ONLINE MUTATION REPORT

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

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Background: *GJA8* encodes connexin-50, a gap junction protein in the eye lens. Mutations in *GJA8* have been reported in families with autosomal dominant cataract.
Objective: To identify the disease gene in a family with congenital cataract of autosomal recessive inheritance.
Methods: Eight candidate genes were screened for pathogenic alterations in affected and unaffected family members and in normal unrelated controls.
Results: A single base insertion leading to frameshift at codon 203 of connexin 50 was found to co-segregate with disease in the family.
Conclusions: These results confirm involvement of *GJA8* in autosomal recessive cataract.

Cataract is defined as any opacity of the lens resulting in partial or total loss of transparency. Hereditary cataracts are clinically and genetically heterogeneous, often presenting as congenital or developmental cataracts that arise 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 Southern India[1] and hereditary cataracts account for about one-fifth of childhood cataracts in this region.[2] The majority of hereditary cataracts that have been genetically characterised to date are of autosomal dominant inheritance.[3] Mutations in six genes (*CRYAA*,[4] *LIM2*,[5] *GCNT2*,[6] *HSF4*,[7] *CRYBB3*,[8] and *BFSP1*[9]) have been associated with the autosomal recessive cataracts.

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 ophthalmic 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 of congenital or developmental type (based on history/examination and age of onset <16 years) as evaluated independently by two examiners. Patients with a history of trauma, or having unilateral (nonfamilial) cataract, co-existing ocular disease, mental retardation, microcephaly, cerebral palsy, systemic syndromes, or a maternal history of intrauterine infections or antenatal steroid use were excluded.

Genomic DNA was isolated from peripheral blood leukocytes using standard protocols. The gene sequence for *GJA8* was retrieved from the Ensembl database (ENSG00000121634). Primers were designed using the Primer 3 software (http://frodo.wi.mit.edu) for PCR amplification of the coding region of *GJA8*, which is present in exon 2. Seven pairs of overlapping primers were used to obtain fragments of <300 bp in length for single-strand conformation polymorphism (SSCP) analysis (listed in table 1). PCR products were mixed with two volumes of formamide, denatured by heating at 90℃, snap-chilled and loaded onto 8% non-denaturing polyacrylamide gels with 5% glycerol. All samples were subjected to electrophoresis at 4℃ and at room temperature. Gels were fixed and subsequently stained with silver nitrate, and DNA visualised under visible light. Variants on the SSCP were subjected to bidirectional sequencing by automated methods. Screening for the observed mutations was performed on 75 ethnically matched unrelated normal controls by SSCP.

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 counting fingers at about 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 1 Primers used for amplification of *GJA8*

Name of primer	Primer sequence (5′ to 3′)
GJA8-2A(F)	CGCGTTAGCAAAAACAGA
GJA8-2A(R)	TCGTAGCAGACGTTCTCG
GJA8-2B(F)	GGATGAGCAATCCGACTT
GJA8-2B(R)	CCAGCCGGAACTTCTTAG
GJA8-2C(F)	ACCAGGGCAGCGTCAA
GJA8-2C(R)	CAGAGGCCACAGACAA
GJA8-2D(F)	CCACGGAGAAAACCATCT
GJA8-2D(R)	TCGGTCAAGGGGAAATAG
GJA8-2E(F)	CTGTCTCCTCCATCCAGAA
GJA8-2E(R)	CGTAGGAAGGCAGTGTCTC
GJA8-2F(F)	TCAGGTCGAGGAGAAGATCA
GJA8-2F(R)	TTTCACCCTCCTTATCCACT
GJA8-2G(F)	GGAGCAGGAGAAGGTG
GJA-2G(R)	TTCCTTTCATCTTGCC

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

Abbreviation: SSCP, single-strand conformation polymorphism

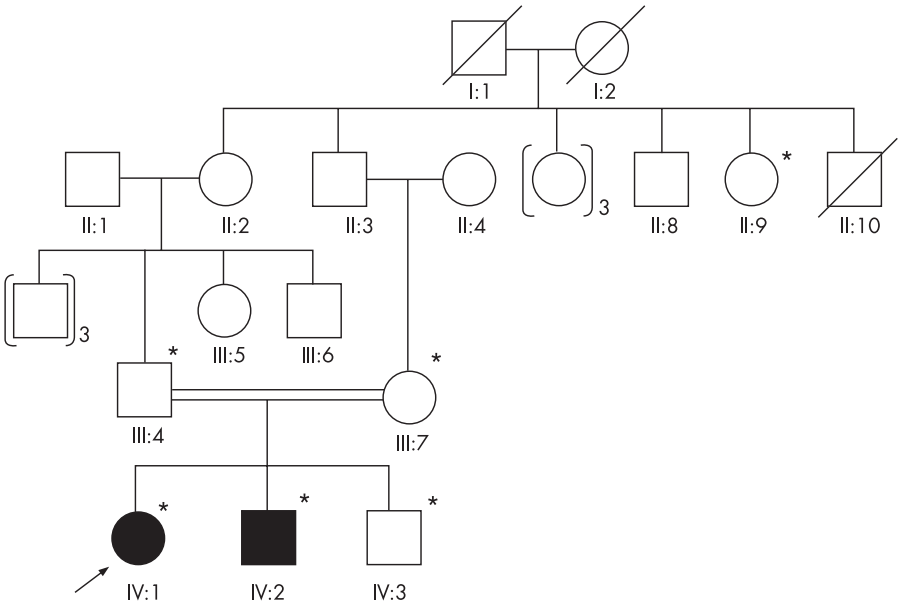

Figure 1 Pedigree of family with autosomal recessive cataract. The dark symbols indicate affected individuals and open symbols indicate unaffected individuals. Symbols marked with asterisks represent individuals who were analysed.

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).

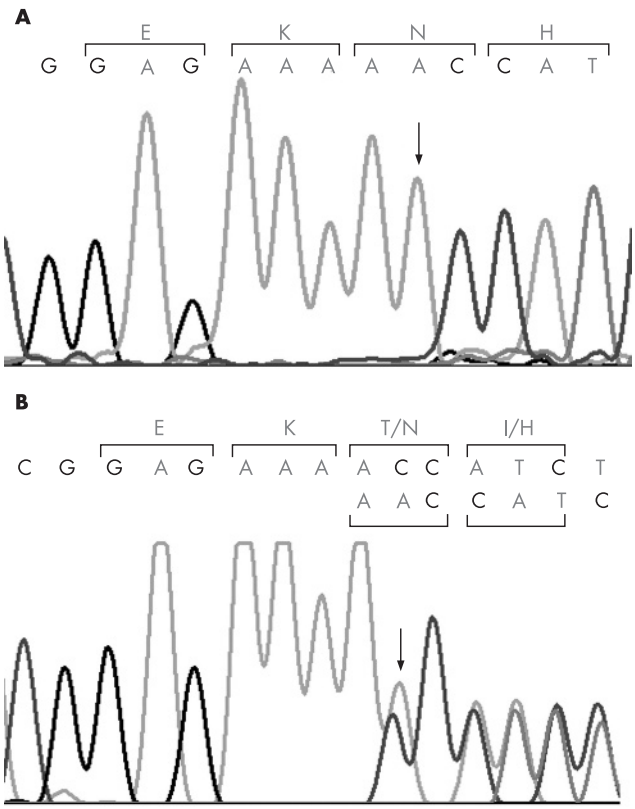

Figure 2 Sequence of the *GJA8* coding region. The sequence of *GJA8* showing the insertion (arrow) of an A at position 670 of the cDNA (c.670insA) (A) homozygous in the proband and (B) heterozygous in unaffected parent. Codons are marked by brackets and amino acids indicated above. Codons and amino acids for both wild type and mutant alleles are shown in (B).

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.[10–14] 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

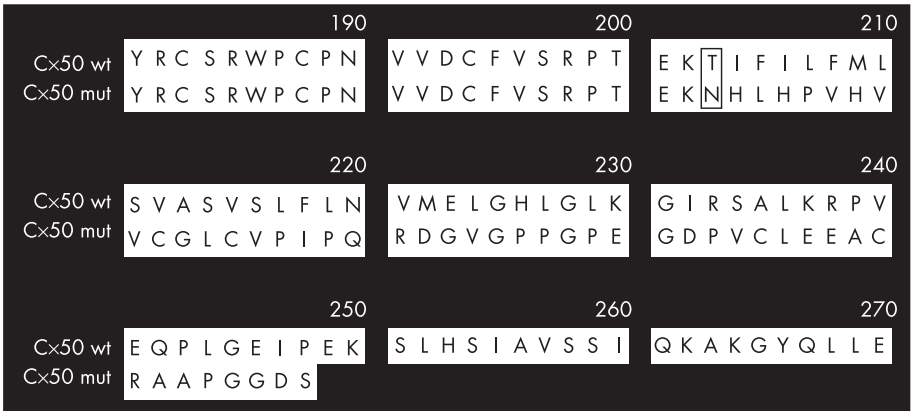

Figure 3 Sequences of wild type and mutant *GJA8* proteins. Partial protein sequences of the wild type *GJA8*/Cx50 (wt) and predicted sequence of the insertion mutant (mut) (c.670insA, p.Thr203AsnfsX47) are shown. The residue (position 203) at the start of the frameshift is boxed. The mutant protein terminates at 248 amino acids. Residues are numbered with respect to the wild-type Cx50 sequence.

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[19 20] and Pro88Gln[21] 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.[22] 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 *No2* mice[23] and two *GJA3* mutants Asn63Ser and fs380, causing cataract in humans.[24] Yet another mechanism of action suggested for the *GJA3* (Cx46) fs380 mutant,[25] upon expression in mammalian cells, is a gain of function, resulting in mislocalisation, caused by the frameshifted protein.[26] Overall, these observations do not point to any unifying mechanisms that may explain how specific connexin mutations could cause dominant versus recessive phenotypes. As has been suggested, interactions between connexin isoforms and the effects of connexins on other lens proteins may determine the phenotype.[16 27]

In the present study, the homozygous *GJA8* insertion mutation was associated with a severe phenotype in both affected siblings as indicated by the presence of opacities evident at birth, as well as nystagmus and amblyopia due to severe visual deprivation. One affected family member also had microcornea and microphthalmia, whereas her affected sibling was normal with respect to these parameters. Although it is not possible to conclude here as to whether the occurrence of microphthalmia is causally linked to deficiency of *GJA8*/Cx50, it is worth noting that microphthalmia was a feature of *GJA8* knockout mice, suggesting that it is required for proper growth and development of the eye.[16] 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 of cataract.

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

Mutation	Phenotype	Reference
Arg23Thr	Progressive congenital nuclear	28
Val44Glu	Congenital or developmental cataract with microcornea	29
Glu48Lys	Zonular nuclear pulverulent	30
Pro88Ser	Zonular pulverulent	19
Pro88Gln	Lamellar pulverulent	21
Val79Leu	''Full moon'' with Y-sutural opacity	31
Arg198Glu	Congenital or developmental cataract with microcornea	29
Ile247Met	Zonular pulverulent	32

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

- Ensembl database: http://www.ensembl.org

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Authors' affiliations

Surya Prakash G Ponnam, Chitra Kannabiran, Kallam Anji Reddy Molecular Genetics Laboratory, Brien Holden Eye Research Centre, L.V. Prasad Eye Institute, Hyderabad, India
Kekunnaya Ramesha, Sushma Tejwani, Balasubramanya Ramamurthy, Jasti V Ramanamma Children's Eye Care Centre, L.V. Prasad Eye Institute, Hyderabad, India

Correspondence to: Dr Chitra Kannabiran, Kallam Anji Reddy Molecular Genetics Laboratory, L.V. Prasad Eye Institute, Road No.2, Banjara Hills, Hyderabad 500 034, India; chitra@lvpei.org

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