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

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/). The likely structure of the mutant Cx46 protein was determined using MEMSAT from the PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/).

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. 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. Deletion of the GJA3 gene (encoding Cx46) leads to the development of cataracts in mice.
| Gene       | Exon | Primers sequence (5’-3’) | Fragment size (bp) |
|------------|------|--------------------------|--------------------|
| CRYAA      | 1    | Forward ACTTGTCCCAGCCACGTTT | 515                |
|            |      | Reverse CTCTGCAAGGAGATGGAATG |                    |
|            | 2    | Forward AACCAGCCACCTAGCATAG | 637                |
|            |      | Reverse ACATAGCTCGGAGATGGTG |                    |
|            | 3    | Forward CAGGGGCAATCTCAAATAA | 551                |
|            |      | Reverse TAAGCTCTCGTGGCATCATC |                    |
| CRYBA1/A3  | 1    | Forward GGCCCTCTGGATTTCTGT | 404                |
|            |      | Reverse GCTAGGGCAATGGTTATGC |                    |
|            | 2    | Forward GCAGAGGTGGCATGGAATG | 516                |
|            |      | Reverse CAATGGCATCCACGTACAC |                    |
|            | 3    | Forward ACTTGTGGCAATGAACACC | 547                |
|            |      | Reverse TCTTTATCCAGCCCCTGAA |                    |
|            | 4    | Forward CCGTTCAACTTCCTCAACTC | 557                |
|            |      | Reverse TGGGCTCTGGATATCCACTT |                    |
|            | 5    | Forward TGTTGGCTGGATTGGTGTA | 609                |
|            |      | Reverse GCATGCTGGGGAGATGAAA |                    |
|            | 6    | Forward CCCGAGACATGCCCTCAT | 648                |
|            |      | Reverse TTACACCTGAGGCGGACCA |                    |
| CRYBB2     | 1    | Forward CAGAGGGAGTGGTGCTCAAG | 540                |
|            |      | Reverse CAAAAGCAGAGGCGTGGTACT | | |
|            | 2    | Forward AGAGGAGGAAATGCAAGCTCA | 600                |
|            |      | Reverse GCAGACAGGAAGCGAGTAGA |                    |
|            | 3    | Forward ATGAAATGGCCACCGCTTA | 586                |
|            |      | Reverse TCCCTGGCCACGAGCCCCTC |                    |
|            | 4    | Forward TAGACACGTAATGGTGTCAC | 405                |
|            |      | Reverse CAGAGGTCAAGAGCACAC |                    |
|            | 5    | Forward TATCACCCCCCTTGGCTGAC | 1105               |
|            |      | Reverse CCCCTGAGAATGCCTTGGT |                    |
| CRYBB3     | 1    | Forward GACGCCTCAGAGTCCCTCT | 512                |
|            |      | Reverse GCAGCAGATCAGCAAGCAA |                    |
|            | 2    | Forward TGAAGTTCTGAGGCTTTGTT | 501                |
|            |      | Reverse AGGTATCTGGGATTTTCTGC |                    |
|            | 3    | Forward TCCCGGTATGTGCTGAGCAG | 511                |
|            |      | Reverse CTGGTGTCGCTGCTGCCAAC |                    |
|            | 4    | Forward ATCACCAGGTTGGGAGGAA | 527                |
|            |      | Reverse CCTGCGCTGAGCCTGACAAG |                    |
|            | 5    | Forward ACGTGTTGAGTGGTGAATGG | 679                |
|            |      | Reverse GGCTCTGACCTGAAAGGATTA |                    |
| CRYGC      | 1    | Forward TTTTGCTGAGATGCGATG | 672                |
|            |      | Reverse TCTGCTGTGGTGGCATGTG |                    |
|            | 2    | Forward CGACGACACAGTGAATCCT | 641                |
|            |      | Reverse ACAAAGTGAGCGCCCTGATC |                    |
| CRYGD      | 1    | Forward GAGAGAATGGCAACGCAAA | 742                |
|            |      | Reverse GCTTATGGGGGAGCAAACCT |                    |
|            | 2    | Forward TGTGCTGCTGATGGAGAGTT | 586                |
|            |      | Reverse CATACCTGTTGGGTCACATTTG |                    |
| GJA3       | 1    | 1 forward TTGTTAGTGGCTGCTCGTC | 711                |
|            |      | 1 reverse AGCTGAGGGGCCAGCAAAA |                    |

Contd...
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 Cxs, 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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