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Molecular Case Studies

RAPID COMMUNICATION

Identification of a novel pathogenic variant in *FBN1* associated with Marfan syndrome

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Abstract Aortic diseases arising in Marfan syndrome (MFS), such as in aneurysms and dissections of the thoracic aorta, are related to genetic alterations in the *FBN1* gene. Databases, such as Universal Mutations-*FBN1*, ClinVar, and The Human Gene Mutation, contain more than a thousand *FBN1* mutations associated with MFS. The *FBN1* gene, which encodes fibrillin-1, is responsible for the integral production of different protein domains. Possible genetic changes may lead to a weakening of blood vessels, leading to the development of aortopathies. In this study, we present the association of a novel *FBN1* variant with MFS. The proband is a man who presented with ascending aortic aneurysm and dissection (TAAD) at 42-yr-old, which was surgically treated. Clinical investigations were performed in all family members enrolled in the study. Marfan signs were observed in the proband, daughters, and granddaughter. Direct sequencing of the *FBN1* gene in the proband identified a novel truncation variant p.(Glu2019Ter), and a cascade screening was done. The variant was classified as pathogenic and causal for MFS according to the American College of Medical Genetics and Genomics (ACMG) criteria and revised Ghent nosology for MFS diagnosis, respectively. Proband's daughter and granddaughter harbor the variant, however, without aortic alteration. This work reports for the first time a patient with the *FBN1*-p.(Glu2019Ter) variant and its association with MFS/TAAD.

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CASE PRESENTATION

Marfan syndrome (MFS; MIM #154700) is a connective tissue disorder with an autosomal dominant pattern of inheritance, conferring phenotypes such as ectopia lentis, arachnodactyly, and thoracic aortic aneurysm/dissection (TAAD) (Araújo et al. 2016). Numerous mutations in the *FBN1* gene, which encodes fibrillin protein, are directly linked to the syndrome, supporting the fact that it is considered the "Marfan gene" (Sakai et al. 2016). The relationship between the phenotype, location, and types of mutations in *FBN1* has been constantly investigated, with the aim of improving treatment and follow-up and establishing an improvement in risk stratification (Takeda and Komuro 2019). Here we report the association of a novel variant in *FBN1* and MFS phenotypes in a Brazilian family.

The proband is a 46-yr-old man who presented acute aortic syndrome (type I aortic dissection) at age 42 yr. He was admitted to an emergency hospital, and a computed tomography (CT) angiography of the chest was performed, which showed dissection of the

ascending aorta with significant aneurysmal dilatation (maximum diameter, 70 mm), extending to the beginning of the abdominal aorta. The echocardiogram showed normal systolic diameter and preserved systolic function without segmental alteration. He underwent emergency surgery. Moreover, he presented Marfan signs, such as wrist and thumb sign, reduced upper/lower extremity ratio, and facial features (Fig. 1A). The proband could not be

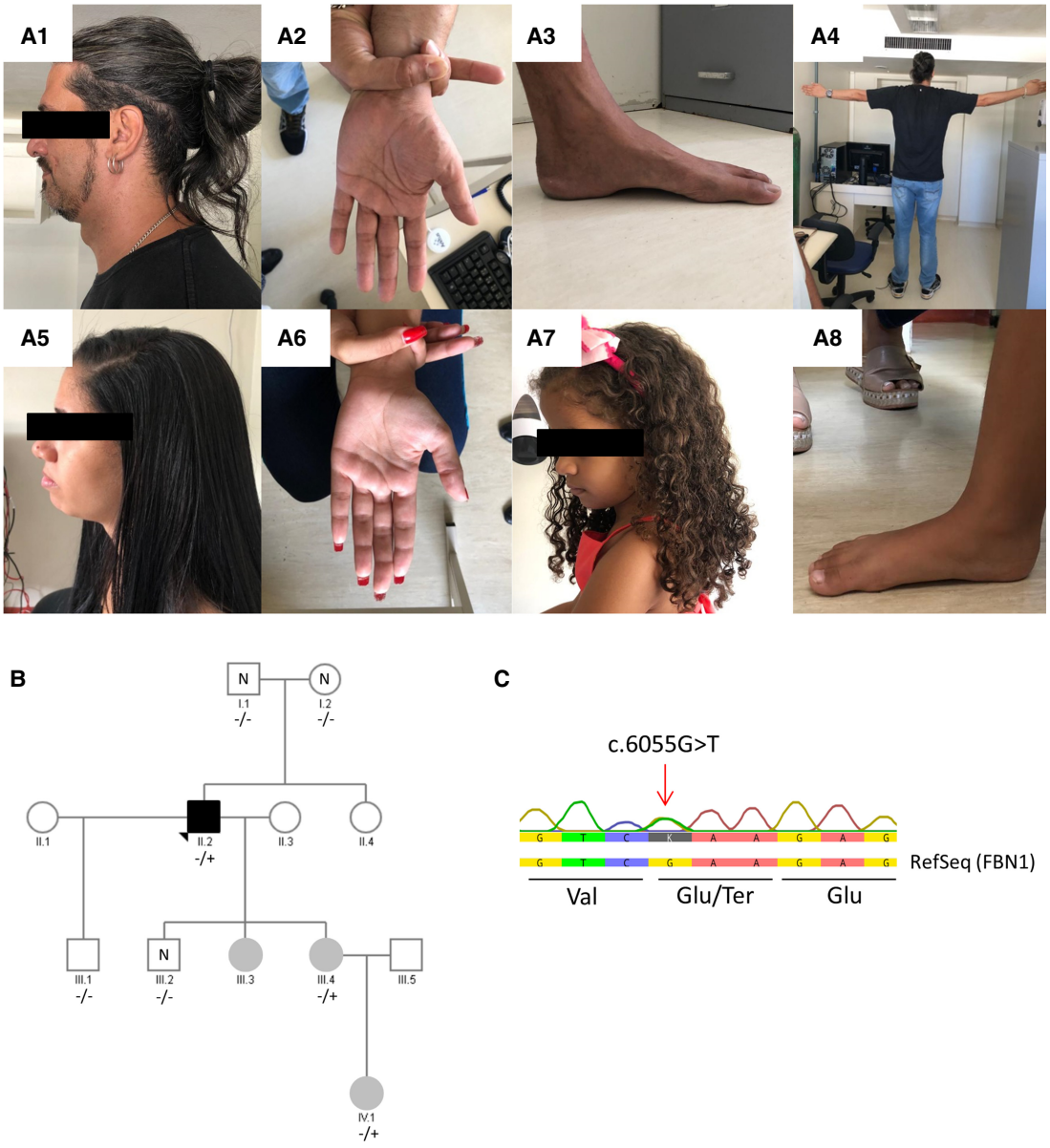

Figure 1. Clinical findings for Marfan syndrome (MFS) in proband and in family members (*A*). (*A1*,*A5*,*A7*) Mandibular retrognathism. (*A2*,*A6*) Positive fist sign. (*A3*,*A8*) Flat feet. (*A4*) Reduced upper/lower segment ratio. (*B*) Pedigree indicating proband with MFS diagnosis (black square), individuals with some MFS physical features, but no aortic disease (gray circles), the p.(Glu2019Ter) carriers (–/+) and noncarriers (–/–). An N indicates unaffected individuals (no signs for MFS and normal aorta) and white symbols indicate not-evaluated individuals. (*C*) Electropherogram of the *FBN1* gene pointing to a variant c.6055G > T p.(Glu2019Ter) in exon 50, resulting in the exchange of a glutamic acid for a premature stop codon.

Table 1. Genetic findings in the *FBN1* gene

Genomic location	HGVS cDNA	HGVS protein	Type	Zigosity	dbSNP	gnomAD MAF	CADD/ mutation taster[a]	Variant interpretation
Chr 15:48734026 (GRCh37) Chr 15:48441829 (GRCh38)	NM_000138.5: c.6055G > T	p.Glu2019Ter	Nonsense	Het	-	-	47/PDC	Pathogenic

(MAF) Minor allele frequency, (PDC) prediction disease causing.
[a]CADD score >20, probably pathogenic.

evaluated by an ophthalmologist; therefore, without an ectopia lentis test, he did not fill the MFS diagnosis criteria, according to the revised Ghent nosology for MFS (Loeys et al. 2010).

Genetic analysis identified a novel variant in the *FBN1* gene, c.6055G > T; (p.(Glu2019Ter)) (Table 1). Nine members of proband's family were clinically evaluated and submitted to cascade screening for variant detection (Fig. 1B).

His parents (77-yr-old father and 68-yr-old mother) have coronary artery disease, but did not present any systemic MFS signs or aorta structural alterations. Cascade screening did not find the variant in his parents; therefore, it is an assumed de novo variant, as no paternity test was performed. Thus, the proband met the criteria for the MFS diagnosis (Loeys et al. 2010): absence of family history of MFS, aorta aneurysm ($Z \geq 2$) and a de novo and nonsense *FBN1* mutation (Table 2).

The proband has four children. Two daughters (25- and 24-yr-old) fill 3 points on the systemic score (wrist sign, reduced upper/lower limb ratio, retrognathia, dolichocephaly, palpebral fissures with inferolateral slope), without aortic aneurysm. They still do not meet the criteria for MFS. The older one inherited the variant *FBN1* allele, and the younger sister did not consent the genetic test (Table 2).

The proband's sons (23-yr-old and 4-yr-old) do not have clinical signs of MFS and presented negative variant screening. The proband's granddaughter is 4-yr-old and does not have an aortic aneurysm, neither does she meet MFS criteria yet, although she meets three points on the systemic score (wrist sign, flat feet, and facial features). She harbors the p.(Glu2019Ter) variant.

TECHNICAL ANALYSIS

Genomic DNA was obtained from peripheral blood samples. For proband, coding regions and intron–exon boundary regions of the *FBN1* gene were amplified by polymerase chain reaction (PCR) and sequenced using the BigDye Terminator v3.1 reagent (Thermo Fisher Scientific) and the genetic analyzer 3500xl (Thermo Fisher Scientific). PCR primers sequences are described in Supplemental Table S1. Cascade screening was performed in all family members who consented. Sequences were analyzed with Geneious software v11.1.5 (BioMatters) and variant classification was done according to criteria from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) (Richards et al. 2015).

VARIANT INTERPRETATION

The alteration c.6055G > T in exon 50 of *FBN1* generates a premature stop codon in place of glutamic acid-2019 [p.(Glu2019Ter)], causing early interruption of mRNA translation. This

Table 2. Revised Ghent criteria for diagnosis of Marfan syndrome and related conditions

Criteria	Pb	I.1	I.2	II.4	III.1	III.2	III.3	III.4	IV.1
FBN1 mutation	Y	N	N	U	N	N	U	Y	Y
Ao ($Z \geq 2$)[a]	Y	N	N	N	N	N	N	N	N
Ectopia lentis	U	U	U	U	U	U	U	U	U
Systemic score[b] (pts)	5	0	0	0	0	0	3	3	3
Wrist and thumb sign (3 pts)	Y	-	-	-	-	-	-	-	-
Wrist or thumb sign (1 pt)	-	-	-	-	-	-	Y	Y	Y
Pectus carinatum deformity (2 pts)	-	-	-	-	-	-	-	-	-
Plain pes planus (1 pt)	-	-	-	-	-	-	-	-	Y
Pneumothorax (2 pts)	-	-	-	-	-	-	-	-	-
Dura ectasia (2 pts)	-	-	-	-	-	-	-	-	-
Protusio acetabuli (2 pts)	-	-	-	-	-	-	-	-	-
Reduced upper/lower segment ratio (1 pt)	Y	-	-	-	-	-	Y	Y	-
Scoliosis or thoracolumbar kyphosis (1 pt)	-	-	-	-	-	-	-	-	-
Reduced elbow extension (1 pt)	-	-	-	-	-	-	-	-	-
Facial features[c] (3/5) (1 pt)	Y	-	-	-	-	-	Y	Y	Y
Skin striae (1 pt)	-	-	-	-	-	-	-	-	-
Myopia > 3 diopters (1 pt)	-	-	-	-	-	-	-	-	-
Mitral valve prolapse (all types) (1 pt)	-	-	-	-	-	-	-	-	-

(Pb) Proband, (Ao) aorta, (N) no, (U) unknown, (Y) yes.
[a]Z-score indicates aorta dilatation (aneurysm, $Z \geq 2$).
[b]Maximum total: 20 points; score ≥7 indicates systemic involvement.
[c]Dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, and retrognathia.

variant is not deposited in gnomAD (https://gnomad.broadinstitute.org/) and ClinVar databases (https://www.ncbi.nlm.nih.gov/clinvar/); neither was it described in any publication until now, being considered a novel variant. It is an assumed de novo variant, because the proband's parents do not carry the altered allele. On the other hand, the variant was transmitted to his daughter and granddaughter, both with physical signs of MFS, but without aortic structural alteration. In silico analyses with the CADD and MutationTaster online prediction tools support for an impact to the protein, resulting in a truncated protein or a haploinsufficiency due to a nonsense-mediated decay (NMD) mechanism. In summary, the p.(Glu2019Ter) meets the PVS1, PM2, PM6, PP3, and PP4 criteria of the ACMG/AMP guideline, which classify it as a pathogenic variant. Moreover, according to the revised criteria for MFS diagnosis (Loeys et al. 2010), the p.(Glu2019Ter) is considered a causal mutation for MFS.

DISCUSSION

Numerous studies have investigated the relationship of phenotypes corresponding to MFS with the involvement of the *FBN1* gene; and in the study of Dietz et al. (1991), the first mutation in the locus corresponding to the fibrillin gene was recorded, establishing the causal relationship between patients with MFS and the *FBN1* gene. Although the identification of mutations in *FBN1* is not a mandatory criterion for the diagnosis of MFS, the *FBN1* gene is, in many cases, crucial in the analysis of mild or incomplete phenotypes of the syndrome (Loeys et al. 2010; Sakai et al. 2016).

The revised Ghent nosology reports that, in the absence of ectopia lentis, the presence of dilation ($Z \geq 2$) or aortic dissection associated with identification of a causal *FBN1* mutation is a sufficient criterion for diagnosis of MFS. For the identified alteration to be considered a causal mutation of the syndrome, some specific criteria, such as a de novo mutation (with proven paternity and absence of disease), and nonsense alterations are required in the analysis (Loeys et al. 2010).

The variant identified in our study fills the Ghent nosology for MFS and the ACMG pathogenicity criteria. The *FBN1* p.(Glu2019Ter) was not deposited in variant databases, and to our knowledge, it was not reported in a MFS/TAAD case. Reporting this variant as a causal mutation for MFS and TAAD can provide valuable information to support informed decision-making in precision medicine context.

The proband's descendants with the identified variant have not yet presented aorta dilatation or closed clinical diagnosis for the MFS, although they have physical signs for syndrome. The genetic evaluation of *FBN1* and the identification of a pathogenic variant in the family can directly influence the management of family clinical follow-up.

ADDITIONAL INFORMATION

Database Deposition and Access

The c.6055G > T; p.(Glu2019Ter) variant was submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number VCV001679424.1.

Ethics Statement

This case report was part of a cohort study approved by the Instituto Nacional de Cardiologia research ethics committee. A written patient consent was obtained from all individuals enrolled in the study.

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Author Contributions

J.P.P. and J.R.F. contributed equally for this work: clinical evaluation of subjects and analyzing and interpreting data. J.P.P. and A.P.A.B. sequenced all samples. M.M.M. reviewed the manuscript. J.P.P., J.R.F., and G.M.D. wrote the manuscript.

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Competing Interest Statement

The authors have declared no competing interest.

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