

A Missense Mutation in the Human Connexin50 Gene (*GJA8*) Underlies Autosomal Dominant “Zonular Pulverulent” Cataract, on Chromosome 1q

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Summary

CZP1, a locus for autosomal dominant “zonular pulverulent” cataract, previously had been linked with the Duffy blood-group-antigen locus on chromosome 1q. Here we report genetic refinement of the *CZP1* locus and show that the underlying mutation is present in *GJA8*, the gene for connexin50. To map the *CZP1* locus we performed linkage analysis using microsatellite markers on two distantly related branches of the original Ev. pedigree, which now spans eight generations. Significantly positive two-point LOD score (Z) values were obtained for markers *D1S2669* (maximum Z [Z_{\max}] = 4.52; maximum recombination frequency [θ_{\max}] = 0) and *D1S514* (Z_{\max} = 4.48; θ_{\max} = 0). Multipoint analysis gave Z_{\max} = 5.22 (θ_{\max} = 0) at marker *D1S2669*. Haplotyping indicated that *CZP1* probably lies in the genetic interval *D1S2746*–(20.6 cM)–*D1S2771*. Sequence analysis of the entire protein-coding region of the *GJA8* gene from the pedigree detected a C→T transition in codon 88, which introduced a novel *MnII* restriction-enzyme site that also cosegregated with the cataract. This missense mutation is predicted to result in the nonconservative substitution of serine for a phylogenetically conserved proline (P88S). These studies provide the first direct evidence that *GJA8* plays a vital role in the maintenance of human lens transparency and identify the genetic defect believed to underlie the first inherited disease to be linked to a human autosome.

Introduction

Inherited cataract is a genetically heterogeneous lens disease that most often presents as a congenital, autosomal dominant Mendelian trait showing considerable inter- and intrafamilial clinical variation and high penetrance (Francois 1982; Merin 1991; Lund et al. 1992; Scott et al. 1994). At least nine independent loci for autosomal dominant cataract (ADC) have been mapped on human chromosomes 1p (Eiberg et al. 1995; Ionides et al. 1997), 1q (Renwick and Lawler 1963; OMIM 1997), 2q (Lubsen et al. 1987; Rogaev et al. 1996), 13q (Mackay et al. 1997), 16q (Marner et al. 1989), 17p (Berry et al. 1996), 17q11-q12 (Padma et al. 1995), 17q24 (Armitage et al. 1995), and 22q (Kramer et al. 1996).

Mutations in crystallin genes, which encode >90% of lens cytoplasmic proteins, have been identified at two of these ADC loci. Novel activation of the γ E-crystallin pseudogene, on 2q, has been associated with the “Cop-pock-like” cataract (Brackenhoff et al. 1994), and a premature chain-termination mutation in the β B2-crystallin gene, on 22q, has been associated with “cerulean” cataract (Litt et al. 1997). In addition, the β A3/A1 crystallin gene, on 17q11 β -q12, represents a strong candidate for “zonular sutural” cataract (Padma et al. 1995). Dominant mutations in the β B2-, γ E-, and ζ -crystallin genes have also been associated with inherited cataract in the Philly mouse (Chambers and Russell 1991), the eye-lens-obsolence mouse (Cartier et al. 1992), and the guinea-pig (Rodriguez et al. 1992), respectively. The discovery, however, of dominant cataract-causing mutations in the mouse genes for lens major intrinsic protein (Shiels and Bassnett 1996) and for lens intrinsic membrane protein 2 (Steele et al. 1997) indicates that lens genes other than those for crystallins may also be involved in human inherited cataract.

More than 30 years ago, a gene for “zonular pulverulent” cataract (*CZP1*; formerly “CAE1” [MIM 116200]) was shown to cosegregate in an autosomal dominant manner with the Duffy blood-group antigen in a six-generation English kindred (Renwick and Lawler 1963). Subsequently, the Duffy gene (*FY*) was linked to

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chromosome 1 (Donahue et al. 1968), making CZP1 the first inherited disease to be linked to a human autosome (Renwick 1970). More recently, *FY* has been cytogenetically localized to 1q22-q23 (Mathew et al. 1994), and *GJA8*, the gene for gap-junction protein alpha-8 (MIM 600897), which encodes a connexin protein (Cx50) that is ~50 kD and that is primarily and abundantly expressed in the lens (White et al. 1992), has also been assigned to human chromosome 1 (Church et al. 1995). In addition, the mouse homologue for *GJA8* (*Gja8*) has been mapped to a chromosome 3 region that is reported to be syntenic with human chromosome 1q12-q21 (Kerscher et al. 1995). Thus, the predicted chromosomal location and the lens preferred expression of *GJA8* suggest that it is a likely candidate gene for CZP1. In order to directly test this hypothesis, we have performed linkage analysis to genetically refine the CZP1 locus and have followed this by sequence analysis to identify the underlying mutation in the *GJA8* gene.

Subjects and Methods

Pedigree and Diagnosis

Using the registers at Moorfields Eye Hospital and Great Ormond Street Hospital (both in London), we traced two distantly related branches (fig. 1) of the original Ev. cataract family first described, in four generations, by Nettleship (1909) and then, in six generations, by Renwick and Lawler (1963). Although all of the affected family members traced in this study had undergone lens surgery, hospital records confirmed that the cataract usually either was present at birth or developed in infancy and that 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 eight generations, equal numbers of affected males and females, and male-to-male transmission.

Historically, the Ev. cataract has been described as “lamellar” (Nettleship 1909) and then, after the invention of the slit lamp, either as zonular pulverulent with innumerable powdery opacities located in the nuclear (central) and perinuclear (lamellar) zones of the lens (Renwick and Lawler 1963) or as total nuclear cataract (Renwick 1970). It has been assumed (Waardenburg 1961) that the zonular pulverulent cataract of the Ev. pedigree is identical to Doyne’s “discoid” cataract, which presents as a circular spotted disk in the center of the lens (Adams 1942) and is characteristic of the genealogically unrelated Coppock pedigree first described by Nettleship and Ogilvie (1906). Indeed the Coppock and Ev. cataracts have become synonymous; however, the former is confined to the tiny embryonic lens, whereas the latter involves the larger fetal lens,

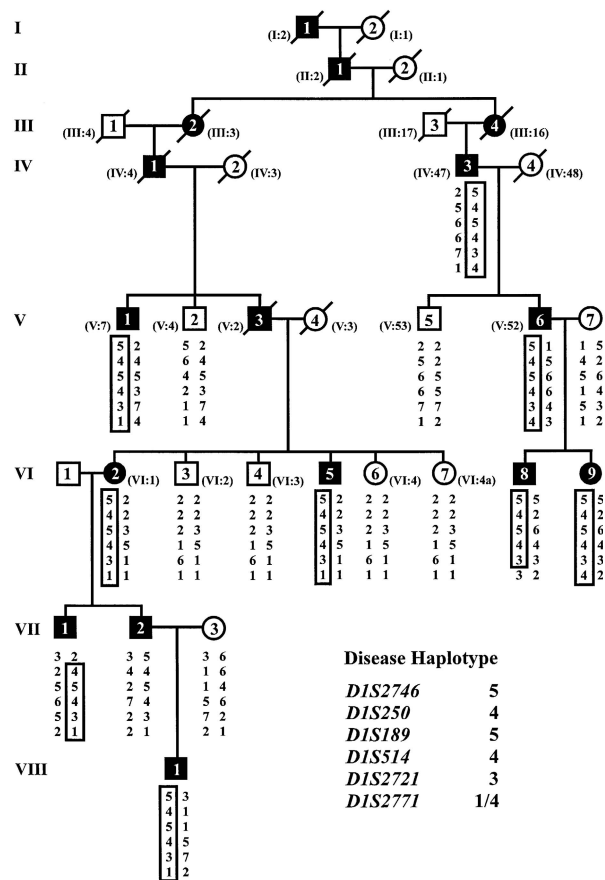


Figure 1 Abridged pedigree of the CZP1 (Ev.) family used in this study, showing segregation of six microsatellite markers on chromosome 1q, listed in descending order from the centromere. Squares and circles symbolize males and females, respectively; and unblackened and blackened symbols denote unaffected and affected individuals, respectively. Bracketed numbers denote individuals from the original Ev. pedigree constructed by Renwick and Lawler (1963).

which is more comparable in size to the nucleus of the adult lens. In addition, there is no conclusive evidence that the Coppock cataract is also linked to *FY* (Renwick 1970; also see MIM 116200).

Genotyping and Linkage Analysis

PCR-based genotyping using Génethon microsatellite markers (Dib et al. 1996) followed by linkage analysis, by means of the LINKAGE package of programs (Lathrop et al. 1984; Atwood and Bryant 1988), was performed essentially as described elsewhere (Mackay et al. 1997).

Sequencing

Exon 2 of the *GJA8* gene was amplified from genomic DNA by PCR using primers for codons 1–7 and 428–stop (table 1). PCR conditions were as follows: 1

Table 1
PCR Primers Used for Mutation Screening of the GJA8 Coding Exon

Codon	Direction	Sequence
1-7	Forward	5'-TATGGGCGACTGGAGTTTCCT
77-83	Forward	5'-CTCTGGGTGCTGCAGATCATC
97-103	Reverse	5'-CTCCATGCGGACGTAGTGAC
102-108	Reverse	5'-TGCTTTTTCGCTTCTCTCCA
169-175	Forward	5'-CACTACTTCTGTACGGGTTTC
209-215	Reverse	5'-CACAGAGGCCACAGACAACAT
257-263	Forward	5'-GTCTCCTCCATCCAGAAAGCC
312-318	Reverse	5'-CTCTTGGTAGCCCCGGGACAA
428-stop	Reverse	5'-TCATACGGTTAGATCGTCTGA

cycle at 94°C for 5 min; 30 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; and then 1 cycle at 72°C for 5 min. PCR products of appropriate size were purified from agarose gels by means of the QIAquick gel-extraction kit (Qiagen) and then were TA subcloned by means of the pGEM-T vector system II (Promega). Plasmid DNA was purified by means of the QIAprep spin miniprep kit (Qiagen), and insert DNA was sequenced by means of the DyeDeoxy-terminator *Taq* FS cycle sequencing kit (Perkin-Elmer) and an ABI 377 automated sequencer. T7 and SP6 primers were used to sequence the 5' and 3' ends of the insert, and internal primers for codons 77-83, 102-108, 169-175, 209-215, 257-263, and 312-318 (table 1) were used to obtain overlapping sequence in both directions.

Restriction Analysis

Flanking primers for codons 77-83 and 97-103 (table 1) were used to amplify the novel *MnII* site in affected individuals and to exclude the 28 other *MnII* sites within the coding exon of the wild-type *GJA8* gene. PCR prod-

ucts of appropriate size (81 bp) were purified by means of the QIAquick PCR kit and then were digested with *MnII* (0.2 U/ml) at 37°C for 1 h, and the resulting restriction fragments (39 bp and 42 bp) were separated in 4% agarose gels stained with ethidium-bromide. The exon 2 fragment (1.3 kb) used for sequencing above was digested with *SfiI* (1 U/ml) at 50°C for 2 h, and the resulting restriction fragments (620 bp and 680 bp) were separated in 1% agarose-ethidium bromide gels.

Results

CZP1 Linkage Analysis

Eighteen members of the pedigree, including 10 affected individuals, 6 unaffected individuals, and 2 spouses (fig. 1), were genotyped by means of 13 microsatellite markers from the Génethon (AC)_n map (Dib et al. 1996) of chromosome 1. The two-point maximum LOD score (Z_{\max}) (table 2) was obtained with marker *D1S2669* ($Z_{\max} = 4.52$; maximum recombination fraction [θ_{\max}] = 0). Multipoint analysis using markers *D1S2746*, *D1S2669*, *D1S442*, and *D1S2771* (fig. 2) yielded $Z_{\max} = 5.22$ ($\theta_{\max} = 0$) at *D1S2669*. These microsatellite marker-derived Z_{\max} values compare favorably with that determined elsewhere ($Z_{\max} = 3.78$; $\theta_{\max} = 0$) with the Duffy blood-group alloantigen markers (Renwick and Lawler 1963).

Haplotype analysis (fig. 1) detected one affected male (VI-8) who was recombinant with regard to marker *D1S2771* and another affected male (VII-1) who was recombinant with regard to marker *D1S2746*. Notably, the disease allele at *D1S2771* differs in each family branch descended from affected females III-2 (allele 1) and III-4 (allele 4), suggesting that a recombination event

Table 2
Two-Point Z Values for Linkage between CZP1 and Chromosome 1q Markers

MARKER	GENETIC DISTANCE ^a (cM)	LOD SCORE AT RECOMBINATION OF								Z_{\max}	θ_{\max}
		.00	.01	.05	.10	.20	.30	.40			
<i>D1S2746</i>	1.1	−∞	1.19	1.66	1.65	1.31	.84	.34	1.68	.07	
<i>D1S250</i>	.6	3.52	3.46	3.19	2.84	2.10	1.31	.52	3.52	.00	
<i>D1S189</i>	2.2	3.34	3.27	3.02	2.69	2.00	1.27	.52	3.34	.00	
<i>D1S2669</i>	1.3	4.52	4.43	4.09	3.65	2.72	2.24	.76	4.52	.00	
<i>D1S514</i>	1.1	4.48	4.40	4.07	3.64	2.73	1.77	.77	4.48	.00	
<i>D1S2696</i>	1.1	3.49	3.44	3.22	2.91	2.21	1.44	.65	3.49	.00	
<i>D1S442</i>	1.2	1.07	1.04	.95	.84	.62	.41	.20	1.07	.00	
<i>D1S2345</i>	3.3	2.23	2.16	1.90	1.57	.90	.30	.00	2.23	.00	
<i>D1S305</i>	1.6	2.34	2.28	2.05	1.76	1.17	.62	.20	2.34	.0	
<i>D1S2721</i>	1.7	3.83	3.75	3.42	2.99	2.08	1.15	.35	3.83	.00	
<i>D1S506</i>	2.7	1.11	1.09	.99	.86	.59	.33	.11	1.11	.00	
<i>D1S2635</i>	2.7	.93	.92	.86	.76	.53	.31	.13	.93	.00	
<i>D1S2771</i>		−∞	-1.84	-.57	-.14	.11	.12	.07	.13	.25	

^a Between the marker directly to the left and that directly below it; values are sex-averaged and are from Dib et al. (1996).

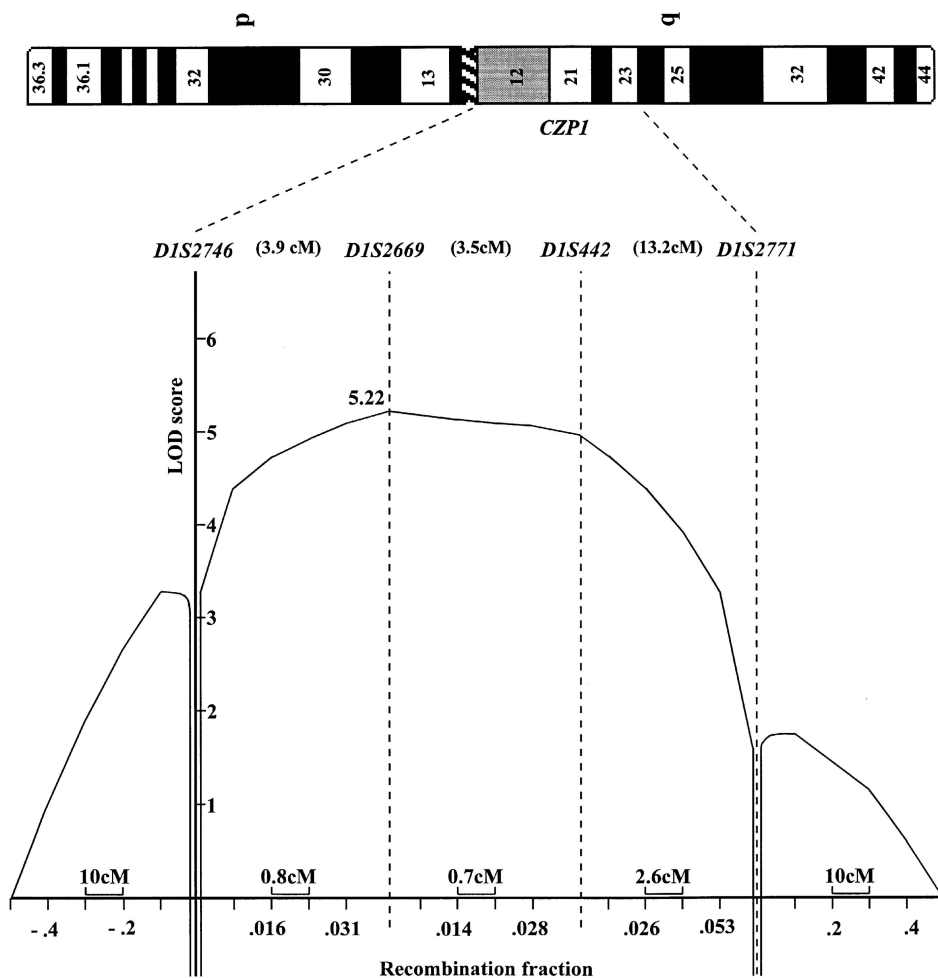


Figure 2 Multipoint linkage analysis between the *CZP1* locus and chromosome 1q microsatellite markers. An ideogram of human chromosome 1 shows the predicted cytogenetic location of *CZP1*, on the basis of both linkage to *FY* at 1q22-q23 (Mathew et al. 1994) and synteny of 1q12-q21 with *Gja8* on mouse chromosome 3 (Kerscher et al. 1995).

had also occurred in an ancestor of those individuals genotyped in this study. However, no individuals recombinant for the cataract locus and five other markers located between *D1S2746* and *D1S2771* were observed. Thus, the combined LOD-score value and haplotype data indicated that *CZP1* probably lies in the genetic interval *D1S2746*–(20.6 cM)–*D1S2771* (fig. 2).

GJA8 Sequence Analysis

In search of the *CZP1* mutation, we amplified the single protein-coding exon of the *GJA8* gene from all 18 haplotyped members of the family who are depicted in figure 1. Sequence analysis of the resulting ~1.3-kb fragments revealed four significant changes from the published human *GJA8* sequence (Church et al. 1995). First, a C→G transversion at nucleotide 330 resulted in the conservative change of aspartate (GAC) to glutamate (GAG) at codon 110 (D110E). Second, an additional

codon for alanine (GCG) was detected at nucleotides 331–333 (A111), extending the published human *GJA8* protein sequence from 432 to 433 amino acids. Both E110 and A111 were also found to be conserved in the other known *GJA8* sequences, from mouse (White et al. 1992), sheep (Yang and Louis 1996), and chicken (Jiang et al. 1994). Furthermore, these changes were present in both affected and unaffected members of the pedigree, indicating that neither D110E nor A111 is the *CZP1* mutation. Third, an A→G transition at nucleotide 673 resulted in both the nonconservative substitution of glycine (GGC) for serine (AGC) at codon 225 (S225G) and the introduction of a unique *Sfi*I restriction-enzyme site (GGCCN₄↓NGGCC). Restriction analysis confirmed that this change was present in both affected and unaffected members of the pedigree, and it was also detected in 25 unrelated normal individuals (data not shown), effectively excluding S225G as the *CZP1* mu-

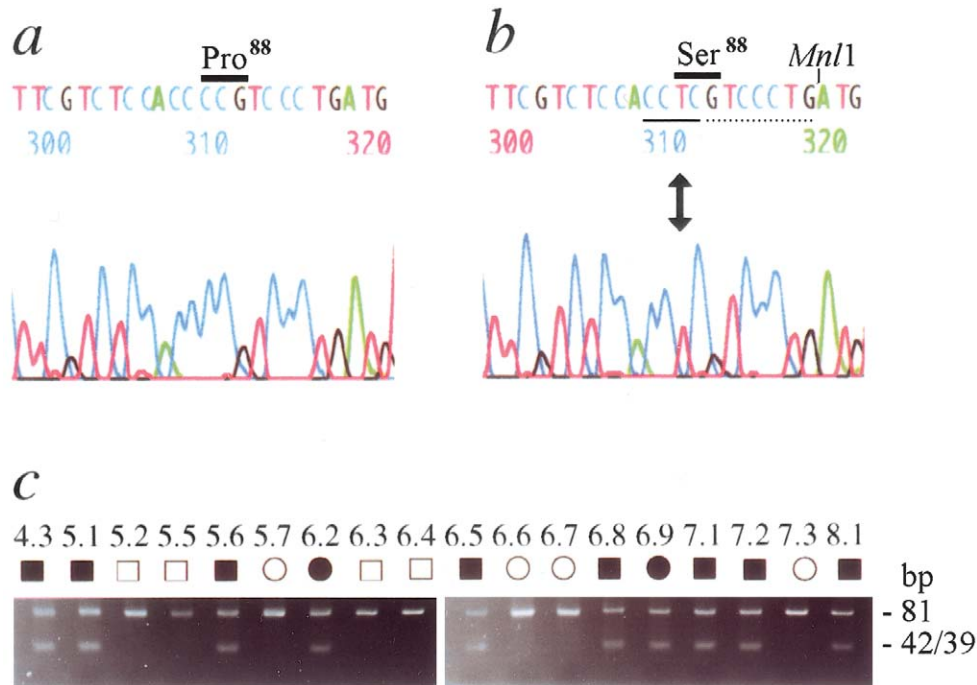


Figure 3 Sequence analysis of the *CJA8* gene from the CZP1 pedigree shown in figure 1. *a*, Wild-type allele showing a phylogenetically conserved proline (CCG) at codon 88. *b*, CZP1 allele showing a C→T transition (arrow) that results in the substitution of serine (TCG) at codon 88. *c*, Restriction-fragment-length analysis showing gain of an *MnlI* site (CCTCN₇₇ in *b*) that cosegregates with affected individuals (blackened symbols) heterozygous for the P88S mutation (81 bp and 39/42 bp) but not with unaffected individuals and spouses (81 bp).

tation. Finally, a C→T transition at nucleotide 262 resulted in both a nonconservative substitution of serine for proline at codon 88 (P88S) and the introduction of an *MnlI* restriction site (fig. 3). Restriction analysis confirmed that this change was present only in affected members of the pedigree (fig. 3), and it was not detected in 102 unrelated normal individuals (data not shown), indicating that P88S is the CZP1 mutation rather than a *GJA8* polymorphism.

Discussion

Using a combination of linkage and sequence analyses we have genetically refined the CZP1 locus to a 20-cM interval between markers *D1S2746* and *D1S2771* on the long arm of chromosome 1 and have identified a P88S missense mutation in *GJA8*, the gene for Cx50, that cosegregates with the cataract. Our data give a genetic-map location for *GJA8* and constitute the first report implicating a connexin gene in human inherited cataract.

The connexin-gene family encodes gap-junction-channel proteins that mediate the intercellular transport of small (<1 kD) biomolecules, including ions, metabolites, and second messengers in diverse vertebrate tissues such as the brain, heart, liver, and lens (for a

recent review, see Kumar and Gilula 1997). At least eight genes for connexins of varying molecular mass (~26–50 kD) have been identified in humans, and mutations in the genes for Cx26 (*GJB2*), Cx32 (*GJB1*), and Cx43 (*GJA1*) have been associated with certain forms of sensorineural deafness (Kelsell et al. 1997; Zelante et al. 1997), Charcot-Marie-Tooth neuropathy (Bergoffen et al. 1993), and viscerotaxial heterotaxia (Britz-Cunningham et al. 1995), respectively.

The P88S mutation identified here is centrally located within the putative second transmembrane domain (M2) of Cx50. Sequence comparison of all the known vertebrate connexins shows that this M2 proline is strictly conserved at either codon 87, codon 88, or codon 89, across species including the zebrafish, frog, chicken, and mouse (GenBank accession numbers 1008909, 117709, 544115, and 117716 <http://ncbi.nlm.nih.gov/entrez/>). In vitro mutagenesis of this phylogenetically conserved proline, supports the view that it functions in voltage gating of gap-junctions (Suchyna et al. 1993). Significantly, inherited P87S (Bort et al. 1997; Janssen et al. 1997) and P87A (Nelis et al. 1997) mutations in the human Cx32 gene have been associated with Schwann-cell dysfunction and peripheral nerve degeneration in X-linked dominant Charcot-Marie-Tooth disease. These obser-

vations raise the possibility that mutation of the M2 proline may impair voltage-dependent opening and closing of gap junctions in electrically excitable tissues. However, both the physiological significance of such voltage gating in the noninnervated lens and its role in cataractogenesis remain to be established.

Note added in proof.—During the preparation of the manuscript of this article, Geyer et al. (1997) reported cytogenetic mapping of the human *Cx50* gene to band region 1q21.1, and White et al. (1997) reported that *Cx50*-deficient mice develop cataract.

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