ONLINE MUTATION REPORT

A novel mutation in the Connexin 46 gene causes autosomal dominant congenital cataract with incomplete penetrance

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Congenital or paediatric cataract is a phenotypically and genetically heterogeneous disorder consisting of lens opacities in early life. Thirteen genes have been described for autosomal dominant congenital cataract (ADCC). These include genes for seven members of the crystallin family,[1 2] which are responsible for the refractive index and transparency of the lens, two connexin genes[3 4] and major intrinsic protein of the lens (MIP)[5] which are involved in the transport directly between cells of small metabolites and water, respectively, the cytoskeletal protein beaded filament structural protein-2 (BFSP2),[6] and transcription factors paired-like homeodomain transcription factor-3 (PITX3)[7] and heat shock factor-4 (HSF4).[8] Five additional loci have been described on chromosomes 1pter-p36.1,[9] 15q21-q22,[10] 17p13,[11] 17q24,[12] and 20p12-q12.[13]

We used a linkage approach to investigate these 13 genes and five loci in a large pedigree from Victoria, Australia, with zonular pulverulent cataract with the aim of identifying the causative mutation.

Key points

- Congenital or paediatric cataract is a highly heterogeneous disorder with 13 known genes and at least five additional loci identified.
- A linkage approach was used to investigate a large pedigree from south-eastern Australia with a faint lamellar opacity surrounding a nuclear pulverulent cataract, with occasional fine needle-like cortical riders.
- Linkage to the Connexin46 locus was identified.
- An R76H mutation was identified in all affected individuals and not found in 100 control chromosomes.
- The mutation was also identified in six unaffected carriers, indicating reduced penetrance of the mutation.

METHODS

Ethics approval for this study was obtained from the Human Research Ethics Committees of the Royal Children's Hospital, Melbourne, Australia, the Royal Victorian Eye and Ear Hospital, Melbourne, Australia, and the University of Tasmania, Hobart, Australia.

Patient ascertainment and collection of genetic material

The pedigree crch13 was identified through a database maintained by the Royal Children's Hospital, Melbourne, Australia and the Royal Victorian Eye and Ear Hospital, Melbourne, comprising paediatric cataract patients from south-eastern Australia with any type of lens opacity.[14] Written informed consent was obtained from all participating individuals or their guardians. When possible, family members were examined by one or more ophthalmologists (MGW, DAM, JEE, JEC, or IR-E). Due to the rural location of most family members, affection status was determined from medical records when direct examination was not feasible. In many cases pre-operative visual acuity was not available. Buccal mucosal swabs were either collected during examination or by mailed kits and DNA extracted using the PureGene DNA Isolation Kit (Gentra Systems). Unaffected controls were ascertained from nursing homes for the elderly in Launceston, Tasmania, Australia, and were found to be free of ophthalmic disorders, including any form of cataract. Blood was collected from control individuals and DNA extracted with the Nucleon BACC3 kit (Amersham Pharmacia Biotech).

Linkage analysis

All individuals were genotyped at microsatellite markers representing known cataract genes and loci by the analysis of fluorescently-tagged PCR products on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Two-point LOD scores were calculated using the FASTLINK package.[15] Allele frequencies were obtained from the population control samples. Penetrance was set at 0.95 in heterozygotes and homozygous variants. Disease gene frequency was set to 0.0001.

Sequencing

The coding region of *CX46* was sequenced using three overlapping PCR fragments. Primer sequences were: 1F: 5′-CGGTGTTCATGAGCATTTTC-3′, 1R: 5′-GACGTAGGTCCGCAGCAG-3′, 2F: 5′-GCAGGACAATCCCTCGTC-3′, 2R: 5′-GGTCAGGGCTAGCAGTTTGA-3′, 3F: 5′-TCGGGTTCCCACCCTACTAT-3′, 3R: 5′-TGCACTTTGGTTTTGGTTTC-3′. PCR products were cycle sequenced with Big Dye Terminator Ready Reaction Mix (Applied Biosystems) and analysed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Computational methods

ClustalW[16] (http://www.ebi.ac.uk/clustalw/) was used to align CX46 protein sequences from five mammalian species represented in GenBank. The likely structure of the CX46 protein was determined using HMMTOP[17 18] (http://www.enzim.hu/hmmtop/index.html) and MEMSAT from the PSIPRED server[19 20] (http://bioinf.cs.ucl.ac.uk/psipred/).

Abbreviations: ADCC, autosomal dominant congenital cataract; BFSP2, beaded filament structural protein-2; HSF4, heat shock factor-4; MIP, major intrinsic protein; PITX3, paired-like homeodomain transcription factor-3

RESULTS

The phenotype in this pedigree is a faint lamellar nuclear opacity surrounding pulverulent nuclear opacities (fig 1), some with fine gold dots or haze and some with needle-like peripheral riders. The median age of diagnosis was 5 years (range 0–73 years), however the 10 patients in the two most recent generations (generations VI and VII) were diagnosed at 6 months to 2 years. Of 42 eyes with cataracts, 20 had not had surgery and have good or minimally (6/9) reduced vision. The median age of surgery was 17–26 years (range 10–67 years). No other systemic or ocular abnormalities, such as nystagmus, strabismus, or iris abnormalities, were noted. One older female diagnosed at age 5 years and operated on at age 67 years, developed pseudophakic glaucoma.

A LOD score of 2.96 was obtained at $\theta = 0.04$ from D13S1236, the marker included to detect linkage to the *CX46* gene. Other candidate loci implicated in ADCC (*CX50, CRYGD/GC, CRYBA1*, 1pter-p36.1, 15q21-q22, 17p13, 17q24) were excluded by linkage analysis (data not shown). An equivocal LOD score of 1.36 at $\theta = 0.08$ from marker D16S496 representing *HSF4* was the only other positive LOD score. Once the significant result at D13S1236 was obtained, additional ADCC loci (*BFSP2, PITX3, CRYAB, MIP, CRYAA, CRYBB2*, and 20p12-q12) were not investigated for this monogenic disorder.

CX46 was sequenced in affected individuals and the variant 226G>A (GenBank reference NM_021954) causing an R76H substitution was identified in all 21 affected individuals but not in 100 control chromosomes by direct sequencing. Six unaffected relatives (IV:4, V:7, V:8, V:9, V:27, and VI:15) were also found to carry the mutation (fig 2). This residue is conserved across species represented in GenBank (fig 3). The residue is predicted by MEMSAT to be at the boundary between extracellular loop 1 and transmembrane loop 2 while HMMTOP predicts that it is within the second transmembrane domain.

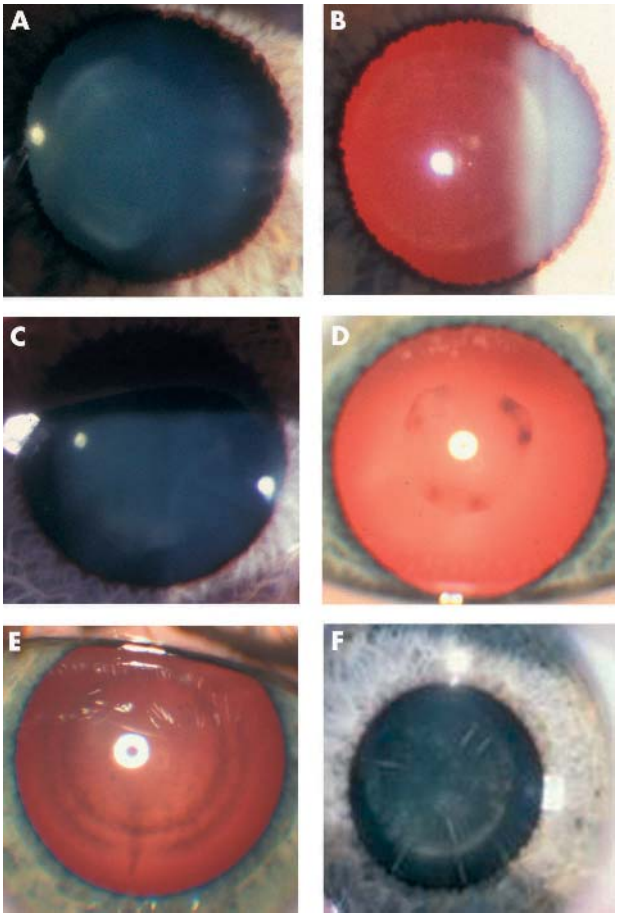

Figure 1 A range of pulverulent cataract phenotypes of pedigree crch13. (A) Mild pulverulent phenotype of the nucleus of individual VI:8, directly illuminated and (B) retroilluminated. (C) Mild pulverulent phenotype of the nucleus with a cortical lamella opacity of individual VII:2, directly illuminated and (D) retroilluminated. (E) Mild pulverulent phenotype of the nucleus with a cortical lamella opacity of individual VII:1, retroilluminated. (F) Pulverulent phenotype in the nucleus with cortical riders of individual VI:3, directly illuminated.

DISCUSSION

The investigation of this large Australian cataract pedigree has revealed a novel mutation, R76H, in the *CX46* gene. The R76H mutation is likely to be causative as it segregates with affected status amongst reasonably distant branches of the pedigree with the same phenotype and was not detected in unaffected, unrelated controls. The inheritance in this pedigree is clearly autosomal dominant, although not fully penetrant.

Connexin proteins form hexamers known as connexons in the cell membranes. Connexons in neighbouring cells dock to form gap junctions which allow the transport of small metabolites directly between cells.[1] Two connexins, *CX46* and *CX50,* are expressed in lens fibre cells. Previously reported mutations of these genes associated with congenital cataracts

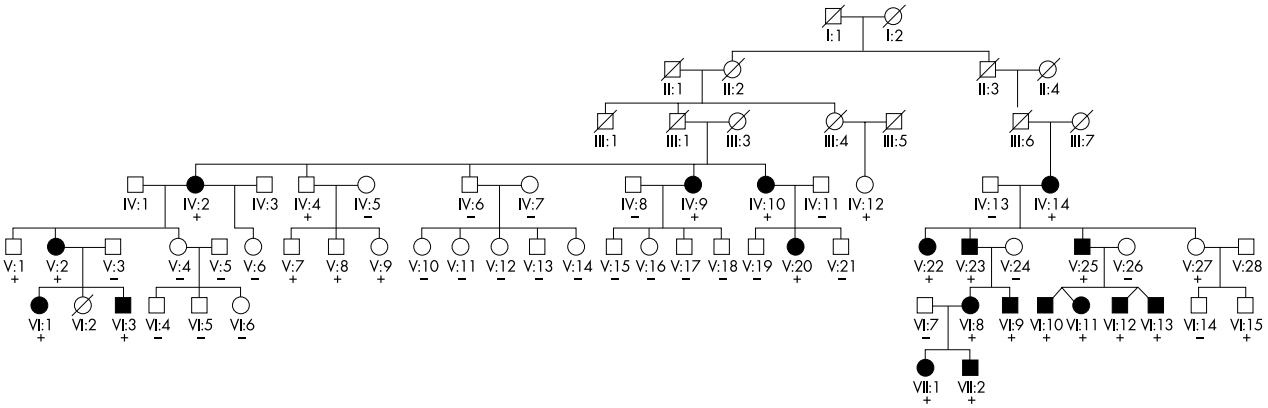

Figure 2 Pedigree diagram of crch13 indicating the presence of Connexin46 R76H mutation. Shaded symbols indicate the presence of an ophthalmologist-confirmed cataract. Squares indicate males, circles females, ''+'' heterozygote, and ''−'' wild type. Individuals with no ''+'' or ''−'' symbol have not been typed.

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Mouse    CENVCYDRAFPISHIRFWALQIIFVSTPTLIYLGHVLHIVRMEEKKKEREEELLRRDNPQ
Rat      CENVCYDRAFPISHIRFWALQIIFVSTPTLIYLGHVLHIVRMEEKKKEREEELLRRDNPQ
Human    CENVCYDRAFPISHIRFWALQIIFVSTPTLIYLGHVLHIVRMEEKKKEREEEEQLK----
Cow      CENVCYDRAFPISHVRFWVLQIIFVSTPTLIYLGHVLHLVRMEEKKKEREEE--------
Danio    CENVCYDEAFPISHIRFWVLQIIFVSTPTLIYLGHVLHIVRMEEKRKEREEELRKASRLQ
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Figure 3 Cross species alignment of Connexin46. ClustalW alignment of residues 61–120 of human Cx46 with mouse, rat, cow, and zebra fish (*Danio rerio*) orthologs. R76 is highlighted in grey.

are shown in table 1. All seven mutations have been linked to zonular pulverulent congenital cataracts which are ''pulverised'' dust-like or punctate opacities in developmental zones of the lens. The phenotype in the Australian pedigree in the present study resembles those previously reported. All mutations of connexin genes described previously appear fully penetrant in the pedigrees in which they were detected.

The R76 residue of CX46 is conserved between species (fig 3), indicating that the arginine is likely to be functionally important and that the mutation may therefore have a detrimental physiological effect. It is not clear whether R76 is located in the first extracellular loop or in the second transmembrane domain. Other cataract mutations have been detected in both these domains (table 1). Mutations in the extracellular domains may affect connexon docking if the conformation of the loop is changed. It is unclear what the affect of variation within the transmembrane domain would be, however, other mutations of transmembrane domains of connexin genes have been reported. The wild type arginine has a positive charge while histidine can be either positively charged or neutral, depending on the microenvironment. This may help explain the incomplete penetrance observed if cataract formation is dependant on the ionisation of this residue.

A lack of gross effects on protein structure is implied by both the range of ages at which surgery was performed, indicating a phenotype of variable severity, and the incomplete penetrance observed in the pedigree. Individuals V:27 and VI:15 have been examined thoroughly by several of the investigators and are clearly unaffected. Carrier individuals IV:4, V:7, V:8, and V:9 have not been examined by a member of our research group and, therefore, it is possible that they may be subtly affected. The only other report of a monogenic paediatric cataract mutation with incomplete penetrance is a 5 bp insertion in the *γ-crystallin* gene also causing a variable zonular pulverulent phenotype.[21] The variability was suggested to be due to environmental factors or modifying genes. Unaffected individuals IV:4 and V:27 have passed on the R76H mutation to their offspring, who also remain unaffected. Environmental factors are unlikely to show this type of pattern, unless there are significant household effects, suggesting the possibility of a second modifying or protective gene.

Investigations of animal models may help elucidate the nature of modifying genes. Mice with a disrupted *cx46* gene develop a nuclear lens cataract. The cataract phenotype and presence of cleaved γ-crystallin in the lens was variable, depending on the genetic background of the mouse, indicating the presence of modifier genes involved in the development of the phenotype.[22] The *Lop10* mutation in mice may also provide some insight. This phenotype is caused by the G22R variant of murine *cx50*,[23] however, the phenotype is variable and dependent on the genetic background of the mouse.[24] These examples provide evidence for modifier genes in the development of congenital cataract and support the hypothesis of modifier genes acting in the pedigree described here.

In summary, a novel mutation of the human *CX46* gene has been found to segregate with a pulverulent phenotype. The mutation is only the second reported congenital cataract mutation with incomplete penetrance in the literature and, as such, provides an opportunity for the investigation of modifying genes and their interactions.

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Conflict of interest: none declared.

Table 1 Previously reported mutations of connexin genes in pedigrees with zonular pulverulent congenital or paediatric cataract

Gene	Mutation	Location	Reference
CX46	1137insC	C-terminal cytoplasmic tail	Mackay *et al*, 1999[4]
	N63S	First extracellular loop	Mackay *et al*, 1999[4]
	P187L	Second extracellular loop	Rees *et al*, 2000[25]
	F32L	First transmembrane domain	Jiang *et al*, 2003[26]
CX50	E48K	First extracellular loop	Berry *et al*, 1999[27]
	I247M	C-terminal cytoplasmic tail	Polyakov *et al*, 2001[28]
	P88S	Second transmembrane domain	Shiels *et al*, 1998[3]

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