Late-onset bilateral lens dislocation and glaucoma associated with a novel mutation in \textit{FBN1}

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**Purpose:** To describe the clinical and genetic findings in one Chinese family with late-onset bilateral lens dislocation and secondary glaucoma.

**Methods:** One family including three affected members and 16 unaffected family members was examined clinically. After informed consent was obtained, genomic DNA was extracted from venous blood of all participants. Linkage analysis was performed with two microsatellite markers around the \textit{fibrillin-1 (FBN1)} gene (D15S992 and D15S126). Mutation screening was performed using direct DNA sequence analysis and single strand conformation polymorphism (SSCP).

**Results:** Clinical examination and pedigree analysis revealed that four members in three generations were affected by late-onset lens dislocation and secondary glaucoma but had no signs of cardiovascular abnormality or abnormal skeletal features. By genotyping, the family showed the linkage to \textit{FBN1} on 15q21.1. After mutation screening analysis on 65 exons of \textit{FBN1}, a novel heterozygous missense mutation, c.2860C>T (R954C), was detected. This mutation cosegregated with the disease phenotype in the family and was not found in 100 normal controls.

**Conclusions:** Late-onset isolated ectopia lentis with secondary glaucoma is consistent with a novel mutation in \textit{FBN1}. Our finding expands the spectrum of \textit{FBN1} mutations and is useful for further genetic consultation and genetic diagnosis.

Ectopia lentis (EL; OMIM 129600) is a dominantly inherited connective disorder characterized by lens dislocation due to stretched or disrupted zonular filaments [1]. It may also occur as a common clinical feature of several different systemic hereditary diseases such as Marfan syndrome. Marfan syndrome (MFS, OMIM 154700) is an autosomal dominant connective tissue disorder involving many systems, but its more cardinal manifestations are cardiovascular, skeletal, and ocular [1]. However, isolated EL or simple EL patients present with no cardiovascular or skeletal features of MFS [1]. By genetic analysis, both EL and MFS are linked to the same gene, \textit{fibrillin-1 (FBN1)} [2-4]. \textit{FBN1} mutations have been identified in patients affected by type I fibrillinopathies, which include MFS, neonatal MFS (nMFS), MASS syndrome (mitral valve, aorta, skeleton, and skin; OMIM 604308), isolated EL, Shprintzen-Goldberg syndrome (OMIM 182212), isolated skeletal features of MFS, and ascending aortic aneurysm [2-10].

\textit{FBN1} contains 65 exons spanning 230 kb of genomic DNA on chromosome 15q21.1. The gene encodes profibrillin-1, a 350 kDa glycoprotein. This glycoprotein is further processed to fibrillin-1, the main component of 10–12 nm extracellular microfibrils that are widely distributed in both elastic and non-elastic tissues including the skin, aorta, periosteum, cartilage, and ciliary zonules [5-8]. Fibrillin-1 contains 47 motifs with homology to the human epidermal growth factor (EGF); 43 of these also contain a consensus sequence for calcium binding (cbEGF). EGF motifs have six conserved cysteine residues that form three disulfide bonds—between C1 and C3, C2 and C4, C5 and C6. It also has seven TGF\(\beta\)-binding protein-like modules containing eight-cysteine motifs (8-Cys/TB), a two-hybrid domain, a NH\textsubscript{2}-terminal domain, one proline rich region, and a COOH-terminal domain [6].

Here, we reported a Chinese family associated with late-onset isolated EL and secondary glaucoma. Molecular genetic analysis of the family revealed a novel heterozygous missense mutation in \textit{FBN1}.

**METHODS**

\textit{Patients and DNA samples collection:} This study was approved by the Beijing Tongren Hospital Joint Committee on Clinical Investigation. After informed consents were obtained, all participants underwent physical, ophthalmologic, and cardiovascular examinations. Ophthalmologic examinations included bilateral visual acuity, slit-lamp biomicroscopy, fundus examination with dilated pupils, and ultrasound biomicroscopy (UBM). Cardiovascular examinations included electrocardiogram and echocardiogram. Peripheral blood was obtained by venipuncture, and genomic DNA was extracted according to standard protocols.

**Linkage analysis:** Genotyping and linkage analysis were performed with two microsatellite markers, D15S992 and D15S126, around \textit{FBN1}. Their detailed genetic and physical distances are shown in Figure 1. The primer sequences of
D15S992 and D15S126 were obtained from the The GDB Human Genome Database. Genotyping and linkage analysis were performed as described elsewhere [11,12]. LOD scores were calculated for the two markers by two-point linkage analysis using linkage package 5.2. We modeled the disease as an autosomal dominant trait with reduced penetrance. Pedigree and haplotype were constructed using Cyrillic V. 2.0 software.

**Mutation screening of FBN1:** The whole coding region of FBN1 was amplified by polymerase chain reaction (PCR) from genomic DNA. Sixty-five pairs of primers for FBN1 were used according to the articles previously published [13, 14]. Nucleotide sequences were compared with the published cDNA sequence of FBN1 (GenBank accession number NM_000138) using DNAssit version 1.0.

**Single strand conformation polymorphism:** Single strand conformation polymorphism (SSCP) was used to exclude the point mutations from the normal controls. PCR amplified DNA fragments were mixed with an equal volume of formamide buffer and electrophoresed on a 12% nondenaturing polyacrylamide gel (12 ml 30% PAGE [acrylamide:bisacrylamide=29:1]; 3 ml 10X TBE; 15 ml distilled water; 600 μl 10% ammonium persulfate, 5 μl tetramethylmethylenediamine). After electrophoresis, gels were silver-stained and analyzed.

**RESULTS**

**Clinical findings:** We have identified a Chinese family with bilateral lens dislocation. The mode of inheritance was autosomal dominant (Figure 1). The family had 20 individuals; four of them were affected (one male and three females). As the mother of the proband passed away several years ago, we did not get her blood sample. However, from her hospital records, we inferred that she suffered the same eye disease. After clinical examinations and reviewing hospital records, we found all affected members shared almost the same clinical manifestations. All of them first experienced the sudden blurring of vision with periorcular pain and congestion, then ophthalmologic examinations showed high intraocular pressure (IOP; 40–80 mmHg), corneal edema, shallow anterior chamber, and lens dislocation. All affected members underwent lens extraction, and their IOP were in the normal range after surgery. Fundus examination for three of the affected individuals showed healthy and pink optic discs with a cup/disc ratio around 0.4 (except the proband’s right eye). Physical and cardiovascular examinations presented no skeletal and cardiovascular features of MFS in any of the affected members. Their detailed clinical information is summarized in Table 1.

**Genotyping results:** This family with isolated EL was genotyped with two microsatellite markers located around FBN1 in the 15q21.1 region. The marker results for D15S992 and D15S126 were fully informative for linkage. Haplotypes were constructed for this family to determine whether the disease was segregating with microsatellite markers. For this family, there were no affected recombinants for either of the two markers (Figure 1). Interestingly, three clinical unaffected individuals (III:1, III:6, and III:7) inherited the affected haplotype as well. As the lens luxation in this family seemed to represent a late-onset feature in the phenotype and all three of them were under 37 years old (the youngest age of onset in this family), they were obligate carriers. Their detailed clinical information was also summarized in Table 1. Therefore, the disease penetrance appeared incomplete in this pedigree. Two-point LOD scores for D15S992 and D15S126 with 60% penetrance were 0.70 (θ=0.0) and 2.36 (θ=0.0), respectively.

**Mutation analysis:** By direct sequencing the 65 exons of FBN1, we identified a novel base change, C>T, at position 2860 of cDNA, replacing arginine acid with cysteine at codon 954 (Figure 2A). Using SSCP analysis, this heterozygous mutation cosegregated with all affected members and affected haplotype carriers in this Chinese family (Figure 2B) but was not detected in 100 unrelated normal controls.

**DISCUSSION**

In this study, we analyzed a Chinese family with four members affected by lens dislocation along with secondary glaucoma. By genotyping, the family showed the linkage to the EL locus on 15q21.1. One novel heterozygous FBN1 mutation, R954C, was identified in this family. The mutation, R954C, was found to cosegregate with the EL phenotype, and where three clinically unaffected members carried the mutation, they were also found to harbor the affected haplotype. This variant was not detected in 100 normal control individuals. All affected individuals showed ocular involvement only and did not meet the Ghent criteria for Marfan syndrome [6]. Diagnosis of isolated ectopia lentis was established for this family. In our
Table 1. Clinical details of three affected members and three carriers in the ectopia lentis family.

|                          | II:1 | II:8 | II:11 | III:1 | III:6 | III:7 |
|--------------------------|------|------|-------|-------|-------|-------|
| Age                      | 67   | 61   | 49    | 36    | 34    | 21    |
| Onset age                | 37   | 54   | 42    | -     | -     | -     |
| Ocular features          |      |      |       |       |       |       |
| Best corrected Visual Acuity (R/L) | LP*/1.5 | 1.0/1.0 | 1.0/1.0 | 1.0/1.0 | 1.0/1.0 | 1.0/1.0 |
| Ectopia lentis           | +    | +    | +     | -     | -     | -     |
| Secondary glaucoma       | +    | +    | +     | -     | -     | -     |
| Eye operation (R)        | LE   | ILV  | ILV   | -     | -     | -     |
| Eye operation (L)        | PI   | ILV  | ILV   | -     | -     | -     |
| Cornea                   | CL (R) | - | - | - | - | - |
| Myopia                   | ?    | +    | +     | +     | +     | +     |
| Retina detachment        | -    | -    | -     | -     | -     | -     |
| Skeletal features        |      |      |       |       |       |       |
| Height (cm)              | 168  | 160  | 165   | 174   | 165   | 162   |
| Pectus carinatum         | -    | -    | -     | -     | -     | -     |
| Pectus excavatum         | -    | -    | -     | -     | -     | -     |
| Scoliosis (>20°)         | -    | -    | -     | -     | -     | -     |
| Arachnodactyly           | -    | -    | -     | -     | -     | -     |
| Joint hypermobility      | -    | -    | -     | -     | -     | -     |
| Cardiovascular features  |      |      |       |       |       |       |
| Mitral valve prolapse    | -    | -    | -     | -     | -     | -     |
| Aortic ascendens dilatation/dissection | -    | -    | -     | -     | -     | -     |

R represents right eye; L represents left eye; + represents positive syndrome; - represents negative syndrome; ? represents syndrome is unknown; LP represents light perception; LE represents lensectomy; PI represents peripheral iridotomy; CL represent corneal leukemia; ILS represent intraocular lens suspension; The asterisk indicates that the lens was completely dislocated into the anterior chamber, which induced corneal endothelial decompensation and corneal opacity.

To date, over 600 mutations in FBN1 have been reported. However, mutations for isolated EL only take a small part (FBN1 Universal Mutation Database [UMD]). Mutation R954C that was detected in this study is the first missense mutation in exon 24 associated with isolated EL. Mutations within the middle region (exons 24–32) of FBN1 are usually associated with a severe form of MFS, neonatal MFS, and define a high-risk group for cardiac manifestations [5-8,16]. Mutation R954C is located in the third 8-Cys/TB modules, which contains eight cysteine residues that form four disulfide bonds—between C1 and C3, C2 and C6, C4 and C7, and C5 and C8. This mutation adds a new cysteine residue, which is review of the literature, isolated EL occurs either as a congenital disorder or as a spontaneous disorder of late onset, which is between the ages of 20 and 65 years [1,7,15]. This family’s late-onset feature (37–51 years old) is in accordance with the secondary condition. As three obligate carriers in this family were still younger than 37 years old (the youngest age of onset in this pedigree), they might develop EL in the future or present reduced penetrance or non-penetrance as reported before [7,15]. They should therefore undergo ophthalmologic surveillance at regular intervals throughout their lives.
might destroy the disulfide bond formation and further influence the structure and function of fibrillin-1.

Early in 2002, [17] Comeglio et al. noted that mutations involving non-conserved arginine to cysteine substitution are usually associated with isolated EL. To date, nine of these types of substitutions have been identified in FBN1 (Table 2) [13,17-29]. Seven of them including the one detected in this study definitively caused isolated EL, and the majority of them are confined to the first 15 exons of FBN1 (5/7). Our results support the prior suggestion that non-conserved arginine to cysteine substitution is highly related to predominant EL regardless of which modules they are located [17,18].

Another clinical feature of this family is glaucoma, which is relatively common and a serious complication of ectopia lentis [1]. According to the literature, glaucoma usually occurs more frequently in spontaneous late subluxation of the lens than in the congenital type [1]. We concluded that the pathogenesis of glaucoma in this family was due to lens-induced pupillary block.

In summary, we described a novel non-conserved arginine to cysteine substitution in exon 24 of FBN1 that is associated with late-onset isolated EL and secondary glaucoma. Our results further expanded the mutation spectrum of FBN1 and provided useful genetic consultation and genetic diagnosis for this family.

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REFERENCES

1. Nelson LB, Maumenee IH. Ectopia lentis. Surv Ophthalmol 1982; 27:143-60. [PMID: 6984233]

2. Lee B, Godfrey M, Vitale E, Horii H, Mattei MG, Sarfarazi M, Tsiourpas P, Ramirez F, Hollister DW. Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 1991; 352:330-4. [PMID: 1852206]

3. Dietz HC, Cutting CR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamaso H, 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]

4. Edwards MJ, Challinor CJ, Colley PW, Roberts J, Partington MW, Halloway GE, Kozman HM, Mulley JC. Clinical and linkage study of a large family with simple ectopia lentis linked to FBN1. Am J Med Genet 1994; 53:65-71. [PMID: 7802039]

5. Robinson PN, Godfrey M. The molecular genetics of Marfan syndrome and related microfibrillopathies. J Med Genet 2000; 37:9-25. [PMID: 10633129]

6. Collobd-Bérard G, Boileau C. Marfan syndrome in the third Millennium. Eur J Hum Genet 2002; 10:673-81. [PMID: 12404097]

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

8. 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]

9. Adès LC, Holman KJ, Brett MS, Edwards MJ, Bennett B. Ectopia Lentis phenotypes and the FBN1 gene. Am J Med Genet A 2004; 126A:284-9. [PMID: 15054843]

10. Faivre L, Gorlin RJ, Wirtz MK, Godfrey M, Dagoneau N, Samples JR, Merrer ML, Collobd-Bérard G, Boileau C, Munnich A, Cormier-Daire V. In frame fibrillin-1 gene deletion in autosomal dominant Weil-Marchesani syndrome. J Med Genet 2003; 40:34-6. [PMID: 12525539]
11. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. SCNA5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 1995; 80:805-11. [PMID: 7889574]

12. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT. Positional cloning of a novel potassium channel gene: KV1.1 mutations cause cardiac arrhythmias. Nat Genet 1996; 12:17-23. [PMID: 8528244]

13. Körkkö J, Kaitila I, Lönnqvist L, Peltonen L, Ala-Kokko L. Sensitivity of conformation sensitive gel electrophoresis in detecting mutations in Marfan syndrome and related conditions. J Med Genet 2002; 39:34-41. [PMID: 11826022]

14. Nijbroek G, Sood S, McIntosh I, Francomano CA, Bull E, Pereira L, Ramirez F, Pyeritz RE, Dietz HC. Fifteen Novel FBN1 Mutations Causing Marfan Syndrome Detected by Heteroduplex Analysis of Genomic Amplicons. Am J Hum Genet 1995; 57:8-21. [PMID: 7611299]

15. Lönnqvist L, Child A, Kainulainen K, Davidson R, Puhakka L, Peltonen L. A novel mutation of the fibrillin gene causing ectopia lentis. Genomics 1994; 19:573-6. [PMID: 8188302]

16. Tiecke F, Katzke S, Booms P, Robinson PN, Neumann L, Godfrey M, Mathews KR, Scheunem L, Hinkel GK, Brenner RE, Hövels-Gürich HH, Hagemeier C, Fuchs J, Skovby F, Rosenberg T. Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype–phenotype correlations in FBN1 exons 24–40. Eur J Hum Genet 2001; 9:13-21. [PMID: 11175294]

17. Comeglio P, Evans AL, Brice G, Cooling RJ, Child AH. Identification of FBN1 gene mutations in patients with ectopia lentis and marfanoid habitus. Br J Ophthalmol 2002; 86:1359-62. [PMID: 12446365]

18. Jin C, Yao K, Jiang J, Tang X, Shentu S, Wu R. Novel FBN1 mutations associated with predominant ectopia lentis and marfanoid habitus in Chinese patients. Mol Vis 2007; 13:1280-4. [PMID: 17679947]

19. Yu R, Lai Z, Zhou W, Ti DD, Zhang XN. Recurrent FBN1 Mutation (R62C) in a Chinese Family with Isolated Ectopia Lentis. Am J Ophthalmol 2006; 141:1136-8. [PMID: 16765689]

20. Katzke S, Booms P, Tiecke F, Palz M, Pletschacher A, Türkmen S, Neumann LM, Pregla R, Leitner C, Schramm C, Lorenz P, Hagemeier C, Fuchs J, Skovby F, Rosenberg T, Robinson PN. TGGE screening of the entire FBN1 coding sequence in 126 individuals with Marfan syndrome and related fibrillinopathies. Hum Mutat 2002; 20:197-208. [PMID: 12203992]

21. Loey 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]

22. Black C, Withers AP, Gray JR, Bridges AB, Craig A, Baty DU, Boxer M. Correlation of a recurrent FBN1 mutation (R122C) with an atypical familial Marfan syndrome phenotype. Hum Mutat 1998; Suppl 1:S198-200. [PMID: 9452085]

23. Loey B, De Backer J, Van Acker P, Wetting K, Pals G, Nuytinck L, Coucke P, De Paepe A. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. Hum Mutat 2004; 24:140-6. [PMID: 15241795]

24. Stuhl-Hallengren C, Ukkonen T, Kainulainen K, Kristoffersson U, Saxne T, Tornqvist K, Peltonen L. An extra cysteine in one of the non-calcium-binding Epidermal Growth Factor-like motifs of the FBN1 polypeptide is connected to a novel variant of Marfan syndrome. J Clin Invest 1994; 94:709-13. [PMID: 8040326]

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

26. Hayward C, Porteous ME, Brock DJ. Mutation screening of all 65 exons of the fibrillin-1 gene in 60 patients with Marfan syndrome: report of 12 novel mutations. Hum Mutat 1997; 10:280-9. [PMID: 9338581]

27. Halliday DJ, Hutchinson S, Lonie L, Hurst JA, Firth H, Handford PA, Wordsworth P. Twelve novel FBN1 mutations in Marfan syndrome and Marfan related phenotypes test the feasibility of FBN1 mutation testing in clinical practice. J Med Genet 2002; 39:589-93. [PMID: 12161601]

28. Colloid-Béroud G, Béroud C, Ades L, Black C, Boxer M, Brock DJ, Holman KJ, de Paepe A, Francke U, Grau U, Hayward C, Klein HG, Liu W, Nuytinck L, Peltonen L, Alvarez Perez AB, Rantamäki T, Junien C, Boileau C. Marfan Database (third edition): new mutations and new routines for the software. Nucleic Acids Res 1998; 26:229-33. [PMID: 9399842]

29. Palz M, Tiecke F, Booms P, Göldner B, Rosenberg T, Fuchs J, Skovby F, Schumacher H, Kaufmann UC, von Kodolitsch Y, Nienaber CA, Leitner C, Katzke S, Vetter B, Hagemeier C, Robinson PN. Clustering of mutations associated with mild Marfan-like phenotypes in the 3’ region of FBN1 suggests a potential genotype-phenotype correlation. Am J Med Genet 2000; 91:212-21. [PMID: 10756346]