Mechanisms of disease

# A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in *MADH4* (*SMAD4*)

*Carol J Gallione, Gabriela M Repetto, Eric Legius, Anil K Rustgi, Susan L Schelley, Sabine Tejpar, Grant Mitchell, Éric Drouin, Cornelius J J Westermann, Douglas A Marchuk*

## Summary

**Background** Juvenile polyposis and hereditary haemorrhagic telangiectasia are autosomal dominant disorders with distinct and non-overlapping clinical features. The former, an inherited gastrointestinal malignancy predisposition, is caused by mutations in *MADH4* (encoding SMAD4) or *BMPR1A*, and the latter is a vascular malformation disorder caused by mutations in *ENG* (endoglin) or *ACVRL1* (*ALK1*). All four genes encode proteins involved in the transforming-growth-factor-β signalling pathway. Although there are reports of patients and families with phenotypes of both disorders combined, the genetic aetiology of this association is unknown.

**Methods** Blood samples were collected from seven unrelated families segregating both phenotypes. DNA from the proband of each family was sequenced for the *ACVRL1*, *ENG*, and *MADH4* genes. Mutations were examined for familial cosegregation with phenotype and presence or absence in population controls.

**Findings** No patient had mutations in the *ENG* or *ACVRL1* genes; all had *MADH4* mutations. Three cases of de-novo *MADH4* mutations were found. In one, the mutation was passed on to a similarly affected child. Each mutation cosegregated with the syndromic phenotype in other affected family members.

**Interpretation** Mutations in *MADH4* can cause a syndrome consisting of both juvenile polyposis and hereditary haemorrhagic telangiectasia phenotypes. Since patients with these disorders are generally ascertained through distinct medical specialties, genetic testing is recommended for patients presenting with either phenotype to identify those at risk of this syndrome. Patients with juvenile polyposis who have an *MADH4* mutation should be screened for the vascular lesions associated with hereditary haemorrhagic telangiectasia, especially occult arteriovenous malformations in visceral organs that may otherwise present suddenly with serious medical consequences.

*Lancet* 2004; **363:** 852–59

**Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA** (C J Gallione BA, D A Marchuk PhD)**; Facultad de Ciencias de la Salud, Universidad del Desarrollo–Clinica Alemana and Hospital Padre Hurtado, Santiago, Chile** (G M Repetto MD)**; Center for Human Genetics** (E Legius MD) **and Department of Gastroenterology, University Hospital Gasthuisberg** (S Tejpar MD)**, Leuven, Belgium; Division of Gastroenterology, Department of Genetics, University of Pennsylvania and Abramson Family Cancer Research Institute and Cancer Center, Philadelphia, PA, USA** (A K Rustgi MD)**; Department of Pediatrics/Genetics, Stanford University, Stanford, CA** (S L Schelley MPH)**; Divisions of Genetics** (G Mitchell MD) **and Gastroenterology** (É Drouin MD)**, Ste Justine Hospital, Montreal, Canada; and Department of Pulmonary Disease, St Antonius Hospital, Nieuwegein, Netherlands** (C J J Westermann MD)

**Correspondence to:** Dr Douglas Marchuk, Department of Molecular Genetics and Microbiology, Duke University Medical Center, Box 3175, Durham, NC 27710, USA
(e-mail: march004@mc.duke.edu)

## Introduction

Juvenile polyposis is an autosomal dominant malignancy predisposition affecting the gastrointestinal epithelium. Classic juvenile polyps consist of stromal elements with a normal epithelial layer and are distinct from both adenomatous polyps and those of the Peutz-Jegher type.[1] Although solitary juvenile polyps are common in children, juvenile polyposis is marked by the presence of many polyps either in the colon or throughout the gastrointestinal tract. Patients with juvenile polyposis typically present with rectal bleeding in the first decade of life and have an increased risk of colon carcinomas later in life. Although juvenile polyps are also a feature of other genetic syndromes, juvenile polyposis is a distinct disorder that is caused by mutations in either *MADH4* or *BMPR1A*.[2,3]

Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu disease) is an autosomal dominant disorder of vascular dysplasia that affects many organs. Characteristic symptoms include skin and mucosal telangiectases, pulmonary, cerebral, and hepatic arteriovenous malformations, and haemorrhage associated with these vascular lesions. Mutations in either *ENG* (endoglin) or *ACVRL1* (*ALK1*), encoding two endothelial-specific receptors for transforming growth factor β (TGF-β), cause this disorder.[4,5]

Although both of these inherited disorders are uncommon, there are many reports of patients and families with both disorders, or of patients with juvenile polyposis who show some symptoms of hereditary haemorrhagic telangiectasia.[6–16] These associations have led some researchers to propose that juvenile polyposis with pulmonary arteriovenous malformations be judged a distinct syndrome.[6,7] The two disorders overlap genetically as well as clinically. The four genes involved in their pathologies encode members of the TGF-β SIGNALLING PATHWAY. Endoglin and ACVRL1 (activin A receptor, type II like 1) are endothelial-specific type III and type I receptors for TGF-β, and BMPR1A is a type I receptor for bone morphogenetic protein, another of the TGF-β superfamily of ligands. SMAD4, encoded by *MADH4*, is the only identified Co-SMAD in human beings. This

## GLOSSARY

CONSENSUS SPLICE SEQUENCE MOTIFS
Conserved nucleotide sequences that flank the 5′ and 3′ ends of exons to direct the splicing of mRNA transcripts.

DE-NOVO MUTATION
A spontaneous mutation occurring during gametogenesis that results in a new mutation in the offspring, which was not present in either parent.

MKK4/JNK PATHWAY
The mitogen-activated protein-kinase kinase (MKK) 4/c-Jun N-terminal kinase (JNK) pathway is involved in differentiation-related cellular processes.

MKK3/P38 PATHWAY
The mitogen-activated protein-kinase kinase (MKK) 3/p38 pathway is stress-responsive and is involved in the appropriate regulation of cellular survival.

TGF-β SIGNALLING PATHWAY
TGF-β is a multifunctional cytokine that mediates many biological processes including cell-cycle control, embryogenesis, growth, development, and differentiation of several cell types. The TGF-β signalling pathway is composed of cell-surface receptors and intracellular effectors (SMADs) that bring about changes in gene expression, which mediate the biological effects of the cytokine.

protein is an integral downstream effector of the TGF-β signal transduction pathway binding to R-SMADs and transporting them to the cell nucleus to induce specific transcriptional events.

We investigated the underlying genetic aetiology of this syndrome. Seven unrelated families or individuals displaying both juvenile polyposis and hereditary haemorrhagic telangiectasia phenotypes were ascertained and characterised clinically and molecularly.

## Methods

### Patients and families

Patients were enrolled in the study with approval from the relevant institutional review boards and after giving informed consent. Diagnosis of juvenile polyposis was based on the accepted diagnostic criteria of the presence of any one of the following: five or more colorectal juvenile polyps; juvenile polyps throughout the gastrointestinal tract; or any juvenile polyps in a patient with a family history of juvenile polyposis.[17] Diagnosis of hereditary haemorrhagic telangiectasia was based on the most recently established criteria.[18] Patients and families in this study were ascertained and collected from various countries (Belgium, Canada, Chile, Surinam, and the USA) and racial groups (Hispanic, Afro-Asian, and non-Hispanic white). The pedigrees of the families are shown in the figure and relevant clinical details of the individual patients in table 1. All family members, both affected and unaffected, who were able and willing to participate were ascertained and included in the study.

Family 25 is a two-generation family of Afro-Asian descent. The proband, II-2, meets diagnostic criteria for both juvenile polyposis and hereditary haemorrhagic telangiectasia. The affected parent (I-1) shows some signs of juvenile polyposis and definite signs of hereditary haemorrhagic telangiectasia. Although I-1 had no history of anaemia or rectal bleeding, when colonoscopy was done a tubular adenoma was found in the caecum. In addition, this individual has telangiectases, epistaxis, both pulmonary and hepatic arteriovenous malformations, and a cerebellar cavernous haemangioma. Three unaffected siblings and half-siblings of the proband have been examined and show none of the signs of either juvenile polyposis or hereditary haemorrhagic telangiectasia. Chest radiography, although not the most sensitive screening method, showed no pulmonary arteriovenous malformations for the three unaffected siblings and II-2.

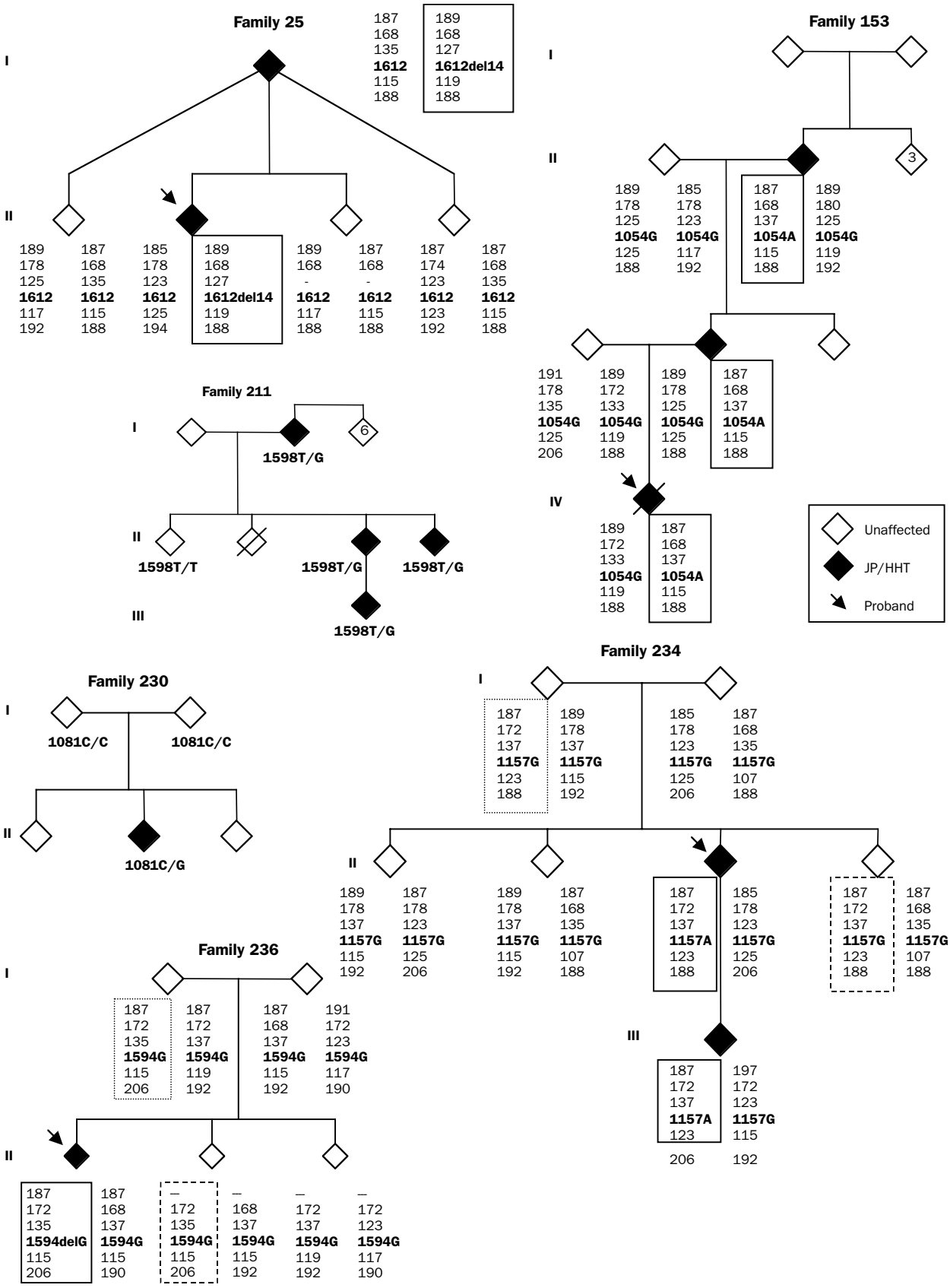
Family 153 is a multi-generation white family. All three affected members of this family have histories of rectal bleeding, severe anaemia, digital clubbing, and polyps. Although none had a history of epistaxis, each showed other features of hereditary haemorrhagic telangiectasia including arteriovenous malformations of the lung and liver, capillary telangiectases of the brain, and oral telangiectases. Chest radiography of IV-1 showed no pulmonary arteriovenous malformations. III-2 had had an intracranial bleed of unknown cause leading to loss of vision in the upper right quadrant. III-2 has an unaffected sibling and II-2 has three siblings who show no signs of either disease. The parents of II-2 likewise show no signs or symptoms of either juvenile polyposis or hereditary haemorrhagic telangiectasia.

Family 211, a three-generation Hispanic family, has four affected members. I-2, II-3, and II-4 have hereditary haemorrhagic telangiectasia with epistaxis and telangiectases. II-4 and III-1 have arteriovenous malformations of the lung, and II-4 has hepatic arteriovenous malformations and digital clubbing. The three older affected individuals have all had gastrointestinal bleeding and have had polypectomies. III-1 is under 3 years old and has not been examined endoscopically for polyps. All four of the affected patients have undergone brain MRI, CT, or both, with normal results. I-2 and II-3 have had normal chest radiography and abdominal CT. II-2 died in early childhood from meningitis. I-1 and II-1 have been examined and are free of symptoms of hereditary haemorrhagic telangiectasia or juvenile polyposis. I-2 has six siblings, none of whom show signs of either disorder.

Family 230 has two generations with only one affected individual. The proband, II-2, has many juvenile polyps in the stomach and colon and a history of gastrointestinal bleeding. This individual has telangiectases and a history of epistaxis but has not been examined for arteriovenous malformations. The parents and the two siblings of the proband have been examined for signs of juvenile polyposis and hereditary haemorrhagic telangiectasia and all are negative for both.

Family 234 is a three-generation family with two affected members. Both II-3 and III-1 are anaemic, have many colonic juvenile polyps, multiple pulmonary arteriovenous malformations, and digital clubbing. Subtotal colectomy was done on both patients, II-3 at age 18 years and III-1 at age 5 years. II-3 was admitted to hospital at age 35 years with intracranial bleeding of unknown cause and right-sided hemiparesis. The proband (II-3) has both cutaneous and visceral manifestations of hereditary haemorrhagic telangiectasia and has been described previously.[8] III-1 had many pulmonary arteriovenous malformations by age 5 years, but without other features at this young age, must be classified as only highly probable for hereditary haemorrhagic telangiectasia. Brain MRI and ultrasonography of the liver did not indicate the presence of either cerebral or hepatic arteriovenous malformations in III-1. The proband's parents (I-1, I-2) have been thoroughly examined for juvenile polyposis and hereditary haemorrhagic telangiectasia and show no signs of either disorder. The proband's three siblings show no signs or symptoms of the disorders.

Family 236 is a two-generation family with only one affected member. At age 11 years, the proband (II-1) was




**Family pedigrees**

Chromosome haplotypes using microsatellite markers flanking *MADH4* are shown (families 25, 153, 234, and 236) as well as the genotypes (in bold) of the altered bases in *MADH4* in each family. Haplotypes outlined with continuous lines represent the alleles with the mutations and the dotted lines mark the unaffected parental allele in which the de-novo mutation occurred. The dashed lines mark the allele without the mutation inherited by an unaffected sibling. De-novo *MADH4* mutations are seen in families 230, 234, and 236. Order of markers (centromeric to telomeric): *D18S460*, *D18S467*, *D18S474*, *MADH4*, *D18S487*, *D18S64*. Sizes are in bp.

| | Symptoms of juvenile polyposis | | | | Symptoms of hereditary haemorrhagic telangiectasia | | | | | Other symptoms | | *MADH4* mutation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Juvenile polyps | | Malignancy | Anaemia | AVM | | Telangiectasia | Epistaxis | Stroke | DC | Other | |
| | Location | Age at diagnosis (years) | | | Location | Age at diagnosis (years) | | | | | | |
| **Family 25** | | | | | | | | | | | | |
| I-1 | Caecum | 41 | + | – | Lung, liver | 32 | + | + | – | – | CCH | 1612-25 del 14 |
| II-2 | Colon | 8 | – | + | None | .. | + | + | – | – | None | |
| **Family 153** | | | | | | | | | | | | |
| II-2 | Colon | 9 | – | + | Lung | 8 | + | – | – | + | Pancytopenia, CCT | 1054G→A, G352R |
| III-2 | Colon | 5 | + | + | Lung | 7 | – | – | + | + | IC bleed | |
| IV-1 | Colon | 3 | – | + | Liver | 11 | – | – | – | + | None | |
| **Family 211** | | | | | | | | | | | | |
| I-2 | Colon | 15 | – | + | None | .. | + | + | – | – | None | 1598T→G, L533R |
| II-3 | Colon | 14 | – | + | None | .. | + | + | – | – | None | |
| II-4 | Colon | 3 | – | + | Lung, liver | 9 | + | + | – | + | None | |
| III-1 | NE | .. | NK | + | Lung | 0·75 | – | – | – | – | None | |
| **Family 230** | | | | | | | | | | | | |
| II-2 | Stomach, colon | 14 | – | + | NE | .. | + | + | – | – | None | 1081C→G, R361G* |
| **Family 234** | | | | | | | | | | | | |
| II-3† | Colon, duodenum | 10 | + | + | Lung | 15 | + | + | + | + | IC bleed | 1157G→A, G386D* |
| III-1 | Colon | 4 | – | + | Lung | 5 | – | – | – | + | Cyanotic | |
| **Family 236** | | | | | | | | | | | | |
| II-1 | Colon | 11 | – | + | NE | .. | + | + | – | – | None | 1594delG* |
| **Individual 2616** | | | | | | | | | | | | |
| Upper GI tract especially duodenum | | <10 | + | – | Liver | NK | – | + | – | + | Seizures, mild mental delay | 1600C→T, Q534X |

AVM=arteriovenous malformations. DC=digital clubbing/osteoarthropathy. CCH=cerebellar cavernous haemangioma. CCT=cerebral capillary telangiectases. IC=intracranial. NE=not examined. GI=gastrointestinal. NK=not known. *De-novo mutations described in this paper. †Patient originally described in Baert et al.[8]

Table 1: **Clinical features of patients with MADH4 mutations**

diagnosed with juvenile polyposis having many juvenile polyps and persistent anaemia. Owing to the anaemia and severe, recurrent epistaxis, II-1 underwent examination of the ears, nose, and throat, which showed telangiectases in the nasopharynx, anterior cavum nasi, lips, and buccal mucosa. This individual has not been examined for pulmonary arteriovenous malformations. Since only two of the three necessary criteria for definite hereditary haemorrhagic telangiectasia are met, this individual is classified as probable for the disorder. The parents have been examined for juvenile polyposis and hereditary haemorrhagic telangiectasia and show no signs of either disorder. The proband has two siblings, neither of whom show signs of either disorder.

Patient 2616 has an extensive number of juvenile polyps in the second portion of the duodenum and in the upper gastrointestinal tract. A subtotal colectomy was done years ago. Since then, polypectomies have yielded hamartomatous as well as adenomatous polyps and one high-grade dysplastic polyp. This person also had chronic epistaxis, hepatic arteriovenous malformations, and digital clubbing. One parent was reported to be affected with juvenile polyposis and hereditary haemorrhagic telangiectasia but is dead.

### Procedures

Genomic DNA was extracted from blood samples by the PureGene extraction protocol (Gentra Systems, Inc, Minneapolis, MN, USA) or the S&S Isocode Stix DNA extraction protocol (Schleicher & Schuell, Keene, NH, USA).

Coding exons from *ENG*, *ACVRL1*, and *MADH4* were amplified from genomic DNA from patients. The primers used were designed to include at least 50 bp of intronic sequence flanking each exon. Any samples showing changes from the reference sequences were reamplified and resequenced to confirm the changes.

Genotyping assays were created for putative missense mutations and done on appropriate population controls. The 1598T→G change alters a restriction endonuclease site in *MADH4* exon 11 (family 211), and was analysed in 99 North American and 89 Chilean control individuals. The two changes in *MADH4* exon 8 each destroy restriction endonuclease sites. More than 100 North American control individuals were assayed for each of these restriction-site changes.

### Role of the funding sources

The funding agencies had no role in study design; collection, analysis, or interpretation of data; writing of the paper; or the decision to submit it for publication.

## Results

The coding exons and at least 50 bp of the flanking intronic regions of the *ENG* and *ACVRL1* genes were sequenced from genomic DNA from representative affected members from each family with the combined juvenile polyposis and hereditary haemorrhagic telangiectasia phenotype. Three exonic sequence changes were identified but none altered the aminoacid sequence of the respective protein (207G→A, L69L, and 1029C→T, T343T in *ENG*, both previously reported as population polymorphisms, and 1131A→G, A377A in *ACVRL1*). As expected, several intronic changes were identified in these genes, most of which have also been previously described by the hereditary haemorrhagic telangiectasia sequencing consortium as population polymorphisms. All of these ranged from 9 to 70 nucleotides from the splice junction and none altered known CONSENSUS SPLICE SEQUENCE MOTIFS.

By contrast, when the *MADH4* gene was sequenced, mutations were identified in all affected individuals with the combined phenotype (table 1). This cohort had four missense, one nonsense, and two frameshift mutations in exons 8, 9, and 11 of *MADH4*. None of the missense mutations was observed in the appropriate population control cohort.

We found that the probands of three of the affected families had DE-NOVO MUTATIONS in *MADH4* (families 230, 234, and 236). Chromosome 18 haplotypes were determined for all three families to confirm that each proband was the biological child of the respective parents. In family 234, the proband's parents and three siblings were clinically unaffected and all were homozygous for the normal sequence (1157G) in *MADH4*. The mutation (1157G→A, G386D) arose on one of the parental gametes that was passed to the proband (figure). This mutation was inherited by the proband's child, who also shows clinical manifestations of both disorders, including multiple pulmonary arteriovenous malformations. The same missense mutation (1157G→A, G386D) has been previously identified as a de-novo mutation in a different patient with juvenile polyposis,[16] who also shows signs of hereditary haemorrhagic telangiectasia, such as pulmonary arteriovenous malformations, but was not diagnosed as having that disorder. The second de-novo mutation was identified in the proband of family 236, who again shows both juvenile polyposis and hereditary haemorrhagic telangiectasia phenotypes. The deletion mutation (1594delG) was not seen in the clinically unaffected parents or siblings. The third de-novo mutation was found in family 230; the mutation (1081C→G, R361G) was seen only in the proband who has many colonic polyps and both telangiectases and epistaxis. The mutation was not found in either unaffected parent.

In each family with more than one affected member, the *MADH4* mutations cosegregated with the combined syndromic phenotype of juvenile polyposis and hereditary haemorrhagic telangiectasia. Each person with a *MADH4* mutation shows phenotypes of both disorders, as would be expected in a mendelian syndrome with autosomal dominant inheritance. If another unlinked gene (eg, *ENG*, *ACVRL1*, or *BMPR1A*) were involved, independent segregation of the juvenile polyposis and hereditary haemorrhagic telangiectasia phenotypes would occur. The identification of three distinct de-novo mutations in *MADH4* shows that mutation of the *MADH4* gene alone is the cause of this combined syndrome.

The symptoms of juvenile polyposis and hereditary haemorrhagic telangiectasia in the 14 patients from seven families are striking (table 1). The most consistent feature of juvenile polyposis is the presence of many juvenile polyps, which were diagnosed in 12 of 14 patients, 11 of whom had colonic involvement. Seven of the 13 patients with reported polyps were diagnosed within the first decade of life, and five others by age 15 years.

All of the patients in this study also have definite features of hereditary haemorrhagic telangiectasia. Nine patients have mucocutaneous telangiectases, nine epistaxis, and seven pulmonary arteriovenous malformations, one patient being diagnosed at age 9 months. One patient has a cerebellar cavernous haemangioma, and two had instances of intracranial bleeding, one resulting in right-sided hemiparesis and the other in vision loss in the

| | Symptoms of juvenile polyposis | | | | Symptoms of hereditary haemorrhagic telangiectasia | | | | Other symptoms | | *MADH4* mutation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Juvenile polyps | | Malignancy | Anaemia | AVM | | Telangiectasia | Epistaxis | DC | Other | |
| | Location | Age at diagnosis (years) | | | Location | Age at diagnosis (years) | | | | | |
| **Cox et al[6]** | | | | | | | | | | | |
| I-1 | Upper/lower GI tract | 12 | – | NS | Lung | 10 | + | NS | + | Cyanotic | NT |
| II-1 | Colon | 5 | – | + | Lung | 8 | + | NS | + | None | NT |
| **Conte et al[7]** | | | | | | | | | | | |
| I-1 | Colon | NS | + | NS | Lung | NS | NS | NS | + | None | NT |
| II-1 | Colon | NS | NS | NS | Lung, brain | NS | NS | NS | + | SAH | |
| II-2 | Colon | NS | NS | NS | Lung | NS | NS | NS | + | None | NT |
| **Baert et al[8]** | | | | | | | | | | | |
| | Colon and duodenum | <15 | - | NS | Lung | 15 | NS | NS | + | None | 1157G→A, G386D* |
| **Prieto et al[9]** | | | | | | | | | | | |
| | Colon (mixed juvenile and adenomatous) | 9 | – | NS | Lung | 6 | + | NS | + | None | NT |
| **Radin[10]** | | | | | | | | | | | |
| | Duodenum, oesophagus | 16 | + | NS | Lung, liver | 32 | – | – | + | None | NT |
| **Desai et al[12]** | | | | | | | | | | | |
| | + | NS | NS | NS | Lung | 34 | + | NS | + | Cyanotic | NT |
| **Inoue et al[13]** | | | | | | | | | | | |
| | Colorectum | 14 | – | + | Lung, liver | 18 | + | + | NS | None | NT |
| **Burger et al[16]** | | | | | | | | | | | |
| | Colon with adenomatous dysplastic changes | 11 | NS | + | Lung | 11 | NS | NS | + | None | 1157G→A, G386D |

AVM=arteriovenous malformations. DC=digital clubbing/osteoarthropathy. GI=gastrointestinal. NS=not stated. NT=not tested. SAH=subarachnoid haemorrhage.
*Reported in this paper.

Table 2: **Features of previously reported juvenile polyposis patients with associated hereditary haemorrhagic telangiectasia phenotypes**

upper right quadrant. Four patients have hepatic arteriovenous malformations, one of whom was only 9 years old when diagnosed. These early ages of onset in several of the patients with the combined syndrome contrast with more typical ages of diagnosis for hereditary haemorrhagic telangiectasia, especially for arteriovenous malformations.[19]

The association of digital clubbing or osteoarthropathy and juvenile polyposis has been previously reported.[6–9,12,16,20,21] Seven patients in this study have digital clubbing or osteoarthropathy. The latter feature has been attributed to the intrapulmonary shunting caused by untreated pulmonary arteriovenous malformations.[10,20] In some of the patients in this study, the clubbing resolved after embolisation of the pulmonary lesions. The osteoarthropathy and digital clubbing previously reported in juvenile polyposis could reflect an underlying hereditary haemorrhagic telangiectasia phenotype of pulmonary arteriovenous malformations.

There does not appear to be an association with sex for this syndrome, because eight of the affected patients are female and six are male. There are four cases of female-to-female transmission of the phenotype and three of female-to-male transmission. Two of the de-novo index cases are male and one is female. There is no discernable difference in severity of symptoms between the male and female patients.

The range of symptoms found in previously reported cases of juvenile polyposis with features resembling hereditary haemorrhagic telangiectasia (table 2) appears similar to those observed in this study. All 11 previously reported patients had arteriovenous malformations in the lungs, two in the liver, and one in the brain. All 11 patients had polyps, in most in the colon and duodenum. Two of the patients had adenomatous changes and two had malignant tumours. Digital clubbing was reported in ten of the patients.

## Discussion

Is this dual-disorder state in fact a distinct genetic syndrome? All affected patients in this study showed clinical features of both juvenile polyposis and hereditary haemorrhagic telangiectasia (table 1) involving both the gastrointestinal epithelium and the vascular endothelium. This pattern of distinct primary malformations affecting various tissues is caused by a single genetic defect, so it meets the definition of a genetic syndrome. The phenotypes of hereditary haemorrhagic telangiectasia in our cohort are similar to those in other cohorts with this disorder with *ENG* or *ACVRL1* mutations, in which no individual patient showed the whole range of features associated with the disorder.[19,22] Similarly, the juvenile polyposis symptoms of our cohort range in age of diagnosis, location of polyps, and severity of bleeding.

Most of the juvenile polyposis patients with reported mutations in *MADH4* have not been described as having the vascular malformations or epistaxis associated with hereditary haemorrhagic telangiectasia. The proportion, if any, of these individuals who were specifically screened for clinically silent vascular lesions is not clear. In light of our findings, we suggest that juvenile polyposis patients with *MADH4* mutations should be actively screened for the vascular lesions associated with hereditary haemorrhagic telangiectasia, especially occult arteriovenous malformations in visceral organs that may otherwise present acutely with serious consequences.

The importance of screening juvenile polyposis patients for arteriovenous malformations of the lungs, brain, and liver due to the potential for serious complications or misdiagnoses of these malformations has been stated previously.[10] Our results suggest that genetic screening might identify individuals most at risk of these vascular lesions. On the basis of the data from the seven families in this study, the penetrance of at least some vascular phenotype in *MADH4* juvenile polyposis appears to be nearly 100%. Aggressive screening protocols for visceral arteriovenous malformations may be warranted for individuals with *MADH4* mutations.

Gastrointestinal bleeding in a patient with known hereditary haemorrhagic telangiectasia may be attributed to the presence of mucosal telangiectases, potentially delaying the diagnosis of juvenile polyposis with its associated risk of malignant disease. We suggest that patients with hereditary haemorrhagic telangiectasia who have early onset of arteriovenous malformations, severe gastrointestinal bleeding, or digital clubbing should be examined for juvenile polyposis to ensure proper clinical management. Genetic testing of patients presenting with either juvenile polyposis or hereditary haemorrhagic telangiectasia phenotypes will reveal those at risk of this combined syndrome due to *MADH4* mutations.

How do mutations in *MADH4* cause this combined syndrome? TGF-β is involved in many biological processes including cell-cycle control, embryogenesis, growth, development, and differentiation of several cell types. The diverse effects of TGF-β are mediated by various cell-surface receptors and intracellular signalling via distinct downstream effectors termed SMADs. A recent model for hereditary haemorrhagic telangiectasia suggests that the vascular phenotype results from an imbalance of signalling via the TGF-β receptors ALK5 and ACVRL1.[23,24] Disruption of endoglin or ACVRL1, both expressed primarily in vascular endothelium, would reduce the signalling through the ACVRL1 pathway, tilting the balance towards the ALK5 pathway, thus causing the vascular remodelling associated with hereditary haemorrhagic telangiectasia. If this model is correct, our genetic data could suggest that a decrease in SMAD4 concentrations would also disrupt this delicate balance regulating vascular remodelling and angiogenesis. Cross-talk between the TGF-β and bone-morphogenetic-protein signalling pathways could also contribute to this syndrome. The existence of individuals with both juvenile polyposis and hereditary haemorrhagic telangiectasia has been predicted because SMAD4 is common to the TGF-β and bone-morphogenetic-protein pathways.[25] Our clinical and genetic findings support this prediction.

The pathogenesis of this syndrome might be due to different effects of lowered concentrations of SMAD4 within specific cell types.[26] Disruption of the cellular localisation of SMAD4 could result in insufficiency of this signal transporter in the cytoplasm. This disequilibrium can lead to an activation of alternative signalling pathways by TGF-β, which would activate aberrant transcription of downstream target genes. In endothelial cells, TGF-β signals through the MKK4/JNK PATHWAY[27] whereas in epithelial cells it signals through the MKK3/P38 PATHWAY.[26,28,29] The activation of these different pathways by the same cytokine shows that molecular responses are cell-type specific. The cell type and microenvironment determine the cell's response to individual proteins and, presumably, to mutant proteins. Whereas *ENG* and *ACVRL1* are expressed primarily in vascular endothelium, *MADH4* is expressed in a broad range of cell types.[30] *MADH4* mutations in endothelial cells might lead to vascular dysplasia, whereas the same mutation in colonic mesenchymal or epithelial cells could lead to polyp formation.

**RELEVANCE OF THIS PAPER TO PRACTICE**

BACKGROUND

Definition of the exact features of disorders, especially rare ones, can be very difficult; syndromes that initially seem to be separate can show some overlap. Sometimes such overlap only becomes obvious when the genetic cause of a disorder becomes clear.

These investigators looked at seven families who had features of two apparently unrelated disorders—juvenile polyposis and hereditary haemorrhagic telangiectasia—believed to be caused by different genes. They found that in all families with both phenotypes there were mutations in one gene—MADH4 (also known as SMAD4), previously thought to be associated only with juvenile polyposis.

IMPLICATIONS

Patients with features of either of these disorders should have genetic screening; individuals who have mutations in SMAD4 should be examined carefully to see whether they have clinical features of more than one disorder. The management of these patients is likely to be more complex than previously realised, and will need to involve different medical specialties. Finally, this overlap syndrome highlights how mutations in one gene can produce a number of complex phenotypes. More work will need to be done to identify the exact pathways involved.

The association of juvenile polyposis and hereditary haemorrhagic telangiectasia might in fact be present only in a subset of patients with *MADH4* mutations. The phenotype could be determined by environmental and genetic modifiers that influence the effects of the mutation on the vascular system. Alternatively, the site of the mutation in *MADH4* might determine the phenotype. The three-dimensional structure of the carboxyl terminus of SMAD4 has been examined in detail,[31] and it is highly conserved between many members of the SMAD family in human beings and in divergent species. SMAD4 is believed to form homo-oligomers, which then form hetero-oligomers with phosphorylated R-SMADs and other cofactors in the nucleus to transduce signals. These specific interactions involve the carboxyl terminus of SMAD4. The mutations in all of our patients are located in that region of SMAD4. Mutations throughout the *MADH4* gene have been reported in other patients and families with juvenile polyposis. Further clinical information on these patients, specifically related to a hereditary haemorrhagic telangiectasia phenotype, might better define the apparent genotype–phenotype correlation between the involvement of the carboxyl terminus of SMAD4 and the juvenile polyposis and hereditary haemorrhagic telangiectasia syndrome.

Diseases and syndromes caused by de-novo mutations pose particular diagnostic challenges for physicians. These cases can appear to be sporadic instances of a disease rather than the inheritable germline mutation that they are. In the past, many patients who had autosomal dominant syndromes with severe symptoms died before being able to reproduce, so the de-novo mutations were not passed on to subsequent generations. With the advances in clinical care now available, many of these patients reach reproductive age, even though very ill, and their children can inherit these mutations. "Sporadic" cases have thus become "familial" with distinct implications for the care and counselling of both patients and their families.

On the basis of our genetic findings, the thorough physical examinations of our study cohort, and similar cases previously reported, our clinical recommendations are as follows. First, patients with hereditary haemorrhagic telangiectasia presenting in childhood with arteriovenous malformations or gastrointestinal bleeding should be screened for juvenile polyps and *MADH4* mutations. Second, patients with juvenile polyposis should be genetically tested for mutations in both the *MADH4* and *BMPR1A* genes. Third, those individuals with juvenile polyposis due to *MADH4* mutations should be screened for the presence of visceral arteriovenous malformations, which could present unexpectedly and lead to serious complications. Systematic screening for visceral manifestations of hereditary haemorrhagic telangiectasia in all juvenile polyposis patients with *MADH4* mutations will reveal the true prevalence of this combined syndrome.

*Contributors*

C J Gallione did the sequencing and haplotype analyses and interpreted the results. G M Repetto, E Legius, A K Rustgi, S L Schelley, G Mitchell, É Drouin, S Tejpar, and C J J Westermann contributed to patients' diagnoses and sample collection. G M Repetto, E Legius, and C J J Westermann interpreted and analysed clinical information. D A Marchuk designed the study and reviewed the results. All investigators contributed to and reviewed the final report.

*Conflict of interest statement*

None declared.

*Acknowledgments*

This work was supported by National Institutes of Health grant HL49171 to DAM. EL is a part-time clinical researcher of the Fonds voor Wetenschappelijk Onderzoek Vlaanderen. GMR thanks M Linares for the referral of a family and P Gavilan for collection of control samples. CJG thanks T Leedom for assistance in family collections. We also thank the families and patients for participating in this study.

**References**

1 Coffin CM, Dehner LP. What is a juvenile polyp? An analysis based on 21 patients with solitary and multiple polyps. *Arch Pathol Lab Med* 1996; **120:** 1032–38.
2 Howe JR, Roth S, Ringold JC, et al. Mutations in the *SMAD4/DPC4* gene in juvenile polyposis. *Science* 1998; **280:** 1086–88.
3 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:** 184–87.
4 McAllister KA, Grogg KM, Johnson DW, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet* 1994; **8:** 345–51.
5 Johnson DW, Berg JN, Baldwin MA, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet* 1996; **13:** 189–95.
6 Cox KL, Frates RC, Jr, Wong A, Gandhi G. Hereditary generalized juvenile polyposis associated with pulmonary arteriovenous malformation. *Gastroenterology* 1980; **78:** 1566–70.
7 Conte WJ, Rotter JI, Schwartz AG, Congleton JE. Hereditary generalized juvenile polyposis, arteriovenous malformations and colonic carcinoma. *Clin Res* 1982; **30:** 93A (abstr).
8 Baert AL, Casteels-Van Daele M, Broeckx J, Wijndaele L, Wilms G, Eggermont E. Generalized juvenile polyposis with pulmonary arteriovenous malformations and hypertrophic osteoarthropathy. *AJR Am J Roentgenol* 1983; **141:** 661–62.
9 Prieto G, Polanco I, Sarria J, Larrauri J, Lassaletta L. Association of juvenile and adenomatous polyposis with pulmonary arteriovenous malformation and hypertrophic osteoarthropathy. *J Pediatr Gastroenterol Nutr* 1990; **11:** 133–37.
10 Radin DR. Hereditary generalized juvenile polyposis: association with arteriovenous malformations and risk of malignancy. *Abdom Imaging* 1994; **19:** 140–42.
11 Schumacher B, Frieling T, Borchard F, Hengels KJ. Hereditary hemorrhagic telangiectasia associated with multiple pulmonary arteriovenous malformations and juvenile polyposis. *Z Gastroenterol* 1994; **32:** 105–08.
12 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:** 476–81.
13 Inoue S, Matsumoto T, Iida M, et al. Juvenile polyposis occurring in hereditary hemorrhagic telangiectasia. *Am J Med Sci* 1999; **317:** 59–62.
14 Roth S, Sistonen P, Salovaara R, et al. SMAD genes in juvenile polyposis. *Genes Chromosomes Cancer* 1999; **26:** 54–61.
15 Ballauff A, Koletzko S. Hereditary hemorrhagic telangiectasia with juvenile polyposis: coincidence or linked autosomal dominant inheritance? *Z Gastroenterol* 1999; **37:** 385–88.

16 Burger B, Uhlhaas S, Mangold E, et al. Novel de novo mutation of *MADH4*/*SMAD4* in a patient with juvenile polyposis. *Am J Med Genet* 2002; **110:** 289–91.
17 Jass JR, Williams CB, Bussey HJ, Morson BC. Juvenile polyposis: a precancerous condition. *Histopathology* 1988; **13:** 619–30.
18 Shovlin CL, Guttmacher AE, Buscarini E, et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). *Am J Med Genet* 2000; **91:** 66–67.
19 Begbie ME, Wallace GM, Shovlin CL. Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu syndrome): a view from the 21st century. *Postgrad Med J* 2003; **79:** 18–24.
20 Simpson EL, Dalinka MK. Association of hypertrophic osteoarthropathy with gastrointestinal polyposis. *AJR Am J Roentgenol* 1985; **144:** 983–84.
21 Erkul PE, Ariyurek OM, Altinok D, Bakkaloglu A, Kotiloglu E. Colonic hamartomatous polyposis associated with hypertrophic osteoarthropathy. *Pediatr Radiol* 1994; **24:** 145–46.
22 Berg J, Porteous M, Reinhardt D, et al. Hereditary haemorrhagic telangiectasia: a questionnaire based study to delineate the different phenotypes caused by endoglin and ALK1 mutations. *J Med Genet* 2003; **40:** 585–90.
23 Goumans MJ, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, ten Dijke P. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *EMBO J* 2002; **21:** 1743–53.
24 Marchuk DA, Srinivasan S, Squire TL, Zawistowski JS. Vascular morphogenesis: tales of two syndromes. *Hum Mol Genet* 2003; **12** (suppl 1)**:** R97–112.
25 Waite KA, Eng C. From developmental disorder to heritable cancer: it's all in the BMP/TGF-beta family. *Nat Rev Genet* 2003; **4:** 763–73.
26 Massague J. How cells read TGF-beta signals. *Nat Rev Mol Cell Biol* 2000; **1:** 169–78.
27 Hocevar BA, Brown TL, Howe PH. TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J* 1999; **18:** 1345–56.
28 Sano Y, Harada J, Tashiro S, Gotoh-Mandeville R, Maekawa T, Ishii S. ATF-2 is a common nuclear target of Smad and TAK1 pathways in transforming growth factor-beta signaling. *J Biol Chem* 1999; **274:** 8949–57.
29 Hanafusa H, Ninomiya-Tsuji J, Masuyama N, et al. Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-beta-induced gene expression. *J Biol Chem* 1999; **274:** 27161–67.
30 Sirard C, de la Pompa JL, Elia A, et al. The tumor suppressor gene Smad4/Dpc4 is required for gastrulation and later for anterior development of the mouse embryo. *Genes Dev* 1998; **12:** 107–19.
31 Shi Y, Hata A, Lo RS, Massague J, Pavletich NP. A structural basis for mutational inactivation of the tumour suppressor Smad4. *Nature* 1997; **388:** 87–93.