A Novel Fibrillin 1 Gene Mutation Leading to Marfan Syndrome with Minimal Cardiac Features

E. Martínez-Quintana^a F. Rodríguez-González^c P. Garay-Sánchez^b
A. Tugores^b

^aCardiology Service and ^bResearch Unit, Complejo Hospitalario Universitario Insular-Materno Infantil, and

^cOphthalmology Service, Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria, Spain

Key Words

Aorta · Ectopia lentis · Fibrillin 1 · Marfan

Abstract

Marfan syndrome is an autosomal dominant disorder of the connective tissue, characterized by early development of thoracic aortic aneurysms and/or dissections, accompanied by ocular and/or skeletal involvement, and is caused by mutations in the fibrillin 1 (*FBN1*) gene. We report on a patient with ectopia lentis and a nonprogressive aortic root dilatation who presented with a novel mutation affecting a conserved cysteine residue present in a calcium-binding epidermal growth factor-like domain of *FBN1* (ENSP00000325527, p.Cys538Phe; Chr15:48,805,751 G>T), as revealed by complete sequencing of the *FBN1* gene exons and flanking sequences. Identification of the mutation led to genetic screening of apparently asymptomatic family members, allowing the detection of characteristic ocular phenotypes in the absence of typical cardiac Marfan features. This finding stresses the importance of genetic screening of asymptomatic relatives for *FBN1* gene mutation carriers.

© 2014 S. Karger AG, Basel

Marfan syndrome (MFS; MIM 154700) is an autosomal dominant disorder of the connective tissue, with an incidence of 2–3 per 10,000, in which cardiovascular, skin, skeletal, ocular, pulmonary, and dura mater abnormalities may be present. The disease is caused by mutations in the fibrillin 1 (*FBN1*) gene on chromosome 15q21, encoding for a glycoprotein that is the major component of extracellular microfibrils.

FBN1 gene mutations are associated with a broad phenotypic continuum, ranging from isolated features of MFS to a neonatal presentation of a severe and rapidly progressing disease in multiple organ systems. More than 1,200 disease-causing mutations have been identified, including missense and nonsense mutations, splice defects, or deletions [Judge and Dietz, 2005]. Genotype-phenotype correlations are difficult to interpret due to the pleiotropic nature of the mutations. Indeed, familial ectopia lentis (MIM 129600), geleophysic dysplasia 2 (MIM 614185), MASS syndrome (Mitral valve prolapse, Aortic enlargement, Skin and Skeletal findings; MIM 604308), stiff skin syndrome (MIM 184900), and dominant Weill-Marchesani syndrome 2 (MIM 608328) are all caused by mutations in the *FBN1* gene.

Due to the variability of clinical presentations, several attempts have been made in order to harmonize diagnos-

tic criteria. The latest Ghent Nosology [Loeys et al., 2010] proposes a decision tree to assist diagnosis in which the focus has shifted from the musculoskeletal signs to the cardiovascular and ocular abnormalities.

Here, we report a patient who presented with ocular manifestations but minimal cardiac features. Genetic analysis revealed a mutation in the *FBN1* gene, therefore, leading to the diagnosis of MFS.

Case Report

A 47-year-old female was referred to our hospital for evaluation. No skeletal (except pes planus), pulmonary or skin involvement was seen according to the old and the revised Ghent diagnostic criteria for MFS [De Paepe et al., 1996; Loeys et al., 2010] (fig. 1a–e). From the ophthalmological point of view, the patient had a supero-temporal subluxation of both lenses in childhood. The right lens dislocated into the vitreous cavity favoring an open-angle glaucoma; the left eye presented a total retinal detachment and secondary glaucoma after an ocular trauma that required enucleation later on. On examination, the right eye showed a thinned retina with peripapillary atrophy, a subretinal neovascular membrane and a posterior staphyloma in relation to her myopia, and aphakia with dislocation of the lens into the vitreous (fig. 1f), requiring latanoprost treatment to reduce the intraocular pressure.

Cardiological examination revealed that the patient was asymptomatic, with no chest pain or syncope, and no murmurs in cardiac auscultation were heard. The 12-lead electrocardiogram revealed sinus rhythm with incomplete right bundle branch block and the echocardiogram showed mild dilatation of the ascending aorta (33 mm) and the aortic root (32 mm) (Z-score <2.0) [Roman et al., 1989] with no mitral valve prolapse. During the last 10 years, repeated echocardiograms have shown no changes in the aortic diameter.

Even though cardiac features were not significant, the eye phenotypes suggested sequencing of the *FBN1* gene. After amplification of the patient's genomic DNA isolated from whole blood [Miller et al., 1988], complete sequencing of all exons and flanking sequences revealed a novel variant at nucleotide 1583 of the transcript (ENST00000316623, c.1583G>T; Chr15; 48,805,751 G>T), leading to a nonsynonymous amino acid change (Cys to Phe) at position 538 of the *FBN1* protein (ENSP00000325527, p.Cys538Phe) (fig. 2b). This amino acid substitution affects one of the 6 highly conserved cysteines within one of the multiple calcium-binding epidermal growth factor-like domains (CaB-EGF; smart00179) of *FBN1* (fig. 2c).

Genetic screening within the family revealed that both the mother of the patient and one of the 2 sons were also carriers of the same mutation (fig. 2a). The child affected by the mutation, a 14-year-old boy, developed supero-temporal subluxation of both lenses at the age of 4 years needing surgical correction, although intraocular pressure was within normal limits. Also, and as seen in his mother, no skeletal, skin (except for the presence of cutaneous abdominal striae), pulmonary or aortic root dilatation (28 mm in diameter) were seen (fig. 3). On the other hand, the mother of our patient only presented with myopia and cataracts with no evidence

Fig. 1. Phenotypic characteristics. **a** Female patient with no characteristic Marfan features (e.g. tall, slender body with long limbs). Height is 176 cm, and the arm-span-to-height ratio is 1.03. **b** Spine radiography, in the postero-anterior position, showing no significant scoliosis or protrusio acetabulae. **c** Normal hands with no signs of arachnodactyly. **d** Normal palate without dental crowding. **e** Pes planus. **f** Ocular ultrasound. **g** Echocardiography, in the parasternal long-axis view, showing mild dilatation of the aortic root.

of significant aortic dilatation. Related family members 2.007, 2.004 and 2.006 presented with neither clinical presentations of Marfan syndrome nor carried the reported genetic variant at the *FBN1* gene.

Discussion

Among a wide variety of phenotypic characteristics, ocular features are often seen in patients bearing *FBN1* gene mutations. Myopia and astigmatism are the most common in MFS and often progress rapidly during childhood due to globe elongation and flat cornea, respectively [Nemet et al., 2006]. However, increased risk for retinal detachment secondary to the weakening of connective

Fig. 2. Genetic characterization of the patient. **a** Family tree showing the situation of the patient (2.003) in relation with other explored relatives (numbered). Black square and circles: mutation carriers. **b** Chromatogram showing the G to T nucleotide change leading to the nonsynonymous aminoacid substitution Cys to Phe at position 528 of the fibrillin 1 polypeptide (ENSP00000325527). **c** Primary structure of the calcium-binding epidermal growth factor-like domain smart00179: EGF_CA. Exon 13 of the *FBN1* gene

(gi 1335064), where the mutation C528P is found (arrow), is shown aligned with similar domains from selected examples: gi 296483077 is mouse *Fbln2*; gi 296439311 is latent-transforming growth factor beta-binding protein 2 (human LTBP-2); gi 290457669 is human nidogen-2 gene (*NID2*), and gi 290457669 corresponds to Protein ZC116.3 (*Caenorhabditis elegans*). Conserved residues are shown in red, while the 6 conserved cysteines are highlighted with an asterisk.

Fig. 3. A 14-year-old male mutation carrier with a height of 187 cm and no skeletal Marfan features. The arm-span-to-height ratio is 1.0. **(a)** Normal hands without long fingers (arachnodactyly) **(b)**. Abdominal skin with cutaneous striae **(c)**. Echocardiography, in the parasternal long-axis view, showing mild dilatation of the aortic root with 28 mm in diameter **(d)**. LV = Left ventricle; LA = left atrium; Ao = aorta.

tissue, glaucoma (mostly open-angle glaucoma) [Izquierdo et al., 1992] and early cataract formation are also seen. Meanwhile, ectopia lentis is the ocular hallmark of MFS, but is only seen in approximately 60% of affected individuals. In these cases, lenses tend to displace supertemporally due to abnormal collagen vascular tissue and faulty lens zonules [Dietz, 2013].

In this study, a novel nonsynonymous amino acid change (Cys538Phe) affecting the sixth conserved cysteine (C6) of the CaB-EGF-like domain, encoded by exon 13 of the *FBN1* gene, has been found. Previous studies have suggested that there is a correlation between ectopia lentis and cysteine substitutions in this type of domain [Schrijver et al., 1999]. Higher frequency and severity of disease symptoms occur when these substitutions affect either C1-C2 or C3-C4, suggesting that correct cysteine localization and disulfide bonding at these positions play an important role in the structural integrity of the suspensory ligaments of the lens [Nemet et al., 2006]. Our observations are in agreement with the view that mutations affecting the C5-C6 disulfide bond are not as severe, as we have identified carriers of the mutation with a mild MFS phenotype, even at the ocular level. The localization of the mutant CaB-EGF domain along the *FBN1* polypeptide also appears to influence the severity of the phenotype observed. Cysteine substitutions in the vicinity of C538P on exon 13 (this study) such as C570R on exon 14 (previously 13) [Schrijver et al., 1999], or C587Y on exon 15 (previously 14) [Booms et al., 1997] both result in ectopia lentis with some skeletal and integument features, but no cardiac symptoms. Likewise, more distant substitutions such as C776G [Katzke et al., 2002], or C1782R [Adés et al., 2004] have been also shown to have little cardiac effect.

The great phenotypic variability observed in patients bearing *FBN1* gene mutations imposes a diagnostic challenge. As we present in this study, even in a single family with the same mutation, the penetrance is highly variable in severity. According to the US National Marfan Foundation, the Ghent Nosology was revised [Loeys et al., 2010], superseding the previous diagnostic criteria [De Paepe et al., 1996]. Currently, a decision tree assists in determining whether a patient has MFS or not, shifting the focus from the musculoskeletal signs to the cardiovascular and ocular abnormalities, so that the presence of a dilated aorta plus ectopia lentis is now sufficient to give an unequivocal diagnosis of MFS without the need of further genetic studies. For patients with either a dilated aorta or ectopia lentis, the presence of a causal *FBN1* gene mutation is a crucial criterion for MFS [Summers et al., 2012].

Furthermore, skeletal, skin, lung, facial, other ocular, and other cardiovascular signs have been included in a new scoring system in which a score of 7 points is associated with a diagnosis of MFS if there is either aortic dilatation and/or dissection, or a family history of the disease. In relation to the aortic diameter, dilatation of the aorta is determined at the sinus of Valsalva and ascending aorta, and measurements must be corrected for age and body size and compared with normative data using a Z-score calculation [Roman et al., 1989].

In the absence of ectopia lentis, an *FBN1* mutation and a family history of MFS, the diagnosis of MFS may only be made in the absence of discriminating features of Shprintzen-Goldberg syndrome, Loeys-Dietz syndrome and vascular Ehlers-Danlos syndrome. Also testing for genes encoding the transforming growth factor beta receptors (*TGFBR2*, *TGFBR1*) and collagen type III alpha 1 (*COL3A1*) mutations, and for abnormalities of collagen biochemistry should be ruled out [Summers et al., 2012]. In the case presented, ectopia lentis, accompanied by a *FBN1* gene mutation and a mild nonprogressive aortic root dilatation/dissection lead to the diagnosis of MFS.

In this context, several studies have compared the old and the revised Ghent criteria in patient groups with suspected MFS and a known *FBN1* mutation. However, and despite the high agreement level between both nosologies criteria, up to 10% of MFS patients were reclassified as ectopia lentis syndrome or MASS syndrome in the absence of aortic dilatation and, conversely, 5% were reclassified as MFS in the presence of aortic dilatation [Faivre et al., 2012]. This is the reason why some authors have criticized that the definition of aortic dilatation is based exclusively on aortic root Z-scores, which may underestimate aortic involvement [Radonic et al., 2011; Devereux et al., 2012].

Since many patients carrying *FBN1* mutations may develop classic MFS over time, genotype information is essential for the diagnosis or exclusion of MFS [Sheikhzadeh et al., 2012], despite the fact that knowledge about a specific *FBN1* mutation seems to have little prognostic value for an individual patient and cannot reliably guide individual management [Hoffjan, 2012]. In our case, although the *FBN1* C538P mutation seems not to favor the progression of aortic root dilatation, periodic echocardiograms should be performed to prevent the risk of aortic dissection in the follow-up.

References

- Adès LC, Holman KJ, Brett MS, Edwards MJ, Bennetts B: Ectopia lentis phenotypes and the *FBNI* gene. *Am J Med Genet A* 126A:284–289 (2004).
- Booms P, Withers AP, Boxer M, Kaufmann UC, Hagemeyer C, et al: A novel de novo mutation in exon 14 of the fibrillin-1 gene associated with delayed secretion of fibrillin in a patient with a mild Marfan phenotype. *Hum Genet* 100:195–200 (1997).
- De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE: Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 62: 417–426 (1996).
- Devereux RB, de Simone G, Arnett DK, Best LG, Boerwinkle E, et al: Normal limits in relation to age, body size and gender of two-dimensional echocardiographic aortic root dimensions in persons ≥ 15 years of age. *Am J Cardiol* 110:1189–1194 (2012).
- Dietz HC: Marfan Syndrome, in Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K (eds): *GeneReviews* (University of Washington, Seattle 1993–2013).
- Faivre L, Collod-Beroud G, Adès L, Arbustini E, Child A, et al: The new Ghent criteria for Marfan syndrome: what do they change? *Clin Genet* 81:433–442 (2012).
- Hoffjan S: Genetic dissection of Marfan syndrome and related connective tissue disorders: an update 2012. *Mol Syndromol* 3:47–58 (2012).
- Izquierdo NJ, Traboulsi EI, Enger C, Maumenee IH: Glaucoma in the Marfan syndrome. *Trans Am Ophthalmol Soc* 90:111–117 (1992).
- Judge DP, Dietz HC: Marfan's syndrome. *Lancet* 366:1965–1976 (2005).
- Katzke S, Booms P, Tiecke F, Palz M, Pletschacher A, et al: TGGE screening of the entire *FBNI* coding sequence in 126 individuals with Marfan syndrome and related fibrillinopathies. *Hum Mutat* 20:197–208 (2002).
- Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, et al: The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 47:476–485 (2010).
- Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16: 1215 (1988).
- Nemet AY, Assia EI, Apple DJ, Barequet IS: Current concepts of ocular manifestations in Marfan syndrome. *Surv Ophthalmol* 51:561–575 (2006).
- Radonic T, de Witte P, Groenink M, de Bruin-Bon RA, Timmermans J, et al: Critical appraisal of the revised Ghent criteria for diagnosis of Marfan syndrome. *Clin Genet* 80: 346–353 (2011).
- Roman MJ, Devereux RB, Kramer-Fox R, O'Loughlin J: Two-dimensional echocardiographic aortic root dimensions in children and adults: biologic determinants and normal limits. *Am J Cardiol* 64:507–512 (1989).
- Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U: Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. *Am J Hum Genet* 65:1007–1020 (1999).
- Sheikhzadeh S, Kade C, Keyser B, Stuhmann M, Arslan-Kirchner M, et al: Analysis of phenotype and genotype information for the diagnosis of Marfan syndrome. *Clin Genet* 82: 240–247 (2012).
- Summers KM, West JA, Hattam A, Stark D, McGill JJ, West MJ: Recent developments in the diagnosis of Marfan syndrome and related disorders. *Med J Aust* 197:494–497 (2012).