Detection of Six Novel FBN1 Mutations in British Patients Affected by Marfan Syndrome

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Marfan syndrome (MFS), an autosomal dominant disorder of the extracellular matrix, is due to mutations in fibrillin-1 (FBN1) gene. Investigations carried out in the last decade, unveiled the unpredictability of the site of the mutation, which could be anywhere in the gene. FBN1 mutations have been reported in a spectrum of diseases related to MFS, with no clear evidence for a phenotype-genotype correlation. In this paper we analysed 10 British patients affected by MFS and we were able to characterise five novel missense mutations (C474W, C1402Y, G1987R, C2153Y, G2536R), one novel frameshift mutation (7926delC), one already described mutation (P1424A) and one FBN1 variant (P1148A) classified as a polymorphism in the Asian population. Four out of the five novel missense mutations involved either cysteines or an amino acid conserved in the domain structure. The mutation yield in this study is calculated at 80.0% (8/10), thus indicating that SSCA is a reliable and cost-effective technique for the screening of such a large gene. Our results suggest that this method is reliable to search for FBN1 mutations and that FBN1 screening could be a helpful tool to confirm and possibly anticipate the clinical diagnosis in familial cases. © 2001 Wiley-Liss, Inc.

KEY WORDS: Marfan syndrome; MFS; FBN1; fibrillin 1; SSCA; Britain

INTRODUCTION

Marfan syndrome (MFS; MIM# 154700) is an autosomal dominantly inherited connective tissue disorder, characterised by highly variable phenotypic manifestations, mainly in cardiovascular (thoracic aortic aneurysm and dissection, mitral valve prolapse), ocular (lens dislocation), and skeletal (pectus deformities, dolichostenomelia) systems (Pyeritz, 1993; De Paepe et al., 1996). The incidence of this pleiotropic disease is estimated at about 1/5,000 in the population world-wide, regardless of ethnicity or gender, and at least 25% of cases are sporadic (Dietz and Pyeritz, 1995; Nijbroek et al., 1995). The cardiovascular system seems to be the most seriously affected, with dilatation of the aortic root that may progress to life-threatening aneurysm or aortic dissection. The locus for this disorder has been mapped on chromosome 15q21.1 and a co-localisation of the MFS locus and the fibrillin-1 gene (FBN1: MIM# 134797; GenBank L13923) has been demonstrated (Lee et al., 1991). The length FBN1 gene is estimated to be 200 kb, and the cDNA, encoded by 65 exons, is 9.7 kb, with a coding region of 8.6 kb (Pereira et al., 1993; Biery et al., 1999).

FBN1 is translated in a ubiquitous connective tissue microfibril, fibrillin-1, a 350 kDa glycoprotein which is one

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of the major structural components of the elastin-associated 10-12 nm microfibrils (Sakai et al., 1986). The protein is mainly composed of cysteine-rich epidermal growth factor (EGF)-like domains, most of them having a consensus sequence for calcium binding (cbEGF-like) (Pereira et al., 1993). The ability to bind calcium is necessary to ensure the interdomain arrangement via intramolecular stabilising forces, to prevent proteolytic degradation, and to maintain conformation and flexibility of the macromolecules (Handford et al., 1995). Interspersed among these domains are Transforming Growth Factor β1-binding protein (TB)-like domains, and other domains exclusive to fibrillin-1, probably originating from a recombination of EGF-like and TB domains (Dietz and Pyeritz, 1995).

FBN1 mutations have been characterised in patients affected by MFS and neonatal MFS, MASS phenotype (MIM 604308), congenital ectopia lentis (MIM 129600), Shprintzen-Goldberg syndrome (MIM 182212), thoracic aortic aneurysms, and atypical MFS phenotype (for reviews see Dietz and Pyeritz, 1995; Hayward and Brock, 1997; Collod-Beroud et al., 1998).

The present study was aimed at elucidating the molecular basis that underlies the clinical abnormalities seen in 10 British patients affected by MFS.

**PATIENTS AND METHODS**

**Clinical evaluation**

We investigated 10 unrelated patients, 6 of which had a family history of MFS, where MFS diagnosis was evaluated following the revised diagnostic criteria of the Gent nosology (De Paepe et al., 1996). Samples were collected after appropriate informed consent. Genomic DNA was extracted from peripheral blood leukocytes.

**Polymerase chain reaction (PCR)**

A set of PCR primers (Sigma-Genosys, Pampisford, Cambridgeshire, UK) was used for routine amplification of all the 65 exons of the FBN1 gene, including flanking splice sites. The oligonucleotide sequences were those described previously (Nijbroek et al., 1995; Hayward et al., 1997), with the exception of the newly designed

- 18F-TAGCTCCCTAAGGTCATTACATT; 18R-ATTATGCAAGGAATTTTTCAG;
- 28F-GCCAAAGTTTGAAGCCTTATGT; 28R-ATAACATAACATAACATATATAAAG;
- 42F-TCCGGTTCCACCTTTTGT; 42R-AAAACCAGAAAGTCTGCAATG;
- 45R-TGAAGCTTTCAACAGCATATG; 48F-ACACCTCTTTATTTTCCC;
- 48R-CCTCATTTGCTACCACTTATGCC;
- 52R-ATTGGATGCTAATGACTGATAA; 52F-GATTAAACACTGAAATGATCATAA;
- 58R-ATTGGATGCTAATGACTGATAA; 58F-GTGGATCCTTCTTTATTTATTTCT;
- 65aF-GCTAAGTGGCATATGTACATT; 65R-GCTGATCCCTTCCTTTTGG;
- 65bF-AGATACTTGATCGAATCTGGA; 65bR-GTTCTACCTATCTATTTTGGT.

**Mutational analysis**

The analysis was carried out employing Single Strand Conformation Analysis (SSCA). In SSCA, an aliquot of each PCR sample was mixed with an equivalent amount of appropriate loading buffer, denatured, and loaded on a gel prepared with 0.5-0.8x Mutation Detection Enhancement 2xMDE gel (BioWhittaker Molecular Applications, Rockland, ME, USA). Gels were run in 0.6x TBE buffer at 250 V for 7-12 h, at temperatures ranging from 4ºC to 20ºC, depending on the PCR product to be analysed, and silver stained.

**Direct sequencing**

Whenever an abnormally migrating band was detected on the MDE gel, direct sequencing analysis was performed on a second PCR sample, using the same oligonucleotides utilised for the PCR. Sequence analysis was carried out using the Big Dye terminators kit (Applied Biosystems, Warrington, Cheshire, UK), and the samples run on an ABI 310 Genetic Analyzer (Applied Biosystems), following the manufacturer’s directions.
Population studies

A hundred chromosomes from 50 unrelated control individuals were tested for the identified mutations, to determine whether they could be considered recurrent mutations or polymorphisms, and to confirm their association with the pathological condition under study.

Detailed protocols for PCR and SSCA are available on request.

RESULTS

In this study 10 patients, whose clinical and molecular findings are schematically reported in Table 1, were analysed. The SSCA and sequence profiles of each abnormal band identified are reported in Figure 1.

Table 1. Molecular and clinical details of 10 British patients affected by MFS.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>FH</th>
<th>Exon</th>
<th>Change</th>
<th>Mutation</th>
<th>OS</th>
<th>SS</th>
<th>CS</th>
</tr>
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<tbody>
<tr>
<td>PB</td>
<td>48</td>
<td>M</td>
<td>+</td>
<td>11</td>
<td>1422T&gt;G</td>
<td>C474W</td>
<td>+</td>
<td>+</td>
<td>AD, AAD, DAD</td>
</tr>
<tr>
<td>JM</td>
<td>27</td>
<td>M</td>
<td>-</td>
<td>52</td>
<td>6458G&gt;A</td>
<td>C2153Y</td>
<td>+</td>
<td>+</td>
<td>AD, AAD, DAD</td>
</tr>
<tr>
<td>AG</td>
<td>41</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>AD</td>
</tr>
<tr>
<td>CW</td>
<td>3</td>
<td>M</td>
<td>-</td>
<td>27</td>
<td>3442C&gt;G</td>
<td>P1148A</td>
<td>+</td>
<td>+</td>
<td>AD</td>
</tr>
<tr>
<td>DG</td>
<td>33</td>
<td>M</td>
<td>+</td>
<td>48</td>
<td>5959G&gt;C</td>
<td>G1987R</td>
<td>-</td>
<td>+</td>
<td>AD, AR, MR, MVP</td>
</tr>
<tr>
<td>SR</td>
<td>32</td>
<td>F</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>AD, MVP</td>
</tr>
<tr>
<td>GH</td>
<td>47</td>
<td>F</td>
<td>+</td>
<td>34</td>
<td>4270C&gt;G</td>
<td>P1424A</td>
<td>-</td>
<td>+</td>
<td>AD, AR, MR, MVP</td>
</tr>
<tr>
<td>HB</td>
<td>64</td>
<td>F</td>
<td>+</td>
<td>61</td>
<td>7606G&gt;A</td>
<td>G2536R</td>
<td>-</td>
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</tr>
<tr>
<td>JG</td>
<td>33</td>
<td>F</td>
<td>-</td>
<td>63</td>
<td>7926delC</td>
<td>Stop at 2681</td>
<td>+</td>
<td>+</td>
<td>MVP</td>
</tr>
<tr>
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<td>32</td>
<td>F</td>
<td>-</td>
<td>33</td>
<td>4205G&gt;A</td>
<td>C1402Y</td>
<td>+</td>
<td>+</td>
<td>AD, MR, MVP</td>
</tr>
</tbody>
</table>


Figure 1. SSCA and sequence analysis in MFS patients. Here are reported the patient identification, the FBN1 exon and the nucleotide change. Comparison between healthy subjects (WT) and patients (M) are showed.
The mutations found were tested in all family members for which a blood sample was available. The mutations were confirmed in affected members only in the families and were absent in 50 controls (data not shown). During the analysis, 3 previously reported polymorphisms (1875T>C, IVS28+15delTTTTA, IVS56+17G>C) have been identified in both patients and controls, where frequencies in controls were 0.23, 0.11 and 0.26 respectively. No significant difference in distribution between the two groups has been observed. No new polymorphisms have been identified in the control population during the screening of all FBN1 65 exons.

In this study 1 frameshift and 7 missense mutations in FBN1 gene (Table 1) have been characterised. Three of the missense mutations predict the substitution of a cysteine involved in disulphide bonds in an EGF-like domain (C474W) or a cbEGF-like domain (C1402Y and C2153Y). Two missense mutations lead to glycine to arginine substitution (G1987R and G2536R) in cbEGF-like domains. The other 2 missense mutations are predicted to result in proline to alanine substitutions (P1148A and P1424A) in cbEGF-like domains. The frameshift mutation (7926delC) results in a Stop codon at position 2681 of a cbEGF-like domain.

DISCUSSION

In this study it is demonstrated that the success rate in identifying causative or putative FBN1 mutation in MFS could be estimated at 8 out of 10 (80.0%), including a DNA variant variably reported as a polymorphism or a predisposing factor. A range of 23.5% to 80.0% of FBN1 mutations in MFS and MFS-related patients has been identified in recent investigations (Nijbroek et al., 1995; Hayward et al., 1997; Halliday et al., 1999; Perez et al., 1999). The results of this study demonstrate that once the conditions have been established for the fragments to be analysed, SSCA is a suitable method for mutation identification, due to its low cost, high efficiency and sensitivity.

Amongst the 8 FBN1 mutations reported in this study, 2 mutations (P1148A and P1424A) have been reported elsewhere (Collod-Beroud et al., 1998). In P1148A, the amino acid substitution has been described as a polymorphism more common in the Asian population (Watanabe et al., 1997), but has not been observed in the cohort of 50 healthy Caucasian subjects in this study. Although it is unlikely that P1148A represents a deleterious change in itself (Whiteman et al., 1998), it may represent a predisposing allele that could be subject to modification by genetic or environmental factors (Dietz and Pyeritz, 1995). In neonatal MFS patient MS4.1, it is the only abnormality observed, and it falls in the neonatal MFS cluster of exons 24 to 32 (Hayward and Brock, 1997). The P1424A substitution has been observed previously (Collod-Beroud et al., 1998), although a lack of clinical information prevents a comparison of the clinical details. There are no reports of this mutation being described as a polymorphism.

Of the 6 novel mutations described, 3 of them predict the substitution of a cysteine in an EGF-like domain (C474W) or in a cbEGF-like domain (C1402Y and C2153Y). Mutations affecting cysteines, calcium-binding amino acids or residues conserved amongst similar domains of the protein, are usually associated with more severe phenotypes (Schrijver et al., 1999). Cysteines are important in establishing the intra- and inter-molecular disulphide bonds necessary to the three-dimensional structure of the microfibrils (Dietz and Pyeritz, 1995). Two of the missense mutations involve the glycine to arginine substitutions (G1987R and G2536R) and, in both cases, the substituted amino acid is not conserved amongst the cbEGF-like domains. The effect of these 2 mutations in term of protein functionality is unpredictable but they have not been observed in any controls, thus allowing us to consider the finding consistent with the diagnosis.

The 7926delC mutation has not been previously reported, and the single base deletion modifies the reading frame resulting in a downstream Stop codon at position 2681. We can postulate that the truncated FBN1 protein product, resulting from the frameshift mutation, could act in a dominant negative fashion (Dietz et al., 1993).

The results of this study suggest how FBN1 mutation screening could be a reliable tool to confirm and possibly anticipate the correct clinical diagnosis in familial cases. MFS diagnosis is still mainly clinical, but molecular diagnosis can be very useful for preimplantation or prenatal diagnosis (Wang and Godfrey, 1998; Sermon et al., 1999). From a clinical point of view, the characterisation of the mutation in a particular family allows us to apply preventive management of thoracic aortic aneurysm rupture or dissection through early diagnosis and follow-up of the members of the family carrying the mutation. Neonatal screening for the family mutation would provide valuable guidance as to whether long-term cardiovascular follow-up is required for each offspring of an affected patient.
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ELECTRONIC DATABASE INFORMATION

Accession numbers and URL for data in this article are as follows:
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for ectopia lentis [MIM 129600], FBN1 [MIM 134797], MFS [MIM 154700], MASS phenotype [MIM 604308], and Shprintzen-Goldberg syndrome [MIM 182212]).

REFERENCES


