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Research article

Molecular genetics of Leber congenital amaurosis in Chinese: New data from 66 probands and mutation overview of 159 probands

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ABSTRACT

Leber congenital amaurosis (LCA) is the most severe form of inherited retinal dystrophy. We have previously performed a mutational analysis of the known LCA-associated genes in probands with LCA by both Sanger and whole exome sequencing. In this study, whole exome sequencing was carried out on 66 new probabds with LCA. In conjunction with these data, the present study provides a comprehensive analysis of the spectrum and frequency of all known genes associated with retinal dystrophy in a total of 159 Chinese probands with LCA. The known genes responsible for all forms hereditary retinal dystrophy were included based on information from RetNet. The candidate variants were filtered by bioinformatics analysis and confirmed by Sanger sequencing. Potentially causative mutations were further validated in available family members. Overall, a total of 118 putative pathogenic mutations from 23 genes were identified in 56.6% (90/159) of probands. These mutations were harbored in 13 LCA-associated genes and in ten genes related to other forms of retinal dystrophy. The most frequently mutated gene in probands with LCA was *GUCY2D* (10.7%, 17/159). A series of mutational analyses suggests that all known genes associated with retinal dystrophy account for 56.6% of Chinese patients with LCA. A comprehensive molecular genetic analysis of Chinese patients with LCA provides an overview of the spectrum and frequency of ethno-specific mutations of all known genes, as well as indications about other unknown genes in the remaining probands who lacked identified mutations.

1. Introduction

Leber congenital amaurosis (LCA; MIM 204000) is the most severe form of inherited retinal dystrophy with the earliest onset, affecting between 1 in 81,000 and 1 in 30,000 subjects (Koenekoop, 2004; Stone, 2007). LCA accounts for over 5% of all forms of retinal dystrophy (Koenekoop et al., 2007) and is characterized by severe vision impairment or blindness during the first year of life, roving nystagmus, sluggish or near-absent pupillary responses, oculo-digital sign, and severely reduced or extinguished electroretinography (ERG) (Weleber et al., 1993). The most common inheritance pattern is autosomal recessive, although some families have been found to have an autosomal dominant trait.

To date, 21 genes have been associated with LCA and listed in RetNet (https://sph.uth.edu/retnet/, an internationally recognized website collecting all genes responsible for retinal diseases) as of January 10, 2015. These genes include three autosomal dominant genes (*CRX*, *IMPDH1*, and *OTX2*) and 18 autosomal recessive genes (*AIPL1*, *CABP4*, *CEP290*, *CRB1*, *DTHD1*, *GDF6*, *GUCY2D*, *IQCB1*, *KCNJ13*, *LCA5*, *LRAT*, *NMNAT1*, *RD3*, *RDH12*, *RPE65*, *RPGRIP1*, *SPATA7*, and *TULP1*).

The highly heterogeneous and complex genetic and clinical features of LCA have led to the observation of phenotypic and molecular genetic overlap in various forms of retinal dystrophy. For example, mutations in eight LCA-associated genes have also been reported as causes of retinitis pigmentosa (RP) (den Hollander et al., 2001; Shen et al., 2015), while a few syndromic ocular disease genes, such as *ALMS1*, *MYO7A* and *BBS4*, have also been described as responsible for nonsyndromic LCA (Wang et al., 2011a, 2011b; Xu et al., 2015). Systematic analyses of all genes responsible for patients with LCA are limited, especially in the Chinese population (Wang et al., 2013). Thus, a comprehensive evaluation of the

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frequency of mutations in all genes responsible for various forms of retinal dystrophy would be valuable.

The purpose of the present study is to investigate the mutation spectrum and frequency of all known genes responsible for monogenic retinal dystrophies in 159 Chinese patients with LCA, based on a comparison of new whole exome sequencing data and the data from our previous studies using Sanger sequencing and whole exome sequencing (Chen et al., 2013; Li et al., 2011; Xu et al., 2015). In the current study, variants in all RetNet genes were analyzed based on the whole exome sequencing dataset of the 66 new Chinese probands with LCA. In addition, the variants in all of these genes except for 19 known LCA-associated genes previously investigated, were also analyzed in 21 of 41 probands determined to lack identified mutations following whole exome sequencing studies [15 out of 41 probands with identified mutations by Chen et al. (Chen et al., 2013), and five probands with *ALMS1* mutations by Xu et al. (Xu et al., 2015)]. Along with the previous Sanger sequencing and whole exome sequencing data, this series of mutational analyses reveals the spectrum and frequency of all known genes associated with retinal diseases in Chinese patients with LCA.

2. Material and methods

2.1. Patients

A total of 159 patients with LCA were recruited from the Pediatric and Genetic Clinic at Zhongshan Ophthalmic Center. Written informed consent was obtained from all probands or their guardians before the collection of clinical data and venous blood samples. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center and adhered to the tenets of the Declaration of Helsinki. Genomic DNA of each participants was extracted from leukocytes of peripheral venous blood as previously described (Wang et al., 2010). All probands were initially diagnosed with LCA based on their clinical features and examinations, including severe vision impairment or blindness from birth or a few months later, wandering nystagmus, sluggish or near-absent pupillary reflex, oculo-digital sign, and severely reduced or extinguished ERG responses.

In the current exome sequencing study, 66 probands with LCA were enrolled, including 46 probands not previously analyzed and 20 probands previously screened by Sanger sequencing but without identified mutations (Li et al., 2011). Of these 66 probands, one had an autosomal dominant trait, six had an autosomal recessive trait, and 59 were sporadic cases.

2.2. Whole exome sequencing

Exome sequencing on the 66 samples was performed by Macrogen (http://www.macrogen.com) using the Agilent SureSelect Human All Exon Enrichment Kit V4 array (51189318 base pairs; Agilent, Santa Clara, CA) and Illumina HiSeq2000 101 PE (San Diego, CA) with a sequencing depth of 125-fold, as previously described (Jiang et al., 2015; Li et al., 2015). Variants of a total of 217 genes known to be responsible for monogenic inherited retinal dystrophies and listed in RetNet (https://sph.uth.edu/retnet/, accessed in January 2015, Table A1) were selected from the whole exome sequencing dataset of the 66 probands. Variants in genes known to be related to retinal dystrophies, except for 19 LCA-associated genes analyzed previously, were selected from 21 identified probands without mutations according to our previous exome sequencing study. The NimbleGen SeqCap EZ Exome (44M) array and Illumina Genome Analyzer II platform with a sequencing depth of 60-fold were used as previously described (Chen et al., 2013). Candidate variants were filtered through the following multiple-step analysis: 1) SNPs and short indels in the exome region were filtered against data from the 1000 Genome Project (http://browser.1000genomes.org/) and Exome Variant Server (http://evs.gs.washington.edu/EVS/), excluding the variants with an allele frequency greater than 0.01; 2) exclusion of non-coding variants without altered splicing sites predicted by BDGP (http://www.fruitfly.org/seq_tools/splice.html); 3) exclusion of the synonymous variants without altered splicing sites in the genes; 4) dbNSFP (version 2.1) (Liu et al., 2013) was used to predict the pathogenicity of missense variants, excluding missense variants predicted to be benign by both Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/); 5) exclusion of one-hit-only heterozygous variants in genes with autosomal recessive traits and heterozygous variants in X-linked genes from female probands; and 6) comparison with the whole exome sequencing data of 633 individuals in ethnicity-matched regions without retinal degeneration. The remaining variants were considered to be potential pathogenic mutations.

2.3. Sanger sequencing

Sanger sequencing was used to confirm the candidate variants. Primers were used to amplify the genomic fragments harboring the variants and designed with Primer3 (http://primer3.ut.ee/) (Koressaar and Remm, 2007; Untergasser et al., 2012). The procedures used for amplification, sequencing, and target fragments analysis were previously described (Chen et al., 2013; Li et al., 2011). The intron region harboring the known mutation in *CEP290* (den Hollander et al., 2006) was also screened by Sanger sequencing in probands in which only one *CEP290* heterozygous mutation was found by whole exome sequencing. Segregation analyses were further validated in available family members.

3. Results

A total of 57 potential pathogenic mutations (42 novel) were identified in 42 out of 87 probands involving 18 genes, including 10 known genes associated with LCA as shown in Table 1, and eight genes related to other forms of retinal dystrophy listed in Table 2. Of the 42 probands, six had heterozygous mutations in autosomal dominant genes, whereas 38 probands had homozygous (12) or compound heterozygous (26) mutations in autosomal recessive genes. Mutations in *LRP5*, *BBS2* and *PEX1* were detected in three of the 21 probands without identified mutations from the previous exome sequencing studies. The segregation analysis was available for 23 out of 87 families in this study, and the mutations were segregated with the disease in those families (Figure A1), including 21 families with mutations in known LCA-associated genes, one family with a mutation in *BEST1* and one with mutations in *CLN3*.

The combination of data from previous studies and the new findings presented here identified the mutation spectrum and frequency of all known genes associated with retinal dystrophies in 159 Chinese probands with LCA. A total of 118 potential pathogenic mutations were identified in 90 of the 159 probands (56.6%) (Tables 1 and 2, and Fig. 1A), including 47.8% (76/159) of probands with mutations in 13 known genes associated with LCA and 8.8% (14/159) with mutations in nine genes responsible for other forms of retinal dystrophy. Mutations in 49.7% (79/159) of probands were found in 16 known autosomal recessive genes, whereas mutations in 6.9% (11/159) were found in six autosomal dominant genes (Fig. 1A). In total, 108 of the 159 probands were analyzed by whole exome sequencing (Fig. 1B). Of the 89 samples in our previous Sanger sequencing studies (Li et al., 2011; Wang et al., 2007; Zhang et al., 2001), the 36 samples without identified mutations were