Identification of FBN1 gene mutations in patients with ectopia lentis and marfanoid habitus

P Comeglio, A L Evans, G Brice, R J Cooling, A H Child

Background: Marfan syndrome (MFS), inherited as an autosomal dominant trait, typically affects the cardiovascular, skeletal, and ocular systems. Ectopia lentis (EL) is an autosomal dominant connective tissue disorder, in which the lenses tend to be dislocated upward and the zonular filaments are stretched or discontinuous. EL belongs to the clinical spectrum of diseases related to Marfan syndrome (MFS, MIM 154700), and it presents with some of the skeletal, but not all of the cardiovascular, skeletal, and ocular manifestations of MFS.

Methods: A consecutive series of 11 patients, affected predominantly by EL, was analysed for FBN1 mutations using PCR, SSCA, and sequencing.

Results: Six mutations were identified, of which three are novel and one is recurrent in two patients, thus establishing a mutation incidence in this group of 7/11 (63%).

Conclusion: The FBN1 variants reported are clustered in the first 15 exons of the gene, while FBN1 mutations reported in the literature are distributed throughout the entire length of the gene. A different type of FBN1 mutation is present in this group of patients, compared with MFS, with arginine to cysteine substitutions appearing frequently.

Ectopia lentis (EL, MIM 129600) is an autosomal dominant connective tissue disorder, in which the lenses are stretched or discontinuous. EL belongs to the clinical spectrum of diseases related to Marfan syndrome (MFS, MIM 154700), and it presents with some of the skeletal, but not all of the cardiovascular, skeletal, and ocular manifestations of MFS.

Mutational analysis

A set of 65 pairs of primers (Sigma-Genosys, Pampisford, Cambridgeshire, UK) was used for polymerase chain reaction (PCR) amplification of all 65 exons of the FBN1 gene. The genomic copy of FBN1 (MIM 134797) has been demonstrated. The genetic copy of the FBN1 gene is 235 kb and encodes for fibrillin-1, a secreted 350 kDa glycoprotein, major structural component of the elastin associated 10–12 nm microfibrils, which are the sole structural element visible by conventional electron microscopy in the suspensory ligaments of the lens. Fibrillin-1 is mainly composed of cysteine rich epidermal growth factor (EGF)-like domains, most of them with a calcium binding consensus sequence (cbEGF-like).

Mutations at the calcium binding sites, and cysteine substitutions seem to affect the structural function of fibrillin-1.

FBN1 mutations have been characterised in patients affected by type I fibrillinopathies, which include MFS, MASS syndrome (MIM 604308), EL, Shprintzen-Goldberg syndrome (MIM 182212), isolated skeletal features of MFS, and thoracic aortic aneurysms. The identified mutations are distributed throughout the FBN1 gene, with limited evidence of genotype-phenotype correlation. In particular, only the cluster of exons 24 to 32 is linked with a severe form of MFS, neonatal MFS.

To date, only four FBN1 mutations have been reported in patients affected by predominant EL, the less severe form of the disease continuum. Clinically, differential diagnosis and overall management of EL patients is problematical. Many patients are never referred for cardiological assessment to rule out MFS.

In this study we characterised the incidence and class of FBN1 mutations in a group of 11 consecutive unrelated British patients affected predominantly by EL.

We identified six causative or putative mutations in the FBN1 gene, three of which have not been previously reported, and one of which is recurrent in two patients, thus establishing an FBN1 mutation incidence of 63% (7/11) in the patients studied, not including three FBN1 variants classified as polymorphisms.

Subjects and methods

We investigated 11 consecutive patients (seven men; four women) with predominant EL, eight of whom had a family history of dominantly inherited lens dislocation, and in whom a diagnosis of MFS was excluded according to the current diagnostic criteria. Physical examinations and investigations are reported in Table 1, and included echocardiography with measurement of aortic root diameter (adult upper limit 39 mm), assessment of valve morphology and function, skeletal features, skin extensibility and lumbar striae, assessment of visual acuity, slit lamp and fundus examination. In the absence of a suitable clinical indication, such as severe chronic low back or abdominal pain, magnetic resonance imaging (MRI) for dural ectasia was not performed. Peripheral blood samples were collected, with appropriate informed consent, from probands and available family members.

Results

We analysed 11 patients, whose clinical findings are schematically reported in Table 1. The six FBN1 mutations, one of which recurred in two patients, are reported in Table 2, together with...
three DNA variants which might represent rare polymorphisms. Several known polymorphisms were identified, without any significant difference in distribution between patients and controls (data not shown). The known polymorphisms and the mutations found were tested in all family members wherever possible to establish, the polymorphisms or the mutations identified being aortic aneurysm and MFS (data not shown). This result led us to exclude the possibility of the mutations identified being de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms and the mutations found were tested in all family members affected by cardiovascular diseases, including thoracic aortic aneurysm and MFS (data not shown). The known polymorphisms were absent in 160 chromosomes from unrelated controls and in 280 chromosomes from unrelated patients affected by cardiovascular diseases, including thoracic aortic aneurysm and MFS (data not shown). The known polymorphisms and the mutations found were tested in all family members wherever possible to establish, the polymorphisms or the mutations identified being de novo changes in the DNA of patients.

Clinical summaries

Patients IBP, VW, and MZ demonstrated only FBN1 variants likely to represent polymorphisms, so that no further blood samples were taken from family members. No family members were available for patients OP and JL.

In patient BM the mutation was demonstrated in the proband’s younger sister, who has bilateral EL and glaucoma, and in this sister’s as yet unaffected 5 year old son. The proband’s unaffected older sister demonstrated the mutation, but is clinically unaffected, presumably an example of reduced penetrance, as reported previously in another EL family. The proband’s unaffected mother and brother did not carry the mutation, presumably inherited from the proband’s deceased father, who appears to have large eyes in his photographs, although he is not known to have had EL or glaucoma.

The mutation discovered in patient GB was also found in an affected brother but not in the brother’s two unaffected adult daughters. Both parents of the proband, including an affected father, died of unrelated causes (cancer) and DNA samples were unavailable.

The echocardiogram of 10 year old patient NS was within normal limits for age. The father of proband NS carries the mutation but does not have dislocated lenses. He demonstrates slight facial asymmetry with simple ears, slight rib sulcus on the left anteriorly, and a non-progressive dilated aortic root (40 mm; upper normal limit 39 mm), unchanged at 42 and 45 years, with mild aortic regurgitation. He has slightly lax skin over the elbow and large hands and feet. The proband’s brother is unaffected on eye examination and does not have dislocated lenses. He demonstrates slight facial asymmetry with simple ears, slight rib sulcus on the left anteriorly, and a non-progressive dilated aortic root (40 mm; upper normal limit 39 mm), unchanged at 42 and 45 years, with mild aortic regurgitation. He has slightly lax skin over the elbow and large hands and feet. The proband’s brother is unaffected on eye examination and does not have dislocated lenses.

### Table 1

**Clinical details of 11 consecutive patients affected by predominant EL**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation site</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Protein domain</th>
<th>References</th>
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<tr>
<td>OP</td>
<td>Exon 4</td>
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<td>R240C</td>
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<td>N164S</td>
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<td>S634P</td>
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<td>MG</td>
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<td>C652Y</td>
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### Table 2

**Results of FBN1 analysis in 11 consecutive patients affected by predominant EL**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation site</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Protein domain</th>
<th>References</th>
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<tr>
<td>MG</td>
<td>Exon 15</td>
<td>G1955A</td>
<td>C652Y</td>
<td>cbEGF-like No 6</td>
<td>13, 17, 18, 19</td>
</tr>
<tr>
<td>VW</td>
<td>Exon 31</td>
<td>A3963G</td>
<td>–</td>
<td>cbEGF-like No 1</td>
<td>21, 18, 19</td>
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<tr>
<td>IBP</td>
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<td>IVS6 del T</td>
<td>–</td>
<td>cbEGF-like No 40</td>
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<td>–</td>
<td>cbEGF-like No 41</td>
<td>14, 18, 19</td>
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</table>

* M = male, F = female; †AS/H = arm span/height ratio; ‡US/LS = upper segment/lower segment ratio.

Abnormal features are highlighted in bold.

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The proband’s unaffected older sister demonstrated the mutation, but is clinically unaffected, presumably an example of reduced penetrance, as reported previously in another EL family. The proband’s unaffected mother and brother did not carry the mutation, presumably inherited from the proband’s deceased father, who appears to have large eyes in his photographs, although he is not known to have had EL or glaucoma.

The mutation discovered in patient GB was also found in an affected brother but not in the brother’s two unaffected adult daughters. Both parents of the proband, including an affected father, died of unrelated causes (cancer) and DNA samples were unavailable.

The echocardiogram of 10 year old patient NS was within normal limits for age. The father of proband NS carries the mutation but does not have dislocated lenses. He demonstrates slight facial asymmetry with simple ears, slight rib sulcus on the left anteriorly, and a non-progressive dilated aortic root (40 mm; upper normal limit 39 mm), unchanged at 42 and 45 years, with mild aortic regurgitation. He has slightly lax skin over the elbow and large hands and feet. The proband’s brother is unaffected on eye examination and does not have dislocated lenses.
significant difference might be indicative of a recurrent type of mutation when compared with the same region (Fig 1) (p<0.0001, Fisher’s test). This statistically significant difference is maintained even when compared with the HGMD (p<0.0001, Fisher’s test), where 31/219 mutations (15%) identified in the same gene length, with just 21/137 (15%) identified in the same region (Fig 1) (p<0.0001, Fisher’s test). This statistically significant difference might be indicative of a recurrent type of mutation in the group of patients affected by predominant EL.

Moreover, the same type of mutation recurs in two out of four reported cases of predominant EL. Considering the three novel mutations described, C652Y causes a cysteine substitution in a cbEGF-like domain in patient MG. The severe nature of the mutation identified, the family history and the borderline aortic root dimension make the fibrillin-1 protein, are highlighted in dark grey, with the others represented in light grey. The exons are numbered from 1 to 65.

DISCUSSION

Analysis of the literature reveals that mutations affecting cysteines, calcium binding amino acids, or residues conserved among similar domains, are usually associated with more severe phenotypes. Considering the six different mutations reported in this study, three mutations (R122C, R240C, R545C) have already been reported, all of them involving the substitution of a cysteine for a non-conserved arginine. Mutations R122C has been reported four times, in all cases the patient presenting with atypical MFS, due to the lack of serious cardiovascular manifestations. Mutation R240C has been reported twice, in a classic MFS patient and in an EL patient, reflecting the interfamilial phenotype variability. Mutation R545C has been reported twice, in patients with cardiovascular involvement. Nevertheless, in our series, these three mutations were characterised in four patients without any serious cardiovascular involvement.

Overall, four out of seven mutations (57%) reported in our study involved the substitution of a cysteine for a non-conserved arginine. This could be driven by the highly probable C to T transitions at CpG dinucleotides, but conversely arginine to cysteine mutations have been reported in only 10 of the 137 entries (∼7%) of the Marfan database (p<0.005, Fisher’s test) and in only four of the 219 entries (∼2%) of the FBN1 Human Gene Mutation Database, Cardiff (HGMD: http://archive.uwcm.ac.uk/uwcm/mg/search/127115.html) (p<0.0001, Fisher’s test). This statistically significant difference might be indicative of a recurrent type of mutation in the group of patients affected by predominant EL.

Moreover, the same type of mutation recurs in two out of four reported cases of predominant EL. Considering the three novel mutations described, C652Y causes a cysteine substitution in a cbEGF-like domain in patient MG. The severe nature of the mutation identified, the family history and the borderline aortic root dimension make long term echocardiogram follow up of all family members necessary. Could possible worsening of the condition be anticipated by the molecular diagnosis, or is this a mildly affected patient carrying an apparently severe mutation?

Mutation N164S (patient BM) causes an asparagine to serine substitution in an EGF-like domain. This type of mutation has been reported five times in the literature, but in all cases it involved an asparagine part of a consensus sequence for calcium binding. The mutation in this study is instead a substitution of a variable residue and is conservative, with both amino acids polar but uncharged. It is not clear if this mutation is causative.

Mutation S634P (patient NS) does not affect a conserved or invariant amino acid for cbEGF-like domains. Although it is not considered classically severe, it remains to be established what is the real effect of a non-conservative substitution of a non-polar for a polar uncharged residue. It should be noted that the proband’s father could be described as a marfanoid patient, thus suggesting a case of variable penetrance.

FBN1 variant A/G 3963 (patient VW) affects the second to last nucleotide of exon 31 and it has previously been reported as a polymorphism. However, the A residue at this position is invariant A/G 3963 (patient VW) affects the second to last nucleotide of exon 31 and it has previously been reported as a polymorphism. However, the A residue at this position is invariant amino acid for cbEGF-like domains. Although it is not considered classically severe, it remains to be established what is the real effect of a non-conservative substitution of a non-polar for a polar uncharged residue. It should be noted that the proband’s father could be described as a marfanoid patient, thus suggesting a case of variable penetrance.

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different role when compared with the middle region of the protein. In our series of patients a relatively mild phenotype, with late onset of stable cardiovascular features in some cases has been observed. In contrast, mutations in the central region, which includes the neonatal cluster of exons 24–32, are usually associated with more severe phenotypes. Furthermore, it has been reported that mutations in the C-terminal end of the protein could also be linked to less severe phenotypes, thus stressing the possibility that involvement of different regions of the FBN1 gene might be partially responsible for phenotype variability. Further studies of patients representing the mild end of the MFS spectrum will help to clarify this issue.

Meanwhile, since a tendency to late onset aortic dilatation and/or dissection is an occasional feature, it is recommended that patients with predominant EL be screened with echocardiography initially and at regular intervals throughout their lifetime.

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REFERENCES

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