ORIGINAL ARTICLE

SMAD4 mutations found in unselected HHT patients

C J Gallione, J A Richards, T G W Letteboer, D Rushlow, N L Prigoda, T P Leedom, A Ganguly, A Castells, J K Ploos van Amstel, C J J Westermann, R E Pyeritz, D A Marchuk

J Med Genet 2006;43:793–797. doi: 10.1136/jmg.2006.041517

See end of article for authors' affiliations

Correspondence to:
Dr D A Marchuk,
Department of Molecular
Genetics and
Microbiology, Box 3175,
Duke University Medical
Center, Durham, NC
27710; march004@mc.
duke.edu

Received 6 February 2006
Revised 28 March 2006
Accepted 30 March 2006
Published Online First
13 April 2006

Background: Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant disease exhibiting multifocal vascular telangiectases and arteriovenous malformations. The majority of cases are caused by mutations in either the endoglin (*ENG*) or activin receptor-like kinase 1 (*ALK1*, *ACVRL1*) genes; both members of the transforming growth factor (TGF)- β pathway. Mutations in *SMAD4*, another TGF- β pathway member, are seen in patients with the combined syndrome of juvenile polyposis (JP) and HHT (JP-HHT).

Methods: We sought to determine if HHT patients without any apparent history of JP, who were undergoing routine diagnostic testing, would have mutations in *SMAD4*. We tested 30 unrelated HHT patients, all of whom had been referred for DNA based testing for HHT and were found to be negative for mutations in *ENG* and *ALK1*.

Results: Three of these people harboured mutations in *SMAD4*, a rate of 10% (3/30). The *SMAD4* mutations were similar to those found in other patients with the JP-HHT syndrome.

Conclusions: The identification of *SMAD4* mutations in HHT patients without prior diagnosis of JP has significant and immediate clinical implications, as these people are likely to be at risk of having JP-HHT with the associated increased risk of gastrointestinal cancer. We propose that routine DNA based testing for HHT should include *SMAD4* for samples in which mutations in neither *ENG* nor *ALK1* are identified. HHT patients with *SMAD4* mutations should be screened for colonic and gastric polyps associated with JP.

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant disease of vascular dysplasia. The symptoms of HHT include epistaxis, telangiectases, and arteriovenous malformations (AVMs), which are most often found in the lungs, brain, liver, and gastrointestinal tract. There is wide variation in penetrance and severity of these symptoms in patients even within the same family,¹ suggesting that environmental or other genetic factors influence the phenotype. Mutations in either one of two genes cause HHT. Mutations in the endoglin (*ENG*) gene are responsible for HHT1² and mutations in the activin receptor-like kinase 1 (*ALK1*) gene for HHT2.³ Both of these genes encode TGF- β binding proteins and play important roles in regulating endothelial cell growth.^{4,5}

Screening for mutations in either *ENG* or *ALK1* in HHT patient cohorts yields mutation detection rates of between 62% and 93%.^{6–14} The failure to achieve a 100% detection rate is not unique to HHT. This range of mutation detection rates is similar to that found for many other mendelian disorders,¹⁵ implying that most, if not all, mutation scans share common detection limitations. In the case of HHT, people may have large deletions or insertions, deep intronic mutations affecting splicing, or regulatory mutations in either *ENG* or *ALK1*. A subset of the mutation negative samples may instead have a mutation in the as yet undiscovered HHT3 gene recently linked to chromosome 5.¹⁶ Because the majority of HHT patients have been found to have mutations in *ENG* or *ALK1*, it is probable that only a small fraction of the remaining cases will be caused by mutations in other genes.

SMAD4 is a key downstream effector of transforming growth factor (TGF)- β signalling. Mutations in *SMAD4* and *BMPRIA* are known to be causative for juvenile polyposis (JP).^{17,18} JP is characterised by the presence of five or more hamartomatous gastrointestinal polyps, or any number of polyps in addition to a family history of polyposis.¹⁹ There is an increased risk of

gastrointestinal cancers associated with these polyps. Recently, *SMAD4* was identified as mutated in a subset of HHT patients with JP, a condition termed JP-HHT syndrome,²⁰ in which juvenile polyps and anaemia are the predominant clinical features. HHT symptoms vary among patients, but all meet the Curaçao criteria for being either definitely or possibly affected.²¹ There is a high penetrance of AVMs in this JP-HHT cohort, particularly in young patients. Importantly, each of these patients has symptoms of both JP and HHT.

Both JP and HHT are diseases known to have a range of clinical presentations and to be variably penetrant.^{1,22} One feature common to both disorders is gastrointestinal (GI) bleeding, potentially confounding the correct diagnosis. Because of this potential for uncertainty in diagnoses, we questioned whether, among a cohort of *ENG* and *ALK1* mutation negative HHT cases, there were occult cases of JP-HHT, diagnosed as only having the HHT component of the combined phenotype. Using sequence analysis for *SMAD4* mutations, we screened 30 people from an unselected group of HHT patients, who had been referred for DNA based testing for HHT and found to be negative for mutations in either *ENG* or *ALK1*.

METHODS

Subjects

Of the the subjects in this cohort, 20 were referred to the genetic diagnostic laboratory at the University of Pennsylvania, five were enrolled through the Dutch HHT Center in the Netherlands, and four were referred through HHT Solutions in Toronto, Canada. Individuals were referred to these centres of DNA based diagnostics because of a

Abbreviations: *ALK1*, activin receptor-like kinase 1; AVM, arteriovenous malformation; *ENG*, endoglin; GI, gastrointestinal; JP, juvenile polyposis; TGF, transforming growth factor

diagnosis or suspicion of HHT (table 1). One patient with HHT was independently assessed at the hospital clinic in Barcelona, Spain, and referred to Duke University for this study after being diagnosed with colon cancer. All participants in this study were enrolled with their informed consent. Clinical subjects also gave consent for their DNA to be used in further research. Enrolment of participants through Duke University and the Dutch HHT Center was given approval by the institutional review boards of the participating institutions (Duke University Health System Institution Review Board Committee, and METC, Utrecht).

Our cohort comprised 30 subjects, of whom 24 (80%) had definite HHT and 6 (20%) had possible HHT, according to the Curaçao diagnostic criteria.²¹ Ages of the participants ranged from 18 to 87 years (mean 51.3, median 49). The majority of the subjects reported having a positive family history for HHT (19/30, 63%) while four people reported a negative family history. The remaining seven did not respond to the query on their family history. Visceral lesions were present in 25 people, absent in 3, and not reported in 2. Five different participants noted GI bleeding, and individual subjects report having stroke, migraines, gastric polyps, and liver shunts. One individual had both anaemia and colon cancer, and another had GI bleeding and colonic polyps.

The samples included in this study are a subset of 102 *ENG*/*ALK1* mutation negative samples from all of the institutions. The 30 participants comprising this cohort had granted consent for additional research using their DNA. They were not selected for *SMAD4* testing based on any reported clinical symptoms.

Diagnostic testing and genetic screening

All 30 subjects were screened by direct DNA sequencing of PCR products consisting of the coding exons and adjacent

intronic regions for *ENG*, *ALK1*, and *SMAD4* from genomic DNA. In all cases, any sequence variations found were reamplified and resequenced to confirm the observed changes. The sequences generated were compared with wild type *ENG* (GenBank accession no. NT_008470 or BC014271), *ALK1* (GenBank NT_029419 or NM_000020), and *SMAD4* (GenBank NM_005359). There were 113 control individuals screened for the presence of the *SMAD4* missense mutation by using a *Sau3A* restriction digest assay. The mutation destroys the single *Sau3A* site in the exon 8 amplicon. In all cases, nucleotides were numbered starting with the A in the initial ATG of the cDNA sequence as c.1, and the starting methionine in the protein sequence as p.1 (<http://www.hgvs.org/mutnomen/>).

Although all centres used direct DNA sequence analysis to identify point mutations, each diagnostic centre employed a different technique to further examine the DNAs for large scale rearrangements. The 20 samples from the genetic diagnostic laboratory at University of Pennsylvania were tested by quantitative Southern blots. Genomic DNA was digested with *RsaI*, because most fragments represent individual exons. cDNA probes covering exons 2–14 for *ENG* and 2–10 for *ALK1* were used for the initial analysis. If a change in dosage for any one or more exons was detected, probing with specific individual exonic clones was performed. In addition, genomic probes for exons 1 of both *ENG* and *ALK1* were tested separately. Whole gene deletions were detected by comparison with an internal control (β -globin gene) run on the same filter.

The four samples from HHT Solutions were tested for changes in exon size and copy number using quantitative multiplex PCR with intronic primers for all 15 *ENG* exons and 9 *ALK1* exons. The fluorescent fragments were analysed using

Table 1 Clinical characteristics of the testing cohort

Sample	Age (years)	Family history	Visceral lesions	Telangiectasia	Epistaxis	Other	HHT status
UP001	18	Yes	Yes	N/A	N/A		Possible
UP002	61	Yes	Yes	N/A	Yes	GI bleeding	Definite
UP003	73	Yes	Yes	Yes	N/A		Definite
UP004	77	Yes	Yes	Yes	Yes		Definite
UP005	21	N/A	Yes	N/A	Yes		Possible
UP006	37	N/A	N/A	Yes, (rectal)	Yes		Possible
UP007	73	Yes	Yes	N/A	Yes	GI bleeding	Definite
UP008	54	Yes	Yes	Yes	Yes		Definite
UP009	23	N/A	Yes	Yes	Yes		Definite
UP010	38	N/A	Yes (lung)	Yes	N/A	Stroke	Possible
UP011	78	N/A	Yes	N/A	Yes	GI bleeding	Possible
UP012	74	Yes	N/A	Yes	Yes		Definite
UP013	52	Yes	Yes	Yes	Yes		Definite
UP014	69	N/A	Yes	Yes	Yes		Definite
UP015*	37	No	Yes (lung, GI)	Yes	Yes	GI bleeding, colonic polyps	Definite
UP016	36	Yes	Yes	Yes	Yes		Definite
UP017	69	Yes	Yes	Yes	Yes		Definite
UP018	32	Yes	Yes	Yes	N/A		Definite
UP019	87	Yes	Yes	Yes	Yes		Definite
UP020	56	N/A	Yes (stomach)	Yes	Yes		Definite
D1827	47	Yes	Yes (liver)	Yes (inc. GI)	Yes	Confirmed HHT	Definite
DKCL331	44	Yes	Yes (lung)	Yes	Yes	Confirmed HHT	Definite
D0883	63	Yes	No	Yes	Yes	Confirmed HHT	Definite
D1488	44	Yes	Yes (lung)	Yes	Yes	Confirmed HHT	Definite
D3748	51	Yes	No	Yes	Yes	Confirmed HHT	Definite
5001	56	Yes	Yes (hip)	Yes	Yes	Confirmed HHT, GI bleeding, gastric polyps	Definite
5031	37	No	Yes (lung, multiple)	No	Yes	Confirmed HHT, migraines	Possible
5051	46	Yes	No	Yes (chest)	Yes	Confirmed HHT	Definite
5068*	39	No	Yes (lung)	Yes	Yes	Confirmed HHT, liver shunts	Definite
3892*	47	Yes (in children) No	Yes (liver)	Yes (lips, tongue)	Yes	Confirmed HHT, anaemia Colon cancer	Definite

HHT status was- as defined by the Curaçao criteria.²¹ **SMAD4* mutation positive patients. N/A, not available; GI, gastrointestinal.

Gene Objects software (version 3.1; Bayer). RNA analysis was used in some cases as an additional test where no mutation was found by sequencing or QM-PCR.

The five samples from the Dutch HHT Center were probed for exonic deletions or duplications in *ENG* and *ALK1* by MLPA. The DNA from patient 3892 was not examined for genomic rearrangements; however, the coding regions and surrounding intronic regions of *BMPRIA* were sequenced and compared with a reference sequence (GenBank accession number NM_004329).

RESULTS

Subjects were referred to four different institutions for DNA based diagnostic testing for HHT based on the clinical diagnosis or suspicion of HHT. Of 374 people sent for DNA testing for HHT, 30 had also consented to allow their DNA to be included in research studies. Of these 30, none was known to have JP, although GI bleeding, a feature in common with HHT and JP, was reported for five of the individuals. All 30 of these subjects had been screened for *ENG* and *ALK1* mutations by direct DNA sequencing but no mutations had been found. As part of the attempted molecular diagnosis for HHT, 29 of these people had also been screened for genomic rearrangements of the *ENG* and *ALK1* genes, but no alterations had been uncovered.

We then sequenced the coding exons and adjacent intronic sequences of *SMAD4* and identified mutations in three subjects. Individual UP015 was referred to the genetic diagnostic laboratory at the University of Pennsylvania. This 37 year old has telangiectases, epistaxis, and AVMs in the lung and gastrointestinal tract. Although no family history of HHT was noted, this patient fulfils the Curaçao criteria for definite HHT.²¹ This person was found to have a frameshift mutation in exon 11 of *SMAD4*, c.1596_97delCCinsT. After this molecular finding, inspection of patient charts revealed that this patient had colonic polyps that had been discovered during endoscopy.

Individual 5068 (family 530) was referred to HHT Solutions in Toronto. This 39 year old has telangiectases, epistaxis, pulmonary AVMs, and liver shunts, and therefore meets diagnostic criteria as definitely affected with HHT. Although there was no reported past family history of HHT, the subject's children show some presumptive signs of HHT (epistaxis and telangiectases). A missense mutation in exon 8 of *SMAD4*, c.1081C→T, R361C, was found.

Individual 3892, 47 years old, was diagnosed at 13 years of age with HHT, and had telangiectases of the lips and tongue, epistaxis, and a hepatic AVM. This person has had iron deficiency anaemia for more than 15 years, requiring blood transfusions on multiple occasions, and is on regular iron supplementation. Signet ring cell type colorectal cancer located in the caecum was diagnosed in June 2004. Upon examination, seven hamartomatous polyps were found in the ascending colon and three in the duodenum. There is no history of either HHT or gastrointestinal cancer in the family. This person harboured the same *SMAD4* exon 8 missense mutation (c.1081C→T, R361C) seen in patient 5068.

All three mutations identified were found within the COOH terminus of *SMAD4*, where all of the mutations in previously reported JP-HHT cases have been identified (fig 1). One mutation, c.1081C→T (R361C) in exon 8, was found in two unrelated members of our study cohort. This same mutation has been reported in a number of people with JP,²³⁻²⁵ and the same base is mutated in a previously reported case of JP-HHT (c.1081C→G, p.R361G).²⁰ The missense mutation at codon 361 was not identified by us in 113 unaffected population controls (226 alleles) or by Houlston *et al* in the 50 controls (100 alleles) tested in the case of a JP patient.²³ Using two different programs that use evolutionary

conservation to investigate the effects of putative missense mutations (SIFT (<http://blocks.fhcrc.org/sift/SIFT.html>) and PolyPhen (<http://genetics.bwh.harvard.edu/cgi-bin/pph/polyphen.cgi>)), the R361C amino acid change is predicted to negatively affect protein function. The third mutation found in this study constitutes a frameshift in exon 11 (c.1596_97delCCinsT). This is a novel change, although frameshift mutations in exon 11 of *SMAD4* have also been identified in JP-HHT patients.²⁰

The total diagnostic testing cohort from the four centres was 374 HHT patients. *ENG* or *ALK1* mutations were found in 272 people, a detection rate of 72.7%. Thus, 102 subjects (27.3%) were considered *ENG/ALK1* mutation negative for this study. The 30 people available to us for research represent approximately 30% of the *ENG/ALK1* mutation negative group and 8% of the total HHT cohort. *SMAD4* mutations were found in three of the 30, a *SMAD4* mutation rate of 10%. Extrapolating to the larger *ENG/ALK1* mutation negative group, up to 10 presumptive HHT patients might harbour *SMAD4* mutations. This would represent 2–3% (10/374) of the original cohort referred for HHT molecular testing.

DISCUSSION

DNA sequencing performed by numerous groups around the world shows that the majority of HHT patients have mutations in either *ENG* or *ALK1*.⁶⁻¹⁴ The mutation detection rates in the scans range from 62% to 93%, a range that matches the detection rates found in scans of other genes in mendelian diseases.¹⁵ There are several explanations why a patient might appear mutation negative in a mutation screen. For diseases such as HHT that have highly variable clinical presentations, it is possible that some of the referred patients may not actually have the disease in question. In the present study, presuming that all members of the cohort are affected HHT patients, another explanation could be that these patients may harbour non-coding mutations, such as alterations in poorly characterised regulatory regions of the gene, including the promoter, introns, and 5' and 3' untranslated regions. Additionally, large scale genomic rearrangements involving whole exons, partial gene loss or duplication, and intrachromosomal or interchromosomal rearrangements will bypass detection by DNA sequencing. It is also possible that some patients will have mutations in other genes that lead to the same disease phenotype. A recent report of two unrelated HHT families that exhibit linkage to a region on chromosome 5 demonstrates that at least one other gene is involved in HHT.¹⁶ Non-*ENG*, non-*ALK1* HHT patients may harbour a mutation in this as yet unidentified gene.

A final explanation for these *ENG/ALK1* mutation negative HHT cases might be that some of these patients harbour mutations in *SMAD4*. Based on a molecular diagnosis, these patients would have the JP-HHT syndrome, but they may have been diagnosed with HHT due to occult or unrecognised manifestations of JP. In this study we queried if *SMAD4* mutations were found in the general HHT population. To

Figure 1 Schematic diagram of exons 8–11 of *SMAD4* showing HHT associated mutations. These four exons encode the MH2 domain of the protein. In confirmed JP-HHT syndrome patients, no mutations have been identified in the first seven exons of the gene. Shaded boxes, exons; triangles, missense mutations; diamonds, frameshift mutation; circles, nonsense mutations; filled symbols above the shaded exons, previously reported mutations in JP-HHT patients;²⁰ open symbols, from our unpublished data; marks below the exons, mutations reported here from people exhibiting symptoms of HHT.

determine the answer, we sequenced the coding exons and adjacent flanking intronic regions of *SMAD4* in a cohort of 30 unrelated people with presumptive HHT. These people had been referred for DNA based diagnostic testing for HHT and all were negative for mutations in *ENG* and *ALK1*. One of them had colon cancer, which is sometimes the first symptomatic manifestation of JP, but none of the others was known or reported to have JP. Although GI bleeding was reported in five of these subjects, this was interpreted as a symptom of HHT.

All of the *SMAD4* mutations identified in JP-HHT patients to date are found in the COOH terminus of the protein. The three mutations identified in the subjects here are in the COOH terminus of *SMAD4* and are nearly identical in location and type of mutation to those seen in JP-HHT patients (fig 1). This strongly suggests that the three HHT subjects with *SMAD4* mutations in this cohort are affected with JP-HHT syndrome. Two of these three *SMAD4*-HHT subjects are known to have colonic polyps, and one of these two has colorectal cancer. The status of the third individual regarding JP symptoms is unknown.

There is growing evidence of distinct phenotypic differences between patients with HHT1 and HHT2.^{26–28} Similarly, other studies have demonstrated phenotypic differences between JP patients with *SMAD4* mutations compared with patients with mutations in *BMPRIA*,^{29–30} the second gene known to be involved in JP. JP is also known to be variably penetrant and is associated with an increased risk of gastrointestinal cancer.^{22–31} Knowing which gene is responsible for the disease in an individual HHT patient will aid in the proper management and care of the patient and, significantly, of related family members.

We recommend that when genetic testing is advised for HHT patients, *SMAD4* should be screened if no mutations are found in either *ENG* or *ALK1*. We also recommend that screening for colonic and gastric polyps be considered in people in whom neither *ENG* nor *ALK1* mutations have been found, in whom *SMAD4* mutations have been uncovered, or in anyone with anaemia that cannot be completely explained by epistaxis or some other cause. This screening will identify those HHT patients with occult polyposis. Early detection of colonic polyps in any patient, but in particular in JP-HHT patients, could prevent the development of colorectal cancer by finding and removing precancerous polyps.

There appears to be a high rate of de novo cases of JP-HHT,²⁰ and all three of the *SMAD4*-HHT patients in this study reported no family history of HHT. Although a positive family history is one of the criteria for HHT diagnosis,²¹ *SMAD4* mutation carriers may often lack this key diagnostic feature, potentially making the clinical diagnosis more difficult. In the cases of presumed HHT without any apparent family history, an argument might be made that *SMAD4* mutation analysis should precede analysis of *ENG* or *ALK1*, as de novo mutations in these other HHT genes appear to be rare.^{8–13–14}

An HHT patient found to harbour a *SMAD4* mutation would be a prime situation where genetic testing can be used to guide clinical management. Because of the increased risk of gastrointestinal cancer associated with JP, it is critically important for a patient with a *SMAD4* mutation to be screened for JP. We suggest that HHT patients harbouring a *SMAD4* mutation should be considered at high risk of JP-HHT, requiring more intensive colorectal cancer screening strategies than those recommended for the average risk population.³² Correspondingly, JP patients with *SMAD4* mutations similar to those seen in JP-HHT patients should be examined for the visceral manifestations of HHT which can present suddenly and catastrophically.

SMAD4, *ENG*, and *ALK1* are all members of the TGF- β signalling pathway, and mutations in the genes encoding

them can cause a broad constellation of phenotypes with both distinct and overlapping clinical features. It has been known for a number of years that mutations in *SMAD4* cause JP,¹⁷ and we have recently shown that certain types of mutations in *SMAD4* cause JP-HHT.²⁰ Here we report that unselected HHT patients have *SMAD4* mutations that are strikingly similar to those seen in JP-HHT patients. In JP cases with the same types of *SMAD4* mutations, it remains to be seen if these JP patients exhibit symptoms of HHT due to the paucity of clinical descriptions in the literature. Mutations in *ENG* have recently been reported in patients with JP,³³ and mutations in *ALK1* cause some cases of primary pulmonary hypertension.^{34–36} Molecular dissection of the interconnections between these genes and the effects of aberrant signalling through the TGF- β and other alternative signalling pathways, will help to further elucidate the pathophysiology of these inherited diseases.

ACKNOWLEDGEMENTS

We would first like to thank the patients for their participation in this study. We also thank K Vandezande at HHT Solutions for helpful discussions. A Castells has received funding from the Ministerio de Educación y Ciencia (SAF 04-07190) and from the Instituto de Salud Carlos III (RC03/02 and RC03/10). D A Marchuk has received funding from the National Institutes of Health (R01 HL49171).

Note added in proof: Further examination of patient records revealed that patient 5068 has colon polyps. This individual also has a liver AVM, migrolines and clubbing of the figures.

Authors' affiliations

C J Gallione, T P Leedom, D A Marchuk, Duke University Medical Center, Durham, NC, USA

J A Richards, A Ganguly, Genetic Diagnostic Laboratory, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

T G W Letteboer, J K Ploos van Amstel, DBG-Department of Medical Genetics, University Medical Center, Utrecht, the Netherlands

D Rushlow, N L Prigoda, HHT Solutions, Toronto Western Hospital, Toronto, Canada

A Castells, Gastroenterology Department, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

C J J Westermann, St. Antonius Hospital, Nieuwegein, the Netherlands

R E Pyeritz, Departments of Medicine & Genetics, Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Competing interests: there are no competing interests

REFERENCES

- 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.
- McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, Helmbold EA, Markel DS, McKinnon WC, Murrell J, McCornick MK, Perieak-Vanee MA, Heutink P, Oostra BA, Haitjema T, Westermann CJ, Porteous ME, Guttacher AE, Letarte M, Marchuk DA. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet* 1994;**8**:345–51.
- Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, Speer M, Pericak-Vance MA, Diamond A, Guttacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous ME, Marchuk DA. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet* 1996;**13**:189–95.
- Lebrin F, Deckers M, Bertolino P, Ten Dijke P. TGF-beta receptor function in the endothelium. *Cardiovasc Res* 2005;**65**:599–608.
- ten Dijke P, Hill CS. New insights into TGF-beta-Smad signalling. *Trends Biochem Sci* 2004;**29**:265–73.
- Letteboer TG, Zewald RA, Kamping EJ, de Haas G, Mager JJ, Snijder RJ, Lindhout D, Hennekam FA, Westermann CJ, Ploos van Amstel JK. Hereditary hemorrhagic telangiectasia: *ENG* and *ALK-1* mutations in Dutch patients. *Hum Genet* 2005;**116**:8–16.
- Schulte C, Geischoff U, Lux A, Kupka S, Zenner HP, Blin N, Pfister M. High frequency of *ENG* and *ALK1/ACVRL1* mutations in German HHT patients. *Hum Mutat* 2005;**25**:595.
- Abdalla SA, Cymerman U, Rushlow D, Chen N, Stoeber GP, Lemire EG, Letarte M. Novel mutations and polymorphisms in genes causing hereditary hemorrhagic telangiectasia. *Hum Mutat* 2005;**25**:320–1.

- 9 Kuehl HK, Caselitz M, Hasenkamp S, Wagner S, El-Harith el HA, Manns MP, Stuhmann M. Hepatic manifestation is associated with ALK1 in hereditary hemorrhagic telangiectasia: identification of five novel ALK1 and one novel ENG mutations. *Hum Mutat* 2005;**25**:320.
- 10 Draghi F, Preeruti M, Danesino GM, Olivieri C, Valacca C, Danesino C, Pagella F, Semino L, Lanzarini L, Buscarini E, Danesino C. Vascular abnormalities in the fingers of patients affected with hereditary hemorrhagic telangiectasia (HHT) as assessed by color doppler sonography. *Am J Med Genet A* 2005;**135**:106–9.
- 11 Lesca G, Plauchu H, Coulet F, Lefebvre S, Plessis G, Odent S, Riviere S, Leheup B, Goizet C, Carrette MF, Cordier JF, Pinson S, Soubrier F, Calender A, Giraud S. Molecular screening of ALK1/ACVRL1 and ENG genes in hereditary hemorrhagic telangiectasia in France. *Hum Mutat* 2004;**23**:289–99.
- 12 Brusgaard K, Kjeldsen AD, Poulsen L, Moss H, Vase P, Rasmussen K, Kruse TA, Horder M. Mutations in endoglin and in activin receptor-like kinase 1 among Danish patients with hereditary haemorrhagic telangiectasia. *Clin Genet* 2004;**66**:556–61.
- 13 Lastella P, Sabba C, Lenato GM, Resta N, Lattanzi W, Gallitelli M, Cirulli A, Guanti G. Endoglin gene mutations and polymorphisms in Italian patients with hereditary haemorrhagic telangiectasia. *Clin Genet* 2003;**63**:536–40.
- 14 Cymerman U, Vera S, Karabegovic A, Abdalla S, Letarte M. Characterization of 17 novel endoglin mutations associated with hereditary hemorrhagic telangiectasia. *Hum Mutat* 2003;**21**:482–92.
- 15 van Ommen GJ, Bakker E, den Dunnen JT. The human genome project and the future of diagnostics, treatment, and prevention. *Lancet* 1999;**354**(Suppl 1):S15–10.
- 16 Cole SG, Begbie ME, Wallace GM, Shovlin CL. A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5. *J Med Genet* 2005;**42**:577–82.
- 17 Howe JR, Roth S, Ringold JC, Summers RW, Jarvinen HJ, Sistonen P, Tomlinson IP, Houlston RS, Bevan S, Mitros FA, Stone EM, Aaltonen LA. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* 1998;**280**:1086–8.
- 18 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 morphogenetic protein receptor 1A in juvenile polyposis. *Nat Genet* 2001;**28**:184–7.
- 19 Jass JR, Williams CB, Bussey HJ, Morson BC. Juvenile polyposis—a precancerous condition. *Histopathology* 1988;**13**:619–30.
- 20 Gallione CJ, Repetto GM, Legius E, Rustgi AK, Schelley SL, Tejpar S, Mitchell G, Drouin E, Westermann CJ, Marchuk DA. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet* 2004;**363**:852–9.
- 21 Shovlin CL, Guttacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, Kjeldsen AD, Plauchu H. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). *Am J Med Genet* 2000;**91**:66–7.
- 22 Stemper TJ, Kent TH, Summers RW. Juvenile polyposis and gastrointestinal carcinoma. A study of a kindred. *Ann Intern Med* 1975;**83**:639–46.
- 23 Houlston R, Bevan S, Williams A, Young J, Dunlop M, Rozen P, Eng C, Markie D, Woodford-Richens K, Rodriguez-Bigas MA, Leggett B, Neale K, Phillips R, Sheridan E, Hodgson S, Iwama T, Eccles D, Bodmer W, Tomlinson I. Mutations in DPC4 (SMAD4) cause juvenile polyposis syndrome, but only account for a minority of cases. *Hum Mol Genet* 1998;**7**:1907–12.
- 24 Woodford-Richens K, Bevan S, Churchman M, Dowling B, Jones D, Norbury CG, Hodgson SV, Desai D, Neale K, Phillips RK, Young J, Leggett B, Dunlop M, Rozen P, Eng C, Markie D, Rodriguez-Bigas MA, Sheridan E, Iwama T, Eccles D, Smith GT, Kim JC, Kim KM, Sampson JR, Evans G, Tejpar S, Bodmer WF, Tomlinson IP, Houlston RS. Analysis of genetic and phenotypic heterogeneity in juvenile polyposis. *Gut* 2000;**46**:656–60.
- 25 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**:484–91.
- 26 Berg J, Porteous M, Reinhardt D, Gallione C, Holloway S, Umasunthar T, Lux A, McKinnon W, Marchuk D, Guttacher A. 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.
- 27 Letteboer TG, Mager HJ, Snijder RJ, Koeleman BP, Lindhout D, Ploos van Amstel HK, Westermann KJ. Genotype - phenotype relationship in Hereditary Hemorrhagic Telangiectasia. *J Med Genet* 2006;**43**:371–7.
- 28 Kjeldsen AD, Moller TR, Brusgaard K, Vase P, Andersen PE. Clinical symptoms according to genotype amongst patients with hereditary haemorrhagic telangiectasia. *J Intern Med* 2005;**258**:349–55.
- 29 Friedl W, Uhlhaas S, Schulmann K, Stolte M, Loff S, Back W, Mangold E, Stern M, Knaebel HP, Sutter C, Weber RG, Pistorius S, Burger B, Propping P. Juvenile polyposis: massive gastric polyposis is more common in MADH4 mutation carriers than in BMPR1A mutation carriers. *Hum Genet* 2002;**111**:108–11.
- 30 Sayed MG, Ahmed AF, Ringold JR, Anderson ME, Bair 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**:901–6.
- 31 Merg A, Howe JR. Genetic conditions associated with intestinal juvenile polyps. *Am J Med Genet C Semin Med Genet* 2004;**129**:44–55.
- 32 Gazelle GS, McMahon PM, Scholz FJ. Screening for colorectal cancer. *Radiology* 2000;**215**:327–35.
- 33 Sweet K, Willis J, Zhou XP, Gallione C, Sawada T, Alhopuro P, Khoo SK, Patocs A, Martin C, Bridgeman S, Heinz J, Pilarski R, Lehtonen R, Prior TW, Frebourg T, Teh BT, Marchuk DA, Aaltonen LA, Eng C. Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. *JAMA* 2005;**294**:2465–2473.
- 34 Harrison RE, Flanagan JA, Sankelo M, Abdalla SA, Rowell J, Machado RD, Elliott CG, Robbins IM, Olschewski H, McLaughlin V, Gruenig E, Kermeeen F, Halme M, Raisanen-Sokolowski A, Laitinen T, Morrell NW, Trembath RC. Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. *J Med Genet* 2003;**40**:865–71.
- 35 Abdalla SA, Gallione CJ, Barst RJ, Horn EM, Knowles JA, Marchuk DA, Letarte M, Morse JH. Primary pulmonary hypertension in families with hereditary haemorrhagic telangiectasia. *Eur Respir J* 2004;**23**:373–7.
- 36 Harrison RE, Berger R, Haworth SG, Tulloh R, Mache CJ, Morrell NW, Aldred MA, Trembath RC. Transforming growth factor-beta receptor mutations and pulmonary arterial hypertension in childhood. *Circulation* 2005;**111**:435–41.

International Forum on Quality & Safety in Health Care

18–20 April 2007, Palau de Congressos, Barcelona.

Why attend?

- Join over 1000 healthcare professionals from over 40 countries worldwide
 - Learn from experienced leaders and improvement experts
 - Gain new skills and tools for creating change in your organisation
 - Take home practical solutions for improvement methods
- <http://www.quality.bmjpg.com>