Original Article

A Novel Missense Mutation in the TGF-β-binding Protein-Like Domain 3 of *FBN1* Causes Weill–Marchesani Syndrome with Intellectual Disability

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Abstract

Background: Weill–Marchesani syndrome (WMS) is a rare connective tissue disorder characterized by locus heterogeneity and variable expressivity. Patients suffering from WMS are described by short stature, brachydactyly, joint stiffness, congenital heart defects, and eye abnormalities. This disorder is inherited in two different modes; the autosomal dominant form of the disease occurs due to a mutation in *FBN1*, and the recessive form results from mutations in *ADAMTS10*, *ADAMTS17*, or *LTP2* genes.

Materials and Methods: The family recruited in this study was a consanguineous Iranian family with an intellectually disabled girl referred to the Sadra Genetics laboratory, Shahrekord, Iran. The clinical history of family members was investigated. Whole-Exome Sequencing (WES) for the proband was performed. Sanger sequencing was used to assess the segregation of candidate variants in the other family members.

Results: Whole-exome sequencing analysis revealed a novel heterozygote mutation in the proband located at the third TGF-β-binding protein-like (TB) domain of the *FBN1* gene (NM000138: c.2066A>G: (p. Glu689Gly), NP_000129.3, in exon 17 of the gene). Co-segregation analysis with Sanger sequencing confirmed this mutation in the affected members of the pedigree.

Conclusion: Our findings represent an autosomal dominant form of specific WMS resulting from a substitution mutation in the *FBN1* gene. In addition to the typical manifestations of the disorder, mild intellectual disability (ID) was identified in the 8-year-old proband. Given the fact that ID is primarily reported in *ADAMTS10* mutated cases, this family was clinically and genetically a novel case.

Keywords: *FBN1*, intellectual disability, Weill-Marchesani syndrome, whole-exome sequencing

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Introduction

Weill–Marchesani syndrome (WMS), with an estimated prevalence of one in 100,000 people, is a genetically heterogeneous disorder with variable expressivity primarily affecting connective tissue.[1] WMS is characterized by short stature, brachydactyly, joint stiffness, taut skin with thickened skin folds, and congenital heart defects.[2,3] Some eye abnormalities reported in WMS patients include microspherophakia, ectopia of the lens, severe myopia, and in many cases, glaucoma.[4] The stature in male patients is in the range of 142 to 169 cm, and it does not typically

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exceed 157 cm in affected females.[5] All types of WMS have the same manifestations, so confirmation of WMS diagnosis needs molecular genetic testing.[2] Mutations in *ADAMTS10 (W*MS1, MIM _277600), *ADAMTS17* (WMS4, MIM _613195), and *LTP2* (WMS3, MIM _608328) genes cause the autosomal recessive form of the disease and are responsible for 45 percent of cases. The autosomal dominant form of the disorder is caused by mutations in the *FBN1* gene (WMS2, MIM _608328), which accounts for 39 percent of cases. The remaining cases occur sporadically. Intellectual disability (ID) has been reported in 11-17 percent of patients, mainly *ADAMTS10* mutated cases.[3] The penetrance of WMS is thought to be complete, but intra and interfamilial variable expressivity has been reported in some cases.[6]

The *FBN1* encodes the fibrillin-1 protein, which is a major structural component of the microfibrillar network. Microfibril, an extracellular matrix (ECM) protein, serves proper structural and regulatory roles in force-bearing connective tissues. Extracellular microfibrils provide a scaffold for elastin deposition in the lung, blood vessels, and skin. In addition, they play an elastin-independent role in eye tissues like the ciliary zonule and cornea, which is compromised in WMS patients.[1] Fibrillin 1-microfibrils contribute to tissue hemostasis mediated by interaction with growth factors such as bone morphogenetic proteins (BMPs), latent transforming growth factor-beta binding proteins (LTBPs), cell surface integrin, and other extracellular matrix proteins. Latent TGF-β binding protein two, which is encoded by *LTBP-2*, is an essential factor for the stability of the microfibrils bundle within the ciliary zonule. *ADAMTS10 an*d *ADAMTS17* encode metalloprotease domain-containing proteases which participate in microfibrils assembly.[7]

Materials and Methods

An Iranian consanguineous family with an 8-year-old intellectually disabled girl referred to Sadra Medical Genetic Laboratory, Shahrekord, Iran, was recruited in this study. This proband girl was affected with mild ID, microcephaly, seizure, developmental delay, speech impairments, and digit (both hands and feet) abnormality. Short stature and bilateral deformities of hands and feet fingers were also detected in some family members in the pedigree. Previous karyotype analysis excluded chromosomal abnormality in the child.

This study was approved by the Ethics Committee of Ilam university of medical sciences (IR.MEDILAM.REC.1401.050), and all participants gave their written informed consent for participation in the current study. The extended pedigree of the family with indicated affected members is provided in Figure 1. All family members were clinically evaluated, and their medical histories were meticulously reviewed. The peripheral blood samples from close family members were collected in EDTA tubes for molecular analysis.

The extraction of genomic DNA was carried out using the standard salting-out method.[8] The concentration and quality

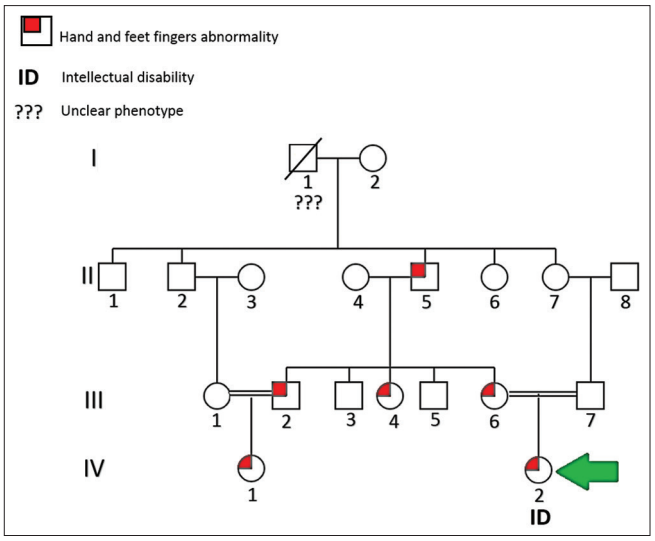

Figure 1: The extended family pedigree indicated affected members with Weill–Marchesani syndrome. The 8-year-old proband girl is marked with a green arrow

of extracted DNA samples were then investigated using a nanodrop spectrophotometer device (Thermo Scientific, Waltham, USA). To identify the genetic cause of symptoms in the family, Whole-exome sequencing (WES) for the proband (VI2) was performed using the NovaSeq6000 platform, Sure Select V-7 Post library type, and 100X depth of Coverage. The workflow of WES data analysis is presented in Figure 2. We analyzed the data based on our previous studies.[9,10] Briefly, sequences were aligned against the human reference genome GRCh37/hg19 build, and variant calling was carried out. Annotation and variant detection were performed using wANNOVAR software. Following steps were subsequently done for prioritizing the most promising variants. The synonymous, intronic, and UTR region variants were excluded. Due to the low prevalence of the disease and the hypothesis of the rare frequency of causative mutation, the reported minor allele frequencies (MAF) of less than 0.01 and variants with unreported MAF were evaluated in databases including Exac (http://exac.broadinstitute.org), genomAD browser (http://gnomad.broadinstitute.org), the 1000 genome project (http://1000genomes.org), Iranome (a genomic database for Iranian populations) (http://iranome.com). Subsequently, the remaining rare variants were classified based on their *in silico* prediction scores in SIFT (http://sift.bii.astar.edu.sg), MutationTaster (www.mutationtaster.org), Polyphen2 (http://genetic.bwh.harvard.edu/pph 2), GERP (https://genome.ucsc.edu/cgi-bin/hgTrackUi?db = hg19&g = allHg19RS_BW) and CADD_phred (http://cadd.gs.washington.edu).

Considering the family pedigree, we took into account variants in homozygous, compound heterozygous states, and dominant states. Afterward, they were finalized by incorporating conservation scores of the variants based on the SiPhy_29way_logOdds score. then, we focused on the gene variants involved in molecular pathways related to ID and skeletal disorders symptoms. Finally, Sanger sequencing was

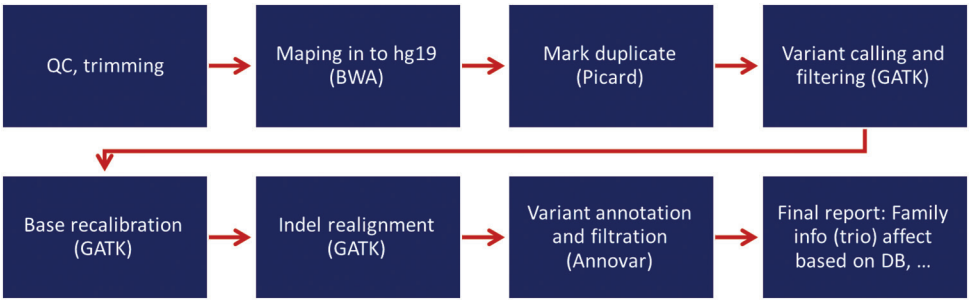

Figure 2: The workflow of WES data analysis was carried out in this study

used as the gold standard of screening to assess the segregation of candidate variants in other family members and confirm the respective causing genes. The sequence of primers and products length are mentioned in Table 1.

Results

Since the proband resulted from a consanguineous marriage, the autosomal recessive pattern of inheritance was first suspected and examined, but no likely pathogenic recessive mutation was found. We also looked for compound heterozygote mutations in the pedigree to rule out the cause of symptoms, but no mutation was found. However, by carefully examining the hypothesis of autosomal dominant inheritance of the disease and considering that some affected people in the pedigree were the result of unrelated marriages, the likely pathogenic variant (NM000138: c.2066A>G: (p. Glu689Gly), NP_000129.3, in exon 17 of the gene) in the *FBN1* gene was identified in the proband. The subsequent Sanger sequencing confirmed the presence of the *FBN1* gene mutation (NM000138: c.2066A>G: (p. Glu689Gly), NP_000129.3, in exon 17 of the gene) in all affected family members and its absence in healthy individuals.

Based on previous reports, mutations in FBN1 only justified the manifestation of skeletal symptoms. We looked into the double heterozygote hypothesis and for de novo mutations to figure out what was causing the mental problems. Two novel mutations were found in NAA15 and KMT2E genes, which were unreported in both genomic projects databases and Iranome (MAF = 0). Sanger sequencing results revealed the presence of these mutations in the proband's father/mother despite the absence of ID symptoms. A medical commission was held and based on that ID has been reported in 13% of Weill-Marchesani patients[2]; we finalized the mutation (NM000138: c.2066A>G: (p. Glu689Gly), NP_000129.3, in exon 17 of the gene) in FBN1 as the cause of symptoms. Based on the American College of Medical Genetics (ACMG) classification, this variant is likely pathogenic. We report this variant in the Varsome database as a pathogenic variant.

Our findings confirmed that the discovered *FBN1* mutation is associated with clinical symptoms in afflicted family members. The chromatogram obtained from Sanger sequencing of pedigree members is shown in Figure 3.

Table 1: list of variants for cosegregation, primers and PCR products length

Gene	variant	Primers	PCR product length
FBN1	NM_000138: exon17:c. A2066G	F: CACACACAT GCGGAGCAC R: GGCACATGG CGTACCTGG	269bp
KMT2E	NM_018682: exon13:c. A1618G	F: GTACAAGGT GGACTGTGCATG R: GCAATTCTG CAAGGATTTGC	485bp
NAA15	NM_057175: exon7:c.C808A	F: CCAGGTCCA TCCACTTAGCAG R: GAATGCAGT GGCACTATGCTG	621bp

Discussion

According to the OMIM database, *FBN1 i*s responsible for eight various kinds of autosomal dominant genetic conditions, including WMS.[11] WMS is an extremely rare systemic connective tissue disorder with extensive interfamilial clinical expressivity. The major Ophthalmological manifestations of WMS in order of observed frequencies include myopia 94%, microspherophakia 84%, ectopia lentis 73%, glaucoma 80%, and cataract 23%. Other features include joint contractures, tight skin, and cardiac abnormalities like pulmonary valve stenosis, aortic valve stenosis, ductus arteriosus, ventricular septal defect, and mitral valve insufficiency.[1,4] In this study, we analyzed an Iranian family with a girl affected with mild Intellectual Disability. She manifested apparent symptoms of WMS, including brachydactyly, short stature, and heart defects (atrial septal defect and mild pulmonary valve stenosis). She also showed microcephaly and misalignment of the teeth [Figure 4]. The audiometry evaluation of the child showed a unilateral and unexplained rupture of the eardrum. She also had a history of kidney stones which has not previously been reported in Weill-Marchesani patients. Remarkably, she showed transient seizures that responded well to treatment and did not recur. MRI examination of the child was normal, and she was diagnosed with mild and autistic-type Intellectual Disability. It is of note that ID has been reported in 13% of Weill-Marchesani patients.[2] The child's grandfather showed the typical symptoms of WMS, including short stature, brachydactyly, joint stiffness, eye and heart abnormalities [Figure 5]. Despite

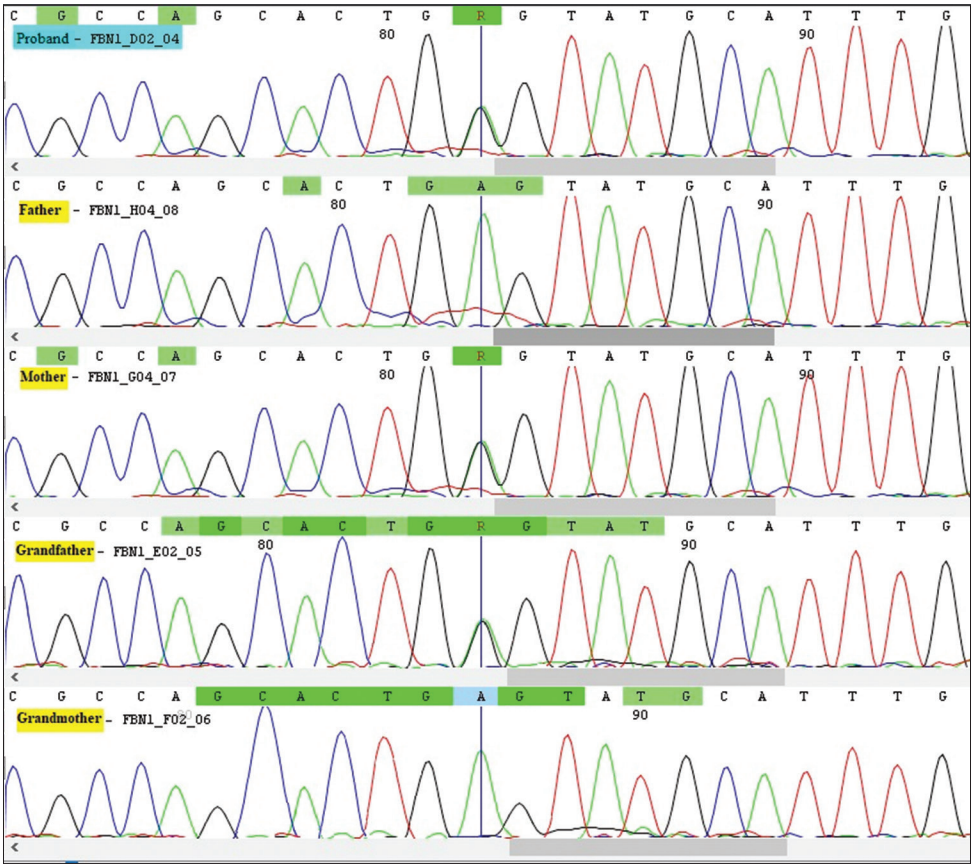

Figure 3: Sanger sequencing chromatograms of family members

Figure 4: (a) Frontal photo of the proband indicating microcephaly. (b) Brachydactyly appearance in hand. (c) Deformity of digits in foot (d) Posture and stature, and (e) Misaligned teeth in the patient

the complete penetrance of the disease, it showed variable expressivity in the family.[2] In the pedigree, the individuals III-5, IV-2, IV-4, IV-6, and V-1 represented brachydactyly, stiffness of joints, and ocular symptoms, and the proband's mother was diagnosed with keratoconus and abnormalities of the hand and foot digits [Figure 5]. The proband had no ocular symptoms. However, it should be noted that the onset of ocular symptoms in affected individuals was age-dependent. Analysis of pedigree demonstrated that the *FBN1* mutation is segregated with WMS clinical characteristics and is compatible with the autosomal dominant pattern of inheritance. Analysis of WES data indicated a substitution mutation in exon 17 of the *FBN1* (NM000138 exon17c.2066A>G: p.E689G). As a consequence of this missense variation, a negatively charged polar amino acid (glutamic acid) is replaced by the smallest and simplest available amino acid (glycine) in the TGF-β-binding protein-like domain 3 of *FBN1*. Fibrillin 1 consists of seven TGF-β-binding protein-like (TB) domains dispersed between calcium-binding epidermal growth factor-like domains.[12] This highly conserved domain (TB) is only found in Fibrillins and LTBPs proteins. This domain plays a broad range of pivotal functions in the ECM structure and protein-protein interactions.[13] Fibrillin1 forms the core component of connective tissue microfibrils. Mutations in this gene are primarily responsible for Marfan syndrome with arachnodactyly, lofty stature, eye abnormality, and cardiovascular dysfunction.[6,14,15] However, mutations of *FBN1* were also reported to be associated with some other syndromes like WMS and acromicric dysplasia.[6,11,16] Faivre *et al*.[1] first

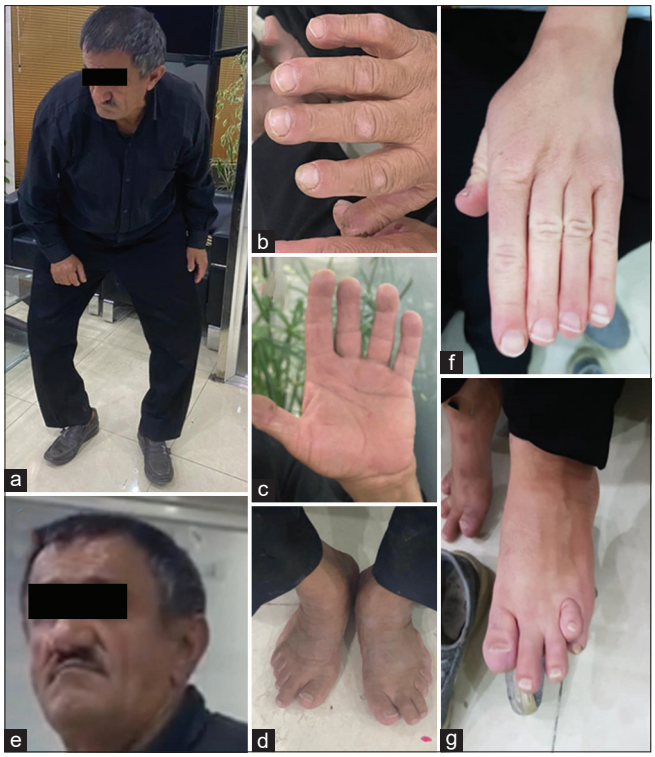

Figure 5: The clinical manifestations in the proband's grandfather. (a) Posture and stature of the patient. (b and c) Dorsal and palmar sides of the hand with brachydactyly appearance. (d) Apparent deformity of foot digits (e) Ocular abnormalities. (f and g) Hand and foot digit abnormalities in the proband's mother

reported a 24-nucleotide in-frame deletion in the TB5 domain in Weill-Marchesani patients. Mutations in the TB4 region of *FBN1* were revealed in scleroderma skin disorder.[17] Moreover, substitution mutations in the TB5 domain of this gene were shown to be associated with acromicric and geleophysic dysplasia with severe short stature.[18] The exact molecular causes of the vast phenotypic differences resulting from fibrillin mutations are unknown, but this observed discrepancy suggests a robust genotype-phenotype correlation of *FBN1* mutants.

Conclusion

In conclusion, we reported an Iranian family with an 8-year-old intellectually disabled girl in this study. Affected members showed typical symptoms of WMS compatible with the autosomal dominant pattern of inheritance. Results of the mutation screening revealed a novel missense mutation in exon 17 of the *FBN1* (NM000138 exon17c.2066A>G: p.E689G), which is assumed to be the causing mutation for the ID and other clinical manifestations in this family.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of Sadra Medical Genetic Laboratory, and all participants gave their written informed consent for participation in the current study.

Consent for publication

The consent for publication was obtained from participants.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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