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# Connexin46 Mutations in Autosomal Dominant Congenital Cataract

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## Summary

**Loci for autosomal dominant "zonular pulverulent" cataract have been mapped to chromosomes 1q (CZP1) and 13q (CZP3). Here we report genetic refinement of the CZP3 locus and identify underlying mutations in the gene for gap-junction protein α-3 (*GJA3*), or connexin46 (Cx46). Linkage analysis gave a significantly positive two-point LOD score (*Z*) at marker *D13S175* (maximum *Z* [$Z_{max}$] => 7.0; maximum recombination frequency [$\theta_{max}$] = 0). Haplotyping indicated that CZP3 probably lies in the genetic interval *D13S1236–D13S175–D13S1316*–cen–13pter, close to *GJA3*. Sequencing of a genomic clone isolated from the CZP3 candidate region identified an open reading frame coding for a protein of 435 amino acids (47,435 D) that shared ~88% homology with rat Cx46. Mutation analysis of *GJA3* in two families with CZP3 detected distinct sequence changes that were not present in a panel of 105 normal, unrelated individuals. In family B, an A→G transition resulted in an asparagine-to-serine substitution at codon 63 (N63S) and introduced a novel *Mwo*I restriction site. In family E, insertion of a C at nucleotide 1137 (1137insC) introduced a novel *Bst*XI site, causing a frameshift at codon 380. Restriction analysis confirmed that the novel *Mwo*I and *Bst*XI sites cosegregated with the disease in families B and E, respectively. This study identifies *GJA3* as the sixth member of the connexin gene family to be implicated in human disease, and it highlights the physiological importance of gap-junction communication in the development of a transparent eye lens.**

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## Introduction

Congenital or infantile cataract is a sight-threatening lens defect that presents with an estimated prevalence of 1–6 cases per 10,000 live births (Lambert and Drack 1996). Approximately 25% of all cases are inherited, most often in a nonsyndromic autosomal dominant fashion, with considerable inter- and intrafamilial clinical variation (Merin 1991; Phelps Brown and Bron 1996). At least 10 independent loci for autosomal dominant congenital cataract (adCC) have been linked to seven human chromosomes (reviewed by Hejtmancik 1998). Mutations in genes for lens crystallins have been identified at adCC loci on 2q (Brackenhoff et al. 1994; Stephan et al. 1999), 17q11–q12 (Kannabiran et al. 1998), 21q (Litt et al. 1997), and 22q (Litt et al. 1998). However, no obvious candidate genes exist at those loci on 1p (Eiberg et al. 1995; Ionides et al. 1997), 16q (Eiberg et al. 1988), 17p (Berry et al. 1996), and 17q24 (Armitage et al. 1995).

Recently, a mutation in the gene for gap-junction protein α8 (*GJA8*), or connexin50 (Cx50), has been linked with a zonular pulverulent form of adCC (CZP1; MIM 116200) on 1q (Shiels et al. 1998). Zonular pulverulent cataract is characterized by numerous powdery or punctate opacities located in different developmental regions of the lens, and a second locus for this phenotype (CZP3; MIM 601885) has been linked to chromosome 13q (Mackay et al. 1997). The gene for gap-junction protein α-3 (*GJA3*), or connexin46 (Cx46), has been localized to chromosome 13q11–q12 (Mignon et al. 1996) and is predominantly expressed in the lens (Paul et al. 1991). The chromosomal location and lens-preferred expression of *GJA3* suggested that it was a strong candidate gene for CZP3. To gain further insight into the role of connexin genes in hereditary cataract, we have isolated *GJA3* and performed mutation analysis in two families of British descent segregating punctate opacities that map to the CZP3 locus on 13q.

## Subjects and Methods

### *Patients and Diagnosis*

The family with cataract (family E) was ascertained at Moorfields Eye Hospital, London, and, for this study,

10 affected and 5 unaffected members underwent a full ophthalmic examination by one of us (A.I.). The opacities (fig. 1) appeared coarse and granular toward the central zone (fetal nucleus) of the lens, whereas fine dust-like opacities predominated in the peripheral zone (juvenile cortex) of the lens. Hospital records confirmed that bilateral cataract was usually present at birth or developed during infancy, and there was no family history of other ocular or systemic abnormalities. Autosomal dominant inheritance of the cataract was supported by the presence of affected individuals in each of the three generations, equal numbers of affected males and females, and male-to-male transmission (fig. 2).

### *Genotyping and Linkage Analysis*

Blood samples were taken from patients after informed consent and with local approval from the ethics committee. Genomic DNA preparation, PCR-based genotyping using Généthon microsatellite markers (Dib et al. 1996), and linkage analysis with the LINKAGE package of programs (Lathrop et al. 1984; Atwood and Bryant 1988) were performed essentially as described elsewhere (Mackay et al. 1997).

### *Cloning and Sequencing*

Primer pairs were designed from the rat Cx46 cDNA sequence (GenBank accession number X57970) with PrimerSelect (DNASTAR) and were used to amplify human and rodent genomic DNA under standard PCR conditions. Sequence homology of resultant PCR products was confirmed by use of BLAST in the GenBank database. A reproducible 214-bp PCR fragment was used to probe conventional Southern blots containing *Hin*dIII digests of a set of 57 P1 artificial chromosomes (PACs) that partially covered the candidate region for both CZP3 and the skin disorder, hidrotic ectodermal dysplasia (HED; Kibar et al. 1996). An arrayed (12 × 8-well) plasmid library of the positive PAC clone (1 $\mu$g) was constructed in pUC18 *Hin*dIII/BAP (Pharmacia Biotech) by standard techniques. The library was transferred to Hybond-N+ nylon membrane (Amersham) by means of a 96-pin replicating tool and was hybridized against the 214-bp probe. Positive plasmid clones were sequenced manually by the dideoxy-chain–termination method by means of a Sequenase Kit (US Biochemical) or with an automated Li-Cor DNA sequencer 4200. DNA sequence assembly was performed by SeqMan (DNASTAR).

### *Mutation Screening*

The entire coding region of the Cx46 gene (exon 2) was amplified from affected individuals by use of gene-specific primers for codons 1–7 and 430–stop (table 1) under standard PCR conditions. The heterozygous PCR product (~1.3 kb) was either directly sequenced or subcloned into the pGEM-T Easy vector (Promega) prior to sequencing of the mutant and wild-type alleles by use of internal gene-specific primers (table 1) and the ABI Prism DyeDeoxy-terminator cycle sequencing kit (PE

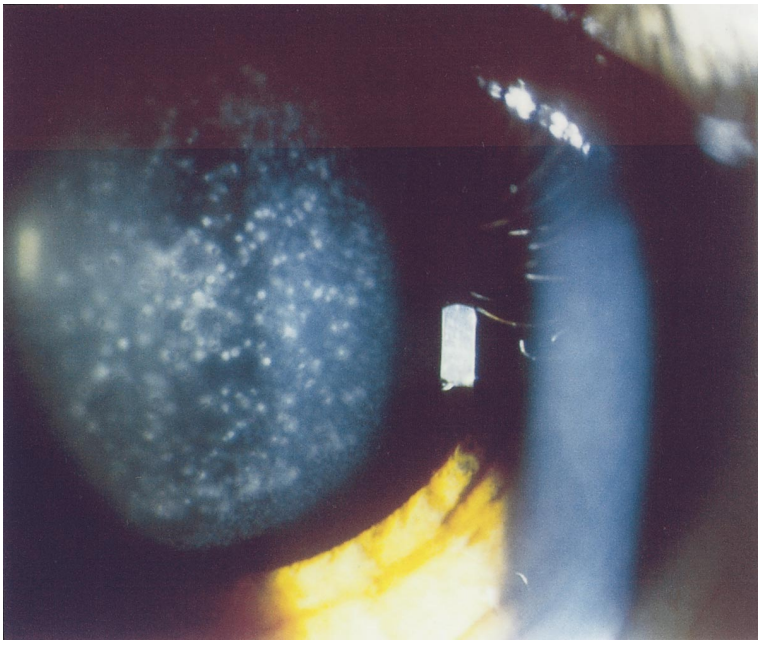

**Figure 1** Slit-lamp photograph showing "punctate" opacities located throughout the lens of individual III:2 in family E (fig. 2)

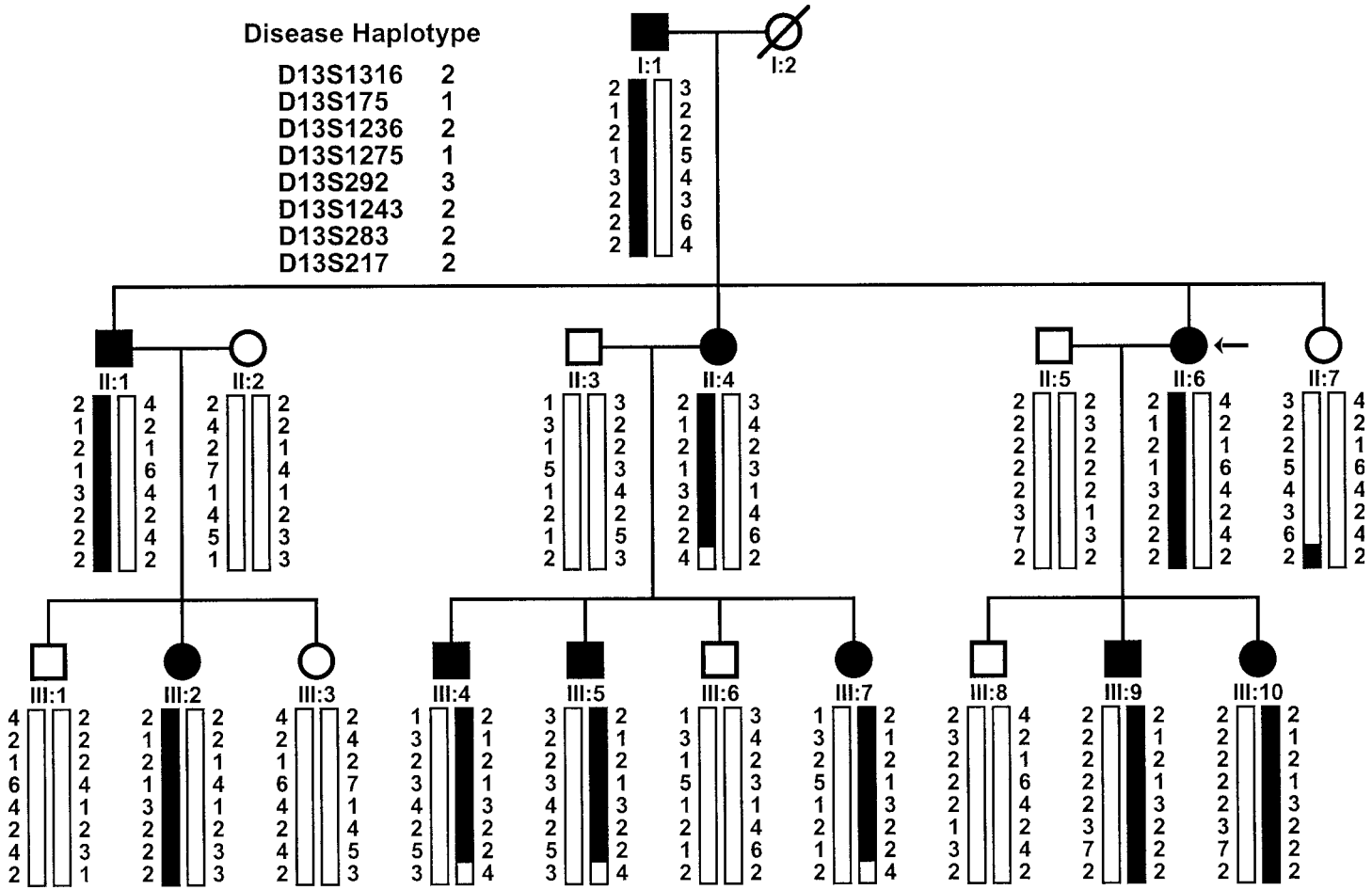


**Figure 2** Pedigree and haplotype analysis of family E showing segregation of eight microsatellite markers on chromosome 13, listed in descending order from the centromeric end. Squares and circles symbolize males and females, respectively. Unblackened and blackened symbols denote unaffected and affected individuals, respectively. The proband is indicated with an arrow.

**Table 1**

**PCR Primers Used for Mutation Screening of the Human Cx46 Coding Region**

| Codon | Strand | Sequence |
|---|---|---|
| 1–7 | Sense | 5′-ATGGGCGACTGGAGCTTTCT |
| 29–36 | Sense | 5′-CTGTTCCTGTTCCGCATTTTGGT |
| 96–103 | Antisense | 5′-TCCATGCGCACGATGTGCAGCA |
| 123–129 | Antisense | 5′-TTGTCCTGCGGTGGCTCCTT |
| 143–150 | Sense | 5′-CGCGCTGCTGCGGACCTACG |
| 185–192 | Sense | 5′-CCTGCCCCAACACGGTGGACTG |
| 207–214 | Antisense | 5′-TGACGCACAGGCCACAGCCAACAT |
| 223–229 | Antisense | 5′-CTTCTTCCAGCCCAGGTGGTA |
| 229–235 | Sense | 5′-AAGCTCAAGCAGGGCGTGAC |
| 316–323 | Sense | 5′-AACGGCCACCACCACCTGCTGAT |
| 329–335 | Antisense | 5′-GCTCGGCCGCCTGGTTGG |
| 430–stop | Antisense | 5′-CTAGATGGCCAAGTCCTCCGG |

Applied Biosystems). In family E, the same ~1.3-kb fragment was used to test for the novel *Bst*XI site in affected individuals. In family B, primers for codons 29–36 and 96–103 (table 1) were used to amplify the novel *Mwo*I site in affected individuals and to exclude 30 other *Mwo*I sites within the coding exon of wild-type *GJA3*. Enzyme digests were performed according to the manufacturer's instructions (New England Biolabs), and the resulting restriction fragments were separated in agarose gels stained with ethidium bromide.

## Results

### *CZP3 Linkage and Refinement*

Eighteen members of family E, including 10 affected individuals, 5 unaffected individuals, and 3 spouses (fig. 2), were genotyped with Généthon $(AC)_n$ microsatellite markers (Dib et al. 1996) that spanned the CZP1 and CZP3 loci. After exclusion of the CZP1 locus, we obtained a maximum two-point LOD score ($Z_{max}$) of 3.91, with no recombination ($\theta_{max} = 0$), at marker *D13S175*. Multipoint analysis yielded $Z_{max} = 3.92$ ($\theta_{max} = 0$) at both *D13S1316* and *D13S175* (data not shown).

Haplotype analysis (fig. 2) detected four affected individuals (II:4, III:4, III:5, and III:7) and one unaffected female (II:7) who were obligate recombinants at the most distal marker *D13S217* in the region. No recombinant individuals were observed between the cataract locus and *D13S1316*, which is the most proximal marker on 13q, suggesting that the cataract locus lies in the genetic interval *D13S217*–(19.1 cM)–*D13S1316*–cen–13pter. This interval contains that defined elsewhere by the original, unrelated CZP3 family (B), that is, *D13S1243*–(11.5 cM)–*D13S1316*–cen–13pter (Mackay et al. 1997). In agreement with recent genetic mapping data (Kibar et al. 1996; Taylor et al. 1998), however, we have placed *D13S1236* distal rather than proximal to *D13S175* (fig. 2) and have identified an individual (IV:8) in family B (Mackay et al. 1997) who carried the disease haplotype at *D13S1316* and *D13S175* but not at *D13S1236*. This individual was previously designated as unaffected; however, our mutation data (fig. 4) suggested that he represented a case of reduced penetrance and was in fact recombinant for the disease at *D13S1236*. Thus, the combined *Z* values (table 2) and haplotytpe data from families B and E gave a $Z_{max}$ of >7.0 ($\theta_{max} = 0$) at *D13S175*, and they indicated that the CZP3 locus lies in the refined genetic interval *D13S1236*–*D13S175*–*D13S1316*–cen–13pter.

### GJA3 *Isolation and Sequencing*

To isolate the human gene *GJA3* for mutation screening, we designed consensus PCR primers based on codons 29–36 and 223–229 of the rat cDNA sequence for Cx46 (GenBank accession number X57970). Sequence alignment of the resulting 603-bp fragment amplified from human genomic DNA in the GenBank database by means of the BLAST algorithim (Altschul et al. 1990) confirmed maximum homology with Cx46 homologues rather than other connexins. We extended this sequence to 687 bp by using a primer to codons 1–7 of human Cx50 (GenBank accession number U34802), and we identified the 5′ coding sequence for Cx46 (fig. 3). Simultaneously, a 214-bp PCR fragment was amplified from a chromosome 13–only somatic cell hybrid by use of consensus primers to codons 143–150 and 207–214 of rat Cx46. Southern blot analysis showed that this Cx46 PCR product hybridized with a 4.7-kb *Hin*dIII restriction fragment (data not shown) derived from a PAC clone that mapped to the candidate region for both CZP3 and HED on 13q (Kibar et al. 1996). Sequencing of this P1 fragment detected a single open reading frame coding for a protein of 435 amino acids with a calculated molecular mass of 47,435 D (fig. 3). Overall, the human Cx46 sequence (GenBank accession number *GJA3*, AFO75290) shared ~88% homology and ~70% identity with that of the rat, particularly in the transmembrane and extracellular domains of the protein.

### GJA3 *Mutation Analysis*

Mutation analysis of the Cx46 gene in the families with cataracts detected two significant changes from wild type (fig. 3). In family B, an adenine to guanine transition at nucleotide 188 of the coding region (188A→G) resulted in the introduction of a novel *Mwo*I restriction enzyme site that cosegregated with the disease (fig. 4). In family E, insertion of a cytosine after coding nucleotide 1137 (1137insC) introduced a novel *Bst*XI restriction site that also cosegregated with the disease phenotype (fig. 5). Neither of these sequence changes was detected in a panel of 105 unrelated, normal individuals (data not shown), implying that 188A→G and

**Table 2**

**Combined Two-Point *Z* Values for Linkage between CZP3 in Families B and E and Chromosome 13 Markers**

| | *Z* AT $\theta$ = | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MARKER | .00 | .01 | .05 | .10 | .20 | .30 | .40 | $Z_{max}$ | $\theta_{max}$ |
| 13pter-cen | | | | | | | | | |
| *D13S1316* | 5.76 | 5.64 | 5.16 | 4.53 | 3.21 | 1.83 | .64 | 5.76 | .00 |
| *D13S175* | 7.44 | 7.31 | 6.78 | 6.08 | 4.55 | 2.86 | 1.18 | 7.44 | .00 |
| *D13S1236* | $-\infty$ | 4.57 | 4.82 | 4.56 | 3.65 | 2.48 | 1.13 | 4.82 | .05 |
| *D13S1275* | $-\infty$ | 4.88 | 5.13 | 4.83 | 3.85 | 2.64 | 1.34 | 5.13 | .05 |
| *D13S292* | 4.86 | 4.83 | 4.64 | 4.29 | 3.42 | 2.36 | 1.19 | 4.86 | .00 |
| *D13S1243* | $-\infty$ | 3.09 | 3.88 | 3.88 | 3.24 | 2.24 | 1.06 | 3.89 | .08 |
| *D13S283* | $-\infty$ | 5.19 | 5.99 | 5.82 | 4.74 | 3.20 | 1.42 | 5.99 | .05 |
| *D13S217* | $-\infty$ | −6.74 | −2.20 | −.54 | .60 | .77 | .49 | .77 | .30 |

1137insC are allelic cataract mutations rather than rare polymorphisms.

## Discussion

The connexin gene family encodes gap-junction channel proteins that mediate the intercellular transport of small biomolecules (<1 kD) including ions, metabolites, and second messengers in diverse vertebrate cell types, including cochlea cells (Kelsell et al. 1997), Schwann cells (Bergoffen et al. 1993), epidermal cells (Richard et al. 1998), and lens fiber cells (White et al. 1994). At least 10 genes for connexins of varying molecular mass (~26–50 kD) have been identified in humans (GenBank). Mutations in the genes for Cx26 (*GJB2*), Cx31 (*GJB3*), Cx32 (*GJB1*), Cx43 (*GJA1*), and Cx50 (*GJA8*) have been associated with certain types of deafness (Kelsell et al. 1997; Xia et al. 1998), skin disease (Richard et al. 1998), peripheral neuropathy (Bergoffen et al. 1993), heart defects (Britz-Cunningham et al. 1995), and cataracts (Shiels et al. 1998), respectively. In the present study, we have refined the locus for zonular pulverulent cataract (CZP3) to the genetic interval *D13S1236*–cen–13pter and have identified distinct mutations in *GJA3,* the gene for Cx46. Our data provide a genetic map location for *GJA3* and constitute the first report implicating Cx46 in human inherited cataract.

The 188A→G missense mutation is likely to result in an asparagine-to-serine substitution at codon 63 (N63S) of Cx46. This represents a relatively conservative amino acid change, as both asparagine (amide side group) and serine (hydroxyl side group) are neutral under physiologic conditions. Asparagine, however, is strictly conserved at codon 61, 62, or 63 within the first extracellular loop (E1) of all vertebrate connexins (GenBank). The extracellular domains of connexins are believed to mediate the intercellular docking of connexon hemichannels (i.e., connexin hexamers) and enable the formation of gap-junction channels composed of connexin dodecamers (reviewed by Simon and Goodenough 1998). Thus, the N63S substitution may induce a defect

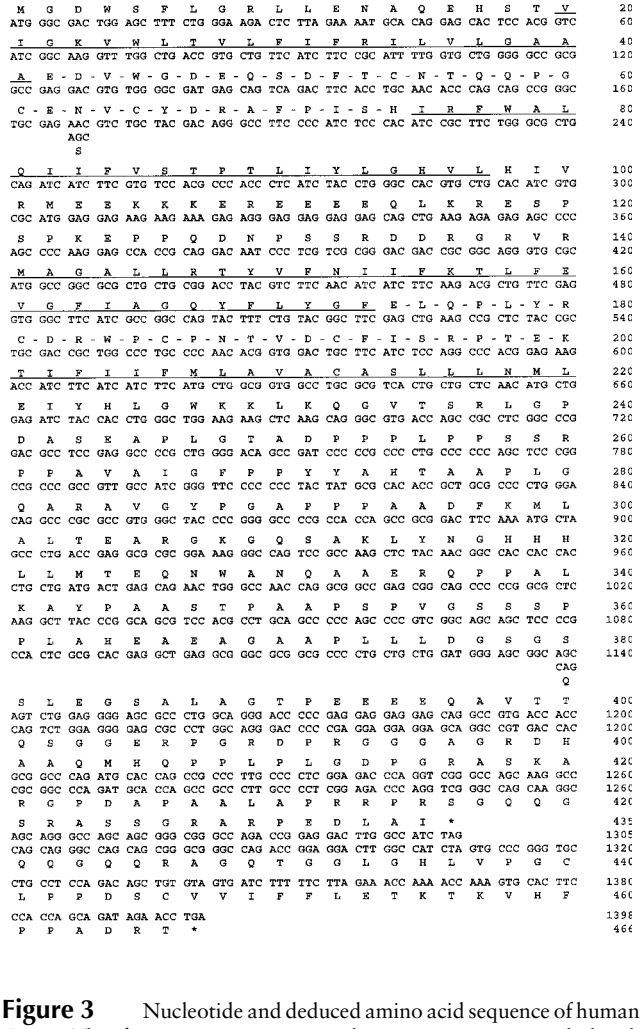

**Figure 3** Nucleotide and deduced amino acid sequence of human Cx46. The four putative transmembrane regions are underlined. Amino acids 42–74 and 174–200 are believed to form the extracellular loops (E1 and E2), and the remainder of the protein is cytoplasmic. The N63S missense mutation (family B) lies in the first extracellular loop (E1), and the 1137insC mutation (family E) causes a frameshift at amino acid 380 in the cytoplasmic carboxy-terminus. In-frame translation stop codons are indicated by asterisks.

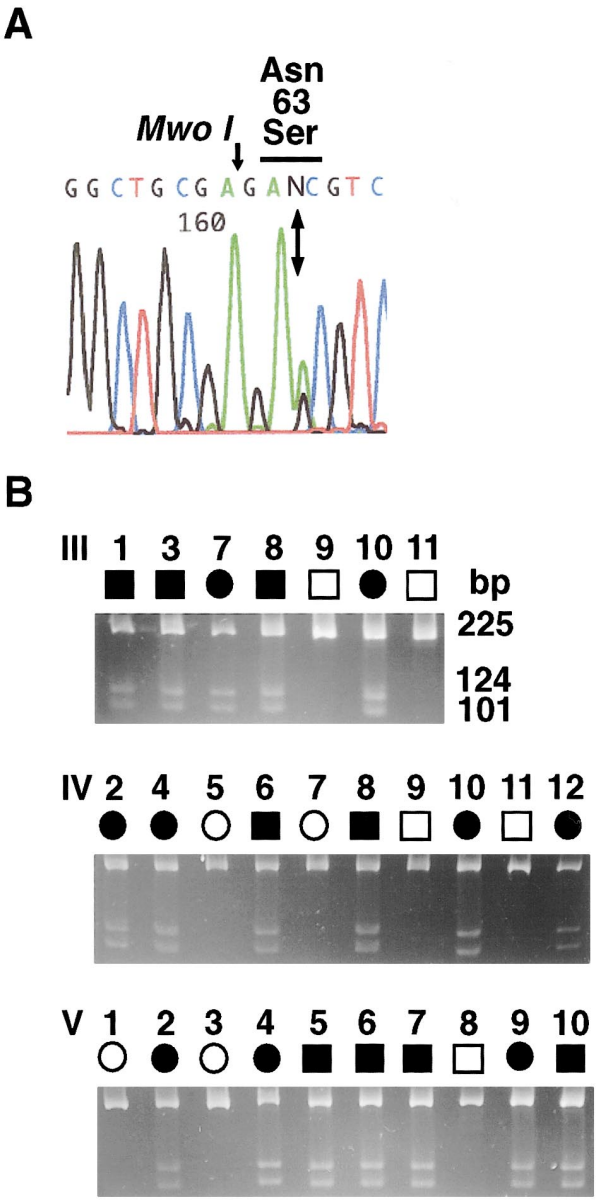


**Figure 4** Mutation analysis of the Cx46 gene in family B. *a*, Heterozygote sequence (sense strand) showing an A→G transition (arrow) in codon 63 that changed asparagine (AAC) to serine (AGC) and introduced an *Mwo*I site ($GCN_5N_2GC$). *b*, Restriction fragment length analysis showing that gain of the novel *Mwo*I site cosegregated with affected individuals (blackened symbols) heterozygous for the A→G transition (225, 124, and 101 bp) but not with unaffected individuals and spouses (225 bp only).

in the E1 secondary structure that impairs Cx46-mediated coupling of lens fiber cells. Similarly, a D47A missense mutation in the E1 domain of Cx50 has been associated with dominant congenital cataract in the *No2* mouse (Steele et al. 1998). Furthermore, mutations in the extracellular domains of human Cx26 (Denoyelle et al. 1998) and Cx31 (Xia et al. 1998) have been implicated in dominant forms of neurosensory deafness, whereas similar mutations in Cx32 are associated with an X-linked form of Charcot-Marie-Tooth neuropathy (Krawczak and Cooper 1997).

The 1137insC mutation is predicted to cause a frameshift immediately after codon 379 of the Cx46 gene. This results in the mistranslation of the final 56 amino acids of the wild-type protein and the addition of 31 amino acids to the carboxy terminus of the mutant protein before an in-frame translation stop codon is detected (fig. 3). Alignment of the 87 novel amino acids in the SWISS-PROT database failed to detect significant homology with other known proteins. An inherited frameshift mutation in the carboxy-terminal domain of Cx26 has been associated with a recessive form of neurosensory deafness (Kelley et al. 1998). However, unlike the 1137insC frameshift mutation in Cx46, this mutation in Cx26 results from a 2-bp deletion that introduces a premature translation stop codon. Notably, the

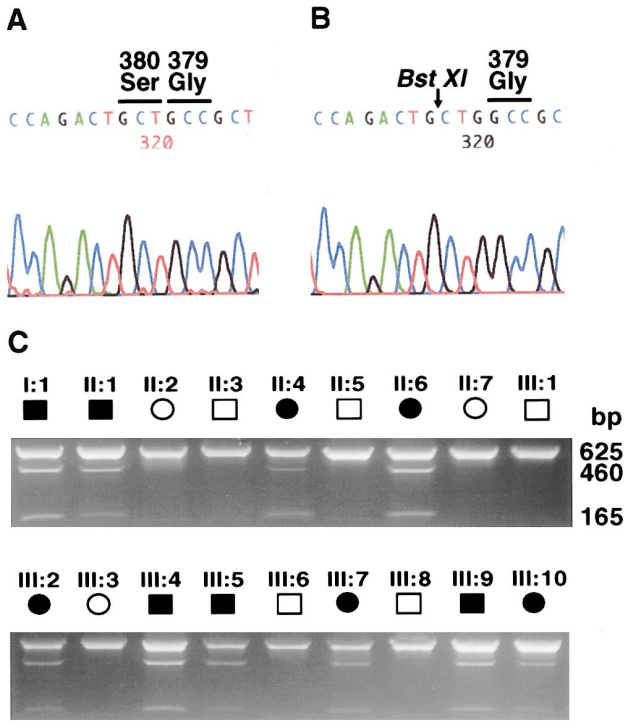


**Figure 5** Mutation analysis of the Cx46 gene in family E. *A*, Wild-type allele (antisense strand) showing translation of an in-frame glycine and serine at codons 379 (5′-GGC) and 380 (5′-AGC), respectively. *B*, Mutant allele (antisense strand) showing that insertion of a G (*arrow*) after coding nucleotide 1137 (i.e., 1137insC on the sense strand) introduced a *Bst*XI site ($CCAN_5NTGG$). *C*, Restriction fragment–length analysis showing that gain of the novel *Bst*XI site cosegregated with affected individuals (blackened symbols) heterozygous for the 1137insC mutation (625, 460, and 165 bp) but not with unaffected individuals and spouses (625 bp only).

1137insC mutation abolished potential phosphorylation sites at the cytoplasmic carboxyl end of Cx46. Recessive mutations at similar sites in Cx43 have been associated with visceroatrial heterotaxia syndrome (Britz-Cunningham et al. 1995), although these findings are controversial (Toth et al. 1998).

We have demonstrated that dominant mutations in the genes for Cx46 (present study) and Cx50 (Shiels et al. 1998) are associated with "punctate" cataracts in humans. Both of these connexins have been shown to colocalize in gap junctions that permeate the lens (Jiang and Goodenough 1996). Such heteromeric channels are believed to facilitate the continuous flow of ions, metabolites, and other small biomolecules (<1 kD) necessary for maintaining lens clarity. Functional expression studies of a dominant deafness mutation in Cx26 (White et al. 1998*a*) suggest that the Cx46 and Cx50 mutants described here and elsewhere (Shiels et al. 1998; Steele et al. 1998) may exert dominant inhibitory effects on their wild-type counterparts in vivo. The recent demonstrations that mice deficient in either Cx46 (Gong et al. 1997) or Cx50 (White et al. 1998*b*) also develop cataracts confirm the vital role of these connexins in lens physiology and provide model systems for elucidating the pathogenetic mechanisms associated with Cx46 and Cx50 defects in humans.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Search/index.html

Généthon, http://www.genethon.fr (for genetic markers and distances and for PCR conditions)

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/BLAST (for BLAST searches of GenBank and SWISS-PROT databases for connexin sequences)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for cataract, deafness, Charcot-Marie-Tooth disease, hidrotic ectodermal dysplasia, EKV skin disorder, and VAH heart defect)

SWISS-PROT, http://www.ebi.ac.uk/ebi_docs/swissprot_db/swisshome.html

## References

Armitage MM, Kivlin JD, Ferrell RE (1995) A progressive early onset cataract gene maps to human chromosome 17q24. Nat Genet 9:37–40

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ (1990) Basic local alignment tool. J Mol Biol 215:403–410

Atwood MM, Bryant SA (1988) A computer program to make analysis with LIPED and LINKAGE easier to perform and less prone to input errors. Ann Hum Genet 52:259

Bergoffen J, Scherer SS, Wang S, Oronzi Scott M, Bone LJ, Paul DL, Chen K, et al (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 262:2039–2042

Berry V, Ionides ACW, Moore AT, Plant C, Bhattacharya SS, Shiels A (1996) A locus for autosomal dominant anterior polar cataract on chromosome 17p. Hum Mol Genet 5: 415–419

Brakenhoff RH, Henskens HAM, van Rossum MWPC, Lubsen NH, Schoenmakers JGG (1994) Activation of the γE-crystallin pseudogene in the human hereditary Coppock-like cataract. Hum Mol Genet 3:279–283

Britz-Cunningham SH, Shah MM, Zuppan CW, Fletcher WH (1995) Mutations of the connexin43 gap-junction gene in patients with heart malformations and defects of laterality. N Engl J Med 332:1323–1329

Denoyelle F, Lina-Granade G, Plauchu H, Bruzzone R, Chaib H, Levi-Acobas F, Weil D, et al (1998) Connexin 26 gene linked to a dominant deafness. Nature 393:319–320

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Milausseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Eiberg H, Lund AM, Warburg M, Rosenberg T (1995) Assignment of congenital cataract Volkmann type (CCV) to chromosome 1p36. Hum Genet 96:33–38

Eiberg H, Marner E, Rosenberg T, Mohr J (1988) Marner's cataract (CAM) assigned to chromosome 16: linkage to haptoglobin. Clin Genet 34:272–275

Gong X, Li E, Klier G, Huang Q, Wu Y, Lei H, Kumar NM, et al (1997) Disruption of the α3 connexin gene leads to proteolysis and cataractogenesis in mice. Cell 91:833–843

Hejtmancik JF (1998) The genetics of cataract: our vision becomes clearer. Am J Hum Genet 62:520–525

Ionides ACW, Berry V, Mackay DS, Moore AT, Bhattacharya SS, Shiels A (1997) A locus for autosomal dominant posterior polar cataract on chromosome 1p. Hum Mol Genet 6:47–51

Jiang JX, Goodenough DA (1996) Heteromeric connexons in lens gap junction channels. Proc Natl Acad Sci USA 93: 1287–1291

Kannabiran C, Rogan PK, Olmos L, Basti S, Rao GN, Kaiser-Kupfer M, Hejtmancik JF (1998) Autosomal dominant zonular cataract with sutural opacities is associated with a splice mutation in the βA3/A1-crystallin gene. Mol Vis 4:21 (http://www.molvis.org/molvis/v4/p21)

Kelley PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, Kimberling WJ (1998) Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet 62:792–799

Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, et al (1997) Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature 387: 80–83

Kibar Z, Der Kaloustian VM, Brais B, Hani V, Clarke Fraser F, Rouleau GA (1996) The gene responsible for Clouston hidrotic ectodermal dysplasia maps to the pericentromeric region of chromosome 13q. Hum Mol Genet 5:543–547

Krawczak M, Copper DN (1997) The human gene mutation database. Trends Genet 13:121–122

Lambert SL, Drack AV (1996) Infantile cataracts. Surv Ophthalmol 40:427–458

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multipoint linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Litt M, Carrero-Valenzuela R, LaMorticella DM, Schultz DW, Mitchell TN, Kramer P, Maumenee IH (1997) Autosomal dominant cerulean cataract is associated with a chain termination mutation in the human $\beta$-crystallin gene CRYBB2. Hum Mol Genet 6:665–668

Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG (1998) Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 7:471–474

Mackay D, Ionides A, Berry V, Moore A, Bhattacharya S, Shiels A (1997) A new locus for dominant 'zonular pulverulent' cataract on chromosome 13. Am J Hum Genet 60: 1474–1478

Mignon C, Fromaget C, Mattei M-G, Gros D, Yamasaki H, Mesnil M (1996) Assignment of connexin 26 (GJB2) and 46 (GJA3) genes to human chromosome 13q11–q12 and mouse chromosome 14D1-E1 by *in situ* hybridisation. Cytogenet Cell Genet 72:185–186

Merin S (1991) Inherited cataracts. In: Merin S (ed) Inherited eye disease: diagnosis and clinical management. Marcel Dekker, New York, pp 86–120

Paul DL, Ebihara L, Takemoto LJ, Swenson KI, Goodenough DA (1991) Connexin 46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of Xenopus oocytes. J Cell Biol 115:1077–1089

Phelps Brown N, Bron AJ (1996) Lens disorders: a clinical manual of cataract diagnosis. Butterworth-Heinemann, Oxford

Richard G, Smith LE, Bailey RA, Itin P, Hohl D, Epstein EH, Digiovanna JJ, et al (1998) Mutations in the human connexin gene GJB3 cause erythrokeratodermia variabilis. Nat Genet 20:366–369

Shiels A, Mackay D, Ionides A, Berry V, Moore A, Bhattacharya S (1998) A missense mutation in the human connexin50 gene (*GJA8*) underlies autosomal dominant "zonular pulverulent" cataract on chromosome 1q. Am J Hum Genet 62:526–532

Simon AM, Goodenough DA (1998) Diverse functions of vertebrate gap junctions. Trends Cell Biol 8:477–483

Steele EC, Lyon MF, Glenister PH, Guillot PV, Church RL (1998) A mutation in the connexin 50 (Cx50) gene is a candidate for the *No2* mouse cataract. Curr Eye Res 17: 883–889

Stephan DA, Gillanders E, Vanderveen D, Freas-Lutz D, Wistow G, Baxevanis AD, Robbins CM, et al (1999) Progressive juvenile-onset punctate cataracts caused by mutation of the $\gamma$D-crystallin gene. Proc Natl Acad Sci USA 96:1008–1012

Taylor TD, Hayflick SJ, McKinnon W, Guttmacher AE, Hovnanian A, Litt M, Zonana J (1998) Confirmation of linkage of Clouston syndrome (hidrotic ectodermal dysplasia) to 13q11-q12.1 with evidence for multiple independent mutations. J Invest Dermatol 111:83–85

Toth T, Hajdu J, Marton T, Nagy B, Papp Z (1998) Connexin43 gene mutations and heterotaxy. Circulation 97: 117–118

White TW, Bruzzone R, Wolfram S, Paul DL, Goodenough DA (1994) Selective interactions among the multiple connexin proteins expressed in the vertebrate lens: the 2nd extracellular domain is a determinant of compatibility between connexins. J Cell Biol 125:879–892

White TW, Deans MR, Kelsell DP, Paul DL (1998*a*) Connexin mutations in deafness. Nature 394:630–631

White TW, Goodenough DA, Paul D (1998*b*) Targeted ablation of connexin50 in mice results in microphthalmia and zonular pulverulent cataracts. J Cell Biol 143:815–825

Xia J-H, Liu C-Y, Tang B-S, Pan Q, Huang L, Dai H-P, Zhang B-R, et al (1998) Mutations in the gene encoding gap junction protein $\beta$-3 associated with autosomal dominant hearing impairment. Nat Genet 20:370–373