Gut Online First, published on January 4, 2008 as 10.1136/gut.2007.142927

LARGE GENOMIC DELETIONS OF *SMAD4*, *BMPR1A* AND *PTEN* IN JUVENILE POLYPOSIS

W. Arnout van Hattem[1*], Lodewijk A. A. Brosens[1*], Wendy W. J. de Leng[1], Folkert H. Morsink[1], Sylvia Lens[2], Ralph Carvalho[2], Francis M. Giardiello[3], G. Johan A. Offerhaus[1]

[1] Department of Pathology, University Medical Center Utrecht, Utrecht, the Netherlands
[2] MRC-Holland B.V., Amsterdam, The Netherlands
[3] Department of Medicine, Division of Gastroenterology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
* These authors contributed equally to this work.

Correspondence to: L.A.A. Brosens, Department of Pathology (H04-312), University Medical Center Utrecht, Postbox 85500, 3508 GA Utrecht, The Netherlands
Tel: +31.88.7556355, Fax: +31.30.2544990, Email: L.A.A.Brosens@umcutrecht.nl

Keywords: Juvenile Polyposis, MLPA, SMAD4, BMPR1A, PTEN

Abbreviations: JPS: Juvenile Polyposis; CS: Cowden syndrome; BRRS: Bannayan-Riley-Ruvalcaba syndrome; MLPA: multiplex ligation-dependent probe amplification

Word count: 1816

ABSTRACT

Introduction

Juvenile polyposis syndrome (JPS) is a rare autosomal dominant disorder characterized by multiple gastrointestinal juvenile polyps and an increased risk of colorectal cancer. This syndrome is caused by germline mutation of either *SMAD4* or *BMPR1A*, and possibly *ENG*. *PTEN*, originally linked to Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, has also been associated with JPS. By direct sequencing, germline mutations are found in only 30-40% of patients with a JPS phenotype. Therefore, alternative ways of inactivation of the known JPS genes, or additional genes predisposing to JPS may be involved. In this study, a comprehensive genetic analysis of *SMAD4*, *BMPR1A*, *PTEN* and *ENG* is performed through direct sequencing and multiplex ligation-dependent probe amplification (MLPA) in JPS patients.

Materials and Methods

Archival material of 29 patients with JPS from 27 families was collected. Direct sequencing and MLPA analysis were performed to search for germline defects in *SMAD4*, *BMPR1A*, *PTEN* and *ENG*.

Results

A germline defect in *SMAD4*, *BMPR1A* or *PTEN* was found in 13 of 27 (48.2%) unrelated JPS patients. Nine mutations (33.3%) were detected by direct sequencing, including six (22.2%) *SMAD4* mutations and three (11.1%) *BMPR1A* mutations. MLPA identified four additional patients (14.8%) with germline hemizygous large genomic deletions, including one deletion of *SMAD4*, one deletion of exons 10 and 11 of *BMPR1A*, and two unrelated patients with deletion of both *BMPR1A* and *PTEN*. No *ENG* gene mutations were found.

Conclusion

Large genomic deletions of *SMAD4*, *BMPR1A* and *PTEN* are a common cause of JPS. Using direct sequencing and MLPA, a germline defect was detected in 48.2% of JPS patients. MLPA identified 14.8% (4/27) of these mutations. Since a substantial percentage of JP patients carry a germline deletion and MLPA is a reliable and user friendly technique, we conclude that MLPA is a valuable adjunct in JPS diagnosis.

INTRODUCTION

Juvenile polyposis (JPS [MIM 174900]) is an autosomal dominant disorder characterized by multiple gastrointestinal juvenile polyps and an increased risk of colorectal cancer.[1] Clinically JPS is defined by the presence of more than 3-5 juvenile polyps, or any number of juvenile polyps and a positive family history of juvenile polyposis. [2, 3] Juvenile polyps are hamartomas with a distinctive histology and most frequently encountered in the colorectum. In the past decade mutations in the *SMAD4* and *BMPR1A* genes were identified as the cause of JPS.[4, 5] However, a germline defect in these genes is found in a minority of JPS patients. The largest study analyzed 77 JPS patients by direct sequencing of *SMAD4* and *BMPR1A* and found a germline mutation of *SMAD4* in 18.2% and of *BMPR1A* in 20.8% of patients.[6] Others have reported similar results.[7-9] Therefore, alternative ways of inactivation of the known JPS genes, or additional, yet unidentified, genes predisposing to JPS may be involved. Other components of the TGF-β/BMP pathway, including *SMAD1*, *SMAD2*, *SMAD3*, *SMAD5*, *SMAD7*, *BMPR2*, *BMPR1B* and *ACVRL1*, were studied but no mutations have been found in these genes.[6, 10, 11] Recently, two patients with JPS were reported to have a germline mutation in the gene encoding the TGB-β co-receptor Endoglin (*ENG*).[12] Therefore, *ENG* was proposed as a potential novel susceptibility gene of JPS,[12] but this has not been confirmed.[13] Also *PTEN*, originally linked to Cowden syndrome (CS [MIM 158350]) and Bannayan-Riley-Ruvalcaba syndrome (BRRS [MIM 153480]), has been associated with JPS.[14, 15] However, others have not found *PTEN* germline mutations in JPS.[16, 17] Consequently, *PTEN* mutations in patients with juvenile polyposis likely represent CS or BRRS patients that have not (yet) expressed the extraintestinal clinical features of these conditions.[18, 19]
Interestingly, germline contiguous deletion of *BMPR1A* and *PTEN* is reported in patients with multiple juvenile polyps. However, it is unclear whether these patients are true JPS patients or BRRS/CS patients that have not yet displayed the clinical features of these conditions. [20-23] Multiplex ligation-dependent probe amplification (MLPA) is a novel technique that can detect copy number changes in genomic DNA sequences.[24] In the current study, direct sequencing and MLPA were combined to perform a comprehensive genetic analysis in a group of well documented JPS patients and to address the question whether large genomic deletions of any of the known JPS genes may cause JPS.

MATERIALS AND METHODS

Patients and patient selection

Archival material from 29 JPS patients from 27 families was collected from The Johns Hopkins Polyposis Registry and clinic (Baltimore, MD, USA) and two academic hospitals in the Netherlands (Academic Medical Center, Amsterdam, and University Medical Center, Utrecht). Patients were defined as having JPS according to the accepted clinical criteria [2, 3] as follows: 1) at least 3-5 juvenile polyps in the colorectum, or 2) juvenile polyps throughout the gastrointestinal tract, or 3) any number of juvenile polyps in a person with a known family history of juvenile polyps. Each case was carefully reviewed by an experienced pathologist (GJAO) to confirm the histopathological diagnosis of JPS. The study was approved by the Johns Hopkins Institutional Review Board and carried out in accordance with the ethical guidelines of the research review committees of the institutions in Amsterdam and Utrecht.

DNA isolation

Genomic DNA was obtained from deparaffinized formalin-fixed paraffin-embedded non neoplastic colorectal tissue from patients with JPS using TK buffer (400 μg/mL of proteinase K and 0.5% Tween 20, 50 mmol/L Tris (pH 9), 1 mmol/L NaCl, 2 mmol/L EDTA). After overnight incubation in 50 μL TK buffer at 56°C, tubes were incubated at 95°C for 10 minutes to inactivate the proteinase K.[25]

Sequencing

Genomic DNA was amplified by PCR using Platinum® *Taq* DNA Polymerase (Invitrogen Corporation, Carlsbad, CA, USA) and specific primers complementary to intronic sequences flanking all exons of *SMAD4*, *BMPR1A*, *ENG* and *PTEN* (Table 1). Amplification was performed using an initial denaturation step at 95°C for 10 min, followed by 35 cycles at 94°C for 15 sec, annealing temperature for 1 min and 72°C for 1 min.
The amplified fragments were first analyzed by agarose-gel electrophoreses. Subsequently, the PCR product was enzymatically purified using Shrimp Alkaline Phosphatase (USB Europe GmbH, Staufen, Germany) and Exonulcease I (New England Biolabs, Ipswich, MA, USA) according to the manufacturers' protocol. Samples were then subjected to direct sequencing of single strand PCR products using the BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and the ABI Prism® 3130 genetic analyzer (Applied Biosystems). All products were sequenced in sense and anti-sense direction. Patient sequences were compared to wild type reference sequences using CodonCode Aligner software. The cDNA bases were numbered according to the reference sequence in Ensembl (*SMAD4*: NM_005359.3; *BMPR1A*: NM_004329.2; *ENG*: NM_000118.1; *PTEN*: NM_000314.3), where nucleotide 1 corresponds to the A of the ATG translation initiation codon. Each mutation/variation was confirmed by a second round of PCR amplification and sequencing. The possibility of missense mutations and intronic variations being polymorphic variants was excluded using a healthy control group. For human mutation nomenclature "Recommendations for Nomenclature System for Human Gene Mutations" were followed.[26, 27]

Table 1. Primers used for PCR amplification and sequencing

Gene	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Annealing Temp. (°C)	$MgCl_2$ conc (mM)	Amplicon (bp)
SMAD4					
Exon 1a	AACGTTAGCTGTTGTTTTTCAC	CTTTACCAAACTTTCAATTGCT	58	1.5	224
Exon 1b	CATAGACAAGGTGGAGAGAGT	AGAGTATGAAGAGATGGAG	53	2.5	292
Exon 2a	TGTATGACATGGCCAAGTTAG	CTATCACATTTTAAGTCAAACGC	60	2.5	285
Exon 2b	CCCGTCTCTGGAGGTGG	CAATACTCGGTTTTAGCAGTC	60	2.5	339
Exon 3	CTGAATTGAAATGGTTCATGAAC	GCCCCTAACCTCAAAATCTAC	60	2.5	309
Exon 4a	ATCTCATGCTGTTACCGCTG	CGATTACTTGGTGGATGCTGGATG	60	1.5	314
Exon 4b	GTGCATGACTTTGAGGGACAGC	GGAGTTTCCCCCCAAGTGACTAC	66	1.5	266
Exon 5	CATCTTTATAGTTGTGCATTATC	GCTTTTATAAAGGCTGCCTAC	58	1.5	294
Exon 6	ACCCATGTGGGCCTTAATTT	GCCCTTACAACAAAAACAAGAG	58	1.5	168
Exon 7	TGAAAGTTTTAGCATTAGACAAC	TGTACTCATCTGAGAAGTGAC	60	1.5	226
Exon 8	TGTTTTGGGTGCATTACATTTC	CAATTTTTTAAAGTAACTATCTGAC	58	1.5	356
Exon 9	TATTAAGCATGCTATACAATCTG	CTTCCACCCAGATTTCAATTC	58	2.5	330
Exon 10	AGGCATTGGTTTTTAATGTATG	CTGCTCAAAGAAACTAATCAAC	60	1.5	294
Exon 11a	CCAAAAGTGTGCAGCTTGTTG	CAATCCAGCAAGGTGTTTC	58	2.5	312
Exon 11b	CCGGATTACCCAAGACAG	CAGTTTCTGTCTGCTAGGAG	60	1.5	288
BMPR1A					
Exon 1	AAATTGGTGAAGTAGCAAGACCA	CACATACATTACTAAAATGAACACTG	60	1.5	165
Exon 2	TTGTCACGAAACAATGAGCTTT	AACTCTTAAGAAGGGCTGCAT	60	1.5	257
Exon 3	AGGCCATCTGTACCTGTTCAC	ATATGGCCCCTCCCTTCTTT	64	1.5	246
Exon 4	TAAAATTTGCAGGCCCCTTT	GAAGCATGCTCCGACTTTTC	60	1.5	250
Exon 5	CCAGGCTACCTAGAATTGAA	AACAGCGGTTGACATCTAAT	62	1.5	236
Exon 6	CCTCAAGGTTTTTCTTAGGG	TCAACACACCATTCATGTCT	62	1.5	254
Exon 7	CCCTTTGCCAGTCTTAATGG	AGGCTTCCACCTGTACCTCA	66	2.5	323
Exon 8	TGAGCATTACTTCTCCCTAGCC	TTCAAAACAGTGGGGCAAAG	61	1.5	394
Exon 9	CAACTTGGACCTTGGCTTTC	CATGGCATGCCTGTATCAAA	62	2.5	362
Exon 10	ATTTTTGTGCCCATGTTTT	AATCACTTCTTCAGGGGACT	56	2.5	198
Exon 11	ACTCAGTCCCCTGAAGAAGT	CTAGAGTTTCTCCTCCGATG	61	1.5	233
ENG					
Exon 1	ACTGGACACAGGATAAGGCCCAG	AATACTTGGGGCCTGGTCCGTG	66	1.5	180
Exon 2	CACCTTATTCTCACCTGGCCTCTT	CTGCCTTGGAGCTTCCTCTGAG	63	1.5	249
Exon 3	GGGTGGCACAACCTATACAAAT	CAGAGATGGACAGTAGGGACCT	63	1.5	269
Exon 4	TTCCTGACCTCCTACATGGG	GGAGCTCAGATTCCTCCTGA	62	1.5	298
Exon 5	TGAGGGAAGGGACTGAGGTG	GTGGGGACTAGTGTCAGGGGC	62	1.5	238
Exon 6	GGCCTGTCCGCTTCAGTGTT	GTTTTGTGTCCCGGGAGCTG	66	2.5	203
Exon 7	CCCCCTGTTCTGCCTCTCTC	CTGATCCAAGGGAGGGGAAG	58	1.5	265
Exon 8	GGGCACACAGTGATCACACA	ATGTCATCCTGAGCCAGAGG	64	1.5	237
Exon 9	CTCCTGATGGTGCCCCTCTCTT	TTGTCTTGTGTTCTGAGCCCCTG	64	1.5	296
Exon 10	ATTGGGTGGGATACCCTCTGGG	GGGTTAGCACGTGACTGTCC	64	1.5	131
Exon 11	ATTGACCAAGTCTCCCTCCC	GAAAGGCGGAGAGGAAGTTC	62	2.5	211
Exon 12	GGTGGGGTGAAGAGCAGCTG	GACCTGGAAGCTCCCACTTGAA	64	1.5	359
Exon 13	GAGTAAACCTGGAAGCCGC	GCCACTAGAACAAACCCGAG	63	1.5	154
Exon 14	AGAGTGGCAGTGCTGATGG	CTCAGAGGCTTCACTGGGCTCC	64	1.5	222
Exon 15	AGGACCCTGACCTCCGCC	CTCTCCTGCTGGGCGAGC	64	2.5	198
PTEN					
Exon 1	GCAGCTTCTGCCATCTCTCT	TCTAAGAGAGTGACAGAAAGGTA	58	2.5	181
Exon 2	GTTTGATTGCTGCATATTTCAG	TCTAAATGAAAACACAACATG	58	1.5	202
Exon 3	GGTGGCTTTTTGTTTGTTTG	ACCTCACTCTAACAAGCAGATA	58	2.5	165
Exon 4	CATTATAAAGATTCAGGCAATG	GACAGTAAGATACAGTCTATC	58	2.5	205
Exon 5	TTCTGAGGTTATCTTTTTACCACA	TCCAGGAAGAGGAAAGGAAAA	58	1.5	304
Exon 6	CATAGCAATTTAGTGAAATAACT	GATATGGTTAAGAAAACTGTTC	58	2.5	274
Exon 7	TGACAGTTTGACAGTTAAAGG	GGATATTTCTCCCAATGAAAG	60	1.5	263
Exon 8	TGTCATTTCATTTCTTTTTCTTTTC	AAGTCAACAACCCCCACAAA	56	2.5	305
Exon 9	GTTCATCTGCAAAATGGA	TTTTCATGGTGTTTTATCCCTC	56	2.5	328

MLPA

Multiplex ligation dependent probe amplification was performed using the Juvenile Polyposis (Kit P158, containing probes for each of the *SMAD4* and *PTEN* exons and for all but one of the *BMPR1A* gene) and Hereditary Hemorrhagic Telangiectasia (Kit P093, containing probes for 13 different *ENG* exons, and for all *ACVRL1* and *BMPR2* exons) probe kits (MRC-Holland B.V., Amsterdam, the Netherlands). MLPA reactions were performed according to the manufacturer's instructions. In brief, 50 ng of genomic DNA in 5 μl TE was heat-denatured (5 min at 98°C) and incubated with the probe set for 16 hours at 60°C. Then the hybridized products were ligated (15 min at 54°C), PCR-amplified (35 cycles: 30 sec at 95°C; 30 sec at 60°C; 60 sec at 72°C; final elongation: 20 min at 72°C) and separated by electrophoresis on an automated sequencer. DNA from healthy individuals was used as normal control. Finally, MLPA data were evaluated using Coffalyser, a Microsoft Excel based program freely available at the MRC-Holland website. All samples were assayed in two independent MLPA reactions.

RESULTS

Sequencing

By direct sequencing of *SMAD4*, *BMPR1A, ENG* and *PTEN* genes, nine germline mutations were found in 27 JPS patients (33.3%), including six (22.2%) *SMAD4* mutations and three (11.1%) *BMPR1A* mutations. (Table 2 and 3)
The *SMAD4* germline mutations included two missense mutations in exon 8 (c.970 T>C, p.C324R and c.989 A>G, p.E330G), and one non-sense mutation in exon 9 (c.1193 G>A, p.W398X). In addition, a 1bp deletion was found in exon 8 (c.971delG, p.C324FfsX12), a 25 bp deletion was found in exon 10 (c.1411_1435del25, p.G471FfsX25), and a single base pair duplication was found in exon 11 (c.1586_1587dupA, p.L529LfsX9).
In *BMPR1A* one missense mutation in exon 10 (c.1483 C>T, p.R480W) and one single base pair deletion in exon 8 (c.1061delG, p.G354EfsX10) were found. In addition, one intronic mutation was found in the splice acceptor site of intron 5 (c.531-2A>G). No mutations were found in *ENG* or *PTEN*. Several polymorphisms were found in *BMPR1A* and *ENG*. (Table 4)

MLPA

Using MLPA, a large genomic deletion was identified in 22.2% (4/18) of patients in whom no point mutation had been detected, or in 14.8% (4/27) of all patients examined. (Table 2 and 3) This included one hemizygous deletion of *SMAD4*, which was also found in an affected family member. In addition, one patient with a hemizygous deletion of exons 10 and 11 of *BMPR1A*, and two unrelated patients with a hemizygous deletion of both *BMPR1A* and *PTEN* were found. No deletions were found in *PTEN* alone, *ENG, ACVRL1* and *BMPR2*.
By sequencing and MLPA combined a germline defect was found in 48.2% (13/27) of JPS patients. MLPA identified 30.8% (4/13) of these germline defects.

Table 2. Mutation detection rates in *SMAD4* and *BMPR1A* in 27 unrelated Juvenile Polyposis patients.

	SMAD4	*BMPR1A*	Total
All patients	27		
Point mutations	6 (22.2%)	3 (11.1%)	9 (33.3%)
Deletions	1 (3.7%)	3 (11.1%)	4 (14.8%)
All mutations	7 (25.9%)	6 (22.2%)	13 (48.2%)

Table 3. Germline defects in *SMAD4*, *BMPR1A* and *PTEN* found in Juvenile Polyposis patients.

Patient	**Gene**	**Exon**	**Nucleotide change**	**Predicted result**	**Controls**	**Detected by**
7[1]	*SMAD4*	8	c.970 T>C	p.C324R	0/118	Sequencing
14	*SMAD4*	8	c.989 A>G	p.E330G	0/117	Sequencing
23	*SMAD4*	8	c.971delG	p.C324FfsX12		Sequencing
19	*SMAD4*	9	c.1193 G>A	p.W398X		Sequencing
4	*SMAD4*	10	c.1411-1435del25	p.G471FfsX25		Sequencing
27	*SMAD4*	11	c.1586_1587dupA,	p.L529LfsX9		Sequencing
11[2]	*SMAD4*	1-11	Hemizygous deletion			MLPA
21	*BMPR1A*	Intron 5	c.531-2A>G	Splice site mutation	0/116	Sequencing
16	*BMPR1A*	8	c.1061delG	p.G354EfsX10		Sequencing
20	*BMPR1A*	10	c.1483 C>T	p.R480W	0/134	Sequencing
3	*BMPR1A*	10 and 11	Hemizygous deletion			MLPA
18	*BMPR1A/PTEN*		Hemizygous deletion			MLPA
24	*BMPR1A/PTEN*		Hemizygous deletion			MLPA

[1] mutation was confirmed in one affected family member
[2] deletion was confirmed in one affected family member

Table 4. Polymorphisms in *BMPR1A* and *ENG*.

	Nucleotide	Amino acid change	Number of patients	Reference	refSNP ID
BMPR1A 1					
Exon 1	c.4 C>A	p.P2T	19/27	Howe et al., 2001 [5]	rs17090779
Exon 5	c.435 G>A	p.P145P	2/27	Pyatt et al., 2006 [7]	rs2230176
ENG					
Exon 1	c.14 C>T	p.T5M	1/27	Howe et al., 2007 [13]	rs35400405
Exon 2	c.207 G>A	p.L69L	6/27	Howe et al., 2007 [13]	rs16930129
Exon 5	c.572 G>A	p.G191D	1/27	Abdalla et al., 2005 [32]	
Exon 8	c.1029 C>T	p.T343T	1/27	Howe et al., 2007 [13]	rs3739817
Exon 8	c.1060 C>T	p.L354L	1/27	Howe et al., 2007 [13]	rs36092484
Exon 11	c.1374 A>G	p.P458P	1/27	Prigoda et al., 2006 [33]	rs34828244
Exon 14	c.1794 T>C	p.G598G	3/27	Abdalla et al., 2005 [32]	

DISCUSSION

SMAD4 and *BMPR1A* are the two best known juvenile polyposis genes. However, by direct sequencing, germline mutation of *SMAD4* or *BMPR1A* is found in only 30-40% of patients, [6-9] indicating that alternative ways of inactivation of these genes, or additional genes causing JPS may exist.

In the current study, a comprehensive genetic analysis of a group of 27 well documented JPS patients was performed using both direct sequencing and MLPA to investigate the role of large genomic deletions in the germline of JPS patients. By direct sequencing, germline mutations were found in 33.3% of JPS patients (22.2% in *SMAD4* and 11.1% in *BMPR1A*). This is consistent with previous studies reporting germline mutation of *SMAD4* in 18-24% and of *BMPR1A* in 11-24% of patients.[6-9]

Using MLPA, four unrelated patients with a large genomic germline deletion were identified, adding 14.8% to the total amount of germline defects in our cohort. Using both sequencing and MLPA a germline defect was identified in 48.2% of patients. Recently, a similar percentage (49%) was reported in a study that also combined sequencing and MLPA in JPS, but the role of *ENG* mutation in JPS was not addressed.[28]

Interestingly, two patients had deletion of both *BMPR1A* and *PTEN*, likely a contiguous gene deletion at 10q22-23. Deletion of this region has been reported in 11 patients.[12, 20-23, 29, 30] *PTEN* was deleted in all of these patients and *BMPR1A* in at least six and probably in another three of these patients. Clinically, most of these patients had juvenile polyposis of infancy as described by Sachatello et al.[31] and some also had symptoms of BRRS.[20, 21] Both patients in the current study had multiple juvenile polyps with the typical associated histology and also dysplasia. One of these patients was also diagnosed with thyroid carcinoma, raising the question of Cowden syndrome. Further studies would be needed to determine the exact size of the genomic deletion on 10q in these individuals.

In 51.8% of JPS patients in this cohort a germline defect was not found. Possibly, mutations in the promotor region or in intronic sequences that affect cryptic splice sites are responsible for some of these cases. However, it seems unlikely that the remaining 51.8% can be explained by undiscovered mutations in *SMAD4* of *BMPR1A* alone, suggesting that other genes predispose to JPS. Recently, germline *ENG* mutation was reported in two JPS patients and proposed as a potential novel JPS susceptibility gene.[12] However, others have not confirmed this finding,[13] and we did not detect any mutations or exon deletions of the *ENG* gene in the current study. Therefore, the role of *ENG* in JPS remains unclear. Several other TGF-β/BMP signalling molecules have also been studied but no mutations have been found.[6, 10, 11] These data suggest that there are other genes responsible for mutation negative JPS cases.

In summary, large genomic deletions in *SMAD4*, *BMPR1A* and *BMPR1A* and *PTEN* are not uncommon causes of JPS and these deletions are detectable using MLPA. In view of the substantial percentage of patients carrying a germline deletion (14.8%) as detected using MLPA, and given the reliability and user friendliness of this technique, we conclude that MLPA is a valuable adjunct in JPS diagnosis.

ACKNOWLEDGEMENTS
We thank Linda Hylind and Jordy Coffa for technical assistance.

FUNDING
Supported by The Netherlands Digestive Disease Foundation (MLDS WS 04-06), The John G. Rangos, Sr. Charitable Foundation, The Clayton Fund, and NIH grants CA 53801, 63721, 51085, and P50 CA 93-16. The study sponsors were not involved in study design, collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

STATEMENT OF COMPETING INTERESTS
None to declare.

STATEMENT

REFERENCES

1. Brosens LA, van Hattem A, Hylind LM, et al. Risk of colorectal cancer in juvenile polyposis. *Gut* 2007;56:965-7.
2. Giardiello FM, Hamilton SR, Kern SE, et al. Colorectal neoplasia in juvenile polyposis or juvenile polyps. *Arch Dis Child* 1991;66:971-5.
3. Jass JR, Williams CB, Bussey HJ, et al. Juvenile polyposis--a precancerous condition. *Histopathology* 1988;13:619-30.
4. Howe JR, Roth S, Ringold JC, et al. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* 1998;280:1086-8.
5. Howe JR, Bair JL, Sayed MG, et al. Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. *Nat Genet* 2001;28:184-7.
6. Howe JR, Sayed MG, Ahmed AF, et al. The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. *J Med Genet* 2004;41:484-91.
7. Pyatt RE, Pilarski R, Prior TW. Mutation screening in juvenile polyposis syndrome. *J Mol Diagn* 2006;8:84-8.
8. Handra-Luca A, Condroyer C, de Moncuit C, et al. Vessels' morphology in SMAD4 and BMPR1A-related juvenile polyposis. *Am J Med Genet A* 2005;138:113-7.
9. Friedl W, Uhlhaas S, Schulmann K, et al. Juvenile polyposis: massive gastric polyposis is more common in MADH4 mutation carriers than in BMPR1A mutation carriers. *Hum Genet* 2002;111:108-11.
10. Bevan S, Woodford-Richens K, Rozen P, et al. Screening SMAD1, SMAD2, SMAD3, and SMAD5 for germline mutations in juvenile polyposis syndrome. *Gut* 1999;45:406-8.
11. Roth S, Sistonen P, Salovaara R, et al. SMAD genes in juvenile polyposis. *Genes Chromosomes Cancer* 1999;26:54-61.
12. Sweet K, Willis J, Zhou XP, et al. Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. *Jama* 2005;294:2465-73.
13. Howe J, Haidle J, Lal G, et al. ENG mutations in MADH4/BMPR1A mutation negative patients with juvenile polyposis. *Clin Genet* 2007;71:91-92.
14. Olschwang S, Serova-Sinilnikova OM, Lenoir GM, et al. PTEN germ-line mutations in juvenile polyposis coli. *Nat Genet* 1998;18:12-4.
15. Huang SC, Chen CR, Lavine JE, et al. Genetic heterogeneity in familial juvenile polyposis. *Cancer Res* 2000;60:6882-5.
16. Marsh DJ, Roth S, Lunetta KL, et al. Exclusion of PTEN and 10q22-24 as the susceptibility locus for juvenile polyposis syndrome. *Cancer Res* 1997;57:5017-21.
17. Woodford-Richens K, Bevan S, Churchman M, et al. Analysis of genetic and phenotypic heterogeneity in juvenile polyposis. *Gut* 2000;46:656-60.
18. Eng C, Peacocke M. PTEN and inherited hamartoma-cancer syndromes. *Nat Genet* 1998;19:223.
19. Eng C, Ji H. Molecular classification of the inherited hamartoma polyposis syndromes: clearing the muddied waters. *Am J Hum Genet* 1998;62:1020-2.
20. Delnatte C, Sanlaville D, Mougenot JF, et al. Contiguous gene deletion within chromosome arm 10q is associated with juvenile polyposis of infancy, reflecting cooperation between the BMPR1A and PTEN tumor-suppressor genes. *Am J Hum Genet* 2006;78:1066-74.
21. Salviati L, Patricelli M, Guariso G, et al. Deletion of PTEN and BMPR1A on chromosome 10q23 is not always associated with juvenile polyposis of infancy. *Am J Hum Genet* 2006;79:593-6; author reply 596-7.

22. Jacoby RF, Schlack S, Sekhon G, et al. Del(10)(q22.3q24.1) associated with juvenile polyposis. *Am J Med Genet* 1997;70:361-4.
23. Tsuchiya KD, Wiesner G, Cassidy SB, et al. Deletion 10q23.2-q23.33 in a patient with gastrointestinal juvenile polyposis and other features of a Cowden-like syndrome. *Genes Chromosomes Cancer* 1998;21:113-8.
24. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 2002;30:e57.
25. Brosens LA, Iacobuzio-Donahue CA, Keller JJ, et al. Increased cyclooxygenase-2 expression in duodenal compared with colonic tissues in familial adenomatous polyposis and relationship to the -765G -> C COX-2 polymorphism. *Clin Cancer Res* 2005;11:4090-6.
26. Antonarakis SE. Recommendations for a nomenclature system for human gene mutations. Nomenclature Working Group. *Hum Mutat* 1998;11:1-3.
27. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000;15:7-12.
28. Aretz S, Stienen D, Uhlhaas S, et al. High proportion of large genomic deletions and genotype-phenotype update in 80 unrelated families with Juvenile Polyposis Syndrome. *J Med Genet* 2007.
29. Arch EM, Goodman BK, Van Wesep RA, et al. Deletion of PTEN in a patient with Bannayan-Riley-Ruvalcaba syndrome suggests allelism with Cowden disease. *Am J Med Genet* 1997;71:489-93.
30. Zigman AF, Lavine JE, Jones MC, et al. Localization of the Bannayan-Riley-Ruvalcaba syndrome gene to chromosome 10q23. *Gastroenterology* 1997;113:1433-7.
31. Sachatello CR, Griffen WO, Jr. Hereditary polypoid diseases of the gastrointestinal tract: a working classification. *Am J Surg* 1975;129:198-203.
32. Abdalla SA, Cymerman U, Rushlow D, et al. Novel mutations and polymorphisms in genes causing hereditary hemorrhagic telangiectasia. *Hum Mutat* 2005;25:320-1.
33. Prigoda NL, Savas S, Abdalla SA, et al. Hereditary haemorrhagic telangiectasia: mutation detection, test sensitivity and novel mutations. *J Med Genet* 2006;43:722-8.

Large Genomic Deletions Of Smad4, Bmpr1a And Pten In Juvenile Polyposis

W. Arnout van Hattem, Lodewijk A.A. Brosens, Wendy W.J. de Leng, Folkert H. Morsink, Sylvia Lens, Ralph Carvalho, Francis M. Giardiello and G. Johan A.O. Offerhaus

Gut published online January 4, 2008

Notes