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Evaluation of Clinical and Molecular Findings in a Group of Turkish Individuals with Marfan Syndrome

Bir Grup Türk Marfan Sendromlu Bireyde Klinik ve Moleküler Bulguların Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to review the clinical and molecular findings of 12 individuals with Marfan syndrome (MS) to identify novel mutations and associated clinical findings.

Method: This study included 12 patients who were diagnosed with MS between January 2018 and January 2021 in a teaching and research hospital in Turkey. The patient files were retrospectively analyzed. A single clinical geneticist evaluated all of the patients. *FBN1* sequencing was performed in all patients.

Results: There were five male and seven female patients. Four of the patients did not meet the MS clinical diagnostic criteria before the molecular analysis. Most of the patients (67%) were referred due to the aortic dilatation; however, none of the patients had aortic aneurysms/dissections. Skeletal findings and MS-related facial features were present in all of the patients. Ectopia lentis was not detected. Only one patient had a history of pneumothorax. Twelve diverse variants were detected in 12 patients. Of these, ten were classified as pathogenic and two as likely pathogenic, and three were novel and nine were previously reported. There were five nonsense (42%), four frameshift (33%), and three missense (25%) variants. *FBN1* variants were distributed within the gene without any hot spots. EGF-like domain was the most commonly affected protein domain.

Conclusion: Elucidating the underlying molecular pathology in MS contributes to expanding the phenotype-genotype correlation in the disease and early diagnosis. Our study has broadened the genotypic and phenotypic spectrum of MS in Turkey by describing the clinical findings of 12 patients and reporting three novel variants.

Keywords: *FBN1*, Marfan Syndrome, phenotype-genotype correlation

ÖZ

Amaç: Bu çalışma ile Marfan sendromundaki(MS) fenotipik genotipik spektrumu genişletmek için Marfan Sendromlu bireylerin klinik ve moleküler bulgularının gözden geçirilmesi amaçlandı.

Yöntem: Çalışmaya Türkiye'de bir eğitim ve araştırma hastanesinde Ocak 2018- Ocak 2021 tarihleri arasında Marfan Sendromu tanısı konulan toplam 12 hasta dahil edildi. Hasta dosyaları geriye dönük olarak incelendi. Tüm hastalar tek bir klinik genetik uzmanı tarafından değerlendirildi. Tüm hastalarda *FBN1* geni dizilendi.

Bulgular: Hastaların beşi erkek, yedisi kadındı. Dört hasta moleküler çalışma sonucu olmadan MS klinik tanı kriterlerini karşılamıyordu. Hastaların çoğu (%67) aort dilatasyonu nedeniyle yönlendirilmişti, bununla birlikte hiçbir hastada aort anevrizması/ diseksiyonu saptanmamıştı. Hastaların tümünde iskelet bulguları ve MS ile ilişkili yüz özellikleri mevcuttu. Ektopia lentis tespit edilen hasta yoktu. Pnömotoraks öyküsü sadece bir hastada mevcuttu. On iki hastada 12 farklı varyant tespit edildi. Bunlardan on tanesi patojenik, ikisi muhtemel patojenik olarak sınıflandırıldı; üçü novel varyanttı, dokuzu ise daha önce bildirilmişti. Varyantlardan beşi (%42) nonsense, dördü (%33) frameshift, üçü (%25) ise missense gruptaydı. Saptanan *FBN1* varyantları herhangi bir hotspot bölge olmaksızın gen içerisinde dağılmıştı. Protein düzeyinde incelendiğinde EGF-like domain, varyantların en sık etkilediği domaindi.

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Sonuç: MS nedeniyle izlenen hastalarda altta yatan moleküler patolojinin aydınlatılması, hastalıktaki fenotip-genotip korelasyonunun genişletilmesi ve hastalara erken tanı konulması açısından çok önemlidir. Çalışmamız tanımlanan ayrıntılı klinik bulgular ve saptanan üç yeni varyant ile sendroma ait genotipik ve fenotipik spektrumun genişletilmesine katkıda bulunmaktadır.

Anahtar kelimeler: *FBN1*, fenotip-genotip korelasyonu, Marfan Sendromu

INTRODUCTION

Marfan syndrome (MS) is a hereditary connective tissue disorder that affects many organs, mainly the cardiovascular system (CVS), skeletal system, and eye.[1] The incidence of the syndrome is 2–3/10,000 in all ethnic groups.[2] Individuals with MS usually have a wide range of symptoms in terms of organ involvement and severity. Some patients may present in the neonatal period with progressive multisystemic findings, while others present with isolated organ involvement.[1,3] CVS involvement, especially aortic dilatation and related dissections, is the leading cause of morbidity and mortality in MS.[4]

Heterozygous mutations in the *FBN1* are responsible for MS.[5] *FBN1* encodes a large multi-domain protein, fibrillin-1. It mainly consisting of hybrid domains and repetitive epidermal growth factor (EGF)-like domains within interspaced transforming growth factor-beta binding protein-like (TB) domains.[6,7] Fibrillin-1 is involved in the formation of microfibrils in the extracellular matrix of the connective tissue as well as in the regulation of the TGF-beta signaling pathway.[8] The multisystemic involvement in MS is observed due to inflammation and fibrosis caused by the disintegration/fragmentation of the mutant fibrillin-1 protein in microfibrils and the dysregulation of the TGF-beta signaling pathway.[9]

Mutations in *FBN1* are inherited from an affected parent in 75% of individuals with MS.[4] Pathogenic variants in *FBN1* are often unique to affected individuals or families; however, variable expressivity, defined as different prevalence and severity of symptoms even among individuals in the same family, is frequently described.[10] To date, more than 3000 MS-associated variants have been reported in *FBN1*, which are mostly missense and create/replace a cysteine residue in EGF-like domains.[11,12]

Early diagnosis is critical in MS, especially in preventing cardiovascular complications, which are the most important cause of mortality and morbidity. The diagnosis of MS is made by using modified Ghent nosology diagnostic criteria, which include various combinations of physical examination findings, aortic root measurements, family history, and molecular analysis results.[2] Among the diagnostic criteria, molecular analysis of *FBN1* allows timely diagnosis, especially in pediatric patients where all clinical findings may not have appeared yet and in patients with variable expression.[4,13,14] Thus, preventing mortality and morbidity in these patient groups becomes possible by appropriate follow-up, treatment, and timely prophylactic interventions.[4,13,14]

In recent years, next-generation sequencing (NGS) has been used widely in routine diagnosis to reach a rapid, cost-effective, and accurate molecular diagnosis in MS as in many diseases. Here, we review the clinical and molecular findings of 12 individuals with MS to identify novel mutations and associated clinical findings.

METHOD

Patients

We reviewed the patients referred to our clinic with a preliminary diagnosis of MS between January 2018 and January 2021. A single clinical geneticist evaluated all of the patients. Molecular analysis was planned for patients who met the diagnostic Ghent nosology criteria or had a family history of MS despite not meeting the diagnostic criteria. A total of 12 unrelated patients were included in the study. Outpatient and inpatient medical records were reviewed from the hospital database. Demographic data, family history, clinical presentation, laboratory, and imaging study results were collected. The local ethics committee approved the protocol of the study with an accession number of B.10.1.TKH.4.34.H.GP.0.01/176. The study was conducted following the Declaration of Helsinki. Informed consent was obtained from all patients and their legal guardians for molecular analysis.

FBN1-Targeted NGS Analysis and Variant Interpretation

Peripheral blood samples were collected from all individuals. Following the standard protocols of the QIAAmp DNA Mini (Qiagen) kit, automatic DNA isolation was performed in EDTA-anticoagulated blood samples. Targeted NGS analysis was performed on Illumina MiSeq (v1.9) platform using Multiplicom Marfan Assay using Illumina V3 chemicals. Sequence analysis covers coding regions of *FBN1* (NM_000138), including all coding exons, +/– 10 base pairs of adjacent intronic sequences, and each nucleotide is read at a depth of at least 50×. Variants that fall outside these

regions and exonic variants with a minor allele frequency of less than 10% were considered false positives and not analyzed. Copy number variations were not examined. The DNA sequences were aligned to the NCBI Build 37 (hg18) version of the human genome. Alignments were confirmed by using Integrative Genomics Viewer (v.2.313). The Sophia-DDM-V5.2 bioinformatics analysis program performed variant calling and data analysis. The interpretation of the variants was performed according to the 2015 American College of Medical Genetics (ACMG) standards and guidelines.[15] Iranome, gnomAD data were used as the control population. The effects of the variants on protein function were investigated using in silico prediction programs such as SIFT, PolyPhen2, Mutation Taster, and M-CAP. Human Gene Mutation Database (HGMD), ClinVar, UMD-*FBN1*, and PUBMED databases were used to investigate mutations previously associated with MS. Only variants of unknown significance, pathogenic (P), and likely pathogenic (LP) are reported in the "Results" section. Segregation analysis was performed by Sangertable sequencing. Primer sequences and reaction conditions are available on request.

RESULTS

There were five male and seven female patients in the study group. The mean age was 13.9±8.9 standart deviation score (SDS) years. The systemic score was ≥7 in 83% of the patients. Family history regarding MS was present in 33%. Most of the patients (67%) were referred due to aortic dilatation detected in the CVS examination. Mitral valve prolapsus (MVP), tricuspid valve prolapsus, and mitral regurgitation were the other CVS findings. None of the patients had aortic aneurysms/dissections; however, the family history of aortic aneurysm/dissection was present in 33%. In physical examination, skeletal findings were present in all of the patients; dolichostenomelia (58%), pectus deformities (92%), and scoliosis (58%) stood out as the main skeletal features. Eye examination revealed myopia in 58% of the patients. However, ectopia lentis was not detected in any of our patients. Only one patient had a history of pneumothorax. All of the patients have at least three of the five MS-related facial features. Clinical features of the patients and the systemic scores are presented in Table 1.

Eight of the patients met the MS clinical diagnostic criteria before the molecular analysis. Family history and clinical findings of the patients who did not fulfill the diagnostic Ghent criteria without a molecular analysis were as follows.

The first patient was a 6-month-old girl (Patient 12). She had been referred to our department with a history of a deceased father with MS. Physical examination revealed enophthalmos, down slanted palpebral fissures, malar hypoplasia, micrognathia, pectus excavatum, and long fingers. Her weight was 7.5 kg (50p), and her height was 70 cm (90p). Eye and CVS examinations were normal. The systemic score was calculated to be 5. In the *FBN1* NGS analysis, the previously reported heterozygous, pathogenic, c.2250C>A, p.(Cys750Ter) variant was detected, and the diagnosis of MS was confirmed.

The second patient was a 7-year-old girl (Patient 7). She had been referred to the genetics department for suspicion of MS due to her family history. Her father had been diagnosed with MS and had been operated on for aortic dilatation and mitral and aortic valve regurgitation at the age of 24. However, molecular analysis had not been performed in the father. In physical exam, enophthalmia, down slanting palpebral fissures, malar hypoplasia, micrognathia, orthodontic problems, pectus excavatum deformity, thumb sign, and cylindrical body structure were detected. Echography revealed MVP. Eye examination was normal. Despite a positive family history, the systemic score was 5; therefore, *FBN1* NGS analysis was planned. The heterozygous, nonsense, pathogenic *FBN1* c.643C>T, p.(Arg215Ter) variant was detected, confirming the diagnosis of MS.

The third patient was a 13-year-old male admitted to the pediatrics department for rapid growth and scoliosis (Patient 12). The physical examination revealed enophthalmia, down slanting palpebral fissures, malar hypoplasia, micrognathia, orthodontic problems, scoliosis, and wrist sign. MVP was detected in the CVS exam regarding MS. Eye examination was normal. Although the systemic score was calculated to be 7, molecular analysis was planned due to the absence of concomitant positive family history, aortic dilatation, and ocular findings. A novel, heterozygous, missense, likely pathogenic *FBN1* c.3172G>A, p.(Gly1058Ser) was detected.

The fourth patient, who could not be diagnosed without molecular analysis, was a 12-year-old girl (Patient 11). She had been referred with the suspicion of MS while being evaluated in the orthopedics department for scoliosis. The prominent findings in her physical exam were MS-related facial features and skeletal findings such as scoliosis, pectus excavatum, pes planus, dolichostenomelia, and thumb and wrist signs. In addition, MVP had been detected in echography, and eye examination had revealed 1 D myopia. The systemic score was 11; however, there was no family history, ectopia lentis, or aortic dilatation to fulfill the diagnostic criteria. The molecular analysis revealed a heterozygous, frameshift, pathogenic *FBN1* c.1571del, p.(Thr524SerfsTer55).

Table 1. Clinical features and the systemic scores of the patients

	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	p11	p12
Age (years)	11	0.5	28	17	16	31	7	5	22	5	12	13
Family history	No	Yes	Yes	No	No	Yes	Yes	No	No	No	No	No
Ocular manifestations												
Myopia	+	–	+	+	–	+	–	+	+	–	+	–
Ectopia lentis	–	–	–	–	–	–	–	–	–	–	–	–
Early cataract	–	–	–	–	–	–	–	–	–	–	–	–
Astigmatism	–	–	–	–	–	–	–	–	–	–	–	–
Microspherophakia	–	–	–	–	–	–	–	–	–	–	–	–
Cardiovascular manifestations												
Aortic root dilatation	+	–	+	+	+	+	–	+	+	+	–	–
Z-score*	2.8	–1	3.02	2.6	2.32	3.14	–1	2.63	2.92	2.33	–1.2	–0.8
Aortic regurgitation	–	–	–	+	–	+	–	–	–	–	–	–
Aortic aneurysm	–	–	–	–	–	–	–	–	–	–	–	–
Aortic dissection	–	–	–	–	–	–	–	–	–	–	–	–
Mitral valve prolapse	+	–	–	+	+	–	+	+	+	+	+	+
Tricuspid valve prolapse	–	–	–	–	+	–	–	–	–	–	–	–
Mitral regurgitation	+	–	–	–	+	–	–	+	–	+	–	–
Tricuspid regurgitation	–	–	–	–	+	–	–	–	–	–	–	–
Skeletal findings												
Thumb sign	+	–	+	+	+	+	+	+	+	+	+	–
Wrist sign	+	+	+	+	+	+	–	+	+	+	+	+
Dolichostenomelia	+	+	+	+	+	+	+	+	+	+	+	+
Pectus excavatum or chest asymmetry	+	+	–	+	–	–	+	+	–	–	+	+
Pectus carinatum	–	–	+	–	+	+	–	–	+	+	–	–
Scoliosis	+	–	+	+	–	+	–	–	+	–	+	+
Reduced elbow extension	+	–	+	–	–	–	–	–	–	–	–	–
Pes planus	+	–	–	+	+	+	–	+	+	+	+	+
Hindfoot deformity	+	–	+	–	+	+	–	–	+	–	–	–
Acetabular protrusion	–	–	–	–	–	–	–	–	–	–	–	–
Long and narrow feet	+	+	+	+	–	–	–	+	+	–	–	–
Facial features												
Long and narrow face	+	+	+	+	+	+	+	+	+	+	+	+
High arched palate	+	+	+	+	+	+	+	+	+	+	+	+
Enophthalmos	–	+	+	+	–	+	+	+	+	+	–	+
Downslanted palpebral fissures	+	+	+	–	+	+	+	+	+	+	+	+
Malar hypoplasia	+	+	+	+	+	+	+	+	+	+	+	+
Micro/retrognathia	+	+	+	+	+	+	+	+	+	+	+	+
Crowding of teeth	+	–	+	+	+	+	+	+	+	–	+	+
Other features												
Skin striae	+	–	+	+	+	+	–	–	+	–	+	–
Pneumothorax	–	–	–	–	–	+	–	–	–	–	–	–
Dural ectasia	–	–	–	–	–	–	–	–	–	–	–	–
Joint laxity	–	–	–	–	–	–	–	+	–	–	–	–
Decreased muscle mass	–	–	–	–	–	–	–	+	+	–	+	+
Systemic score**	14	4	13	10	11	13	5	9	13	9	11	7

*Z-scores were calculated using the Z-score calculator available at https://www.marfan.org/dx/zscore, according to the aortic diameter measurements from the sinus Valsalva level. **The systemic scores were calculated according to the revised Ghent criteria

Variant Analysis

A total of 12 diverse variants were detected in 12 patients. Of these, ten were classified as pathogenic and two as likely pathogenic, and three were novel and nine were previously reported. No biallelic variants were detected. There were four frameshift (33%), five nonsense (42%), and three missense (25%) variants. Eight (67%) of the detected variants affected the EGF-like domain of the fibrillin-1 protein, three (25%) affected one of the TB domains, and one (8%) was located in the interdomain region (Fig. 1). A summary of the molecular findings is presented in Table 2.

Missense Variants

FBN1 c.3302A>G, c.7754T>C, and c.3172G>A were the detected missense variants. All of them were affecting the calcium-binding (cb) EGF-like domain; however, only the c.3302A>G, p.(Tyr1101Cys) was affecting the conserved cysteine residue. The *FBN1* c.3302A>G, p.(Tyr1101Cys) and c.7754T>C, p.(Ile2585Thr) were previously reported as pathogenic in MS patients.

The only novel missense variant was *FBN1* c.3172G>A, p.(Gly1058Ser), detected in Patient 12. The variant was not reported in the gnomAD and Iranome databases (PM2). Most of the in silico prediction tools (M-CAP, MutationTaster, and SIFT) showed that the variant had a damaging effect on the protein (PP3). Alternative variants chr15:48780600C>A, p.(Gly1058Val) and chr15:48780600C>T, p.(Gly1058Asp) had been classified as pathogenic by ClinVar and UniProt[16,17] (PM5). The glycine amino acid (a.a.) at the position of 1058 was conserved among species (Fig. 2a). The variant was classified as likely pathogenic with the ACMG evidence of PM2, PM5, PP2, PP3. Biomolecular modeling of the variant is shown in Figure 2b.

Frameshift Variants

FBN1 c.1526_1530dupGTTCG, p.(Tyr511ValfsTer70); c.2967dupT, p.(Thr990TyrfsTer2); c.4969del, p.(Ile1657SerfsTer25); and c.1571del, p.(Thr524SerfsTer55) were the detected frameshift variants. All frameshift variants affected the cbEGF-like domain except the *FBN1* c.2967dupT, p.(Thr990TyrfsTer2) that affected the TB domain. Among these, the *FBN1* c.4969del, p.(Ile1657SerfsTer25) was the only novel variant that was detected in a 5-year-old female with aortic dilatation, MVP, and skeletal findings (Patient 10). It was a null variant creating a termination codon and not reported in the gnomAD and Iranome databases. The isoleucine a.a at the position of 1657 was conserved among species (Fig. 2a). With the ACMG evidence of PVS1, PM2, the variant was classified as likely pathogenic. Biomolecular modeling of the variant is shown in Figure 2b.

Nonsense Variants

Five nonsense variants were detected in the study group. Four of the variants were previously reported. Three of them (*FBN1* c.2250C>A, c.4429G>T, and c.850C>T) affected the EGF-like domain, while one (*FBN1* c.643C>T) affected the TB domain (Fig. 1). The FBN1 c.1264G>T, p.(Gly422Ter) was the only novel nonsense variant and located in the interdomain area (Fig. 1). This novel variant was present in a 22-year-old male patient who presented with aortic dilatation, MVP, tall stature, and dysmorphic facial features (Patient 9). It was not reported in the gnomAD and Iranome databases. The variant was affecting the conserved glycine residue (Fig. 2a). As it is a null variant with the ACMG evidence of PVS1, PM2, the variant was classified as likely pathogenic.

DISCUSSION

In this study, we described nine reported and three novel variants in twelve patients with MS. In eight (67%) of the patients, the modified Ghent clinical diagnostic criteria enabled the diagnosis of MS without a molecular diagnosis. Ghent diagnostic criteria stand out as the most important tool for diagnosing MS.[2,18,19] As it has been reported that molecular confirmation can be performed in up to 97% of

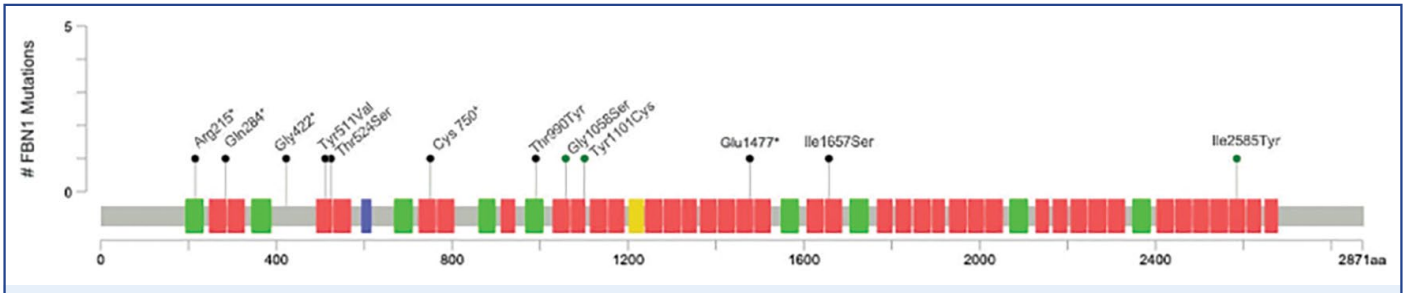

Figure 1. Scale model of the human fibrillin-1 domains. All of the protein level variations of the study group are presented. The image was generated using cBioPortal mutation mapper (https://www.cbioportal.org/mutation_mapper)

Table 2. Summary of the molecular findings of the patient group

Patient ID	Gender (F/M)	Position	Variation type	Zygosity	Protein exchange	Domain	HGMD/ UMD	Cystein substitution	Variant class	ACMG evidence	Reference (PMID)
Patient 1	M	ex13 c.1526_1530 dupGTTCG	Fs	Het	p.(Tyr511ValfsTer70)	EGF-like 7; calcium binding	–	No	Pathogenic	PVS1, PM2, PP3	33483584
Patient 2	F	ex19 c.2250C>A	Ns	Het	p.(Cys750Ter)	EGF-like 11; calcium binding	CM055253/2487, 1095	Yes	Pathogenic	PVS1, PM2, PP3	16220557
Patient 3	F	ex25 c.2967dupT	Fs	Het	p.(Thr990TyrfsTer2)	TB5	–	No	Pathogenic	PVS1, PM2, PP3	–
Patient 4	F	ex27 c.3302A>G	Ms	Het	p.(Tyr1101Cys)	TB5	CM013925/2774, 419, 602, 696, 714, 918, 1001, 1274, 2097	Yes	Pathogenic	PP5, PM1, PM2, PP2, PP3	11700157, 12402346, 14695540, 19293843, 16222657, 16596670, 19863550
Patient 5	F	ex36 c.4429G>T	Ns	Het	p.(Glu1477Ter)	EGF-like 25; calcium binding	CM972810/244	No	Pathogenic	PVS1, PM2, PP3, PP5	10464652
Patient 6	M	ex63 c.7754T>C	Ms	Het	p.(Ile2585Thr)	EGF-like 45; calcium binding	CM972820/3282, 258, 313, 412, 418, 483, 509, 882, 883, 884, 1018, 1041, 1056, 1442, 1602, 1805, 1979, 2258, 2286, 2434, 2516, 2539, 2648, 2677, 2701, 2736, 2742, 2786, 3043, 3044	No	Pathogenic	PP5, PM1, PM2, PP2, PP3	24161884, 10464652, 11700157, 12938084, 17657824, 19293843, 17627385, 19159394, 21907952
Patient 7	F	ex7 c.643C>T	Ns	Het	p.(Arg215Ter)	TB1	CM003136/2599, 294, 741, 756, 821, 1090, 1358, 2512	No	Pathogenic	PVS1, PM2, PP3, PP5	11139245, 15241795, 17657824, 16220557, 19293843
Patient 8	M	ex8 c.850C>T	Ns	Het	p.(Gln284Ter)	EGF-like 4; calcium binding	CM074792/1365	No	Pathogenic	PVS1, PM2, PP3	17657824
Patient 9	M	ex11 c.1264G>T	Ns	Het	p.(Gly422Ter)	Interdomain area	–	No	Likley pathogenic	PVS1, PM2,	–
Patient 10	F	ex41 c.4969del	Fs	Het	p.(Ile1657SerfsTer25)	EGF-like 28; calcium binding	–	No	Likely pathogenic	PVS1, PM2, PP3	–
Patient 11	F	ex13 c.1571del	Fs	Het	p.(Thr524Serfs*55)	EGF-like 7; calcium binding	–	No	Pathogenic	PVS1, PM2, PP3, PP5	–
Patient 12	M	ex26 c.3172G>A	Ms	Het	p.(Gly1058Ser)	EGF-like 15; calcium binding	–	No	Likely pathogenic	PM2, PM5, PP2, PP3	–

**The systemic scores were calculated according to the revised Ghent criteria. HGMD: Human Gene Mutation Database; UMD: The Universial Mutation Database; ACMG: American College of Medical ; PMID: PubMed - Indexed for MEDLINE; Fs: Frameshift; Ns: Nonsense; Ms: Missense; EGF: Epidermal Growth Factor Genetics

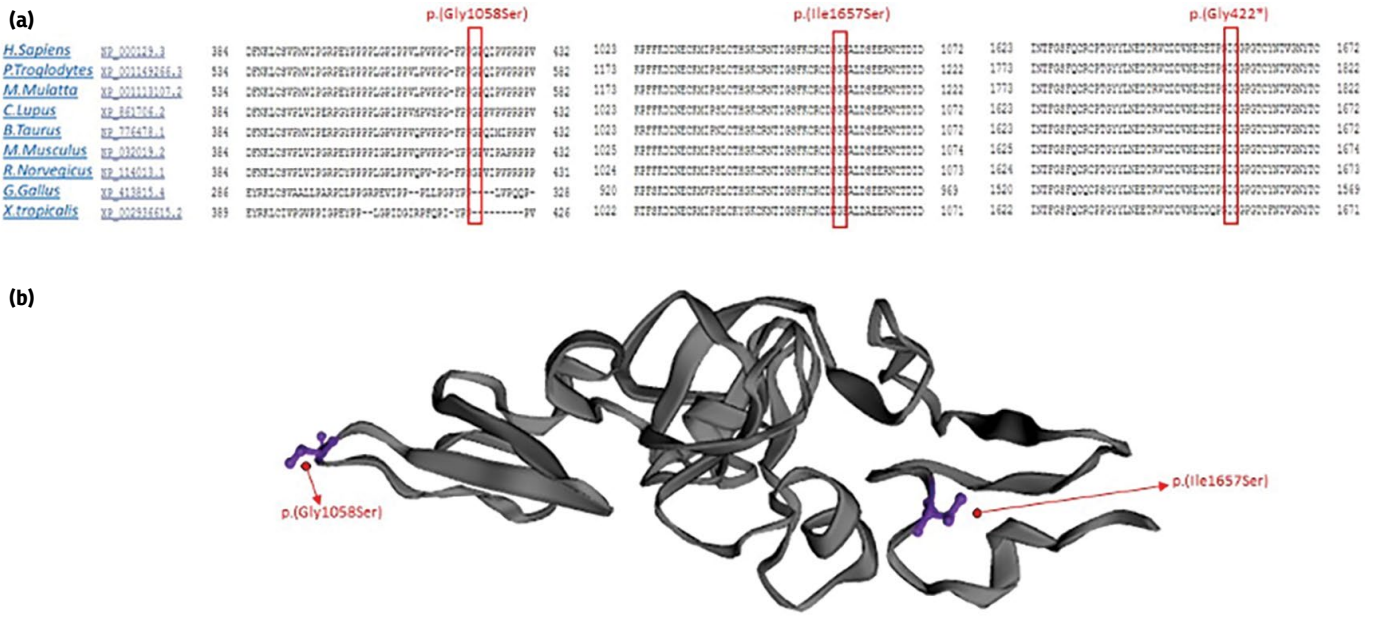

Figure 2. Interspecies conservation status of amino acids affected by novel variants detected in the study **(a)**, biomolecular modeling (PDB1uzj: integrin binding cbegf22-tb4-cbegf33 fragment of human fibrillin-1, holo form. ChainA: fibrillin-1) of the two novel variants p.(Ile1657SerfsTer25), and p.(Gly1058Ser) **(b)**, The other novel variant p.(Gly422Ter) could not be modeled due to its location (interdomain area)

the patients who meet the clinical diagnostic criteria,[18,20] Ghent criteria also guide the identification of patients with *FBN1* variants, as in our study. However, using those clinical criteria may be insufficient for diagnosis in childhood when some features of the syndrome have not yet emerged.[2,20,21] In addition, one should also keep in mind that clinical findings may not be fully manifested in some patients due to variable expression and intrafamilial variability. Considering the importance of early diagnosis in the disease, it is undeniable that molecular analysis of the *FBN1* is a valuable tool for diagnosis, especially in this group of patients. In our study, the definitive diagnosis was reached by detecting pathogenic variants in *FBN1* by NGS analysis in four patients who did not meet the clinical diagnostic criteria. All of those patients were under the age of 13, and the definitive diagnosis achieved by molecular studies has greatly benefited, monitoring the MS-related complications and raising awareness in both family and caregivers.

Identifying the underlying molecular pathology in individuals with MS is also valuable in establishing phenotype-genotype correlations and predicting the course of the disease. The most frequently reported phenotype-genotype correlation in MS is the severe phenotype with neonatal-onset that is seen mainly in patients with variants in *FBN1* exon 24–32.[21–24] Three of the variants detected in our study were located in this region, and none of those patients (Patient 3, Patient 4, and Patient 12) had been diagnosed with neonatal-onset MS. However, the presence of early-onset severe aortic dilatation in two patients (Patient 3 and Patient 4) supports the association of exon 24–32 variants with severe clinical findings. The other patient with a variant located in this region was a 13-year-old male (Patient 12). Although the absence of cardiac problems in this patient is inconsistent with this phenotype-genotype correlation, it is hard to reach a definitive conclusion considering the patient's young age. Thus, in Patient 12, close follow-up was planned due to aortic dilatation, which can develop rapidly, especially during adolescence, based on this phenotype-genotype correlation.

Another phenotype-genotype correlation frequently reported in the literature is the presence of ectopia lentis in patients with missense variants affecting cysteine residues (ms-cys).[21,25–27] There was only one ms-cys variant in our study; however, ectopia lentis was absent in this patient (Patient 4). This finding might be attributed to the variable expressivity in MS. In addition, ectopia lentis was not detected in any patient in our study. We think that this is due to the scarcity of ms-cys variants and indirectly supports the genotype-phenotype correlation reported in the literature.

Contradictory data are reported in genotype-phenotype association studies on the severity of cardiological involvement and variant types. Although it is controversial, recent studies suggest that the frequency of aortic dissection is higher in patients with variants that cause premature termination.[21,27–29] We observed that the variants detected in four of the five patients with aortic dilatation under 17 years of age were in the premature termination (PTC) group. In addition, among the patients with PTC variants, two pediatric patients had no cardiac involvement; however, their parents had a history of aortic rupture. Therefore, we concluded that variants in these patients might also be associated with severe aortic dilatation. Although statistical analysis could not be performed due to the small number of patients, these findings supported the suggestion that the cardiological involvement would be more severe in individuals carrying variants that cause premature termination.

Twenty-five percent of the *FBN1* variants are de novo, while 75% are inherited from the affected parent in MS.[4] Although it could not be molecularly confirmed, MS-related family history had been found in only 33% of the patients in the present study. The higher incidence of de novo variants in our study, unlike the literature, may be related to the small number of the study group.

Variants in *FBN1* are usually specific to affected individuals or families. Novel variants have been described in 25–30% of patients in the literature.[2,30] Variants in all patients were different from one another in our study, and novel variants were detected in 25% of the patients following the literature data.

Among the variants in MS, it is reported that pathogenic/likely pathogenic variants are mostly missense, followed by frameshift, nonsense, and splice variants, respectively.[11,12] We found that missense variants were detected less often than frameshift and nonsense variants. In addition, while it has been reported that missense variants usually affect the cysteine residue,[12,20] only one missense variant affected the preserved cysteine residue in the present study. This difference in our result might be due to geographical region or the small number of patients. There is a need for more studies to be conducted on individuals with MS in Turkey to clarify this finding.

The localization of the *FBN1* variants is reported to distribute within the gene without any hot spots, as in our study.[11,12] At the protein level, studies have shown that the *FBN1* variants reported so far frequently affect the EGF-like domains of fibrillin-1.[20,21,31] The majority of the variants in the present study also similarly affected the EGF-like domains, followed by the TB domain (Fig. 1).

We identified three novel and nine previously reported variants. *FBN1* c.1264G>T, p.(Gly422Ter), c.4969del, p.(Ile1657SerfsTer25), and c.3172G>A, p.(Gly1058Ser) were the novel variants reported in our study. The first two variants, which create PTC, are predicted to activate the nonsense-mediated decay mechanism and completely inhibit fibrillin-1 production. On the other hand, the missense variant *FBN1* c.3172G>A, p.(Gly1058Ser) is predicted to affect a highly conserved embedded glycine residue. Therefore, the substitution of glycine with another a.a. is predicted to be damaging.[32]

Among the previously reported variants, patients with *FBN1* c.1526_1530dupGTTCG (Patient 1), c.2967dupT (Patient 3), c.3302A>G(Patient 4), c.7754T>C (Patient 6), and c.643C>T (Patient 7) variants had classical MS findings, including eye, skeletal, and CVS involvement, similar to the patients previously reported in the literature. However, the clinical findings of the patients with the variants *FBN1* c.850C>T (Patient 8) and c.2250C>A (Patient 2) differed from the previously reported patients. Patient 8 was a 5-year-old male with myopia, aortic dilatation, mitral, and tricuspid valve prolapse, as well as pectus anomaly and dolichostenomelia. The *FBN1* c.850C>T variant was previously reported by Comeglio et al.,[30] in a patient only with CVS involvement. Although the widespread manifestation of the syndrome in Patient 8 could be explained by the variable expression defined in MS, it should be kept in mind that additional genomic variations in individuals may also cause this variability. The *FBN1* c.2250C>T variant was detected in a 6-month-old female patient who presented with arachnodactyly and facial dysmorphic features (Patient 2). Although she did not have any CVS findings, her father died at the age of 24 due to an aortic rupture. The same variant was reported by Rommel et al.,[27] in a patient with aortic dilatation, mitral valve prolapse, and skeletal features. Even if the present findings of the patient were not as extended as the findings in the previous patient,[27] it was thought that some findings may not have emerged yet due to her young age.

The *FBN1* c.1571del and c.4429G>T variants were also among the previously reported variants in the ClinVar database (ClinVarID:263469/429983); however, data on clinical findings in patients were not available. In our study, the patient with the *FBN1* c.1517del variant was a 12-year-old girl with a systemic score of 11. The clinical findings of this patient were presented in detail in the result section (Patient 11). Skeletal involvement was the most prominent finding in the patient. The *FBN1* c.4429G>T variant was identified in a 16-year-old female patient (Patient 5) with aortic dilatation, mitral

valve prolapse, and mitral and tricuspid regurgitation. The detailed phenotypic information about these variants was presented for the first time through our study.

In conclusion, in MS, where the timely diagnosis is the most critical step in follow-up and treatment, molecular analysis of the *FBN1* by NGS is beneficial for accurate diagnosis, especially in patients whose clinical findings do not meet diagnostic criteria. Also, elucidating the underlying molecular pathology in all patients contributes to expanding the phenotype-genotype correlation in the disease. Our study has broadened the genotypic and phenotypic spectrum of MS in Turkey by describing detailed clinical findings of twelve patients and reporting three novel variants.

Disclosures

Ethics Committee Approval: The study was approved by the Umraniye Training and Research Hospital Clinical Research Ethics Committee (No: B.10.1.TKH.4.34.H.GP.0.01/176, Date: 27/05/2021).

Informed Consent: Informed consent was obtained from all patients before molecular analysis.

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