Novel non-synonymous mutation in the transforming growth factor β binding protein-like (TB) domain of the fibrillin-1 (FBN1) gene in a Han Chinese family with Marfan syndrome (MFS)

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Abstract

In order to further understand the role of fibrillin-1 (FBN1, OMIM 134797) perturbations plays in the pathogenesis of Marfan syndrome (MFS, OMIM 154700) we studied a Han Chinese family in which MFS was segregating. In the Chinese family with 5 affected members, mutation screening for FBN1 was performed using direct sequencing. A novel non-synonymous mutation in the transforming growth factor β binding protein-like (TB) domain of the FBN1 gene was found. The missense mutation c.3022T>C (C1008R) located in exon 24. This mutation was present in the proband and in two other affected family members, but in neither unaffected family members nor unrelated control subjects. The novel non-synonymous mutation, c.3022T>C (C1008R) in the TB domain of FBN1 gene, may be involved in the pathogenesis of MFS in a Han Chinese family.

ORIGINAL ARTICLE

Abbreviations:

cbEGF	- calcium-binding epidermal growth factor domain
ECM	- extracellularmatrix
FBN1	- fibrillin-1
MFS	- Marfan syndrome
PGD	- preimplantation genetic diagnosis
TB	- transforming growth factor β binding protein-like domain

INTRODUCTION

Marfan syndrome (MFS, OMIM 154700) is an autosomal dominant disorder involving connective tissue. Variable phenotypic manifestations mainly affect the cardiovascular, ocular, and skeletal systems (De Paepe *et al*. 2004). The incidence of this multi-organ system disorder is estimated at about 1/3 000–5 000, without gender or ethnic predisposition. At least 25% of the cases are sporadic, thought to result from parental germinal mutations (Nollen & Mulder, 2004). Marfan syndrome shows a high penetrance but its clinical spectrum and severity vary greatly both between and within families (Judge & Dietz, 2005).

MFS is caused by perturbation of Fibrillin-1, a 350 kD calicium-binding protein that assembles 10-12 nm microfibrils in the extracellularmatrix (ECM). Fibrillin-1 consists of two types of disulfide-rich motifs: the calcium-binding epidermal growth factor-like (cbEGF) and transforming growth factor β binding protein-like (TB) domains (Sakai *et al*. 1986). Fibrillin-1 (FBN1, OMIM 134797) is located on human chromosome 15q21.1, and consists of 65 exons with a coding region of 8.6 kb and a 5'-UTL region of 1.1 kb. Mutations in the FBN1 were first discovered in the patients with MFS in 1991(Dietz *et al*. 1991). Since then, many sequence variations in the regions 5' upstream, cbEGF and TB domains of the FBN1 gene have been associated with the development of this syndrome (Singh *et al*. 2006). There have been in excess of 600 mutations described. Several large series of MFS patients have been reported, seemingly almost exclusively Caucasians. None of these series mention Chinese subjects. In these series mutations reported mainly affect one of the 43 cbEGF modules of fibrillin-1.

Very few molecular studies have been reported among Chinese MFS (Table 1) (Lo *et al*. 2001; XU & Hu 2001; Wang *et al*. 2003; Huang *et al*. 2004). We are aware of only 6 affected Chinese probands known to have a discernable mutation. We therefore studied a Chinese Han family in which MFS was segregating. We report a novel non-synonymous mutation in the TB domain of the FBN1 gene in the Chinese family. To our knowledge this mutation has not previously been described in either Chinese or other populations (UMD-FBN1 database: http://www.umd.be: 2030/W_FBN1/Mutations_liste.html).

MATERIALS AND METHODS

The proband (III1 in Figure 1) was a 25-year-old female with ascending aortic dilatation, aortic regurgitation, bilateral lens dislocation and skeletal manifestations typical for MFS. She had dolichostenomelia and was 175 cm tall. Pedigree revealed 6 affected family members in this family, all of Han Chinese ancestry. Clinical examination of affected cases was confirmed by the authors, and summarized in Figure 1. Subject II 2 died of rupture of aortic aneurysms at an age of 54 yrs. Subjects II3, II 7 and III 6 displayed clinical features of MFS. Informed consent for molecular studies was obtained from the 3 living affected family members and from 11 unaffected family members. The study was approved by the Shandong University Ethics Committee. Controls consisted of 50 unrelated healthy healthy subjects.

Peripheral blood was obtained for genomic DNA, and extracted using a DNA purification kit (Tiangen Inc, Beijing, China). All exons and exon-intron boundaries were PCR-amplified using an optimized PCR protocol. Oligonucleotides used as primers were synthesized and PAGE purified by Sangong Biotechnology Co. (Shanghai, China). For amplification, genomic DNA (50 ng) was added to a final volume of 50 µl PCR solution containing 0.5 µM of each primer, 0.25 mM dNTPs, 2 mM $MgCl_2$ and 1.25 U Taq DNA polymerase (TaKaRa Biotechnology Co, Beijing, China). Reactions were performed using the Gene Amp thermocyclers (Gene Amp® PCR System 9700, Singapore, USA). Detailed information of the primer sequences and PCR conditions are available upon request.

All PCR products were purified with a DNA Extraction Kit (Fermentas UAB, Vilnius, Lithuania). Subsequent sequencing analysis protocols for mutation screening used a BigDye terminator kit with the ABI 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, USA). Mutations are described according to accepted nomenclature recommendations for human gene mutations (Antonarakis, 1998).

RESULTS

Direct sequencing in the proband revealed a novel transition, T to C at nucleotide 3022 in exon 24 of the FBN1 gene. This causes substitution of arginine to cysteine in amino acid 1008 i.e. c.3022T>C (C1008R). Figure 2 shows partial chromatogram images indicating wild type sequence in exon 24 of the FBN1 gene (A) and a heterozygous mutation of c.3022T>C (C1008R) in the proband (B). C1008R was found in 3 affected family members (II7, III1, III6) but in neither unaffected family members nor any of the 50 Chinese controls. We also found a synonymous mutation [c.6855T>C (D2285D)] in exon 56 in the proband. However, this was observed in both affected (III1) as well as unaffected family members (II1 and III7).

DISCUSSION

The novel mutation (C1008R) in this Chinese family provides evidence that perturbation in TB domain of the FBN1 gene may contribute to the pathogenesis of MFS.

Table 1. Summary of FBN1 Mutation Analysis in Chinese Patients.

Location	Sequence variation	Amino acid variation	Protein Domain	Patient Identification	Author
24	c.3037G>A	p. Gly1013Arg	TGF-β like #3	CHI01HKO F01 I01	Lo IF (2001)
25	c.3083A >T	p. Asp1028Val	cbEGF #11	CHI01HKO F02 I01	Lo IF (2001)
25	c.3131G >A	p. Cys1044Tyr	cbEGF#11	Proband	Dong Xu (2001)
25	c.3243_3256 del 13 gcctctgcaccc		cbEGF #11	MFS (A) II1	Bing Wang (2003)
34	c.4307_4308 Ins 4 tcgt		cbEGF #20	Case 7	Xiaoli Huang (2004)
43	c.5309G>T	p. Cys1770Phe	cbEGF #25	Case 6	Xiaoli Huang (2004)
24	c.3022T>C	p. Cys1008Arg	TGF-β like #3	III1	Yingying Qin Present study

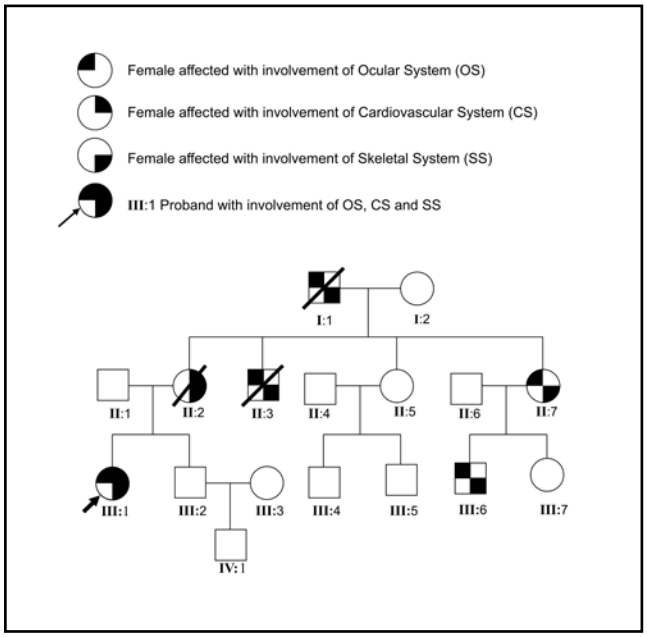

Figure 1. The pedigree of a Chinese family with MFS.

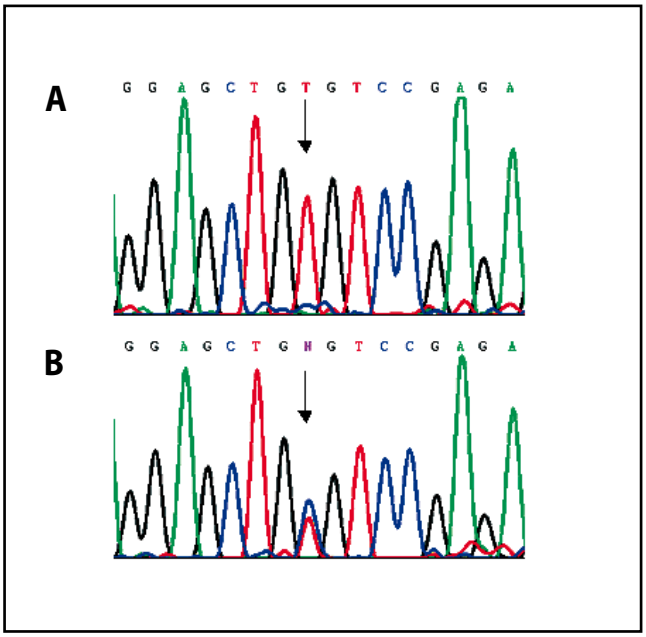

Figure 2. A partial chromatogram images indicating wild type (A) and heterozygous mutant (B) sequences of c.3022T>C (C1008R) in the FBN1 gene.

C1008R causes substitution of arginine, a strong basic hydrophilic amino acid to cysteine, a sulfur-containing hydrophobic one, which may influence overall stability of the tertiary structure and biological activity of TB domain. Future functional studies are additionally required to evaluate the effect of C1008R on Fibrillin protein.

This particular mutation [c.3022T>C (C1008R)] has not reported previously in any other ethnic group. Five of seven Chinese subjects with MFS whose molecular basis has been elucidated have been shown to involve perturbations of exons 24 or 25 (Table 1). Undoubtedly, genetic studies will contribute to the clinical consultation, early diagnosis and surgical treatment for relatives. Moreover, with the development of preimplantation genetic diagnosis (PGD), it is implementary for MFS patients whose causative mutation has been identified to bear healthy offspring. However, finding mutations in the FBN1 gene is currently very laborious and time-consuming. Further screening of larger number of Chinese MFS patients is necessary to confirm the mutational hotspots for Chinese. If this impression is confirmed, one could identify mutations in Chinese more efficiently by screening the hotspots. Initially, a less onerous approach than evaluating the entire FBN1.

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