Original Article

Further evidence for P59L mutation in *GJA3* associated with autosomal dominant congenital cataract

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Context: Congenital cataracts are one of the common eye disorders leading to visual impairment or blindness in children worldwide. We found a Chinese family with autosomal dominant pulverulent cataract. **Aims:** To identify the pathogenic gene mutation in a Chinese family with autosomal dominant inherited pulverulent cataract. **Subjects and Methods:** After obtained informed consent, detailed ophthalmic examinations were carried out; genomic DNAs were obtained from seven family members in a three-generation Chinese family with three affected. All exons of candidate genes were amplified by polymerase chain reaction and were sequenced performed by bidirectional sequencing. **Results:** By sequencing the encoding regions of the candidate genes, a missense mutation (c. 176C>T) was detected in gap junction protein alpha 3 genes (*GJA3*), which resulted in the substitution of highly conserved proline by leucine at codon 59 (p.P59L). The mutation co-segregated with all patients and was absent in 100 normal Chinese controls. **Conclusions:** The study identified a missense mutation (c. 176C>T) in *GJA3* gene associated with autosomal dominant congenital pulverulent cataract in a Chinese family. It gave further evidence of phenotype heterogeneity for P59L mutation in *GJA3* associated with congenital cataract.

Key words: Congenital cataract, *GJA3*, mutation

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Congenital cataracts are one of the common eye disorders leading to visual impairment or blindness in children worldwide. Congenital cataract may be inherited or familial, either as an isolated form or as a part of a syndrome, such as Nance–Horan syndrome. Along with the development of molecular genetics, more than 20 genes have been identified to be involved in isolated cataract formation.[1]

The lens is an avascular organ which is composed of a monolayer of cuboidal epithelial cells covering the anterior surface of elongated fibers, which transmits and focuses light images onto the retina. Interior fiber cells, including both primary and secondary fiber cells, undergo a maturation process to eliminate all intracellular organelles, such as the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus, thereby minimizing light scattering and ensuring lens transparency.[2] The interior mature fibers have an extremely low metabolic activity and depend mainly on the epithelium and peripheral differential fibers for maintenance. Therefore, the lens has developed as a syncytium and a sophisticated cell-cell communication network, which facilitates both an active metabolism and transport of small metabolites, such as ions, water, and secondary messengers.[3] Intercellular gap junction channels provide pathways for metabolic and electrical coupling between cells in the lens. Gap junction channels consist of connexin (*Cx*) protein subunits. To date, many *Cx* genes have been found in the mouse genome and the human genome.[4]

Mutations in *Cx* have been identified with various inherited diseases,[5] including *Cx32* mutation in X-linked Charcot-Marie tooth disease, *Cx26* and *Cx30* mutations in deafness and skin diseases, *Cx46* and *Cx50* mutations in hereditary cataracts, and *Cx31* mutation in erythrokeratodermia variabilis and hearing impairment with/without peripheral neuropathy.

In our study, we found a missense mutation the substitution of proline to leucine of the codon 59 (p.P59L) in *GJA3* (*Cx46*) associated with autosomal dominant pulverulent cataract in a Chinese family.

Subjects and Methods

Clinical evaluation and DNA specimens

A three-generation family with autosomal dominant congenital cataract was ascertained [Fig. 1]. After explanation of nature and possible consequences of the study, seven individuals participated in the study. The study was performed with informed consent in accordance with the Declaration of Helsinki and following all the guidelines for experimental investigations required by the Institutional Review Board. The ophthalmologic examinations, including visual function and dilated slit-lamp examination, were carried out by ophthalmologists. Blood samples were collected, and leukocyte genomic DNA was extracted.

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Mutation detection

All the exons of candidate genes which associated with autosomal dominant congenital cataract were amplified by polymerase chain reaction (PCR) method, including *CRYAA*, *CRYBA1/A3*, *CRYBB2*, *CRYBB3*, *CRYGC*, *CRYGD*, *GJA3*, and *GJA8*. The primers are listed in Table 1. The PCR products were sequenced on both directions with an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were analyzed using Chromas (version 2.23) software (http://www.technelysium.com.au/chromas.html) and compared with the reference sequences in the NCBI (http://www.ncbi.nlm.nih.gov/) gene bank.

Bioinformatics analysis

GJA3 amino-acid sequences were retrieved from the Entrez protein database and aligned using the ClustalW multiple sequence alignment web servers (http://www.ebi.ac.uk/clustalw/).[6] The likely structure of the mutant *Cx46* protein was determined using MEMSAT from the PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/).[7]

Results

Clinical evaluations

We studied a three-generation Chinese pedigree segregating autosomal dominant cataract in the absence of other ocular or systemic defects [Fig. 1]. Ophthalmic records described the cataract as congenital bilateral irregular pulverulent cataract in three affected individuals (I: 2, II: 2, and III: 2) with corrected visual acuities <20/200, and the lens opacities were found at birth and progressed slowly with age; however, no slit-lamp images of the lens opacities presurgery were available. The affected individuals have had cataract surgery. Autosomal dominant inheritance mode of the cataract was supported by the presence of affected individuals in each of the three generations and equal opportunities to develop disease in female and male of each generation.

Mutation detection

By bidirectional sequencing of amplified exons of the candidate genes, we found a heterozygous missense mutation, C>T at position 176 in *GJA3* (NM_021954) in affected individuals, but not in unaffected individuals. The c. 176C>T transition occurred at the second base of codon 59 (CCG > CTG) and was predicted to result in the missense substitution of proline to leucine (p. P59L) at the level of protein translation [Fig. 2]. This mutation was not found in 100 unrelated control individuals. No other sequence variant was found.

Bioinformatics analysis

Based on the hydrophobicity profile of *GJA3*, the p.P59L substitution is likely located in the first extracellular (EC-1) loop. Cross-species alignment of *GJA3* amino-acid sequences revealed that p.P59 is phylogenetically conserved [Fig. 3].

Conclusion and Discussion

In a Chinese family with congenital pulverulent cataract, we identified a missense mutation c. 176C>T in *GJA3*, leading to the substitution of proline by leucine (p.P59L). This mutation co-segregated with the phenotype and was not found in 100 unrelated control individuals.

Cx channels play essential roles in maintaining lens cell homeostasis, metabolic coupling and preventing accumulation of reactive oxidants. *Cx* proteins have four transmembrane domains with three intracellular regions (amino terminus, cytoplasmic loop, and carboxyl terminus) and two extracellular loops (EC-1 and EC-2). Six *Cx* protein subunits oligomerize to form one connexon. A gap junction channel is formed by the docking of extracellular loops of two opposing connexons (hemichannels) in the plasma membrane. Three isoforms of the *Cx* gene family are expressed abundantly in the vertebrate lens: *GJA1* (*Cx43*), *GJA3* (*Cx46*), and *GJA8* (*Cx50*). *Cx46* is abundantly expressed in the differentiating lens fiber cells.[8] Electrophysiological studies of intact lenses confirm that *Cx46* is essential for the coupling of interior fiber cells, especially in mature fiber in the central core of the lens.[9,10] Deletion of the *GJA3* gene (encoding *Cx46*) leads to the development of cataracts in mice.[11]

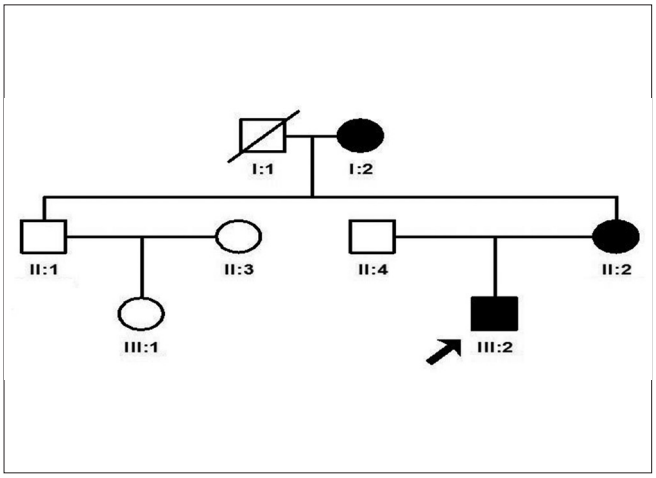

Figure 1: Pedigree of inherited cataract. Squares and circles symbolize males and females, respectively. Clear and blackened symbols denote unaffected and affected individuals, respectively. The arrow indicates the proband

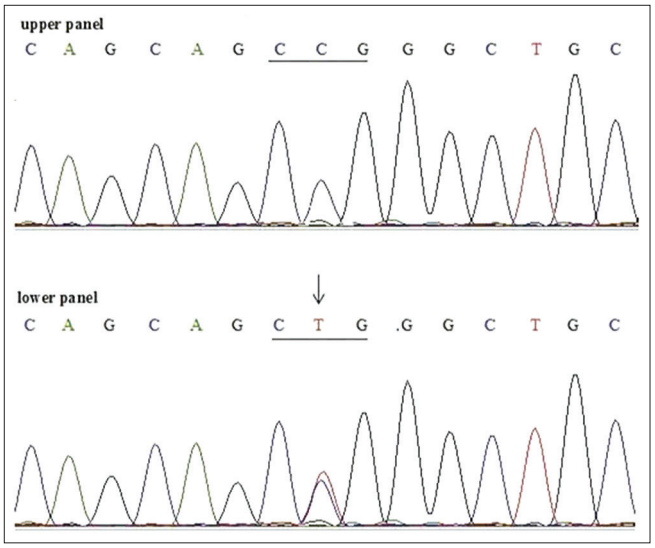

Figure 2: Forward sequence chromatogram of *GJA3*. The arrow indicates the C>T transition. The upper panel is unaffected, the lower panel is affected. The encoded amino acid at codon 59 (underlined) is indicated, CCG encodes proline (P), and CTG encodes leucine (L)

Table 1: Primers for polymerase chain reaction amplification of exons of candidate genes and the size of the polymerase chain reaction products

Gene	Exon		Primers sequence (5'-3')	Fragment size (bp)
CRYAA	1	Forward	ACTTGTCCCAGCCACGTTT	515
		Reverse	CTCTGCAAGGGGATGAAGTG	
	2	Forward	AACCAGCCACCTGACCATAG	637
		Reverse	ACATTAGCTCGGGAATGGTG	
	3	Forward	CAGGGGCATGAATCCATAAA	551
		Reverse	TAAGCTCTCCTGGCTGCTCT	
CRYBA1/A3	1	Forward	GGGCCTCTCTGGATTTCTGT	404
		Reverse	GCTAGGGCAGTGGTTATTGC	
	2	Forward	GCAGAGGTTGCAGTGAAGTG	516
		Reverse	CAATGGCATCCACAGTCATC	
	3	Forward	ACTCTGGGCAAATGAACACC	547
		Reverse	TCTTTATCCAGCCCCTGAAA	
	4	Forward	CCTGTCAACTCATTCCTCAACTC	557
		Reverse	TGGGCTCTTGAGTATCCACTT	
	5	Forward	TGGTTGGCTGCATTTTGTTA	609
		Reverse	GCATGTCTGGGGGAGATAAA	
	6	Forward	CCCCCAGACATGCCTCTAT	648
		Reverse	TTACACTCCAGCCTGAGCAA	
CRYBB2	1	Forward	CAGAGGGGAGTGGTCTCAAG	540
		Reverse	CAAAGCCAGAGGCTGGTACT	
	2	Forward	AGAGGAGAAATGCAGGCTCA	600
		Reverse	GCAGACAGGAGCAAGGGTAG	
	3	Forward	ATGGAAATTGGCAAACGCTA	586
		Reverse	TCCTGGTCCCCAGACCTCCA	
	4	Forward	TAGACACGTAGTGGGTGCAC	405
		Reverse	CAGAGGTCAGCAGAGCACAC	
	5	Forward	TATCACCCCCTTGCTCTGAC	1105
		Reverse	CCCCTGAGAGTGACTGTGCT	
CRYBB3	1	Forward	GAGCCTCAGAGTTCCCCTCT	512
		Reverse	GCAGCAAAGTCATGAAGCAA	
	2	Forward	TGAAGTTCCTGAAGGCGTTT	501
		Reverse	AGGTATCCTGGGATTTTCTGC	
	3	Forward	TTCCCGGTATGTCCTAGCAG	511
		Reverse	CTGGTGTCTCAATCCCCAAC	
	4	Forward	ATCAACCAGCTTTGGAGGAA	527
		Reverse	CTTGCAGTGAGCTGAGATCG	
	5	Forward	ACGGTGTTGAGTGTGAATGG	679
		Reverse	GGCTCTGCCTGAAAGGATTA	
CRYGC	1	Forward	TTTCCAGTGAATGCAGGATG	672
		Reverse	TCTGCTGTTTTTGTGCATGTT	
	2	Forward	CGCAGCAACCACAGTAATCT	641
		Reverse	CAACGTCTGAGGCTTGTTCA	
CRYGD	1	Forward	GAGAGAATGCGACCAAAACC	742
		Reverse	GCTTATGTGGGGAGCAAACT	
	2	Forward	TGTGCTCGGTAATGAGGAGTT	586
		Reverse	CACATCTTGGTTGCCATTTG	
GJA3	1	1 forward	TTGTGTAGTGCCTGCTCGTC	711
		1 reverse	AGCTCGAAGCCGTACAGAAA	

Contd...

Table 1: Contd...

Gene	Exon		Primers sequence (5'-3')	Fragment size (bp)
		2 forward	AAAGAGAGGGAGGAGGAGGA	668
		2 reverse	GCCCAGTTCTGCTCAGTCAT	
		3 forward	GCTGGAAGAAGCTCAAGCAG	803
		3 reverse	AAGCATTGAACACGGAAACC	
GJA8	1	1 forward	TCTGCACAAAGGAAGCACTG	690
		1 reverse	CGGAACCCGTACAGGAAGTA	
		2 forward	GTGCTGCAGATCATCTTCGT	825
		2 reverse	TGCTTCCTCCTTCTTCTCTCC	
		3 forward	TGAGAAATCCCTCCACTCCA	752
		3 reverse	GTAGCCCTTATGCTGGATGC	

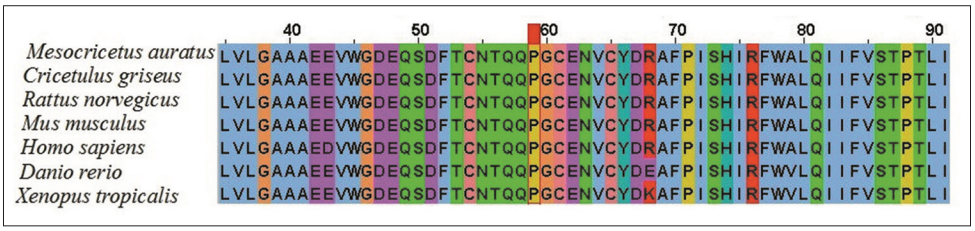

Figure 3: Clustal 2.1 multiple sequence alignment of *Cx46*

Many mutations of *Cx46* have been reported to be associated with congenital cataract with different phenotype in human. To date, 25 mutations in the different domain of *Cx46* have been identified to contribute to human inherited cataracts. Interestingly, most of the *Cx46* mutations associated with cataracts are located in transmembrane and extracellular loop domains. Based on the hydrophobicity profile of *GJA3*, the p.P59L substitution is located in EC-1. The amino-acid positions are highly conserved in humans. Extracellular domains of C*xs*, containing EC-1 and EC-2, play a key role in both mediating hemichannel docking[12,13] and regulating of voltage gating of the channel.[14] Electrophysiological studies of *Cx* mutants in *Xenopus* oocytes showed charged residues in EC-1 facing the channel lumen and playing an important role in determining *Cx* channel conductance and selectivity.[15]

The p.P59L substitution was reported in USA, Danish, and Chinese family. Bennett *et al*. reported first the c. 176C>T mutation in *GJA3* underlying autosomal dominant nuclear punctate cataracts in a six-generation Caucasian American pedigree in 2004.[16] They speculated that the p.P59L substitution operated at the *Cx* (monomer) level prior to connexon (hexamer) formation, perhaps as a result of impaired targeting to the cell surface, accelerated degradation in a manner similar to that of the N63S mutant.[8,17] Then, Hansen *et al*. found this mutation in a Danish family with hereditary congenital cataract in a cohort study by comprehensive mutational screening in 2009.[18] Sun *et al*. analyzed 12 genes in Chinese families with congenital cataracts and found the c. 176C>T in *GJA3* that was not present in 96 controls and was predicted to be pathogenic with online bioinformatics tools - polymorphism phenotyping 2 and sorting intolerant from tolerant at the protein level.[19] The p.P59L mutation of *Cx46* might impair gap junction between lens fiber cells and lead to the development of cataract. The detailed phenotype was not described in the last two families. In the American family, the phenotype was described as nuclear punctate cataracts. In this study, the phenotype was described as bilateral irregular pulverulent cataracts according to the ophthalmic records. Our study gave further evidence of phenotype heterogeneity for P59L mutation in *GJA3* associated with congenital cataract.

Minogue *et al*. found p.G46V in EC-1 of *Cx50* associated with congenital total cataracts, which forms normal gap junctions.[20,21] Expression of this mutant increases the proportion of apoptotic cells and causes cell death,[21] suggesting that opening of the hemichannels would also cause severe cell damage *in vivo*. Moreover, Ren *et al*. found that the p.G143R mutation on *Cx46* associated with congenital Coppock cataracts has the increase of hemichannel activity, except for the reduction of gap junction.[22] It is inferred that the increased hemichannel activity of this mutant is associated with decreased cell viability by disruption of intracellular microenvironment through a complex sequence of events including activation of kinases, imbalances of redox potentials, and accumulation of calcium.[23,24] This combination of the reduction of gap junction channel function and the increased hemichannel function is contributed to the development of human congenital cataracts.

The hemichannel function of the p.P59L mutation in *Cx46* should be further detected. The precise way in which this kind of mutations of *Cxs* causing cataract represents the next challenge in understanding the basis of *Cx*-mediated cataractogenesis.

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Conflicts of interest

There are no conflicts of interest.

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