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Mutation of the gap junction protein alpha 8 (GJA8) gene causes autosomal recessive cataract

Surya Prakash G Ponnam, Kekunnaya Ramesha, Sushma Tejwani, Balasubramanya Ramamurthy, Chitra Kannabiran

MATERIALS AND METHODS

The study protocol was approved by the institutional review board of the L. V. Prasad Eye Institute and followed the tenets of the Declaration of Helsinki. A family of southern Indian origin was recruited for the study. The proband and five available family members underwent a complete ophthalmologic evaluation and blood samples were obtained after informed consent. Diagnosis of hereditary cataract was based on the presence of a bilateral, familial, lenticular opacity of any size, even at birth or during the first decade of life, respectively. As these opacities can cause blurring of the vision during form vision development, they are clinically very important. Cataracts may account for about one-tenth of total childhood blindness in South India and hereditary cataracts account for about one-fifth of childhood cataracts in this region. The majority of hereditary cataracts that have been genetically characterised to date are of autosomal dominant inheritance. Mutations in six genes (CRYAA, LIM2, GCNT2, HSF4, CRYBB3, and BFSP1) have been associated with the autosomal recessive cataracts.

RESULTS AND DISCUSSION

The proband (IV: 1 in fig 1) presented at our institution at 12 years of age. She had a history of poor vision, white opacities in both eyes and nystagmus since birth. She had undergone cataract surgery elsewhere, and had an unaided visual acuity in both eyes of 1 metre. Her brother (IV: 2 in fig 1), who was similarly affected, had a history of decreased vision since birth and on examination, had total cataracts, nystagmus and a visual acuity in both eyes of counting fingers. The pedigree obtained upon examination of all available members of the family was suggestive of autosomal recessive inheritance (fig 1).

We employed a candidate gene approach consisting of SSCP-based screening and sequencing. We screened eight genes including six crystallin genes and two connexin genes to identify mutations. Screening of the coding regions of GJA8 revealed a single base insertion causing a frameshift at codon 203 (c.670insA; p.Thr203AsnfsX47; shown in fig 2) that was

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Primer sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJA8-2A(F)</td>
<td>CGCGTACAGAAAAACAGA</td>
</tr>
<tr>
<td>GJA8-2A(R)</td>
<td>TCGTACAGACGTCGCTCG</td>
</tr>
<tr>
<td>GJA8-2B(F)</td>
<td>GGAGGCAATCCGCACCT</td>
</tr>
<tr>
<td>GJA8-2B(R)</td>
<td>CGACCCGAAACTTCAG</td>
</tr>
<tr>
<td>GJA8-2C(F)</td>
<td>ACCAGGCGACGCTCAA</td>
</tr>
<tr>
<td>GJA8-2C(R)</td>
<td>CAGAGGCCACAGACAA</td>
</tr>
<tr>
<td>GJA8-2D(F)</td>
<td>TCGTCAAGGCGGAAAGAG</td>
</tr>
<tr>
<td>GJA8-2D(R)</td>
<td>TCGTCAAGGCGGAAAGAG</td>
</tr>
<tr>
<td>GJA8-2E(F)</td>
<td>CGAAGGACACGCTCATCT</td>
</tr>
<tr>
<td>GJA8-2E(R)</td>
<td>CGAAGGACACGCTCATCT</td>
</tr>
<tr>
<td>GJA8-2F(F)</td>
<td>TCAGGGCGAGGAAAGATCA</td>
</tr>
<tr>
<td>GJA8-2F(R)</td>
<td>TTTCCACCTCATCTAC1</td>
</tr>
<tr>
<td>GJA8-2G(F)</td>
<td>GGAGGCGGAAAGAGTG</td>
</tr>
<tr>
<td>GJA8-2G(R)</td>
<td>TTTCCACCTCATCTAC1</td>
</tr>
</tbody>
</table>

Primers used for amplification of the coding regions of GJA8 within the exon 2 are listed above. F and R in parentheses refer to forward and reverse primers respectively.

Abbreviation: SSCP, single-strand conformation polymorphism
homozygous in the two affected members IV:1 and IV:2 (fig 1), and heterozygous in the parents (III:4 and III:7 in fig 1), sibling (IV:3 in fig 1) and a second-degree relative of the proband (II:9 in fig 1), all of whom were unaffected. This change was not found in 75 unrelated controls. The mutation is predicted to result in a frameshift at codon 203 with a stop codon after 46 amino acids of altered reading frame, producing a truncated protein consisting of 248 amino acid residues (fig 3).

GJA8 encodes the gap junction protein connexin 50 (Cx50), which is one of the major lens connexins along with connexins 43 (locus GJA1) and 46 (locus GJA3). Connexins 50 and 46 are expressed in differentiating lens fibres and persist in mature fibres, and connexin 43 is expressed in lens epithelial cells. Connexins form intercellular channels consisting of two halves or hemichannels, the connexons, each made up of six connexin monomers. Mutations in GJA3 and GJA8 are known to result in autosomal dominant cataract. Eight different mutations have been reported in the GJA8 gene (table 2), all of which are missense changes.

The insertion described here is located in codon 203, which is predicted to be in the second extracellular domain of connexin 50; a frameshift at this position would be expected to lead to the disruption of the C-terminal half of the protein (amino acids 203–433) and thereby produce a functionally null allele. Possible consequences could be instability or non-functionality of the mutant protein, or degradation of the mRNA through the nonsense-mediated decay pathway. A mechanism of disease involving loss of function at connexin loci has also been suggested in mouse models of recessive cataract. GJA3 or GJA8 homozygous knockout mice are reported to have a cataractous phenotype, whereas heterozygous knockout mice (GJA3+/−, GJA8+/−) have normal lenses.15 16

DeRosa et al.17 studied the properties of Cx50 proteins with C-terminal truncations at residue 290 that correspond to physiological truncations occurring during lens maturation. Such truncated Cx50 proteins were found to be expressed and localised to the cell membrane effectively when transiently expressed in HeLa cells.17 Interestingly, they also retained the ability to form channels, but had significantly impaired conductance compared with wild-type connexin 50.17 Truncation at residue 290 would be expected to result in loss of the C-terminal cytoplasmic domain of the protein (residues 228–433),18 with all putative transmembrane domains intact.

In comparison, the mutation identified in the present study would be predicted to result in the loss of the second extracellular domain and the subsequent transmembrane and cytoplasmic domains. Heterologous expression in cell lines and...
in *Xenopus* oocytes would be required to determine the level of inactivation of the protein.

Studies on various mutant connexin proteins causing dominant cataract in humans and mice, have suggested varied mechanisms of action. Dominant negative effects have been proposed for the *GJA8* mutant proteins Pro88Ser and Pro88Gln based on studies in *Xenopus* oocytes. Studies on the effect of the *GJA8* mutation Gly22Arg (found in Lop10 mice), in mouse lenses also revealed dominant negative effects. In that study, the mutant proteins were found to interfere with the formation of gap-junction channels. In contrast, other mutants of both *GJA3* and *GJA8*, when tested in *Xenopus* oocytes, have been observed to result in loss of function without any dominant negative effects. These are the *GJA8* mutant Asp47Ala (D47A) in the *GJA8* knockout mice, suggesting that it is required for proper growth and development of the eye. This study adds to the range of phenotypes associated with *GJA8* mutations and to our knowledge, describes the first mutation in this gene to be associated with autosomal recessive inheritance.

**Table 2**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg23Thr</td>
<td>Progressive congenital nuclear</td>
<td></td>
</tr>
<tr>
<td>Val44Glu</td>
<td>Congenital or developmental cataract with microcornea</td>
<td></td>
</tr>
<tr>
<td>Glu48Lys</td>
<td>Zonular nuclear pulverulent</td>
<td></td>
</tr>
<tr>
<td>Pro88Ser</td>
<td>Zonular pulverulent</td>
<td></td>
</tr>
<tr>
<td>Pro88Gln</td>
<td>Lamellar pulverulent</td>
<td></td>
</tr>
<tr>
<td>Val79Glu</td>
<td>‘Full moon’ with Y-sutural opacity</td>
<td></td>
</tr>
<tr>
<td>Arg198Glu</td>
<td>Congenital or developmental cataract with microcornea</td>
<td></td>
</tr>
<tr>
<td>ile247Met</td>
<td>Zonular pulverulent</td>
<td></td>
</tr>
</tbody>
</table>

**KEY POINTS**

- Candidate gene analysis on an Indian family with autosomal recessive cataract showed an insertion (c.670insA) in *GJA8* that segregated with disease in the family and was consistent with recessive inheritance.
- The mutation is predicted to lead to a frameshift at codon 203 of *GJA8*/connexin 50 with termination after 46 amino acids, giving rise to a protein of 248 residues.
- This study is the first to demonstrate the involvement of connexin 50 in recessive cataract.

**ELECTRONIC DATABASE INFORMATION**

- Ensemble database: [http://www.ensembl.org](http://www.ensembl.org)

**ACKNOWLEDGEMENTS**

We thank all the patients and their family members for their consent to participate in the project. Thanks are also due to Drs Archana Bhargava and Sheik Fazal Hussain for systemic evaluation of patients and Dr Ravi Thomas for his valuable suggestions. This study was supported by Hyderabad Eye Research Foundation. S. P. G. Ponnam was supported by junior research fellowships from the ICMR and CSIR, Government of India.

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**Figure 3** Sequences of wild type and mutant *GJA8* proteins. Partial protein sequences of the wild type *GJA8/Cx50* (wt) and predicted sequence of the insertion mutant (mut) (c.670insA, pThr203AsnX47) are shown. The residue (position 203) at the start of the frameshift is boxed. The mutant protein terminates at 248 amino acids. Residues are numbered with respect to the wild-type Cx50 sequence.

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**Table 2** *Cx50 (GJA8)* mutations reported in human cataracts

- **Mutation**: Arg23Thr, Val44Glu, Glu48Lys, Pro88Ser, Pro88Gln, Val79Glu, Arg198Glu, Ile247Met
- **Phenotype**: Progressive congenital nuclear, Congenital or developmental cataract with microcornea, Zonular nuclear pulverulent, Zonular pulverulent, Lamellar pulverulent, ‘Full moon’ with Y-sutural opacity, Congenital or developmental cataract with microcornea, Zonular pulverulent
- **Reference**: Further details are available in the reference list at the end of the document.

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**Figure 3** Sequences of wild type and mutant *GJA8* proteins. Partial protein sequences of the wild type *GJA8/Cx50* (wt) and predicted sequence of the insertion mutant (mut) (c.670insA, pThr203AsnX47) are shown. The residue (position 203) at the start of the frameshift is boxed. The mutant protein terminates at 248 amino acids. Residues are numbered with respect to the wild-type Cx50 sequence.
REFERENCES


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