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, characterised by variable phenotypic manifestations mainly in cardiovascular, skeletal, and ocular systems.č

Ectopia lentis (EL, MIM 129600) is an autosomal dominant connective tissue disorder, in which the lenses tend to be dislocated upward and the zonular filaments are stretched or discontinuous.č EL belongs to the clinical spectrum of diseases related to Marfan syndrome (MFS, MIM 134797), and it presents with some of the skeletal, but not serious cardiovascular, features of MFS.č MFS is an autosomal dominant connective tissue disorder, characterised by variable phenotypic manifestations mainly in cardiovascular, skeletal, and ocular systems.č

Co-localisation of MFS and EL loci and the gene FBN1 (MIM 134797) has been demonstrated.č The genomic 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,č 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).č Mutations at the calcium binding sites, and cysteine substitutions seem to affect the structural function of fibrillin-1.č

FBN1 mutations have been characterised in patients affected by type I fibrillinopathies,č 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.č In particular, only the cluster of exons 24 to 32 is linked with a severe form of MFS, neonatal MFS.č

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

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.

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%).

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

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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.č 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.

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 oligonucleotide sequences were those previously describedč or reported in the literature.č All PCR reagents except primers were supplied by Gibco-BRL Life Technologies (Paisley, UK). The amplifications were performed in a DNA Thermal-Cycler (Hybaid, Ashford, Middlesex, UK) under conditions depending on the exon analysed.

Single strand conformation analysis (SSCA) was carried out as previously describedč Electrophoresis and silver staining chemicals were from Merck (Poole, Dorset, UK) and Sigma-Aldrich (Poole, Dorset, UK).

Sequencing was carried out on an ABI310 using the Big Dye chemistry (Applied Biosystems, Warrington, Cheshire, UK).

Detailed protocols are available on request.

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

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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 available. Wherever possible to establish, the polymorphisms were not the results of de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms and the mutations found were tested in all family members available. Wherever possible to establish, the polymorphisms were not the results of de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms were not the results of de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms were not the results of de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms were not the results of de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms were not the results of de novo changes in the DNA of patients. Wherever possible to establish, the polymorphisms were not the results of 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

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex*</th>
<th>Ectopia lentis family history</th>
<th>FBN1 change</th>
<th>Skeletal system</th>
<th>Cardiovascular system</th>
<th>Other manifestations</th>
<th>Ocular system</th>
<th>Abnormal features</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBP</td>
<td>33</td>
<td>M</td>
<td>-</td>
<td>R122C</td>
<td>189</td>
<td>0.81</td>
<td>+</td>
<td>±</td>
<td>−−−−−−−−</td>
</tr>
<tr>
<td>VW</td>
<td>32</td>
<td>M</td>
<td>-</td>
<td>R240C</td>
<td>187</td>
<td>0.79</td>
<td>+</td>
<td>±</td>
<td>−−−−−−−−</td>
</tr>
<tr>
<td>MZ</td>
<td>32</td>
<td>M</td>
<td>-</td>
<td>R545C</td>
<td>189</td>
<td>0.93</td>
<td>+</td>
<td>±</td>
<td>−−−−−−−−</td>
</tr>
</tbody>
</table>

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

Abnormal features are highlighted in bold.

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation site</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Protein domain</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>Exon 4</td>
<td>C364T</td>
<td>R122C</td>
<td>EGF-like No 2</td>
<td>13, 18, 19</td>
</tr>
<tr>
<td>BM</td>
<td>Exon 5</td>
<td>A491G</td>
<td>N164S</td>
<td>EGF-like No 3</td>
<td></td>
</tr>
<tr>
<td>RWT</td>
<td>Exon 6</td>
<td>C718T</td>
<td>R240C</td>
<td>Hybrid Mutif No 1</td>
<td>13, 14, this report</td>
</tr>
<tr>
<td>GB</td>
<td>Exon 13</td>
<td>C1633T</td>
<td>R545C</td>
<td>cbEGF-like No 4</td>
<td>13, 17, this report</td>
</tr>
<tr>
<td>JL</td>
<td>Exon 13</td>
<td>C1633T</td>
<td>R545C</td>
<td>cbEGF-like No 4</td>
<td>13, 17, this report</td>
</tr>
<tr>
<td>NS</td>
<td>Exon 15</td>
<td>T1900C</td>
<td>S634P</td>
<td>cbEGF-like No 6</td>
<td>13, 17, this report</td>
</tr>
<tr>
<td>MG</td>
<td>Exon 15</td>
<td>G1955A</td>
<td>C652Y</td>
<td>cbEGF-like No 6</td>
<td>13, 17, this report</td>
</tr>
<tr>
<td>VW</td>
<td>Exon 31</td>
<td>A3963G</td>
<td>–</td>
<td>cbEGF-like No 1</td>
<td>21, this report</td>
</tr>
<tr>
<td>IBP</td>
<td>Intron 6</td>
<td>IVS6 del T 26</td>
<td>–</td>
<td>cbEGF-like No 40</td>
<td>22, this report</td>
</tr>
<tr>
<td>MZ</td>
<td>Intron 62</td>
<td>F</td>
<td>–</td>
<td>cbEGF-like No 41</td>
<td>14, this report</td>
</tr>
<tr>
<td>SR</td>
<td>No mutation identified</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
not carry the mutation. This family demonstrates intrafamilial phenotype variability.

The family of patient MG demonstrates six affected members in three generations, with dominantly inherited EL. The proband's affected mother and two affected children also carry the mutation. Affected family members do not fulfill the criteria for MFS. No unaffected relatives were available for analysis. Two affected adult siblings of the mother were uncooperative. The proband's mother died unexpectedly aged 72 of undiagnosed ruptured ascending thoracic aortic aneurysm.

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. Mutation 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 predominant in FBN1 exon “donor sequences” (56%) and the same mutation has been observed in a patient with late onset aortic aneurysm, EL and marfanoid build (P Johnson, personal communication), where it affects the splicing of exon 31 through activation of a cryptic splice site. The unavailability of RNA from patient VW does not permit further analysis.

In this study we report a success rate of 7/11 (63%) in identifying causative or putative FBN1 mutations in patients affected by predominant EL with marfanoid habitus. These results are within the 23%–86% range of mutation identification rate in MFS and MFS related patients in recent investigations. These results confirm that SSCA, although not 100% successful, is a suitable method and a viable alternative to direct sequencing for mutations identification, because of its low cost, simplicity, and efficiency, once the optimal conditions have been established.

Although the group studied is small, it is the largest predominant EL consecutive series yet reported. All mutations described in this paper are in the first 15 exons of the gene, while the Marfan database mutations are distributed over the gene length, with just 21/137 (15%) identified in 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 (14%) are within the first 15 exons.

The N-terminal domains of fibrillin-1 have a role in directing the formation of dimer intermediates which aggregate to form the functional microfibril. Clustering of the mutations identified in this study at the N-terminus may underline its
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.\(^\text{12}\) Furthermore, it has been reported that mutations in the C-terminal end of the protein could also be linked to less severe phenotypes,\(^\text{10}\) 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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