Received: 5 February 2020 | Revised: 16 March 2020 | Accepted: 20 March 2020

DOI: 10.1002/mgg3.1274

ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine Open Access WILEY

Three Novel Variants identified in *FBN1* and *TGFBR2* in seven Iranian families with suspected Marfan syndrome

Fatemeh Bitarafan[1] | **Ehsan Razmara**[2] | **Mehrnoosh Khodaeian**[3] | **Mohammad Keramatipour**[4] | **Alireza Kalhor**[5] | **Ehsan Jafarinia**[2] | **Masoud Garshasbi**[2]

[1]Department of Cellular and Molecular Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran

[2]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Teheran, Iran

[3]Department of Medical Genetics, DeNA Laboratory, Tehran, Iran

[4]Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[5]Department of medical science, Qom Branch, Islamic Azad University, Qom, Iran

Correspondence
Masoud Garshasbi, Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
Email: masoud.garshasbi@modares.ac.ir

Abstract

Background: Marfan syndrome (MFS) is a multi-systemic autosomal dominant disease of the connective tissue characterized by the early development of thoracic aneurysms/dissections, along with various manifestations of the ocular and skeletal systems. Due to the genetic and clinical heterogeneity, the clinical diagnosis of this disorder is challenging. Loss-of-function mutations in *FBN1* (encodes fibrillin-1) lead to MFS type 1. Also, similar mutations in transforming growth factor β receptor 2 (*TGFBR2)* gene cause MFS type 2. Both proteins involve in TGF-β signaling.

Methods: In this study, genetic screening using a panel involving 14 genes, especially *FBN1* and *TGFBR2*, were performed on seven representatives affected members of seven unrelated Iranian families suspected with MFS. To confirm the variants, Sanger sequencing was applied to other affected/unaffected members of the families.

Results: A total of 13 patients showed MFS manifestations. Using genetic screening, two novel and three previously reported variants in *FBN1* were identified. We also detected two variants (a novel and a previously reported variant) in the *TGFBR2* gene.

Conclusion: In this study, we introduce three novel variants identified through gene screening in seven Iranian MFS families. This report is expected to considerably improve genetic counseling for Iranian MFS families. Early precise molecular diagnosis can be helpful for better management and improving the life expectancy of these patients.

KEYWORDS

FBN1, Marfan syndrome, next-generation sequencing, novel variants, *TGFBR2*

1 | INTRODUCTION

Marfan syndrome (MFS; OMIM: 154700) and Marfan-associated disorders such as Loeys–Dietz syndrome (LDS; OMIM: 609192) are autosomal dominant connective tissue diseases (Cao et al., 2018). MFS with an incidence rate of at least one per 5,000 individuals is a pleiotropic genetic disorder with systemic involvement (Coucke, Van Acker, & De Paepe, 2006; Summers et al., 2012). The clinical diagnosis is primarily based on well-defined clinical

manifestations often involving cardiovascular, ocular, and also skeletal systems with broad clinical variability (Coucke et al., 2006).

Over 1,800 mutations in the fibrillin-1 (*FBN1*) gene (NM_000138.4) associated with MFS have been described. Various studies reported that around 91% of MFS cases were associated with *FBN1* mutations (MFS-1; OMIM: 134797) (B. Loeys et al., 2004), while a further small percentage of the patients had a mutation in the transforming growth factor β receptor 1 or 2 (*TGFBR1 or 2*) (MFS-2; OMIM 190182) (Waldmüller et al., 2007). MFS-2 is also known as LDS type II (Cao et al., 2018). The estimated incidence of LDS is not clear but it seems to be less frequent than MFS-1.

FBN1 encodes a large cysteine-rich glycoprotein known as fibrillin-1; this protein is a principal component of microfibril macromolecules of the extracellular matrix (ECM), which can be associated with elastin fibers (Frédéric et al., 2009). Fibrillins play essential roles as calcium-binding microfibrillar structural molecules and also function as a regulator of TGF-β signaling (Cao et al., 2018). Loss-of-function mutations, especially those that affect cysteine residues, cause disulfide-bond disruption and subsequently misalignment of microfibrils which lead to the aberrant TGF-β signaling (Shi et al., 2011). Misregulation of the TGF-β signaling pathway is also involved in MFS-2 (Cao et al., 2018; Dong et al., 2012; Kirby, Dietz, & Sponseller, 2018).

TGF-β receptor 2, encoded by *TGFBR2* (NM_003242.6), is a transmembrane serine-threonine kinase. To function appropriately, TGFBR2 forms a homodimer. Mutations affecting the intracellular protein kinase domains can disturb TGF-β signaling and subsequently lead to similar clinical manifestations to MFS-1. In actual fact, TGF-β signaling regulates many key cellular processes such as proliferation, cell differentiation, apoptosis, cell cycle arrest, ECM formation, and cell remodeling (Cao et al., 2018). Bone abnormalities in MFS and MFS-related disorders can cogently be associated with the deficits of the TGF-β signaling pathway which is crucial for chondro- and osteogenesis (Kirby et al., 2018; Razmara et al., 2019).

Although clinical evaluations have remained a cornerstone in the diagnostic decision-making process (Baetens et al., 2011), molecular genetic testing is becoming increasingly important. In other words, the clinical diagnosis of MFS and Marfan-related diseases can be challenging due to the age-related entity of some of the clinical manifestations, the variable phenotypic expression of the diseases, and their phenotypic similarities. Besides, it has been reported that the identified mutations scattered over the entire *FBN1* gene, are nearly always specific to each family and there are not located in a particular hot spot region (Coucke et al., 2006). Studies have revealed that around 25% of the patients show de novo mutations, often with more severe phenotypes in comparison with familial cases (Cao et al., 2018). Thus, molecular analysis can be helpful to make an accurate robust diagnosis (Coucke et al., 2006; Seo et al., 2018).

In this study, by using targeted next-generation sequencing, seven likely causative variants in seven unrelated Iranian families were identified in *FBN1* and *TGFBR2*, of which three variants were novel yet have not been reported. Further clinical assessments confirmed MFS in the patients.

2 | MATERIALS AND METHODS

2.1 | Patients and ethics statement

In this study, we enrolled seven unrelated Iranian families suspected with MFS who were referred to the Department of Medical Genetics, DeNA Laboratory, Tehran, Iran. The study protocol was approved by the local medical ethics committee of Tarbiat Modares University, Tehran, Iran. Written informed consent obtained either from all subjects or from their legal representatives. Furthermore, informed consent was obtained from the parents when patients were under 18.

Some cases were sporadic in which the familial history and also other family members' genotypes were negative for MFS and the variants, while other families had multiple affected members with a positive MFS history. The diagnosis of the disease was made based on the revised Ghent nosology for MFS (Cao et al., 2018; Chandra et al., 2015; B. L. Loeys et al., 2010). All of the clinical information and the medical histories were collected at the Department of Medical Genetics, DeNA Laboratory, Tehran, Iran.

2.2 | DNA extraction

Genomic DNAs were extracted from the peripheral blood of the patients and also all available family members by the High Pure PCR template preparation kit (Roche: Product No. 11814770001). Genetic screening using a panel to test 14 genes including *ACTA2, CBS, FBN1, FBN2, MYH11, COL3A1, SMAD3, TGFBR1, TGFBR2, MYLK, MSTN, COL5A2, TGFB2,* and *SLC2A10* was performed on the probands from seven unrelated Iranian families in BGI clinical laboratories.

2.3 | Targeted next-generation sequencing

The sequencing was performed according to a custom-designed Nimblegen chip capturing the aforementioned genes. In general, the test platform examined >95% of the target gene with sensitivity >99%. Point variants, micro-insertions, deletions, and duplications (<20 bp) could be simultaneously detected. For analysis of the sequencing results, the

TABLE 1 Identified variants in seven unrelated families. We summarized some of the key clinical findings

F	ID	Age	Sex	Gene	Exon	Nucleotide	Protein	Domain	F.H	N/R	Ref.	C	S	O	SK	O.M	Cys	Diagnosis
1	II.1	4	F	*TGFBR2*	4	c.1085A>G	p.(His362Arg)	PKD VIA	—	N	—	+	+	+	–	–	–	MFS-2
2	II.1	5	F	*TGFBR2*	5	c.1258G>A	p.(Gly420Arg)	PKD DVIII	—	R	Zarate, Sellars, Lepard, Tang, and Collins II (2016)	+	+	+	–	–	–	MFS-2
3	II.2	48	M	*FBN1*	15	c.1879C>T	p.(Arg627Cys)	cb EGF-like 06	—	R	Yang, Guo, Jiang, Gong, and Qu (2019)	+	+	+	–	–	+	MFS-1
4	II.5	43	M	*FBN1*	6	c.643C>T	p.(Arg215*)	Hyd module 01	NA	R	Jin et al. (2007)	+	–	+	–	–	–	MFS-1
5	II.2	8	F	*FBN1*	30	c.3833G>A	p.(Cys1278Tyr)	cb EGF-like 16	—	N	—	+	–	+	–	–	+	MFS-1
6	II.2	33	M	*FBN1*	50	c.6288C>A	p.(Cys2096*)	TGFBP 06	—	N	—	+	+	+	–	–	+	MFS-1
7	II.1	24	F	*FBN1*	55	c.6793T>G	p.(Cys2265Gly)	cb EGF-like 35	—	R	Franken et al. (2016)	+	+	+	–	–	+	MFS-1

Abbreviations: C, cardiovascular; Cys, affected Cysteine; F, Family ID; F.H, Family history; Hyd, hybrid module; N/R, Novel/Reported; O, Ocular; O.M, Other manifestations; PKD, protein kinase domain; Ref. , Reference; S, Skeletal; SK, Skin.

international Publicly available mutation and polymorphism databases such as 1000 genome project, ExAC (Exome Aggregation Consortium), ESP (Exon Sequencing Projects), Iranome (http://www.iranome.ir/), and also BGI self-developed local database were utilized. Only variants with minor allele frequency below 1 percent (MAF <1%) were selected. Previously reported mutations that have been described in HGMD (Human Gene Mutation Database) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) as pathogenic or likely pathogenic were given the highest priority.

2.4 | Co-segregation analysis

Samples from all available family members were subjected to Sanger sequencing to show whether the potential variants in the causative genes co-segregates with the MFS phenotype. Primers surrounding the region of the identified variant were designed by using Primer3.0 (version 0.4.0; http://bioinfo.ut.ee./primer3-0.4.0) (Table S1). PCR reaction was included 10–40 ng of genomic DNA with Taq DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany). PCR was performed in a standard condition (Esmaeilzadeh-Gharehdaghi, Razmara, Bitaraf, Mahmoudi, & Garshasbi, 2019), then PCR products were sequenced by ABI 3130XL, using the conventional capillary system; sequence traces were analyzed using the Sequencher 4.7 program (Gene Codes Corporation).

2.5 | In silico prediction

Prediction for the consequence of the variants was obtained from at least three online databases including SIFT, PolyPhen, and Provean. In terms of nonsense variants, we used ENTPRISE-X (http://cssb2.biology.gatech.edu/entprise-x/) to predict the pathogenicity of the variants. Additionally, to better show the potential pathological identity of the variants, the ConSurf (http://www.consurf.tau.ac.il) was applied to check the evolutionary conservation profile for FBN1 and TGFBR2 proteins. Additionally, UCSC databases (https://genome.ucsc.edu) were used to check the genomic conservation.

To predict the possible effects of the missense variants on protein conformation, the protein families and domains were analyzed using ScanProsite (Gattiker, Gasteiger, & Bairoch, 2002). Sequence alignments of the human TGFBR2 and FNB1 were recruited by using ClustalW (http://www.ebi.ac.uk/clustalw). Also, a BLAST sequence search against the protein data bank (PDB) was performed to select a template structure with the closest sequence similarity to the domains of TGFBR2 and FNB1. The protein structures were depicted by PyMOL (DeLano, 2002) after the structures were

recruited from SWISS-PROT (Boutet, Lieberherr, Tognolli, Schneider, & Bairoch, 2007) in a distinct PDB file.

2.6 | Prediction of single-point variation on protein stability

The I-Mutant2.0, which computes the ΔΔG values of protein variant, was utilized to predict and annotate the effect of the single nonsynonymous variants on the stability of the proteins from their sequences. I-Mutant2.0 (https://www.folding.biofold.org/i-mutant/i-mutant2.0.html) is based on the Support Vector Machine and ProTherm database (Bava, Gromiha, Uedaira, Kitajima, & Sarai, 2004) and is trained to predict the thermodynamic free energy change upon single-point variations in protein sequences.

2.7 | Prediction of effects of the variants on disulfide bonding state

Some of the identified variants influence Cysteine amino acids; to predict the possible effects of such substitutions on disulfide bonding state, the DISULFIND server (Ceroni, Passerini, Vullo, & Frasconi, 2006) was utilized. Just in case, DiANNA 1.1 webserver (Ferrè & Clote, 2006) was applied as well.

3 | RESULTS

3.1 | Clinical outcomes

We evaluated seven unrelated Iranian families suspected with MFS according to the revised Ghent criteria. Clinical manifestations are summarized in Table 1. All patients showed typical clinical features of MFS. Additionally, they underwent mutational screening using a panel involving 14 genes. The list of the identified variants in all patients is shown in Table 2. Blood samples from at least one other affected family member(s) available in seven families were investigated to find the segregated variants (Figures 1 and 2).

3.2 | In silico prediction

Various in silico predicting tools such as SIFT, Polyphen, Mutation taster, Provean, and ENTPRISE-X (Zhou, Gao, & Skolnick, 2018) were used to evaluate the possible pathogenicity of all variants. All detailed results are described in Table 2.

All variants were located in the extremely conserved protein regions, while p.(Arg627Cys) was an exception.

TABLE 2 Various online databases that used to predict the pathogenicity of the variant in the *FBN1* and *TGFBR2* genes. The annotation was performed according to the human genome 19 (hg19) dataset

Gene	NM	Nucleotide change	AA change	Variant Type	dbSNP ID	SIFT	Polyphen	Mutation Taster	Pmut	Mutation Assessor	Provean	I-Mutant2.0 (DDG)	PhastCons	ENTPRISE-X	Iranome	1k Genome	EXAC
TGFBR2	NM_003242.6	c.1085A>G	p.(His362Arg)	Nonsynonymous	—	D	P.D	D	D	M	D	−0.59	1	—	N.R	N.R	N.R
TGFBR2	NM_003242.6	c.1258G>A	p.(Gly420Arg)	Nonsynonymous	—	D	P.D	N	D	H	D	−0.83	1	—	N.R	N.R	N.R
FBN1	NM_000138.4	c.1879C>T	p.(Arg627Cys)	Nonsynonymous	—	D	P.D	D	D	—	D	−0.52	1	—	N.R	N.R	N.R
FBN1	NM_000138.4	c.643C>T	p.(Arg215*)	Stop Gain	rs111687884	D	—	D	—	—	—	—	1	D	N.R	N.R	N.R
FBN1	NM_000138.4	c.3833G>A	p.(Cys1278Tyr)	Nonsynonymous	—	D	P.D	D		—	D	−0.15	1	—	N.R	N.R	N.R
FBN1	NM_000138.4	c.6288C>A	p.(Cys2096*)	Stop Gain	—	D	—	D	—	—	—	—	1	D	N.R	N.R	N.R
FBN1	NM_000138.4	c.6793T>G	p.(Cys2265Gly)	Nonsynonymous	rs1057522272	D	P.D	D		—	D	−0.32	1	—	N.R	N.R	N.R

Abbreviations, D, Damaging; P.D, probable Damaging; M, moderate; H, high function; N, Neutral; N.R, Not Reported.

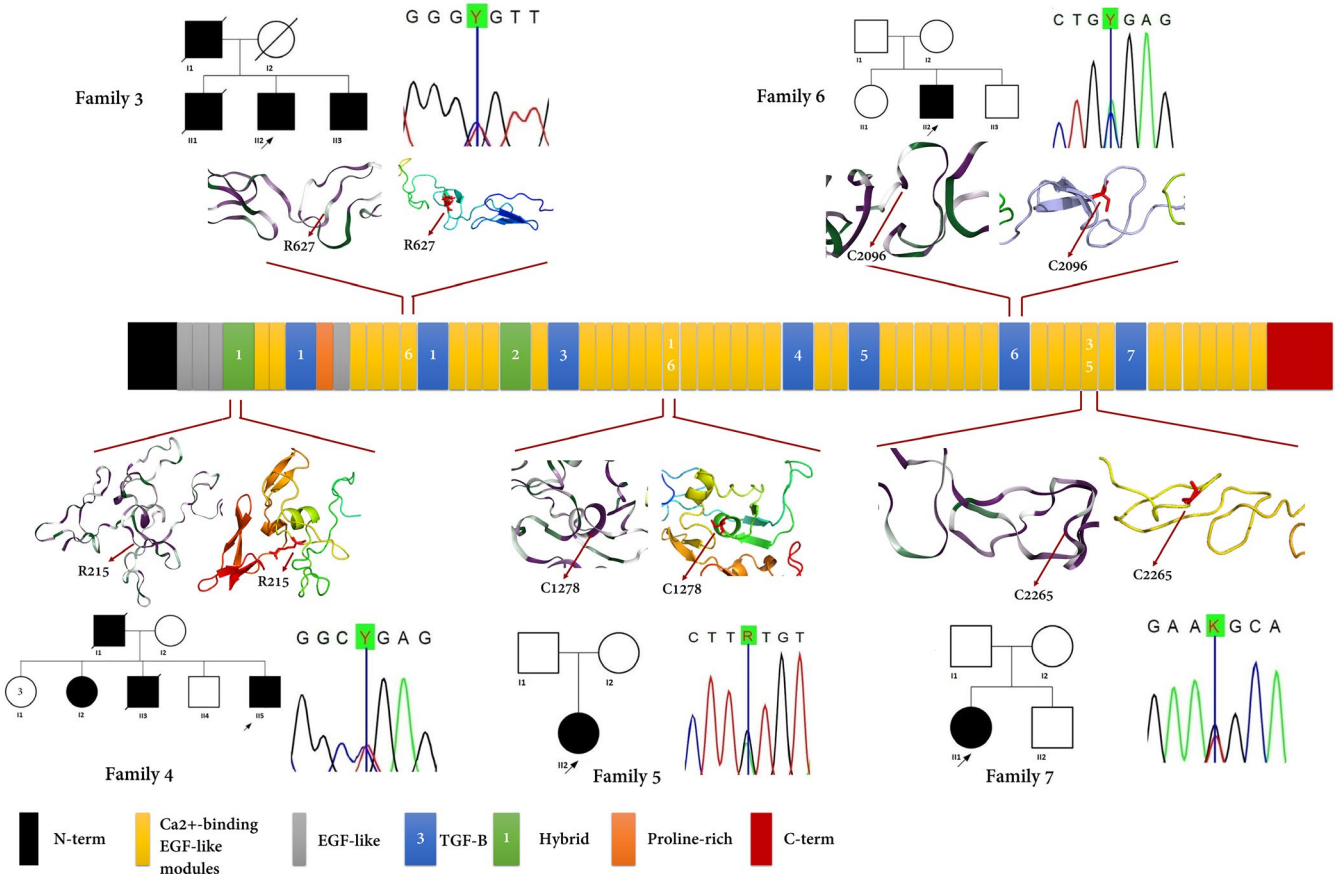

FIGURE 1 Pedigree information, variants spectrum, and three-dimensional (3D) protein modeling of fibrillin-1. This protein is composed of different domains as shown. The originally affected amino acids are shown in a 3D structure. The conservation structures are also indicated by ConSurf in 3D. Domains TGF-B3 to Ca^{2+} -binding EGF like module-18 form the "neonatal" region, in which substitutions leading to the severe neonatal form of MFS. In the figure, C: Cytosine, R: Arginine, C-term: C-terminal, and N-term: N-terminal domain (the protein domains are redrawn from [Handford, 2000]). In the pedigrees, white symbols: unaffected; black symbol: affected; squares: men; circles: females; Chromatograms showing substitutions in the probands.

p.(Arg627Cys) is a highly conserved amino acid in primates, not invertebrates. All identified variants change the high conserved nucleotides, confirmed by the UCSC database, although in amino acid levels there were some fluctuations (Figure 3).

Data of I-Mutant2.0 demonstrated all missense identified variants cause to reduce protein flexibility and stability (DDG < 0; Table 2).

DISULFIND and DiANNA 1.1 web servers were used to predict the possible effects of the variants on the disulfide bonding state. Data showed that p.(Cys1278Tyr), p.(Cys2096*), and p.(Cys2265Gly) variants may cause to disrupt the conformation of fibrillin-1. Thus, these cysteines are essential in forming disulfide bonds.

4 | DISCUSSION

According to the UMD-FBN1 mutation database (Collod-Béroud et al., 2003), which is the currently the most extensive *FBN1* mutation database, in total 1,847 different mutations in *FBN1* have been reported in which missense, small deletions, nonsense, and splice sites mutations with 1,015, 268, 210, and 200 reports, respectively, are the frequent alternation types (www.umd.be/FBN1). Studies have revealed that mutations are scattered throughout the entire length of the FBN1 gene (Coucke et al., 2006). Some mutations in fibrillin lead to the disruption of microfibril formation and fibrillin protein abnormalities, so causing the connective tissue disorders (Child, Aragon-Martin, & Sage, 2016; Ferrè & Clote, 2006). Moreover, to date, 111 different mutations including 93 missense mutations have been reported in the UMD-TGFBR2 mutations database (www.umd.be/TGFBR2). Spontaneous cases of MFS justifies around 25% of the total MFS cases; it has been proven that these spontaneous cases show severe clinically important phenotypes compared to the familial ones (reviewed in [Child et al., 2016]).

The fibrillin-1 is a multi-domain protein composed of 47 epidermal growth factor (EGF)-like domains, 43 calcium-binding (cb-EGF), seven TGF (transforming growth

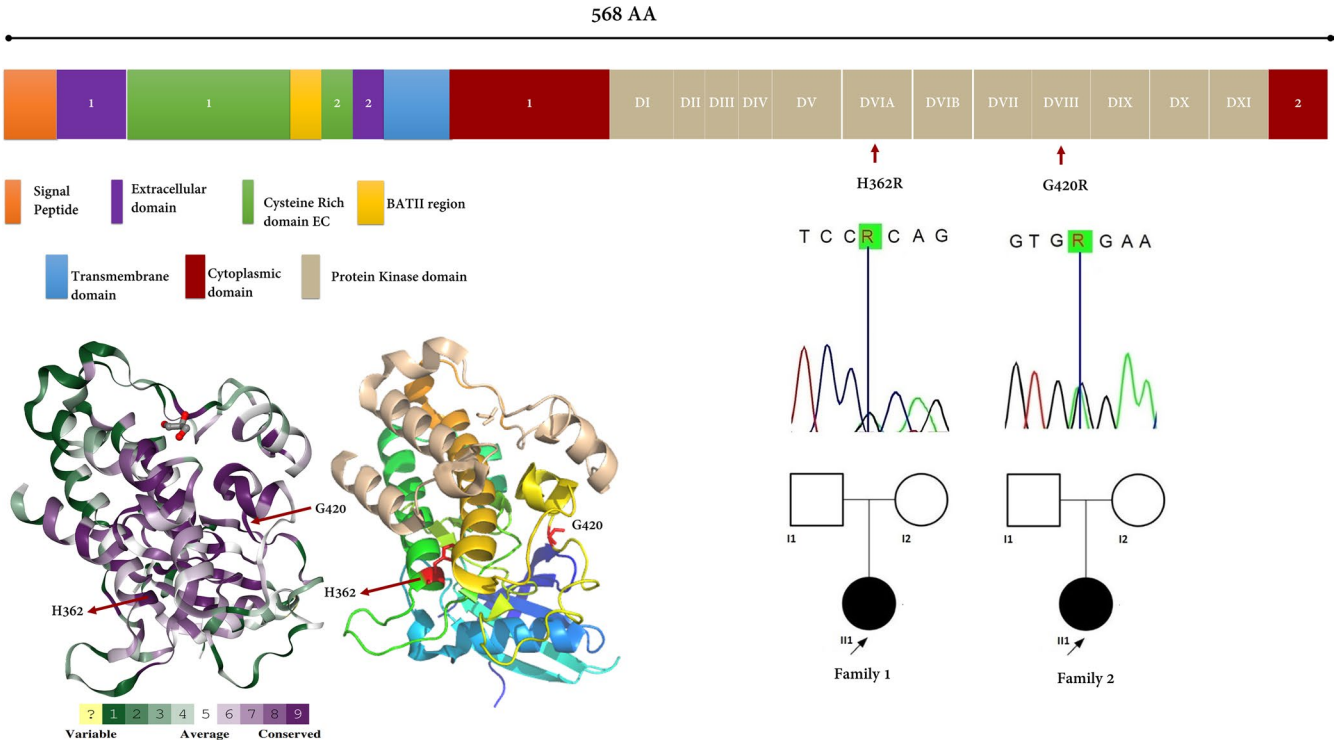

FIGURE 2 Organization of the TGFBR2 protein (redrawn from [Wang et al., 2013]) showing the position of the variants. The conservational structure provided by the ConSurf server is shown. The position of the identified variants is shown in red in the 3D structure of TGFBR2. Pedigrees and chromatograms are also indicated.

factor)-β-binding (TB) domains, two hybrid domains, an N-terminal, and a C-terminal domain (Figure 2). Each cb-EGF and TGF-beta binding domain contains six and eight highly conserved cysteine residues, respectively. Disulfide bonds between these cysteines play a key role in forming a proper three-dimensional structure of the protein. It has been shown that mutations affecting these cysteines and consequently the disulfide bonds most likely affect the function of the protein and make it more vulnerable to proteolysis (Liu et al., 2015; Muiño-Mosquera et al., 2018). Thus, substitutions in which cysteines would be removed or added have detrimental functional consequences. The loss or addition of cysteine residues will result in misfolded protein which may be degraded by intracellular proteases or retained within the cell (reviewed in [Child et al., 2016]).

All the missense variants found in the *FBN1*, namely p.(Arg627Cys), p.(Cys1278Tyr), and p.(Cys2265Gly), affect the cysteine amino acids which are important to the three-dimensional structure of fibrillin proteins. It has been reported that p.(Cys2265Gly) is associated with MFS (Franken et al., 2016) and is categorized as likely pathogenic variant. To support this notion, we provided further clinical evidence and data derived from in silico prediction. According to the in silico predictions, both p.(Cys1278Tyr) and p.(Cys2265Gly) are potentially disrupting disulfide bonds with high predicted confidence. This leads to changing the conformation of TGFBR2 which in turn can affect the proper functions of this protein (Dietz, Saraiva, Pyeritz, Cutting, & Francomano, 1992). Although according to the DiANNA and DISULFIND databases p.(Arg627Cys) could not affect any structural cysteines, it seems that this variant is located adjacent to Ca^{2+} binding EGF-like domain which in turn may affect the protein's affinity to the Ca^{2+} (Collod-Béroud et al., 2003); however, further investigations are necessary to validate the kinds of conclusions that can be drawn from this study.

The cb-EGF domains contain specific calcium-binding sites playing crucial roles such as maintaining the structural stability of the protein, protecting against degradation, and regulating interaction with other components of the extracellular matrix (Muiño-Mosquera et al., 2018). Thus, they can lead to the formation of an unstable protein with a tendency to undergo degradation. Accordingly, the majority of the missense mutations reported in previous studies affect highly conserved cysteines in the cb-EGF modules (Dong et al., 2012; Seo et al., 2018).

Consistent with previous studies, variants identified in this study were spreading throughout all exons of *FBN1*, not in a hotspot region (Coucke et al., 2006). The two nonsense variants, p.(Arg215*) and p.(Cys2096*), affect the Hybrid module and TGFBP domains of FBN1, respectively. We propose two mechanisms by which these variants can impose their effects: haploinsufficiency and impaired downstream functional protein. The variants may lead to the production of a truncated protein with loss of downstream functional domain

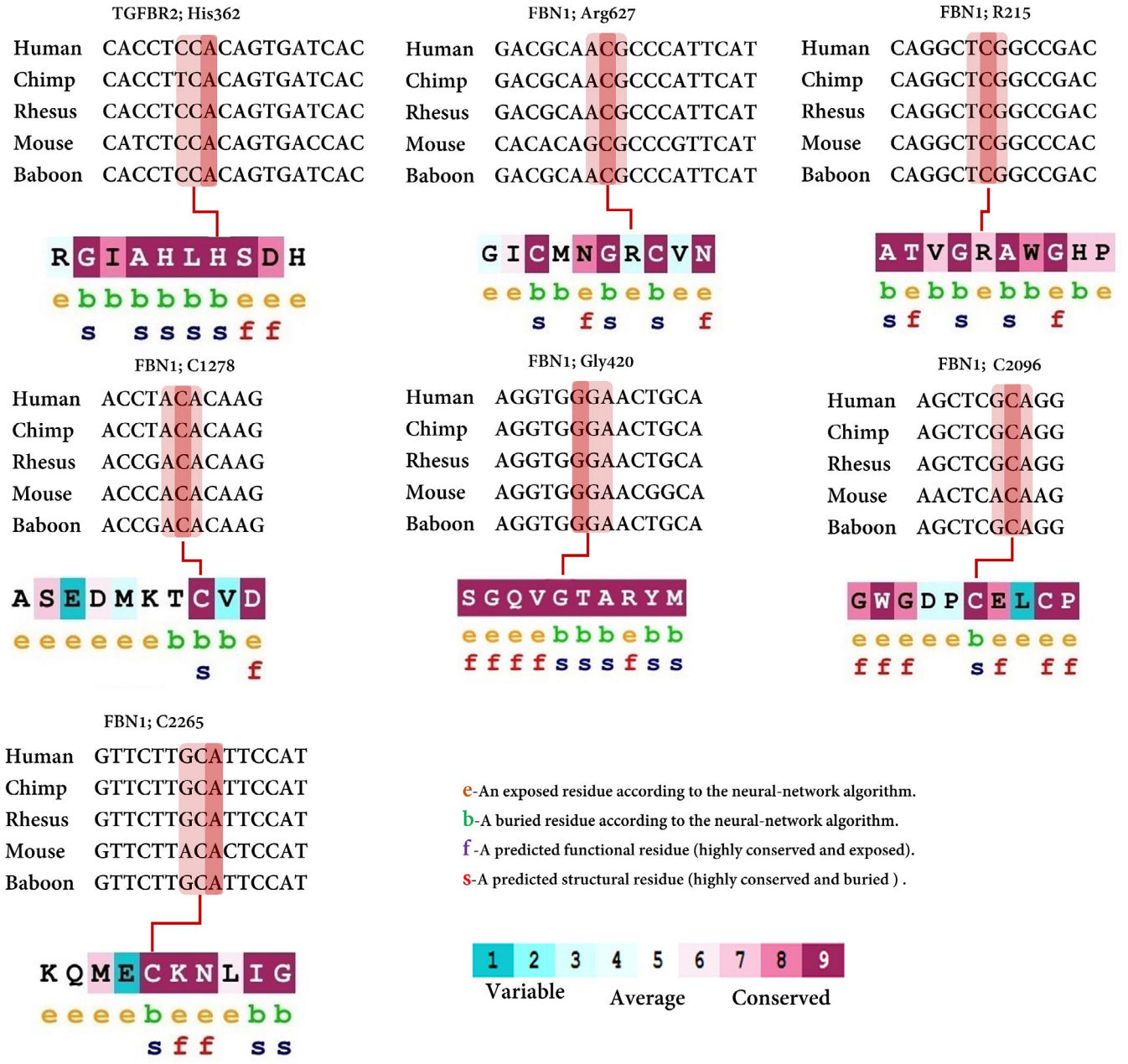

FIGURE 3 Nucleotides alignment using the UCSC database showing high conservation of the codon residue in vertebrates regarding the variants. The ConSurf server was used to calculate conservation scores for the amino acid residue affected by the missense variants. Scores ranged from 1–9, where a score of 9 represented a highly conserved residue. ConSurf demonstrates evolutionary conservation profiles for proteins of known structure in the protein data bank (PDB) according to the phylogenetic relations between homologous sequences as well as amino acid's structural and functional importance. The affected nucleotides are highlighted in red.

in the cell; as a result, cells should forestall this process by Nonsense-Mediated Decay response (NMD response) which is increasingly appreciated as one of the central mechanisms of RNA surveillance, with a great role in the physiological control of gene expression. Secondly, the main cause of MFS is thought to be haploinsufficiency of fibrillin proteins; these proteins provide stretch and elasticity to the connective tissues and are also involved in TGF- β1 signaling. Decreased fibrillin in the ECM leads to weakening the tissues and causes MFS phenotypes (Cao et al., 2018).

TGFBR2 encodes a receptor that plays a substantial role in TGF-β signaling. The TGFBR2 protein composed of several subdomains including the N-terminal domain or a signal peptide from codon 1 to 22, a cysteine-rich extracellular domain from codon 51 to 142, a transmembrane serine/threonine-protein kinase catalytic domain from codons 246–544, and the C-terminal. The kinase catalytic domain is encoded by exons 4–7. Seven kinase subdomains, I to VII, are coded by exon 4, two kinase subdomains, VIII and IX are coded by exon 5, and subdomains X and XI are coded by exon 6 and

7, respectively (Takenoshita et al., 1996). Los-of-function mutations in the *TGFBR2* cause disturbing TGF-β signaling. Mutations, affecting the intracellular kinase domain of this protein, lead to similar clinical features to those of MFS-1 (Cao et al., 2018).

The two identified variants in the *TGFBR2* gene, p.(His-362Arg) and p.(Gly420Arg), affect the evolutionarily conserved amino acids of TGFBR2 and can influence protein stability or affect the protein functions (Pannu et al., 2005). We conjectured that these variants may affect the serine/threonine-protein kinase catalytic domains (codons 246–544). The p.(His362Arg) is located inside the protein kinase domain VI, while the p.(Gly420Arg) is placed in protein kinase domain VIII. They may disrupt the serine-threonine kinase domain of the protein which in turn leads to diminishing TGF-β signaling (Takenoshita et al., 1996) and causes MFS-2. On the other hand, these variants can also change the protein's affinity to bind each other and form a homodimer that is required for the proper function. However, further investigations (functional analysis) are necessary to find the underlying mechanisms.

We believe that the molecular characterization of patients with MFS would give novel insights into the complex role of fibrillins and TGF-β signaling in other human disorders. It can also help to improve the development of drugs by better understanding the pathophysiology of MFS (van der Feltz,).

5 | CONCLUSION

In this study, we investigated seven families suspicious of MFS-1, even though molecular studies indicated that two of them affected by MFS-2. This study identified two novel variants in *FBN1* and also a novel variant in *TGFBR2*. We believe that these variants can broaden the mutation spectrum of Iranian patients. We also are optimistic that early precise molecular diagnosis can be helpful to reduce morbidity and mortality from life-threatening manifestations such as cardiovascular complications.

ACKNOWLEDGEMENT

We thank the families for their contribution to this study. We are especially grateful to the staff of the DeNA laboratory for helping us in this research. This research received no specific grant from any funding agency, commercial or not for profit sectors.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

MG and FB conceived and designed the experiments. MG and MK contributed to data collection. FB, ER, MKH, AK, and EJ wrote the paper. MG supervised the work. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

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

ORCID

Fatemeh Bitarafan https://orcid.org/0000-0002-4489-2923
Ehsan Razmara https://orcid.org/0000-0001-9926-3975
Mohammad Keramatipour http://orcid.org/0000-0002-5803-4209
Masoud Garshasbi https://orcid.org/0000-0002-5508-7903

REFERENCES

Baetens, M., Van Laer, L., De Leeneer, K., Hellemans, J., De Schrijver, J., Van De Voorde, H., … Coucke, P. J. (2011). Applying massive parallel sequencing to molecular diagnosis of Marfan and Loeys-Dietz syndromes. *Human Mutation*, *32*(9), 1053–1062. https://doi.org/10.1002/humu.21525

Bava, K. A., Gromiha, M. M., Uedaira, H., Kitajima, K., & Sarai, A. (2004). ProTherm, version 4.0: Thermodynamic database for proteins and mutants. *Nucleic Acids Research*, *32*(suppl_1), D120–D121.

Boutet, E., Lieberherr, D., Tognolli, M., Schneider, M., & Bairoch, A. (2007). Uniprotkb/swiss-prot. In D. Edwards (Ed.), *Plant Bioinformatics*, Methods in Molecular Biology, *406*, 89–112).: Humana Press. https://doi.org/10.1007/978-1-59745-535-0_4

Cao, Y., Tan, H., Li, Z., Linpeng, S., Long, X., Liang, D., & Wu, L. (2018). Three novel mutations in FBN1 and TGFBR2 in patients with the syndromic form of thoracic aortic aneurysms and dissections. *International Heart Journal*, *59*, 1059–1068. https://doi.org/10.1536/ihj.18-046

Ceroni, A., Passerini, A., Vullo, A., & Frasconi, P. (2006). DISULFIND: A disulfide bonding state and cysteine connectivity prediction server. *Nucleic Acids Research*, *34*(suppl 2), W177–W181. https://doi.org/10.1093/nar/gkl266

Chandra, A., Patel, D., Aragon-Martin, J. A., Pinard, A., Collod-Béroud, G., Comeglio, P., … Arno, G. (2015). The revised ghent nosology; reclassifying isolated ectopia lentis. *Clinical Genetics*, *87*(3), 284–287. https://doi.org/10.1111/cge.12358

Child, A. H., Aragon-Martin, J. A., & Sage, K. (2016). Genetic testing in Marfan syndrome. *British Journal of Hospital Medicine*, *77*(1), 38–41. https://doi.org/10.12968/hmed.2016.77.1.38

Collod-Béroud, G., Le Bourdelles, S., Ades, L., Ala-Kokko, L., Booms, P., Boxer, M., … Hyland, J. C. (2003). Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Human Mutation*, *22*(3), 199–208.

Coucke, P., Van Acker, P., & De Paepe, A. (2006). Mutation analysis of the FBN1 gene in patients with Marfan syndrome. In M. Kearns-Jonker (Ed.), *Congenital Heart Disease*, Methods in Molecular Medicine, *126*, 81–95). Humana Press. https://doi.org/10.1385/1-59745-088-X:81.

DeLano, W. L. (2002). Pymol: An open-source Molecular Graphics Tool. *CCP4 Newsletter on Protein Crystallography*, *40*(1), 82–92.

Dietz, H. C., Saraiva, J. M., Pyeritz, R. E., Cutting, G. R., & Francomano, C. A. (1992). Clustering of fibrillin (FBN1) missense mutations in Marfan

syndrome patients at cysteine residues in EGF-like domains. *Human Mutation*, *1*(5), 366–374. https://doi.org/10.1002/humu.1380010504

Dong, J., Bu, J., Du, W., Li, Y., Jia, Y., Li, J., … Zhou, A. (2012). A new novel mutation in FBN1 causes autosomal dominant Marfan syndrome in a Chinese family. *Molecular Vision*, *18*, 81.

Esmaeilzadeh-Gharehdaghi, E., Razmara, E., Bitaraf, A., Mahmoudi, M., & Garshasbi, M. (2019). S3440P substitution in C-Terminal region of human reelin dramatically impairs secretion of reelin from HEK 293T cells. *Cellular and Molecular Biology*, *65*(6), 12. https://doi.org/10.14715/cmb/2019.65.6.3

Ferrè, F., & Clote, P. (2006). DiANNA 1.1: An extension of the DiANNA web server for ternary cysteine classification. *Nucleic Acids Research*, *34*(suppl 2), W182–W185. https://doi.org/10.1093/nar/gkl189

Franken, R., Groenink, M., deWaard, V., Feenstra, H. M. A., Scholte, A. J., van denBerg, M. P., … Mulder, B. J. M. (2016). Genotype impacts survival in Marfan syndrome. *European Heart Journal*, *37*(43), 3285–3290. https://doi.org/10.1093/eurheartj/ehv739

Frédéric, M. Y., Monino, C., Marschall, C., Hamroun, D., Faivre, L., Jondeau, G., … Binquet, C. (2009). The FBN2 gene: New mutations, locus-specific database (Universal Mutation Database FBN2), and genotype-phenotype correlations. *Human Mutation*, *30*(2), 181–190. https://doi.org/10.1002/humu.20794

Gattiker, A., Gasteiger, E., & Bairoch, A. M. (2002). ScanProsite: A reference implementation of a PROSITE scanning tool. *Applied Bioinformatics*, *1*(2), 107–108.

Handford, P. (2000). Fibrillin-1, a calcium binding protein of extracellular matrix. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research*,, *1498*(2–3), 84–90. https://doi.org/10.1016/S0167-4889(00)00085-9

Jin, C., Yao, K., Jiang, J., Tang, X., Shentu, X., & Wu, R. (2007). Novel FBN1 mutations associated with predominant ectopia lentis and marfanoid habitus in Chinese patients. *Mol vis*, *13*(1280), 4.

Kirby, D. J., Dietz, H. C., & Sponseller, P. D. (2018). Spondylolisthesis is common, early, and severe in Loeys-Dietz syndrome. *Journal of Pediatric Orthopaedics*, *38*(8), e455–e461. https://doi.org/10.1097/BPO.0000000000001203

Liu, W., Zhao, N., Li, X.-F., Wang, H., Sui, Y. U., Lu, Y.-P., … Jiang, M. (2015). A novel FBN2 mutation in a Chinese family with congenital contractural arachnodactyly. *FEBS Open Bio*, *5*(1), 163–166. https://doi.org/10.1016/j.fob.2015.02.005

Loeys, B., De Backer, J., Van Acker, P., Wettinck, K., Pals, G., Nuytinck, L., … De Paepe, A. (2004). Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. *Human Mutation*, *24*(2), 140–146. https://doi.org/10.1002/humu.20070

Loeys, B. L., Dietz, H. C., Braverman, A. C., Callewaert, B. L., De Backer, J., Devereux, R. B., … De Paepe, A. M. (2010). The revised Ghent nosology for the Marfan syndrome. *Journal of Medical Genetics*, *47*(7), 476–485. https://doi.org/10.1136/jmg.2009.072785

Muiño-Mosquera, L., Steijns, F., Audenaert, T., Meerschaut, I., De Paepe, A., Steyaert, W., … Renard, M. (2018). Tailoring the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines for the Interpretation of Sequenced Variants in the FBN1 Gene for Marfan Syndrome: Proposal for a disease-and gene-specific guideline. *Circulation: Genomic and Precision Medicine*, *11*(6), e002039.

Pannu, H., Fadulu, V. T., Chang, J., Lafont, A., Hasham, S. N., Sparks, E., … Milewicz, D. M. (2005). Mutations in transforming growth factor-β receptor type II cause familial thoracic aortic aneurysms and dissections. *Circulation*, *112*(4), 513–520. https://doi.org/10.1161/CIRCULATIONAHA.105.537340

Razmara, E., Bitaraf, A., Yousefi, H., Nguyen, T. H., Garshasbi, M., Cho, W.-C.-S., & Babashah, S. (2019). Non-coding RNAs in cartilage development: An updated review. *International Journal of Molecular Sciences*, *20*(18), 4475. https://doi.org/10.3390/ijms20184475

Seo, G. H., Kim, Y.-M., Kang, E., Kim, G.-H., Seo, E.-J., Lee, B. H., Yoo, H.-W. (2018). The phenotypic heterogeneity of patients with Marfan-related disorders and their variant spectrums. *Medicine*, *97*(20), e10767. https://doi.org/10.1097/MD.0000000000010767

Shi, M., Zhu, J., Wang, R., Chen, X., Mi, L., Walz, T., & Springer, T. A. (2011). Latent TGF-β structure and activation. *Nature*, *474*(7351), 343. https://doi.org/10.1038/nature10152

Summers, K. M., West, J. A., Hattam, A., Stark, D., McGill, J. J., & West, M. J. (2012). Recent developments in the diagnosis of Marfan syndrome and related disorders. *Medical Journal of Australia*, *197*(9), 494–497. https://doi.org/10.5694/mja12.10560

Takenoshita, S., Hagiwara, K., Nagashima, M., Gemma, A., Bennett, W. P., & Harris, C. C. (1996). The genomic structure of the gene encoding the human transforming growth factor β type II receptor (TGF-β RII). *Genomics*, *36*(2), 341–344. https://doi.org/10.1006/geno.1996.0471

Yu, C., Jeremy, R. W. (2018). Angiotensin, transforming growth factor β and aortic dilatation in Marfan syndrome: Of mice and humans. *IJC Heart & Vasculature*, *18*, 71–80. http://dx.doi.org/10.1016/j.ijcha.2018.02.009

Waldmuller, S., Muller, M., Warnecke, H., Rees, W., Schols, W., Walterbusch, G., … Scheffold, T. (2007). Genetic testing in patients with aortic aneurysms/dissections: A novel genotype/phenotype correlation?*European Journal of cardio-thoracic Surgery*, *31*(6), 970–975. https://doi.org/10.1016/j.ejcts.2007.02.027

Wang, W.-J., Han, P., Zheng, J., Hu, F.-Y., Zhu, Y., Xie, J.-S., … Tian, X.-L. (2013). Exon 47 skipping of fibrillin-1 leads preferentially to cardiovascular defects in patients with thoracic aortic aneurysms and dissections. *Journal of Molecular Medicine*, *91*(1), 37–47. https://doi.org/10.1007/s00109-012-0931-y

Yang, L., Guo, X., Jiang, L., Gong, B., & Qu, C. (2019). Analysis of FBN1 gene mutations in two pedigrees affected with Marfan syndrome. *Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese Journal of Medical Genetics*, *36*(6), 566–570.

Zarate, Y. A., Sellars, E., Lepard, T., Tang, X., & Collins, R. T.II (2016). Aortic dilation, genetic testing, and associated diagnoses. *Genetics in Medicine*, *18*(4), 356–363. https://doi.org/10.1038/gim.2015.88

Zhou, H., Gao, M., & Skolnick, J. (2018). ENTPRISE-X: Predicting disease-associated frameshift and nonsense mutations. *PLoS ONE*, *13*(5), e0196849. https://doi.org/10.1371/journal.pone.0196849

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Bitarafan F, Razmara E, Khodaeian M, et al. Three Novel Variants identified in *FBN1* and *TGFBR2* in seven Iranian families with suspected Marfan syndrome. *Mol Genet Genomic Med*. 2020;8:e1274. https://doi.org/10.1002/mgg3.1274