Original research

Phenotypic characterisation of *SMAD4* variant carriers

Claire Caillot,[1] Jean-Christophe Saurin,[2,3] Valérie Hervieu,[4] Marie Faoucher,[5,6] Julie Reversat,[5,6] Evelyne Decullier,[3,6] Gilles Poncet,[6,7] Sabine Bailly,[8] Sophie Giraud,[5] Sophie Dupuis-Girod [1,8]

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[1]Service de Génétique et Centre de référence pour la maladie de Rendu-Osler, Femme-Mère-Enfants Hospital, Hospices Civils de Lyon, Bron, France
[2]Service de Gastroenterologie, Hôpital E. Herriot, Hospices Civils de Lyon, Lyon, France
[3]Pôle Santé Publique, Hospices Civils de Lyon, Lyon, France
[4]Institut de Pathologie Est, Hospices Civils de Lyon, Lyon, France
[5]Service de génétique, Centre de Biologie et Pathologie Est, Hospices Civils de Lyon, Lyon, France
[6]Université Claude Bernard Lyon 1, Villeurbanne, France
[7]Service de Chirurgie Digestive, Hôpital E. Herriot Lyon, Hospices Civils de Lyon, Lyon, France
[8]Biosanté Lab, Unit U1292, Health Department of IRIG, CEA de Grenoble, Grenoble, France

Correspondence to
Dr Sophie Dupuis-Girod, Service de Génétique et Centre de référence pour la maladie de Rendu-Osler, Femme-Mère-Enfants Hospital, Hospices Civils de Lyon, BRON, France; sophie.dupuis-girod@chu-lyon.fr

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ABSTRACT

Background Both hereditary haemorrhagic telangiectasia (HHT) and juvenile polyposis syndrome (JPS) are known to be caused by *SMAD4* pathogenic variants, with overlapping symptoms for both disorders in some patients. Additional connective tissue disorders have also been reported. Here, we describe carriers of *SMAD4* variants followed in an HHT reference centre to further delineate the phenotype.

Methods Observational study based on data collected from the Clinical Investigation for the Rendu-Osler Cohort database.

Results Thirty-three participants from 15 families, out of 1114 patients with HHT, had an *SMAD4* variant (3%). Regarding HHT, 26 out of 33 participants (88%) had a definite clinical diagnosis based on Curaçao criteria. Complication frequencies were as follows: epistaxis (n=27/33, 82%), cutaneous telangiectases (n=19/33, 58%), pulmonary arteriovenous malformations (n=17/32, 53%), hepatic arteriovenous malformations (AVMs) (n=7/18, 39%), digestive angiodysplasia (n=13/22, 59%). No cerebral AVMs were diagnosed. Regarding juvenile polyposis, 25 out of 31 participants (81%) met the criteria defined by Jass *et al* for juvenile polyposis syndrome. Seven patients (21%) had a prophylactic gastrectomy due to an extensive gastric polyposis incompatible with endoscopic follow-up, and four patients (13%) developed a digestive cancer. Regarding connective tissue disorders, 20 (61%) had at least one symptom, and 4 (15%) participants who underwent echocardiography had an aortic dilation.

Conclusion We describe a large cohort of *SMAD4* variant carriers in the context of HHT. Digestive complications are frequent, early and diffuse, justifying endoscopy every 2 years. The HHT phenotype, associating pulmonary and hepatic AVMs, warrants systematic screening. Connective tissue disorders broaden the phenotype associated with *SMAD4* gene variants and justify systematic cardiac ultrasound and skeletal complications screening.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Both hereditary haemorrhagic telangiectasia (HHT) and juvenile polyposis syndrome (JPS) are known to be caused by *SMAD4* pathogenic variants, with overlapping symptoms of both disorders in some patients.

WHAT THIS STUDY ADDS

⇒ This study highlights that symptoms associated with connective tissue disorders, including not only aortic dilation but also skeletal and skin symptoms, may be linked to SMAD4-HHT.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Thus, in addition to HHT and JPS monitoring, annual aortic ultrasound should be performed to screen for aortic dilatation and careful clinical examination should be performed to screen for and treat skeletal complications such as scoliosis.

INTRODUCTION

Hereditary haemorrhagic telangiectasia (HHT) (OMIM#187300, 600376, 175050) is an autosomal dominant vascular disease associating epistaxis, telangiectasia and arteriovenous malformations (AVMs) of the lungs, the gastrointestinal tract, the liver and less frequently, the brain. HHT affects 1 patient in 6000 and has a large phenotypical range. The main sign is spontaneous and induced nosebleeds which are often the most upsetting symptom for patients in their everyday life.[1]

Two genes were originally known to be associated with HHT: *ENG* coding for endoglin[2] and *ACRLV1* coding for the activin receptor-like kinase 1, *ALK1*.[3] A pathogenic variant in one of these two genes is identified in most cases. In 2004, Gallione *et al* described pathogenic variants in *SMAD4*, a gene coding for a cytoplasmic protein involved in the transforming growth factor-beta (TGFβ) signalling pathway. These variants are responsible for juvenile polyposis/HHT overlap syndrome,[4] which is present in approximately 2% of patients with HHT.

Juvenile polyposis syndrome (JPS) (OMIM#174900) is a rare autosomal dominant disease which affects 1 patient in 100 000[5] and causes hamartomatous polyps in the gastrointestinal tract. JPS also predisposes to gastrointestinal cancer[6] and is associated with upper gastrointestinal polyps.[5 7] It is caused by pathogenic variants in *BMPR1A* and *SMAD4*.[8] The prevalence of *SMAD4* variants in JPS is around 30%.[9–11]

SMAD4 heterozygous loss-of-function mutations have been described in isolated JP[5 12] and combined JP-HHT syndrome.[4 13] In addition to those main phenotypes, connective tissue abnormalities such has aortic root dilation, mitral valve abnormalities and 'Marfan-like' phenotype with skeletal and cutaneous abnormalities have been described.[14–19]

The main genes involved in connective tissue disorders such as *TGFβR1* and *TGFβR2*, as well as genes involved in HHT and JPS, are members

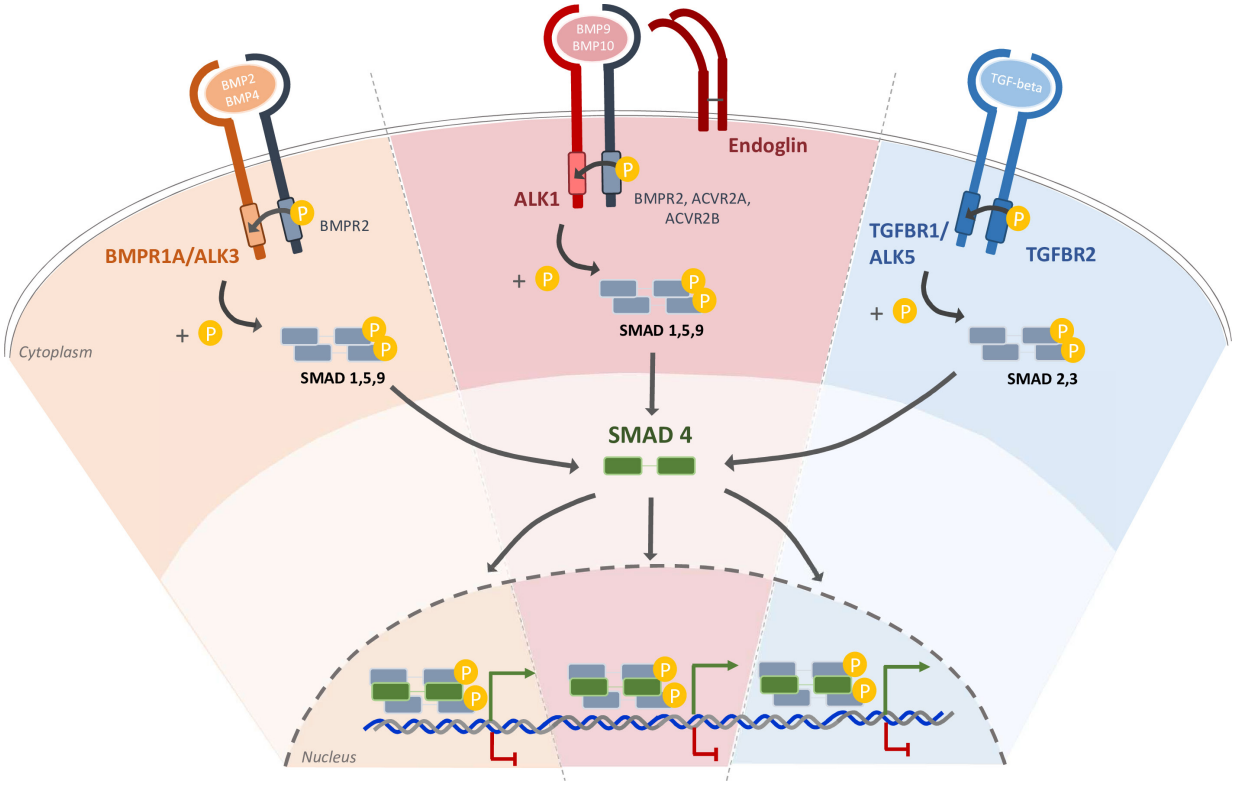

Figure 1 SMAD4 in the transforming growth factor-beta (TGFβ) signalling pathway. SMAD4 is a central element in SMAD-dependent TGFβ signalling. For each ligand, specific receptors and receptor-associated SMADs are activated. BMPR1A and SMAD4 have been identified in juvenile polyposis syndrome, ALK1/ACVRL1, ENG and SMAD4 in hereditary haemorrhagic telangiectasia, TGFBR1, TGFBR2, SMAD2 and SMAD3 in connective tissue disorders (Loeys-Dietz syndrome).

of the TGFβ superfamily signalling pathway. The superfamily ligands in the TGFβ family (TGFβs, BMPs, GDFs) bind to heteromeric complex of type I and type II transmembrane serine/threonine kinase receptors.[20–22] On ligand binding, the type II receptor phosphorylates and activates the type I receptor. The activated type I receptor then propagates the signal by phosphorylating a family of transcription factors, known as SMADs (SMAD1, 2, 3, 5, 9). These receptor-associated SMADs bind to SMAD4 and translocate to the nucleus, where they modulate transcription in association with other transcription factors. ALK1, encoded by *ACVRL1*, BMPR1A and TGFBR1 are type I receptors, TGFBR2, a type II receptor and ENDOGLIN, a co-receptor facilitating ligand binding. Depending on the ligand, specific receptors and receptor-associated SMADs are activated, but SMAD4 is at the intersection of all the pathways used in SMAD-dependent TGFβ signalling (figure 1).

The aim of this study was to present a detailed clinical description of all patients with HHT with an *SMAD4* variant in a French HHT reference centre, to provide a thorough description of the *SMAD4*-associated phenotype.

MATERIALS AND METHODS

All patients with known variants followed at the French Reference Centre for HHT disease in Lyon, France, were identified (online supplemental figure 1). Only patients with *SMAD4* pathogenic variants were included in this study. Data regarding HHT phenotype were also extracted for patients with *ENG* and *ACVRL1* variants.

Clinical diagnosis

HHT was defined using the Curaçao criteria[23] as follows: (1) spontaneous recurrent epistaxis, (2) multiple telangiectases at characteristic sites (lips, oral cavity, fingers, nose), (3) family history (a first-degree relative with HHT) and (4) visceral lesions (gastrointestinal telangiectases with or without bleeding, pulmonary, hepatic, cerebral or spinal AVMs). The diagnosis was certain if at least three criteria were present, possible or suspected if two criteria were present and unlikely if fewer than two criteria were present.

Pulmonary AVMs (PAVMs) were investigated using CT scans and/or transthoracic contrast echocardiography. Liver involvement was assessed with Doppler sonography and/or CT scans. Cerebral AVMs were screened for with cerebral MRI (screening was discussed with asymptomatic patients but not systematically performed). Digestive telangiectases and polyps were assessed with gastric and colonoscopic endoscopy (recommended to all patients with *SMAD4* variants starting from early adolescence). Videocapsule was performed in case of digestive bleeding.[24]

The diagnosis of juvenile polyposis was based on clinical criteria by Jass *et al*[25] (at least one criterion from: >5 colorectal juvenile polyps, juvenile polyps throughout the gastrointestinal tract or any number of juvenile polyps and a positive family history). However, as expert opinion regarding the diagnostic criteria for JPS has evolved, we described all digestive lesions.

Database

A database called Clinical Investigation for the Rendu-Osler Cohort, accessible online since 2007, was created by the French

HHT reference centre to collect clinical and biological data.[26] Clinical data were extracted from this database.

Molecular analysis

Genetic analyses were performed on DNA extracted from peripheral blood lymphocytes. DNA samples analysed before 2016 were processed as follows: *ALK1* and *ENG* genes were first analysed using denaturating HPLC (dHPLC) screening for a single nucleotide variant followed by direct sequencing and quantitative multiplex PCR of fluorescent short fragments to detect large rearrangements. For patients with no variants in *ALK1* and *ENG*, dHPLC screening followed by direct sequencing of *SMAD4* was performed. Multiplex ligation-dependent probe amplification (MLPA) (kit P158C1 MRC Holland) was performed for patients with no variants identified in *SMAD4*. DNA samples of new patients analysed after 2016 underwent high-throughput panel sequencing, exploring *ALK1*, *ENG* and *SMAD4*. MLPA for those three genes was used for patients with no identified variant. Sanger sequencing was performed directly for patients with an identified family variant. We verified that new variants had not previously been described on Alamut (available online at http://www.interactive-biosoftware.com/alamut.html). All variants were named according to the international standard reference nomenclature (Human Genome Variation Society, http://www.hgvs.org/mutnomen/). The NM_005359.6 sequence was used as the mRNA reference for the *SMAD4* gene. Variant nomenclature was validated using Variant Validator.[27]

Chromosomal microarray analysis

QIAamp DNA Blood Midikit (Qiagen, Courtaboeuf, France) was used for DNA extraction. Oligonucleotide aCGH was performed with a 180 000-oligonucleotide microarray (SurePrint G3 Human CGH Microarray Kit, 4×180K, AMADID: 022060, Agilent Technologies, Santa Clara, California, USA). The median probe spacing of this microarray is about 13 kb. The aCGH procedures were performed in accordance with the manufacturer's instructions. A CNV was validated if an abnormal log 2 ratio was obtained for at least three contiguous probes. The aCGH results were analysed using the UCSC hg19 assembly (https://genome-euro.ucsc.edu/). Deletion was named after the international standard reference nomenclature for copy number variation.[28]

Statistics

Descriptive data were reported as median (minimum (min)–maximum (max)) for the quantitative variables and number of patients, and percentages for the qualitative variables. Qualitative variables were compared using a χ^2 test and quantitative variables were compared using Kuskal-Wallis test.

For significant comparisons, we performed post hoc tests using pairwise comparisons with Bonferroni correction ($\alpha=0.05/2$) for qualitative variables and Dwass-Steel-Critchlow-Fligner multiple comparison analysis (based on pairwise comparisons) for quantitatives.

All statistical analyses were performed using SAS V.9.3 (SAS Institute, Cary, North Carolina, USA). Statistical significance was defined as $p<0.05$.

RESULTS

Of the 1114 patients with HHT with known mutations and followed at the HHT Reference centre, 666 had the *ACVRL1* variant (60%), 415 had the *ENG* variant (37%) and 33 had an *SMAD4* mutation (3%) (table 1). The variants identified are described in figure 2 and online supplemental file 1. Patients with *SMAD4* variants were from 15 different families. Patient characteristics are summarised in table 1. The mean follow-up duration was 8 years (min=0, max=43).

Hereditary haemorrhagic telangiectasia phenotype

Of the 33 participants, 29 (88%) had a definite diagnosis of HHT according to the Curaçao criteria, and 4 (12%) had a possible HHT diagnosis.

Thirty-three patients (82%) had spontaneous epistaxis (table 1). Nineteen patients (58%) had skin telangiectases. Out of 32 patients screened for at least one type of visceral AVM or digestive telangiectasia, 17 (53%) had at least one.

Seventeen patients out of 32 screened had PAVMs (53%). Among them, seven had one lesion, eight had more than one lesion and two had diffuse lesions. Clinical symptoms or complications of these PAVMs were present in 12 cases (digital clubbing

Table 1 General characteristics and HHT phenotype in *SMAD4*, along with *ACVRL1* and *ENG* patients

Patients	*SMAD4* N=33		*ACVRL1* N=666		*ENG* N=415		P value
General characteristics							
Median age at first consultation—years (minimum–maximum)	29 (9–66)		47 (0–87)		36 (0–87)		<0.0001(•)
Sex (F–M)							
Number of females (%)	14 (42)		393 (59)		227 (55)		0.09
HHT phenotype	**Screened**	**Presence N (%)**	**Screened N**	**Presence N (%)**	**Screened N**	**Presence N (%)**	
Skin and oral telangiectases	33	19 (58)	656	560 (85)	412	321 (78)	<0.001
Epistaxis	33	27 (82)	666	604 (91)	415	368 (89)	0.18
PAVMs	32	17 (53)	558	104 (19)	385	248 (64)	<0.001
HAVMs	18	7 (39)	378	251 (66)	211	47 (22)	<0.001
CAVMs	17	0	233	17 (7)	190	48 (25)	<0.001
Digestive telangiectases	22	13 (59)	148	41 (49)	83	41 (49)	0.77

P values: χ^2 test for qualitative variables and (•) Kruskal-wallis for quantitatives variables.
CAVMs, cerebral arteriovenous malformations; HAVMs, hepatic arteriovenous malformations; HHT, hereditary haemorrhagic telangiectasia; PAVMs, pulmonary arteriovenous malformations.

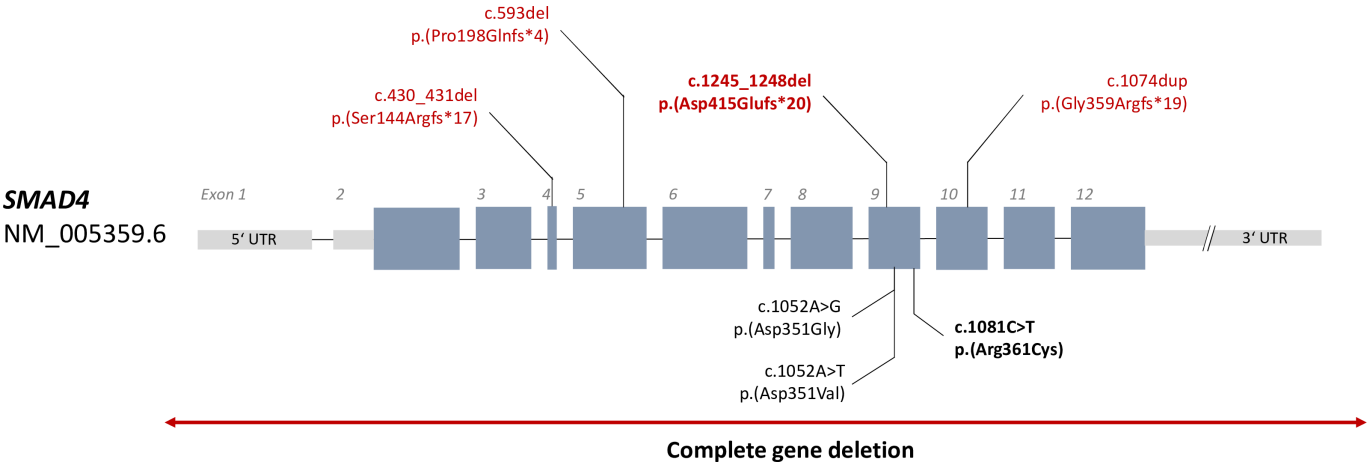

Figure 2 Identified heterozygous *SMAD4* variants. Variants in red are frameshift variants. Variants in black are missense variants. Variants in bold have been found in multiple families. The c.1245_1248del variant was present in 19 patients from 5 different families. c.1081C>T was found in three patients from three different families, with one proven de novo occurrence. Two gene deletions were identified, one by MLPA, the other with chromosomal microarray analysis. UTR, untranslated region.

(n=8), cyanosis (n=3), cerebral abscess (n=1), transient ischaemic attack (n=4), stroke (n=2)).

Eighteen patients were screened for hepatic AVMs (HAVMs), and seven had HHT liver involvement (39%) with vascular tortuosity or hepatic artery enlargement. Seven patients had focal nodular hyperplasia, two of whom had no associated HAVMs. Three had hepatic shunts with a high cardiac index (median=4.27, min=4.08, max=5.45 L/min/m^2), two of which were in the context of identified HAVMs.

Out of 17 patients who underwent cerebral MRI, none had cerebral AVMs. No patient presented with neurological symptoms related to cerebral or spinal AVMs.

Thirteen patients out of the 31 who underwent at least one duodenogastroscopy had digestive telangiectases of duodenal or jejunal localisations. No colonic telangiectases were reported. One participant had a history of severe digestive bleeding since early childhood. He had been supplemented with oral iron since early childhood, and with intravenous iron since early adolescence. Juvenile polyposis was then diagnosed and the video-capsule revealed duodenal and jejunal angiodysplasias which were treated endoscopically, making it possible to improve his anaemia.

The HHT clinical criteria for *ACVRL1* and *ENG* variant carriers are shown in table 1.

Post hoc tests revealed that patients with *SMAD4* mutations had significantly fewer telangiectases (p=0.008) and CAVMs (p=0.014) than patients with *ENG* mutations and had significantly fewer telangiectases (p<0.0001), more PAVMs (p<0.0001) and fewer HAVMs (p=0.017) than patients with *ACVRL1* mutations.

Juvenile polyposis syndrome phenotype

Twenty-five participants (81%) had a diagnosis of JP confirmed with the criteria defined by Jass *et al*, but all patients, except the two who did not undergo a digestive endoscopy, despite the recommendations, had digestive polyps (detailed in table 2).

The median age at first duodenogastroscopy and colonoscopy was 29 years (min=10, max=67) and 30 years (min=8, max=58), respectively. Two patients had no digestive explorations. Of the 31 patients who had digestive endoscopies, 23 (74%) had gastric polyps, 12 had small intestine polyps (39%) and 30 (97%) had lower intestinal tract polyps (table 2). All patients had digestive polyps of either the lower or the upper digestive tract.

Of the 23 patients with gastric polyps, 12 (39% of the patients explored) were reported to have extensive gastric polyposis, and the 11 others had fewer than 5 polyps. Patients with pathology results available were mainly reported to have juvenile or hamartomatous polyps (11 out of 14), and half of them hyperplastic or glandular cystic polyps. Seven patients had a prophylactic gastrectomy due to extensive gastric polyposis incompatible with endoscopic follow-up, with a specific surgical procedure allowing duodenal follow-up. The preventive gastrectomies were performed at a mean age of 50 years (41–55 years). One participant had a gastrectomy following a diagnosis of gastric adenocarcinoma. Of the patients with no upper gastrointestinal

Table 2 JPS phenotype in patients with *SMAD4*

Patient (n=33)	Screened (n)	Presence n (%)
Criteria defined by Jass *et al* for JPS	31	25 (81)
Stomach	31	
Presence of polyps		23 (74)
Confluent aspect of polyps		12 (41)
Gastrectomy		7 (21)
Small intestine	31	
Presence of polyps		12 (39)
Median age at first polyp (years—min–max)		47 (13–58)
Colon and rectum		30 (97)
Median age at first polyp (years—min–max)		31 (8–58)
Hemicolectomy/Colectomy		8 (29)
Prophylactic		4
Curative		4
Digestive cancer		4 (13)
Colonic adenocarcinoma		3 (10)
Gastric adenocarcinoma		1 (3)
Duodenal adenocarcinoma		1 (3)
Pancreatic adenocarcinoma		1 (3)

Stomach and small intestine were explored with duodenogastroscopy. Colon and rectum were explored with colonoscopy.
JPS, juvenile polyposis syndrome; max, maximum; min, minimum.

tract polyps, four had an abnormal gastric mucosa, with an endoscopic aspect of gastritis.

Regarding the small intestine polyps, 12 patients had duodenal polyps, and 1 patient also had jejunal polyps. Pathology data were available for six of them and described hamartomatous polyps in four cases.

Twenty-two patients (71%) had 10 or fewer polyps of the lower intestinal tract, 9 (29%) had >10 polyps. Pathology reports mentioned juvenile or hamartomatosis polyps in 15 of the 21 cases (71%) with available data. Adenomatous and hyperplastic polyps were found in 13 out of 21 cases (62%).

Eight patients underwent a hemicolectomy or colectomy (29%): four because of excessive polyp burden, three in a context of colonic adenocarcinoma and one patient had a total colectomy due to associated severe Crohn's disease.

Four patients had digestive cancers (13%) at a mean age of 36 years for the first tumour. One patient had multiple cancers: a colonic adenocarcinoma before the age of 40 years, and after the age of 60 years, a stomach adenocarcinoma (in the context of extensive gastric polyposis) and a pancreatic adenocarcinoma. The patient had previously been diagnosed with intraductal papillary mucinous tumours of the pancreas and treated with distal pancreatectomy. Two other patients had colonic adenocarcinoma, and one patient had a duodenal adenocarcinoma during late adolescence.

Connective tissue abnormalities

At least one connective tissue finding was present in 20 patients (61%), with isolated mitral insufficiency in 5 patients (15%).

Cardiovascular findings

Of the 26 patients with *SMAD4* variants who had a cardiac echography, 4 (15%) had an aortic root dilation at the sinuses of Valsalva measured at a median value of 42.3 mm (mean z-score=3.1, SD=1.5) (table 3). One of these patients also had a tubular dilation of 41 mm. No patients from this cohort presented an aortic dissection.

Mitral insufficiency was present in 10 out of 26 patients, with a dystrophic aspect noted in 4 cases, and prolapse in 1 case. The severity was grade I for eight patients, grade II for two patients.

Left atrial dilation was observed in five patients with no high cardiac output, but four of them had mitral insufficiency with a dystrophic aspect and bivalvular prolapse in one case. Three patients had patent foramen ovale.

Table 3 Cardiac, skeletal and cutaneous findings in patients with *SMAD4*

Patients	Screened (n)	Presence n (%)
Cardiovascular findings	26	12 (46)
Aortic root dilation*		4 (15)
Mitral insufficiency		10 (39)
Left atrial dilatation		5 (19)
Skeletal findings	33	7 (21)
Pectus carinatum/excavatum		5 (15)
Scoliosis		2 (6)
Dolichostenomelia		1 (3)
Early arthritis		2 (6)
Cutaneous findings	33	5 (15)
Inguinal or crural hernia		5 (15)
Skin striae		0

*Aortic root dilation was defined as a z-score >2 at the Valsalva sinus.

Other connective tissue findings

Regarding the skeletal phenotype (table 3), two patients had isolated pectus excavatum, two patients had pectus deformity and scoliosis and one patient had pectus excavatum along with dolichostenomely, pes planus and early osteoarthritis. One patient had early osteoarthritis at multiple sites.

Regarding the skin, repeated episodes of inguinal hernias were noted for three patients, and a single episode of inguinal or crural hernia for four patients. No skin striae were noted.

Regarding the ocular phenotype, one patient had myopia >3 D. No ectopia lentis was observed.

SMAD4 heterozygous variants

The variants identified are described in figure 2 and supplemental table. Four different heterozygous microdeletions and microduplications resulting in frameshifts were identified, one of which was found in five independent families. They were all classified as pathogenic or most likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) classification criteria.[29]

Three heterozygous missense variants were identified, one of which was present in three independent families. Two of the missense variants affected the same nucleotide. The variants were classified as pathogenic or most likely pathogenic according to the ACMG classification criteria.[29]

Two complete heterozygous *SMAD4* gene deletions were found, one with MLPA, the other by performing chromosomal microarray analysis. Both events were classified as pathogenic.

Twenty-four patients were from seven different families with at least two cases with positive genetic testing. Three patients had proven de novo variants. Data were not available for six patients.

DISCUSSION

This study describes the detailed phenotype of a French cohort of 33 patients with JP-HHT syndrome related to *SMAD4* variants, reporting their vascular and digestive follow-up, and confirming data reported in other studies regarding the connective tissue abnormalities.[14–17]

Regarding the HHT phenotype, a high proportion of patients (88%) in our study had a definite clinical diagnosis. Three out of four patients without a definite diagnosis are adolescents, which is in line with the age-dependent penetrance of HHT clinical symptoms.[30 31] Pulmonary AVMs were frequent in the cohort, with 53% of patients with at least one lesion. HAVMs were present in 39% of patients, and severe in two cases, with high cardiac output. These proportions coincide with the other published *SMAD4* cohorts.[4 11 16 18 32–34] Digestive telangiectases, found in 29% of patients, were not systematically reported in the published cohorts but have been reported in some patients. No cerebral AMVs were diagnosed in this study, but they have already been reported at a low level in other *SMAD4* cohorts.[11 16 33 34] Seventy-five per cent of patients with at least one explored organ had a visceral AVM. Overall, these data are in line with published data on *SMAD4* variant carriers and underlines the frequency of visceral AVMs in patients with *SMAD4*, as well as the need for systematic screening.

Regarding the juvenile polyposis phenotype, pathology results were not systematically available for all the endoscopic follow-ups and may have led to underestimating the number of patients with confirmed JP diagnosis with the criteria defined by Jass *et al*. Other types of polyps than hamartomatous/juvenile polyps were frequently found, as reported in other studies.[8 11 18 33] Gastric

polyps were frequent (74%), and extensive polyposis was present in 39%, in line with published data describing the higher prevalence of gastric lesions and extensive polyposis in *SMAD4* JPS compared with *BMPR1A* JPS.[5 35–37] In addition, small intestine polyps are not described as being frequent in patients with JPS[8] in the literature, and seem to be found only in *SMAD4* variant carriers, mainly, if not exclusively, in the duodenal location.[38] Thirty-nine per cent of our cohort had lesions localised in the duodenal location, and only one patient also had jejunal polyps (with no histological data). One of these lesions was an in situ duodenal carcinoma. These data confirm that *SMAD4* variant carriers tend to develop small intestine polyps preferentially in the duodenal location, and that they are susceptible to degenerate into cancer. Thirteen per cent of our cohort was diagnosed with cancer, which is less than in the literature,[6 8 11] and can be partly explained by the young age of our cohort (median age 37 years), the regular digestive endoscopic follow-up and a bias of recruitment with elder family members deceased from digestive cancer and not included in the study. These data emphasise the importance of digestive follow-up as recommended for cancer prevention in JPS,[24] with a specific focus on gastroduodenal endoscopies in patients with *SMAD4*.

Regarding the connective tissue disorder, at least one finding was made in 61% of patients, and even if the patients with isolated mitral insufficiency (18%) were not taken into account, it was still higher than the 20% previously reported.[16] Vascular findings, such as aortic dilation in 15% of patients, and mitral insufficiency in 39%, were noted, and reinforces the association already reported with *SMAD4*,[8 14–16] although frequencies are variable, probably due to the small numbers. Left atrial dilation was present in five patients, with no high cardiac output, and has not been reported in patients with *SMAD4* to date. Skeletal findings (joint hyperlaxity, pectus deformities, scoliosis, dolichostenomelia) and cutaneous findings (inguinal hernia, skin hyperlaxity) have been published in few patients, and are sometimes described as a 'Marfan-like' phenotype,[15 16] and our study reinforces this association. A recent study estimated the prevalence of pectus excavatum at 0.4% in a large adult population.[39] The prevalence of 15% is clearly higher. Furthermore, as this study is based on retrospective data collection, and patients were not initially screened for skeletal and cutaneous findings, their frequency is probably underestimated. Complete prospective data collection for global connective tissue disorders should be done to clarify their frequency, with systematic cardiac ultrasound screening for aortic dilation, as already recommended.

At the molecular level, SMAD4 is a central component of the TGFβ pathways involved in HHT, JPS and connective tissue disorders such as Marfan syndrome, and gives a plausible biological explanation for the association of the symptoms. As there are multiple frameshift variants, it seems that the loss of function of SMAD4 leads to the phenotype, as was previously thought for the mechanism.[32] Very similar missense variants to those identified here on codon 351 and 361 have been described previously in ovarian and colonic cancers (p.(Asp351His) and p.(Arg361Cys)) and it has been demonstrated that these variants disrupt hetero-oligomerisation of SMAD4 with SMAD2 and thus block signal transduction.[40] Gain-of-function variants in the SMAD4 gene lead to a completely different pathology known as Myhre syndrome, a neurodevelopmental disorder[41] that does not share any common phenotypic elements with HHT or JPS. Interestingly, patients present with contrasting connective tissue abnormalities such as aortic stenosis, bone size and morphology abnormalities, joint stiffness and thickened skin.[42]

Regarding the global phenotype of patients with *SMAD4*, most patients have a definite clinical diagnosis for both pathologies according to the criteria used, showing a high penetrance for both diseases. Recruiting patients in an HHT reference centre might have introduced a bias regarding the frequency of HHT symptoms, although other studies tend to show that patients with *SMAD4* express signs for both.[16 33] We thus reinforce the association with connective tissue abnormalities previously mentioned in the literature as part of the phenotype.[14–16] Screening for AVMs, digestive polyps, aortic dilation and skeletal complications should be recommended to all *SMAD4* pathogenic variant carriers.

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Contributors Conceptualisation: SD-G, CC, J-CS, VH, GP, SB. Data curation: CC, SD-G, MF, JR, ED. Investigation: SG. Writing—original draft: CC, SD-G. Writing—review and editing: SD-G, CC, J-CS, VH, GP, SB, SG. SDG is responsible for the overall content as the guarantor, accepts full responsibility for the work and the conduct of the study.

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ORCID iD

Sophie Dupuis-Girod http://orcid.org/0000-0002-8834-5526

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