Exome Sequencing of 18 Chinese Families with Congenital Cataracts: A New Sight of the NHS Gene

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Introduction

Congenital cataract is the most common cause of childhood blindness. Its prevalence is 1–6 per 10,000 live births, but it can be as high as 5–15 per 10,000 in less developed areas of the world [1–3]. Congenital cataracts can occur independently or be accompanied by other ocular and/or systemic abnormalities, and are thus designated as “nonsyndromic” or “syndromic” forms [4]. Genetic defect is a common cause of congenital cataract and was estimated to account for about 23% of patients [5]. Autosomal dominant inheritance is the most common form, although other forms of inheritance have also been described. To date, at least 34 genes have been reported to cause congenital cataracts (Cat-Map; http://cat-map.wustl.edu/) [6] (Table 1). Identification of the genetic basis is a great challenge in congenital cataracts, as it is highly heterogeneous in term of genetic and clinical phenotypes. Some of the known genes were selected for analysis in a cohort of patients in few studies and the results showed that the mutation frequencies have great differences [7–12]. We previously conducted similar studies and were able to detect mutation in 14 of 34 (41%) families with congenital cataracts [13–15]. In order to identify the genetic cause of the remaining and newly recruited families with congenital cataracts, whole exome sequencing was used in this study to screen the mutations, and then the detected variants were confirmed by Sanger sequencing. Nine mutations were identified in the 18 families; of those NHS mutations were found in four families.

Methods

Patients

Written informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (863-plan) by the Ministry of Public Health of China were obtained from all participating individuals or their guardians of the 18 families enrolled in this study. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. Of the 18 families, ten were from our previous studies with no mutations identified [13–15], while the other eight families had not been analyzed before. Thirteen families with family history showed autosomal dominant inheritance, two were sporadic patients, while the other three were not clear if they had family histories. None of them were recorded to have systemic abnormalities. Ocular examinations were...
performed by ophthalmologists in Zhongshan Ophthalmic Center. Congenital cataract means that cataracts were noticed at birth or in the first few months. Microcornea was defined as a cornea whose horizontal diameter was less than 10 mm. The procedures for obtaining written informed consent and for preparing of genomic DNA were the same as previously described [13,14,16].

### Exome Sequencing

Exome sequencing was performed by Macrogen ([http://www.macrogen.com/](http://www.macrogen.com/)), a commercial service. The criteria to select samples for exome sequencing included: 1. The total amount of genome DNA can’t be less than 5 μg; 2. There was no smear by running on an agarose gel. All the 18 samples of probands from 18 unrelated families were involved in exome sequencing. Exome capture was carried out using an Illumina TruSeq Exome Enrichment Kit (62 M) array. The kit included 340,427 probes (95 mer DNA probes) and could enrich about 201,121 exons and cover about 97.2% CCDS region. Exome-enriched DNA fragments were sequenced by an Illumina HiSeq2000; the average sequencing depth was 125-fold. Over 99% base call accuracy was up to Q20, which means that the probability of an incorrect base call is 0.01. After the low quality reads were filtered, the clean data will be aligned to the consensus sequence (UCSC hg19) to detect variants by SAMtools. Additional bioinformatics analysis of all the variants were provided from dbSNP ([http://www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)), 1000 Genome ([http://browser.1000genomes.org/index.html](http://browser.1000genomes.org/index.html)), PolyPhen-2 ([http://genetics.bwh.harvard.edu/pph2/](http://genetics.bwh.harvard.edu/pph2/)), SIFT ([http://sift.jcvi.org/](http://sift.jcvi.org/)), and GERP ([http://mendel.stanford.edu/SidowLab/downloads/gerp/](http://mendel.stanford.edu/SidowLab/downloads/gerp/)) online tools.

### Table 1. Information of the 34 genes referred in this study.

| Gene | Inheritance | Genomic DNA | mRNA | Protein |
|------|-------------|-------------|------|---------|
| EPHA2 | AD/AR | NC_000001.10 | NM_004431.3 | NP_004422.2 |
| GJA8  | AD/AR | NC_000001.10 | NM_005267.4 | NP_005258.2 |
| CRYGC | AD | NC_000002.11 | NM_020989.3 | NP_066269.1 |
| CRYGD | AD | NC_000002.11 | NM_006891.3 | NP_008822.2 |
| FYCO1 | AR | NC_000003.11 | NM_024513.2 | NP_078789.2 |
| BFS2  | AD/AR | NC_000003.11 | NM_003571.2 | NP_003562.1 |
| CRY5S  | AD | NC_000003.11 | NM_017541.2 | NP_060011.1 |
| GCNT2 | AR | NC_000006.11 | NM_001491.2 | NP_001482.1 |
| GALT  | AR | NC_000009.11 | NM_000155.3 | NP_000146.2 |
| TDRD7 | AR | NC_000009.11 | NM_014290.2 | NP_055105.2 |
| VIM   | AD | NC_000010.10 | NM_003380.3 | NP_003371.2 |
| SLC16A12 | AD | NC_000010.10 | NM_213606.3 | NP_998771.3 |
| PITX3 | AD/AR | NC_000010.10 | NM_050203.2 | NP_050203.2 |
| CRYAB | AD/AR | NC_000011.9 | NM_001885.1 | NP_001876.1 |
| MIP   | AD | NC_000012.11 | NM_012064.3 | NP_036196.1 |
| GJA3  | AD | NC_000013.10 | NM_021954.3 | NP_068773.2 |
| TMEM114 | AD | NC_000016.9 | NM_001146336.1 | NP_001139808.1 |
| HSF4  | AD/AR | NC_000016.9 | NM_001040667.2 | NP_001035757.1 |
| MAF   | AD | NC_000016.9 | NM_005360.4 | NP_005351.2 |
| CRYBA1 | AD | NC_000017.10 | NM_005208.4 | NP_005199.2 |
| GALK1 | AR | NC_000017.10 | NM_000154.1 | NP_000145.1 |
| FTL   | AD | NC_000019.9 | NM_000146.3 | NP_000137.2 |
| LIM2  | AR | NC_000019.9 | NM_030657.3 | NP_085915.2 |
| BFS1  | AR | NC_000020.10 | NM_001278607.1 | NP_001265336.1 |
| CHMP4B | AD | NC_000020.10 | NM_176812.4 | NP_789782.1 |
| CRYAA | AD/AR | NC_000021.8 | NM_000394.2 | NP_000385.1 |
| CRYBB2 | AD | NC_000022.10 | NM_000496.2 | NP_000487.1 |
| CRYBB3 | AD/AR | NC_000022.10 | NM_000496.2 | NP_000487.1 |
| CRYBB1 | AD/AR | NC_000022.10 | NM_001887.3 | NP_001878.1 |
| CRYBA4 | AD | NC_000022.10 | NM_001886.2 | NP_001877.1 |
| NHS   | XL | NC_000023.10 | NM_198270.2 | NP_938011.1 |
| AGK   | AR | NC_000007.13 | NM_018238.3 | NP_060708.1 |
| EYA1  | AD | NC_000008.10 | NM_005034.3 | NP_000494.2 |
| FOXE3 | AD/AR | NC_000001.10 | NM_012186.2 | NP_036318.1 |

Note: AD = autosomal dominant; AR = autosomal recessive; XL = X-linked. doi:10.1371/journal.pone.0100455.t001
### Table 2. The 9 mutations identified in 9 of 18 Chinese families with congenital cataract.

| Family ID | Gene  | Nucleotide | Amino acid | Status | Polyphen-2 | SIFT | Splice | Variations in patients | controls | Note          |
|-----------|-------|------------|------------|--------|------------|------|--------|------------------------|----------|---------------|
| Family 2  | CRYB2 | c.326T>A   | p.I109N    | Hetero  | PD         | D    | /      | 1/18                   | 0/96     | Novel         |
| Family 3  | EPHA2 | c.1046C>T  | p.T349M    | Hetero  | PD         | D    | /      | 1/18                   | 0/96     | rs200490325   |
| Family 4  | GJA8  | c.130G>A   | p.V44M     | Hetero  | PD         | D    | /      | 1/18                   | 0/96     | Novel         |
| Family 6  | MAF   | c.880C>T   | p.R294W    | Hetero  | PD         | D    | /      | 1/18                   | 0/96     | Novel         |
| Family 7  | MIP   | c.500delC  | p.A169Pfs*15| Hetero | /          | /    | /      | 1/18                   | 0/96     | Novel         |
| Family 8  | NHS   | c.556G>T   | p.E186*    | Hetero  | /          | /    | /      | 1/18                   | 0/96     | Novel         |
| Family 9  | NHS   | c.853-1G>A | /          | Hemi    | /          | /    | DSA    | 1/18                   | 0/96     | Novel         |
| Family 10 | NHS   | c.1117C>T  | p.R373*    | Hemi    | /          | /    | /      | 1/18                   | 0/96     | Reported (rs132630322) |
| Family 11 | NHS   | c.2716_2719delTAG | p.L906fs*24 | Hetero | /          | /    | /      | 1/18                   | 0/96     | Novel         |

Note: Hetero = heterozygosity; Hemi = hemizygosity; D = damaging; PD = probably damaging; DSA = donor site abolished.

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### Table 3. The two variants that can't be excluded.

| Family ID | Gene  | Nucleotide | Amino acid | Status | Polyphen-2 | SIFT | Splice | Variations in patients | controls | Note          |
|-----------|-------|------------|------------|--------|------------|------|--------|------------------------|----------|---------------|
| Family 1  | CRYBA4| c.26C>T    | p.A9V      | Hetero  | Benign     | Toleate | /      | 1/18                   | 0/96     | Novel         |
| Family 5  | GJA8  | c.367G>A   | p.G123S    | Hetero  | Benign     | Toleate | /      | 1/18                   | 0/96     | COSM1333689   |

Note: Hetero = heterozygosity.

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Variants Analysis

From the exome sequencing results of the 18 probands, detected variants in the 34 known causative genes were summarized. Then we excluded the variants which we don’t considered pathogenic as the following criteria: 1. Minor allele frequency (MAF) $\leq 0.01$ from 1000 Human Genome Project database; 2. Located in non-coding region without affecting splicing site; 3. Synonymous variants without affecting splicing site; 4. Only one single heterozygous variation detected in recessive genes. All the other variants were considered pathogenic and summarized for validation.

Sanger Sequencing

Sanger sequencing was used to confirm the potential pathogenic variants detected by exome sequencing, including missense, nonsense, indels, and splice site variants. Primers used to amplify the sequence with each variant were either referred to in previous studies [13,15,17] or designed by online tool Primer3 (http://frodo.wi.mit.edu/ primer3/) (Table S1). The nucleotide sequences of amplicons were analyzed using an ABI BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA) on an ABI 3130 Genetic Analyzer (Applied Biosystems). Sequencing results were aligned with consensus sequences from the National Center for Biotechnology Information (NCBI) human genome database (http://www.ncbi.nlm.nih.gov/), using the SeqManII program of the Lasergene package (DNASTar Inc. Madison, WI). Confirmed variants were further sequenced in the available family members and 96 unrelated control individuals. The descriptions of the variants followed the nomenclature recommended by the Human Genomic Variation Society (HGVS; http://www.hgvs.org/mutnomen/). The effects of missense variations were evaluated by the PolyPhen-2 [18] and SIFT [19] online tools, and the effects of intronic variants on splicing site changes were predicted by the Berkeley Drosophila Genome Project (BDGP; http://www.fruitfly.org/seq_tools/splice.html [20].

Results

From the whole exome sequencing results of the 18 probands, a total of 1545 variants were identified in the 34 known genes. Twelve variants of them were considered potential pathogenic after we excluded all the variants which were not pathogenic. And 11 of them were confirmed while one was false positive by Sanger sequencing. The 11 variants were present in seven genes and were identified in 11 of the 18 families with congenital cataracts (Table 2, Table 3, Figure 1, and Figure S1). Two of the 11 variants were predicted to be benign (Table 3), while the other 9 were likely to be pathogenic (Table 2). None of the 11 variants was found in 96 normal controls. Four of the 11 variants were identified in $NHS$ in four of the 18 (22.2%) families. The other seven mutations were
identified in six genes, including two mutations in \textit{GJA8} (gap junction protein, alpha-8; MIM: 600897), and one mutation each in \textit{CRYBA4} (crystallin, beta-A4; MIM: 123631), \textit{CRYBB2} (crystallin, beta-B2; MIM: 123620), \textit{EPHA2} (ephrin receptor EphA2; MIM: 176946), \textit{MAF} (v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog; MIM: 177075), and \textit{MIP} (major intrinsic protein of lens fiber; MIM: 154050). The four mutations in \textit{NHS} included two nonsense, one frameshift, and one splice site mutations. Analysis of available family members in three families demonstrated cosegregation of the mutation with the disease (Figure 1; families 4, 6, and 9). Conservation alignment analysis of the six missense mutations showed relatively conserved residues (Figure 2). None of the 11 variants was present in the 96 normal controls.

The clinical data of the probands with mutations are listed in Table 4. Of the four families with mutations in \textit{NHS}, an X-linked gene, three (family 9, family 10, and family 11) showed congenital cataracts, as well as microcornea and nystagmus. The fourth one, family 8, with \textit{NHS} c.556G>T (p.E186*) mutation, showed punctate cataract and high myopia (−27.5D for the right eye and −28.0D for the left eye). There were no records of other abnormalities in the patients with \textit{NHS} mutations. Clinical re-examination was only possible for two affected individuals and one unaffected member (II:1, II:2, and III:1) of family 9, with the c.853-1G>A mutation in \textit{NHS}. The proband (III:1) harbored a hemizygous mutation and his affected mother (II:2) harbored a heterozygous mutation, while his unaffected father (II:1) did not carry the mutation. The proband (Figure 1; family 9-II:1) had undergone cataract surgery nine years prior to the study, so that cataract was no longer observed (Figure 3A). His affected mother (Figure 1; family 9-II:2) had posterior subcapsule opacification in her left eye; her right eye underwent cataract surgery when she was a teenager (Figure 3D). The horizontal corneal diameter of the proband (III:1) was 10 mm in both eyes (Figure 3A) while those of his affected mother (II:2) were 9 mm in the left eye (Figure 3E) and 8 mm in the right eye (Figure 3F). Both the mother and son had bilateral nystagmus. In addition, the proband (III:1) showed bilateral tigroid retinal change in the temporal region of the optic disc (Figure 3B). The proband (III:1) had abnormal teeth (figure 3C), but atypical for Nance-Horan syndrome. His mother had lost all her teeth when she was forty.

**Discussion**

In this study, we performed whole exome sequencing on probands from 18 families with congenital cataracts. Analysis of the sequencing information for 34 genes known to be associated with congenital cataract revealed 12 potential pathogenic mutations. Sanger sequencing confirmed the 11 of them and nine mutations in six genes were considered to be pathogenic in 9 (50.0%) of the 18 families.

To date, about 34 genes have been reported to be associated with congenital cataract (Cat-Map). About half of the mutations were from genes encoding crystallins and 15% of the mutations were from genes encoding connexins [21]. Some screening studies on a set of the two groups of genes in a cohort of patients showed that the frequencies were much lower than expected. Hansen et al. selected 28 Danish families with hereditary congenital cataracts to screen 17 genes and found that mutations in genes encoding crystallins and connexins accounted for 53.5% [7]. Other studies have screened only a few genes in different populations and all of their results showed that the mutation frequencies were no more than 20% [7–10,12,22]. In our previous study, we screened all of...
the 12 genes encoding crystallins and connexins in 25 Chinese families, and 40% of the families were found to carry mutations [13]. Regarding other genes, most reports were based on an individual gene in one family. Therefore, it is still not clear about the mutation frequencies of the known genes in a group of patients with congenital cataracts, especially it can be different in specific populations.

In the 9 potential variants identified in the current study, four were in NHS gene and were detected in four X-linked families, which we previously considered as autosomal dominant inheritance. Combining our previous studies with the current study, we identified mutations in 23 of 34 recruited families with congenital cataract, accounting for 67.6% (23/34) of the families [13–15]. Mutation frequencies in genes encoding crystallins, genes encoding connexins, and the NHS gene were 29.4% (10/34), 14.7% (5/34), and 11.8% (4/34), respectively. Our results indicated that the NHS gene is also a major causative gene besides the above two groups of genes, especially in some congenital cataracts with pseudo-autosomal dominant inheritance.

NHS gene is located in Xp22.13, and mutations in NHS can cause X-linked dominant Nance–Horan syndrome, which is also known as X-linked congenital cataract [23,24] or X-linked cataract–dental syndrome [25]. Affected males with Nance–Horan syndrome typically show severe congenital cataracts and dental abnormalities, with occasional dysmorphic features and mental retardation, while females have milder symptoms [26]. While 100% of patients with Nance–Horan syndrome have bilateral congenital cataracts, only 65% have typical dental anomalies, including screwdriver incisors and mesiodens [26]. In some cases, Nance–Horan syndrome was diagnosed after mutations were identified in the NHS gene of affected males who were first noted to suffer from severe congenital cataracts [27]. In our study, all patients in the four families with NHS gene mutations were recruited as congenital cataract, and only three (family 9, family 10, and family 11) had microcornea and nystagmus, which are major manifestations of Nance–Horan syndrome. However, there were no records regarding abnormal dental features. Although it has been demonstrated in a study that Nance–Horan syndrome and X-linked cataract are allelic disorders, there have been no studies to date regarding mutation frequency in congenital cataracts [24]. Therefore, we strongly suggest that the NHS gene

Table 4. Clinical features of affected individuals with variants identified in this study.

| Variations | Gene  | Nucleotide  | Other abnormalities |
|------------|-------|-------------|---------------------|
| Family ID  | Gene  | Nucleotide  | Other abnormalities |
| Family 1   | CRYBA4| c.26C>T     | NA                  |
| Family 2   | CRYBB2| c.326T>A    | Lamellar, punctate  |
| Family 3   | EPHA2 | c.1046C>T   | AD                  |
| Family 4   | GJA8  | c.500delC   | NA                  |
| Family 5   | MAF   | c.880C>T    | Membranous cataract |
| Family 6   | MIP   | c.500delC   | NA                  |
| Family 7   | NHS   | c.853-1G    | NA                  |
| Family 8   | NHS   | c.1117C>10  | NA                  |
| Family 9   | NHS   | c.2716_2719delTTAG | NA   |

Note: NA = not available; S = sporadic; FMB = first few month; AD = autosomal dominant; XL = X-linked; FC = finger counting; PL = pursuit of light; OD = right eye; OS = left eye.

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Figure 3. The clinical examination details of patients from the family 9. A–C showed that the member III:1 had bilateral 10 mm of the horizontal corneal diameter (A), bilateral mild tigroid retinal change in the temporal region of the optic disc (B), and dental condition without obvious screwdriver incisors or mesiodens (C). D–F showed that the member II:2 had posterior capsule opacification in her left eye and postoperative pupil shift in her right eye (D), 10 mm of the horizontal corneal diameter in her left eye (E), and 8 mm in her right eye (F).

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should be considered one of the major genes associated with congenital cataract.

In conclusion, combined with our previous studies [13–15], based on a total of 34 analyzed families, the results showed that mutations in the 34 known genes were responsible for about 67.6% of this set of Chinese families with congenital cataracts. And mutations in the NHS gene were identified in 11.8% of the families, in whom congenital cataract was the only recorded sign in the other three families with NHS mutations except for cataract. Therefore, we supposed that maybe it was not taken too much attention on the abnormal dental and face features caused by NHS mutations, especially in Chinese patients. We suggest that mutations in NHS are a common cause of congenital cataract, both syndromic and nonsyndromic.

Supporting Information

Figure S1 Sequence chromatography. The family number was shown in the left column. Sequences with mutations from patients and normal controls were shown in the middle and right column, respectively. Each mutation was noted under the corresponding sequence. For the Family 9, the proband and his affected mother showed the hemizygous mutant sequence (upper one in the middle column) and the heterozygous mutant sequence (the lower one in the middle column), respectively.

| Family 1 | Family 2 | Family 3 | Family 4 | Family 5 |
|----------|----------|----------|----------|----------|
| X         | Y         | Z         | X         | Y         |

Table S1 Primers used to amplify and sequence the variants regions in this study. This table listed 12 pairs of primers which were used to amplify the genomic fragments with variants detected by exome sequencing.

| Primer Name | Sequence (5'→3') |
|-------------|------------------|
| Forward     | Reverse           |

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Author Contributions

Conceived and designed the experiments: QZ. Performed the experiments: WS XX SL XG. Analyzed the data: WS ZQ. Contributed reagents/materials/analysis tools: XX SL XG QZ. Wrote the paper: WS.

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