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Identification of a *GJA3* mutation in a Chinese family with congenital nuclear cataract using exome sequencing

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Congenital cataract, a clinically and genetically heterogeneous lens disorder is defined as any opacity of the lens presented from birth and is responsible for approximately 10% of worldwide childhood poor vision or blindness. To identify the genetic defect responsible for congenital nuclear cataract in a four-generation Chinese Han family, exome and direct Sanger sequencings were conducted and a missense variant c.139G>A (p.D47N) in the gap junction protein-alpha 3 gene (*GJA3*) was identified. The variant co-segregated with patients of the family and was not observed in unaffected family members or normal controls. The above findings indicated that the variant was a pathogenic mutation. The mutation p.D47N was found in the first extracellular loop (E1) domain of GJA3 protein. Our data suggest that exome sequencing is a powerful tool to discover mutation(s) in cataract, a disorder with high genetic heterogeneity. Our findings may also provide new insights into the cause and diagnosis of congenital nuclear cataract and have implications for genetic counseling.

Keywords: Congenital nuclear cataract, *GJA3* gene, Gap junction, p.D47N, Mutation, Exome sequencing

Congenital cataract is defined as any opacity of the lens present at birth. It is responsible for approximately 10% of worldwide childhood poor vision or blindness[1,2]. The incidence is estimated to be 2.2-2.49 per 10,000 live births[2]. Congenital cataract is a clinically and genetically heterogeneous lens disorder and one third of isolated congenital cataracts are genetically determined[1,3]. Clinically, it can be classified as polar/subcapsular, nuclear, lamellar/zonular, suture, cortical, capsular, and total cataract according to the position of the suffered lens[4,5]. Nuclear cataract refers to the opacification limited to the embryonic and/or fetal nuclei of the lens[6,7]. Most of them often occur in a non-syndromic autosomal dominant fashion, although autosomal recessive and X-linked inheritance are also known to exist[1,8].

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Abbreviations: E1, the first extracellular loop; *GJA3*, gap junction protein-alpha 3 gene; indels, insertions or deletions; SIFT, sorting intolerant from tolerant; YH1, Yan Huang1.

The first three authors contributed equally to this work.

To date, at least 22 disease loci and 17 disease-causing genes for congenital nuclear cataract are reported, including eight crystallin genes (alpha-A-crystallin gene *CRYAA*, alpha-B-crystallin gene *CRYAB*, beta-A1-crystallin gene *CRYBA1*, beta-B1-crystallin gene *CRYBB1*, beta-B2-crystallin gene *CRYBB2*, beta-B3-crystallin gene *CRYBB3*, gamma-C-crystallin gene *CRYGC*, and gamma-D-crystallin gene *CRYGD*); one cytoskeletal protein gene (beaded filament structural protein 2 gene *BFSP2*); three membrane protein genes (gap junction protein-alpha 3 gene *GJA3*, gap junction protein-alpha 8 gene *GJA8* and major intrinsic protein of lens fiber gene *MIP*); one growth and transcription factor gene (v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog gene *MAF*); ferritin light chain gene *FTL*; galactokinase 1 gene *GALK1*; FYVE and coiled-coil domain containing 1 gene *FYCO1*; and Nance-Horan syndrome gene *NHS*[6,9-18]. Structural proteins belonging to the gap junction protein family make up the intercellular channels presented in gap junctions and three distinct gap junction protein genes (*GJA1*, *GJA3* and *GJA8*) are expressed in the lens[3].

Earlier, Hansen *et al.*[9] detected crystallin and connexin mutations in 35.71% (10/28) and 21.43%

(6/28) Danish families, respectively. Here, we report a heterozygous c.139G>A transition (p.D47N) in the *GJA3* gene associated with congenital nuclear cataract in a Chinese Han family. The results show that the transition co-segregates with disease phenotype in the family and is absent in controls, indicating it is a pathogenic mutation for congenital nuclear cataract.

Materials and Methods

Clinical evaluation and DNA specimens

A 4-generation, 19-member Chinese Han family from Hunan province, China with autosomal dominant nuclear cataract was recruited from the Third Xiangya Hospital, Central South University (Fig. 1). Thirteen members of the pedigree were involved in this study, including six affected individuals (II:3, II:6, II:8, III:1, III:2 and IV:1) and seven unaffected ones (II:5, II:7, II:9, III:4, III:5, III:6 and III:7). Both affected and unaffected individuals underwent detailed ophthalmic examinations, including visual acuity, slit lamp examination, fundus examination with the dilated pupil and intraocular pressure measurement. The phenotypes were documented by slit lamp photography[1]. One hundred unrelated ethnically-matched subjects (male/female: 50/50, age 40.2 ± 8.3 yrs) without diagnostic features of congenital cataract were recruited from the same region of Mainland China served as normal controls.

Exome capture

Genomic DNA was extracted from peripheral blood using standard phenol-chloroform extraction method[19]. Three micrograms of genomic DNA was used to extract the exome library. DNA of one patient (III:2, Fig. 1) was sheared by sonication and then hybridized to the Nimblegen SeqCap EZ Library for enrichment, according to the instructions from manufacturer. The enriched library targeting the exome was sequenced on the HiSeq 2000 platform to obtain paired-end reads with read length of 90 bp[20]. A mean exome coverage of 78.87× was obtained and such deep coverage provided sufficient depth to accurately call variants at 99.34% of targeted exome[21].

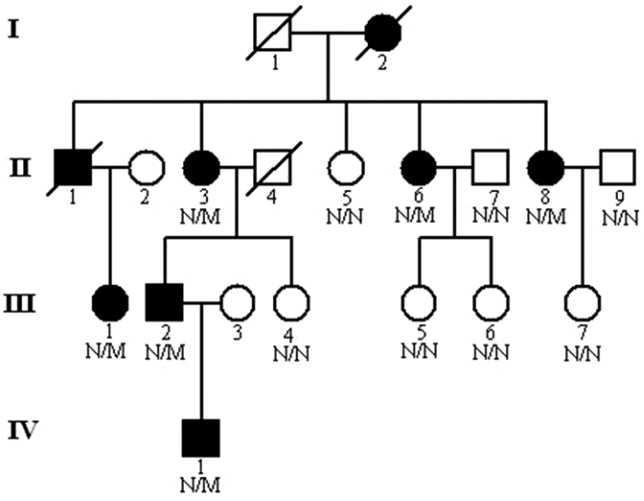

Fig. 1—Pedigree of the family with congenital nuclear cataract showing affected cases (fully shaded) [N: Normal; M: *GJA3* p.D47N mutation]

Read mapping and variant analysis

The human reference genome was obtained from the UCSC database (http://genome.ucsc.edu/), version hg19 (build 37.1). Alignment of the sequences from the patient was performed using SOAPaligner and SNPs were called using SOAPsnp set with the default parameters after the duplicated reads (produced mainly in the PCR step) were deleted[21,22].

Insertions or deletions (indels) affecting coding sequence or splicing sites were identified[23]. The thresholds for calling SNPs and short indels included the number of unique mapped reads supporting a SNP ≥ 4 and the consensus quality score ≥20. The quality score is a Phred score, generated by the program SOAPsnp 1.05, quality = -10log (error rate). All candidate mutations were filtered against the Single Nucleotide Polymorphism database (dbSNP build 135, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), 1000 genomes data (1000genomes release_20100804, http://www.1000genomes.org/), hapmap (2010-08_phase I + II + III, http://hapmap.ncbi.nlm.nih.gov/) and Yan Huang1 (YH1) project.

Sorting intolerant from tolerant (SIFT) prediction (http://sift.jcvi.org/) was performed to evaluate whether an amino acid substitution affects protein function[21]. Sanger sequencing was employed to validate the identified potential disease-causing variants with ABI3500 sequencer (Applied Biosystems, Foster City, CA, USA)[24]. Sequences of the primers were as follows: 5′-CGGTGTTCATGAGCATTTTC-3′ and 5′- GTGG CCCAGGTAGATGAGG-3′.

Results

Clinical findings

The proband (II:3) was 56 yrs old and was diagnosed with bilateral nuclear cataract by slit lamp examination (Fig. 2). According to the medical records, the other five patients (II:6, II:8, III:1, III:2 and IV:1) were diagnosed with bilateral nuclear cataract and had cataract extraction performed. From the hospital

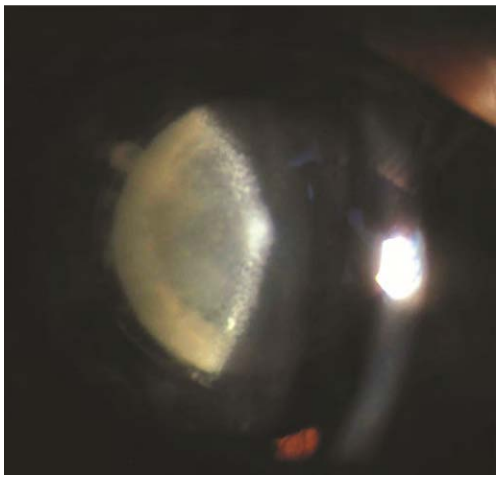

Fig. 2—Slit lamp photograph showing congenital nuclear cataract of the proband of the pedigree

records, bilateral cataract of all patients was present at birth or developed during infancy, the best corrected visual acuity of the affected eye varied from 0.1 to 0.5 before cataract extraction.

Mutation screening

We sequenced the exome of one patient (III:2, Fig. 1) of the Chinese Han family with congenital nuclear cataract. We generated 7.91 billion bases of sequence from the patient as paired-end 90-bp reads. 7.54 billion bases (95.37%) passed the quality assessment, 6.91 billion bases (91.56%) were aligned to the human reference sequence and 64.55% of the total bases were mapped to the targeted bases with a mean coverage of 78.87-fold[20]. 107,890 genetic variants, including 97,408 non-synonymous changes were identified in the patient in the coding regions or the canonical dinucleotide of the splice sites.

A prioritization scheme was applied to identify the pathogenic mutation in the patient, similar to two recent studies[23,25]. Given that the frequency of congenital nuclear cataract is less than 0.005, we excluded known variants identified in 1000 genome project, HapMap and dbSNP135 with MAF >0.50%. By doing so, we reduced the number of candidate genes by more than 94.32%.

A c.139G>A variant (p.D47N) in *GJA3* gene was observed in the patient, while other known disease-causing gene mutations for congenital cataract were excluded (Fig. 3B). The same heterozygous mutation was subsequently identified in all patients with congenital nuclear cataract in the family. The variant co-segregated with patients in the family and was absent in unaffected individuals in this family and in

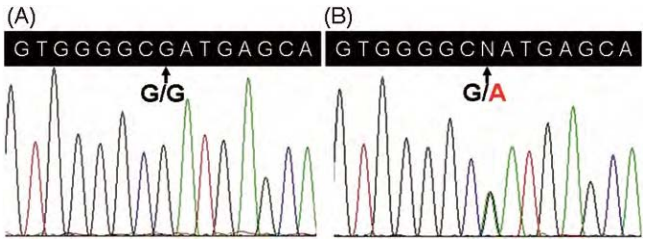

Fig. 3—Sequencing analysis of p.D47N mutation in the *GJA3* gene (DNA) [(**A**): Unaffected member (III:4) of the family; (**B**): Heterozygous p.D47N mutation patient (III:2)]

the 100 ethnically-matched unrelated controls. A multiple amino acid sequence alignment has shown that aspartic acid at position 47 is phylogenetically conserved among different species (*Bos taurus*, *Homo sapiens*, *Danio rerio*, etc.) and gap junctions (human GJA4, GJA8 and GJA10) and SIFT predicts the mutation to be possibly damaging. Our data indicated that p.D47N variant in *GJA3* gene was the pathogenic mutation in the family with congenital nuclear cataract.

Discussion

The *GJA3* gene is located on 13q11 and consists of two exons encoding a 435-amino acid protein. GJA3 protein is predominantly expressed in the lens, which is essential for maintaining lens transparency[1,8]. Connexins are a family of structurally-related transmembrane proteins that assemble to form gap junctions and transport metabolites, ions and water in the lens[26]. All connexins have four transmembrane domains (M1, M2, M3 and M4), two extracellular loops (E1 and E2), a cytoplasmic loop and the NH_2-terminus and COOH-terminus located in the intracellular region[27].

GJA3 functions as a gap junction that oligomerizes into intercellular channels mediating the transportation of low molecular weight metabolites, ions and second messengers between adjacent cells[28]. The lens is an avascular structure and lens fiber cells eliminate all intracellular organelles during development to achieve optical clarity. The survival of fiber cells highly depends on intercellular communication[29], which is formed mainly by gap junctions. This extensive intercellular communication network is vital for maintaining osmotic and metabolic homeostasis in lens fiber cells and ultimately for transparency of lens[30].

In the animal model study, the targeted replacement of *GJA8* gene with *GJA3* gene in mice has demonstrated that *GJA8* gene is required for cell

growth, whereas non-specific restoration of communication by *GJA3* gene maintains differentiation[29]. Mutations of *GJA3* gene might induce the appearance of a cleaved form of γ-crystallin. The cleavage of γ-crystallin might result in a change in the normal 3-D conformational structure of γ-crystallin, leading to abnormal interactions with other lens proteins[31]. Knock-out of *GJA3* gene in different mice strains leads to various degrees of cataracts, similar to the features in this family and other pedigrees, depending on genetic background[1,31].

To date, at least 28 *GJA3*-related pedigrees with congenital cataract, including ours and 23 mutations, including missense, deletion and insertion mutations have been described[1,3,4,8,9,30,32-48] (Table 1). Clinically, the cataracts share several genotype-phenotype similarities with some inter- and intra-familial differences with respect to the appearance and location of opacities within the lens[48]. Patients from 20 out of 28 families (71.43%) presented with nuclear opacities in the lens[1,3,8,9,30,32-34,36-41,43-48]. Phenotypes in the other three families were not described[4,9,41]. The differences in the *GJA3*-related cataract phenotypes are reported in different families, even in a same family, which may be explained by the interaction of modifier genes or background environmental factors affecting the expression of the gene and hence resulting in different cataract types[48].

The *GJA3* c.139G>A variant (p.D47N), previously reported in a Chinese congenital nuclear cataract family from Northeast of China[3] was also identified in our congenital nuclear cataract family from South of China, indicating that the D47 might be a mutation hotspot in Chinese population. This variant seemed to be a pathogenic mutation because it segregated with patients with congenital nuclear cataract in our family

Table 1—Mutations in the *GJA3* gene associated with conenital cataract

Nucleotide change	Amino acid change	Location	Cataract phenotype	Geographic distribution/ ethnic background	References
c.5G>A	p.G2D	NH_2-terminus	Nuclear pulverulent and posterior polar	Chinese	32
c.7G>T	p.D3Y	NH_2-terminus	Zonular pulverulent	Hispanic Central American	33
c.32T>C	p.L11S	NH_2-terminus	Ant-egg	Danish	9,34
c.56C>T	p.T19M	NH_2-terminus	Posterior polar	Indian	35
c.82G>A	p.V28M	M1	Variable	Indian	36
c.96C>A	p.F32L	M1	Nuclear pulverulent	Chinese	37
c.98G>T	p.R33L	M1	Finely granular embryonal	Indian	38
c.130G>A	p.V44M	E1	Nuclear/-	Chinese/Caucasian American	4,30
c.134G>C	p.W45S	E1	Nuclear	Chinese	39
c.139G>A	p.D47N	E1	Nuclear	Chinese	3, this study
c.176C>T	p.P59L	E1	Nuclear punctate/-/-	Caucasian American/Danish/Chinese	9,40,41
c.188A>G	p.N63S	E1	Zonular pulverulent	British	42
c.226C>G	p.R76G	Boundary of E1 and M2	Total	Indian	36
c.227G>A	p.R76H	Boundary of E1 and M2	Nuclear pulverulent/ lamellar and suture	Australian/Danish	9,43
c.260C>T	p.T87M	M2	Pearl box	Indian	44
c.427G>A	p.G143R	Cytoplasmic loop	Coppock-like	Chinese	45
c.559C>T	p.P187S	E2	Nuclear pulverulent	Chinese	46
c.560C>T	p.P187L	E2	Zonular pulverulent	Caucasian	47
c.563A>C	p.N188T	E2	Nuclear pulverulent	Chinese	8
c.563A>T	p.N188I	E2	Nuclear coralliform (zonular pulverulent)	Chinese	48
c.616T>A	p.F206I	M4	Nuclear	Chinese	1
c.1137insC	p.S380fs	COOH-terminus	Zonular pulverulent	British	42
c.1143_1165del23	p.S381fs	COOH-terminus	Punctate nuclear	Chinese	41

M1, first transmembrane domain; E1, first extracellular loop; M2, secondary transmembrane domain; E2, secondary extracellular loop; M4, fourth transmembrane domain.

and was absent in both unaffected family members and the 100 unrelated ethnically-matched controls.

The mutation p.D47N in the E1 domain of *GJA3* may influence the hemi-channel docking and subsequently may lead to damaging interference with conformation and function of GJA3[3,30]. Except p.D47N, four other reported mutations (p.V44M, p.W45S, p.P59L and p.N63S) located in the E1 domain of GJA3 have been reported in seven pedigrees with congenital cataract[3,30]. The high frequency mutations (32.14%, 9/28) in the E1 domain of GJA3 protein by meta-analysis of *GJA3*-related congenital cataract suggested that this domain was the hot mutation region.

In summary, our study shows that exome sequencing is a powerful tool to discover mutation(s) in cataract, a disorder with high genetic and clinical heterogeneity. Our findings may also provide new insights into the cause and diagnosis of congenital cataract and may have implications for genetic counseling in the future.

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