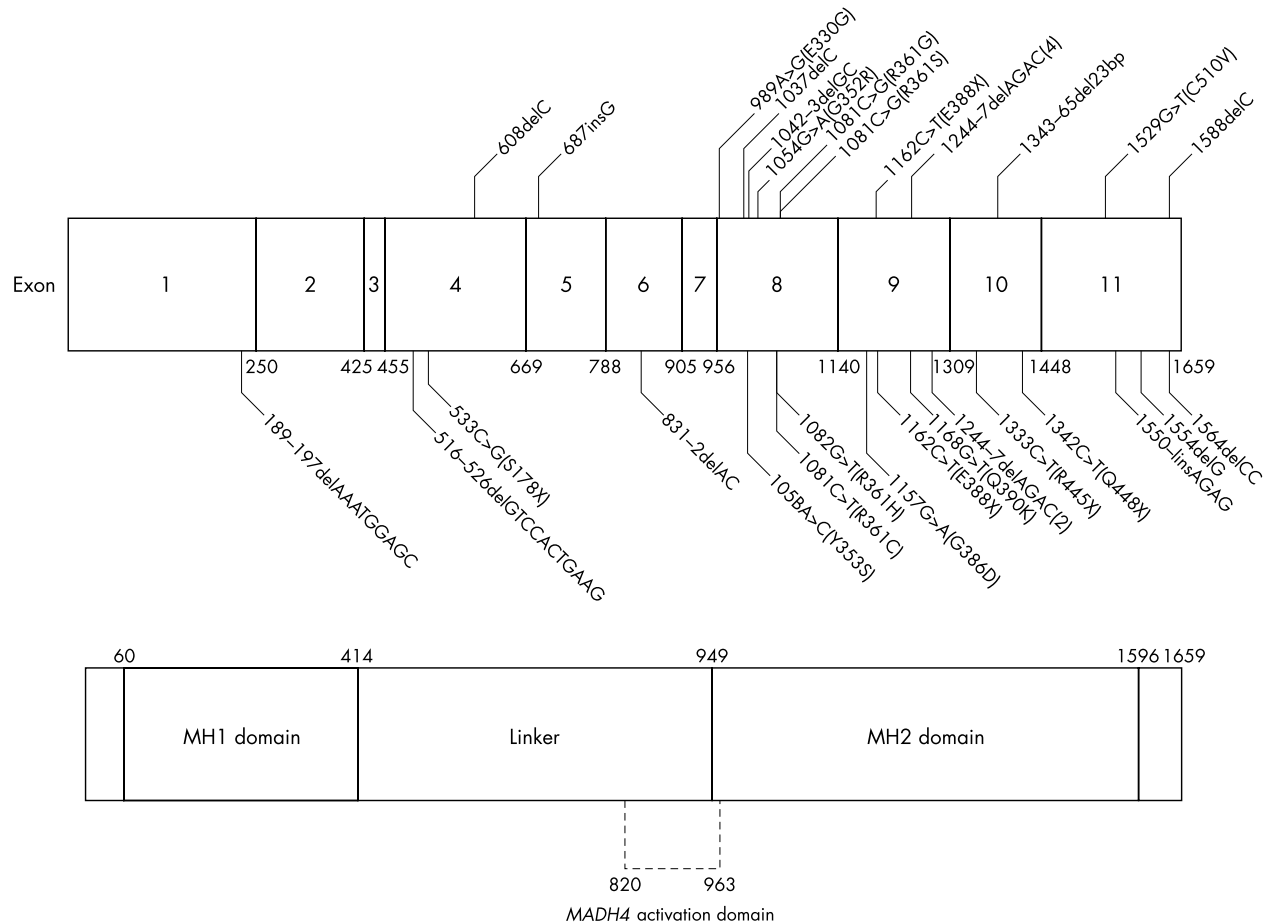


Figure 1 The upper rectangle represents the *MADH4* gene, with the exons shown within the rectangle and nucleotides above and below. Lines designate mutations identified in JP cases, with those shown above each exon identified in our laboratory and those below from the reports of Friedl *et al*,⁴ Roth *et al*,¹² Kim *et al*,⁵ and Woodford-Richens *et al*.⁶ The lower rectangle represents the different known domains of the *MADH4* gene.

receptor gene within the TGF- β superfamily has also been implicated in human disease. Germline mutations in *ACVRI*, a type I activin receptor, have been described in hereditary hemorrhagic telangiectasia,¹⁵⁻⁴⁴ and JP patients with pulmonary osteoarthropathy have also had features reminiscent of HHT.¹⁷ In this study, 32 *MADH4* or *BMPRIA* mutation negative JP cases were negative for mutations in *BMPR1B*, *BMPR2*, or *ACVRI* 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. She had both upper and lower GI juvenile polyps, requiring both colectomy and gastrectomy. 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 *ACVRI*, *BMPRIA*, *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,⁴⁵ but no mutations of *MADH4*, *BMPRIA*, *BMPR1B*, *BMPR2*, or *ACVRI* were found in this patient. No clear history of PPH or HHT

was found in the other 31 cases sequenced for *BMPR1B*, *BMPR2*, or *ACVRI*. 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 *ACVRI* or endoglin, confirming *MADH4* as a third predisposing gene for HHT.⁴⁶

A controversial issue in JP genetics is whether *P TEN* mutations cause JP. Two reports have described four patients with JP who had *P TEN* mutations,⁴⁷⁻⁴⁸ but some of these patients could have represented cases of undiagnosed Cowden syndrome.⁴⁹ Furthermore, no mutations were found by two groups who sequenced *P TEN* in 36 JP cases.⁵⁰⁻⁵¹ We found only one case of *P TEN* mutation in 32 *MADH4* and *BMPRIA* mutation negative JP cases and in retrospect this patient had a family history of goitre more suggestive of Cowden syndrome than JP.⁷ 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.⁵² We believe that JP is not caused by *P TEN* 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.⁴⁹ Therefore, we recommend that initial genetic screening in JP patients include *MADH4* and *BMPRIA* sequencing, but if negative, *P TEN* sequencing may be of value to detect undiagnosed

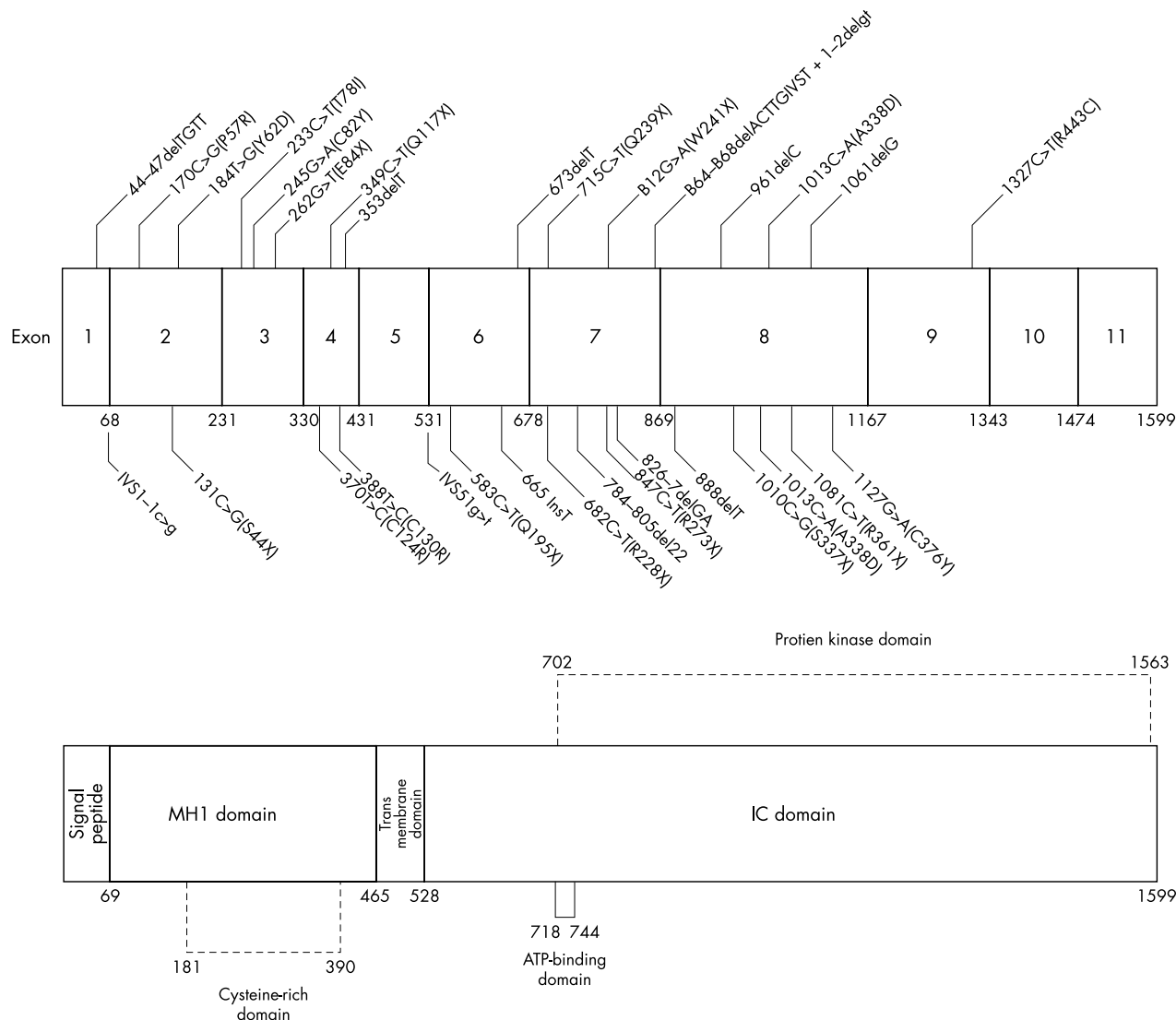


Figure 2 The upper rectangle represents the *BMPR1A* gene, with the exons shown within the rectangle and nucleotides above and below. Lines designate mutations identified in JP cases, with those shown above each exon identified in our laboratory and those below from the reports of Zhou et al.⁹ and Friedl et al.¹⁰ The lower rectangle represents the different known domains of the *BMPR1A* gene.

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 *MADH4* and *BMPR1A* germline mutations in JP. These data suggest there are other genes which predispose to JP and we have excluded *BMPR1B*, *BMPR2*, and *ACVR1* in this study by sequencing. It is also possible that alternate methods of inactivation of *MADH4* and *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.³³ 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.³⁴ At present, it is unclear whether the JP phenotype results specifically from altered BMP signal transduction or disruption of other TGF-β superfamily mediated pathways. The discovery of new JP genes, careful analysis of JP cases for alternate methods of *MADH4* and *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



Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>; GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/index.html>; BLAST, <http://www.ncbi.nlm.nih.gov/BLAST/>; Primer3 Program, <http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>; Celera genome sequence, <http://public.celera.com/humanpub/index.jsp>

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