Mutation analysis of congenital cataract in a Chinese family identified a novel missense mutation in the connexin 46 gene (GJA3)

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Purpose: To identify the genetic defects in a three-generation Chinese family with congenital nuclear cataract.

Methods: Four patients and three healthy members from the family underwent complete physical and ophthalmic examinations. Genomic DNA was extracted from peripheral blood leukocytes of the family members as well as from 100 healthy normal controls. Polymerase chain reaction (PCR) amplification and direct sequencing of all coding exons of candidate genes were performed. The functional consequences of the mutation were analyzed with biology softwares.

Results: A novel mutation (c.130G>A) was identified in the connexin 46 gene (GJA3), which resulted in the substitution of valine by methionine at the highly conserved codon 44 of connexin 46. This mutation co-segregated among the affected members of the family and was not observed in either unaffected members or the 100 normal controls.

Conclusions: This is a novel missense mutation identified in the first extracellular loop of connexin 46; this expands the mutation spectrum of GJA3 in association with congenital cataract.

Congenital cataract is a significant cause of poor vision or blindness in children worldwide and is responsible for 10.7%–14.0% of the children who are blind [1]. It is a clinically and genetically heterogeneous lens disorder, with autosomal dominant inheritance being most common. Currently, more than 22 genes have been identified to be associated with various forms of congenital cataract, including ten crystalline genes (CRYAA [2], CRYAB [3], CRYBA1/A3 [4], CRYBA4 [5], CRYBB1 [6], CRYBB2 [7], CRYBB3 [8], CRYGC [9], CRYGD [10], and CRYGS [11]), three transcription factor genes (HSF4 [12], PITX3 [13], and MAF [14]), two cytoskeletal protein genes (BFSP1 [15] and BFSP2 [16]), four membrane transport protein genes (MIP [17], GJA8 [18], GJA3 [19], and LIM2 [20]), glucosaminyl (N-acetyl) transferase 2 (GCNT2) [21], chromatin-modifying protein-4B (CHMP4B) [22], and transmembrane protein 114 (TMEM114) [23]. Knowledge of the structure and function of these candidate genes as well as the pathophysiological effect of their disease-associated mutations on their functions will aid in understanding the mechanisms of cataractogenesis.

Here, we report a heterozygous 130G>A transition in the connexin 46 gene (GJA3) associated with congenital nuclear cataract in a Chinese family, while it co-segregated completely with the disease phenotype. This is a novel mutation and has not been reported previously with congenital cataract.

METHODS

Clinical data and sample collection: A three-generation Chinese Han family (Figure 1) with congenital nuclear cataract was recruited from the Second Affiliated Hospital of Harbin Medical University, Harbin, China. Seven members of the pedigree were involved in this study, including four affected individuals (II:3, II:5, III:2, and III:3) and three unaffected ones (II:4, II:6, and III:4). All participants underwent full physical and ophthalmic examinations. Phenotype was documented by slit-lamp photography (Figure 2). One hundred subjects without diagnostic features of congenital cataract were recruited from the Chinese Han
population to serve as normal controls. After informed consent, 5 ml venous blood from family members and controls was collected in a BD Vacutainer (BD, San Jose, CA) containing EDTA. Genomic DNA was extracted by QIAamp DNA Blood Mini Kits (QIAGEN Science, Germantown, MD). The research was approved by the Institutional Review Board of Harbin Medical University and followed the clauses of the Declaration of Helsinki.

**Mutation detection:** All coding exons and their flanking regions of the known candidate genes associated with autosomal dominant congenital nuclear cataract, such as CRYAA, CRYAB, CRYBA1, CRYBB2, CRYGC, CRYGD, CRYGS, GJA3, and GJA8, were amplified by PCR with primers listed in Table 1. The PCR products were sequenced from both directions with the ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA). The sequencing results were analyzed using Chromas (version 2.3) and compared with the reference sequences in the NCBI database.

**Bioinformatics analysis:** The wild-type and mutant connexin 46 (Cx46) protein sequences were analyzed with computer programs.
assistance for better understanding the effects of the mutation on its biochemical properties. We used PolyPhen (polymorphism phenotyping), which is based on the position-
specific independent counts score derived from multiple
sequence alignments of observations [24], to predict whether
the amino acid substitution affects protein function. An online
bio-software program Misc Protein Analysis was used to
compute the hydrophilicity of the wild-type and mutant Cx46.

RESULTS

Clinical data: There were five affected people in 13 members
of this family (Figure 1). The proband (III:2) was a 5-year-old
girl whose grandmother (I:1), mother (II:3), aunt (II:5), and
male cousin (III:3) also had poor vision in their childhood.
Among them, one (I:1) passed away and two (II:3, II:5) had
had cataract extractions before examination. The other
subjects had no operations and showed bilateral cataract
characterized as a central nuclear opacity involving
embryonic and fetal nucleus with punctate cortical opacities
(Figure 2). There was no history of other ocular or systemic
abnormalities in the family. To date, all of the affected
individuals have had cataract surgery.

Mutation analysis: Direct sequencing of candidate genes
revealed a heterozygous G>A transition in GJA3 at position
130 that led to the replacement of the highly conserved valine
with methionine at codon 44 (Figure 3). This mutation was
detected in all affected members but was not observed in either
the unaffected family members or the normal controls. There
was no noticeable nucleotide polymorphism in other
candidate genes.

Bioinformatics analysis: With PolyPhen, substitution in Cx46
at position 44 from V to M scored 1.654 and was confidently
predicted to be “possibly damaging.” The obvious decrease in
hydrophilicity in the mutant form is shown in Figure 4.

DISCUSSION

The GJA3 gene, coding a 435-amino acid protein, was first
reported by Willecke et al. [25] in 1990 and is located on

Figure 2. Slit-lamp photograph of the proband. Slit-lamp
examination of the proband (III:2) showed a central nuclear cataract
involving embryonic and fetal nucleus with punctate cortical
opacities.

Figure 3. Mutation analysis of the connexin 46 gene (GJA3). The
sequence chromatogram (forward strand) shows a heterozygous
G>A transition that changes valine to methionine at codon 44. The
red arrows show the wild-type (normal) and mutant point,
respectively.

Figure 4. The hydrophilicity of the wild-type and mutant connexin
46 (Cx46). The x-axis represents the position of amino acids. The y-
axis represents the hydrophilicity value in a default window size of
nine. The regions of interest are marked by black circles. The
decrease in hydrophilicity in the mutant form is evident.
| Mutation | Amino acid change | Location | Cataract type | Family origin | Reference |
|----------|------------------|----------|---------------|---------------|-----------|
| c.7G>T   | p.D3Y            | NH2-terminus | Zonal pulverulent | Hispanic Central American | [35]       |
| c.32T>C  | p.L11S           | NH2-terminus | Ant-egg        | Danish        | [36]      |
| c.82G>A  | p.V28M           | First transmembrane domain (M1) | Variable | Indian | [37] |
| c.96C>A  | p.F32L           | First transmembrane domain (M1) | Nuclear pulverulent | Chinese | [38] |
| c.98G>T  | p.R33L           | First transmembrane domain (M1) | Embryonal nuclear granular | Indian | [39] |
| c.130G>A | p.V44M           | First extracellular loop (E1) | Nuclear | Chinese | Present study |
| c.134G>C | p.W45S           | First extracellular loop (E1) | Bilateral nuclear | Chinese | [40] |
| c.176C>T | p.P59L           | First extracellular loop (E1) | Nuclear punctate | American | [41] |
| c.188A>G | p.N63S           | First extracellular loop (E1) | Zonal pulverulent | Caucasian | [18] |
| c.226C>G | p.R76G           | First extracellular loop (E1) | Total | Indian | [37] |
| c.227G>A | p.R76H           | First extracellular loop (E1) | Nuclear pulverulent | Australian | [42] |
| c.269C>T | p.T87M           | Second transmembrane domain (M2) | Pearl box | Indian | [43] |
| c.560C>T | p.P187L          | Second extracellular loop (E2) | Zonal pulverulent | Caucasian | [44] |
| c.563A>C | p.N188T          | Second extracellular loop (E2) | Nuclear pulverulent | Chinese | [45] |
| c.1137insC | p.S380fs       | COOH-terminus | Zonal pulverulent | Caucasian | [18] |

Summary of the mutations identified in *GJA3* provide the different congenital cataract phenotypes with different families belonging to different ethnic groups. Most of these mutations are in accord with autosomal dominant, and the cataract phenotypes are nuclear pulverulent types.
chromosome 13q11. Cx46, which is encoded by GJA3, is mainly expressed in lens fiber cells. Like other connexins, Cx46 has four transmembrane domains (M1, M2, M3, and M4), two extracellular loops (E1 and E2), an intracellular loop (CL), and intracellular NH2 and COOH termini. Cx46 functions as a gap junction that mediates the intercellular transport of small molecules (<1 kDa), including ions, metabolites, and second messengers between elongated fiber cells [26]. Since the lens is an avascular structure and lens fiber cells lose all intracellular organelles during development, the fiber cells are highly dependent on intercellular communication for their survival [27]. The intercellular communication network is formed mainly by the gap junctions. This extensive network is vital since it maintains osmotic and metabolic homeostasis in lens fiber cells and ultimately maintains lens transparency [28].

However, extracellular domains of connexins that contain two extracellular loops (E1 and E2) play a key role in both mediating hemichannel docking [29,30] and regulating voltage gating of the channel [31]. The two extracellular loops are the most conserved domains among connexins and are the sites that provide the strong interaction between the two hemichannels that enable the formation of an intercellular channel with no leakage of current and molecules to the extracellular environment [32]. Furthermore, the first extracellular loop (E1) has been proven to be a major determinant of charge selectivity in Cx46 channels [33].

In this study we identified a new mutation (130G>A) in GJA3. This variation seems to be disease causative as it segregated with the phenotype and was absent in both unaffected pedigrees and the 100 unrelated controls from a similar ethnic background. This substitution resulted in the replacement of valine to methionine at codon 44 (V44M), localized in the first extracellular loop (E1) of Cx46. A multiple amino acid sequence alignment showed that valine at position 44 is phylogenetically conserved in different species and gap junctions (Figure 5), and Polyphen predicted the mutation to be possibly damaging. These results suggest that valine may be functionally important and the mutation may lead to damaging interference with conformation and function of Cx46. The decline of hydrophilicity in the mutant (Figure 4) might alter the charge on the surface of the extracellular loop, thereby affecting hemichannel docking [34]. The mutation may also affect the charge selectivity in Cx46 channels, disturbing the charge balance inside the lens fiber cells [33]. These changes would disorder intercellular homeostasis in the lens fiber cells and result in lens nucleus opacity.

To date, 15 mutations in GJA3 have been reported to be associated with congenital cataract in humans (Table 2) [35-45]. Most of these are described as nuclear or zonular pulverulent types and share genotype–phenotype similarities to some extent. In this study the phenotype also shows a conspicuous nuclear cataract but one that is surrounded with punctate opacities. The difference in the cataract phenotypes associated with GJA3 may be attributed to the action of modifier genes or environmental factors that could affect the expression of GJA3 and thus resulting cataract types.

In summary, we described a novel missense mutation (V44M) in GJA3 that causes congenital cataract in a three-generation Chinese family. This study further confirms that Cx46 plays a vital role in the maintenance of human lens transparency and expands the mutation spectrum of GJA3 in association with congenital cataract.

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