OPEN ACCESS

Original research

Pathogenic variants affecting the TB5 domain of the fibrillin-1 protein: not only in geleophysic/acromicric dysplasias but also in Marfan syndrome

Pauline Arnaud ,[1,2,3] Zakaria Mougin ,[2] Genevieve Baujat,[4] Valérie Drouin-Garraud,[5] Salima El Chehadeh,[6] Laurent Gouya,[3] Sylvie Odent,[7,8] Guillaume Jondeau,[2,3] Catherine Boileau,[1,2] Nadine Hanna,[1,2,3] Carine Le Goff [2]

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/jmg-2023-109646).

[1]Département de Génétique, Assistance Publique - Hopitaux de Paris, Paris, France
[2]U1148 LVTS, INSERM, Paris, Île-de-France, France
[3]Centre de Référence Maladies Rares Syndrome de Marfan et apparentés, Hôpital Bichat, APHP, Paris, Île-de-France, France
[4]Département de Génétique, AP-HP, Hôpital Necker-Enfants malades, AP-HP, Paris, Île-de-France, France
[5]Département de Pédiatrie, CHU de Rouen, Rouen, Normandie, France
[6]Service de Génétique Médicale, Hôpital de Hautepierre, CHU de Strasbourg, Strasbourg, Grand Est, France
[7]Service de Génétique Clinique, CLAD Ouest, CHU Rennes, Rennes, Bretagne, France
[8]UMR 6290, IGDR, Rennes, Bretagne, France

Correspondence to
Dr Carine Le Goff, U1148 LVTS, INSERM, Paris, Île-de-France, France; carine.le-goff@inserm.fr

Received 18 September 2023
Accepted 18 January 2024
Published Online First 8 March 2024

To cite: Arnaud P, Mougin Z, Baujat G, *et al*. *J Med Genet* 2024;**61**:469–476.

ABSTRACT

Background Marfan syndrome (MFS) is a multisystem disease with a unique combination of skeletal, cardiovascular and ocular features. Geleophysic/acromicric dysplasias (GPHYSD/ACMICD), characterised by short stature and extremities, are described as 'the mirror image' of MFS. The numerous *FBN1* pathogenic variants identified in MFS are located all along the gene and lead to the same final pathogenic sequence. Conversely, in GPHYSD/ACMICD, the 28 known heterozygous *FBN1* pathogenic variants all affect exons 41–42 encoding TGFβ-binding protein-like domain 5 (TB5).

Methods Since 1996, more than 5000 consecutive probands have been referred nationwide to our laboratory for molecular diagnosis of suspected MFS.

Results We identified five MFS probands carrying distinct heterozygous pathogenic in-frame variants affecting the TB5 domain of FBN1. The clinical data showed that the probands displayed a classical form of MFS. Strikingly, one missense variant affects an amino acid that was previously involved in GPHYSD.

Conclusion Surprisingly, pathogenic variants in the TB5 domain of FBN1 can lead to two opposite phenotypes: GPHYSD/ACMICD and MFS, suggesting the existence of different pathogenic sequences with the involvement of tissue specificity. Further functional studies are ongoing to determine the precise role of this domain in the physiopathology of each disease.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ *FBN1* pathogenic variations have been described all along the gene in Marfan syndrome (MFS), whereas the *FBN1* variations associated with geleophysic/acromicric dysplasias, which is 'the mirror image' of MFS, affect a specific domain, called TGFβ-binding protein-like domain 5 (TB5).

WHAT THIS STUDY ADDS

⇒ Surprisingly, we describe five distinct MFS probands carrying a pathogenic variation in the TB5 domain, including one variation affecting an amino acid that was previously involved in a case of geleophysic dysplasia.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The knowledge that variations affecting the same domain of FBN1 can lead to two opposite phenotype is essential since it opens the way to further studies that may clarify a missing part in the pathophysiology of each disease.

INTRODUCTION

FBN1 encodes the fibrillin-1, a large glycoprotein that is a critical actor in the biomechanical function of connective tissue but also a regulator of the bioavailability of various molecules involved in signalling pathways, notably TGF-β (transforming growth factor β). FBN1 is the major component of microfibrils in the extracellular matrix. It is a multidomain protein, comprising 47 epidermal growth factor-like domains, among which 43 are calcium-binding (cbEGF-like), 7 TGF-β-binding protein-like domains (TB), 2 hybrid domains, and the N-terminal and C-terminal domains. The seven TB domains are characterised by an eight-cysteine motif that forms four disulfide bonds with a 1–3, 2–6, 4–7 and 5–8 arrangement (figure 1, adapted from Robertson *et al*[1]). The domains also harbour some highly conserved non-cysteine residues which may participate in the stabilisation of the domain or inter-domain structure. In the fifth TB domain (TB5), two asparagine residues (Asn1703 and Asn1713) are N-glycosylated.

Marfan syndrome (MIM#154700, MFS) is a hereditary connective tissue disorder with a unique combination of clinical features. Indeed, in this disease, many systems are affected with great phenotypic variability and life-threatening complications, such as the cardiovascular system, with thoracic aortic aneurysms (TAA) and dissections, ocular system with *ectopia lentis*, and the skeletal system with recognisable features such as scoliosis, long bone overgrowth, arachnodactyly and pectus deformity. The clinical diagnosis is based on the revised Ghent nosology.[2] MFS is the best known fibrillinopathy, with heterozygous pathogenic variants in the *FBN1* gene, encoding fibrillin-1 found in the majority of patients with MFS (1850 different pathogenic variants described in the UMD-FBN1 database).[3]

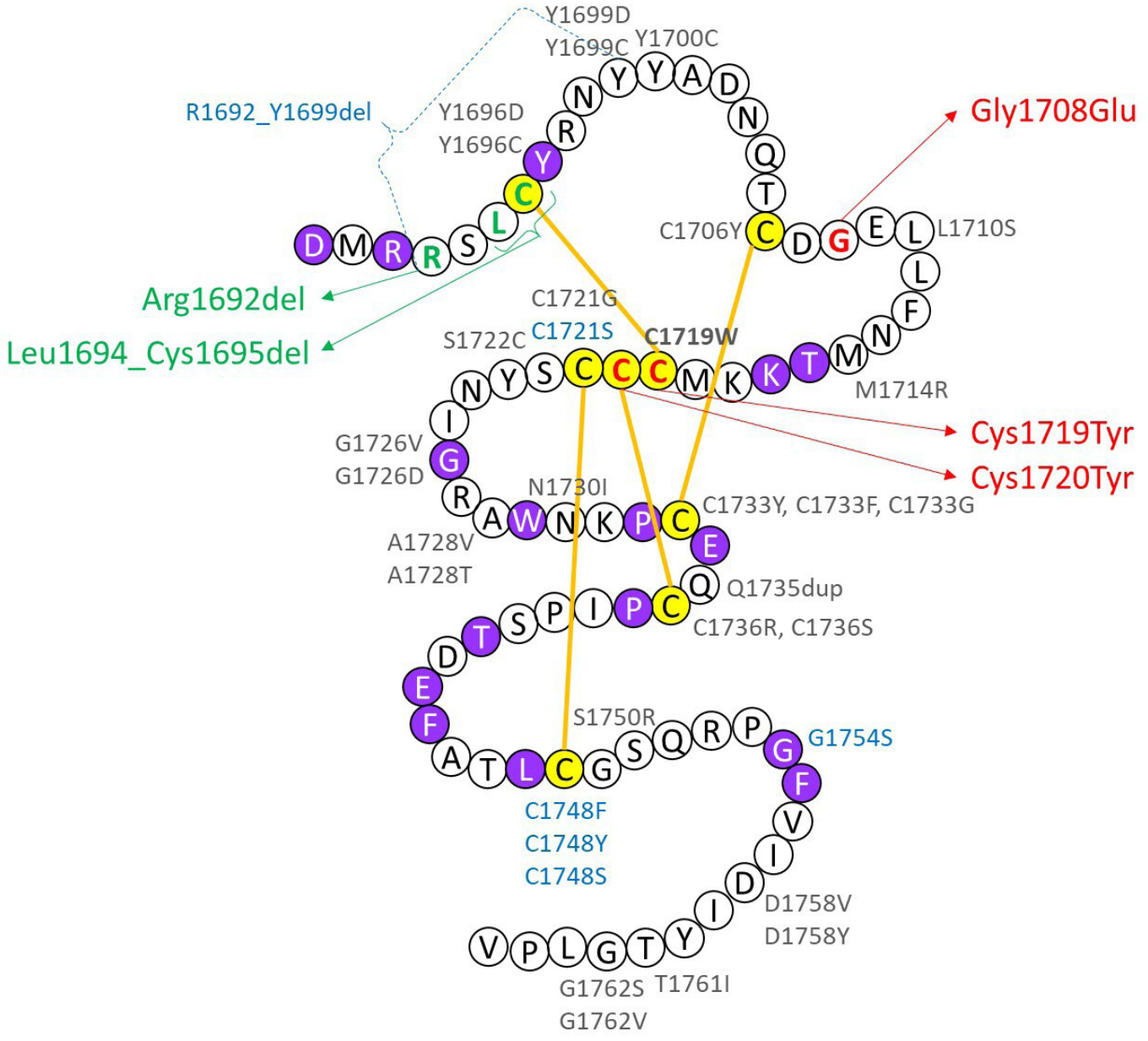

Figure 1 Schematic representation of the variants identified in TB5 domain in fibrillin-1 protein. Cysteine residues are shown in yellow, with disulfide bonds illustrated as yellow lines. Residues in purple correspond to highly conserved residues in human TB domains (conserved in at least four out of seven TB domains in fibrillin-1). Pathogenic variants previously reported in Weill-Marchesani syndrome are shown in blue, pathogenic variants previously reported in geleophysic dysplasia or acromicric dysplasia are shown in grey. Missense variants from this report associated with MFS are shown in red. In-frame deletions from this report associated with MFS are shown in green. The figure was adapted from Robertson *et al*.[1]

Some pathogenic variants in the *FBN1* gene are also found in acromelic dysplasias, a group of genetic skeletal disorders[4] that comprises 15 distinct skeletal disorders among which Weill-Marchesani syndrome (WMS (MIM#608328)), geleophysic dysplasia (GPHYSD (MIM#231050)) and acromicric dysplasia (ACMICD (MIM#102370)). As a 'mirror image' of MFS, the acromelic dysplasia group is characterised by short stature and shortening of the limbs, mainly in the acromelic segments (brachydactyly) and restrictive joint mobility. Although pathogenic variants in other genes (*ADAMTS10*,[5] *ADAMTS17*,[6] *ADAMTSL2*,[7] *LTBP2*,[8] *LTBP3*[9]) have also been linked to these disorders, all these three phenotypes can be caused by pathogenic variants in the *FBN1* gene. The *FBN1*-related acromelic dysplasias share short stature, brachydactyly, joint stiffness, contractures and thick skin as common features. However, some disorder-specific clinical features have been suggested. In WMS, severe eye abnormalities such as microspherophakia and sometimes ectopia lentis are present.[10] Distinguishing GPHYSD features are progressive thickening of the cardiac valves, tracheal stenosis and respiratory insufficiency which may lead to the death of young patients. They present also toe walking and a 'happy' face characterised by full cheeks, a short nose, hypertelorism and a thin upper lip.[10 11] A recessive form of GPHYSD is linked to homozygous or compound heterozygous variations in *ADAMTSL2*.[7] At the molecular level, WMS is linked to heterozygous pathogenic variations in the *FBN1* gene, including six pathogenic variations in the TB5 domain[10 12 13] but also others in the TB1-EGF4 region.[14] Other variations in *ADAMTS10*,[5] *ADAMTS17*[6] and *LTBP2*[8] genes have been reported in WMS with an autosomal recessive transmission. Conversely, there is a

hotspot for GPHYSD/ACMICD[15–23] variants in the *FBN1* gene. Indeed, 28 different heterozygous pathogenic variants were reported in patients, all within the TB5 motif encoded by exons 41 and 42. All these GPHYSD/ACMICD and WMS variants are in-frame, with a majority of missense variants and two small in-frame amino acid deletion/duplication.

The present study reports on the original identification in our genetic laboratory of five MFS probands with in-frame pathogenic variants in the TB5 domain, molecular events highly comparable to those seen in GPHYSD/ACMICD and WMS probands.[24]

MATERIAL AND METHODS

Patients and clinical data

All the patients included were followed up either by the Centre National Maladies Rares-Syndrome de Marfan et apparentés, the French National Reference Centre in Paris or an affiliated regional Centre of Expertise. Clinical diagnosis was established according to the revised Ghent nosology.[2] In brief, patients were examined by several physicians: cardiologists, ophthalmologists, geneticists, rheumatologists or paediatricians (depending on their age) with specific evaluation of clinical features included in MFS. Systematic slit-lamp examination, cardiac ultrasonography and radiological investigations were also performed. The aortic diameter was evaluated at the root and the tubular portion of the ascending aorta at end-diastole. The aortic aneurysm was defined as a measure above mean+2 SDs (Z score >2 SD), as described by Campens *et al*.[25] Dural ectasia was looked for by imaging. The systemic score was calculated as described in the revised Ghent nosology.[2] Between 1996 and 2023, blood samples were obtained for more than 5000 consecutive unselected probands referred nationwide to our laboratory for molecular diagnosis of suspected MFS.

Molecular analysis

Genomic DNA was isolated from peripheral blood leucocytes with a DNA Blood 4K kit (PerkinElmer) on Chemagicstar (Hamilton) according to the manufacturer's instructions. Originally, the *FBN1* gene was systematically screened in patients suspected of MFS by bidirectional Sanger sequencing as previously reported.[26] Since 2014, the *FBN1* gene has been screened on MiSeq (Illumina) by Next Generation Sequencing (on more than 4000 patients) using MARFAN MASTR Assay (Multiplicom) or a custom capture array (NimbleGen, Roche) designed to capture *FBN1* gene (NM_000138.4; genome build hg19) and 27 other genes already known to be associated with MFS and related diseases (total size of the target: 132 kb). Variant calling is performed through CLC Genomics Workbench V.10.1.1 (Qiagen Bioinformatics). Once a single-nucleotide or a small insertion/deletion pathogenic variant is found, it is systematically confirmed by bidirectional Sanger sequencing of the altered exon. When the pathogenic variant alters the regional restriction map, the presence of the variation is also checked by PCR/digestion using the appropriate restriction enzyme. When possible, familial segregation of pathogenic variants is investigated. Description of sequence variants is performed according to Human Genome Variation Society nomenclature.[27] In brief, cDNA numbering with +1 corresponds to the A of ATG, the translation initiation codon in the reference sequence (*FBN1*: NM_000138.4). Exon numbering is historically made considering that exon 1 carries the initiation codon.

In silico variant analysis

Three consensual bioinformatics programs (Polyphen-2,[28] VarSome,[29] REVEL[30]) were used to predict the pathogenicity of missense variants. The UMD locus-specific databases and ClinVar database were queried for each identified variant. The existence of each molecular event was looked for in the Genome Aggregation Database (gnomAD V.2.1.1).[31] However, the laboratory has been performing molecular diagnosis of MFS and TAA for over 20 years and a population of over 5000 probands has been sequenced for the disease-causing genes. This has led to an in-house reference database of molecular events identified in each gene for which we have robust French population frequencies. Variants were classified according to recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[32]

Protein structure predictions

Using FBN1 domain sequences of cb-EGF-like 14–TB5–cb-EGF-like 15 and introducing variants found in patients with MFS, GPHYSD and WMS (sequences used can be found in online supplemental material), the domain structure predictions were performed using NovaFold AI (DNASTAR). Briefly, this method prediction uses a network-based model, AlphaFold 2,[33] to visualise models, to analyse structure data (eg, Karplus-Schulz flexibility plot), and to predict binding sites and protein functions. Structural data were visualised using the library Matplotlib.

RESULTS

Molecular aspects

Of the 5000 patients with suspected MFS, 2160 probands were shown to harbour at least one heterozygous pathogenic variant in the *FBN1* gene (yield of 43%).[34] Among these molecular events, 14 affect the TB5 domain in the fibrillin-1 protein. Nine are premature termination codon variants and typical of MFS. Interestingly, five variants are in-frame variants, including two small amino-acid deletions (p.(Arg1692del) and p.(Leu1694_Cys1695del)) and three missense variants (p.(Gly1708Glu), p.(Cys1719Tyr) and p.(Cys1720Tyr)). All the variants were absent or present at a very low frequency in the gnomAD population database. The three consensual bioinformatics programs (Polyphen-2,[28] VarSome,[29] REVEL[30]) were in favour of the pathogenicity of the three missense variants. A summary of the molecular aspects of these variants is available in table 1. Interestingly, four of these variants were previously described in the UMD-FBN1 database and/or in the literature. In the two cases (UMD-FBN1 entry for p.(Gly1708Glu) and p.(Cys1719Tyr)[35]), it corresponds to previous reports from our team. Three variations (p.(Arg1692del) and p.(Cys1720Tyr)) were previously reported in patients with clinical MFS[36] and familial TAA.[37] A schematic view of the different variations in the TB5 domain is available in figure 1.

Clinical aspects

The five MFS proband carriers of a pathogenic variant in the TB5 domain had ages ranging from 15–20 to 40–45 years old at the last physical examination. All of them were addressed for suspected MFS with several degrees of severity. Four out of five had a major ocular involvement with *ectopia lentis*. Three out of five had a major cardiovascular involvement with two probands who underwent aortic surgery: mitral valve replacement at 10–15 years old (patient 7) and a Tirone-David procedure for TAA at 20–25 years old (patient 10). The systemic score according to Ghent nosology[2] of probands ranged from 2 to 8.

Table 1 Molecular aspects of the pathogenic variants identified in patients with MFS in this report

	c.5076_5078del p.(Arg1692del)	c.5079_5084del p.(Leu1694_Cys1695del)	c.5123G>A p.(Gly1708Glu)	c.5156G>A p.(Cys1719Tyr)	c.5159G>A p.(Cys1720Tyr)
gnomAD	Abs.	Abs.	2/251 348 alleles	Abs.	Abs
UMD-FBN1	1 entry: Comeglio *et al*[36] (clinical MFS)	Abs.	1 entry: this patient	1 entry: this patient in Stheneur *et al*[35]	2 entries: this patient and Comeglio *et al*[36] (clinical MFS)
Literature	Comeglio *et al*[36] (clinical MFS)	Abs.	Ziganshin *et al*[37] (familial TAA)	This patient in Stheneur *et al*[35]	Comeglio *et al*[36] (clinical MFS)
ClinVar	7 entries: 5 LP (no clinical data/MFS/FTAAD), 2 VUS (MFS and FTAAD)	Abs.	4 entries: 4 VUS (2 MFS, 1 FTAAD)	2 entries: 1 P (MFS), 1 LP (MFS)	Abs.
PolyPhen-2	NA	NA	0.998 (probably damaging)	0.996 (probably damaging)	0.996 (probably damaging)
REVEL	NA	NA	0.6539 pathogenic	0.972 pathogenic	0.9499 pathogenic
VarSome (metascores)	NA	NA	4/4 damaging	4/4 damaging	4/4 damaging
VarSome (individual predictions)	NA	NA	14/17 damaging	17/17 damaging	23/23 damaging

Abs, absent; FTAAD, familial thoracic aortic aneurysm and dissection; LP, likely pathogenic variant; MFS, Marfan syndrome; NA, not available; VUS, variant of unknown significance.

Familial screening allowed us to identify a total of seven relatives carrying the familial pathogenic variant. A summary of the clinical features that could be obtained for these 12 variant carriers is available in table 2 and in the online supplemental material. Taken together, all present variable clinical features. Six out of 9 carriers had a thoracic aortic aneurysm, including notably 2 patients out of 12 who underwent cardiovascular surgery at a young age. Regarding the other systems, 8 out of 11 had *ectopia lentis*, 6 out of 9 had striae, and 5 out of 10 had scoliosis (>20°). All patients' heights were in the normal range for adult patients, with two women with low heights (patients 4 and 8 in the online supplemental material).

Protein structure predictions

As a variation affecting p.Cys1719 was already described in the literature in a patient with another phenotype,[15] we predicted structural changes in the presence of the different variants impacting the same residue. Prediction for the WT sequence of the TB5 domain surrounded by both cb-EGF-like domains illustrates one β-hairpin (ββ) and a bulge believed to be stabilised by the four disulfide bonds (figure 2A). Substitution of the cysteine residue (p.Cys1719) by a tyrosine residue (MFS variant) seems to lead to a loss of the β-hairpin besides the complete loss of the bulge while the substitution of the cysteine residue for a tryptophan residue (GPHYSD-1 variant) seems to lead to complete loss of the bulge but with the β-hairpin maintained. The same findings are observed in the predictions when another cysteine residue (p.Cys1721), also involved in a disulfide bond, is substituted for a glycine residue (GPHYSD-2 variant) or a serine residue (WMS variant).

The flexibility (Karplus-Schulz) plot predicts backbone chain flexibility using the approach of Karplus and Schulz.[38] Briefly, values oscillate between 0 and 2, the more a residue is flexible, the more the value is close to 2; conversely, the more a residue is rigid, the more the value is close to 0. One of the substitutions occurs on the 67th residue (p.Cys1719), the flexibility of the predicted peptide at this residue is 0.93 for the WT sequence, vs 0.90 and 0.89 for the MFS and GPHYSD-1 sequences, respectively. The second substitution, on the 69th residue (p.Cys1721), leads to an increase in the flexibility of the region, with 0.98 for both variants (GPHYSD-2 and WMS) vs 0.92 for the WT sequence (figure 2B).

DISCUSSION

We report here an original discovery of five patients with MFS harbouring in-frame pathogenic variants in the TB5 domain of fibrillin-1 (two deletions and three missense variations). Interestingly, two of the missense variants affect a cysteine residue. This type of molecular event is frequent since it is observed in approximately half of all the in-frame variants in our cohort of 2160 MFS probands with a pathogenic variant in the *FBN1* gene.[39] The two variants affecting a cysteine in our study are loss of cysteine (-Cys, in patients 9, 10, 11 and 12). This type of variant has been found to provide the poorest prognosis in patients with MFS as they are associated with particularly severe cardiovascular (more aortic dissection or surgery; $p<0.0001$), skeletal and ophthalmologic phenotypes.[39] One of the two probands of this study with (-Cys) pathogenic variant (patient 10) was a man who had surgery for TAA at 20–25 years old, which is considered as a severe involvement of the cardiovascular system. The other (-Cys) variant carriers were three women (patients 9, 11 and 12), who did not display particularly severe cardiovascular features but they all had significant skeletal involvement with elevated systemic scores between 7 and 9. These observations were in line with what was observed in the French cohort,[39] in which the expression of the disease tends to be more severe at the skeletal level for women.

Missense pathogenic variants in the TB5 domain are classically involved in three acromelic dysplasias: GPHYSD, ACMICD and WMS. A recent study on GPHYSD/ACMICD reveals that a greater proportion of patients with a missense *FBN1* variant involving a cysteine had a poor outcome with life-threatening complications,[22] with, in particular, a higher probability of heart valve disease.

Interestingly, Cys1719 is involved in two different pathogenic variants with these opposite phenotypes. On one hand, p.(Cys1719Tyr) was identified in a Caucasian patient from this report with MFS (patient 9). This young girl was diagnosed with *ectopia lentis* and skeletal features (systemic score according to Ghent nosology[2]) at the age of

Table 2 Clinical features according to the revised Ghent nosology for MFS[2] for the five probands carrying a *FBN1* pathogenic variant in the TGFBP#5 domain

		Patient 1	Patient 3	Patient 7	Patient 9	Patient 10
	FBN1 pathogenic variant	**c.5076_5078del p.(Arg1692del)**	**c.5079_5084del p.(Leu1694_Cys1695del)**	**c.5123G>A p.(Gly1708Glu)**	**c.5156G>A p.(Cys1719Tyr)**	**c.5159G>A p.(Cys1720Tyr)**
	Gender	F	F	M	F	M
	Age range at diagnosis (years old)	40–45	10–15	10–15	5–10	20–25
	Age range at consultation (years old)	40–45	15–20	25–30	15–20	30–35
	Height at last physical examination (cm)	171.5	176	175	183	198
Cardiovascular	Thoracic aortic aneurysm	NA	Y	NA	N	Y
	Valsalva diameter (z-score (Campens *et al*[25])	NA	31 mm (+2.8 SD)	NA	30 mm (+0.9 SD)	NA
	Ascending aortic dissection	N	N	N	N	N
	Aortic surgery	N	N	Mitral valve replacement	N	Y
	Age/type/indication of surgery	–	–	10–15 years old/ mitral valve replacement/severe endocarditis	–	20–25 years old/ Tirone-David/TAA
	Mitral valve prolapse	N	Y	NA	N	Y
	Ascending aortic dilatation or dissection before 40 years old	N	N	N	N	N
Ocular	Ectopia lentis	Y	Y	N	Y	Y
	Myopia (>3 dioptries)	N	Y	N	Y	N
	Flat cornea	N	Y	N	Y	N
Musculoskeletal	Pectus carinatum	N	N	Y	N	N
	Severe pectus excavatum	Y	N	N	N	Y
	Dolichostenomelia=Reduced US/LS AND increased arm/height AND no severe scoliosis	N	N	Y	Y	N
	Positive wrist and thumb signs (arachnodactyly)	N	N	Y	Y	N
	Positive wrist or thumb signs	N	Y	N	N	Y
	Scoliosis >20° or spondylolisthésis	N	Y	Y	Y	Y
	Limited elbow extension <170°	Y	N	N	N	N
	Joint hypermobility	Y	N	Y	Y	N
	Protrusio acetabulae	NA	N	N	NA	N
	Hindfoot deformity	N	N	N	N	N
	Plain pes planus	N	Y	N	N	N
	Typical facial appearance (3/5: dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)	N	N	Y	Y	N
	Highly arched palate with crowding	Y	Y	Y	N	N
Other	Pneumothorax	N	N	NA	N	N
	Striae	N	Y	NA	N	Y
	Recurrent herniae	N	Y	NA	N	N
	Dural ectasia	NA	N	NA	NA	N
	Systemic score	2	6	8	7	5
	Diagnosis	EL+SK-m	MFS	Mitral valve replacement+SK-M	EL+SK-M	MFS

The clinical data for the 12 patients (5 probands and their relatives) can be found in the supplemental material.
F, female; M, male; N, no; NA, not available; SK-M, major skeletal involvement; SK-m, minor skeletal involvement; Y, yes.

5–10 years old; at 15–20 years old, her height was 183 cm (+3 SD). On the other hand, p.(Cys1719Trp) was identified in a 14-year-old Lebanese patient presenting GPHYSD.[15] Interestingly, this second proband had no cardiac involvement and his height was 133 cm (−3.5 SD). In both cases, the molecular events arose *de novo*. This cysteine residue is the third cysteine of the TB5 domain and is involved in a disulfide bond with the first cysteine of the domain Cys1695 (figure 1). The amino acid change with tyrosine and tryptophan involves both aromatic residues, with a hydroxyl group for tyrosine and an amino group for tryptophan. At the molecular level, a tyrosine residue is more hydrophilic

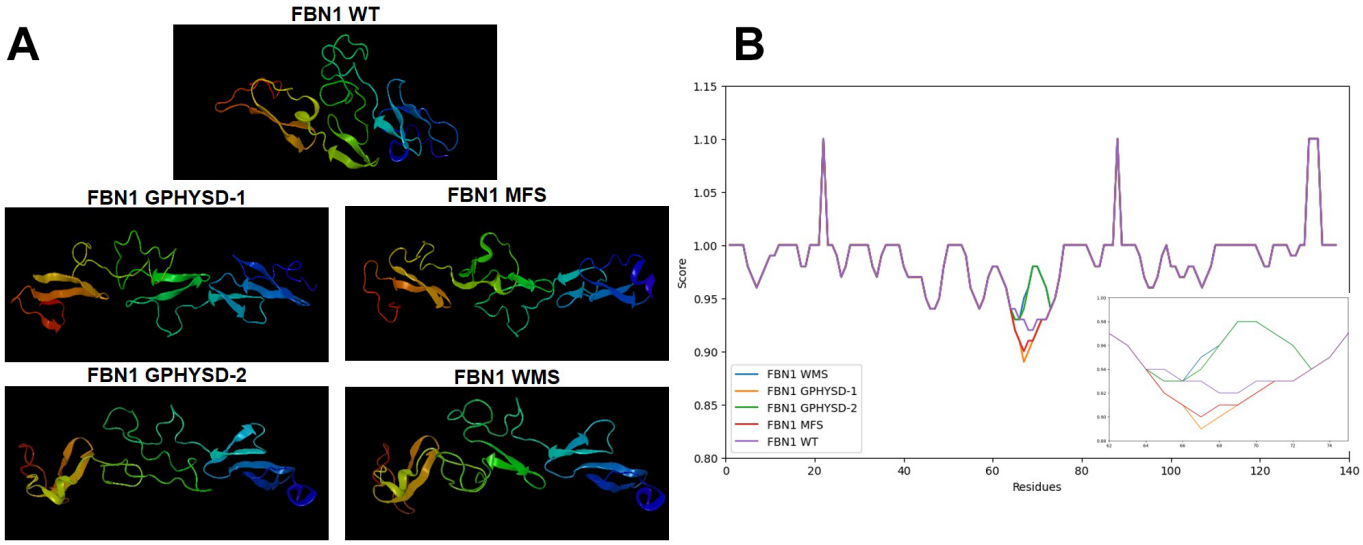

Figure 2 Protein structure predictions of the variants impacting p.Cys1719 and p.Cys1721 residues. (A) Using NovaFold AI, protein structure predictions were obtained using the WT cb-EGF-like 14/TB5/cb-EGF-like 15 peptidic sequences (FBN1 WT), introducing the variations, respectively, leading to MFS (FBN1 MFS–p.(Cys1719Tyr)), GPHYSD [FBN1 GPHYSD-1–p.(Cys1719Trp); and FBN1 GPHYSD-2: p.(Cys1721Gly)] and WMS (FBN1 WMS–p.(Cys1721Ser)). (B) Karplus-Schulz flexibility plot, depicting the rigidity of a residue, was obtained using the corresponding protein structure predictions. A zoom on the differences is shown at the bottom right of the graph. The substitutions occur at the 67th and 69th residues (see supplementary data for peptidic sequences used).

than a cysteine residue while a tryptophan is more hydrophobic. Interestingly, while the substitution of a tyrosine for a cysteine residue is relatively frequent, the substitution of a tryptophan for a cysteine is a rare event in our database. Moreover, the protein structure predictions performed showed a complete loss of the peptidic bulge into the TB5 domain, which may be associated with the loss of a disulfide bond in the MFS case. Curiously, it appears that the β-hairpin of the TB5 domain is yet present in the presence of the GPHYSD variant, whereas this β-hairpin seems to be abolished in the presence of the MFS variant. This structural difference suggests that the TB5-GPHYSD may keep a part of its function but not enough to completely fulfil its function. The generation of the Karplus-Schulz flexibility plot on the predicted structures seems to indicate that the presence of both variants leads to a more rigid TB5 domain in the area of the substitutions. The loss of the secondary and tertiary structures and the change in the physicochemical characteristics of the TB5 domain demonstrate the primordial role of the p.Cys1719 residue. More importantly, the two distinct substitutions seem to have two different impacts on the TB5 domain. It could be hypothesised that beyond the disruption of a disulfide bond and the generally accepted dominant negative effect, the introduction of the tryptophan residue results in a 'gain of function' and a novel interaction with other extracellular proteins. Interestingly, the substitution of another cysteine residue involved in a disulfide bond (eg, p.Cys1721) also leads to a loss of the secondary/tertiary structure of the domain, but the resulting flexibility is increased. This observation suggests that, depending on the cysteine residue, but also the disrupted disulfide bond, the resulting defect seems to variate. Altogether, this notion suggests that the pathomechanism may not only be linked to the impact of the structural consequence of amino acid substitution. Further investigation is warranted to elucidate the different pathogenic effects of these different substitutions within the TB5 module. Furthermore, some genetic or environmental factors such as ethnicity may also contribute to patient phenotypes as already described in a previous study on Hispanic patients with MFS and mild skeletal manifestations.[40]

Sun *et al*[18] recently notably reported that acromelic dysplasia was caused only by in-frame missense variants affecting TB5 in FBN1 while truncating variants have been reported only in MFS. Our results suggest a more complex picture with missense variants associated with both phenotypes.

The exact function of the TB5 domain is not known. It was demonstrated that this domain interacts with heparan sulfate (HS) motifs and heparin. The impact of ACMICD mutations on the TB5/heparin interaction has been studied previously. In all cases, a significant reduction of the interaction of the mutated TB5 domain with heparin has been observed.[41] To date, the interaction of the MFS TB5 domain with heparin has to be performed to better understand and decipher the role of HS in the pathomechanisms of MFS.[41] It was also shown that ACMICD-causing substitutions in TB5 do not prevent fibrillin-1 from being secreted or assembled into microfibrils, whereas MFS-associated substitutions in the domain result in a loss of recombinant protein in the culture medium and no association with microfibrils.[42] In 2011, Le Goff *et al* showed in patient skin fibroblasts that the mutations leading to GPHYSD and ACMICD impaired the microfibrillar network of FBN1, with a reduced number of fibrils, and enhanced TGF-β signalling pathway.[15] The impact of the FBN1 TB5 variant was confirmed in the case of GPHYSD with the generation of the *Fbn1*^{Y1698C} mouse model mimicking GPHYSD. In this model, thicker fibrils with a decreased network density have been observed.[43] Interestingly, the global protein level of Fbn1 was not altered, and neither was the activation of the TGF-β signalling pathway. The abnormal structure of the microfibrillar network may be explained by the interaction of TB5 with HS. Indeed, Yadin *et al* showed that HS sequesters fibrillin monomers at the

cell surface preventing premature N-terminal-C-terminal domain interactions.[44] Moreover, via the TB5 domain, it is known that fibronectin is the origin point of ECM assembly regulating pericellular FBN1 microfibril assembly.[45]

The TB5 domain may be central to other interactions between FBN1 and other extracellular molecules which are not yet known. ADAMTSL-2 in GPHYSD, ADAMTS-17 and ADAMTS-10 in WMS may be considered potential interacting proteins with the TB5 domain. All these three ADAMTS seem involved in FBN1 microfibrillar biogenesis. With the help of these ADAMTS, the TB5 domain may be involved in the polymerisation or microfibril assembly of FBN1. Recently, another ADAMTS has been implicated in a fibrillinopathy, ADAMTSL-6. This ADAMTS is involved in a marfanoid phenotype[46] suggesting that each ADAMTS has its tissue specificity and its own function but they may all have a common domain of interaction with FBN1.

Given the fact that the present study describes different missense variants in the same domain for two opposite phenotypes, we hypothesise that the structural impact of the amino acid substitutions and the potential role of the TB5 domain as a regulator of ECM assembly may be involved in the two different pathomechanisms in these diseases. An interactome of the FBN1 TB5 domain should be performed to define the distinct molecules interacting with this domain and highlight the potential tissue specificity mechanism. On the other hand, we can not exclude the role of (a) modifier gene(s) in these mechanisms.

Acknowledgements We thank the patients for their participation. This work was previously presented as an abstract at the 54th European Society of Human Genetics (ESHG) Conference.[24]

Contributors Conceptualisation: PA, ZM, CLG. Data curation: PA, NH. Formal analysis: PA, ZK, CLG. Funding acquisition: GJ, CB, CLG. Investigation: GB, VDG, SEC, LG, SO, GJ. Methodology: PA, ZM, CLG. Resources: CB, CLG. Supervision: CLG. Writing original draft: PA, ZM, CLG. Writing—review and editing: CB, CLG.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval A specific informed consent was obtained for all patients in agreement with the requirements of French bioethics laws (institutional review board (IRB) approval from Comité de Protection des Personnes number 11008, 13 January 2011).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iDs
Pauline Arnaud http://orcid.org/0000-0001-9727-1592
Zakaria Mougin http://orcid.org/0000-0001-9429-2865
Carine Le Goff http://orcid.org/0000-0002-7788-708X

REFERENCES

1 Robertson I, Jensen S, Handford P. TB domain proteins: evolutionary insights into the Multifaceted roles of Fibrillins and Ltbps. *Biochem J* 2011;433:263–76.
2 Loeys BL, Dietz HC, Braverman AC, *et al*. The revised Ghent Nosology for the Marfan syndrome. *J Med Genet* 2010;47:476–85.
3 Collod-Béroud G, Le Bourdelles S, Ades L, *et al*. Update of the UMD-Fbn1 Mutation database and creation of an Fbn1 polymorphism database. *Hum Mutat* 2003;22:199–208.
4 Mortier GR, Cohn DH, Cormier-Daire V, *et al*. Nosology and classification of genetic Skeletal disorders: 2019 revision. *Am J Med Genet A* 2019;179:2393–419.
5 Dagoneau N, Benoist-Lasselin C, Huber C, *et al*. Adamts10 mutations in Autosomal Recessive weill-Marchesani syndrome. *Am J Hum Genet* 2004;75:801–6.
6 Morales J, Al-Sharif L, Khalil DS, *et al*. Homozygous mutations in Adamts10 and Adamts17 cause Lenticular myopia, Ectopia Lentis, glaucoma, Spherophakia, and short stature. *Am J Hum Genet* 2009;85:558–68.
7 Le Goff C, Morice-Picard F, Dagoneau N, *et al*. Adamtsl2 mutations in Geleophysic dysplasia demonstrate a role for ADAMTS-like proteins in TGF-beta Bioavailability regulation. *Nat Genet* 2008;40:1119–23.
8 Haji-Seyed-Javadi R, Jelodari-Mamaghani S, Paylakhi SH, *et al*. Ltbp2 mutations cause weill-Marchesani and weill-Marchesani-like syndrome and affect disruptions in the extracellular matrix. *Hum Mutat* 2012;33:1182–7.
9 McInerney-Leo AM, Le Goff C, Leo PJ, *et al*. Mutations in Ltbp3 cause Acromicric dysplasia and Geleophysic dysplasia. *J Med Genet* 2016;53:457–64.
10 Faivre L, Gorlin RJ, Wirtz MK, *et al*. In frame Fibrillin-1 gene deletion in Autosomal dominant weill-Marchesani syndrome. *J Med Genet* 2003;40:34–6.
11 Sakai LY, Keene DR. Fibrillin protein Pleiotropy:Acromelic Dysplasias. *Matrix Biol* 2019;80:6–13.
12 Newell K, Smith W, Ghoshhajra B, *et al*. Cervical artery dissection expands the cardiovascular phenotype in Fbn1-related weill-Marchesani syndrome. *Am J Med Genet A* 2017;173:2551–6.
13 Cecchi A, Ogawa N, Martinez HR, *et al*. Missense mutations in Fbn1 Exons 41 and 42 cause weill-Marchesani syndrome with Thoracic aortic disease and Marfan syndrome. *Am J Med Genet A* 2013;161A:2305–10.
14 Sengle G, Tsutsui K, Keene DR, *et al*. Microenvironmental regulation by Fibrillin-1. *PLoS Genet* 2012;8:e1002425.
15 Le Goff C, Mahaut C, Wang LW, *et al*. Mutations in the TGFβ binding-protein-like domain 5 of Fbn1 are responsible for Acromicric and Geleophysic Dysplasias. *Am J Hum Genet* 2011;89:7–14.
16 Wang Y, Zhang H, Ye J, *et al*. Three novel mutations of the Fbn1 gene in Chinese children with Acromelic dysplasia. *J Hum Genet* 2014;59:563–7.
17 Wang T, Yang Y, Dong Q, *et al*. Acromicric dysplasia with stiff skin syndrome-like severe cutaneous presentation in an 8-year-old boy with a Missense Fbn1 Mutation: case report and literature review. *Mol Genet Genomic Med* 2020;8.
18 Sun C, Xu D, Pei Z, *et al*. Separation in genetic pathogenesis of mutations in Fbn1-Tb5 region between Autosomal dominant Acromelic dysplasia and Marfan syndrome. *Birth Defects Res* 2020;112:1834–42.
19 Globa E, Zelinska N, Dauber A. The clinical cases of Geleophysic dysplasia:one gene, different phenotypes. *Case Rep Endocrinol* 2018.
20 Jin HS, Song HY, Cho SY, *et al*. Acromicric dysplasia caused by a novel heterozygous Mutation of Fbn1 and effects of growth hormone treatment. *Ann Lab Med* 2017;37:92–4.
21 Lee T, Takeshima Y, Okizuka Y, *et al*. A Japanese child with Geleophysic dysplasia caused by A novel Mutation of Fbn1. *Gene* 2013;512:456–9.
22 Marzin P, Thierry B, Dancasius A, *et al*. Geleophysic and Acromicric Dysplasias: natural history, genotype-phenotype correlations, and management guidelines from 38 cases. *Genet Med* 2021;23:331–40.
23 Shan Y-C, Yang Z-C, Ma L, *et al*. A review of three Chinese cases of Acromicric/ Geleophysic dysplasia with Fbn1 mutations. *Int J Gen Med* 2021;14:1873–80.
24 Abstracts from the 54th European society of human Genetics (ESHG) conference: E-posters. *Eur J Hum Genet* 2022;30:88–608.
25 Campens L, Demulier L, De Groote K, *et al*. Reference values for echocardiographic assessment of the diameter of the aortic root and ascending aorta spanning all age categories. *Am J Cardiol* 2014;114:914–20.
26 Arnaud P, Hanna N, Aubart M, *et al*. Homozygous and compound heterozygous mutations in the Fbn1 gene: unexpected findings in molecular diagnosis of Marfan syndrome. *J Med Genet* 2017;54:100–3.
27 den Dunnen JT, Dalgleish R, Maglott DR, *et al*. HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat* 2016;37:564–9.
28 Adzhubei IA, Schmidt S, Peshkin L, *et al*. A method and server for predicting damaging Missense mutations. *Nat Methods* 2010;7:248–9.
29 Kopanos C, Tsiolkas V, Kouris A, *et al*. Varsome: the human Genomic variant search engine. *Bioinformatics* 2019;35:1978–80.
30 Ioannidis NM, Rothstein JH, Pejaver V, *et al*. REVEL: an ensemble method for predicting the Pathogenicity of rare Missense variants. *Am J Hum Genet* 2016;99:877–85.
31 Lek M, Karczewski KJ, Minikel EV, *et al*. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–91.

32 Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical Genetics and Genomics and the Association for molecular pathology. *Genet Med* 2015;17:405–24.
33 Jumper J, Evans R, Pritzel A, *et al*. Highly accurate protein structure prediction with Alphafold. *Nature* 2021;596:583–9.
34 Arnaud P, Morel H, Milleron O, *et al*. Unsuspected somatic Mosaicism for Fbn1 gene contributes to Marfan syndrome. *Genet Med* 2021;23:865–71.
35 Stheneur C, Collod-Béroud G, Faivre L, *et al*. Identification of the minimal combination of clinical features in Probands for efficient Mutation detection in the Fbn1 gene. *Eur J Hum Genet* 2009;17:1121–8.
36 Comeglio P, Johnson P, Arno G, *et al*. The importance of Mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 Fbn1 mutations. *Hum Mutat* 2007;28.
37 Ziganshin BA, Bailey AE, Coons C, *et al*. Routine genetic testing for Thoracic aortic aneurysm and dissection in a clinical setting. *Ann Thorac Surg* 2015;100:1604–11.
38 Karplus PA, Schulz GE. Prediction of chain flexibility in proteins: a tool for the selection of peptide antigens. *Naturwissenschaften* 1985;72:212–3.
39 Arnaud P, Milleron O, Hanna N, *et al*. Clinical relevance of genotype–phenotype correlations beyond vascular events in a cohort study of 1500 Marfan syndrome patients with Fbn1 pathogenic variants. *Genet Med* 2021;23:1296–304.
40 Villamizar C, Regalado ES, Fadulu VT, *et al*. Paucity of skeletal manifestations in Hispanic families with Fbn1 mutations. *Eur J Med Genet* 2010;53:80–4.
41 Cain SA, McGovern A, Baldwin AK, *et al*. Fibrillin-1 mutations causing weill-Marchesani syndrome and Acromicric and Geleophysic Dysplasias disrupt Heparan sulfate interactions. *PLoS ONE* 2012;7:e48634.
42 Jensen SA, Iqbal S, Bulsiewicz A, *et al*. A Microfibril assembly assay identifies different mechanisms of dominance underlying Marfan syndrome, stiff skin syndrome and Acromelic Dysplasias. *Hum Mol Genet* 2015;24:4454–63.
43 Delhon L, Mougin Z, Jonquet J, *et al*. The critical role of the TB5 domain of fibrillin-1 in endochondral ossification. *Hum Mol Genet* 2022;31:3777–88.
44 Yadin DA, Robertson IB, McNaught-Davis J, *et al*. Structure of the Fibrillin-1 N-terminal domains suggests that Heparan sulfate regulates the early stages of Microfibril assembly. *Structure* 2013;21:1743–56.
45 Sabatier L, Chen D, Fagotto-Kaufmann C, *et al*. Fibrillin assembly requires fibronectin. *Mol Biol Cell* 2009;20:846–58.
46 Elbitar S, Renard M, Arnaud P, *et al*. Pathogenic variants in Thsd4, Encoding the ADAMTS-like 6 protein, Predispose to inherited Thoracic aortic aneurysm. *Genet Med* 2021;23:111–22.