Two novel mutations of fibrillin-1 gene correlate with different phenotypes of Marfan syndrome in Chinese families

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Purpose: To identify the causative mutations in two Chinese families with autosomal dominant Marfan syndrome and to describe the associated phenotypes.

Methods: Complete physical, ophthalmic, and cardiovascular examinations were given to the patients and unaffected individuals in the two families. Exclusive linkage mapping was performed for transforming growth factor beta receptor II (TGFBR2) and fibrillin-1 (FBN1) loci in both families. The entire coding region and flanking splice sites of the FBN1 gene were screened for mutations in the two families with Sanger sequencing. The potential mutations of FBN1 were tested in 100 normal controls.

Results: Lens dislocation was observed in two out of ten patients in the MF1 family and all patients in the MF2 family. However, the MF1 family displayed more severe cardiovascular and skeletal system involvement compared with the MF2 family. The transforming growth factor beta receptor II locus was excluded in both families by linkage analysis. A maximum multipoint lod score score of 2.83 was obtained for marker D15S992 (located in the FBN1 gene) in the MF1 family and 1.51 for the same marker in the MF2 family. Two novel mutations of FBN1, p.C271* and p.C637Y, were identified in the MF1 and MF2 families, respectively.

Conclusions: Genotype-phenotype correlations in this study indicate that nonsense mutations of FBN1 may correlate with relatively severe systemic phenotypes when compared with cysteine substitutions, the most common type of FBN1 mutations. Genetic diagnosis for patients with Marfan syndrome would help with genetic counseling, clinical intervention, and prognosis.

Marfan syndrome (MFS; MIM #154700) is a pleiotropic, autosomal dominant inherited disorder of connective tissue with an estimated prevalence of up to 1:5,000 [1]. MFS is characterized by variable phenotypic manifestations mainly in cardiovascular, ocular, and skeletal systems. Thus far, two causative genes have been demonstrated for MFS: fibrillin-1 (FBN1) and transforming growth factor beta receptor II (TGFBR2). Approximately 90% of MFS cases are caused by mutations in the FBN1 gene (15q21.1) [2]; whereas a second MFS causative gene, the TGFBR2 (3p22) gene, was identified in a French family with MFS originally found not to be associated with FBN1 in 2004 [3].

Fibrillin-1 is the major constitutive element of extracellular microfibrils encoded by the large FBN1 gene that contains 66 exons spanning 235 kb of genomic DNA on chromosome 15q21.1. Fibrillin-1 is a 350-kDa glycoprotein and has a modular structure comprising 47 epidermal growth factor-like (EGF) domains, seven transforming growth factor β1-binding protein-like (TB) domains, a proline-rich region, hybrid modules, and unique N- and C-termini. EGF modules are approximately 45 residues long and are characterized by six conserved cysteine residues that form three intramodule disulfide bonds [4,5]. Of the 47 EGF domains, 43 contain a consensus cysteine-rich sequence for calcium binding (cb-EGF), which is predicted to play an important role in microfibril stability and assembly [6]. All cysteines in fibrillin-1 are evolutionarily conserved, emphasizing their essential function. Fibrillin-1 mutations break microfibril formation, weaken the connective tissue, and result in fibrillin protein abnormalities [7].

In this study, we performed exclusive linkage analysis on two families with MFS with distinct phenotypes by using microsatellite markers spanning the critical regions of the two known MFS loci. Consistent with the positive linkage found on the FBN1 locus in the two families, we identified two novel FBN1 mutations, each of which correlated with distinct phenotypes presented by the corresponding pedigree.
METHODS

Patients and controls: Fifteen patients (ten from kindred MF1 and five from kindred MF2) were recruited in Tianjin Chest and Heart Hospital and Tianjin Eye Hospital. The fifteen patients were clinically diagnosed as MFS at the time of recruitment (Figure 1). The patients’ gender and ages are shown in the Table 1. Eleven individuals in kindred MF1 (ten affected members and one unaffected sibling) and seven individuals in kindred MF2 (five affected members and two presently unaffected siblings) underwent ophthalmic examinations, including assessment of best-corrected visual acuity, slit-lamp and direct fundus examinations, and systemic evaluations including physical examinations, echocardiography with measurement of the aortic root diameter, assessment of valve morphology and function, skeletal features, and skin extensibility.

Peripheral blood samples were drawn from median cubital vein from 15 individuals of kindred MF1, including ten affected members, one unaffected member, and four spouses, and ten individuals of kindred MF2, including five affected members, two unaffected siblings, and three spouses (Figure 1A). In addition, peripheral blood samples were collected from 100 normal Chinese volunteers with neither history of MFS nor related fibrillinopathy and were...
| Family    | Individual | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF1 | MF2 | MF2 | MF2 | MF2 | MF2 | MF2 |
|-----------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MF1       | II:1       | F/61| F/39| M/36| M/37| F/34| F/32| M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | III:1      | F/39| M/36| M/37| F/34| F/32| M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | III:2      | M/36| M/37| F/34| F/32| M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | III:7      | M/37| F/34| F/32| M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | III:8      | M/37| F/34| F/32| M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | III:9      | M/37| F/34| F/32| M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | IV:1       | M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | IV:2       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | IV:3       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF1       | IV:4       | F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | I:1        | M/17| F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | II:2       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | II:3       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | III:1      | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | III:2      | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | III:3      | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | IV:1       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | IV:2       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | IV:3       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |
| MF2       | IV:4       | F/15| F/15| F/10| M/57| F/35| M/32| M/13| M/9 |

**Cardiovascular system**
- Aortic root dimension (mm):
  - MF1: 58, 65, 62, 60, 52, 38, 28, 27, 35, 25
  - MF2: 25, 24, 21, 20
- Mitral valve prolapse: (-) for (-), (+) for (+)

**Ocular system**
- Lens dislocation: (-) for (-), (+) for (+)
- Myopia: (-) for (-), (+) for (+)
- Strabismus: (-) for (-), (+) for (+)
- Glaucoma: (-) for (-), (+) for (+)

**Skeletal system**
- Height (cm): 184, 190, 187, 188, 185, 187, 188, 180, 176, 140
- Arm span (cm): 192, 191, 186, 188, 190, 189, 190, 180, 173, 138
- Pectys deformities: (+) for (+)
- Scoliosis: (+) for (+)
- Joint hypermobility: (-) for (-)

**Other manifestations**
- Hyperextensible skin: (-) for (-), (+) for (+)
- Striae: (+) for (+)

F, female; M, male.
used as normal controls in the mutation study. All the peripheral blood samples were stored at -80°C before extraction of genomic DNA. Informed consent was obtained from all cases for sample collection and molecular analysis, and human studies were prospectively reviewed and approved by local institutional ethical review boards according to the Declaration of Helsinki. Genomic DNA was isolated from peripheral leukocytes.

**Genotyping of microsatellite markers and linkage analysis:**

Exclusive linkage analyses were performed on the two families by genotyping microsatellite markers spanning two known loci for MFS: FBN1 (D15S659, D15S992, D15S1016, and D15S648) and TGFBR2 (D3S2336, D3S2335, D3S1283, D3S3727, and D3S2432). Amplification of these microsatellite markers with PCR was performed using primers labeled with FAM, TET, or HEX and experimental conditions as previously described [8]. Briefly, the PCR program consists of an initial denaturation for 10 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C and 60 s at 72°C, and a final extension at 72°C for 7 min. The polymerase chain reactions were carried out in 10μl reactions containing 10 ng genomic DNA, 1xPCR buffer, 200 μM of each deoxyribonucleoside triphosphate (dNTP), 2.5 mM MgCl₂, 0.15 μM of each primer (labelled with Fam, Tet or Hex) and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). The PCR products were appropriately pooled according to allele size and labeling, mixed with GeneScan 500 TAMRA standard (Applied Biosystems, Foster City, CA), denatured, loaded onto 6% standard denaturing polyacrylamide gels, and run in the ABI 377XL sequencer (Applied Biosystems) for fluorescent detection. Genotyping data were collected using GeneScan Analysis 3.1 (PerkinElmer Inc., Waltham, MA) and analyzed using the Genotyper 2.0 software package (PerkinElmer).

Linkage analyses were performed by calculating multipoint lod scores using the LINKAGE software package Simwalk2, Version 3.35. MFS in both families was modeled as an autosomal dominant trait with a disease-allele frequency of 0.0001 and a penetrance of 99%. The allele frequencies for an autosomal dominant trait with a disease-allele frequency of 0.0001 and a penetrance of 99%. The allele frequencies for

**RESULTS**

Clinical evaluations: All 15 patients from both families (kindred MF1 and MF2) were clinically evaluated, and the two families were compared. According to the clinical findings schematically outlined in Table 1, the MF1 family has more severe systemic involvement than the MF2 family does. Notably, the ultrasonic examination revealed that the adult affected members in the MF1 family had a significantly increased diameter of the aortic root compared with age-matched controls (p<0.001), and compared with the adult affected members in the MF2 family (p=0.003; Table 1, Figure 2A). Moreover, the skeletal system was commonly and severely involved in patients in the MF1 family, whereas patients in the MF2 family mainly manifest increased height and arm span, and only one patient has joint hypermobility (Table 1). Unlike the systemic involvements, bilateral lens dislocation was observed in all affected members in the MF2 family (Figure 2B), but only in two out of ten patients in the MF1 family (Table 1).

**Linkage analysis:** The only two known loci related to MFS were initially screened with nine microsatellite markers.
(D15S659, D15S992, D15S1016, D15S648, D3S2336, D3S2335, D3S1283, D3S3727, and D3S2432). After the TGFBR2 locus on chromosome 3 was excluded (data not shown), positive linkage was found with markers of the FBN1 locus in the 15q21.1 region in both families. A maximum multipoint limit of detection score of 2.83 was obtained for marker D15S992 in the MF1 family, and 1.51 for the same marker in the MF2 family (Table 2). A haplotype between D15S659 and D15S648 was cosegregated with disease phenotype in each family (Figure 1A). Because D15S992 is located within the FBN1 gene, our genetic analysis of both families then turned to mutation analysis of FBN1.

**Mutation analysis:** With direct sequencing of FBN1, one novel mutation was identified in each of the two studied families. In the MF1 family, a heterozygous base substitution, c.813C>A, was detected in exon 8. This single base substitution generates a premature stop codon (TGA) instead of a cysteine (TGC) at codon 271 (p.C271*). In the MF2 family, a heterozygous variation, c.1910G>A was found in exon 16, resulting in a cysteine substitution by tyrosine at codon 637 (p.C637Y). These two allelic variations were cosegregated with the disease phenotypes in the two families, respectively, and were absent in 200 chromosomes from 100 unrelated control individuals, probably excluding the possibility of a rare polymorphism.

**Discussion:** Since the first causative FBN1 mutation was reported in MFS [2], over thousand of mutations have been described (HGMD). These mutations are spread throughout the gene without obvious predilection for any given region [9]. According to Robinson et al., causation could be suggested by the type of mutation: involvement of a highly conserved cysteine residue, nonsense, frameshift, or splice site mutation [10]. For instance, several cysteine substitutions in the FBN1 gene were correlated with ectopia lentis as the sole or predominant manifestation [11]. In this study, we report the identification of two novel mutations in the FBN1.
gene, including one nonsense mutation leading to a premature termination codon (PTC) and one missense mutation resulting in the substitution of a cysteine residue. The two types of mutation correlate with distinct phenotypes observed in two families with MFS. Further evidence of the mutations’ causality is suggested by the evidence that both mutations segregated with the disease phenotypes in two families whereas neither was found in 200 chromosomes from 100 unrelated control individuals.

The MF1 family displayed rather severe involvement of the organ system but with significant intrafamilial phenotypic variability (Table 1). All adult patients in the MF1 family exhibited remarkable cardiovascular system phenotypes, and the proband’s brother (III:4) died of aortic dissection at the age of 30 years. All patients presented various skeletal phenotypes, but interestingly, only two patients had lens dislocation. The rather severe cardiovascular and skeletal system phenotypes with less occurrence of lens dislocation observed in the MF1 family were linked to a PTC mutation (c.813C>A) of the FBN1 gene. PTC mutations predict the production of truncated fibrillin 1 monomers, which could interfere with the assembly of normal monomers into extracellular microfibrils [12]. However, the existence of putative truncated proteins has not been proved due to the possibility of nonsense-mediate decay of the mutant mRNA transcript. As the transcripts harboring PTC could be detected by the mRNA quality control system and eliminated before translation by nonsense-mediate decay (NMD), therefore, the heterozygous mutation c.813C>A of FBN1 could cause haplo-insufficiency of fibrillin 1 by 50%, which in turn results in more severe systemic involvement in the MF1 family. If this is the case, it would potentially suggest that loss of fibrillin 1 function by 50% can lead to remarkable phenotypes in multiple organs involving the cardiovascular and skeletal systems. Nevertheless, the mechanism by which PTC mutations exert an effect on extracellular fibrillin monomers is not yet clear.

Patients in the MF2 family had relatively mild phenotypes compared with those carried by the MF1 family. The MF2 family mainly manifested lens dislocation, with some mild symptoms of the skeletal system, but no obvious cardiovascular features of MFS. This mild phenotype was the consequence of a missense mutation (c.1910G>A) of the FBN1 gene. This mutation is located in cb-EGF modules and substituted a conserved cysteine residue potentially implicated in disulfide bonding. As three disulfide bonds are required to maintain cb-EGF module-fold, the mutation would likely disrupt one of the three disulfides bonds of the affected cb-EGF modules and probably cause domain misfolding with secondary deleterious effects on the global structure of fibrillin or microfibrils [13]. However, the effects of this mutation at the protein level are complicated: Impaired trafficking [14], delayed secretion [15], and enhanced protease susceptibility [16] have been described.

At the phenotypic level, our study showed that patients with MFS with the PTC mutation (the MF1 family) had more common aortic dilatation, more striking skeletal features, and large joint laxity, coupled with a much lower risk of serious ocular manifestations. However, the cysteine substitution mutation in the MF2 family causes a mild phenotype of MFS lacking aortic dilatation, but with major involvement of the ocular system and mild skeletal manifestations. Similar correlations have been suggested previously [12,15,17], and these data collectively emphasize the importance of these correlations referring to the clinical consequence of specific types of FBN1 mutations.

In general, FBN1 mutations have been associated with a broad spectrum of phenotypes, ranging from single connective tissue manifestations to lethal neonatal MFS [18,19]. The intrafamilial variability shown in the MF1 family also confirmed that the allelic mutation was not the only determinant of clinical severity; circumstances and other modified factors might contribute to the expression of a certain mutation as well.

Our results further expand the spectrum of FBN1 mutations, and, perhaps more importantly, confirm the potential relationship between mutation type and phenotype. This insight about these relationships will allow more precise assessment of the clinical diagnosis, management, prognosis
at early stage [20], and genetic counseling. Moreover, this insight may potentially lead to tailor-made patient follow-up.

**APPENDIX 1. PRIMERS FOR MUTATION SCREENING OF FBN1.**

To access the data, click or select the words “Appendix 1.”

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REFERENCES

1. Pyeritz RE. The Marfan syndrome.  Annu Rev Med  2000; 51:481-510.  [PMID: 10774478].

2. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EJ, Curristin SM, Stetten G, Meyers DA, Francomano CA.  Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene.  Nature  1991; 352:337-9.  [PMID: 1852208].

3. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, Allard D, Varret M, Claustres M, Morisaki H, Ihara M, Kinoshita A, Yoshiura K, Junien C, Kajii T, Jondeau G, Ohta T, Kishino T, Furukawa Y, Nakamura Y, Niikawa N, Boileau C, Matsumoto N.  Heterozygous TGFB2 mutations in Marfan syndrome.  Nat Genet  2004; 36:855-60.  [PMID: 15235604].

4. Corson GM, Chalberg SC, Dietz HC, Charbonneau NL, Sakai LY.  Fibrillin binds calcium and is coded by cDNAs that reveal a multidomain structure and alternatively spliced exons at the 5′ end.  Genomics  1993; 17:476-84.  [PMID: 7691719].

5. Pereira L, D’Alessio M, Ramirez F, Lynch JR, Sykes B, Pangilinan T, Bonadio J.  Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome.  Hum Mol Genet  1993; 2:1762-72.  [PMID: 8268958].

6. Handford PA, Downing AK, Reinhardt DP, Sakai LY.  Fibrillin: from domain structure to supramolecular assembly.  Matrix Biol  2000; 19:457-70.  [PMID: 11068200].

7. Dong J, Bu J, Du W, Li Y, Jia Y, Li J, Meng X, Yuan M, Peng X, Zhou A, Wang L.  A new novel mutation in FBN1 causes autosomal dominant Marfan syndrome in a Chinese family.  Mol Vis  2012; 18:81-6.  [PMID: 22262941].

8. Zhao C, Lu S, Zhou X, Zhang X, Zhao K, Larsson C.  A novel locus (RP33) for autosomal dominant retinitis pigmentosa mapping to chromosomal region 2cen-q12.1.  Hum Genet  2006; 119:617-23.  [PMID: 16612614].

9. Boileau C, Jondeau G, Mizuguchi T, Matsumoto N.  Molecular genetics of Marfan syndrome.  Curr Opin Cardiol  2005; 20:194-200.  [PMID: 15861007].

10. Robinson PN, Booms P, Katzke S, Ladewig M, Neumann L, Palz M, Pregla R, Tiecke F, Rosenberg T.  Mutations of FBN1 and genotype-phenotype correlations in Marfan syndrome and related fibrillinopathies.  Hum Mutat  2002; 20:153-61.  [PMID: 12203987].

11. Vanita V, Singh JR, Singh D, Varon R, Robinson PN, Sperling K. A recurrent FBN1 mutation in an autosomal dominant ectopia lentis family of Indian origin.  Mol Vis  2007; 13:2035-40.  [PMID: 18079676].

12. Schrijver I, Liu W, Odom R, Brenn T, Oefner P, Furthmayr H, Francke U.  Premature termination mutations in FBN1: distinct effects on differential allelic expression and on protein and clinical phenotypes.  Am J Hum Genet  2002; 71:223-37.  [PMID: 12068374].

13. Downing AK, Knott V, Werner JM, Cardy CM, Campbell ID, Handford PA. Solution structure of a pair of calcium-binding epidermal growth factor-like domains: implications for the Marfan syndrome and other genetic disorders.  Cell  1996; 85:597-605.  [PMID: 8653794].

14. Whiteman P, Handford PA.  Defective secretion of recombinant fragments of fibrillin-1: implications of protein misfolding for the pathogenesis of Marfan syndrome and related disorders.  Hum Mol Genet  2003; 12:727-37.  [PMID: 12651868].

15. Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U.  Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes.  Am J Hum Genet  1999; 65:1007-20.  [PMID: 10486319].

16. Vollbrandt T, Tiedemann K, El-Hallous E, Lin G, Brinckmann J, John H, Bätge B, Notbohm H, Reinhardt DP.  Consequences of cysteine mutations in calcium-binding epidermal growth factor modules of fibrillin-1.  J Biol Chem  2004; 279:32924-31.  [PMID: 15161917].

17. Loeyes B, Nuytinck L, Delvaux I, De Bie S, De Paepe A.  Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome.  Arch Intern Med  2001; 161:2447-54.  [PMID: 11700157].

18. Putnam EA, Cho M, Zinn AB, Towbin JA, Byers PH, Milewicz DM.  Delineation of the Marfan phenotype associated with mutations in exons 23–32 of the FBN1 gene.  Am J Med Genet  1996; 62:233-42.  [PMID: 8882780].

19. Kainulainen K, Karttunen L, Puhakka L, Sakai L, Peltonen L.  Mutations in the fibrillin gene responsible for dominant ectopia lentis and neonatal Marfan syndrome.  Nat Genet  1994; 6:64-9.  [PMID: 8136837].

20. Meng B, Li H, Yang T, Huang S, Sun X, Yuan H. Identification of a novel FBN1 gene mutation in a Chinese family with Marfan syndrome.  Mol Vis  2011; 17:2421-7.  [PMID: 21976953].
