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Mutation Screening in Juvenile Polyposis Syndrome

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Juvenile polyposis syndrome (JPS) is an autosomal dominant cancer predisposition syndrome characterized by congenital anomalies, hamartomatous polyps in the gastrointestinal tract, and the development of tumors in these tissues. The diagnosis of JPS is often difficult because of the phenotypic overlap with other hamartomatous polyposis syndromes. Germline mutations have been identified in *MADH4* and *BMPR1A*, aiding in presymptomatic genetic testing. In this study, we describe the results from 3 years of molecular diagnostic screening in JPS. Seventy unrelated individuals referred to our lab for JPS testing were examined through the sequence analysis of coding regions and exon-intron boundaries in both genes. Germline mutations were identified in 30% of cases, with 11.4% in *BMPR1A* and 18.6% in *MADH4*. All mutation-positive individuals were negative for cancer at testing, and a single pulmonary valve stenosis was the only congenital anomaly reported. A majority of mutations identified were novel including the first splice site alteration in *MADH4*. Based on the limited number of exons in each gene, low polymorphism frequency, and high frequency of frameshift or nonsense mutations identified, direct sequence analysis is a suitable methodology for mutation screening if all coding regions and exon-intron boundaries are examined in both genes. *(J Mol Diagn 2006, 8:84–88; DOI: DOI:10.2353/jmoldx.2006.050072)*

Juvenile polyposis syndrome (JPS) (OMIM no. 174900) is one of four hamartomatous-polyposis syndromes including Puetz-Jeghers, Cowden, and Bannayan-Riley-Ruvalcaba syndromes.[1] Individuals with JPS develop juvenile polyps primarily in the colorectal region but also throughout the upper gastrointestinal tract.[2] Histologically, these polyps are characterized by a smooth appearance with cystic glands embedded in hyperplastic stroma and inflammatory infiltrate. Most juvenile polyps are benign, but individuals are at risk for developing cancers of the gastrointestinal system. Colorectal cancer is by far the most common cancer, with risk estimates ranging from 9 to 68%,[3–5] but stomach, duodenal, and pancreatic cancers have also been described.[4] The molecular mechanisms by which the juvenile polyps seen in JPS give rise to this spectrum of tumors are still unknown. Congenital heart defects, cleft lip or palate, and malrotations have also been described in 11 to 20% of individuals with JPS.[6]

JPS is inherited in an autosomal dominant manner with a prevalence of 1 in 100,000.[7] In 1974, the syndrome was subdivided into three groups based on the age of involvement and polyp location, including 1) JPS of infancy characterized by bloody diarrhea, anemia, anasarca, and death before 1 year; 2) JPS coli in which polyps are limited to colon and rectum; and 3) generalized JPS in which polyps occur from the stomach to the rectum.[8] It still remains to be elucidated if these distinctions truly represent real clinical subsets of JPS or are instead due to the variable expression of the underlying molecular defects.[4]

The diagnosis of JPS is typically made first through the exclusion of the other hamartomatous polyposis syndromes. Once that is established, a diagnosis can be made based on meeting any one of the three following sets of criteria: 1) more than five juvenile polyps in the colorectal region, 2) juvenile polyps throughout the gastrointestinal tract, or 3) any number of juvenile polyps and a family history of JPS.[9]

In 1998, a locus was identified on 18q21 through linkage in a large family from Iowa, and germline mutations in the *MADH4* gene (SMAD4, DPC4) were subsequently found.[10,11] MADH4 forms cytoplasmic complexes with other phosphorylated MADH family members, which then translocate to the nucleus and act in transcriptional regulation of cell-cycle genes as part of the transforming growth factor-β signaling pathway.[12] Mutations in *MADH4* have recently been identified in individuals who express features of both JPS and hereditary hemorrhagic telangiectasia (HHT) (OMIM no. 175050), an autosomal dominant disorder of vascular malformation.[13]

The discovery of *MADH4* mutations was followed in 2001 with the identification of germline mutations in bone morphogenic protein receptor 1A (*BMPR1A*) in another subset of JPS cases.[14] BMPR1A is a serine-threonine kinase receptor and member of the transforming growth factor-β superfamily. Activation is achieved through phosphorylation by type II receptors, and BMPR1A can then phosphorylate MADH1,[15,16] MADH5, and MADH8, which then associate with cytoplasmic MADH4.[17] Although the products of these genes all function in the common transforming growth factor-β pathway, no addi-

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Table 1. Summary of *MADH4* Mutation-Positive Cases

Case	Location	Nucleotide change	Result	Clinical notes	Age at polyp Dx	Number of polyps	Polyp type and location
1	Ex2	403 C>T	R135X		35	~20	
2	Ex3	430-431delTC	Stop 150-151		Infancy	>5	Juvenile–rectum, stomach
3	Ex5	729-730insCCGC	Stop 262-263		8	>140	Juvenile–colon, terminal ileum
4	Ex7	925-928dupGCAT	Stop 320-321		17	>10	Juvenile–transverse, descending, sigmoid, rectum
5	Ex8	982-983insT	Stop 329-330		9.5	>9	Juvenile–hepatic flexure, rectum
6	Ex8	1088-1090delGTT	del363C	HHT	11		Colon
7	Ex8	1091 T>G	L364W		2.5	>15	Juvenile–colon
8	Ex8	1113delC	Stop 383-384		33	Many	Juvenile–colon, stomach
9	Ex9	1242-1245delAGAC	Stop 435-436		10	>6	Sigmoid
10	Ex9	1244-1247delACAG	Stop 435-436		21	Innumerable	Juvenile–colon, stomach Hyperplastic-stomach
11	Ex10	1361-1364delCACA	Stop 475-476		23	>20	Cecum, ascending, descending, sigmoid
12	Intron 10	Ivs9 + 1 G>A	Loss of SD		30	>15	Juvenile (two with focal adenomatous changes) colon
13	Ex11	1596delC	Stop 536-537	HHT	7	Colectomy at 7	

Dx, diagnosis; SD, splice donor; HHT, hereditary hemorrhagic telangiectasia.

tional germline mutations have been identified in other MADH family members[18,19] or in other bone morphogenic proteins and receptors[20] in patients with JPS.

The identification of the underlying genetic causes in other cancer predisposition syndromes, such as familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, have moved molecular testing into the standard of care for these disorders. We consequently sought to examine the molecular testing for JPS in a similar light. In this report, we describe the results of 3 years of experience in screening a series of 70 unrelated cases for germline mutations in *MADH4* and *BMPR1A* by direct sequence analysis of all coding exons and exon-intron boundaries. Mutations were identified in 30% of this group, with 38% occurring in *BMPR1A* and 62% occurring in *MADH4*. All *BMPR1A* mutations identified were novel, as were 11 of 13 in *MADH4* including the first description of a splice site alteration in that gene.

Materials and Methods

The cases in this report represent 70 sequential patients referred to the Molecular Pathology Laboratory at Ohio State University for JPS gene testing based on medical and/or family history. DNA was extracted from patient peripheral blood samples using a standard salting-out procedure. All coding regions and exon-intron boundaries for *MADH4*[11] and *BMPR1A*[14] were amplified using previously described primers. Amplification was confirmed by loading 8 μl of product on a 2.5% agarose gel, and products were purified for sequencing using Sephacryl S-400 microspin columns (Amersham Biosciences, Uppsala, Sweden). Cycle sequencing was conducted in both forward and reverse directions using ABI-Prism dye terminators on either an ABI model 377 DNA Sequencer or 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Alterations from the wild-type sequence were identified using Mutation Surveyor (SoftGenetics, State College, PA) and confirmed in both directions. All individuals, regardless of mutation status, were sequenced for both genes. Medical information, including mutation-positive patient and family histories, was obtained through interviews with either the referring physician for molecular testing or the individual's genetic counselor.

Results

All mutation-positive individuals met the diagnostic criteria for JPS based on the number of polyps, anatomical location, or family history (data not shown). Additionally, a majority of cases were classified as familial with the exception of two in which no information was available (Table 1, *MADH4* case 1; Table 2, *BMPR1A* case 5) and two sporadic cases (*BMPR1A* case 6 and *MADH4* case 11). A single case of pulmonary valve stenosis was the only congenital anomaly reported (*BMPR1A* case 8), and all mutation-positive individuals were negative for cancer at the time of molecular testing (data not shown). The mean age of polyp diagnosis was similar for individuals harboring mutations in either gene with an average age of 17.25 years in *MADH4* mutation-positive individuals and 18.14 years in those with *BMPR1A* mutations. The numbers of polyps ranged from five to innumerable or extensive and were typically juvenile, while hyperplastic and adenomatous polyps were also reported. Gastric polyposis was identified in 17% (1 of 6) of the familial cases with *BMPR1A* mutations and in 27% (3 of 11) of familial *MADH4* mutation-positive cases.

Of the 70 individuals examined in our laboratory, 30% (21 of 70) had detectable mutations in either *BMPR1A* (Table 2) or *MADH4* (Table 1). Of those mutations, 38% were found in *BMPR1A* (8 of 21), 62% (13 of 21) in *MADH4*, and all were private mutations. All individuals were sequenced for both genes, and no patients were identified harboring alterations in both *MADH4* and *BMPR1A*. A majority of *MADH4* mutations clustered toward the 3′ portion of the gene with 9 of 13 located in the

Table 2. Summary of *BMPR1A* Mutation-Positive Cases

Case	Location	Nucleotide change	Result	Clinical notes	Age at polyp Dx	Number of polyps	Polyp type and location
1	Ex 4	351-352insG	Stop 130-131		8	Innumerable	Juvenile–rectal and elsewhere
2	Ex 4	405-406insA	Stop 147-148		7	30	Juvenile–colon, stomach
3	Intron 4	Ivs 4-1G>A	Loss of SA		26		
4	Ex 6	674delT	Stop 260-261		23	>5 (Juvenile)	Juvenile–cecum and rectum hyperplastic and adenomatous–cecum, ascending, transverse
5	Ex 8	1061delGinsCA	Stop 379-380				
6	Ex 9	1231 G>A	E411K		8	Extensive	Juvenile–colon
7	Ex 9	1275 C>A	Y425X		25	5	Juvenile–cecum, rectum
8	Ex 11	1552-1554delAAG	del519K	Congenital pulmonary valve stenosis	30	40	Juvenile and adenomatous–colon, gastric fundus

Dx, diagnosis; SA, splice acceptor.

MH2 domain of that protein (Figure 1). No such clustering was seen in *BMPR1A* in which mutations were distributed across both the MH1 and IC protein domains (Figure 2). In addition to these alterations, sequence analysis revealed four nonpathological, single nucleotide polymorphisms within the coding regions of these genes. These included two silent alterations in *BMPR1A* exons 5 and 8, a silent change in *MADH4* exon 2, and proline to threonine substitution in *BMPR1A* exon 1 (Table 3). Both the silent base changes identified in *MADH4* exon 2 and *BMPR1A* exon 8 are previously undescribed.

Small deletions were the most common type of mutation identified in *MADH4*, accounting for more than half the alterations described in that gene (Table 1 and Figure 1). The inversion G>A in intron 10, which results in the loss of a splice donor site, is the first splice site alteration described in *MADH4* (Table 1). Small insertions and deletions were also common in *BMPR1A* accounting for half the described mutations. Overall, a similar spectrum of mutations was identified in both genes including small insertions, deletions, duplications, splice site alterations, missense mutations, and nonsense mutations (Tables 1 and 2). Additionally, all *BMPR1A* mutations and 11 of 13 *MADH4* mutations are also novel and not previously reported.

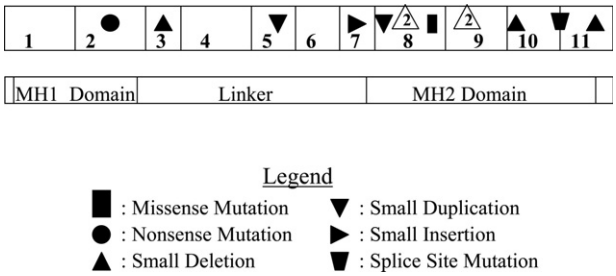

Figure 1. Mutation map of *MADH4* with each division depicting an exon and all mutations designated by legend symbols. The lower figure shows all corresponding domains within the MADH4 protein.

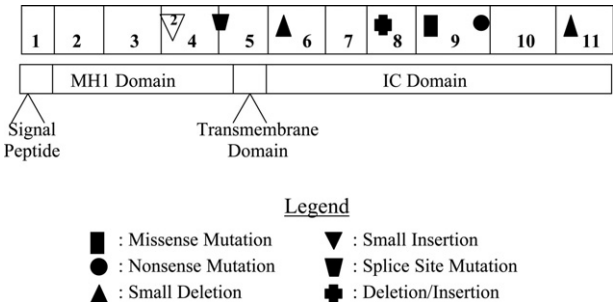

Figure 2. Mutation map of *BMPR1A* with each division depicting an exon and all mutations designated by legend symbols. The lower figure shows all corresponding domains within the BMPR1A protein.

Discussion

This report describes the experiences in our laboratory regarding 3 years of molecular diagnostic screening for JPS in a series of 70 unrelated individuals. By direct genomic sequencing of all coding regions, germline mutations were identified in 30% of screened cases with 11.4% having mutations in *BMPR1A* and 18.6% in *MADH4*. This distribution is similar to the two other reports in the literature examining large cohorts of patients, the first of which reported 16.7% with *MADH4* mutations in a series of 54 JPS patients and 24.1% with mutations in *BMPR1A*.[21] With the further addition of 23 JPS cases to this group, a total of 18.2% had germline mutations in *MADH4* and 20.8% in *BMPR1A*.[20] All of the *BMPR1A* mutations and 85% of *MADH4* mutations reported here are novel, and a majority of these alterations are predicted to result in a truncated protein due to a frameshift or the introduction of a stop codon. The four-base deletion in *MADH4* exon 9 was observed in two individuals, but all other mutations were novel and unique to single patients. This deletion is the most common mutation described thus far in JPS[19–21] and appears to be a mutational hotspot with no common haplotype shared among the unrelated individuals examined with it.[22] Because of the repetitive sequence in this region, the deletion can

Table 3. Single Nucleotide Polymorphisms

Gene	Location	Nucleotide	Change	Result	dbSNP
MADH4	Ex2	354	G>A	A118A	
BMPR1A	Ex1	4	C>A	P2T	rs11528010
BMPR1A	Ex5	435	G>A	P145P	rs11818239
BMPR1A	Ex8	1140	C>T	D380D	

begin at either base 1242 or 1244 and generate the same mutant allele with a new stop site spanning codons 435 to 436.

Sayed and colleagues[21] previously described a majority (86%) of familial cases with upper gastrointestinal polyps as *MADH4* mutation-positive whereas only 10% had *BMPR1A* mutations. Although this study did not include the specific anatomical location of polyposis, 70% of probands with *MADH4* germline mutations had upper gastrointestinal involvement. Our data support a similar prevalence of gastric polyposis in familial *MADH4* mutation-positive individuals, but the trend was not as strong as previously described with ~27% (3 of 11) of probands having gastric polyps in this study. Data on the status of gastric polyposis in other family members was not available to us. Additionally, 15% (2 of 13) of *MADH4* mutation-positive individuals in our series displayed features of both JPS and HHT. Although neither mutation described in these two cases has been previously linked with HHT, both reside in the MH2 domain as do all previously reported HHT-associated *MADH4* mutations.[13]

Direct genetic analysis can currently identify germline mutations in ~30% of unrelated JPS cases, with mutations found in *BMPR1A* and *MADH4* at a 1:1.6 ratio. Germline alterations have been identified across *MADH4* and in nearly all exons of *BMPR1A*.[20] The mutation detection rate may improve with the examination of regulatory elements and introns by direct sequencing and addressing the presence of large, genomic alterations. However, at least one study has failed to find large mutations in *MADH4* by Southern blot analysis.[23] It stands to reason then that additional predisposition genes for JPS have yet to be identified or that alternative methods for the inactivation of *MADH4* and *BMPR1A* need to be investigated.

On a clinical level, the diagnosis of JPS can be difficult to make because of phenotypic overlap with the other hamartomatous-polyposis syndromes. But because germline mutations in *MADH4* and *BMPR1A* are exclusive to JPS or JPS-HHT, the mutation analysis of these genes in individuals with hamartomatous polyposis can support clinical findings and establish a diagnosis at the molecular level. Direct sequence analysis provides a straightforward and cost-effective method for testing based on the limited number of exons, low frequency of polymorphisms, and high frequency of frameshift or nonsense mutations recorded in either gene. However, the large number of unique mutations and the absence of mutational hotspots mandate that all coding regions and exon-intron boundaries must be examined. Based on the similar frequencies of mutations found in *MADH4* and *BMPR1A*, both genes must be examined in this manner.

Approximately 20 to 50% of JPS cases will have a family history of polyposis.[24] The identification of a causative mutation in a proband subsequently allows for the clarification of mutation status in at-risk relatives. Mutation-positive patients can then be counseled regarding the appropriate surveillance regiment for early detection of polyps or cancers specific to malignant risks seen in JPS. With the identification of germline mutations in 21 probands in our series, 39 additional family members were referred to our laboratory to determine their mutation status. Molecular testing was then able to provide these families with presymptomatic testing through a molecular diagnosis, thus facilitating appropriate management.

References

1. Hampel H, Peltomaki P: Hereditary colorectal cancer: risk assessment and management. Clin Genet 2000, 58:89–97
2. Jarvinen HJ: Genetic testing for polyposis: practical and ethical aspects. Gut 2003, 52(Suppl II):ii19–ii22
3. Jarvinen HJ, Franssila KO: Familial juvenile polyposis coli: increased risk of colorectal cancer. Gut 1984, 25:792–800
4. Howe JR, Mitros FA, Summers RW: The risk of gastrointestinal carcinoma in familial juvenile polyposis. Ann Surg Oncol 1998, 5:751–756
5. Murday V, Slack J: Inherited disorders associated with colorectal cancer. Cancer Surv 1989, 8:139–157
6. Desai DC, Murday V, Phillips RK, Neale KF, Milla P, Hodgson SV: A survey of phenotypic features in juvenile polyposis. J Med Genet 1998, 35:476–481
7. Burt RW, Bishop DT, Lynch HT, Rozen P, Winawer SJ: Risk and surveillance of individuals with heritable factors for colorectal cancer. WHO Collaborating Centre for the Prevention of Colorectal Cancer. Bull World Health Organ 1990, 68:655–665
8. Sachatello CR, Hahn IL, Carrington CB: Juvenile gastrointestinal polyposis in a female infant: report of a case and review of the literature of a recently recognized syndrome. Surgery 1974, 75:107–114
9. Jass JR, Williams CB, Bussey HJR, Morson BC: Juvenile polyposis—a precancerous condition. Histopathology 1988, 13:619–630
10. Howe JR, Ringold JC, Summers RW, Mitros FA, Nishimura DY, Stone EM: A gene for familial juvenile polyposis maps to chromosome 18q21.1. Am J Hum Genet 1998, 62:1129–1136
11. Howe JR, Roth S, Ringold JC, Summers RW, Jarvinen HJ, Sistonen P, Tomlinson IPM, Houlston RS, Bevan S, Mitros FA, Stone EM, Aaltonen LA: Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science 1998, 280:1086–1088
12. de Caestecker MP, Piek E, Roberts AB: Role of transforming growth factor-beta signaling in cancer. J Natl Cancer Inst 2000, 92:1388–1402
13. Gallione CJ, Repetto GM, Legius E, Rustgi AK, Schelley SL, Tejpar S, Mitchell G, Drouin E, Westermann CJJ, Marchuk DA: A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). Lancet 2004, 363:852–859
14. Howe JR, Bair JL, Sayed MG, Anderson ME, Mitros FA, Petersen GM, Velculescu VE, Traverso G, Vogelstein B: Germline mutations of the gene encoding bone morphogenic protein receptor 1A in juvenile polyposis. Nat Genet 2001, 28:184–187
15. Kretzschmar M, Liu F, Hata A, Doody J, Massague J: The TGF-beta family mediator Smad1 is phosphorylated directly and activated functionally by the BMP receptor kinase. Genes Dev 1997, 11:984–995
16. Liu F, Hata A, Baker JC, Doody J, Carcamo J, Harland RM, Massague J: A human Mad protein acting as a BMP-regulated transcriptional activator. Nature 1996, 381:620–623
17. Nakao A, Afrakhte M, Moren A, Nakayama T, Christian JL, Heuchel R, Itoh S, Kawabata M, Heldin NE, Heldin CH, ten Dijke P: Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signaling. Nature 1997, 389:631–635
18. Bevan S, Woodford-Richens K, Rozen P, Eng C, Young J, Dunlop M, Neale K, Phillips R, Markie D, Rodriguez-Bigas M, Leggett B, Sheridan E, Hodgson S, Iwama T, Eccles D, Bodmer W, Houlston R, Tomlinson I: Screening SMAD1, SMAD2, SMAD3, and SMAD5 for germline mutations in juvenile polyposis syndrome. Gut 1999, 4:406–408
19. Roth S, Sistonen P, Salovaara R, Hemminki A, Loukola A, Johansson M, Avizienyte E, Cleary KA, Lynch P, Amos CI, Kristo P, Mecklin JP, Kellokumpu I, Jarvinen H, Aaltonen LA: SMAD genes in juvenile polyposis. Genes Chromosom Cancer 1999, 26:54–61
20. Howe JR, Sayed MG, Ahmed AF, Ringold J, Larsen-Haidle J, Merg A,

Mitros FA, Vaccaro CA, Petersen GM, Giardiello FM, Tinley ST, Aaltonen LA, Lynch HT: The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. J Med Genet 2004, 41:484–491

21. Sayed MG, Ahmed AF, Ringold JR, Anderson ME, Blair JL, Mitros FA, Lynch HT, Tinley ST, Petersen GM, Giardiello FM, Vogelstein B, Howe JR: Germline SMAD4 or BMPR1A mutations and phenotype of juvenile polyposis. Ann Surg Oncol 2002, 9:901–906
22. Howe JR, Shellnut J, Wagner B, Ringold JC, Sayed MG, Ahmed AF, Lynch PM, Amos CI, Sistonen P, Aaltonen LA: Common deletion of SMAD4 in juvenile polyposis is a mutational hotspot. Am J Hum Genet 2002, 70:1357–1362
23. Woodford-Richens KL, Rowan AJ, Poulsom R, Bevan S, Salovaara R, Aaltonen LA, Houlston RS, Wright NA, Tomlinson IPM: Comprehensive analysis of SMAD4 mutations and protein expression in juvenile polyposis. Am J Pathol 2001, 159:1293–1300
24. Desai DC, Neale KF, Talbot IC, Hodgson SV, Phillips RKS: Juvenile polyposis. Br J Surg 1995, 82:14–17