A novel FBN1 variant in a large Marfan family with high penetrance of aortic dissection or rupture

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ABSTRACT

INTRODUCTION: Marfan syndrome is an autosomal, dominantly inherited disorder of the connective tissue. We report the clinical data and results of a genetic analysis of a large Danish Marfan family.

METHODS AND MATERIAL: Sanger sequencing of *FBN1* was initially performed on genomic DNA from the index patient. Subsequently, four affected family members and three non-affected family members were tested for the variant identified in the index patient.

RESULTS: A novel variant (c.701G>T) in the *FBN1* segregated with Marfan features in the family.

CONCLUSION: In the majority of the family members, this novel variant seems to cause a uniform and very detrimental set of disease characteristics including fatal aortic dissection.

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TRIAL REGISTRATION: not relevant.

Marfan syndrome is an autosomal, dominantly inherited connective tissue disorder caused by mutation in the FBN1 gene [1]. More than 1,700 different variants have currently been reported in the Universal Mutation Database (UMD)-FBN1 [2]. The majority of these variants are private, which complicates the interpretation of the pathogenicity of the many variants detected in *FBN1* [3].

Identification of the pathogenic variant is a prerequisite for reproductive decision-making when there is a family history of Marfan syndrome and for conducting predictive testing of family members not yet meeting the diagnostic criteria. Also, genetic testing is required to confirm a clinical suspicion when there is no family history of Marfan syndrome, since the diagnostic Ghent criteria depend heavily on genetic testing [4]. As genetic testing is not always feasible, it can be impossible to establish the Marfan diagnosis in time. This may prevent some patients from attending appropriate surveillance. If left untreated, Marfan syndrome can have fatal consequences such as aortic dissection or aortic rupture. So far, reported genotype-phenotype correlations have not been clinically applicable.

Here, we report a large Danish Marfan family with a high penetrance of aortic dissection or rupture whose size offered the opportunity of segregation analysis and thereby determination of the pathogenicity of the identified variant.

METHODS AND MATERIAL

Patients

The six family members clinically diagnosed with Marfan syndrome were evaluated at the Department of Clinical Genetics, Aarhus University Hospital, Denmark, and their hospital records were studied.

This retrospective case report does not require ethics committee approval at our institution. Written informed consent was obtained from all family members who underwent genetic testing.

Mutation screening and evaluation

Sanger sequencing of the exons and exon-intron boundaries of *FBN1* was initially performed on genomic DNA from the index patient (arrow in **Figure 1**) by DNA Diagnostica. The previously unclassified variant c.701G>T in *FBN1* (NM_000138.3) was detected in heterozygous form. Thereafter, four family members clinically thought to have Marfan syndrome and three unaffected family members were tested for presence of the variant by Sanger sequencing. Briefly, exon seven of genomic DNA was amplified by polymerase chain reaction (PCR) using gene specific primers (P1: tgt aaa acg acg gcc agt. P2: cag gaa aca gct atg ac) and Advantage GC Genomic LA Polymerase (Clontech). Sequencing using the BigDye® Terminator v1.1 Cycle Sequencing Kit was carried out according to the manufacturer's description (Applied Biosystems, Life Technology) and analysed using the ABI 3130xl Genetic Analyzer (Applied Biosystems, CA, USA) and MutationSurveyor vs. 4.0 (Softgenetics).

The twin brother of the index patient was not tested, as a previous testing of multiple DNA markers had indicated that he was a monozygotic twin (III4).

To determine the possible pathogenic effects of the variant, we used BlastP, Translate Tool [5], PolyPhen2 [6], the Ensemble Variant Effect Predictor tool [7] and HGMD Professional vs. 2014.

Trial registration: not relevant.

RESULTS

In the index patient, heterozygosity for a single variant

ORIGINAL ARTICLE

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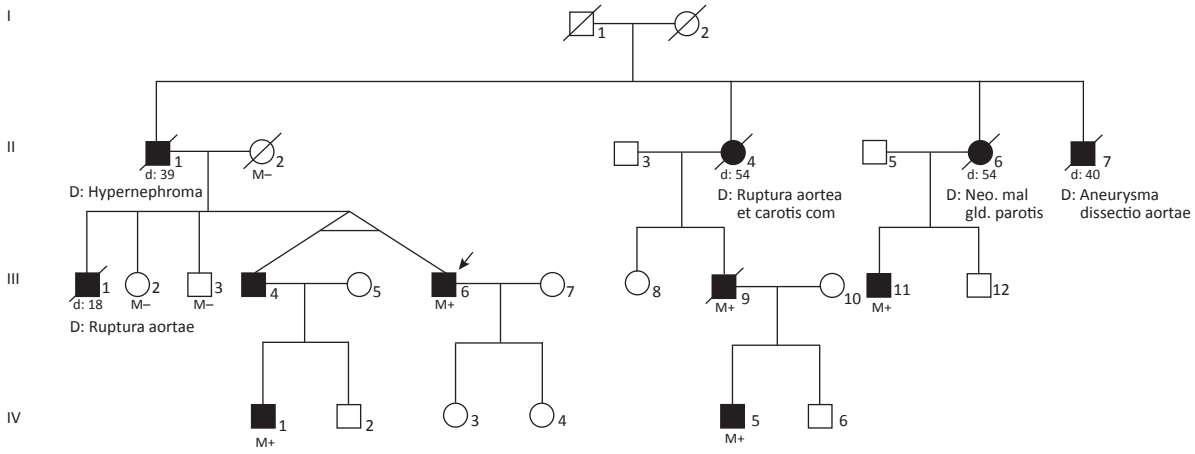
FIGURE 1

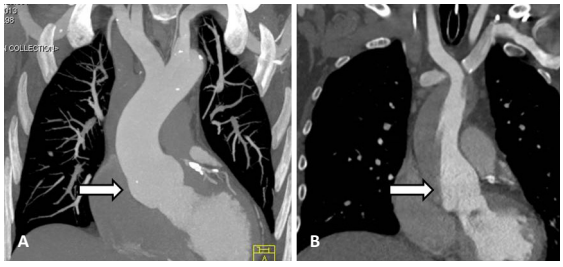
Pedigree of the family. The mutation was initially detected in the index patient (arrow).



Circle: female; square: male; open symbol: unaffected; solid symbol: affected; symbol with diagonal line: deceased;
M+: harbouring the c.701G>T mutation; M–: not harbouring the mutation; D: information from death certificate: d: age at death.

FIGURE 2

Computed tomographies of the 67-year-old male harbouring the c.701G>T variant (III9). The initial scan (**A**) shows aortic root dilatation of 4.8 cm (arrow). Two months later, he was admitted to the local hospital due to severe chest pain. A new computed tomography (**B**) showed a type A dissection (arrow). Please notice that only half of the aorta is filled with contrast, whereas the false lumen is darker grey. Acute aortic surgery was performed, but the patient did not survive the operation.



in the FBN1 gene was identified. This variant, c.701G>T, was also identified in four other affected family members. It was not detected in three non-affected relatives (age > 60 years).

To our knowledge, this variant has not previously been reported or seen in the 2,000 Danish genomes; nor has any other variants at the same position been identified. The predicted change in the amino acid sequence, p. (Gly234Val), affects a highly conserved amino acid in species as different as humans, sea squirt and honey bee (blast analysis). Gly and Val are both hydrophobic residues, but still the Grantham distance is 109 [8]. Also, PolyPhen in silico prediction reports that the variant is damaging (score 1.0) [6]. The results of the prediction software are supported by the observations in previous structural studies on the hybrid domain. Jensen and co-workers suggested that the Gly flanked by either Phe or Tyr at position p.234-235 functions as an interdomain packing region [9]. Even the relatively small change in residue induced by the identified variant (Gly→Val) may therefore result in changes of the protein structure and hence cause disease in the patients.

Clinical evaluation of the six family members diagnosed with Marfan syndrome and review of their hospital records revealed Marfan features, as summarised in **Table 1**.

A 67-year-old male family member with the c.701G>T variant (III9) was found to have aortic dilatation of 4.8 cm (**Figure 2**A). He was offered preventive aortic root surgery, but died from a fatal aortic dissection shortly before the planned operation (Figure 2B).

Evaluation of death certificates showed that three of five previously deceased family members thought to have had Marfan syndrome died from aortic rupture or aortic dissection. The other two family members were obligate mutation carriers. Two died from cancer, one at the age of 39 and the other at the age of 50 (Figure 1).

DISCUSSION

The in-silico analysis pointed towards the mutation being possibly damaging and the c.701G>T variant co-segregated with Marfan features in nine meioses. Thus, it is highly likely that this variant is a disease-causing variant.

Clinical evaluation of the six living affected family members showed that hypermobility was not a major feature in this family. None of these patients had positive wrist sign or flat feet, and only one patient (III6) had unilateral positive thumb sign. Though all patients were tall (well above the 95 percentile), they did not suffer from significant skeletal morbidity, and only III11 met

TABLE 1

Marfan features in a six-person family with FBN1 c.701G>T. The presence of dural ectasia and protrusio acetabulae were not examined in any of the family members.

	Family member					
	1	**2**	**3**	**4**	**5**	**6**
Pedigree position	III6	III4	IV1	III11	III9	IV5
Age at evaluation, yrs	57	57	29	58	67	44
Cardiovascular features						
Age, yrs	49	Information on dilatation before dissection was not available	29	57	66	44
Aortic root dilatation, mm	50		37	55	48	50
Z-score[a]	–		2.09	6.48	4.60	4.03
Age, yrs	51	41	–	58	67	44
Aortic dissection	Type A-dissection, (composite graft)	Type A-dissection (composite graft)	–	Prophylactic surgery (David procedure)	Fatal type A-dissection	Prophylactic surgery (David procedure)
Skeletal features						
Wrist sign, right/left	– /–	– /–	– / –	–/–	– / –	– /–
Thumb sign, right/left	– / +	– / –	– / –	–/–	– / –	– / –
Pectus carinatum	–	–	–	-	–	–
Pectus excavatum	–	–	Discrete	Discrete	–	–
Hindfoot deformity	–	–	–	–	–	–
Flat foot	–	–	–	–	–	–
Reduced elbow extension	–	–	–	–	–	–
Scoliosis or thoracolumbar kyphosis	–	–	–	–	–	–
Height, cm	206.5	206	197.5	196	192	195
Upper/lower segment ratio	0.86	0.85	0.89	0.83	0.90	0.98
Arm span/height ratio	1.02	1.04	1.02	1.07	1.04	1.02
BMI, kg/m^2	23.2	25.9	20.3	24.7	26.1	32.9
Ocular features						
Ectopia lentis	–	–	–	+	+	+
Other ocular findings	–	–	Lens coloboma	Retinal detachment	–	Cataract
Facial features						
Dolichocephali	–	–	+	–	–	–
Malar flattening	–	–	–	+	–	–
Enophthalmos	–	–	+	–	–	–
Retrognathia	–	–	–	–	+	+
Downslanted palpebral fissures	+	+	+	–	–	–
Other features						
Pneumothorax	–	–	–	–	–	–
Hernia	–	+	+	–	–	–
Skin striae	+	+	+	–	–	–

BMI = body mass index
a) www.marfan.org/dx/zscore

the criteria for disproportionate skeletal growth. However, evaluation of hospital journals and death certificates showed a high penetrance of aortic rupture or aortic dissection in the family. Three of six affected family members had lens dislocation, which confirms that the clinical entity inherited in this family was not "isolated ascending aortic aneurysm and dissection", but rather Marfan syndrome.

Only one family member (III1) had a significantly exacerbated course of disease. According to his death certificate, he had pectus excavatum surgically corrected at the age of 14 years, and at the age of 19 years he died from an 8-millimeter rupture in a severely dilated ascending aorta. One could speculate that he harboured variants in his genome not inherited by the other clinically affected family members and that this modulated his phenotype. Unfortunately, no DNA was available from this patient.

The monozygotic twins showed very similar phenotypes except that their aortic dissections of type A, which includes the ascending aorta [10], occurred ten years apart. This difference in age at A-dissection indi-

cates that the clinical course of Marfan syndrome is not only influenced by hereditary factors, but also by environmental or stochastic factors.

CONCLUSION

We report the novel pathogenic *FBN1* variant c.701G>T, located in a hybrid domain of the *FBN1*. To our knowledge, specific genotype-phenotype correlations for variants affecting this hybrid domain have not been reported. In 2007, Faivre et al reported that patients with an FBNI premature termination codon had a more severe skeletal and skin phenotype than did patients with an in-frame mutation. This is in line with observations in the present family. In the reported family, the missense variant was rarely associated with significant skeletal morbidity or disproportionate skeletal growth, but its presence implied high penetrance of aortic dissection or rupture.

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