Mutation analysis of congenital cataract in a Chinese family identified a novel missense mutation in the connexin 46 gene (*GJA3*)

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Purpose: To identify the genetic defects in a three-generation Chinese family with congenital nuclear cataract.
Methods: Four patients and three healthy members from the family underwent complete physical and ophthalmic examinations. Genomic DNA was extracted from peripheral blood leukocytes of the family members as well as from 100 healthy normal controls. Polymerase chain reaction (PCR) amplification and direct sequencing of all coding exons of candidate genes were performed. The functional consequences of the mutation were analyzed with biology softwares.
Results: A novel mutation (c.130G>A) was identified in the connexin 46 gene (*GJA3*), which resulted in the substitution of valine by methionine at the highly conserved codon 44 of connexin 46. This mutation co-segregated among the affected members of the family and was not observed in either unaffected members or the 100 normal controls.
Conclusions: This is a novel missense mutation identified in the first extracellular loop of connexin 46; this expands the mutation spectrum of *GJA3* in association with congenital cataract.

Congenital cataract is a significant cause of poor vision or blindness in children worldwide and is responsible for 10.7%–14.0% of the children who are blind [1]. It is a clinically and genetically heterogeneous lens disorder, with autosomal dominant inheritance being most common. Currently, more than 22 genes have been identified to be associated with various forms of congenital cataract, including ten crystalline genes (*CRYAA* [2], *CRYAB* [3], *CRYBA1/A3* [4], *CRYBA4* [5], *CRYBB1* [6], *CRYBB2* [7], *CRYBB3* [8], *CRYGC* [9], *CRYGD* [10], and *CRYGS* [11]), three transcription factor genes (*HSF4* [12], *PITX3* [13], and *MAF* [14]), two cytoskeletal protein genes (*BFSP1* [15] and *BFSP2* [16]), four membrane transport protein genes (*MIP* [17], *GJA8* [18], *GJA3* [19], and *LIM2* [20]), glucosaminyl (N-acetyl) transferase 2 (*GCNT2*) [21], chromatin-modifying protein-4B (*CHMP4B*) [22], and transmembrane protein 114 (*TMEM114*) [23]. Knowledge of the structure and function of

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these candidate genes as well as the pathophysiological effect of their disease-associated mutations on their functions will aid in understanding the mechanisms of cataractogenesis.

Here, we report a heterozygous 130G>A transition in the connexin 46 gene (*GJA3*) associated with congenital nuclear cataract in a Chinese family, while it co-segregated completely with the disease phenotype. This is a novel mutation and has not been reported previously with congenital cataract.

METHODS

Clinical data and sample collection: A three-generation Chinese Han family (Figure 1) with congenital nuclear cataract was recruited from the Second Affiliated Hospital of Harbin Medical University, Harbin, China. Seven members of the pedigree were involved in this study, including four affected individuals (II:3, II:5, III:2, and III:3) and three unaffected ones (II:4, II:6, and III:4). All participants underwent full physical and ophthalmic examinations. Phenotype was documented by slit-lamp photography (Figure 2). One hundred subjects without diagnostic features of congenital cataract were recruited from the Chinese Han
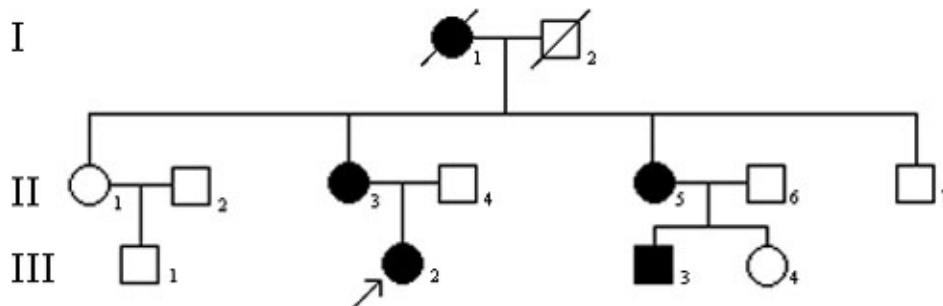
Figure 1. Pedigree of the family. Pedigree of the family with five affected individuals: the proband (III:2), her grandmother (I:1), mother (II:3), aunt (II:5), and male cousin (III:3). Circles represent females, while squares indicate males. Shaded shapes indicate affected individuals. A slash through the symbol indicates the person is deceased. The arrow points to the proband.

TABLE 1. THE PRIMERS USED FOR PCR.

Exon	Forward (5′-3′)	Reverse (5′-3′)	Product length (bp)
CRYAA-1	5′-AGCAGCCTTCTTCATGAGC-3′	5′-CAAGACCAGAGTCCATCG-3′	584
CRYAA-2	5′-GGCAGGTGACCGAAGCATC-3′	5′-GAAGGCATGGTGCAGGTG-3′	550
CRYAA-3	5′-GCAGCTTCTCTGGCATGG-3′	5′-GGGAAGCAAAGGAAGACAGA-3′	511
CRYAB-1	5′-AACCCCTGACATCACCATTC-3′	5′-AAGGACTCTCCCGTCCTAGC-3′	250
CRYAB-2	5′-CCATCCCATTCCCTTACCTT-3′	5′-GCCTCCAAAGCTGATAGCAC-3′	350
CRYAB-3	5′-TCTCTCTGCCTCTTTCCTCA-3′	5′-CCTTGGAGCCCTCTAAATCA-3′	400
CRYBA1–1	5′-GGCAGAGGGAGAGCAGAGTG-3′	5′-CACTAGGCAGGAGAACTGGG-3′	550
CRYBA1–2	5′-AGTGAGCAGCAGAGCCAGAA-3′	5′-GGTCAGTCACTGCCTTATGG-3′	508
CRYBA1–3	5′-AAGCACAGAGTCAGACTGAAGT-3′	5′-CCCCTGTCTGAAGGGACCTG-3′	463
CRYBA1–4	5′-GTACAGCTCTACTGGGATTG-3′	5′-ACTGATGATAAATAGCATGAACG-3′	355
CRYBA1–5	5′-GAATGATAGCCATAGCACTAG-3′	5′-TACCGATACGTATGAAATCTGA-3′	597
CRYBA1–6	5′-CATCTCATACCATTGTGTTGAG-3′	5′-CATCTCATACCATTGTGTTGAG-3′	528
CRYBB2–1	5′-GTTTGGGGCCAGAGGGGAGTGGT-3′	5′-TGGGCTGGGGAGGGACTTTCAGTA-3′	350
CRYBB2–2	5′-CCTTCAGCATCCTTTGGGTTCTCT-3′	5′-GCAGTTCTAAAAGCTTCATCAGTC-3′	330
CRYBB2–3	5′-GTAGCCAGGATTCTGCCATAGGAA-3′	5′-GTGCCCTCTGGAGCATTTCATAGT-3′	360
CRYBB2–4	5′-GGCCCCCTCACCCATACTCA-3′	5′-CTTCCCTCCTGCCTCAACCTAATC-3′	230
CRYBB2–5	5′-CTTACCCTTGGGAAGTGGCAATGG-3′	5′-TCAAAGACCCACAGCAGACAAGTT-3′	600
CRYGC-1	5′-TGCATAAAATCCCCTTACCG-3′	5′-CCTCCCTGTAACCCACATTG-3′	514
CRYGC-2	5′-TGGTTGGACAAATTCTGGAAG-3′	5′-CCCACCCCATTCACTTCTTA-3′	430
CRYGD-1	5′-CAGCAGCCCTCCTGCTAT-3′	5′-GGGTCCTGACTTGAGGATGT-3′	550
CRYGD-2	5′-GCTTTTCTTCTCTTTTTATTTCTGG-3′	5′-AAGAAAGACACAAGCAAATCAGT-3′	308
CRYGS-2	5′-GAAACCATCAATAGCGTCTAAATG-3′	5′-TGAAAAGCGGGTAGGCTAAA-3′	575
CRYGS-3	5′-AATTAAGCCACCCAGCTCCT-3′	5′-GGGAGTACACAGTCCCCAGA-3′	479
CRYGS-4	5′-GACCTGCTGGTGATTCCAT-3′	5′-CACTGTGGCGAGCACTGTAT-3′	974
GJA3–1	5′-CGGTGTTCATGAGCATTTTC-3′	5′-CTCTTCAGCTGCTCCTCCTC-3′	450
GJA3–2	5′-GAGGAGGAGCAGCTGAAGAG-3′	5′-AGCGGTGTGCGCATAGTAG-3′	450
GJA3–3	5′-TCGGGTTCCCACCCTACTAT-3′	5′-TATCTGCTGGTGGGAAGTGC-3′	300
GJA8–1	5′-CCGCGTTAGCAAAAACAGAT-3′	5′-CCTCCATGCGGACGTAGT-3′	420
GJA8–2	5′-GCAGATCATCTTCGTCTCCA-3′	5′-GGCCACAGACAACATGAACA-3′	330
GJA8–3	5′-CCACGGAGAAAACCATCTTC-3′	5′-GAGCGTAGGAAGGCAGTGTC-3′	350
GJA8–4	5′-TCGAGGAGAAGATCAGCACA-3′	5′-GGCTGCTGGCTTTGCTTAG-3′	500

Summary of the primers and products length used for the amplification of the all exons of candidate genes related with nuclear cataract.

population to serve as normal controls. After informed consent, 5 ml venous blood from family members and controls was collected in a BD Vacutainer (BD, San Jose, CA) containing EDTA. Genomic DNA was extracted by QIAamp DNA Blood Mini Kits (QIAGEN Science, Germantown, MD). The research was approved by the Institutional Review Board of Harbin Medical University and followed the clauses of the Declaration of Helsinki.

Mutation detection: All coding exons and their flanking regions of the known candidate genes associated with autosomal dominant congenital nuclear cataract, such as *CRYAA*, *CRYAB*, *CRYBA1, CRYBB2*, *CRYGC*, *CRYGD*, *CRYGS*, *GJA3*, and *GJA8,* were amplified by PCR with primers listed in Table 1. The PCR products were sequenced from both directions with the ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA). The sequencing results were analyzed using Chromas (version 2.3) and compared with the reference sequences in the NCBI database.

Bioinformatics analysis: The wild-type and mutant connexin 46 (Cx46) protein sequences were analyzed with computer

assistance for better understanding the effects of the mutation on its biochemical properties. We used PolyPhen (polymorphism phenotyping), which is based on the position-specific independent counts score derived from multiple sequence alignments of observations [24], to predict whether the amino acid substitution affects protein function. An online bio-software program Misc Protein Analysis was used to compute the hydrophilicity of the wild-type and mutant Cx46.

RESULTS

Clinical data: There were five affected people in 13 members of this family (Figure 1). The proband (III:2) was a 5-year-old girl whose grandmother (I:1), mother (II:3), aunt (II:5), and male cousin (III:3) also had poor vision in their childhood. Among them, one (I:1) passed away and two (II:3, II:5) had had cataract extractions before examination. The other subjects had had no operations and showed bilateral cataract characterized as a central nuclear opacity involving embryonic and fetal nucleus with punctate cortical opacities (Figure 2). There was no history of other ocular or systemic abnormalities in the family. To date, all of the affected individuals have had cataract surgery.

Mutation analysis: Direct sequencing of candidate genes revealed a heterozygous G>A transition in *GJA3* at position 130 that led to the replacement of the highly conserved valine with methionine at codon 44 (Figure 3). This mutation was detected in all affected members but was not observed in either the unaffected family members or the normal controls. There was no noticeable nucleotide polymorphism in other candidate genes.
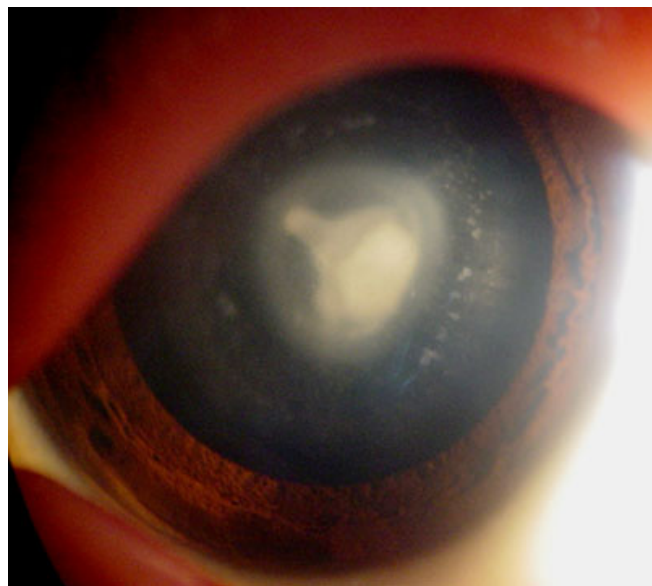

Figure 2. Slit-lamp photograph of the proband. Slit-lamp examination of the proband (III:2) showed a central nuclear cataract involving embryonic and fetal nucleus with punctate cortical opacities.

Bioinformatics analysis: With PolyPhen, substitution in Cx46 at position 44 from V to M scored 1.654 and was confidently predicted to be "possibly damaging." The obvious decrease in hydrophilicity in the mutant form is shown in Figure 4.

DISCUSSION

The *GJA3* gene, coding a 435-amino acid protein, was first reported by Willecke et al. [25] in 1990 and is located on
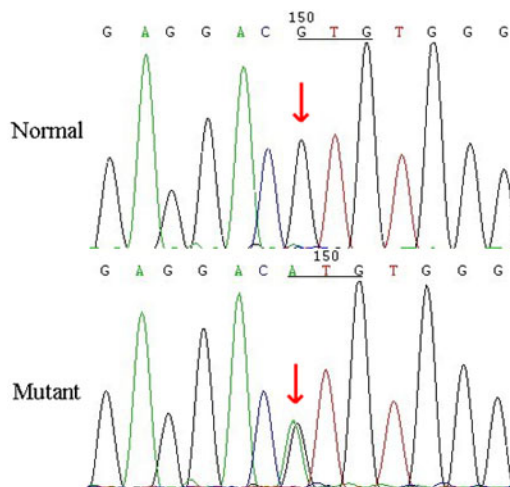

Figure 3. Mutation analysis of the connexin 46 gene (*GJA3*). The sequence chromatogram (forward strand) shows a heterozygous G>A transition that changes valine to methionine at codon 44. The red arrows show the wild-type (normal) and mutant point, respectively.
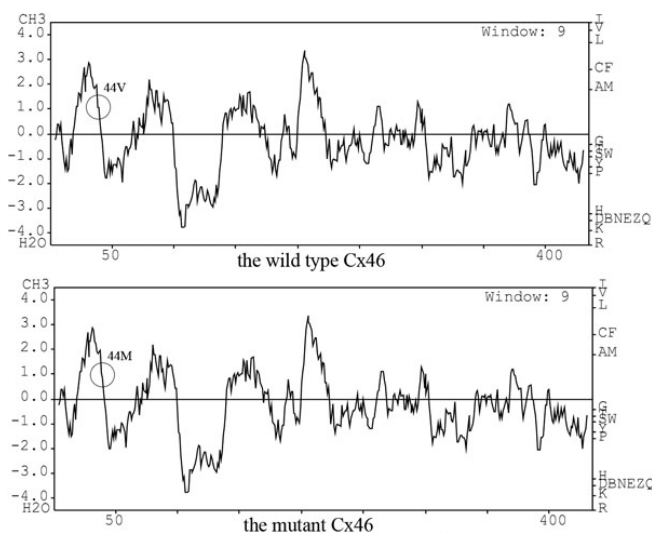

Figure 4. The hydrophilicity of the wild-type and mutant connexin 46 (Cx46). The *x*-axis represents the position of amino acids. The *y*-axis represents the hydrophilicity value in a default window size of nine. The regions of interest are marked by black circles. The decrease in hydrophilicity in the mutant form is evident.

TABLE 2. THE SUMMARY OF PREVIOUS STUDIES OF CONGENITAL CATARACT ASSOCIATED WITH *GJA3*.

Mutation	Amino acid change	Location	Cataract type	Family origin	Reference
c.7G>T	p.D3Y	NH2-terminus	Zonular pulverulent	Hispanic Central American	[35]
c.32T>C	p.L11S	NH2-terminus	Ant-egg	Danish	[36]
c.82G>A	p.V28M	First transmembrane domain (M1)	Variable	Indian	[37]
c.96C>A	p.F32L	First transmembrane domain (M1)	Nuclear pulverulent	Chinese	[38]
c.98G>T	p.R33L	First transmembrane domain (M1)	Embryonal nuclear granular	Indian	[39]
c.130G>A	p.V44M	First extracellular loop (E1)	Nuclear	Chinese	Present study
c.134G>C	p.W45S	First extracellular loop (E1)	Bilateral nuclear	Chinese	[40]
c.176C>T	p.P59L	First extracellular loop (E1)	Nuclear punctate	American	[41]
c.188A>G	p.N63S	First extracellular loop (E1)	Zonular pulverulent	Caucasian	[18]
c.226C>G	p.R76G	First extracellular loop (E1)	Total	Indian	[37]
c.227G>A	p.R76H	First extracellular loop (E1)	Nuclear pulverulent	Australian	[42]
c.260C>T	p.T87M	Second transmembrane domain (M2)	Pearl box	Indian	[43]
c.560C>T	p.P187L	Second extracellular loop (E2)	Zonular pulverulent	Caucasian	[44]
c.563A>C	p.N188T	Second extracellular loop (E2)	Nuclear pulverulent	Chinese	[45]
c.1137insC	p.S380fs	COOH-terminus	Zonular pulverulent	Caucasian	[18]

Summary of the mutations identified in *GJA3* provide the different congenital cataract phenotypes with different families belonging to different ethnic groups. Most of these mutations are in accord with autosomal dominant, and the cataract phenotypes are nuclear pulverulent types.

chromosome 13q11. Cx46, which is encoded by *GJA3*, is mainly expressed in lens fiber cells. Like others connexins, Cx46 has four transmembrane domains (M1, M2, M3, and M4), two extracellular loops (E1 and E2), an intracellular loop (CL), and intracellular NH_2 and COOH termini. Cx46 functions as a gap junction that mediates the intercellular transport of small molecules (<1 kDa), including ions, metabolites, and second messengers between elongated fiber cells [26]. Since the lens is an avascular structure and lens fiber cells lose all intracellular organelles during development, the fiber cells are highly dependent on intercellular communication for their survival [27]. The intercellular communication network is formed mainly by the gap junctions. This extensive network is vital since it maintains osmotic and metabolic homeostasis in lens fiber cells and ultimately maintains lens transparency [28].

However, extracellular domains of connexins that contain two extracellular loops (E1 and E2) play a key role in both mediating hemichannel docking [29,30] and regulating voltage gating of the channel [31]. The two extracellular loops are the most conserved domains among connexins and are the sites that provide the strong interaction between the two hemichannels that enable the formation of an intercellular channel with no leakage of current and molecules to the extracellular environment [32]. Furthermore, the first extracellular loop (E1) has been proven to be a major determinant of charge selectivity in Cx46 channels [33].

In this study we identified a new mutation (130G>A) in *GJA3*. This variation seems to be disease causative as it segregated with the phenotype and was absent in both unaffected pedigrees and the 100 unrelated controls from a similar ethnic background. This substitution resulted in the replacement of valine to methionine at codon 44 (V44M), localized in the first extracellular loop (E1) of Cx46. A multiple amino acid sequence alignment showed that valine at position 44 is phylogenetically conserved in different species and gap junctions (Figure 5), and Polyphen predicted

the mutation to be possibly damaging. These results suggest that valine may be functionally important and the mutation may lead to damaging interference with conformation and function of Cx46. The decline of hydrophilicity in the mutant (Figure 4) might alter the charge on the surface of the extracellular loop, thereby affecting hemichannel docking [34]. The mutation may also affect the charge selectivity in Cx46 channels, disturbing the charge balance inside the lens fiber cells [33]. These changes would disorder intercellular homeostasis in the lens fiber cells and result in lens nucleus opacity.

To date, 15 mutations in *GJA3* have been reported to be associated with congenital cataract in humans (Table 2) [35-45]. Most of these are described as nuclear or zonular pulverulent types and share genotype–phenotype similarities to some extent. In this study the phenotype also shows a conspicuous nuclear cataract but one that is surrounded with punctate opacities. The difference in the cataract phenotypes associated with *GJA3* may be attributed to the action of modifier genes or environmental factors that could affect the expression of *GJA3* and thus resulting cataract types.

In summary, we described a novel missense mutation (V44M) in *GJA3* that causes congenital cataract in a three-generation Chinese family. This study further confirms that Cx46 plays a vital role in the maintenance of human lens transparency and expands the mutation spectrum of *GJA3* in association with congenital cataract.
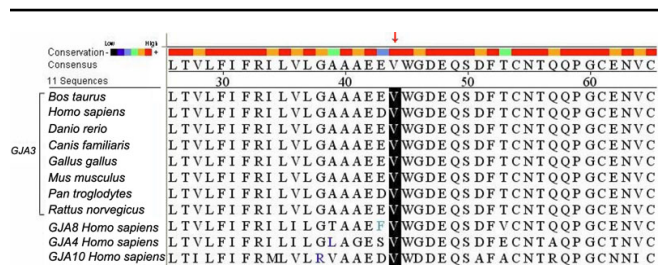
Figure 5. Phylogenetic conservation analysis. Amino acid sequences of connexin 46 (Cx46) from different species and other human connexins were downloaded from the NCBI and automatically aligned by Lasergene MegAlign (DNASTAR, Madison, WI). Multiple alignment indicates that valine at position 44 (black bar highlight) is highly conserved. The red arrow indicates high conservation.

REFERENCES

1. Gralek M, Kanigowska K, Seroczynska M. Cataract in children - not only an ophthalmological problem. Med Wieku Rozwoj 2007; 11:227-30. [PMID: 17965473]

2. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG. Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 1998; 7:471-4. [PMID: 9467006]

3. Berry V, Francis P, Reddy MA, Collyer D, Vithana E, MacKay I, Dawson G, Carey AH, Moore A, Bhattacharya SS, Quinlan RA. Alpha-B crystallin gene (CRYAB) mutation causes dominant congenital posterior polar cataract in humans. Am J Hum Genet 2001; 69:1141-5. [PMID: 11577372]

4. Kannabiran C, Wawrousek E, Sergeev Y, Rao GN, Kaiser-Kupfer M, Hejtmancik JF. Mutation of beta A3/A1 crystallin gene in autosomal dominant zonular cataract with sutural

opacities results in a protein with single globular domain. Invest Ophthalmol Vis Sci 1999; 40:S786.

5. Billingsley G, Santhiya ST, Paterson AD, Ogata K, Wodak S, Hosseini SM, Manisastry SM, Vijayallakshmi P, Gopinath PM, Graw J, Heon E. CRYBA4 a novel human cataract gene is also involved in microphthalmia. Am J Hum Genet 2006; 79:702-9. [PMID: 16960806]

6. Mackay DS, Boskovska OB, Knopf HLS, Lampi KJ, Shiels A. A nonsense mutation in CRYBB1 associated with autosomal dominant cataract linked to human chromosome 22q. Am J Hum Genet 2002; 71:1216-21. [PMID: 12360425]

7. Litt M, Carrero-Valenzuela R, LaMorticella DM, Schultz DW, Mitchell TN, Kramer P, Maumenee IH. Autosomal dominant cerulean cataract is associated with a chain termination mutation in the human beta-crystallin gene CRYBB2. Hum Mol Genet 1997; 6:665-8. [PMID: 9158139]

8. Riazuddin SA, Yasmeen A, Yao W, Sergeev YV, Zhang Q, Zulfiqar F, Riaz A, Riazuddin S, Hejtmancik JF. Mutations in beta-B3-crystallin associated with autosomal recessive cataract in two Pakistani families. Invest Ophthalmol Vis Sci 2005; 46:2100-6. [PMID: 15914629]

9. Heon E, Priston M, Schorderet DF, Billingsley GD, Girard PO, Lubsen N, Munier FL. The gamma-crystallins and human cataracts: a puzzle made clearer. Am J Hum Genet 1999; 65:1261-7. [PMID: 10521291]

10. Stephan DA, Gillanders E, Vanderveen D, Freas-Lutz D, Wistow G, Baxevanis AD, Robbins CM, VanAuken A, Quesenberry MI, Bailey-Wilson J, Juo S-HH, Trent JM, Smith L, Brownstein MJ. Progressive juvenile-onset punctate cataracts caused by mutation of the gamma-D-crystallin gene. Proc Natl Acad Sci USA 1999; 96:1008-12. [PMID: 9927684]

11. Sun H, Ma Z, Li Y, Liu B, Li Z, Ding X, Gao Y, Ma W, Tang X, Li X, Shen Y. Gamma-S crystallin gene (CRYGS) mutation causes dominant progressive cortical cataract in humans. J Med Genet 2005; 42:706-10. [PMID: 16141006]

12. Bu L, Jin Y, Shi Y, Chu R, Ban A, Eiberg H, Andres L, Jiang H, Zheng G, Qian M, Cui B, Xia Y, Liu J, Hu L, Zhao G, Hayden MR, Kong X. Mutant DNA-binding domain of HSF4 is associated with autosomal dominant lamellar and Marner cataract. Nat Genet 2002; 31:276-8. [PMID: 12089525]

13. Berry V, Yang Z, Addison PKF, Francis PJ, Ionides A, Karan G, Jiang L, Lin W, Hu J, Yang R, Moore A, Zhang K, Bhattacharya SS. Recurrent 17 bp duplication in PITX3 is primarily associated with posterior polar cataract (CPP4). J Med Genet 2004; 41:e109. [PMID: 15286169].15286169

14. Jamieson RV, Perveen R, Kerr B, Carette M, Yardley J, Heon E, Wirth MG, van Heyningen V, Donnai D, Munier F, Black GCM. Domain disruption and mutation of the bZIP transcription factor MAF associated with cataract ocular anterior segment dysgenesis and coloboma. Hum Mol Genet 2002; 11:33-42. [PMID: 11772997]

15. Ramachandran RD, Perumalsamy V, Hejtmancik JF. Autosomal recessive juvenile onset cataract associated with mutation in BFSP1. Hum Genet 2007; 121:475-82. [PMID: 17225135]

16. Conley YP, Erturk D, Keverline A, Mah TS, Keravala A, Barnes LR, Bruchis A, Hess JF, FitzGerald PG, Weeks DE, Ferrell RE, Gorin MB. A juvenile-onset progressive cataract locus on chromosome 3q21-q22 is associated with a missense

17. mutation in the beaded filament structural protein-2. Am J Hum Genet 2000; 66:1426-31. [PMID: 10729115]

17. Berry V, Francis P, Kaushal S, Moore A, Bhattacharya S. Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q. Nat Genet 2000; 25:15-7. [PMID: 10802646]

18. Shiels A, Mackay D, Ionides A, Berry V, Moore A, Bhattacharya S. A missense mutation in the human connexin50 gene (GJA8) underlies autosomal dominant "zonular pulverulent" cataract, on chromosome 1q. Am J Hum Genet 1998; 62:526-32. [PMID: 9497259]

19. Mackay D, Ionides A, Kibar Z, Rouleau G, Berry V, Moore A, Shiels A, Bhattacharya S. Connexin46 mutations in autosomal dominant congenital cataract. Am J Hum Genet 1999; 64:1357-64. [PMID: 10205266]

20. Pras E, Levy-Nissenbaum E, Bakhan T, Lahat H, Assia E, Geffen-Carmi N, Frydman M, Goldman B, Pras E. A missense mutation in the LIM2 gene is associated with autosomal recessive presenile cataract in an inbred Iraqi Jewish family. Am J Hum Genet 2002; 70:1363-7. [PMID: 11917274]

21. Yu LC, Twu YC, Chang CY, Lin M. Molecular basis of the adult i phenotype and the gene responsible for the expression of the human blood group I antigen. Blood 2001; 98:3840-5. [PMID: 11739194]

22. Shiels A, Bennett TM, Knopf HLS, Yamada K, Yoshiura K, Niikawa N, Shim S, Hanson PI. CHMP4B a novel gene for autosomal dominant cataracts linked to chromosome 20q. Am J Hum Genet 2007; 81:596-606. [PMID: 17701905]

23. Jamieson RV, Farrar N, Stewart K, Perveen R, Mihelec M, Carette M, Grigg JR, McAvoy JW, Lovicu FJ, Tam PPL, Scambler P, Lloyd IC, Donnai D, Black GCM. Characterization of a familial t(16,22) balanced translocation associated with congenital cataract leads to identification of a novel gene TMEM114 expressed in the lens and disrupted by the translocation. Hum Mutat 2007; 28:968-77. [PMID: 17492639]

24. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002; 30:3894-900. [PMID: 12202775]

25. Willecke K, Jungbluth S, Dahl E, Hennemann H, Heynkes R, Grzeschik K-H. Six genes of the human connexin gene family coding for gap junctional proteins are assigned to four different human chromosomes. Eur J Cell Biol 1990; 53:275-80. [PMID: 1964417]

26. Paul DL, Ebihara L, Takemoto LJ, Swenson KI, Goodenough DA. Connexin46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of Xenopus oocytes. J Cell Biol 1991; 115:1077-89. [PMID: 1659572]

27. White TW. Unique and redundant connexin contributions to lens development. Science 2002; 295:319-20. [PMID: 11786642]

28. Goodenough DA. The crystalline lens: a system networked by gap junctional intercellular communication. In: Gilula NB, editor. Seminars in Cell Biology. London; Academic Press; 1992. p. 49–58.

29. Simon AM, Goodenough DA. Diverse functions of vertebrate gap junctions. Trends Cell Biol 1998; 8:477-83. [PMID: 9861669]

30. Jiang JX, Goodenough DA. Heteromeric connexons in lens gap junction channels. Proc Natl Acad Sci USA 1996; 93:1287-91. [PMID: 8577756]

31. Verselis VK, Ginter CS, Bargiello TA. Opposite voltage gating polarities of two closely related connexins. Nature 1994; 368:348-51. [PMID: 8127371]

32. Scemes E, Suadicani SO, Dahl G, Spray DC. Connexin and pannexin mediated cell-cell communication. Neuron Glia Biol 2007; 3:199-208. [PMID: 18634611]

33. Trexler EB, Bukauskas FF, Kronengold J, Bargiello TA, Verselis VK. The First Extracellular Loop Domain Is a Major Determinant of Charge Selectivity in Connexin46 Channels. Biophys J 2000; 79:3036-51. [PMID: 11106610]

34. Yeager M, Nicholson BJ. Structure of gap junction intercellular channels. Curr Opin Struct Biol 1996; 6:183-92. [PMID: 8728651]

35. Addison PK, Berry V, Holden KR, Espinal D, Rivera B, Su H, Srivastava AK, Bhattacharya SS. A novel mutation in the connexin 46 gene (GJA3) causes autosomal dominant zonular pulverulent cataract in a Hispanic family. Mol Vis 2006; 12:791-5. [PMID: 16885921]

36. Hansen L, Yao W, Eiberg H, Funding M, Riise R, Kjaer KW, Hejtmancik JF, Rosenberg T. The congenital "ant-egg" cataract phenotype is caused by a missense mutation in connexin46. Mol Vis 2006; 12:1033-9. [PMID: 16971895]

37. Devi RR, Reena C, Vijayalakshmi P. Novel mutations in GJA3 associated with autosomal dominant congenital cataract in the Indian population. Mol Vis 2005; 11:846-52. [PMID: 16254549]

38. Jiang H, Jin Y, Bu L, Zhang W, Liu J, Cui B, Kong X, Hu L. A novel mutation in GJA3 (connexin46) for autosomal dominant congenital nuclear pulverulent cataract. Mol Vis 2003; 9:579-83. [PMID: 14627959]

39. Guleria K, Sperling K, Singh D, Varon R, Singh JR, Vanita V. A novel mutation in the connexin 46 (GJA3) gene associated with autosomal dominant congenital cataract in an Indian family. Mol Vis 2007; 13:1657-65. [PMID: 17893674]

40. Ma ZW, Ma Z, Zheng JQ, Zheng J, Yang F, Li J, Ji J, Li XR, Li X, Tang X, Yuan XY, Yuan X, Zhang XM, Zhang X, Sun HM, Sun H. Two novel mutations of connexin genes in Chinese families with autosomal dominant congenital nuclear cataract. Br J Ophthalmol 2005; 89:1535-7. [PMID: 16234473]

41. Bennett TM, Mackay DS, Knopf HL, Shiels A. A novel missense mutation in the gene for gap-junction protein alpha3 (GJA3) associated with autosomal dominant "nuclear punctate" cataracts linked to chromosome 13q. Mol Vis 2004; 10:376-82. [PMID: 15208569]

42. Burdon KP, Wirth MG, Mackey DA, Russell-Eggitt IM, Craig JE, Elder JE, Dickinson JL, Sale MM. A novel mutation in the Connexin 46 gene causes autosomal dominant congenital cataract with incomplete penetrance. J Med Genet 2004; 41:e106. [PMID: 15286166]

43. Guleria K, Vanita V, Singh D, Singh JR. A novel "pearl box" cataract associated with a mutation in the connexin 46 (GJA3) gene. Mol Vis 2007; 13:797-803. [PMID: 17615540]

44. Rees MI, Watts P, Fenton I, Clarke A, Snell RG, Owen MJ, Gray J. Further evidence of autosomal dominant congenital zonular pulverulent cataracts linked to 13q11 (CZP3) and a novel mutation in connexin 46 (GJA3). Hum Genet 2000; 106:206-9. [PMID: 10746562]

45. Li Y, Wang J, Dong B, Man H. A novel connexin46 (GJA3) mutation in autosomal dominant congenital nuclear pulverulent cataract. Mol Vis 2004; 10:668-71. [PMID: 15448617]

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