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RESEARCH ARTICLE

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Overcoming challenges associated with identifying *FBN1* deep intronic variants through whole-genome sequencing

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Abstract

Background: Marfan syndrome (MFS), caused by pathogenic variants of *FBN1* (fibrillin-1), is a systemic connective tissue disorder with variable phenotypes and treatment responsiveness depending on the variant. However, a significant number of individuals with MFS remain genetically unexplained. In this study, we report novel pathogenic intronic variants in *FBN1* in two unrelated families with MFS.

Methods: We evaluated subjects with suspected MFS from two unrelated families using Sanger sequencing or multiplex ligation-dependent probe amplification of *FBN1* and/or panel-based next-generation sequencing. As no pathogenic variants were identified, whole-genome sequencing was performed. Identified variants were analyzed by reverse transcription-PCR and targeted sequencing of *FBN1* mRNA harvested from peripheral blood or skin fibroblasts obtained from affected probands.

Results: We found causative deep intronic variants, c.6163+1484A>T and c.5788+36C>A, in *FBN1*. The splicing analysis revealed an insertion of in-frame or out-of-frame intronic sequences of the *FBN1* transcript predicted to alter function of calcium-binding epidermal growth factor protein domain. Family members carrying c.6163+1484A>T had high systemic scores including prominent skeletal features and aortic dissection with lesser aortic dilatation. Family members carrying c.5788+36C>A had more severe aortic root dilatation without aortic dissection. Both families had ectopia lentis.

Conclusion: Variable penetrance of the phenotype and negative genetic testing in MFS families should raise the possibility of deep intronic *FBN1* variants and the need for additional molecular studies. This study expands the mutation spectrum of *FBN1* and points out the importance of intronic sequence analysis and the need for integrative functional studies in MFS diagnosis.

KEYWORDS
FBN1, Marfan syndrome, messenger RNA, phenotype, whole-genome sequencing

Jee Ah Kim and Mi-Ae Jang contributed equally to this work.

1 | INTRODUCTION

Marfan syndrome (MFS; MIM 154700) is a systemic connective tissue disorder with a high degree phenotypic variability, ranging from mild (features of MFS in one or a few systems) to severe and even rapidly progressive neonatal multi-organ disease.[1] Pathogenic variants of fibrillin-1 (*FBN1*; MIM 134797) which is the causative gene can lead to highly penetrant thoracic aortic aneurysms and dissections with ocular and skeletal complications.[2] Currently, more than 2800 pathogenic or likely pathogenic variants in *FBN1* have been reported in ClinVar, a public archive of human genetic variants (last accessed August 11, 2023).[3] Although there has been controversy regarding genotype-phenotype correlation, the phenotypic variability is attributed to pathogenic variants. For example, variants responsible for haploinsufficiency have been known to be more responsive to losartan therapy and associated with more severe cardiovascular phenotypes than in-frame variants.[4,5] Therefore, identifying the genetic cause is of utmost importance for patients with suspected MFS. It is not only relevant for diagnosis confirmation and genetic counselling but also increasingly useful for personalized medicine approaches including preventing drug treatment and aortic surgery planning.[5–8]

Diagnostic genetic testing such as sequencing for coding exons and canonical splice sites and deletion/duplication analyses have identified a pathogenic variant of *FBN1* in the majority of patients who meet the clinical diagnostic criteria for MFS.[1] However, a significant number of individuals with MFS, up to 8% of cases, remain genetically unexplained.[9] Genetic workup performed to find the genetic cause of MFS typically focuses on exonic region and therefore variants located in non-coding regions, such as the promotor region, 5′ untranslated region (UTR), 3′ UTR, and deep intronic region, are likely to be missed. In addition, as it is cumbersome to perform functional studies in the laboratory on variants suspected of having deleterious effects, only a few variants have been functionally proven by RNA studies. Nowadays, whole-genome sequencing (WGS) is increasingly recognized as a vital technique in diagnostic genetic screening of rare genetic diseases. Compared to whole exome sequencing and panel-based next-generation sequencing (NGS), WGS showed improved diagnostic yield by enabling identification of pathogenic variants in deep intronic regions,[10,11] structural variants,[12] and variants located in regulatory region.

Here, we report two unrelated non-consanguineous families with MFS associated with different novel pathogenic intronic variants in *FBN1*. These pathogenic variants could not be detected by Sanger sequencing or multiplex ligation-dependent probe amplification (MLPA) for *FBN1* and/or panel-based NGS but were identified through WGS. Further characterization of effects of these variants on mRNA allowed us to support their pathogenicity and possible use for patient and family management.

2 | MATERIALS AND METHODS

2.1 | Study population

Two unrelated families who had MFS-associated phenotypes were admitted to our institution for investigation in October 2013 and March 2020, respectively. Informed consent for genetic testing and research use of biological and related clinical data was obtained from all investigated subjects (IRB No.: SMC 2020-10-042 and SMC 2016-11-039).

2.2 | Sanger sequencing

Genomic DNA was extracted from individual's peripheral blood leukocytes. All 65 coding exons of *FBN1* were amplified by PCR using primers that we designed (available on request). PCR products were sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator Cycle sequencing kit (Applied Biosystems). Sequences were analyzed using Sequencher software (Gene Codes Corp., Ann Arbor, MI, USA) and compared with the reference sequence for *FBN1* (NM_000138.5).

2.3 | MLPA

To detect deletions or duplications in genes of *FBN1* and *TGFBR2* (MIM 190182), MLPA was performed using SALSA MLPA P065/P066 Marfan syndrome probe mixes (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions.

2.4 | Panel-based NGS assay

DNA underwent sequencing with gene panel designed to selectively capture known genes of *FBN1*, *TGFBR1* (MIM 190181), *TGFBR2*, *TGFB2* (MIM 190220), *TGFB3* (MIM 190230), *ACTA2* (MIM 102620), *MYH11* (MIM 160745), *SMAD3* (MIM 603109), *COL3A1* (MIM 120180), *SKI* (MIM 164780), and *SLC2A10* (MIM 606145) associated with syndromic and non-syndromic thoracic aneurysms and/or Marfanoid habitus. Targeted fragments were sequenced on a NextSeq 550 platform (Illumina, San Diego, CA, USA). Data analysis was performed on variants located within +/− 25 base pairs of the coding exon, considering population frequency, impact on the encoded protein, and conservation and expression of variant.

2.5 | WGS

Genomic DNA from peripheral blood was collected for WGS. After DNA fragmentation, preparation of the library was performed

without amplification. The library was paired-end sequenced using NovaSeq 6000 platform (Illumina) at a mean depth of 35×. The sequence reads were aligned to a human reference genome GRCh38 (hg38) using Burrows-Wheeler Alignment software (BWA-MEM, version 0.7.17). Subsequent preprocessing and variant calling were performed using Genome Analysis Toolkit (GATK, version 4.2.0). Variant annotation was performed using ANNOVAR and SnpEff for all genome sequences, including introns. In silico prediction for missense variant was based on REVEL, and in silico prediction for synonymous or intronic variants was based on SpliceAI. The 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology variant interpretation guidelines were used to determine the pathogenicity of variants.[13]

2.6 | Reverse transcription-PCR (RT-PCR) and RNA sequencing

Total RNA was extracted from lymphocyte fraction isolated from peripheral blood or skin fibroblasts using TRIzol method. Total RNA was reverse-transcribed into cDNA using an Omniscript reverse transcriptase kit (Qiagen, Hilden, Germany) and amplified using Platinum II Hotstartaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) with specific primers that we designed (Table S1).

3 | RESULTS

3.1 | Case reports

In Family A, the proband (III:2 of family A, Figure 1A) was an 18-year-old female, the second child of unrelated parents. Her mother (II:6) was 169 cm tall and affected by dissection of the ascending aorta, which required surgery at 46 years. Her father (II:7) and elder sister (III:1) were healthy. The proband (III:2) visited our institution for full cardiac assessment due to a previous diagnosis of aortic disease in her mother. Echocardiography and computed tomography angiography of the proband showed dilatation of the aortic root, 32.8 mm with *Z*-score of 2.2. An ophthalmologic examination revealed bilateral ectopia lentis and severe myopia. Physical examination of the proband included a height of 177.3 cm, a weight of 58 kg, an arm span of 176.5 cm, an arm span/height ratio of 0.98, positive wrist and thumb signs, reduced extension at elbows, joint hypermobility, and skin striae. On skeletal and central nervous system examinations, she revealed bilateral protrusio acetabuli and sacral dural ectasia. The combination of aortic root dilatation and 11 points of systemic features led to the clinical suspicion of MFS. Since then, she has been under preventive pharmacologic therapy. Current treatment includes atenolol 25 mg/day without significant side effects. The last echocardiography at 28 years showed a stable dilatation of the aortic root with normal ascending aorta and mild insufficiency of the tricuspid and mitral valve.

In the second family (family B), the proband (II:5, Figure 1B) was a 46-year-old female, the fifth child of unrelated parents. All siblings were healthy and none of them were tall. Her father (I:1) and mother (I:2) were healthy with heights of 165 and 150 cm, respectively. She had a 19-year-old daughter (III:1) who was 177 cm tall with a history of bilateral ectopia lentis diagnosis around the age of six. The proband (II:5) visited our institution for evaluation of chest and back pain that had persisted for a year. Echocardiography showed dilated aortic root with diameters of 73 mm (*Z*-score 15.9) and dilated left ventricular and atrial cavities. She underwent cardiac surgery involving composite graft replacement of the aortic valve, aortic root, and ascending aorta. Routine ophthalmologic assessments revealed bilateral ectopia lentis and severe myopia. Physical examination showed a height of 175 cm, a weight of 68 kg, an arm span of 180 cm, an arm span/height ratio of 1.03, positive thumb sign, plain flat foot, protrusion acetabuli, and skin striae. The presence of 7 points of systemic features, marked dilated aortic root diameter, and positive family history of ectopia lentis suggested a diagnosis of MFS. Current treatment includes carvedilol 8 mg/day and warfarin 6 mg/day without significant side effects. The last echocardiography at 48 years showed well-functioning prosthetic aortic valve and normalized left ventricular cavity size.

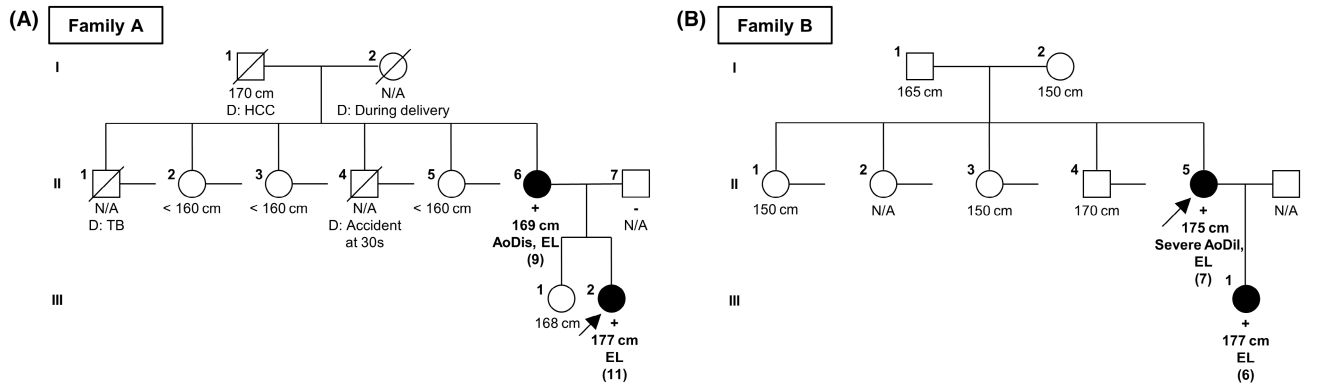
3.2 | Genetic investigations

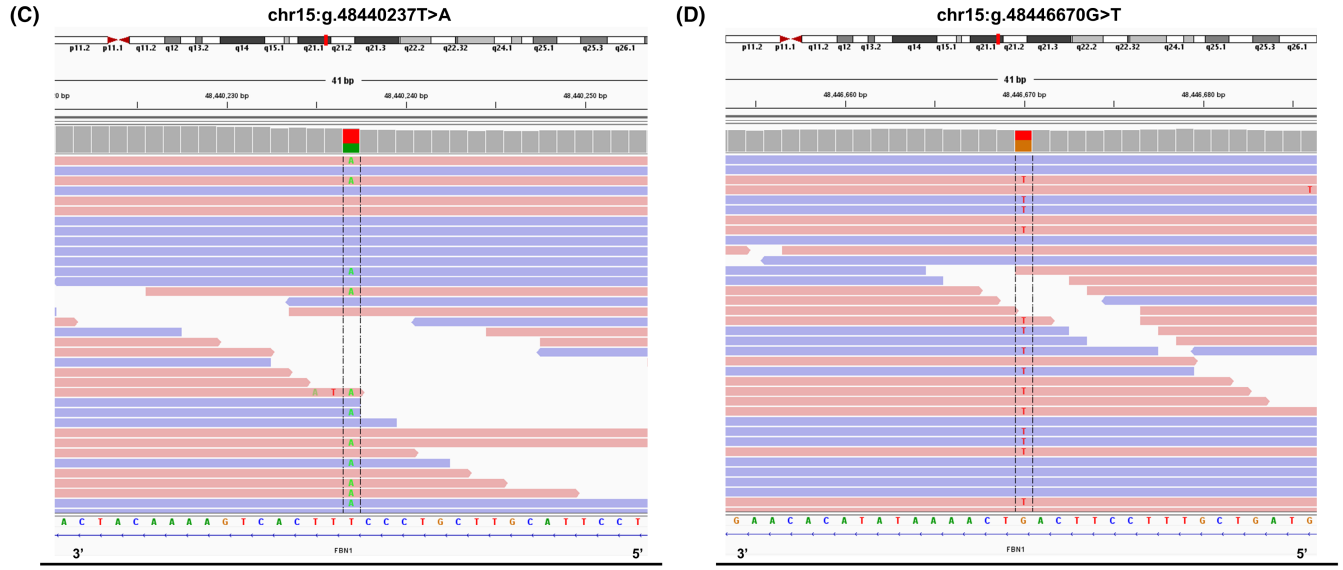
Sanger sequencing and MLPA for *FBN1* were performed for individuals suspected for MFS in Family A and B. No pathogenic variants nor significant pathogenic deletions or duplications of target genes were detected. In addition, we found no significant evidence of a genetic cause through panel-based NGS assay. We performed WGS for three individuals with suspected MFS, including II:6 and III:2 from family A and II:5 from family B, to explore the unexplained genetic cause underlying MFS.

In family A, a deep intronic variant of [NM_000138.5 (*FBN1*):c.6163+1484A>T] in intron 51 was the most interesting one as it was absent from the Genome Aggregation Database (gnomAD). In silico analysis (SpliceAI) predicted the creation of a cryptic acceptor splice site with a high prediction score of 0.69. Likewise, a cryptic donor splice site had a score of 0.87. The resulting mRNA was predicted to retain a 191 bp pseudoexon in intron 51 (Figure 1C).

In family B, we identified a variant in intron 47 of *FBN1*, NM_000138.5:c.5788+36C>A through WGS. It was absent from the gnomAD. SpliceAI predicted that this intronic variant, c.5788+36C>A, could lead to the creation of a cryptic donor splice site with a high prediction score of 0.97 (Figure 1D). Sanger sequencing of the target variant (c.5788+36C>A) was performed for III:1 and the variant was detected.

Based on RT-PCR and Sanger sequencing in family A (II:6, III:2), wild-type cDNA revealed a band of the expected size of 337 bp. However, the mutant cDNA had three thick bands of 337, 436, and 524 bp in size with other faint bands. The presence of the abnormal transcript of 436 bp in size indicated that the variant





variant	gene	Δ type	Δ score	pre-mRNA position
15-48440237-T-A	*FBN1* (ENSG00000166147.15 / ENST00000316623.10 / NM_000138.5)	Acceptor Loss	0.00	
		Donor Loss	0.00	-174 bp
		Acceptor Gain	0.69	192 bp
		Donor Gain	0.87	2 bp

variant	gene	Δ type	Δ score	pre-mRNA position
15-48446670-G-T	*FBN1* (ENSG00000166147.15 / ENST00000316623.10 / NM_000138.5)	Acceptor Loss	0.00	305 bp
		Donor Loss	0.10	36 bp
		Acceptor Gain	0.00	
		Donor Gain	0.97	3 bp

FIGURE 1 Two novel pathogenic *FBN1* intronic variants found in two families with Marfan syndrome by whole-genome sequencing. (A) Pedigree of family A. (B) Pedigree of family B. For individuals who have undergone a genetic test, their results are described with either "+" (variant detected) or "−" (variant not detected). For affected members, height, cardiovascular and ophthalmologic features, and systemic score (with bracket) are reported at the bottom of the symbol. (C) Integrative Genomics Viewer snapshot of a deep intronic variant of *FBN1* [NM_000138.5 (*FBN1*):c.6163+1484A>T] found in family A and in silico splice prediction. (D) An intronic variant of *FBN1* [NM_000138.5 (*FBN1*):c.5788+36C>A] found in family B and in silico splice prediction. AoDil, aortic root dilatation; AoDis, aortic dissection; D, cause of death; EL, ectopia lentis; HCC, hepatocellular carcinoma; N/A, not applicable; TB, tuberculosis.

NM_000138.5:c.6163+1484A>T has caused abnormal splicing of the mRNA, resulting in retention of 99 bp in length within intron 50 of the *FBN1* transcript. Another novel abnormal transcript of 524 bp in size indicated a pseudoexon with a length of 187 bp in intron 50 into the mature mRNA. This pseudoexon introduced a premature termination codon (PTC), p.(Asp2055AlafsTer7) that was predicted to lead to nonsense-mediated decay of the *FBN1* mRNA (Figure 2A,B).

In family B, RT-PCR for the proband (II:5)'s dermal fibroblast confirmed the alternative splicing in *FBN1* by showing an extra band that was approximately 30 bp longer than the wild-type cDNA. Sequencing of this fragment demonstrated retention of 33 bp between exon 47 and exon 48. This retention resulted in addition of 11 amino acids, p.(Asp930delinsGlyAlaCysAlaLysLeuCysIleSerLysGlyAsn), in the calcium-binding epidermal growth factor (cbEGF) domain of the protein that may alter its function (Figure 2C,D).

4 | DISCUSSION

With WGS and subsequent splicing analysis, we were able to finally establish the suspected genetic causes for two unrelated families

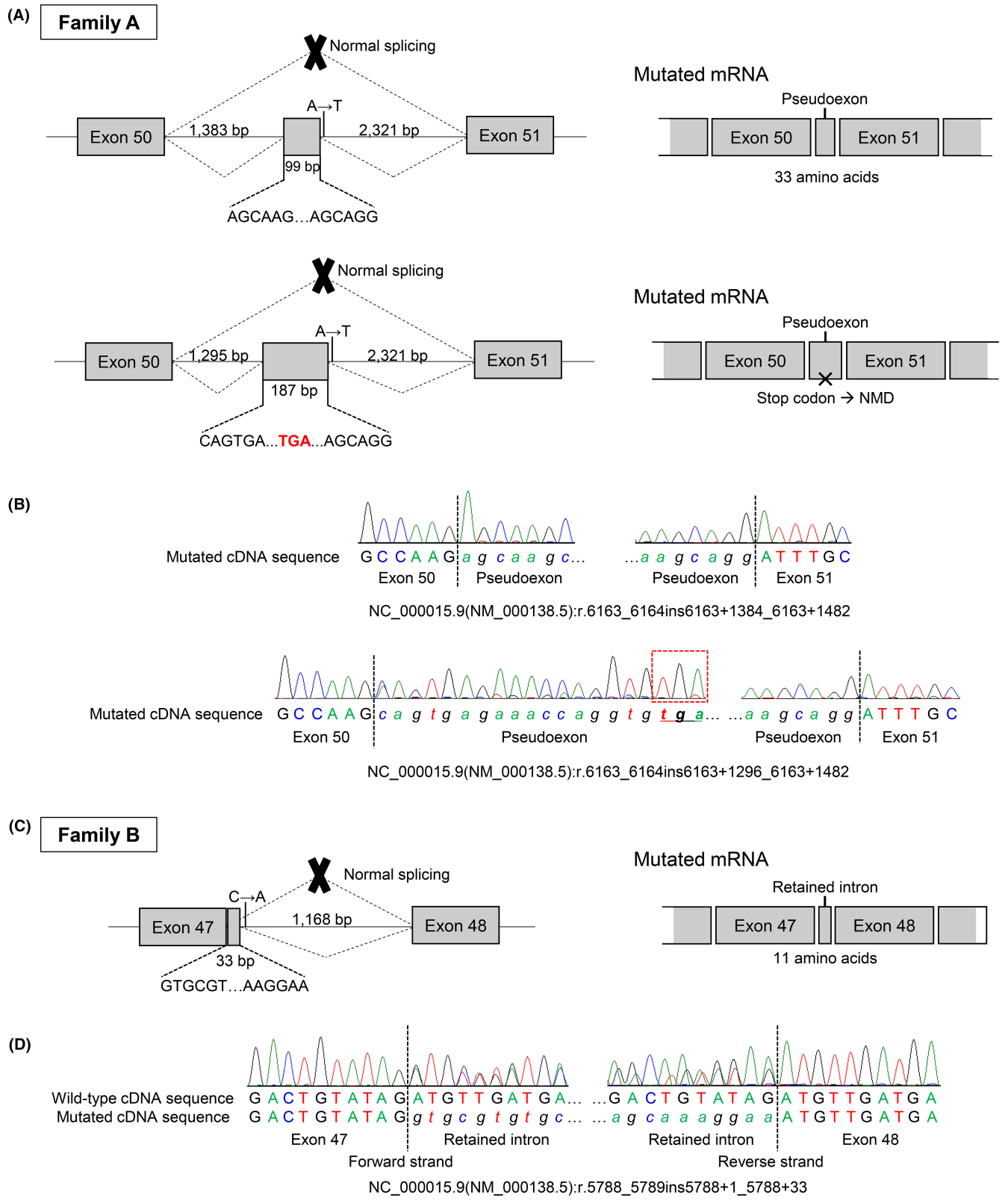


FIGURE 2 Schematic diagrams of targeted RNA sequencing and cDNA sequences of aberrant *FBN1* transcripts in two families. (A) In Family A, a 436 bp-sized band contained the 99 bp pseudoexon (the higher) and 524 bp-sized band contained the 187 bp pseudoexon (the lower). (B) The 99 bp pseudoexon (the higher) leads to the addition of 33 amino acids. The 187 bp pseudoexon (the lower) introduces a premature termination codon, which is predicted to lead to nonsense-mediated decay of the FBN1 mRNA. (C) In Family B, an aberrant *FBN1* transcript contained a 33 bp-long partial intronic retention. (D) Retained intronic sequence leads to addition of 11 amino acids in the calcium-binding epidermal growth factor domain.

with MFS and discovered novel pathogenic intronic variants leading to alternative splicing and retention of in-frame or out-of-frame intronic sequences. Non-coding variants that lead to pseudoexon formation, intron retention, and regulatory changes are not typically identified by exome sequencing or gene panels that focus on coding and canonical splice site regions.[14] In this report, we show that the detection of intronic *FBN1* pathogenic variants by WGS has important clinical implications in that it not only provides a definitive genetic diagnosis for patient but also enables initiation of medications to prevent progressive aortic root dilatation and proper care of complications such as ectopia lentis in patients and their family members with the same pathogenic variant.

TABLE 1 Systemic and clinical characteristics of patients with Marfan syndrome included in this study.

	Family A		Family B	
Category	**Proband (III:2)**	**Mother (II:6)**	**Proband (II:5)**	**Daughter (III:1)**
Observed *FBN1* variant (NM_000138.5)	c.6163+1484A>T		c.5788+36C>A	
General characteristics				
Current age (year)	28	56	48	21
Age at event or last aortic imaging (year)	18	46	46	19
Sex	Female	Female	Female	Female
Height (cm)	177	169	175	177
Arm span (cm)	174	182	180	184
Arm span/height ratio	0.98	1.08	1.03	1.04
Systemic and clinical features				
Cardiovascular				
Aortic root diameter (mm)	33	50	73	41
Z-score	2.2	7.0	15.9	4.4
Aortic dissection	−	+	−	−
Mitral valve prolapse	−	+	−	−
Preventive aortic surgery	−	+	+	−
Mitral valve surgery	−	+	+	−
Ophthalmologic				
Ectopia lentis (Rt./Lt.)	+/+	+/+	+/+	+/+
Ectopia lentis surgery	−	−	−	−
Severe myopia	+	−	+	+
Skeletal				
Wrist or thumb sign	+	+	+	+
Reduced elbow extension	+	N/A	−	+
Scoliosis	−	+	−	+
Pectus carinatum deformity	−	−	−	−
Pectus excavatum or chest asymmetry	−	−	−	−
Acetabular protrusion	+	+	+	−
Facial dysmorphia[a]	+	+	+	+
Hindfoot deformity	−	−	−	−
Flat feet	−	+	+	+
Skin				
Skin striae	+	+	+	N/A
Lung				
Spontaneous pneumothorax	−	−	−	−
Central Nervous System				
Dural ectasia	+	−	−	−
Systemic Score	11	9	7	6

Abbreviations: Lt., left; N/A, not applicable due to non-evaluation; Rt., right.

[a]Facial dysmorphia (3/5: dolichocephaly, malar hypoplasia, enophthalmos, retrognathia, downslanting palpebral fissures).

Intronic *FBN1* variants leading to introduction of aberrant transcripts are rare causes of MFS. However, the overall frequency of such pathogenic non-coding variants might have been underestimated because it is difficult to identify them by DNA sequencing of coding exons and their flanking sequences of *FBN1*.[15] Regarding the interpretation of these intronic variants, in silico tools such as SpliceAI, which we used in this study, might be the first approach to screen alternative splicing events. Recent recommendations suggest utilizing SpliceAI as a splice prediction tool as it has been demonstrated to have a wide range of applicability.[16–18] As for functional tests, the gold standard is analysis of patient RNA from the tissue of interest to confirm alternative splicing.[19] However, specific analysis of each identified variant can be time-consuming and difficult. Moreover, RNA analysis remains challenging given the need to obtain tissue samples. Based on our study results, SpliceAI accurately predicted changes in cryptic splice sites. However, for a deep intronic variant such as NM_000138.5 (*FBN1*):c.6163+1484A>T, prediction of the cryptic splice acceptor site (c.6163+1292) made by SpliceAI showed some differences (4 bp) from the actual cryptic splice acceptor site (c.6163+1296) proven by mRNA study. Screening for alternative splicing events using in silico tools such as SpliceAI for deep intronic variants can increase the diagnostic yield for Marfan syndrome. However, confirmation by functional testing remains necessary.

In our study, we found two novel intronic variants that introduced aberrant transcript of *FBN1*. Several studies have suggested a correlation between genotype and phenotype in Marfan syndrome patients with *FBN1* variants.[20,21] The proband (III:2) in family A carrying a deep intronic variant (c.6163+1484A>T) predicted to have PTC leading to nonsense-mediated decay had a high systemic score of 11, predominantly showing skeletal and central nervous system features (Table 1). The proband's mother (II:6) had cardiovascular event of aortic dissection with lesser aortic dilatation. On the other hand, the proband (II:5) in family B carrying an intronic variant (c.5788+36C>A) predicted to have amino acids elongation had more severe aortic root dilatation without aortic dissection. These findings are consistent with previous findings showing that patients with PTC variants have a higher frequency of skeletal involvement than those with in-frame variants.[20] Patients with PTC had a significantly higher risk of aortic dissection or surgery than those with in-frame variants as lower amounts of fibrillin-1 due to haploinsufficiency play an important role in the aortic wall.[21] Phenotypic effects of in-frame variants, particularly located in the well-conserved cbEGF domain, depend on cysteine content associated with disulfide-bridge formation. Correct cysteine localization and disulfide bonding are essential for structural integrity of fibrillin-1 in the aortic wall and the extracellular matrix surrounding the lens of the eye. These in-frame variants that create additional cysteine residues are responsible for the mild cardiovascular phenotype. The dominant negative effect of cysteine-producing or cysteine-disrupting variants increases the risk of ectopia lentis in patients with in-frame variants. However, in our cases, ectopia lentis appeared in both eyes regardless of the type of variant. The molecular mechanism by which *FBN1* intronic variants exert their deleterious effects through regulation of gene expression needs to be further elucidated.

Taken together, results from our work show that various penetrance of phenotype and negative genetic testing in MFS families require additional molecular workup, bearing in mind the possibility of deep intronic pathogenic variants. This study enlarges the mutation spectrum of *FBN1* and points out the importance of intronic sequence analysis and the need for integrative functional studies in MFS diagnosis.

AUTHOR CONTRIBUTION

JAK and M-AJ: Data curation, investigation, validation, visualization, writing-original draft; SYJ, D-KK, Y-GK, and J-WK: Sample collection, investigation; TKP and J-HJ: Conceptualization, supervision, writing-review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest relevant to this study to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Dietz H. FBN1-related Marfan syndrome. GeneReviews. [Cited 2023 Aug 11]. https://www.ncbi.nlm.nih.gov/books/NBK1335/
2. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet*. 2010;47:476-485.
3. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46:D1062-d1067.
4. Franken R, Teixido-Tura G, Brion M, et al. Relationship between fibrillin-1 genotype and severity of cardiovascular involvement in Marfan syndrome. *Heart*. 2017;103:1795-1799.
5. Wagner AH, Zaradzki M, Arif R, Remes A, Müller OJ, Kallenbach K. Marfan syndrome: a therapeutic challenge for long-term care. *Biochem Pharmacol*. 2019;164:53-63.

6. Franken R, den Hartog AW, Radonic T, et al. Beneficial outcome of losartan therapy depends on type of FBN1 mutation in Marfan syndrome. *Circ Cardiovasc Genet*. 2015;8:383-388.
7. Becerra-Muñoz VM, Gómez-Doblas JJ, Porras-Martín C, et al. The importance of genotype-phenotype correlation in the clinical management of Marfan syndrome. *Orphanet J Rare Dis*. 2018;13:16.
8. Kim N, Kong SY, Yoo J, Kim DH, Seo SH, Kim J. Current issues, challenges, and future perspectives of genetic counseling in Korea. *Ann Lab Med*. 2022;42:314-320.
9. Baetens M, Van Laer L, De Leeneer K, et al. Applying massive parallel sequencing to molecular diagnosis of Marfan and Loeys-Dietz syndromes. *Hum Mutat*. 2011;32:1053-1062.
10. Gillis E, Kempers M, Salemink S, et al. An FBN1 deep intronic mutation in a familial case of Marfan syndrome: an explanation for genetically unsolved cases? *Hum Mutat*. 2014;35:571-574.
11. Guo DC, Duan X, Mimnagh K, et al. An FBN1 deep intronic variant is associated with pseudoexon formation and a variable Marfan phenotype in a five generation family. *Clin Genet*. 2023;103:704-708.
12. Meester JAN, Hebert A, Loeys BL. Structural genomic variants in thoracic aortic disease. *Curr Opin Cardiol*. 2023;38:157-161.
13. 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;17:405-424.
14. Zhong Y, Xu F, Wu J, Schubert J, Li MM. Application of next generation sequencing in laboratory medicine. *Ann Lab Med*. 2021;41:25-43.
15. Guo DC, Gupta P, Tran-Fadulu V, et al. An FBN1 pseudoexon mutation in a patient with Marfan syndrome: confirmation of cryptic mutations leading to disease. *J Hum Genet*. 2008;53:1007-1011.
16. Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, et al. Predicting splicing from primary sequence with deep learning. *Cell*. 2019;176:535-548.e524.
17. Walker LC, Hoya M, Wiggins GAR, et al. Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing: recommendations from the ClinGen SVI splicing subgroup. *Am J Hum Genet*. 2023;110:1046-1067.
18. Ha C, Kim JW, Jang JH. Performance evaluation of SpliceAI for the prediction of splicing of NF1 variants. *Genes (Basel)*. 2021;12:12.
19. Gu H, Hong J, Lee W, Kim SB, Chun S, Min WK. RNA sequencing for elucidating an Intronic variant of uncertain significance (SDHD c.314+3A>T) in splicing site consensus sequences. *Ann Lab Med*. 2022;42:376-379.
20. Faivre L, Collod-Beroud G, Loeys BL, et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. *Am J Hum Genet*. 2007;81:454-466.
21. 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;23:1296-1304.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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