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Fibrillin 3 Gene Mutation in Chinese Patients with Marfan Syndrome

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Abstract.- Marfan syndrome (MFS) is an autosomal dominant disorder of the extracellular matrix. Though the variations in the gene for fibrillin-1 (FBN1) have been shown to cause MFS, many MFS cases do not harbor any fibrillin-1 mutation suggesting that the disorder may be genetically heterogeneous. Weill-Marchesani syndrome is also a connective tissue disorder characterized by short stature, brachydactyly, joint stiffness, and eye anomalies including ectopia lentis, which is also one of the clinical symptoms of MFS. Fibrillin-3 gene has been associated with Weill-Marchesani syndrome. To explore if mutations in FBN3 have some relation with MFS, we analyzed FBN3 allelic variants in twelve Chinese patients with MFS by direct sequencing analysis. We identified six single nucleotide polymorphisms (SNPs) that have already been reported for FBN3 and five novel mutations including two frameshift mutations (c.3648delC and c.3686insC) and three missense mutations (p.454T>P, p.1642G>P and p.1876G>C). These novel mutations were found in FBN3 of MFS patients only while forty healthy individuals showed none of the mutations indicating that these mutations might be the cause for MFS. In order to know if these mutations can disrupt the function of FBN3, further studies are needed.

Keywords: Marfan syndrome, fibrillin-3, sequence analysis.

INTRODUCTION

Marfan syndrome is an inherited connective tissue disorder transmitted as an autosomal dominant trait. It is noteworthy for its worldwide distribution, relatively high prevalence, clinical variability, and pleiotropic manifestations, some of which are life threatening (Caglayan and Dundar, 2009). The disorder results from molecular defects in the fibrillin genes, which are responsible for the impaired structural integrity of the skeletal, ocular, and cardiovascular system. Cardinal features of the disorder include tall stature, ectopia lentis, mitral-valve prolapsed, aortic-root dilatation, and aortic dissection (Collod-Beroud and Boileau, 2002). About three quarters of patients have an affected parent (Corson *et al*., 2004); new mutations account for the remainder.

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Marfan syndrome (MFS) is fully penetrated with marked interfamilial and intra-familial variability (Aalberts *et al.,* 2010). The wide spectrum of variability of MFS is not wholly explained solely by fibrillin-1 gene (FBN1) mutations (Faivre *et al.,* 2002). It is possible that another unknown candidate gene or genes causing or modifying the disease might exist.

Fibrillin is a family of matrix glycoprotein (350 kDa) that comprises multiple epidermal growth factor (EGF)-like motifs interspersed with eight cysteine repeats with homology to the transforming growth factor-1 (TGF-1) binding protein and several apparently unique ~cysteine-rich motifs (Lee *et al.,* 1991). For example, the fibrillin-2 gene (FBN2; MIM 121050) on human chromosome 5q23-q31 shares a high degree of homology with FBN1 (Mizuguchi and Matsumoto, 2007). The fibrillin-3 (FBN3) gene exists on human chromosome 19p13 and shows high homology to other fibrillin family members (Nagase *et al*., 2001). The FBN3 gene is fragmented into 63 exons, transcribed in a 9 kb mRNA that encodes a 2809 amino acid protein

which has overall homology of greater than 60% with either FBN1 or FBN2, and contains multiple EGF-hike domains. The expression of FBN3 is highest in brain. The spectrum of overlapping disorders like EL (ectopia lentis), associated with FBN1 mutations, defines the molecular group of type1 fibrillinopathies (Oklu and Hesketh, 2000). Interestingly, the autosomal recessive WMS locus has recently been mapped to chromosome 19p13.3-p13.2, where the FBN3 gene is located (Reinhardt *et al*., 1995). This suggests that FBN3 is a candidate gene for WMS which is a connective tissue disorder just like MFS, thus FBN3 may have some connection with the pathogenesis of MFS. Therefore, we analyzed genetic variation in the FBN3 gene using DNA sequencing in Chinese Han population patients with MFS and in normal healthy individuals from the same area.

MATERIALS AND METHODS

Subjects

Twelve MFS patients from the department of ophthalmology of No. 1 affiliated hospital, Xi'an Jiaotong University, were enrolled into the present study. All these patients fulfilled the diagnostic criteria for the disease. Forty healthy Chinese volunteers who were examined in No. 2 affiliated Hospital of Xi'an Jiaotong University, served as control.

Mutation scanning

Genomic DNA was isolated from peripheral blood using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). We amplified exons 11, 22, 25, 30, 38 and 44 of FBN3 by polymerase chain reaction (PCR) using the primer sequences given in Table 1. PCR amplifications were performed using 100 ng of genomic DNA, 5 pmol of forward and reverse primers, l9µl of dd water and 25µl of Master Mix. For all exons, one cycle with denaturation at 95°C for 10 min was performed that was followed by 35 cycles with denaturation at 95°C for 30s, annealing at varying temperatures (63.9, 56, 63.7, 63.7, 59 and 58°C respectively) for 45s, extension at 72°C for 45s and final extension for 10 min at 72°C. The PCR products were stored at 4°C. The size of amplified products was 353, 470, 277, 264, 252 and 442 bp, respectively. PCR product (10µl) was combined with 10µl of 95% deionized formamide with 2µl bromophenol blue and 0.5µl EDTA. The samples were heated to 99°C for 10 min, and then placed immediately on crashed ice. Once cooled, the samples were loaded onto 16cm long, 1mm thick 15% polyacrylamide gels, and run for 16 h in TBE at a constant current determined by the PCR fragment size (15-30 mA per gel). The gels were then silver stained. Samples with abnormal CSGE band patterns were sequenced in both directions by means of the ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Forward and reverse primers were the same as used for amplification.

RESULTS

Clinical findings

All 8 affected patients, presenting at least one frameshift or missense mutation, had heart diseases and ocular symptoms. Most of them had lens dislocation, high intra-ocular pressure, ectopia lentis and severe ophthalmic complications like vitreous hemorrhage and retina detachment with vision for only hand movements or light perception (Table II).

SNP analysis

We sequenced exons 11, 22, 25, 38 and 44 in patients and in normal individuals, aligning the sequences from these individuals with standard FBN3 sequence showed different polymorphisms (Table III). These polymorphisms can not be considered causative for the phenotype as they were present in both the patients' and normal samples.

Two new frameshift mutations in exon 25 of FBN3 were also identified. A deletion of C at position 3648 (c.3648delC) resulted in a frameshift mutation giving rise to a predicted truncated protein (1079 amino acids) shorter than the normal protein (2809 amino acids). The patient in whom this mutation was detected was a 24-years old female with vision loss in both eyes since childhood. She had strong family history of Marfan syndrome. She got lens dislocation with retinal detachment in both eyes.

Table I.- List of primers for amplification and sequencing of FBN3 gene.

S #	Forward Primer	Reverse Primer	Exon#
1	5′TCTGCCGACACTTCACCAACCTGT	5′CCCCAACAACATCACCCCTACTCT	11
2	5′CTGGCCCTCGACCTGACTAC	5′TTCTTAGTGCCTCGCCTTACCCA	22
3	5′CCAGCATGAGCCTCATCACC	5′CACATTCACCAGACATCCACTCAC	25
4	5′AGAGGAAGAGTCCCTGCCCTTTC	5′CCCCACCTACCTCTACTGCT	30
5	5′GAAGTGGCCTTGTGCTGGGTCTC	5′TGACTGCCAGTTCCCTAGGTCATC	38
6	5′GTAAATGGTTGATCTGCCTCCCTG	5′GGACTTTCTGGGCATCTTTCTGTG	44

Table II.- Clinical and genetic profile of the patients.

Patient	Vision	Lens dislocation	Intra-ocular pressure	Vitreous hemorrhage	Retinal detachment	Mutation	Exons
F	OD=H.M/30cm Os = LP	OD = no Os = yes	OD=normal Os = high	OD = yes Os = yes	OD = yes Os = yes	c.5339G>C	38
D	OD = LP Os = 0.02	OD = yes Os = yes	OD = high Os = high	OD = yes Os = no	OD = yes Os = yes	c.5339G>C	38
DB	OD=0.03 Os = 0.5	OD = yes Os=normal	OD = high Os= normal	OD =yes Os = no	OD = yes Os = no	c.5339G>C	38
PGF	OD=NLP Os = HM	OD=yes Os=aphakic	OD=high Os =high	OD=yes Os =yes	OD = no Os = yes	c.1775C>A, c.3648delC, c.5339G>C	11, 25, 38
PGM	OD=LP Os=0.02	OD=yes Os= normal	OD=high Os=normal	OD = yes Os = no	OD = yes Os = no	c.1775C>A	11
PGT	OD=HM Os =0.02	OD=yes Os=no	OD=normal Os= normal	OD = no Os = no	OD = no Os = no	c.1775C>A	11
PHW	OD=0.03 Os =LP	OD = yes Os = yes	OD = high Os = high	OD = yes Os = yes	OD = yes Os = yes	c.3686insC	25
PY	OD = 0.03 Os = LP	OD = IOL Os = yes	OD = high Os= normal	OD = yes Os = no	OD = yes Os = no	c.6039G>C	44

Table III.- SNPs Identified in FBN3 gene.

	Polymorphism	Amino acid	Genotype frequency in patients	Genotype frequency in normal
Exon 11	c.1833G>A homozygous	p.473R>Q	2/12	5/40
Exon 22	c.3219G>T homozygous	p.935R>L	3/12	7/40
Exon 22	c.3227G>T homozygous	p.l938V>F	1/12	4/40
Exon 25	c.3662C>T homozygous	p.1083R>W	3/12	6/40
Exon 38	c.5255G>A homozygous	p.1614G>S	2/12	3/40
Exon 44	c.6022C>G heterozygous	p.1869N>K	2/12	2/40

Another frameshift mutation, an insertion of C at position 3686 (c.3686insC) is predicted to give rise to a truncated protein shorter (1090 amino acids) than the normal protein (2809 amino acids). The patient in whom this FBN 3 mutation was detected was a two-years-old boy with familial history of Marfan syndrome. Her mother was tall and with low vision in both eyes but still lens was in normal position. Her fingers and arms were longer than normal. The baby's grandfather got operation of lens dislocation and got silicon oil due to retinal tear. This two year old baby is taller than a normal baby, has long skull and elevated jaws. Visual acuity was not clear because baby was not cooperative. We screened FBN3 for c.3686insC in all the family members of the patients and found that all the

patients in this family harbored the insertion that was not found in the normal members of the family.

In exon 11, a C to A substitution at nucleotide 1775 (c.1775C>A) was identified. This mutation replaced T454 in the first TB domain with P (p.454T>P). This mutation was found in three patients (Table 3). Two of these patients were 40 to 50 years old while one was 16 years old. These patients belonged to typical Marfan syndrome families. All severely affected family members had been operated for lens removal and retinal tear.

In exon 38, a G to C substitution at nucleotide 5339 (c.5339G>C) was identified. This mutation replaced G1642 in the fourth TB domain with P (p.1642G>P). This mutation was found in four patients. All these patients were 40-55 years old, except one baby who was 2 years old. All showed clinical symptoms like lens dislocation and retinal tear but the baby was normal showing only mild Marfan like features.

In exon 44, a G to C substitution at nucleotide 6039 (c.6039G>C) was identified. This mutation replaced G1876 in bEGF domain 11 with C (p.1876G>C). This mutation was found in only one patient who was a 55 years old male. He had lens dislocation with retinal tear and typical skeletal manifestations. Also, dilatation of the root of the aorta was documented.

DISCUSSION

Because no mutation of the FBN1 gene has been found in nearly half of MFS patients, there may be some other genes carrying mutations responsible for MFS. FBN3 is important extracellular matrix macromolecules that perform architectural functions in most connective tissues as FBN1, and it has been proved a cause of Weill-Marchesani syndrome that is caused by the disruption of extracellular matrix macromolecules (Robinson and Godfrey, 2000). In order to investigate the possibility that a portion of MFS might be caused by mutations in other fibrillin genes, we scanned mutations in 11th, 22nd, 25th, 38th and 44th exons encoding several conserved domains of the FBN3 protein.

We found some SNPs (c.1833G>A, c.3219G>T, c.3227G>T, c.3227C>T, c.3662G>A, c.6022C>G) that have already been identified. All SNPs showed homozygosity except c.6022C>G that was heterozygous in both patients and normal controls. As these SNPs were found in FBN3 of both patients and normal controls, so they may not be responsible for MFS (Uyeda *et al.,* 2004).

Except these identified SNPs, we identified five novel mutations in the FBN3 gene, including two frameshift mutations (c.3648delC and c.3686insC) and three missense mutations (p.454T>P, p.1642G>P and p.1876G>C). According to bioinformatics predication, the two frame shift mutations would lead to truncated protein precursors of lengths 1079 and 1090 amino acids, respectively. The length of normal protein is 2089 amino acids. Because the sequence of isoform FBN3 is not included in the truncated protein precursor, the activity of FBN3 gene must be lost. The mutation p. 454T>P was located in the site near first TB domain in protein precursor; the mutation p.1642G>P was located in the site near the fourth TB domain just before where the isoform of FBN3 start, and the mutation p.1876G>C was located in the site near the fourth metal ion-dependent adhesion site domain. As these amino acid changes were not detected in the Chinese normal controls and metal ion-dependent adhesion site and TGF binding domain are very critical to the functional integrity of the molecule (Uyeda *et al*., 2004), we presumed that they might be the genetic cause for MFS.

Conclusively, FBN3 might be another Marfan syndrome-associated gene but further studies on genetic characterization and functional relevance of the identified mutations are necessary because of the small sample size and high rate of mutation detection in this study.

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