Three Novel Mutations in \textit{FBN1} and \textit{TGFBR2} in Patients with the Syndromic Form ofThoracic Aortic Aneurysms and Dissections

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\textbf{Summary}

There are many inherited disorders associated with thoracic aortic aneurysms and dissections (TAADs), like Marfan syndrome and Loeys-Dietz syndrome (LDS). The 4 patients in this study all had TAADs and were initially diagnosed with suspected Marfan syndrome. We collected peripheral blood samples from the patients and their family members and then attempted to identify the causal mutation using different methods including PCR, Sanger sequencing, and next generation sequencing. We identified 3 novel heterozygous mutations including 2 splicing mutations of \textit{FBN1} and 1 missense mutation of \textit{TGFBR2} in our patients. Although these mutation sites have been reported in the Human Gene Mutation Database, the nucleotide changes are different. All novel mutations found in this study were confirmed to be absent in 50 unrelated normal individuals of the same ethnic background. The RT-PCR results of 2 splicing mutations verified that the mutations can lead to the skipping of exons. The RT-qPCR results indicated that \textit{FBN1} mRNA levels were nearly 50 percent lower in the patients than in normal controls, indicating that there is almost no expression of truncated fibrillin-1 because of the nonsense-mediated mRNA decay (NMD) mechanism. To the best of our knowledge, we are the first to report these 3 novel mutations. However, the pathogenicity of these mutations still needs further confirmation. Our study has confirmed or corrected the clinical diagnosis, and enlarged the mutation spectrum of \textit{FBN1} and \textit{TGFBR2}. The results should be helpful for prenatal diagnosis and genetic counseling.

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\textbf{Key words:} Marfan syndrome, Loeys-Dietz syndrome, Splicing mutation, Missense mutation, Exon skipping, RT-PCR, RT-qPCR, Mosaicism

There are many inherited disorders associated with thoracic aortic aneurysms and dissections (TAADs), like Marfan syndrome and Loeys-Dietz syndrome (LDS).\textsuperscript{3} Marfan syndrome (MFS) (OMIM 154700) is an autosomal dominant inherited connective tissue disorder with high penetrance and variable phenotypes. It was first described by the French pediatrician Antoine Marfan in 1896.\textsuperscript{4} MFS primarily affects the cardiovascular, skeletal and ocular systems, with skin, fascia, lung and adipose tissue occasionally involved.\textsuperscript{5} The disproportionate long bone overgrowth, ectopia lentis and aortic root aneurysm are the main features of this syndrome.\textsuperscript{6} The major life-threatening manifestation of MFS is aortic aneurysm with eventual dissection.\textsuperscript{7} Aneurysm of the aortic root and dissection were described in 1943.\textsuperscript{8} Loeys-Dietz syndrome (LDS) is a connective tissue disorder that is characterized by a high risk for aneurysm and dissection throughout the arterial tree and phenotypically resembles Marfan syndrome.\textsuperscript{9} Thus Loeys-Dietz syndrome was also known as Marfan syndrome type II. Patients mainly exhibit progressive vascular diseases such as dilation of the aortic root, aortic dissection and valvular insufficiency. They also have anomalies of other systems, such as ocular hypertelorism, high-arched palate, b陋id uvula, scoliosis and clubfoot. Vascular complications in Loeys-Dietz syndrome progress more rapidly than those in Marfan syndrome.\textsuperscript{10}

The clinical diagnosis of MFS is based on a set of major and minor clinical criteria called the “Ghent nosology”.\textsuperscript{11} Ghent nosology, which was established in 1996 and requires the evaluation of family history; \textit{FBN1} mutations; and skeletal, ocular, cardiovascular, and pulmonary organ systems; skin; and dura. The diagnostic criteria were revised in 2010 (revised Ghent nosology) to place more emphasis on genetic analyses, the presence of aortic root aneurysm/dissection, and ectopia lentis.\textsuperscript{12} Even based on this, it is very difficult to establish a definitive diagnosis because of the variable phenotypic expression among patients.\textsuperscript{13}

Mutations in the \textit{FBN1} and \textit{TGFBR2} genes have been identified in probands with MFS and related phenotypes.\textsuperscript{14} \textit{FBN1} (chr.15q21.1) is the gene responsible for
most cases of MFS.\textsuperscript{13} $FBN1$ (RefSeq NM_000138) contains 65 exons encoding profibrillin-1 with 2871 amino acids, a 350 kDa glycoprotein that is processed to fibrillin-1.\textsuperscript{14} Fibrillin-1 is a large, extracellular matrix glycoprotein that not only serves as an important calcium-binding microfibrillar structural molecule, but also serves as a regulator of TGF-$\beta$ signaling.\textsuperscript{15} In the extracellular matrix, fibrillin acts as a reservoir for transforming growth factor-$\beta$ (TGF-$\beta$). Decreased deposition of fibrillin in the extracellular matrix leads to enhanced TGF-$\beta$ signaling and the resultant Marfan phenotype.\textsuperscript{16} The protein is modular comprising 47 epidermal growth factor-like (EGF) domains, 43 of which have calcium-binding consensus sequences, 7 transforming growth factor-$\beta$ (TGF-$\beta$) binding protein-like domains, 2 hybrid domains, 1 consensus sequences, 7 transforming growth factor-$\beta$ module comprising 47 epidermal growth factor-like domains. Mutations affecting the intracellular kinase domain of this protein are able to disturb TGF-$\beta$ signaling, which subsequently leads to features similar to those of MFS.

The 4 patients in our study all had TAADs and had been clinically diagnosed with suspected Marfan syndrome. The genetic testing was performed with mutation screening techniques like Sanger sequencing and next generation sequencing. Reverse transcription-PCR was used to explore the pathologic mechanisms of the 2 splice variants. Here we have reported 4 mutations including 2 novel splicing mutations, 1 novel missense mutation, and another missense mutation which has been reported in the HGMD database. The 3 novel mutations have not been registered in the Human Gene Mutation Database (HGMD) or in the Universal Mutation Database (UMD). The purpose of this study was twofold: first, to confirm the 2 novel splicing mutations as pathogenic mutations by testing the splicing change and the mRNA level; and second, to make a definite diagnosis for these patients and enlarge the mutation spectrum of $FBN1$ and $TGFBR2$. The findings will hopefully be helpful for prenatal diagnosis and genetic counseling.

**Methods**

**Subjects:** The subjects included 4 clinically affected individuals who had TAADs and were diagnosed with suspected Marfan syndrome and 50 randomly selected normal controls. The 4 patients requested molecular genetic testing because of a need for prenatal diagnosis. All of the participants were recruited at the Hunan Jiahui Genetic Hospital. Informed consent was obtained from all participants. The study was approved by the Ethics Committee of Hunan Jiahui Genetics Hospital.

**Patient 1:** Patient 1 is a 24-year-old male. He first went to the hospital because of chest pain. UCG revealed aortic dissection and an ascending aortic aneurysm. The aorta including Z-score is 2.67. The Z-score $\geq 2$ is a sign of aortic root dilatation and is one of the important features in the revised Ghent nosology. Thus, we need to calculate the aortic Z-score, which indicates the aortic root diameter at the sinuses of Valsalva when standardised to age and body size. Patient 1 was 190 cm in height, 75 kg in weight, and had an arm span of 1.88 cm. The US/LS of this patient was 0.70 (< 0.86) which is low. He also had scoliosis and high myopia. Upon initial examination, it was obvious he was thin and tall with elongated limbs, which is consistent with the features of MFS. His systemic score was 3 according to the revised Ghent nosology. The systemic score $\geq 7$ is a criteria required for the diagnosis of MFS. In addition to the two cardinal features of MFS (aortic root aneurysm/dissection and ectopia lentis), the systemic score system of the revised Ghent nosology includes all other manifestations of MFS, such as the skeleton, dura, skin and lungs. The systemic score $\geq 7$ is the presence of sufficient systemic findings which indicates systemic involvement.

**Patient 2:** Patient 2 is a 36-year-old female. The patient has always been taller than other children of the same age since a very young age. She felt pain in the chest and back when she was 28 years old. She was then diagnosed with suspected MFS because of aortic dissection and aortic regurgitation. She had undergone Bentall surgery and the CTA revealed aortic dissection. The patient also presented with mitral regurgitation and tricuspid regurgitation. Her aorta including Z-score is -0.28. She was 173 cm tall, weighed 55 kg, and had an arm span of 184 cm. Thus, the arm span to height ratio was almost 1.06 (> 1.05). She also presented with dolichocephaly, high myopia with strabismus and astigmatism, ptosis, skin striae, slender fingers and toes, and positive ‘thumb signs’ and ‘wrist signs’. The systemic score of this patient was 7.

**Patient 3:** Patient 3 is a 36-year-old female. Her initial presentation was chest pain and swallowing difficulty. She had undergone surgery because of aortic dissection. She presented with an enlarged left ventricle, ascending aortic aneurysm, and aortic valve insufficiency with regurgitation. Her aorta including Z-score is 13.95. She was 168 cm tall, weighed 65 kg, and had an arm span of 184 cm. The arm span to height ratio was almost 1.06 (> 1.05). She also presented with dolichocephaly, high myopia with strabismus and astigmatism, ptosis, skin striae, slender fingers and toes, and positive ‘thumb signs’ and ‘wrist signs’. The systemic score of this patient was 1. Her father had similar symptoms and died of a cardiovascular accident.

**Patient 4:** Patient 4 is a 9-year-old girl. She was clinically diagnosed with suspected Marfan syndrome according to the first impression. She was 144 cm tall and weighed 24 kg. Her father was 176 cm tall and weighed 53 kg. The phenotypes of this patient include strabismus, refractive error, pectus carinatum, scoliosis, arachnodactyly, and camptodactyly. Her ‘thumb signs’ and ‘wrist signs’ were positive. The patient’s main cardiovascular abnormalities were Sinus of Valsalva aneurysm, aortic root dilation, aortic regurgitation, atrial septal defect, patent fo-
ramen ovale, pulmonary artery dilatation, and tricuspid valve prolapse with regurgitation. Her aorta including Z-score is 4.25. Craniofacial abnormalities of the patient include bifid uvula, malar hypoplasia, and micrognathia (Figure 1). The systemic score in the revised Ghent nosology of the patient was 8. The father of this patient has mild craniofacial abnormalities, as well as mild aortic regurgitation, mitral regurgitation, and tricuspid regurgitation. He has an atrial septal aneurysm and his aorta including Z-score is 0.65.

**Methods:** Sanger sequencing or next generation sequencing (each variant needs to be subsequently confirmed by the gold standard Sanger sequencing) was used to test the mutations directly. Aiming at the likely pathogenic variants, qualitative analysis was performed with reverse transcription-PCR (RT-PCR) and gel electrophoresis, and quantitative analysis was used to determine the *FBN1* mRNA levels.

**Mutation analysis by direct sequencing:** Genomic DNA of all subjects was extracted from peripheral blood cells by the phenol/chloroform method. All 65 exons (RefSeq NM_000138) and intron-exon boundaries of *FBN1* in patient 1 were sequenced by Sanger sequencing. The primers are listed in the Supplemental Table. Comparatively long turnaround times and/or high cost are the major disadvantages of Sanger sequencing and screening of the *FBN1* gene is an arduous task because of its large size and complex mutational spectrum. Furthermore, *TGFBR2* aberrations were also found in a subset of MFS. We began to adopt next generation sequencing as a rapid and cost-effective method for genetically diagnosing. Patient 2 and patient 3 were tested by targeted next generation sequencing. A minimum of 3 μg gDNA from these patients was used to create the indexed Illumina DNA libraries. All the exons of the candidate genes involved in MFS and its related disorders were enriched using a GenCap custom enrichment kit (MyGenostics Inc., Beijing, China). The paired-end sequencing (100 bp) was performed on an Illumina HiSeq2000 sequencer (Illumina, San Diego, CA, USA) to provide a mean sequence coverage of more than 150 ×, with more than 95% of the target bases having at least 20× coverage. With the rapid development of biotechnology, we now prefer to use whole exome sequencing to raise the diagnostic detection rate. The exon-containing fragments of patient 4 were enriched with an xGen® Exome Research Panel (Integrated DNA Technologies, USA) and paired-end sequencing (150 bp) was performed on a Novaseq6000 (Illumina, San Diego, CA, USA) to provide a mean sequence coverage of more than 100 ×, with more than 95% of the target bases having at least 20 × coverage.

**Reverse transcription-polymerase chain reaction (RT-PCR) and sequencing:** Total RNA was isolated from cultured lymphocyte cell lines from patient 1, patient 2, and random control samples using TRIzol reagent as indicated in the manufacturer’s protocol. About 1 μg of total RNA from each sample was reverse transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Carlsbad, CA, USA). The cDNA was amplified using 2 pairs of primers (Table I) which were

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**Figure 1.** Special phenotypes of patient 4 and her father. A: Patient 4: bifid uvula (severe). B: Father of patient 4: bifid uvula (mild). C: Patient 4: arachnodactyly and camptodactyly.
were calculated according to the 2−ddCT method and the manufacturer’s protocol. The primer sequences of the gene exon5-6 are shown in Table I. Amplification levels of FBN1 mRNA levels were nearly 50 percent lower in patients than in normal controls. The splicing pattern of patient 1 and patient 2 by agarose gel electrophoresis (Figure 4A) and found that both had additional amplification products. Sanger sequencing of the PCR products verified the skipping of exons. There were 2 additional amplification products in patient 1; one skips exons 11 and 12 and the other skips exons 10, 11, and 12 (Figure 4B). The additional amplification product of patient 2 was a skipping of exon 58 (Figure 4B). Figure 4C shows a schematic diagram of the pattern of exon skipping.

RT-qPCR results: We performed RT-qPCR to compare the expression levels in the patients with FBN1 splicing mutations and in the normal controls. FBN1 mRNA levels were nearly 50 percent lower in patients than in normal controls, indicating that the splicing site mutations triggered NMD (P = 0.0021 and 0.0027, respectively; Student’s t-test; Figure 4D).

Somatic mosaicism results: When we analyzed the parental blood samples of patient 4 in order to confirm whether the change was de novo or inherited, we determined that patient 4 inherited the mutation from her father. Since there were complete heterozygous patterns of typical equal double peaks at an SNP (rs2228048) (Figure 3C) downstream of the mutation c.1084C>T in TGFBR2 of the father while the mutation still showed a relatively lower peak (Figure 3B) in all tissues tested (peripheral blood cells, buccal cells, hair root cells, nails and skin), this suggests the mosaicism was specific to the mutation.

Discussion

Currently, the clinical diagnosis of MFS mainly depends on the Ghent nosology. However, there are still...
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THREE NOVEL MUTATIONS IN FBN1 AND TGFBR2

Figure 2. Sanger sequencing results of 4 patients and their parents. A: Patient 1 is heterozygous for FBN1 splicing mutation c.1148-2A > T and his parents are normal. B: Patient 2 is heterozygous for FBN1 splicing mutation c.7204 + 1G > A and her parents are normal. C: Patient 3 is heterozygous for FBN1 missense mutation c.3725G > A and her mother is normal (the father’s DNA sample was not available). D: Patient 4 is heterozygous for TGFBR2 missense mutation c.1084C > T, her mother is normal while her father’s result implied mosaicism for the mutation.

some concerns that some of the diagnostic criteria have not been sufficiently validated because they are not applicable in children or necessitate expensive and specialized investigations. Especially taking into account that a wide variability of phenotypes in connective tissue disorders is well known and that a lot of MFS-related disorders share the same symptoms with MFS, thus, it is very important for clinicians to recognize this disease through a few main manifestations and to give the patients induction to further confirm it. The patients in our study were diagnosed with suspected Marfan syndrome at first. On the basis of genetic test results, patients 1 to 3 did receive an accurate diagnostic decision of MFS according to the revised Ghent nosology.

Most cases of MFS are caused by FBN1 mutations. However, TGFBR2 mutations were identified in a subset of patients with MFS; this syndrome was termed Marfan syndrome type 2 (MFS2). MFS type 2 (OMIM 154705) has now been moved to LDS2 (OMIM 610168) in “Online Mendelian Inheritance in Man” and TGFBR2 is its main disease-causing gene. This term is no longer in use because the term “LDS” rather than “MFS2” is more efficient to health care providers.

In our study, we identified 4 mutations, 3 of which are novel. The pathogenicity analysis is listed in Table II. The same sites with the 3 novel mutations of our study have been reported, but the nucleotide changes are different. The reports are from the screening of large numbers
of patients with no functional research to support the pathogenicity.\textsuperscript{21-23}

The 2 novel \textit{FBN1} splicing mutations in our study can cause truncated transcripts because of exon-skipping. The splicing mutation of patient 1 produced 2 types of truncated fibrillin-1 with the loss of TB2 and EGF-like 6 domains; patient 2 produced 1 truncated fibrillin-1 with the loss of TB9 domain. Truncated transcripts are usually degraded by the nonsense-mediated mRNA decay (NMD) mechanism, which would then result in almost no expression of truncated fibrillin-1 and thus ameliorating the negative effects of microfibrils on ECM. The mRNA levels of the patients are significantly lower than those of the normal controls, implying that haplo-insufficiency of \textit{FBN1} is the major disease mechanism due to NMD. There was a study which proved that true haplo-insufficiency is sufficient to cause MFS. Their mouse models suggested a critical threshold of functional microfibrils in the disease presentation of MFS and provided evidence for a critical contribution of haplo-insufficiency by showing that half-normal amounts of fibrillin-1 can be insufficient to initiate productive microfibrillar assembly.\textsuperscript{24}

The mutation of patient 3 is a missense mutation located at EGF-like 20 domain leading to cysteine being replaced by tyrosine. Missense mutations mainly influence the conformation of fibrillin-1 and matrix deposition, and cysteine residues are critical to those functions. Thus, missense mutations, especially cysteine-involved mutations leading to disorganized microfibrils, tend to cause ectopia lentis. This is maybe why only our patient 3 presented with lens subluxation. Another point we need to mention is that the primers designed for the amplicon \textit{FBN1}-exon56-60 were

\begin{figure}[h]
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\caption{Sanger sequencing results for other family members of patient 4. \textbf{A:} All family members from the father’s side of patient 4 showed no mutation at c.1084. \textbf{B:} The mutation c.1084C > T of TGFB2 in the father’s different tissues — relatively lower peak. \textbf{C:} The SNP (rs2228048) downstream of the mutation in the father’s different tissues—typical equal double peaks.}
\end{figure}
Patients with TGFBR2 and Marfan syndromes. Although group fulfilled the diagnostic criteria for both Loeys-Dietz TGFBR2 for Marfan syndrome. Three patients in the FBN1 patients with patients with study compared the clinical features and outcomes of 71 TGFBR2. A previous

Figure 4. RT-PCR and RT-qPCR results of two splicing mutations. A: Amplification result by using primer FBN1-exon8-13 shows that patient 1 has 2 additional amplification products compared with control. The 738 bp band represents the normal product, the 417 bp band represents the deletion of 2 exons, and the 258 bp band represents the deletion of 3 exons. Amplification result by using primer FBN1-exon56-60 shows that patient 2 has 1 additional amplification product compared with control. The 539 bp band represents the normal product, and the 332 bp band represents the deletion of 1 exon. B: Sanger sequencing results of cDNA product in patient 1 compared with normal show that the two additional amplification products skip 2 exons (exon 11, 12) or 3 exons (exon 10, 11, 12), respectively. The Sanger sequencing result of cDNA product in patient 2 compared with normal shows that the additional amplification product skips exon 58. C: Schematic diagram showing the pattern of exon skipping in patient 1 and patient 2. The arrows point out the locations of the splicing mutations which produce new truncated transcripts. D: RT-qPCR results of the 2 patients with splicing mutations and the normal control. FBN1 mRNA levels were nearly 50 percent lower in patients than in normal controls (Patient 1: ***P = 0.0021; Patient 1: **P = 0.0027).

verified using UCSC In-Silico PCR, and the result of the entire target sequence showed there are 2 isoforms while FBN1 only has 1 transcript. The lengths of the products were 539 bp and 601 bp, respectively. The latter product contains a 62 bp cryptic exon from intron 57, and this isoform has been reported in another study and in the Ensembl database. However, the proportion of this isoform varies strikingly between tissues and it represents a major FBN1 isoform in the brain. Maybe this is the reason why we have not observed the isoform in lymphocyte cell lines. Also, the protein encoded by the isoform is highly likely to be non-functional.

Patient 4, who was initially diagnosed with suspected MFS, now is regarded as having LDS2. The next generation result for her showed only a polymorphism in FBN1 and a likely pathogenic mutation in TGFBR2. A previous study compared the clinical features and outcomes of 71 patients with TGFBR2 mutations with those of 243 patients with FBN1 mutations. Seven (10%) of the 71 patients with TGFBR2 mutations fulfilled the Ghent criteria for Marfan syndrome. Three patients in the TGFBR2 group fulfilled the diagnostic criteria for both Loeys-Dietz and Marfan syndromes. Although TGFBR1/2 mutations have been identified in patients fulfilling the previous Ghent criteria who did not carry FBN1 mutations, after a deeper evaluation of the clinical data, some of these patients showed manifestations of LDS in addition to features of classical MFS. So when we made a further inquiry for patient 4 and took all phenotypes into consideration, we were inclined to diagnose the proband as LDS2 rather than MFS. Interestingly, the proband inherited the mutation from her father in whom no major symptoms but only mild abnormalities were recognized. This confused us at first because LDS2 is an autosomal dominant inherited disorder. We then found a report that described paternal somatic mosaicism of a TGFBR2 mutation and was similar with our case. Based on the genetic testing of different tissues of her father, we confirmed that the father carried the somatic mosaicism of a TGFBR2 mutation and passed it to the proband. The mosaicism may be the reason why the father showed no severe symptoms.

There have been few reports of MFS and LDS associated with mosaicism. In addition to the report mentioned above, the author also summarized 3 other mosaicism cases for MFS and 1 for LDS. A proband’s mother with 43% mutant cells in lymphoblasts and 51% mutant cells in fibroblasts only showed joint hypermobility, pes planus, and striae distensae. Another patient’s father with somatic
mosaic in blood leukocytes only showed discreet dilatation of the ascending aorta and minimal aortic regurgitation. The third patient’s father with somatic mosaicism in his blood, saliva, and semen did not show any MFS symptoms. As for LDS, somatic mosaicism of TGFBR2 in a father was only briefly mentioned. The father required aortic root replacement and had no craniofacial manifestations. In addition, a previous study described a mosaic splicing mutation in FBN1. The patient’s mother with somatic mosaicism showed aortic dissection.27) A neonatal form of MFS patient’s genetic examination documented a mosaicism in the maternal blood cells (10-25% of genomic DNA) and a clinical examination showed unilateral lens ectopy.28) Another patient’s father was asymptomatic, and approximately 25% of his body cells carry the mutation.29) The literature on mosaicism reveals that parent carriers have less severe phenotypes than probands or express no manifest phenotype.

The mutation of patient 4 was a missense mutation which has not been reported, and it has not been found in 1000 G or ExAC. To determine likely pathogenic novel mutations, the missense substitution of patient 4 was predicted and scored using the Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping v2 (PolyPhen-2) prediction algorithms. The predictions of this mutation from SIFT were “Deleterious” and “Damaging”. The prediction from PolyPhen-2 was “PROBABLY DAMAGING” and the sequence where the mutation occurred is highly conserved in different species (Figure 5A). We also used Combined Annotation Dependent Depletion (CADD) to predict the mutation and the score was 27.1. Considering the mutation located at the protein kinase domain of TGFBR2, we predicted the 3D structural change using Swiss-Model online software and compared it to the wild type, and the amino acid polarity was changed because histidine was substituted by tyrosine (Figure 5B). In vitro studies suggested that mutations in the exons encoding the protein kinase domain disturbed the kinase activity of

Figure 5. Conservation analysis and 3D structure prediction for the TGFBR2 mutation. A: The sequence where the mutation occurred is highly conserved in different species. The letters in different colors represent the protein differences. The location in the yellow background is where the mutation occurred. B: The 3D structure prediction of the wild type TGF-beta receptor type-2 and the mutation type TGF-beta receptor type-2. The amino acid polarity was changed because histidine was substituted by tyrosine.
TGFBR2 and internalization process of the receptor, suggesting the dominant effects of heterozygous mutations. Therefore, our conclusion is that the mutation is “likely pathogenic” for this family.

All patients in our study share the same main symptom - aortic disorder. TGF-β signaling regulates many cellular processes, including proliferation, cell cycle arrest, apoptosis, differentiation, and extracellular matrix (ECM) formation and remodeling. FBN1, TGFBR1, TGFBR2, and SMAD3 are the genes encoding proteins of the transforming growth factor-β (TGF-β) signaling pathway. And dysregulated TGF-β signaling likely contributes to the pathogenesis of thoracic aortic diseases. FBN1 encodes an extracellular matrix protein (fibrillin-1) that is a component of the elastic fibers in the medial layer of the aorta. TGF-β signaling is increased within the aortic wall of patients with MFS and this activation is one of the core mechanisms of MFS pathogenesis, causing metalloproteinase activation and ECM proteolysis. Cardiovascular pathologies are the most important clinical manifestations of MFS and MFS-related disorders. It can cause life-threatening events, such as acute aortic dissections and aortic rupture, which play important roles in the life expectancy of patients. The survival of patients has been significantly improved with innovations in the surgical management of aortic disease during the last 4 decades. Therefore, it is very important for doctors or genetic counselors to educate patients about these symptoms, especially those who have a family history.

Conclusion

Our study identified 3 novel mutations in patients with Marfan syndrome and Loeys-Dietz syndrome. We also revealed the pathogenicity of the 2 splicing mutations. This would expand the mutation spectrums of FBN1 and TGFBR2 and help us better understand the molecular basis of these diseases, as well as improve our understanding of genotype-phenotype correlations in the disease. The pathogenicity evaluation of all the novel mutations was made according to the latest standards and guidelines for the interpretation of sequence variants.31)

Our study has confirmed or corrected the clinical diagnosis. It will be helpful for the clinical diagnosis, prenatal diagnosis, genetic counseling, and medical care in patients with the same mutations. With respect to accurate diagnostic decision making, on the basis of our study, doctors can allay concerns regarding delayed or ambiguous diagnoses by providing specific recommendations for counseling and follow-up. Since all mutations tested are from Han Chinese patients, the pathogenicity of the mutations may be restricted to this specific ethnic group.

We also reported a somatic mosaicism of a TGFBR2 mutation. The information described should be of interest because it is quite useful for the counseling of patients or their families who have somatic mosaicism. This study also confirmed that parental mosaicism may be the cause of the varied phenotypic expression of these connective tissue disorders.

Disclosures

Conflicts of interest: The authors have no financial conflicts of interest to report.

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Supplemental Files
Supplemental Table
Please see supplemental file; https://doi.org/10.1536/ihj.18-046