The 208delG Mutation in \textit{FSCN2} Does Not Associate with Retinal Degeneration in Chinese Individuals

Qingjiong Zhang, Shiqiang Li, Xueshan Xiao, Xiaoyun Jia, and Xiangming Guo

\textbf{PURPOSE.} The 208delG (c.72delG, p.Thr25GlnfsX120) mutation in the \textit{FSCN2} gene was reported to cause autosomal dominant retinitis pigmentosa (ADRP) and autosomal dominant macular degeneration (ADMD). The purpose of this study was to detect the 208delG mutation in Chinese individuals, with or without hereditary retinal degeneration.

\textbf{METHODS.} DNA fragments encompassing the 208delG mutation were amplified by polymerase chain reaction (PCR). The amplicons were analyzed by sequencing or and heteroduplex-single-strand conformational polymorphism (SSCP) analysis. An ophthalmic evaluation was conducted in those individuals with the 208delG mutation.

\textbf{RESULTS.} The 208delG mutation was detected in 8 of 242 unrelated probands: 175 with retinitis pigmentosa (RP), 20 with Leber congenital amaurosis (LCA), and 47 with cone–rod dystrophy (CORD). Of the eight, the retinal diseases were RP in six probands, LCA in one proband, and CORD in one proband. The disease was transmitted as an autosomal dominant (one family), autosomal recessive (two families), or sporadic (five families) trait. The mutation did not cosegregate with retinal degeneration in three families, whereas five normal family members also had the mutation. In addition, this mutation was also detected in 13 of 521 unrelated control subjects.

\textbf{CONCLUSIONS.} The 208delG mutation in \textit{FSCN2} is not associated with hereditary retinal degeneration in the Chinese individuals examined, which contradicts the original report about mutation in \textit{FSCN2} as a cause of ADRP and ADMD. This finding reminds us that great care is needed in making mutation–disease associations. (\textit{Invest Ophtalmol Vis Sci.} 2007;48: 530–533) DOI:10.1167/iovs.06-0669

Hereditary retinal degeneration is a group of severe disorders affecting vision, which can be transmitted as an autosomal dominant, autosomal recessive, or X-linked trait. Several loci or genes responsible for retinal degeneration have been reported (RetNet, http://www.sph.uth.tmc.edu/Retnet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX).

\textit{FSCN2} (OMIM 607643; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD).

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\textbf{METHODS}

\textbf{Patient Samples and Pedigrees}

Patients with RP, CORD, or LCA were ascertained from our Pediatric and Genetic Clinic, Zhongshan Ophthalmic Center as part of our 863-project to identify genes responsible for genetic eye diseases. This study was approved by IRB of the Zhongshan Ophthalmic Center and adhered to the tenets of the Declaration of Helsinki and the Guidance of Sample Collection of Human Genetic Diseases (863-Plan) by the Ministry of Public Health of China. Informed consent was obtained from the participating individuals or their guardians before the study. Medical and ophthalmic histories were obtained, and ophthalmic examination (by QZ and XG) included visual acuity, slit lamp and funduscopic examinations. Electroretinogram (ERG) responses were recorded in selected probands consistent with ISCEV standards.

\textbf{Detection of the 208delG Mutation in \textit{FSCN2}}

DNA fragments encompassing the 208delG mutation in \textit{FSCN2} (human genome build 35.1, NC_000017 region between nucleotides 710017 and 77114632 for genomic DNA, NM_012418 for mRNA, NP_036550 for protein; http://www.ncbi.nlm.nih.gov/ provided by the National Center for Biotechnology Information [NCBI], Bethesda, MD) were

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amplified by polymerase chain reaction with two pairs of primers as follows: (1) for DNA sequencing: FSCN2-1AF (forward) 5'−CCCCGCCGGCCCTCTGCT−3', FSCN2-R (reverse) 5'−CACGGCCCGGCTGCTTGGC−3'; (2) for heteroduplex-SSCP (single-strand conformational polymorphism) analysis: FSCN2-HAF 5'−CCCCGGCCAGCTGTGAGATG−3', FSCN2-HAR 5'−CACAGGCGTGGCGCTTGC−3'. The DNA sequences in additional patients with RP, CORD, or LCA as well as control subjects were screened by heteroduplex-SSCP analysis, as we have described elsewhere.6

RESULTS

In initial sequence analysis of 96 probands with RP, heterozygous 208delG mutation in FSCN2 was detected in 3 probands (Fig. 1). Heteroduplex-SSCP analysis of an additional 146 probands with RP, CORD, or LCA disclosed another five probands (Fig. 1). Heteroduplex-SSCP analysis of an additional 146 probands with RP, CORD, or LCA disclosed another five probands (Table 1). In the eight probands with the mutation, sequence analysis of these five probands revealed a heterozygous 208delG mutation.

In total, the heterozygous 208delG (c.72delG, p.Thr25GlnfsX120) mutation was detected in 8 of 242 unrelated probands with RP (n = 175), LCA (n = 20), or CORD (n = 47). Of the eight probands, the retinal diseases were RP in six, LCA in one, and CORD in one (Table 1). In the eight probands with the mutation, the disease was transmitted as an autosomal dominant (one family), autosomal recessive (two families), or sporadic (five families) trait (Fig. 2).

The mutation did not cosegregate with the disease in three families in which five unaffected family members also had the mutation (Fig. 2). In family A with LCA, the 208delG mutation was detected in only one (Fig. 2, IV:4) of the six affected individuals. This mutation was also present in three unaffected individuals of family A (II:5, II:6, and III:9). In family B, the 208delG mutation was identified in an unaffected mother and her son who had CORD. In family C, the 208delG mutation was found in a patient with RP and in his elder sister, without any sign of retinal degeneration. Ophthalmoscopic observation revealed a normal fundus in all five unaffected family members in families A, B, and C (Supplementary Fig. S1, online at http://www.iovs.org/cgi/content/full/48/2/530/DC1). Electoretinograms demonstrated normal retinal rod–cone function in three unaffected family members with the 208delG mutation (Fig. 3). As these five unaffected family members were older than the corresponding proband in each family and were more than 40 years old except one, who was 20 years old, a normal ocular phenotype was unlikely due to the late expression of the retinal diseases (Table 2).

In addition, the 208delG mutation was detected in 13 of 521 unrelated control subjects (Tables 1, 2; Fig. 4), including 9 of 329 normal control subjects, and 4 of 192 individuals with Leber hereditary optic neuropathy (LHON) who harbored one.

![FIGURE 1. Sequence chromatograms around the 208delG mutation. Both forward and reverse sequences were shown. Wild: normal sequence. Family A IV:4: individual IV:4 with LCA from family A had the 208delG mutation. C1: a normal control subject also had the 208delG mutation. Arrow: the site where a normal sequence overlaps with the shifted mutant sequence due to the 208delG mutation.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932945/)

![FIGURE 2. The 208delG mutation was identified in five families with retinal degeneration. Arrow: proband in each family. Filled symbols: individuals affected with retinal degeneration. +−, normal sequence around the 208delG region. +−, presence of the heterozygous 208delG mutation.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932945/)
of the three common mtDNA primary mutations for LHON. None of the 13 control individuals had night blindness. Signs for retinal degeneration, such as attenuation of retinal vessels and pigment deposits on fundus, were not present in these individuals. Clinical information on the 26 individuals with the 208delG mutation, including 8 patients with retinal degeneration, 5 unaffected family members, and 13 unrelated control subjects is shown in Table 2.

**DISCUSSION**

In this study, the 208delG mutation in *FSCN2* was detected in 8 of 242 patients with retinal degeneration. The mutation in three families is apparently not associated with the disease, in that five normal family members also had the mutation. The mutation was also detected in 13 of 521 control individuals.

Previously, the 208delG mutation in *FSCN2* was detected in only six Japanese families: four with ADRP and two with ADMD. Subsequently, a mouse model involving targeted disruption of the *FSCN2* gene was constructed by replacing exon 1 of *FSCN2* with the cDNA of a green fluorescent protein. It was suggested that haploinsufficiency of the *FSCN2* gene results in retinopathy in the *FSCN2* knockout mice. Unfortunately, the phenotype of mice with homozygous knockout of *FSCN2* has not been reported.

*FSCN2* was excluded as a candidate gene for RP17 mapped to this region. The 208delG mutation was not detected in 458 families with retinal degeneration from other ethnic groups so far reported. This mutation was not detected in 215 Spanish probands with ADRP (200 cases) or ADMD (15 cases). It was not detected in 43 Italian families with ADRP or in 200 U.S. families with ADRP in a recent study. In addition, no other mutation, other than 208delG, has been

**Table 2. Clinical Information on Individuals with the 208delG Mutation**

<table>
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<tr>
<th>ID</th>
<th>Gender</th>
<th>Age (y)</th>
<th>Age at Onset</th>
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<th>Visual Acuity</th>
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<th>ERG Recording</th>
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<td>Cone Response</td>
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**Affected probands**

A M 13 Infancy Poor vision 0.02; 0.01 LCA* None identifiable None identifiable
B M 16 9 y Night blindness 0.04; 0.1 RP Severely reduced Severely reduced
C M 18 Early childhood Night blindness 0.2; 0.1 RP None identifiable None identifiable
D M 50 Early childhood Night blindness 0.2; 0.2 CORD Normal NormalNormal
E F 7 Early childhood Night blindness 0.07; 0.4 RP N/A N/A
F M 24 Early childhood Night blindness 0.07; 0.4 RP N/A N/A
G F 62 Early childhood Night blindness 0.07; 0.4 RP N/A N/A
H F 39 Early childhood Night blindness 0.07; 0.4 RP N/A N/A

**Unaffected family members**

A-I6 M 65 — No 0.8; 0.8 Normal N/A N/A
A-II6 F 56 — No 0.9; 0.9 Normal Normal Normal
A-III9 M 46 — No 1.0; 1.0 Normal Normal Normal
B-I1 F 40 — No 1.0; 1.0 Normal N/A N/A
C-II1 F 26 — No 1.0; 1.0 Normal N/A N/A

**Control subjects**

C1 M 32 — No 1.5; 1.5 Normal N/A N/A
C2 F 40 — No 1.5; 1.5 Normal N/A N/A
C3 M 35 — No 1.5; 1.5 Normal N/A N/A
C4 M 59 — No 0.6; 1.0 Normal N/A N/A
C5 M 66 — No 0.7; 1.0 Normal N/A N/A
C6 M 39 — No 1.2; 1.2 Normal N/A N/A
C7 M 60 — No 1.0; 1.0 Normal N/A N/A
C8 F 44 — No 0.5; 0.6 Normal N/A N/A
C9 F 17 — No 0.7; 0.7 Normal N/A N/A
LHON1 M 20 20 y Reduced vision 0.1; 0.1 LHON N/A N/A
LHON2 M 11 11 y Reduced vision 0.2; 0.15 LHON N/A N/A
LHON3 M 18 18 y Reduced vision 0.1; 0.2 LHON N/A N/A
LHON4 M 15 15 y Reduced vision 0.1; 0.1 LHON N/A N/A

N/A. Not available.

* Searching nystagmus present in all patients in this family.

**FIGURE 3.** Electroretinogram recording of four individuals with the 208delG mutation. Individual family A-IV4, affected with LCA, had no appreciable rod and cone responses. The other three were unaffected family members from families A and C, who had normal rod and cone responses.
identified in the FSCN2 gene of patients with retinal degeneration.\textsuperscript{3,4,9,10} It is unusual that a gene is responsible for disease in one ethnic group but not in many others, if a reasonable number of cases have been studied. It is highly unusual that the same mutation can cause both rod–cone and cone–rod retinal degeneration, although different mutations in the same gene have been reported to cause both types of retinal degeneration. In this case, careful and extensive re-evaluation of a larger number of control subjects and unaffected family members is of the first priority. It is almost impossible to claim a disease-causing mutation if it is equally distributed in normal individuals and in patients. Our results indicate that the 208delG mutation was not associated with RP, CORD, and LCA in the Chinese population studied. Further studies in other populations are needed to clarify the different findings in Japanese and Chinese populations. If our result is supported by further studies, it is advised that care be taken in correlating a mutation with a disease until confirmed by multiple findings.

\textbf{Acknowledgments}

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\textbf{References}


