A novel mutation in GJA3 (connexin46) for autosomal dominant congenital nuclear pulverulent cataract

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Purpose: Autosomal dominant congenital cataract (ADCC) is a clinically and genetically heterogeneous lens disorder that usually presents as a sight-threatening trait in childhood. Here, we described a six-generation Chinese family presenting with morphologically homogeneous “nuclear pulverulent” cataracts.

Methods: A whole genome scan was performed with polymorphic microsatellites in the Human MapPairs marker set, with special attention paid to known ADCC loci. Then we screened for mutations by direct sequencing.

Results: A significantly positive two-point LOD score was obtained at marker D13S175 (Zmax = 7.83, θ = 0). Haplotype analysis indicated this disease was located at 13q11, close to GJA3. Upon screening for mutations in GJA3 in this family, we found a novel transition mutation (TTC → TTA) resulting in a Phenylalanine-Leucine substitution at the highly conserved codon 32 of the GJA3 protein. This mutation segregated with the affected members of the family.

Conclusions: This finding is the first report of a mutation in the first transmembrane region of GJA3. Our study further confirmed that GJA3 plays a vital role in the maintenance of human lens transparency.

Congenital or infantile cataract causes 10-30% of all blindness in children, with one third of cases estimated to have a genetic cause [1]. Currently, more than twelve independent loci for autosomal dominant congenital cataract (ADCC) have been mapped on human chromosomes 1p, 1q, 2q, 3q, 11q, 13q, 16q, 17p, 17q11-q12, 17q24, 20p, and 22q [2-15]. Those loci for which mutations in candidate genes have been identified reveal that the biological causes of congenital cataract can be partly attributed to mutations in crystallin genes. There are, however, recent reports that implicate homeostatic and development genes as the underlying basis of cataractgenesis within a proportion of congenital cataract families [16]. Dominant mutations have been revealed in HSF4 (16q21-q22), which encodes heat-shock transcription factor 4 [17]; MIP, which encodes the major intrinsic protein of the lens [18], and a lens-specific gap-junction protein (GJA8) that mediates intercellular trafficking and communication [19], and a human homeobox gene PITX3 [20]. Indeed, two mutations in the GJA3 (connexin46) gene mapped on 13q11-1q3 were firstly identified in 1999 in two zonular pulverulent cataract families [21]. Ress et al. [16] later found another mutation in GJA3 segregating with in a four-generation caucasian family. Using polymorphic markers scanning the whole human genome, including the markers near all known autosomal dominant congenital cataract loci, we mapped a congenital nuclear pulverulent cataract locus to 13q11, close to the GJA3 gene. Subsequently, we screened this family for base changes by direct sequencing of the GJA3 gene. A novel mutation in GJA3 was detected in this family.

METHODS

Clinical data and sample collection: All participating family members received careful clinical examination, including tests of visual function such as Snellen visual acuity and corrected visual acuity, in addition to slit-lamp and fundus examinations with the dilated pupil. After informed consent was obtained from all participating individuals, venous blood samples were collected for genomic DNA extraction using the QIAamp Blood kit.

Genotyping and linkage analysis: Genome-wide screening was performed using 366 fluorescent microsatellite markers from autosomes, at a resolution of approximately 10 cM (Human MapPairs Version 9, Research Genetics). The fine mapping primer sequences were obtained from the Genome Database. The primers are M13 tailed. The M13 primers were labeled IRD700 or IRD800. In the linkage analysis, we modeled the disease as an autosomal dominant inheritance with complete penetrance, setting the affected allele frequency to 0.00001 and the marker allele frequencies to be uniformly distributed. We carried out two-point linkage analysis using the MLINK program from the LINKAGE v. 5.10 software package [22]. We carried out pedigree and haplotype construction using Cyrillic v. 2.02 software.
Sequencing of the GJA3 gene: To detect base changes in GJA3, we designed 4 pairs of primers to sequence the entire exon of GJA3 from both directions using an ABI3100 sequencer. The primer sequences were based on the genomic sequence available in the Genebank (NT_009799). All affected and unaffected members of this family and at least 250 unrelated control individuals were examined for GJA3 gene mutations.

RESULTS
Clinical findings: In affected members of this family, the lens opacity was characterized as pulverulent cataract (Figure 1). The “punctate” opacities were located in the central zone (2 mm) of the lens, which is limited to the embryonic nucleus. Among affected members, the visual activity of the affected eye varies from 0.1 to 1.2 and associated with the degree of lens opacity.

Linkage and haplotype analysis: We performed a whole genome scan using Human MapPairs polymorphic markers, especially investigating all regions of known ADCC susceptibility. As a result, positive two-point lod scores were obtained at five markers within the 13q11-q13 interval (Table 1). Two-point linkage analysis revealed a maximum lod score of 7.83 at D13S175 with a recombination fraction (θ) equal to 0.00. Haplotype analysis (Figure 2) indicates this cataract family was mapped to 13q, consistent with the findings of Mackay et al. [9].

<table>
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<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Zmax</th>
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<td>0.89</td>
<td>0.81</td>
<td>0.7</td>
<td>0.49</td>
<td>0.3</td>
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<tr>
<td>D13S1120</td>
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<td>0.58</td>
<td>0.54</td>
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<td>D13S175</td>
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<td>7.69</td>
<td>7.11</td>
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<td>0.94</td>
<td>0.7</td>
<td>0.94</td>
<td>0.2</td>
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Two-point lod scores for linkage in chromosome 13 markers. Genome-wide screening with 366 fluorescent microsatellite markers mapped the nuclear pulverulent cataract locus to chromosome 13q11. The maximum two-point lod score was achieved at D13S1275 at θ=0. Zmax is the maximum lod score achieved.

Figure 1. Slit-lamp photograph of individual V:7 of this six-generation Chinese family. Slit-lamp photograph of individual V:7 of this six-generation Chinese family. Slit-lamp photograph showed punctate opacities located in the in the central zone of the lens.

Figure 2. Family structure and haplotype analysis of the six-generation Chinese family. Pedigree and haplotype analysis of this six-generation Chinese family show segregation five microsatellite markers on chromosome 13, listed in descending order from the centromeric end. Squares and circles symbolize males and females, respectively. Unblackened and blackened symbols denote unaffected and affected individuals, respectively.
Mutation analysis: By direct sequencing of GJA3 with four pairs of primers (Table 2), we found a novel base change in this six-generation Chinese family. All affected members carried a TTC to TTA (96C>A) transversion (NM_021954). This missense mutation caused an F32L substitution in GJA3 (Figure 3A).

Our mutation analysis showed a complete segregation of the GJA3 mutations with the affected members of this family. This mutation was not observed in any unaffected members of this family, and also not found in the 300 unrelated normal controls.

Multiple-sequence alignment: Using the NCBI and Incyte websites, we obtained multiple-sequence alignment of the connexin family proteins in various species, including *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus* (Figure 3B).

We found the mutation (F32L) in codon 32 located in a highly conserved region. Furthermore, we used the bioinformatics software Peptool Lite to predict possible secondary structure changes induced in GJA3 by the missense mutation. This mutation (F32L) in the family was predicted to transform the surrounding sequence from a β-strand into an α-helix.

**DISCUSSION**

The connexin gene family encodes gap-junction channel proteins that mediate the intercellular transport of small biomolecules (<1 kDa) including ions, metabolites, and second messengers in diverse vertebrate cell types, including cochlea cells, Schwann cells, epidermal cells, and lens fiber cells [23-26].

In mice, targeted deletion of the GJA3 or GJA8 gene produces lens opacity and targeted replacement of GJA8 with GJA3 by genetic knock-in can prevent these cataracts [27]. Mutations of GJA3 may induce the appearance of a cleaved form of γ-crystallin. The cleavage of γ-crystallin may result in a change in the normal 3-D conformational structure of γ-crystallin, leading to abnormal interactions with other lens proteins. [28] Furthermore, knock out of the GJA3 gene in different mouse strains leads a various degree of cataract depending on genetic background [27], consistent with the clinical finding in this Chinese family.

Recently, Mackay et al., Shiels et al., and Ress et al. have demonstrated that dominant mutations in the genes for GJA3

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**Table 2. Primer pairs used for sequencing**

<table>
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<tr>
<th>Name</th>
<th>Strand</th>
<th>Sequence</th>
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<td>GJA3 EXON1A</td>
<td>Forward</td>
<td>5'-CTGCGATGCTGTCCCTGTTG-3'</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>5'-TGTCTCTGCGGGTGGCTCTT-3'</td>
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<tr>
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<td>5'-CAAGGGGCGCTGGTGCATCT-3'</td>
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<tr>
<td>GJA3 EXON1D</td>
<td>Forward</td>
<td>5'-CCCAGGCCCTCAAGGCTTAC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-AACCTCTGTCCGCGCAACC-3'</td>
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</tbody>
</table>

Primer pairs used for sequencing. GJA3 gene contains only one exon. For mutation analysis, four pairs of primers were designed covering the entire exon. The genomic DNA PCR fragments, which are overlapped, were then sequenced using ABI3100 sequencer.

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Figure 3. DNA sequence chromatograms and multiple protein sequences alignment. A: The DNA sequence chromatograms of the unaffected members and affected members in an autosomal dominant congenital nuclear pulverulent cataract family. B: Multiple-sequence alignment in the connexin family and connexins of different species.
and GJA8 are associated with “Zonular pulverulent” cataracts in human [9,16,19,21]. They found two missence mutations of GJA3 in the extracellular domain and a frameshift mutation of GJA3 in the intracellular domain [16,21]. Subsequently, mutations of the βB1- and γC-crystallin genes were found to result in autosomal dominant “Zonular pulverulent” cataracts [29,30]. In our study, a novel mutation (F32L) was detected in connexin 46 (GJA3) in a six-generation Chinese family. Sequence comparison of GJA3 from various species shows Phenylalanine and Arginine in codon 32 and 33 of GJA3. These amino acids were located in the first transmembrane region and were strictly conserved in the connexin family from various species such as Mus musculus, Rattus norvegicus, etc. This indicates that Phe32 and Arg33 may play a key role in connexin function. A mutates Arginine in GJB2 or GJB3 has been shown to cause deafness [23,31]. Until now, no mutation of Phenylalanine was detected in the connexin family. Our finding is the first report of a mutated phenylalanine in the connexin family and is also the first report that a mutation is located in the first transmembrane region of GJA3. In summary, our data further confirmed that GJA3 is important in the maintenance of optical clarity.

ACKNOWLEDGEMENTS

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REFERENCES


