Genetic variations in GJA3, GJA8, LIM2, and age-related cataract in the Chinese population: a mutation screening study

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Purpose: To investigate the role of genetic variations in three known cataract-associated genes, gap junction protein α3 (GJA3), gap junction protein α8 (GJA8), lens intrinsic membrane protein 2 (LIM2), encoding lens fiber cell membrane proteins in the development of age-related cataracts.

Methods: One hundred and forty-five sporadic age-related cataract patients and one hundred and fifty-six unrelated random healthy controls participated in this study. Genomic DNA was extracted from peripheral blood leukocytes. All exons of GJA3, GJA8, and LIM2 were sequenced after being amplified by polymerase chain reaction (PCR). The functional consequences of the mutations were analyzed using PolyPhen.

Results: We found five novel variations in 145 patients and none of them presented in the 156 controls. There are two variations in GJA3 (c.-39C>G, c.415G>A); one in GJA8 (c.823G>A), and two in LIM2 (c.57G>A, c.67A>C). PolyPhen predicted that the LIM2 c.67A>C mutation may have potential pathogenicity.

Conclusions: The genetic mutation in GJA3, GJA8, and LIM2 may slightly contribute to the development of age-related cataracts. This study showed a potential relationship between lens fiber cell membrane protein genes and the development of age-related cataracts in the Chinese population.

Age-related cataract (ARC) is the leading cause of low vision and blindness in Asia [1,2]. As the world’s population ages, cataract-induced visual dysfunction and blindness is on the increase [3]. Several studies have identified the risk factors for cataract, including being of the female sex, having lower socioeconomic status, having diabetes mellitus, smoking, and lower body mass index [4-6]. Recently, some twin studies provided evidence for the contribution of genetic factors in the pathogenesis of ARC. In 2000 and 2001, Hammond et al. recognized that the heritability for age-related cortical cataract was 53%–58% [7] and approximately 48% for nuclear cataract [8].

In the past few years, attempts have been made to identify new loci for ARC. Until now, at least nine genes associated with congenital cataract were proven to link to ARC, including EPH receptor A2 (EPHA2, 1p) [9], gap junction protein α8 (GJA8, 1q) [10], galactose-1-phosphate uridyltransferase (GALT, 9p) [11], solute carrier family 16, member 12 (monocarboxylic acid transporter 12; SLC16A12, 10q) [12], heat shock transcription factor 4 (HSF4, 16q) [13], galactokinase 1 (GALK1, 17q) [14], ferritin light polypeptide (FTL, 19q) [15], crystallin αA (CRYAA, 21q) [16], and crystallin βB2 (CRYBB2, 22q) [17]. However, the genes associated clearly with adult-onset cataract are still few compared to those with congenital cataract. There was a theory that mutations which severely disrupted the lens cell architecture or environment might produce congenital cataract, while other relatively mild mutations might contribute to age-related cataract [18]. According to this hypothesis we selected some candidate genes from those associated with congenital cataract as our objects. Gap junction protein α3 (GJA3), gap junction protein α8 (GJA8), and lens intrinsic membrane protein 2 (LIM2) are genes that encode proteins on the lens fiber cell membrane. These proteins play a decisive role in the growth and differentiation of lens fiber cells as well as in the maintenance of eye lens transparency.

In this work, we screened all exons and the flanking sequences of GJA3, GJA8, and LIM2 in a total of 301 case-control individuals and found some novel mutations. Meanwhile, some of the mutations may have potential pathogenicity, since they may disrupt the process of transcription or translation or the normal function of the translation product.

METHODS

Patients and controls: One hundred and forty-five patients with age-related cataracts were collected during our clinical work. Cataract diagnosis was determined according to lens
We conducted according to the principles in the Declaration of Helsinki. All experiments were approved by the Institutional Review Board of Harbin Medical University (Harbin, China) and were performed in accordance with the principles set out in the Declaration of Helsinki. Informed consents were obtained from all subjects explaining the nature and possible consequences of the study. We paid a return visit to the ophthalmic examination.

DNA analysis: Five milliliters samples of venous blood were collected in EDTA vascutainers (BD, San Jose, CA) from ARC patients and control subjects. Genome DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kits (QIAGEN Science, Germantown, MD). The primers (Table 1) for polymerase chain reaction (PCR) were designed using Primer3 according to the reference sequences in the NCBI Gene database. We sequenced the PCR products with an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA), and analyzed the sequencing results using Lasergene SeqMan (DNASTAR, Madison, WI).

**Simulation for functional change in coding nonsynonymous SNPs (nsSNPs):** PolyPhen online software, which is based on the position-specific independent counts (PSIC) score derived from multiple sequence alignments of observations [20], was used to investigate the possible impact of all nonsynonymous changes on the structure and function of GJA3, GJA8, and LIM2. PolyPhen scores of >2.0 indicate that the variation is likely damaging to protein function; scores of 0.5–2.0 are possibly damaging; and scores of <0.5 are likely benign.

**Cross species conservation analysis:** We downloaded the amino acid sequences from the NCBI HomoloGene database and evaluated the cross species conservation of the nonsynonymous mutations using Lasergene MegAlign (DNASTAR).

### RESULTS

**Characteristics of patients:** A total of 301 unrelated subjects were recruited in this study. The cohort consisted of 145 unrelated cases with age-related cataracts and 156 random control subjects. The mean age of the cases was 70.77±8.46 and that of controls was 50.72±11.82.

**Identification of novel mutations:** We found five novel sequence changes in 145 patients and none of them presented in the 156 controls. In **GJA3**, we found two variations c.−39C>G and c.415G>A lying in the 5′ UTR and the exon, respectively (Figure 1A). Among them, the nonsynonymous change c.415G>A leads to p.V275I. In **GJA8**, a 70-year-old woman with cortical cataracts was found to carry a heterozygous missense mutation c.823G>A (Figure 1B) that leads to p.V275I. In **LIM2**, a 56-year-old women with cortical cataracts was found to carry a heterozygous sequence alteration c.67A>C that leads to p.M23L; meanwhile, another 62-year-old woman carried a heterozygous synonymous mutation c.57G>A (Figure 1C). There was no other mutation in these three candidate genes.

The number, gender, age, and clinical type (both eyes) of patients who carried genetic variations were listed in Table 2. After the laboratory work, we paid a return visit to the mutation carrying patients and confirmed they had no family history of cataracts.

**Effects of nonsynonymous changes and cross species conservation by in silico analysis:** Using PolyPhen, p.M23L in **LIM2** is predicted to be possibly damaging, while p.V139M in **GJA3** and p.V275I in **GJA8** are predicted to be benign. The scores and results of PolyPhen are listed in Table 3.

We aligned the amino acid sequences of **GJA3**, **GJA8**, and **LIM2** from several species. The valine at codon 139 in **GJA3** is medium conserved (Figure 2A) and the valine at position 275 in **GJA8** is highly conserved (Figure 2B). In **LIM2**, tyrosine is more conservative in other species compared with methionine at codon 23 in human (Figure 2C).

| Primer name | Forward (5′-3′) | Reverse (5′-3′) |
|-------------|----------------|----------------|
| GJA3–1      | TGGCCATACGCCCATCCCCATCCAGTA | AGCCACCTCGAACAGTCTTTGA |
| GJA3–2      | CTACCTGCGGCACTGGCTGC | GCTTGGCCGACTGGCCTTTT |
| GJA3–3      | TCGGGTTCAACTCCCTACTAT | TATCTGTTGGGAAATGTC |
| GJA8–1      | CCGGTATAGCAAAAAACAGAT | GCTGCTTACAGCCCTTTC |
| GJA8–2      | TGGCCCTCTGTGCTCCCTATTC | GTTGCCACCTCTCTTCA |
| LIM2–1      | CATCCTCCTTTCTCCAAGCAC | ACCTCTGAAGCGTCGAGAA |
| LIM2–2      | GGTGTGGGGGTTGTTATGAC | GGTTGAGTTGAGGAGGAG |
| LIM2–3      | CACCCCTTTCCCACACCTTA | CACAAACCCACAGTCCAGAA |
DISCUSSION

In general, maintenance of an intact, transparent lens requires the balanced homeostasis of metabolic components [21]. The proteins on the membrane of lens fiber cells, such as connexins, are crucial for the maintenance of the homeostasis in the whole lens.

**GJA3** and **GJA8** encode gap junction protein α3 (connexin 46) and α8 (connexin 50), which express on the lens fiber cell membrane. Gap junctions construct an extensive network, which is vital for the maintenance of osmotic and metabolic balance in the avascular lens [22]. Defects in **GJA3** and **GJA8** have previously been reported to cause cataracts in humans and mice [23,24] whereas previous studies of congenital cataract associated with connexin 46 did not find any change in the intracellular loop (CL) of the protein. In this study, we found a 76-year-old man with cortical cataracts carried a heterozygous mutation c.415G>A that lead to p.V139M lying in the middle of CL. It is the first report about an amino acid residue change in the intracellular loop of connexin 46. However, we still need more experiments to figure out how this mutation affects cataract formation.

In **GJA8**, the mutation we detect is located in the COOH-terminus. Now, few mutations in connexin genes that have been reported to be associated with cataracts lay in the COOH-terminus. Indeed, removing the COOH-terminus (139–150 amino acids) of connexin 50 did not inhibit the formation of homotypic or heterotypic channels, it only caused a loss of pHi sensitivity and a decrease in conductance [25,26]. In 2008, Schmidt et al. [27] found a homozygous insertion of one G after position 776 of **GJA8**, leading to a frame shift and 123 novel amino acids, causing a recessive triangular cataracts in a German family. Polyakov et al. [28] found a mutation (I274M) in a Russian family with zonular pulverulent cataracts in 2001, but in 2009 this allele was thought to be a rare polymorphism, not a cataract-causing mutation [29]. Yan et al. [30] found a mutation (S276F) causing a dominant congenital pulverulent nuclear cataract in a Chinese family in 2008. These findings suggested that mutation in the COOH-terminus of connexin 50 may somehow interfere with the
normal function of the gap junction and lead to cataract. The mutation V275I we found in the present study is between I274M and S276F in the COOH-terminus of connexin 50. The high conservation of valine at codon 275 from zebrafish to humans (Figure 2B) indicates the importance of this residue.

The product of LIM2 is a 173 amino acids membrane protein, with four intramembrane domains [31], expressed mainly in the cortical region of the lens [32]. The function of this protein remains unknown. Taylor et al. [33] suggested that it has an important role in the switch from proliferation to differentiation and in the maintenance of the cells in the differentiated state. Mutations in LIM2 were related to autosomal recessive congenital cataracts in humans. Simultaneously, the heterozygous To3 mice developed congenital total cataracts, but in the homozygous state they also appear microphthalmia [34]. The semidominant pattern of the To3 mutation highlights the genetic complexity of LIM2 in the formulation of cataracts [35]. In our study, the synonymous and nonsynonymous mutations were found in both female patients with cortical cataracts; their ages were 62 and 56 years old. The nonsynonymous mutation (c.67A>C) that leads to the alteration of methionine to leucine at codon 23 of LIM2 was predicted as potentially damaging to the protein’s function by PolyPhen, although the methionine was not a conserved amino acid residue among species (Figure 2C).

On one hand, the mutations in the coding region may affect the normal function of the protein more obviously; on the other hand, the variations in untranslated region are also important to the translational regulation [36,37].

Figure 2. Cross species conservation analysis. The black bars highlight the interesting positions of the proteins. A: Multiple alignment indicates that valine at position 139 in gap junction protein α3 (GJA3) is medium conserved. B: Valine at codon 275 in gap junction protein α8 (GJA8) is highly conserved. C: At lens intrinsic membrane protein 2 (LIM2) position 23, methionine was not a conserved amino acid residue compared to threonine.
of the regulation of the translational machinery leads to perturbed cellular metabolism and may shift the physiologic balance from healthy to diseased states, such as breast cancer, Alzheimer disease, bipolar affective disorder, fragile X-syndrome, or others [38]. Meanwhile, synonymous changes may also contribute to the development of human diseases [39]. As such, the variation c. −39C>G in the 5′UTR of GJA3 and the synonymous mutation c.57G>A in LIM2 may affect the normal process of translation. Unfortunately, due to a lack of lens tissues, we could not examine the effect of these mutations on the RNA level.

Nonetheless, ARC is considered a multi-factorial disease, where environmental components as well as genetic predisposition contribute to the development of the pathological condition. This study revealed a slight association between the three lens fiber cell membrane proteins genes (GJA3, GJA8, and LIM2) and age-related cataract development in the Chinese population. We need further studies to find out the precise mechanisms by which genetic mutations of these genes influence the natural history of age-related cataract development.

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REFERENCES

1. Wong TY, Loon SC, Saw SM. The epidemiology of age related eye diseases in Asia. Br J Ophthalmol 2006; 90:506-11. [PMID: 16547337]
2. Xu L, Wang Y, Li Y, Wang Y, Cui T, Li J, Jonas JB. Causes of blindness and visual impairment in urban and rural areas in Beijing: the Beijing Eye Study. Ophthalmology 2006; 113:1134.e1-11. [PMID: 16647133]
3. Brian G, Taylor H. Cataract blindness—challenges for the 21st century. Bull World Health Organ 2001; 79:249-56. [PMID: 11222516]
4. Tsai SY, Hsu WM, Cheng CY, Liu JH, Chou P. Epidemiologic study of age-related cataracts among an elderly Chinese population in Shih-Pai, Taiwan. Ophthalmology 2003; 110:1089-95. [PMID: 12799231]
5. Nirmalan PK, Krishnadas R, Ramakrishnan R, Thulasiraj RD, Katz J, Tielsch JM, Robin AL. Lens opacities in arual population of southern India: the Aravind Comprehensive Eye Study. Invest Ophthalmol Vis Sci 2003; 44:4639-43. [PMID: 14578379]
6. Krishnaiah S, Vilas K, Shamanna BR, Rao GN, Thomas R, Balasubramanian D. Smoking and its association with cataract: results of the Andhra Pradesh eye disease study from India. Invest Ophthalmol Vis Sci 2005; 46:58-65. [PMID: 15623755]
7. Hammond CJ, Duncan DD, Snieder H, de Lange M, West SK, Spector TD, Gilbert CE. The heritability of age-related cortical cataract: the twin eye study. Invest Ophthalmol Vis Sci 2001; 42:601-5. [PMID: 11222516]
8. Hammond CJ, Snieder H, Spector TD, Gilbert CE. Genetic and environmental factors in age-related nuclear cataracts in monoyzotic and dizygotic twins. N Engl J Med 2000; 342:1786-90. [PMID: 10853001]
9. Jun G, Guo H, Klein BE, Klein R, Wang JJ, Mitchell P, Miao H, Lee KE, Joshi T, Buck M, Chugha P, Bardenstein D, Klein AP, Bailey-Wilson JE, Gong X, Spector TD, Andrew T, Hammond CJ, Elston RC, Iyengar SK, Wang B. EPHA2 Is Associated with Age-Related Cortical Cataract in Mice and Humans. PLoS Genet 2009; 5:e1000584. [PMID: 19649315]
10. Liu Y, Ke M, Yan M, Guo S, Mothobi ME, Chen Q, Zheng H. Association between gap junction protein-alpha 8 polymorphisms and age-related cataract. Mol Biol Rep 2011; 38:1301-7. [PMID: 20582632]
11. Karas N, Gobec L, Pfeifer V, Mlinar B, Battelino T, Lukac-Bajal J. Mutations in galactose-1-phosphate uridytransferase gene in patients with idiopathic presenile cataract. J Inherit Metab Dis 2003; 26:699-704. [PMID: 14707519]
12. Zuercher J, Neidhardt J, Magyar I, Labs S, Moore AT, Tanner F, Waseem N, Schorderet DF, Munier FL, Bhattacharya S, Berger W, Kloeckener-Gruissem B. Alterations of the 5′untranslated leader region of SLC16A12 lead to age-related cataract. Invest Ophthalmol Vis Sci 2010; 51:3354-61. [PMID: 18941546]
13. Shi Y, Shi X, Jin Y, Miao A, Bu L, He J, Jiang H, Lu Y, Kong X, Hu L. Mutation screening of HSF4 in 150 age-related cataract patients. Mol Vis 2008; 14:1850-5. [PMID: 18941546]
14. Okano Y, Asada M, Fujimoto A, Ohtake A, Murayama K, Hsiao KJ, Cheo K, Yang Y, Cao Q, Reichardt JK, Nihiira S, Immamura T, Yamano T. A genetic factor for age-related cataract: identification and characterization of a novel galactokinase variant, “Osaka,” in Asians. Am J Hum Genet 2001; 68:1036-42. [PMID: 11231902]
15. Faniello MC, Di Sanzo M, Quaresima B, Nistico A, Fregola A, Grosso M, Cuda G, Costanzo F. Bilateral cataract in a subject carrying a C to A transition in the L ferritin promoter region. Clin Biochem 2009; 42:911-4. [PMID: 19254706]
16. Bhagyalaxmi SG, Srinivas P, Barton KA, Kumar KR, Vidyavathi M, Petrasch JM, Bhanuprakash Reddy G, Padma T. A novel mutation (F71L) in alphaB-crystallin with defective chaperone-like function associated with age-related cataract. Biochim Biophys Acta 2009; 1792:974-81. [PMID: 19595763]
17. Zhang J, Li J, Huang C, Xue L, Peng Y, Fu Q, Gao L, Zhang J, Li W. Targeted knockout of the mouse betaB2-crystalline gene (Crybb2) induces age-related cataract. Invest Ophthalmol Vis Sci 2008; 49:5476-83. [PMID: 18719080]
18. Hejtmanck J, Kantoorow M. Molecular genetics of age-related cataract. Exp Eye Res 2004; 79:3-9. [PMID: 15183095]
19. Chylack LT Jr, Wolfe JK, Singer DM, Leske MC, Bellmore MA, Bailey IL, Friend J, McCarthy D, Wu SY. The Lens
Opacities Classification System III. Arch Ophthalmol 1993; 111:831-6. [PMID: 8512486]

20. Sunyaev S, Ramensky V, Koch I, Lathe W III, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Hum Mol Genet 2001; 10:591-7. [PMID: 11230178]

21. Truscott RJ. Age-related nuclear cataract: a lens transport problem. Ophthalmic Res 2000; 32:185-94. [PMID: 10971179]

22. Mathias RT, White TW, Gong X. Lens gap junctions in growth, differentiation, and homeostasis. Physiol Rev 2010; 90:179-206. [PMID: 20086076]

23. Xu X, Ebihara L. Characterization of a mouse Cx50 mutation associated with the No2 mouse cataract. Invest Ophthalmol Vis Sci 1999; 40:1844-50. [PMID: 10393059]

24. Arora A, Minogue PJ, Liu X, Reddy MA, Ainsworth JR, Bhattacharya SS, Webster AR, Hunt DM, Ebihara L, Moore AT, Beyer EC, Berthoud VM. A novel GJA8 mutation is associated with autosomal dominant lamellar pulverulent cataract: further evidence for gap junction dysfunction in human cataract. J Med Genet 2006; 43:e2. [PMID: 16397066]

25. Xu X, Berthoud VM, Beyer EC, Ebihara L. Functional role of the carboxyl terminal domain of human connexin 50 in gap junctional channels. J Membr Biol 2002; 186:101-12. [PMID: 11944087]

26. DeRosa AM, Mui R, Srinivas M, White TW. Functional characterization of a naturally occurring Cx50 truncation. Invest Ophthalmol Vis Sci 2006; 47:4474-81. [PMID: 17003442]

27. Schmidt W, Klopp N, Illig T, Graw J. A novel GJA8 mutation causing a recessive triangular cataract. Mol Vis 2008; 14:851-6. [PMID: 18483562]

28. Polyakov AV, Shagina IA, Khlebnikova OV, Evgrafov OV. Mutation in the connexin 50 gene (GJA8) in a Russian family with zonular pulverulent cataract. Clin Genet 2001; 60:476-8. [PMID: 11846744]

29. Graw J, Schmidt W, Minogue PJ, Rodriguez J, Tong JJ, Klopp N, Illig T, Ebihara L, Berthoud VM, Beyer EC. The GJA8 allele encoding Cx50I247M is a rare polymorphism, not a cataract-causing mutation. Mol Vis 2009; 15:1881-5. [PMID: 19756179]

30. Yan M, Xiong C, Ye SQ, Chen Y, Ke M, Zheng F, Zhou X. A novel connexin 50 (GJA8) mutation in a Chinese family with a dominant congenital pulverulent nuclear cataract. Mol Vis 2008; 14:418-24. [PMID: 18334966]

31. Arneson ML, Louis C. Structural arrangement of lens fiber cell plasma membrane protein MP20. Exp Eye Res 1998; 66:495-509. [PMID: 9593642]

32. Tenbroek E, Arneson M, Jaris L, Louis C. The distribution of the fiber cell intrinsic membrane proteins MP20 and connexin 46 in the bovine lens. J Cell Sci 1992; 103:245-57. [PMID: 1331134]

33. Taylor V, Welcher AA, Program AE, Suter U. Epithelial membrane protein-1, peripheral myelin protein22, and lens membrane protein 20 define a novel gene family. J Biol Chem 1995; 270:28824-33. [PMID: 7499407]

34. Steele EC Jr, Kerscher S, Lyon MF, Glenister PH, Favor J, Wang J, Church RL. Identification of a mutation in the MP19 gene, Lim2, in the cataractous mouse mutant To3. Mol Vis 1997; 3:5. [PMID: 9238094]

35. Steele EC Jr, Wang JH, Lo WK, Saperstein DA, Li X, Church RL. Lim2(To3) transgenic mice establish a causative relationship between the mutation identified in the lim2 gene and cataractogenesis in the To3 mouse mutant. Mol Vis 2000; 6:85-94. [PMID: 10851259]

36. Reynolds PR. In sickness and in health: the importance of translational regulation. Arch Dis Child 2002; 86:322-4. [PMID: 11970919]

37. Scheper GC, van der Knaap MS, Proud CG. Translation matters: protein synthesis defects in inherited disease. Nat Rev Genet 2007; 8:711-23. [PMID: 17680008]

38. Chatterjee S, Pal JK. Role of 5′- and 3′-untranslated regions of mRNAs in human diseases. Biol Cell 2009; 101:251-62. [PMID: 19275763]

39. Kimchi-Sarfaty C, Oh JN, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MMA. “Silent” polymorphism in the MDR1 gene changes substrate specificity. Science 2007; 315:525-8. [PMID: 17185560]