

# A novel genetic interplay in marfan syndrome: a brief review of literature and presentation of a case

## Abstract

**Problem statement:** Marfan syndrome (MFS) is an inherited connective tissue disorder with an autosomal dominant inheritance pattern and has an incidence rate of 4 – 17 per 100,000 births worldwide. Until recently, majority of the cases were attributed to mutations in genes such as *FBN1* and *FBN2*, whereas interplay of numerous other genes has been recently documented. MFS is characterized by its effects on multiple organ systems such as cardiovascular, ocular and skeletal anomalies.

**Methods:** We investigated the mutations in *FBN1*, *FBN2*, *COL6A2* and *TGFBR1* genes using whole genome sequencing of a patient with Marfan syndrome. For this purpose, we performed sequence analysis of the genes implicated to be involved in the onset and progression of Marfan Syndrome, followed by whole genome sequencing of the patient genome. This was followed by expression analysis of the fibrillin-1, fibrillin-2, collagen alpha 2 (VI), TGF-beta receptor type-1 proteins.

**Results:** According to whole genome sequencing data; there are seven mutations in the *FBN1* gene, eight mutations in the *FBN2* gene, thirty-seven mutations in the *COL6A2* gene, and two mutations in the *TGFBR1* gene. Expression analysis showed that the expression of the *FBN1* gene was reduced by 50%, whereas the expression of the *FBN2* gene was overexpressed by 150%. Additionally, a slight decrease was observed in the expressions of *COL6A2* and *TGFBR1* genes.

**Conclusion:** Exome analysis revealed that, albeit none identified as clinically important, all but two of the genetic alterations observed in our patient was among documented variations. However, these two alterations were presumed to be located at potentially important locations that may affect the function of the proteins. We, therefore, believe that this intriguing presence or interplay of these two variations might provide us insight into mechanisms for the development and potentially formulation of therapeutic strategies of Marfan Syndrome.

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## Introduction

Despite the recent advances in diagnosis and treatment of patients with Marfan Syndrome (MFS, OMIM 154700), an autosomal dominant connective tissue disease shown to be associated with numerous mutations in fibrillin (*FBN1* and *FBN2*) and TGF $\beta$  receptor genes (*TGFBR1* and *TGFBR2*), the number and variety of genetic factors leading to the disease continues to astonish researchers working on the syndrome. Clinical prognosis of MFS is challenging due to complex interplay of these genetic factors leading to puzzling clinic profiles that can involve ocular, skeletal, and cardiovascular systems.<sup>1</sup> Even though early detection along with meticulous echocardiographic follow-up and multidisciplinary assessment are proven to be essential for the care of the MFS patients, it is becoming more important to underline the importance of genetic counseling as the importance of genetic heterogeneity is further recognized in the progression of the disease.

MFS has an incidence rate of 4 – 17 per 100,000 births, where 25 to 30% of cases exhibit significant skeletal abnormalities.<sup>2-4</sup> In addition to problems with the skeletal system, cardinal manifestations of MFS include ectopis lentis, myopia, aortic root aneurysm, mitral valve prolapses in heart and striae in skin.<sup>5</sup> Mitral valve disease may be among earliest cardiovascular symptoms of MFS, and leading causes of premature death in MFS patients are aortic root dilation, aortic regurgitation and dissection.<sup>6</sup> Clinical heterogeneity of the disease makes it challenging to formulate a common set of criteria for

the diagnosis of MFS, which has been suggested in the International Nomenclature of Heritable Disorders of Connective Tissue Meeting in Berlin and revised in 2010.<sup>7</sup> Clinical diagnosis of MFS is established by a complete skeletal examination, cardiovascular examination including echocardiography, MRI, computer tomography, X-ray and ophthalmologic examination such as biomicroscopic eye examination.<sup>5</sup> It is also important to note that etiology of disease has not shown any significant dependence on gender or ethnic background of the patients regarding the onset, progression or the severity of the disease.<sup>8,9</sup> Even though treatment might not completely reverse symptoms, it can slow down progression of the disease, where effective management of the disease benefits most from a multidisciplinary team of experts in cardiology, ophthalmology, orthopedics, and genetics.<sup>9</sup>

## Molecules of marfan syndrome

### Fibrillin 1

Fibrillin-1 gene (*FBN1*; MIM 134797), located on 15q21.1, spans 235 kb DNA allocated in 65 exons and encodes a 350 kDa cysteine rich microfibrillar glycoprotein, fibrillin 1.<sup>10</sup> Majority of the mutations are located in the 47 tandemly repeated epidermal growth factor like domains, where cysteine residues direct formation of disulfide bonds enabling the accurate folding of the domains and their calcium binding capacity that is crucial for its function.<sup>9,10</sup> It is important to note the pleiotropic nature of these variations, as they have been documented to be associated with ectopia lentis, familial aortic aneurysm and Marfan-like other skeletal anomalies.<sup>10</sup>

Fibrillin-1 forms the core component of extracellular microfibrils, which are thought to play an important role in formation of elastic fibers of connective tissue.<sup>11</sup> Microfibril assembly relies on rod-like domains located in the middle section of fibrillin-1, where 12 calcium binding EGF-like (cbEGF) repeats are located. Despite the independent nature of its structural assembly, disulfide bonds between cysteine residues in these repeats direct formation of the final structure. Overall, fibrillin-1 has 47 EGF-like modules, 43 of which form the cbEGF domain, which has been documented to contribute calcium binding property of the protein.<sup>12</sup> This interplay underlies the structural properties of the protein, where calcium binding has a pivotal role in limiting structural flexibility of the protein, which in turn directs interactions as well as susceptibility of the microfibrils to proteolytic cleavage.<sup>13</sup> Recent studies show impact of these variations on onset and progression of MFS, mostly by disrupting extracellular matrix homeostasis. Reduced amount of functional fibrillin-1, which can be attributed to hampered expression or distribution, impact structural and functional characteristics of connective tissue, such as cell disarray, elastolysis, inflammation, matrix metallo-proteinase (MMP) upregulation, stiffening of aortic wall, and dysregulation of TGF $\beta$  activity.<sup>14</sup>

### Fibrillin-2

*FBN2*, located on 5q23.3, encodes a protein which forms the structural components of 10-12 nm extracellular calcium-binding microfibrils.<sup>15</sup> Despite significant similarities to fibrillin-1, fibrillin-2 features a proline-rich region instead of glycine-rich domain at the N-terminal end of the protein. In addition, fibrillin-1 has a single RGD sequence, whereas fibrillin-2 protein has 2 of these motifs.<sup>15</sup> Association of fibrillin 1 and 2 constructs, is a complex and multistage process where problems associated with this mechanism can yield to various anomalies. Fibrillins interact in N to C-terminal fashion to form homotypic fibrillin-1 or heterotypic fibrillin-1/fibrillin-2 microfibrils and this interaction was stabilized by disulfide bonds.<sup>16,17</sup> Fibrillin-1 supports the structure of fiber playing a supportive and force-bearing role, whereas fibrillin 2 has a role in the early stages of elastic fiber formation.<sup>18</sup> The importance of specific domain organization was clearly shown by the detection of exon-skipping mutations which lead to the deletion of fibrillin-1 domains and lead to the formation of like MFS indications. The first hybrid domain is located at the N-terminal of the proline-rich domain, which is shown to be unstructured,<sup>1</sup> and deletions located in this domain has important effects on the proline-rich domain, which are regularly structured with extensive interdomain contacts. It is also possible that fibrillin-2 could play a compensatory role in the assembly of fibrillin-1 molecules lacking the first hybrid domain.<sup>19</sup>

### Transforming growth factor beta receptor 1

*TGFBR1*, located on 9p22.23, encodes aserine/threonine protein kinase that consists of 503 amino acids and as a complex with TGFBR2 protein, transducing signals of TGFB1, TGFB2 and TGFB3, affecting numerous physiological and pathological phenomena including cell growth, differentiation, extracellular matrix production, pluripotency and apoptosis. It is important to note that genes in that pathway have been associated with several kidney and lung diseases, Duchenne Muscular Dystrophy, Camurati-Engelmann Disease, cancer, obesity, cardiac fibrosis and Marfan Syndrome.<sup>20</sup>

TGF $\beta$ 1 is a member of cytokine family of proteins and plays a crucial role in cardiac and vascular morphogenesis through regulating resilience of extracellular matrix homeostasis.<sup>21</sup> Bioavailability of TGF $\beta$ 1 protein in extracellular matrix is associated

with increased protease activity. Reduced Fibrillin-1 protein leads to increased release and activation of TGF $\beta$ 1.<sup>14</sup> TGF $\beta$ 1 interacts with extracellular matrix and modulates activity of fibrillin, which is how it is implicated in MFS.<sup>22</sup> Selected MFS manifestations reflect excessive TGF $\beta$ 1 signaling, which can be regulated by TGF $\beta$ -neutralizing antibody or angiotensin II type 1 receptor blockers.<sup>23</sup> TGF $\beta$ 1 and fibrillin1 relationship has been shown in mouse studies, where it was revealed that TGF $\beta$ 1 was partially responsible for the disease profile. In these studies, the relationship between TGF $\beta$ 1 and fibrillin-1 has been shown in various fibrilopathies.<sup>24,25</sup>

### Collagen

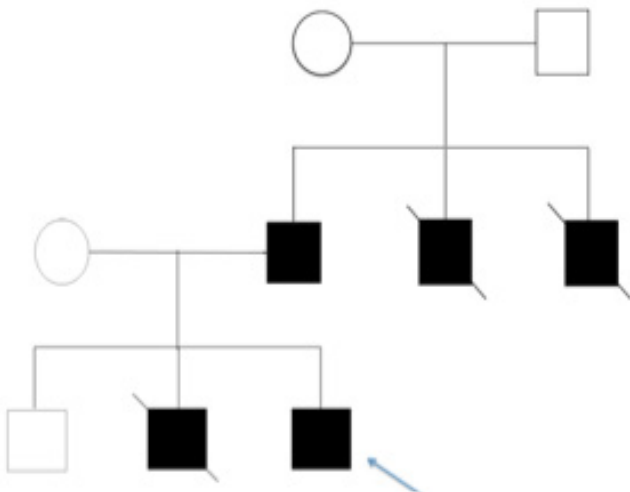
Collagen is a fibrous structural protein that consists of 3 parallel, left-handed polyproline II helix motifs, which functions in providing rigidity, organization, strength and form of tissues.<sup>26,27</sup> The most common types of collagen in aortic wall are type I and III.<sup>14,28</sup> Type IV is present in the basal membrane layer where endothelial cells adhere.<sup>14</sup> Type V is essential for type I and III interaction and plays a central role in determining quality and optimal fibrillation of the tissue.<sup>29</sup> Collagen type VI has recently drawn considerable attention due to its role for the pathophysiology of cardiomyocytes, myofibrils, neurons, fibroblasts and chondrocytes.<sup>30</sup>

*COL6A2* gene, located at 21q22.3, encodes for one of three building blocks of full collagen and its variants has been shown to be associated with Bethlem Myopathy, Ullrich Congenital Muscular Dystrophy, Atrio ventricular Septal Defect, Autosomal Recessive Myosclerosis. Even though mutations of type I, II and III collagens have been shown to be associated with MFS, the link between *COL6A2* variations and MFS is still controversial.<sup>31-34</sup>

### Case presentation

This case report retrospectively presents clinical and genetic findings of a single patient, which were gathered upon collecting informed patient consent from the patient and used in performing advanced genetic and expression level analysis. A 26-year-old male is presented with a family history of Marfan Syndrome, as shown in the family pedigree (Figure 1). He is 65 kg in weight and 190 cm tall. His medical history, which was previously taken by several physicians in different hospitals of Turkey clearly revealed that he suffered from numerous indications of Marfan Syndrome, one of which has previously led to his operation due to pneumothoraces. Patient has been reported to have a history of shortness of breath and dyspnea. He also has frequent sore throat, cough, middle ear infections and a mild difficulty in hearing. He appeared thin and slender with his body weight measuring less than average for his age and sex. He had disproportionately long arms and legs as compared with the trunk and his arm span was more than his height about 3 inches with an increased floor to pubis measurement/pubis to vertex measurement. His hands were elongated with thickening at the phalange joints. He also presented positive with Wickett Murdoch wrist sign and a positive Steinberg thumb sign.

His prior examination of the feet revealed that he had flat feet with mild pronation along with elongated toes. Another finding was a subtly indented chest and mild hunching of the back. Extraoral examination revealed a narrow cranium with dolicocephaly and leptoproscopic facial features. Malar hypoplasia, mandibular retrognathia, macrostomia and downward slating palpebral fissures were evident. Despite of the above-mentioned clinical indications, his electrocardiography did not show any signs of problems related to cardiac system, which is not uncommon for MFS patients, mostly attributed to the genetic heterogeneity of the disease.



**Figure 1** Pedigree of the affected family.

**Family history**

Patient’s family history is summarized in the pedigree below (Figure 1). Proband’s elder brother, who was 184 cm tall and 55 kg in weight, had MFS and has died at the age of 26 years old due to aortic aneurysm caused by the disease. Father of the proband, who is 184 cm tall and 75 kg in weight, also has MFS and is alive, whereas both of his two uncles have lost their lives due to this disease. Proband also has a healthy brother and a healthy mother, who both does not have any clinical manifestations related to a connective tissue disorder (Figure 1). Further information about the family is not present at the time of the preparation of this manuscript.

**Clinical examination**

Proband has received the Marfan Syndrome diagnosis with a Ghent score of 7+ points, cataract formation, -3.5 and -9.0 degree of myopia in the left and right eyes, respectively. Currently, he has pneumothoraces, and has been operated twice in the last 10 years. He has been found to have arachnodactyly and wrist anomalies visible upon physical examination (Figures 2, 3, 4A and 4B). Upon examination with chest computed tomography, both of his lungs have air cysts on the apex side and emphysematous on the left. Albeit numerous complications in the physical examination, his cardiograph did not show any anomaly related to any cardiovascular problems.



**Figure 2** Arachnodactyly in the patient.



**Figure 3** Arachnodactyly in the patient.



**Figure 4**

The Refractive error was -9.00 diopter myopia in the right eye and -3.50 diopter myopia in the left eye, before cataract surgery. His axial length was 29.12 mm in the right eye and 25.71 mm in the left eye (NIDEK AL Scan optical biometry). The patient had cataracts in his both eye and underwent bilateral cataract surgery. According to present examination best-corrected visual acuities according to Snellen chart were 10/10 in the right eye and 4/10 in the left eye. The refractive errors were -1.25 diopter astigmatism in 65-degree axis in the right eye and -1.00 diopter myopia, and -0.75 diopter astigmatism in 140-degree axis in the left eye. He was subjected to slit lamp biomicroscope (Topcon SL-D4). Anterior segment examination revealed posterior chamber intraocular lenses in both eyes. Retinal periphery was evaluated after dilatation of the pupil by indirect ophthalmoscopy and scleral depression. Tropicamid 0.5% (TROPAMID®) was instilled for pupillary dilatation in his eyes. In fundus examination 360°panretinal photocoagulation in his peripheral retina bilaterally due to the lattice degeneration and a retinal tear surrounded by bridge laser in the superior retina of left eye was seen and no other pathology was detected in his fundus. His intraocular pressure was 14 mmHg/16 mmHg, respectively (Non-Contact Tonometer CT-800-TOPCON).

**Genetic screening**

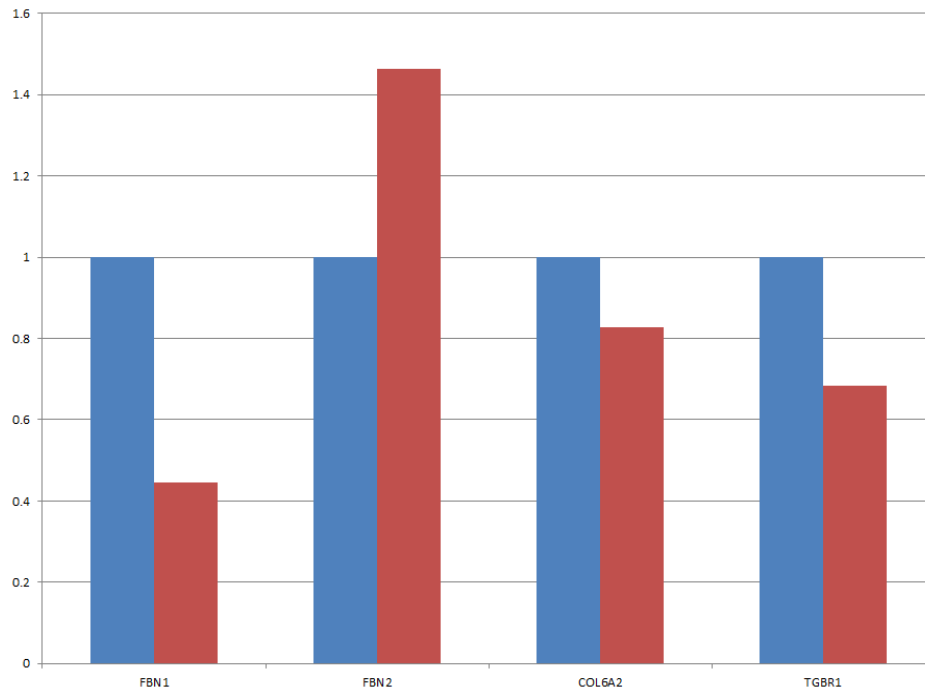
Even though the initial sequencing of *FBN1* and *FBN2* exomes, did not reveal any clinical mutations in the sample, a second cardiac panel screening of the patient identified two mutations; *TXNRD2* (c.1066G>A) and *TGFBR1* deletion (Ala24\_ Ala26del). However, when we performed a whole genome sequence analysis of the sample, we identified a third clinically relevant mutation in the *COL6A2* gene (c.2611G>A, p.Asp871Asn) and numerous other mutations in the intronic regions of these genes, as detailed in the following section. List of mutations that we identified using whole genome sequencing results are presented in Table 1.

**Protein expression analysis**

The expression profiles of the *FBN1*, *FBN2*, *TGFBR1* and *COL6A2* proteins were determined using qRT-PCR analysis. Control primers were prepared for actin protein (other primer sequences are presented as supplementary figure). Analysis were performed at Atlas Biotechnology (Turkey). Our results, as presented in Figure 5, showed a clear reduction in the *FBN1* production, and an over expression of the *FBN2* protein, which were in agreement with the knowledge that the expression *FBN2* occurs earlier than *FBN1*. This is in agreement with the prior knowledge that *FBN2* transcripts accumulate before

tissue differentiation, then rapidly decrease or disappear, whereas FBN1 transcripts are then gradually increased. Any failure of accurate FBN1 physiology may lead to overexpression of FBN2.<sup>18</sup> Fibrillin-2 is found in the elastic cartilage, the tunica media layer of the aorta and the elastic tissues along the bronchial tree. Fibrillin-2 has a large

functional role during early morphogenesis in directing the elastic fiber assembly, the predominance of fibrillin-1 in stress and load-bearing structures, such as aortic adventitia, ciliary zonules and skin, proves that fibrillin-1 provides mainly structural support; where fibrillin-2 mainly regulates the initial treatment of the elastic fiber assembly.<sup>10,35</sup>



**Figure 5** Expression profiles for FBN1, FBN2, COL6A2 and TGFB1R for normal (blue) and case (red) samples.

**Table 1** Mutations identified based on gene sequencing and whole genome sequencing analysis

| Gene           | Position       | dbSNP ID     | Genotype | Consequence | ClinVar      | Disease Name |
|----------------|----------------|--------------|----------|-------------|--------------|--------------|
| FBN1           | chr15:34614315 | rs1820448    | G/T      | None        | NR           |              |
|                | chr15:48428329 | rs363832     | G/C      | IV          | Benign       | NS           |
|                | chr15:48437451 | rs2042746    | T/C      | IV          | NR           |              |
|                | chr15:48444487 | rs2303502    | A/T      | IV          | NR           |              |
|                | chr15:48515440 | rs4775765    | C/T      | MSV         | Benign       | NS           |
|                | chr15:48520887 | unidentified | C/T      | IV          | unidentified | unidentified |
|                | chr15:48610929 | rs1018148    | A/G      | IV          | NR           |              |
| FBN2           | chr5:128278780 | rs190450     | A/G      | SV          | Benign       | CVP, CCA     |
|                | chr5:128287291 | rs2042327    | C/T      | IV          | Benign       | CCA          |
|                | chr5:128289867 | rs56131649   | T/C      | IV          | Benign       | CCA          |
|                | chr5:128305091 | rs27713      | G/A      | IV          | Benign       | CCA          |
|                | chr5:128336232 | rs32221      | A/G      | IV          | NR           |              |
|                | chr5:128349443 | rs154001     | C/T      | MSV         | Benign       | CVP, CCA     |
|                | chr5:128376648 | rs1182771803 | C/G      | IV          | NR           |              |
| chr5:128442235 | rs17697995     | A/G          | IV       | NR          |              |              |

Table Continued...

| Gene          | Position                | dbSNP ID     | Genotype    | Consequence | ClinVar      | Disease Name |
|---------------|-------------------------|--------------|-------------|-------------|--------------|--------------|
|               | chr21:46117753-46117755 | rs5844252    | _/G         | IV          | NR           |              |
|               | chr21:46122741          | rs5844253    | _/C         | IV          | NR           |              |
|               | chr21:46119876          | rs3737362    | A/G         | IV          | Benign       | NS           |
|               | chr21:46119970          | rs114994097  | A/C         | IV          | NR           |              |
|               | chr21:46121639          | rs1077182    | A/G         | IV          | Benign       | NS           |
|               | chr21:46122947          | rs915786     | A/G         | IV          | Benign       | MS, CRM      |
|               | chr21:46124748          | rs55933135   | A/G         | IV          | Benign       | NS           |
|               | chr21:46125241          | rs2839113    | A/G         | IV          | Benign       | NS           |
|               | chr21:46132216          | rs9977394    | A/G         | SV          | Benign       | GFD, MS, CRM |
|               | chr21:46132671          | rs1043962    | A/G         | NCV         | Benign       | GFD, MS, CRM |
|               | chr21:46112630          | rs1010688    | C/T         | IV          | NR           |              |
|               | chr21:46116632          | rs762438     | C/T         | IV          | Benign       | NS           |
|               | chr21:46122306          | rs2070578    | C/T         | IV          | NR           |              |
|               | chr21:46122464          | rs17272651   | C/T         | IV          | Benign       | NS           |
|               | chr21:46122865          | rs17357592   | C/T         | IV          | Benign       | MS, CRM      |
|               | chr21:46124627          | rs3746995    | C/G         | IV          | Benign       | NS           |
|               | chr21:46125912          | rs13046639   | C/T         | SV          | Benign       | MS, CRM      |
|               | chr21:46126468          | rs16978878   | C/A         | IV          | Benign       | NS           |
| <b>COL6A2</b> | chr21:46131919          | rs7279622    | C/T         | IV          | Benign       | NS           |
|               | chr21:46132471          | rs6652       | C/T         | SV          | Benign       | GFD, MS, CRM |
|               | chr21:46117968          | rs7279347    | G/A         | IV          | Benign       | NS           |
|               | chr21:46118777          | rs9917540    | G/T         | IV          | NR           |              |
|               | chr21:46119046          | rs2839110    | G/A         | MSV         | Benign       | MS, CRM      |
|               | chr21:46120928          | rs55964853   | G/C         | IV          | NR           |              |
|               | chr21:46124614          | rs79205686   | G/T         | IV          | Benign       | NS           |
|               | chr21:46125854          | rs1042917    | G/A         | MSV         | Benign       | MS, CRM      |
|               | chr21:46125909          | rs13052956   | G/A         | SV          | Benign       | MS, CRM      |
|               | chr21:46125999          | rs2839114    | G/A         | SV          | Benign       | MS, CRM      |
|               | chr21:46132103          | rs387906610  | G/A         | MSV         | Benign       | BMI, CRM     |
|               | chr21:46132640          | rs369124688  | G/C         | NCV         | Benign       | MS, CRM      |
|               | chr21:46119619          | rs9980483    | T/C         | IV          | NR           |              |
|               | chr21:46120507          | rs73159701   | T/C         | IV          | Benign       | MS, CRM      |
|               | chr21:46122072          | rs9976026    | T/C         | IV          | Benign       | NS           |
|               | chr21:46122720          | rs17272671   | T/C         | IV          | NR           |              |
|               | chr21:46125396          | rs16978870   | T/C         | IV          | NR           |              |
|               | chr21:46126420          | rs17272947   | T/C         | IV          | NR           |              |
|               | chr21:46132668          | rs3087667    | T/C         | NCV         | Benign       | GFD, MS, CRM |
| <b>TGFBR1</b> | chr9:99105275-99105280  | unidentified | GCGGCGGCG/- | IV          | unidentified | unidentified |
|               | chr9:99149166           | rs201772204  | A/T         | IV          | NR           |              |

IV, intron variant; MSV, missense variant; SV, synonymous variant; NCV, non coding transcript variant; CVP, cardiovascular phenotype; CCA, congenital contractural arachnodactyly; MS, myosclerosis; CRM, collagen vi-related myopathy; GFD, glutamate formiminotransferase deficiency; BMI, bethlem myopathy 1; NR, not reported; NS, not specified

**Supplementary Table 1** Primer Sequences used for qTR-PCR in expression analysis

|          |                         |
|----------|-------------------------|
| FBN1-F   | CGTGAAGGAAACCAGAGCCA    |
| FBN1-R   | ATCCAGGGCAACAGTAAGCA    |
| FBN2-F   | TGAGTAGAGCAGACATTCCA    |
| FBN2-R   | CTAGAATTGCCTATGCCTTTACC |
| COL6A2-F | AGATCGACCAGGACACCATC    |
| COL6A2-R | GGTCTCCCTGTCTTCCCTTC    |
| TGFBR1-F | GATGGGCTCTGCTTTGTCTC    |
| TGFBR1-R | CAAGGCCAGGTGATGACTTT    |
| ACTB-F   | ACTCTCCAGCCTTCCTTC      |
| ACTB-R   | ATCTCCTTCTGCATCCTGTG    |

## Discussion

It is of utmost interest to accurately understand the phenotype-genotype correlation in MFS, especially for rare genetic variations, to formulate effective and accurate diagnostic and therapeutic tools. In a recent publication, it has been shown that the expression of *FBN1* was also reduced in fibroblast cell cultures derived from MFS patients.<sup>36</sup> Similarly, the expression levels of *FBN1* was also reduced in our sample and understanding the possible correlation of this reduction to identified mutations is of utmost importance for formulation of a diagnostic and therapeutic strategy for MFS.

Similarly, fibrillin-1 expression has been in the focus of numerous studies, which led to the conclusion that genetic alterations that affect the expression or stability of the fibrillin-1 protein generally leads to the early onset of clinical conditions of MFS.<sup>15</sup> It has been clearly shown that cysteine substitutions in EGF-like domains, which disrupt disulfide bonds, are among cardinal causes of the pathophysiology of MFS. For instance, p.Cys476Gly mutation of *FBN1*, which located on the EGF like-5 domain spanning amino acids 449-489, is one of such mutations.<sup>37</sup>

Based on sequencing results, we identified 7 mutations, 6 of which were previously observed mutations. Six of these variants, rs1820448, rs363832, rs2042746, rs2303502, rs4775765, and rs1018148 have been identified previously, and their impact on pathophysiology has been somewhat documented. Among these, rs4775765 involves introduction of a Cysteine residue, in the proximity of the cysteine residue on the 476<sup>th</sup> position. Despite its close proximity to a present disulfide bond, it is not plausible to draw a conclusion about the impact of this variation on the protein function. On the contrary, we believe that the seventh variant that we identified holds a bigger premise in clarification of the alterations for our sample. The location of our novel alteration has previously been reported, however in our sample the alteration is different. In a previous report, rs978058890, an intronic variant of the *FBN1* was reported as a g.456C>G alteration. In our sample we identified a C to T alteration, which has not been reported previously. We, therefore, believe that this novel alteration may also hold premise in explaining the decreased level of *FBN1* expression.

We identified eight mutations in the intronic region of the *FBN2* gene, all of which were previously identified and have somewhat been found to be implicated in the onset and progression of the MFS pathogenesis. For instance, rs190450 (c.7200T> C) synonymous variant occurring at the exon of *FBN2* 57 is not expected to have

clinical significance because it does not alter an amino acid residue and is not included in the fusion consensus sequence.

Genes that code for Collagen VI family of proteins have been found to be implicated in the diseases of Bethlem myopathy and Ullrich congenital muscular dystrophy (UCMD), especially COL6A1, COL6A2 and COL6A3. UCMD is a characterized by congenital muscle weakness, proximal joint contractures, and significant distal joint hyperexcitability. In contrast, lighter Bethlem myopathy disorder is predominant and originates from mutations in COL6A1, COL6A2 and COL6A3.<sup>38</sup> The occurrence of two missense mutations in the COL6A2 gene in the patient is thought to be associated with these diseases rs2839110 (p.Ser399Asn, c.1196G>A) and rs1042917 (p.Arg680His, c.2039G>A). Four types of collagen proteins interact with triple-helical regions and the alpha 1 domain of integrins. p.Ser399 Asn mutation is located in the triple-helical region of the protein.<sup>39</sup> Another mutation in the Col6a2 gene, p.Arg680His, is found in the VWFA2 domain and the non-helical domain.

The TGFβR1 gene is located on Chromosome 9, which has shown to be important in the development of MFS spectrum of syndromes. Our sequencing results showed that our sample had a deletion that is similar to rs886038783 p.Ala24Gly mutation which includes a GCGGCG/G deletion. Our sample had GCGGCGGCG/- deletion and based on our knowledge this is the first documentation of this variation. The absence of three Alanine residues in the repeat region of the protein can lead to the disruption of the signal region of the receptor. It is well known that the biosynthesis of the receptor protein is tightly linked to the rate of transport of the protein to the cell membrane through its interaction with the proteins on the membrane surface or the ligand.<sup>40</sup> Therefore, it is logical to assume that in our patient the absence of the Alanine residues leads to the disruption of this localization and in turn hampering of the receptor function in the cells. One of the possible explanations for this effect can be the impact of Alanine and Glycine replacement in a protein within a signaling domain, which leads to the hampering of the stability of the protein. Glycine normally is a residue that is preferentially located at the G and N caps of the proteins. On the other hand, Alanine stabilizes the helix structure by 0.4 to 2 kcal mol<sup>-1</sup> compared to Glycine in the hydrophobic regions. Therefore, the replacement of Glycine at a position originally occupied by Alanine affects the conformation of possibly a hydrophobic region of the protein, which in turn leads to further conformational changes.<sup>41</sup>

In conclusion, our results point to a novel genetic interplay which can be used to shed light on the intriguing and complex clinic outcome of MFS molecular pathogenesis in our patient. It is of utmost importance to incorporate the expression difference of these proteins, in relationship to the genetic variations in these patients and correlate the clinical variations in relation to the genetic alterations. This may hold promising clues for deciphering the molecular pathogenesis of the disease in different patients, which in turn might lead to formulation of personalized therapeutical strategies for the patients.<sup>42,43</sup>

## Acknowledgments

None.

## Conflicts of interest

The authors declare that there is no conflict of interest.

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