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Novel pathogenic SMAD2 variants in five families with arterial aneurysm and dissection: further delineation of the phenotype

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ABSTRACT

Background: Missense variants in *SMAD2*, encoding a key transcriptional regulator of TGF- β signaling, were recently reported to cause arterial aneurysmal disease.

Objective: The study aim was to identify the genetic disease cause in families with aortic/arterial aneurysmal disease and to further define *SMAD2* genotype-phenotype correlations.

Methods and Results: Using gene panel sequencing, we identified a *SMAD2* nonsense variant and four *SMAD2* missense variants, all affecting highly conserved amino acids in the MH2 domain. The premature stop codon (c.612dup; p.(Asn205*)) was identified in a marfanoid patient with aortic root dilatation and in his affected father. A p.(Asn318Lys) missense variant was found in a Marfan syndrome (MFS)-like case who presented with aortic root aneurysm and in her affected daughter with marfanoid features and mild aortic dilatation. In a man clinically diagnosed with Loeys-Dietz syndrome (LDS) that presents with aortic root dilatation and marked tortuosity of the neck vessels, another missense variant, p.(Ser397Tyr), was identified. This variant was also found in his affected daughter with hypertelorism and arterial tortuosity as well as affected in his mother. The third missense variant, p.(Asn361Thr), was discovered in a male presenting with coronary artery dissection. Variant genotyping in three unaffected family members confirmed its absence. The last missense variant, p.(Ser467Leu), was identified in a male with significant cardiovascular and connective tissue involvement.

Conclusion: Taken together, our data suggest that heterozygous loss-of-function *SMAD2* variants can cause a wide spectrum of autosomal dominant aortic and arterial aneurysmal disease, combined with connective tissue findings reminiscent of MFS and LDS.

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102 INTRODUCTION

103 Aortic and arterial aneurysmal disease is a prominent cause of morbidity and mortality in the
104 Western population ¹. Especially, in thoracic aortic aneurysm and dissection (TAAD), genetic
105 factors play an important role ². TAAD most commonly segregates in an autosomal dominant
106 mode and either presents as an isolated condition (non-syndromic TAAD) or as part of a syndrome
107 (syndromic TAAD). Non-syndromic forms are typically caused by variants in genes encoding

proteins involved in vascular smooth muscle cell contractility (e.g. *MYH11*, *ACTA2*)³⁻⁷, whereas the causal genes for syndromic TAA such as Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS) are linked to extracellular matrix integrity and/or transforming growth factor beta (TGF- β) signaling (e.g. *FBNI*, *LOX*, *MFAP5*, *TGFB2/3*, *SMAD2/3*, *TGFBR1/2*)^{2 8-12}. Syndromic TAAD cases present with a pleiotropy of cardiovascular (e.g. aortic aneurysm and dissection), ocular (e.g. ectopia lentis), cutaneous (e.g. thin and translucent skin, skin hyperextensibility, inguinal/umbilical hernia) and skeletal manifestations (e.g. joint hypermobility or contractures, scoliosis, clubfoot)^{11 13}. In 1991, Dietz et al. pinpointed the *FBNI* gene, encoding the microfibrillar protein fibrillin-1, as the causal gene for MFS. Subsequent studies in MFS mice revealed increased TGF- β signaling as a key pathomechanistic event¹⁴. More evidence for involvement of TGF- β in the pathogenesis of syndromic TAAD was obtained through the identification of LDS-causing variants in other genes coding for key components of the canonical TGF- β pathway, i.e. *TGFBR1*, *TGFBR2*, *TGFB2*, *TGFB3* and *SMAD3*¹³⁻¹⁸. Loeys-Dietz syndrome is typically characterized by the triad of hypertelorism, bifid uvula or cleft palate and aortic aneurysm with widespread arterial aneurysms and tortuosity¹³. Although initially the more severe syndromic LDS patients were identified, there is now a broad phenotypical spectrum with variable to no systemic involvement of other organ systems. Although pathogenic *SMAD3* variants were initially associated with aortic aneurysm osteo-arthritis syndrome (AOS,¹⁸), it is now well accepted that the *SMAD3* phenotype fits within the LDS spectrum as many patients do not have osteo-arthritis but do have other LDS clinical characteristics.

In 2015, heterozygous missense variants in the *SMAD2* gene, encoding a key intracellular downstream effector of the TGF- β pathway, were first described to cause arterial aneurysms and

dissections with or without connective tissue features¹⁹. Zhang et al. more recently also reported on a novel *SMAD2* missense variant in a Chinese family with early-onset aortic aneurysms²⁰. All (likely) pathogenic variants published to date are located in the MH2 domain, a region that is extremely well-conserved among species and other human SMAD proteins (**Figure 1**)^{19 20}. The MH2 domain mediates the recognition of SMAD proteins by type I receptors as well as the interaction with cytoplasmic retention proteins and several transcription factors²¹. Moreover, it

Table 1. Overview of *in silico* evidence of the five identified (likely) pathogenic *SMAD2* variants

	<i>gnomAD</i>	<i>1000 Genomes Project</i>	<i>dbSNP 147</i>	<i>SIFT</i>	<i>PolyPhen2</i>	<i>MutationTaster</i>
<i>p.(Asn318Lys)</i>	absent	absent	absent	Damaging	Probably damaging	Disease causing
<i>p.(Asn361Thr)</i>	absent	absent	absent	Damaging	Probably damaging	Disease causing
<i>p.(Ser397Tyr)</i>	absent	absent	absent	Damaging	Probably damaging	Disease causing
<i>p.(Ser467Leu)</i>	absent	absent	absent	Damaging	Probably damaging	Disease causing
<i>p.(Asn205*)</i>	absent	absent	absent	/	/	Disease causing

regulates SMAD2/SMAD3/SMAD4 oligomerization²¹.

METHODS

All subjects and their family members provided written informed consent for participation in this project. Next-generation TAAD gene panel (Supplementary Table S1) sequencing was performed on DNA of probands according to previously described methods^{22 23}. Analysis of the raw data was performed using Seqpilot (JSI) or a Galaxy pipeline, followed by variant calling with the Genome Analysis Toolkit (GATK) and variant annotation using an in-house developed tool, VariantDB²⁴. Identified (likely) pathogenic variants were genotyped in both affected and unaffected available family members using Sanger sequencing.

RESULTS

We report on the identification of five novel *SMAD2* (likely) pathogenic variants (Supplementary Table S2), i.e. the first nonsense variant and four missense variants affecting the functionally important MH2 domain (Table 1). Our phenotypic data indicate that *SMAD2* (likely) pathogenic variants can cause a wide spectrum of aortic and arterial aneurysmal disease, either in the presence or absence of connective tissue findings reminiscent of MFS and LDS.

In family 1, the diagnosis of MFS was initially suspected in the proband (III-1) (Figure 2A) at age 20 during a hospitalization for psychosis, because of aortic root dilatation (42mm) and marfanoid habitus with tall stature and large hands (Table 2). The diagnosis of MFS could not be confirmed after thorough clinical examination, owing to the absence of ectopia lentis, insufficient systemic features and negative *FBNI* mutation testing. Over the past ten years, the patient's aortic root size has gradually increased to 49 mm (Z-score = 5,1).

The proband's father (II-1; 70 years old) was also diagnosed with dilatation of the aortic root (47 mm; Z-score = 3.2). Furthermore, he presented with mild dysmorphic features, retinal detachment, tall stature (195 cm) and large hands. His father and mother (I-1 & I-2) died at old age, respectively 90 and 88 years, and no cardiovascular anomalies were reported in other family members. The sister of the proband (III-2) had normal echocardiographic evaluation at age 29 years.

A *SMAD2* nucleotide duplication (c.612dup) leading to the introduction of a premature stopcodon p.(Asn205*) was found in the affected father (II-1) of the index patient (Figure 1, Figure 2A, Table 1). Genotype analysis of the proband (III-1) confirmed the presence of the father's *SMAD2* pathogenic variant. Since the nonsense variant is located before the penultimate exon of *SMAD2*, the transcript is predicted to undergo nonsense-mediated mRNA decay.

The proband (II-2) of family 2 is a 65-year old female (**Figure 2B, Table 2**). She was diagnosed with MFS at age 32 years based on the presence of aortic root dilatation and skeletal features such as a tall stature (182 cm, with armspan 185 cm), arachnodactyly (**Figure 3A**), pectus excavatum and scoliosis, with the latter two requiring surgery at ages 11 and 36, respectively. Marked facial features were also apparent, including dolichocephaly, downslanting palpebral fissures, a high and narrow palate and a broad uvula (**Figure 3B, C**). Ectopia lentis was absent. Later in life, severe cardiovascular complications developed; at age 53, her aortic root and valve were replaced by a composite aortic graft. At the latest check-up, she presented with an enlarged left atrium, mild tricuspid valve/pulmonary valve insufficiency and mild dilatation of the aorta ascendens above the prosthesis (34 mm). Other health issues include poly-articular arthritis, a diaphragmatic hernia and gallbladder stones. Currently, she is under long-acting metoprolol (200 mg daily), losartan (50 mg daily) and acenocoumarol treatment.

The proband's now 37-year old daughter (III-1) was diagnosed with MFS at the age of three because of a marfanoid habitus and scoliosis. She underwent an inguinal hernia repair during childhood and has a history of pyelo-uretero junctional stenosis with hydronephrosis and hemicolectomy due to malrotation. Recent echocardiography revealed mild dilatation of the aortic root (42 mm) and aortic valve insufficiency. Arthritis of the hands and migraine have also been reported. She was initially put on propranolol treatment but, due to beta-blockade-related fatigue, converted to 50 mg losartan daily.

TAAD gene panel sequencing in the proband identified a novel *SMAD2* missense variant, p.(Asn318Lys) (c.954T>A), located in the MH2 domain (**Figure 1, Table 1**). The likely pathogenic variant was also found in the affected daughter (III-1, **Figure 2B**).

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195 The 44-year old proband of the third family (III-2, **Figure 2C, Table 2**) was born at 35 weeks of

196 gestation to non-consanguineous parents. A LDS diagnosis was made based on the appearance of

197 characteristic facial (hypertelorism, blue sclerae, high arched palate, retrognathia) (**Figure 3D, E**),

198 mild skeletal (reduced upper to lower segment ratio = 0.79, joint hypermobility), skin (striae and

199 easy bruising) and suggestive cardiovascular features: borderline aortic root measurement (39 mm,

200 Z-score 1.3 for 178 cm and 102 kg) with torn chordae tendinae and marked tortuosity and enlarged

201 caliber of the neck vessels, including vertebral, basilar and internal carotid artery, as well as mild

202 tortuosity of the proximal middle cerebral artery and anterior communicating arteries. The latter

203 were only noted after the proband experienced several episodes with slurred speech and

204 dizziness. Medical history is significant for migraine and left-sided hearing loss.

205 The proband's eldest daughter (IV-2), age 9, has hypertelorism and tortuosity of the neck vessels

206 (**Figure 2C, Figure 3E, Table 2**). His youngest daughter (IV-3), age 6, has a bifid uvula and

207 displays joint hypermobility, but no cardiovascular anomalies. His mother (II-2), age 64, has a

208 round face with mild dolichocephaly and hypertelorism as well as marked skin anomalies, i.e. skin

209 translucency and hyperelasticity, varicose veins and easy bruising. She was also diagnosed with

210 diabetes, arthritis, hiatal hernia and diverticulosis. She has been taking beta-blockers for

211 hypertension since her 20s. At age 62 years, she had a perfectly normal aortic root (2.7cm) and

212 ascending aorta (2.7cm). MRA of the brain, neck, thoracic and abdominal aorta did not reveal any

213 tortuosity or aneurysm. A maternal aunt (II-4) also had normal aortic measurements but MRA of

214 the neck vessels demonstrated a carotid (2.2 mm) and a basilar (1.8 mm) artery aneurysm. A

215 maternal great uncle (I-3) died from a ruptured brain aneurysm at 42 years of age.

216 A novel *SMAD2* MH2-located missense variant (p.(Ser397Tyr), c.1190C>A) was found in the

proband of this family (**Figure 1, Figure 2C, Table 1**). Variant genotyping in gDNA samples of relatives confirmed its presence in the eldest daughter with hypertelorism and arterial tortuosity (IV-2) as well as in the affected mother (II-2) and the affected maternal aunt (II-4). The proband's unaffected sister (III-1), niece (IV-1) and youngest daughter (IV-3) were negative for the p.(Ser397Tyr) variant. No genomic DNA was available of the maternal great-uncle (I-3).

The index patient of family 4, a 53-year old male (III-2, **Figure 2D, table 2**), came to medical attention because of a spontaneous dissection of the left coronary artery for which percutaneous transluminal coronary angioplasty was conducted with extensive stenting from the left main coronary artery to the distal left anterior descending. Additional imaging studies revealed no aortic dilatation (aortic sinus 35 mm, ascending aorta 38 mm), but identified tortuosity of the common iliac arteries and circle of Willis. He had a history of arterial hypertension (treated with calcium channel antagonist and an ACE inhibitor) but no smoking or dyslipidemia. His medical history is significant for generalized arthralgia with tendinopathies, possible enteric associated arthritis, inguinal hernia, varicocoele, and stomach ulcers. Physical exam revealed normal stature (180 cm) but enlarged armspan (190 cm; ratio 1.055) and reduced upper to lower segment ratio (0.84), mild downslanting of the palpebral fissures, broad uvula, pes plani, pectus asymmetry and mild scoliosis (**Figure 3F, G, H**). No hypertelorism or joint hypermobility were noted.

His father (II-2) died at age 75 due to complications of Parkinson's disease and a medical history of acute myocardial infarction (**Figure 2D**). His mother (II-3) also suffered from angina pectoris but died from metastatic breast cancer at 85 years of age. His sister (III-1) is also known with medically treated hypertension. The two sons of the proband (IV-1 & IV-2) appear healthy at age 17 and 18.

In the MH2 domain of *SMAD2*, we identified a novel missense variant c.1082A>C (p.(Asn361Thr)) in the proband. This variant was absent in his unaffected sister (III-1) and sons (IV-1 & IV-2) (**Figure 1, Figure 2D, Table 1**). Genotyping in the proband's parents (II-2 & II-3) was impossible due to the unfortunate event of death before diagnosis of the proband.

The 64-year old proband of family 5 (**Figure 2E, Table 2**) was suspected to suffer from MFS. Because of a family history of sudden cardiac death, cardiovascular screening was performed and revealed prominent manifestations, including aortic root (52mm, Z-score=5.6 for 187.5 cm and 87.6 kg), ascending aorta (42 mm) and aortic arch (33 mm) dilatation, mitral valve prolapse and moderate mitral insufficiency. Medical treatment with losartan was initiated. Surgical treatment, however, was delayed because of the concomitant diagnosis of anal carcinoma. Other connective tissue anomalies include enlarged armspan (197.5 cm), pectus excavatum, scoliosis and easy bruising. Medical history reveals knee replacement for gonarthrosis at age 63, inguinal hernia surgery (52 years) and mild cataract. Ectopia lentis was not present.

Two brothers (II-1 & II-2) of the index patient died of a so-called “acute myocardial infarction” at ages 17 and 19, respectively (**Figure 2E, Table 2**) but no autopsy was performed. The three other brothers (II-3 & II-4 & II-6), now 68, 67 and 62 years old, are healthy. The proband's father (I-1) suffered from unspecified heart problems and died at the age of 65. The proband's mother (I-2) suffered from Parkinson's disease and died at age 79. One maternal nephew (son of sister of mother) had unexplained sudden cardiac death at age 13.

The novel *SMAD2* p.(Ser467Leu) (c.1400C>T) missense variant located in the MH2 domain was identified in the proband of family 5 (**Figure 1, Figure 2E, Table 1**). Unfortunately, the patient

262 lost contact with his family members and no DNA samples of relatives are available to check for
263 the presence or absence of the variant.

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Table 2. Clinical features of patients with *SMAD2* variants

	<i>p.Asn205*</i> Family 1 II-1 III-1		<i>p.Ala278Val</i> (*)	<i>p.Asn318Lys</i> Family 2 II-2 III-1		<i>p.Asn361Thr</i> Family 4 III-2	<i>p.Gln388Arg</i> (**) P3-1 P3-2		<i>p.Ser397Tyr</i> Family 3 III-2 IV-2 II-2 II-3				<i>p.Leu449Ser</i> (**) P1-1	<i>p.Gly457Arg</i> (**) P2-1	<i>p.Ser467Leu</i> Family 5 II-5	Total n = 15
Gender	M	M	M	F	F	M	F	F	M	F	F	F	F	F	M	M/F:6/9
Age at diagnosis (years)	70	30	51	65	30	53	54	59	44	9	64	51	47	17	64	
<i>Arterial anomalies</i>																
Thoracic aortic aneurysm	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	10
Abdominal aortic aneurysm	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	1
Aneurysm cerebral arteries	NA	NA	NA	-	-	-	-	-	+	-	-	+	+	NA	NA	3
Arterial tortuosity of cerebral arteries	NA	NA	NA	-	-	+	-	NA	+	+	-	-	-	NA	NA	3
Coronary artery dissection	NA	NA	NA	-	-	+	-	-	-	-	-	-	-	-	-	1
Iliac artery abnormalities	NA	NA	NA	-	-	+	-	-	-	-	-	-	-	-	-	1
Valve abnormalities (M, A, T, U)	NA	NA	NA	+ ^{AT}	+ ^A	-	+ ^M	-	-	-	-	NA	+ ^U	-	+ ^M	5
<i>Joint anomalies</i>																
Arthritis	NA	NA	NA	+	+	+	+	+	-	-	+	+	NA	+	NA	8
Painful joints	NA	NA	NA	-	-	+	+	+	-	-	+	+	+	+	NA	7
Disc degeneration	NA	NA	+	NA	NA	NA	+	+	NA	NA	NA	NA	NA	+	NA	4
<i>Skeletal anomalies</i>																
Pes planus	NA	NA	NA	-	-	+	-	-	-	NA	NA	-	+	+	-	3
Scoliosis	NA	NA	NA	+	+	+	-	-	-	-	-	-	-	+	+	5
Pectus deformity	NA	NA	NA	+	-	-	-	-	-	-	-	-	-	+	+	3
Long fingers	+	+	NA	+	+	-	-	-	-	-	-	-	+	+	-	6
Tall stature	+	+	NA	+	+	-	-	-	+	NA	-	-	-	+	-	6
<i>Skin anomalies</i>																
Striae	NA	NA	NA	NA	NA	NA	+	+	+	-	-	-	-	-	-	3
Hernia D/I/U	NA	NA	NA	+ ^D	+ ^I	+ ^I	+ ^A	-	-	-	+ ^D	-	-	+ ^I	+ ^I	7
Skin translucency	NA	NA	NA	NA	NA	NA	-	-	-	-	+	-	-	-	-	1
Skin hyperelasticity	NA	NA	NA	NA	NA	NA	NA	NA	-	-	+	-	NA	-	-	1
Easy bruising	NA	NA	NA	NA	NA	NA	-	+	+	-	-	+	-	-	+	5
<i>Craniofacial anomalies</i>																
Hypertelorism	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	4
Abnormal palate	-	-	+	+	-	-	+	+	+	-	-	-	-	+	-	6
Abnormal uvula	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	2
Downslanting palpebral fissures	-	-	-	+	-	+	NA	NA	-	-	-	-	NA	+	-	3
Dolichocephaly	-	-	+	+	-	-	-	-	-	-	+	-	-	+	-	4
<i>Other phenotypic anomalies</i>																
Transient ischaemic attacks	-	-	NA	NA	NA	NA	-	-	-	-	-	-	+	-	NA	1
Migraine	NA	NA	NA	-	+	NA	+	-	+	-	-	+	+	-	NA	5
Spontaneous pneumothorax	-	-	NA	-	-	NA	-	-	-	-	-	-	+	-	-	1
Varices	NA	NA	NA	-	-	+	-	+	-	-	+	+	-	-	-	4

265 Valve abnormalities: M = mitral valve; A = aortic valve; T = tricuspid valve; U = unspecified. Hernia: D = diaphragmatic; I = inguinalis; U = umbilicalis;
 266 A=abdominal wall. (*) (**) Represent (likely) pathogenic variants previously identified in *SMAD2* by Zhang et al (2017)²⁰ and Micha et al (2015)¹⁹, respectively

DISCUSSION

In 2015, (likely) **pathogenic variants** in *SMAD2* were described as a genetic cause for arterial aneurysm and dissection¹⁹. All three initially identified missense **variants** were located in the MH2 domain of *SMAD2* (**Figure 1A**). This C-terminal domain is highly conserved between *SMAD* proteins, and mediates *SMAD*-dependent transcription and *SMAD2*/*SMAD3*/*SMAD4* complex formation²⁵. Recently, Zhang and colleagues identified a fourth *SMAD2* **variant** in a Chinese family (p.(Ala278Val)), also located in the MH2 domain²⁰. Of note, two *de novo SMAD2* **variants** (one missense affecting the first amino acid of the MH2 domain (p.(Trp274Cys)) and one splice site **variant** (c.997+1 G>A) (predicted to lead to in-frame exon skipping)) have also been described in pediatric cases with complex congenital heart disease²⁶, including dextrocardia with complete atrio-ventricular canal defect, double outlet right ventricle, transposition of the great arteries and pulmonic stenosis. We here describe the first disease-associated *SMAD2* nonsense **variant**. Occurring before the penultimate exon, p.(Asn205*) is predicted to lead to nonsense-mediated mRNA decay, suggesting that disease is caused by a loss-of-function mechanism. The four additional, novel missense **variants** identified in this report all affect highly conserved amino acids in the MH2 domain (**Figure 1A**). The genetic architecture of *SMAD2*-related disease is reminiscent of what has been described for *SMAD3*. In *SMAD3* patients, more than 60% of the **pathogenic variants** are missense while just a small fraction is nonsense (7%) and the remainder are frameshift, splice site or partial or whole gene deletions²⁷. No mutational hotspot is observed for *SMAD3*, but *SMAD3* missense **variants** also tend to cluster in the functionally important MH1 and MH2 domains²⁷. Although still limited numbers are available, a similar mutational pattern is emerging for *SMAD2*, as hitherto, only **pathogenic SMAD2 missense variants** affecting the evolutionary conserved MH2 domain were identified in TAAD patients.

291 In total, 15 *SMAD2* (likely) pathogenic variants carriers, including 5 from the literature and 10 in
 292 the current report, have now been described (**Table 2**). In line with the findings of Micha and
 293 Zhang et al.^{19 20}, variant carriers present with aortic/arterial aneurysm and dissection in addition
 294 to connective tissue findings reminiscent of MFS and/or LDS. Nearly all described *SMAD2* (likely)
 295 pathogenic variants carriers in our series display thoracic aortic aneurysm (10/15; ascending aorta
 296 and/or aortic root), but the cerebral (3/10) or iliac arteries (1/12) can also be affected. In addition,
 297 one abdominal aortic aneurysm has been reported in a 51 years old patient (p.(Ala278Val) from
 298 Zhang et al.²⁰. Importantly, our study further expands the phenotypic spectrum of *SMAD2*-related
 299 disease. So far, no aortic dissections had been described in proven *SMAD2* variant carriers, but
 300 aortic dissection was reported in family histories: the maternal uncle of the proband of family 1
 301 died of abdominal dissection at age 50 years in the paper by Micha et al.¹⁹ and the father of the
 302 proband in the paper by Zhang et al.²⁰ died of thoracic aortic dissection at age 40. Also in our
 303 series, two brothers of the proband of family 5 died at young age, 17 and 19 years respectively.
 304 We describe in this report for the first time a coronary artery dissection (proband III-2 of family
 305 4). This observation confirms that dissections may occur in any segment of the arterial tree as
 306 carotid artery dissection was also previously reported (proband of family 1 in Micha et al.¹⁹).
 307 Arterial tortuosity, present in three patients of our cohort, was previously only reported once in
 308 *SMAD2* mutant patients (proband of family 1 by Micha et al.¹⁹). The index patient (III-2) of family
 309 3 (**Figure 3D-E**) and his affected daughter (IV-2) were diagnosed with tortuosity of the cerebral
 310 vessels, whereas proband III-2 of family 4 (**Figure 3F-H**) had tortuosity of the iliac arteries. These
 311 findings highlight the need for extended arterial imaging, as recommended in other subtypes of
 312 LDS linked to pathogenic variants in other genes coding for component of the TGF- β signaling
 313 (*TGFBRI/2*, *SMAD3*, *TGFB2/3*)²⁸ (**Table 3**).

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Table 3. Comparison of the clinical manifestations of patients with *TGFBRI/2*, *SMAD2/3*, *TGFB2/3* and *FBN1* mutations.

	<i>TGFBRI</i>	<i>TGFB2</i>	<i>SMAD2</i>	<i>SMAD3</i>	<i>TGFB2</i>	<i>TGFB3</i>	<i>FBN1</i>
Dissection at young age	+	+	-	+	+	+	-
Arterial tortuosity	+	+	+	+	+	+	-
Osteo-arthritis	+	+	+	+	+	+	-
Club foot	+	+	-	+	+	+	-
Scoliosis	+	+	+	+	+	+	+
Craniosynostosis	+	+	-	+	-	-	-
Cervical spine instability	+	+	-	+	-	+	-
Hernia	+	+	+	+	+	+	+
Dural ectasia	+	+	+	+	+	?	+
Hypertelorism	+	+	+	+	+	+	-
Bifid uvula / cleft palate	+	+	-	+	+	+	-
Exotropia	+	+	-	+	+	+	+
Retrognathia	+	+	-	+	+	+	+
Pneumothorax	+	+	+	+	+	-	+
Ectopia lentis	-	-	-	-	-	-	+

315 The presence of the clinical feature is indicated with a “+” sign, a “-“ sign indicates absence of the clinical features or
 316 population frequency. This table is adapted from Schepers et al. (2018) ²⁹.
 317
 318

319 Valve abnormalities, including prolapse and insufficiency of either the aortic, tricuspid or mitral
 320 valve, have been observed in 5 out of the 15 variant carriers. Recurrent skeletal anomalies include
 321 scoliosis (5/12), long slender fingers (6/14), tall stature (6/13), pectus deformity (3/12), and pes
 322 plani (3/10). Osteo-arthritis (8/10) and hernias (7/12; diaphragmatic, inguinal and umbilical) are
 323 quite common. Other phenotypic features that were frequent in these patients were hypertelorism
 324 (4/15), an abnormal palate or uvula (8/15), striae (3/9) and easy bruising (5/9). Other findings
 325 included varices (4/12) and migraine (5/10). Transient ischemic attack was reported in one patient
 326 ¹⁹.

Overall, the emerging clinical spectrum of *SMAD2* variant-positive patients seems similar to the one previously described for *SMAD3*. The cardiovascular phenotype in *SMAD2* patients is comparable to patients with *SMAD3* variants. Both groups tend to be vulnerable to widespread arterial aneurysms with tortuosity and aortic aneurysms²⁷. Although, dissection at young age is not yet observed in the *SMAD2* variant-positive cohort, significant family histories were reported. As such, regular cardiovascular examination is recommended, since TAA can still evolve at any time later in life.

Similarly, the observed skeletal features in the *SMAD2* cohort show overlap with those of *SMAD3* variant carriers. Osteo-arthritis, which is considered a hallmark for *SMAD3* mutant-positive patients, is also present in at least 50% of the currently known *SMAD2* patients. Additionally, hernias were also frequently observed (42%) in the *SMAD3* population.

Due to the similarity between the protein structure of *SMAD2* and *SMAD3*, Micha et al. (2015) suspected that the TGF- β signaling would be increased as seen in other TGF- β depending pathologies. Western blot analysis of patient dermal fibroblasts indeed showed an increase in phosphorylated *SMAD2* and *SMAD3* upon exogenous TGFB1 treatment and no difference in total *SMAD2* and *SMAD3* compared to controls. These findings confirm the suspected enhancement of the TGF- β signaling cascade¹⁹.

Taken together, *SMAD2* variant screening should be considered in patients with aortic/arterial aneurysm and dissection with or without connective tissue findings of MFS and LDS. Inclusion of *SMAD2* in existing TAAD gene panels for next-generation sequencing is strongly recommended.

Conclusion

We report on five novel *SMAD2* (likely) pathogenic variants; four missense variants in the highly

conserved and functionally important MH2 domain and one nonsense variant. Variant carriers can present with a broad range of features, including aneurysms and tortuosity of the entire arterial tree, coronary artery dissections and prominent connective tissue characteristics. It is recommended to screen patients with aortopathy or more widespread aneurysmal disease, with or without connective tissue features, for (likely) pathogenic variants in the *SMAD2* gene.

Web resources

gnomAD: <http://gnomad.broadinstitute.org/>; 1000 Genome Project: <http://www.internationalgenome.org/>; dbSNP build 147: <https://www.ncbi.nlm.nih.gov/SNP/>; Sift: <http://sift.jcvi.org/>; PolyPhen2: <http://genetics.bwh.harvard.edu/pph2/>; MutationTaster: <http://www.mutationtaster.org/>

Declarations

Ethics approval and consent to participate

Approval for this study was obtained from the Ethics Committee of the Antwerp University Hospital/University of Antwerp.

Consent for publication

We obtained written consent for publication of photographs from each subject or their legal representative whose images were included in this manuscript.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EC carried out sequencing and analysis of DNA samples and drafted the manuscript. MK, AM, CM, TG, JR, DM, LB, JT, NM and BL collected DNA samples and contributed to accumulation and interpretation of clinical data. NP and GB carried out sequencing and analysis of DNA samples. DS, LVL, AV, BL contributed to writing the manuscript. All authors read and approved the final manuscript.

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Figure Legends

Figure 1. (Likely) pathogenic variant overview of the SMAD2 gene and evolutionary conservation in SMAD2 and its related proteins. (A) Schematic representation of the SMAD2 domains defined by their corresponding start and end amino acid position. All identified missense variants are located in the MH2 domain. The variant indicated with (*) were identified by Zhang et al. (2017)²⁰. Variants identified with (**) were discovered by Micha et al. (2015)¹⁹. Variants represented in **bold** and *italic* are reported in this manuscript. (B) Schematic overview of evolutionary conservation of the mutated amino acid positions (p.(Asn318Lys); p.(Asn361Thr); p.(Ser397Tyr); p.(Ser467Leu)).

Figure 2 Pedigrees of the families and their respective (likely) pathogenic SMAD2 variant. (A) Pedigree of family 1 with nonsense variant p.(Asn205*). (B) Pedigree of family 2 with missense variant p.(Asn318Lys). (C) Pedigree of family 3 with missense variant p.(Ser397Tyr). (D) Pedigree of family 4 with missense variant p.(Asn361Thr). (E) Pedigree of family 5 with missense variant p.(Ser467Leu). Proband is indicated with an arrow. Females are indicated with a circle and males are represented by a square. Black filled symbols represent affected family members, clear symbols indicate unaffected family members. Black spots in symbols indicate the presence of cerebral aneurysm. Deceased persons are indicated with a diagonal line. The variant status is indicated by a plus and minus sign.

Figure 3. Phenotypic characteristics of patients with a (likely) pathogenic SMAD2 variant. (A) Observed clinical features of the proband (II-2) of family 2 include arachnodactyly (B, C) dysmorphic facial features (dolichocephaly, downslanting palpebral fissures, retrognathia). (D) Proband (III-2) of family 3 with his two daughters. Observed facial features (hypertelorism, retrognathia) and (E) his eldest daughter (IV-2) present with hypertelorism. (F, G, H) Proband of family 4 (III-2) has an enlarged armspan, but no distinctive facial features.