Exome Sequencing of 47 Chinese Families with Cone-Rod Dystrophy: Mutations in 25 Known Causative Genes

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Abstract

**Objective:** The goal of this study was to identify mutations in 25 known causative genes in 47 unrelated Chinese families with cone-rod dystrophy (CORD).

**Methods:** Forty-seven probands from unrelated families with CORD were recruited. Genomic DNA prepared from leukocytes was analyzed by whole exome sequencing. Variants in the 25 genes were selected and then validated by Sanger sequencing.

**Results:** Fourteen potential pathogenic mutations, including nine novel and five known, were identified in 10 of the 47 families (21.28%). Homozygous, compound heterozygous, and hemizygous mutations were detected in three, four, or three families, respectively. The 14 mutations in the 10 families were distributed among CNGB3 (three families), PDE6C (two families), ABCA4 (one family), RPGRIP1 (one family), RPGR (two families), and CACNA1F (one family).

**Conclusions:** This study provides a brief view on mutation spectrum of the 25 genes in a Chinese cohort with CORD. Identification of novel mutations enriched our understanding of variations in these genes and their associated phenotypes. To our knowledge, this is the first systemic exome-sequencing analysis of all of the 25 CORD-associated genes.

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Introduction

Cone-rod dystrophy (CORD) refers to a series of hereditary retinal disorders with a predominantly cone involvement [1]. Rod impairment may occur at the same time as the cone impairment or appear later. Patients with CORD usually have reduced visual acuity, photophobia, and color vision defects.

CORD may be transmitted as an autosomal dominant (adCORD), autosomal recessive (arCORD), or X-linked trait (xCORD). To date, mutations in at least 25 genes have been reported to be associated with different forms of CORD, including the following: aryl hydrocarbon receptor interacting protein-like 1 (AIPL1) [1], the cone-rod homeobox containing gene (CRX) [2], guanylate cyclase activator 1A (GUCA1A) [3], guanylate cyclase 2D (GUCA2D) [4], polyadenylation factor family member 3 (PTPN3) [5], prominin 1 (PROM1) [6], peripherin 2 (PRPH2) [7], regulating synaptic membrane exocytosis 1 (RIMS1) [8], sema domain, immunoglobulin domain, transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A (SEMA4A) [9], unc-119 homolog (UNC119) [10], ATP-binding cassette, sub-family A (ABC1) member 4 (ABCA4) [11], ADAM metallopeptidase domain 9 (ADAM9) [12], chromosome 8 open-reading frame 37 (C8orf37) [13], calcium channel voltage-dependent alpha 2/delta subunit 4 (CACNA2D4) [14], cadherin-related family member 1 (CDHRL1) [15], ceramide kinase-like (CERKL) [16], cyclic nucleotide-gated channel beta 3 (CNGB3) [17], cyclin M4 (CNNM4) [18], potassium channel subfamily V member 2 (KCNV2) [19], phosphodiesterase 6C, cGMP-specific, cone, alpha prime (PDE6C) [20], retina and anterior neural fold homeobox 2 (RAHX2) [21], retinol dehydrogenase 5 (RDH5) [22], retinitis pigmentosa GTPase regulator interacting protein 1 (RPGRIP1) [23], calcium channel voltage-dependent L type alpha 1F subunit (CACNA1F) [24], and retinitis pigmentosa GTPase regulator (RPGR) [25].

In our previous study on CORD, mutations were only detected in 7 of 130 (5.38%) Chinese families with CORD by using cycle sequencing of all coding exons of five genes (CRX, GUCA2D,
GUCA1A, PRPH2, and KCN2) as well as of all exons harboring reported mutations in other 17 CORD-associated genes [26,27,28,29]. Of the seven families, all mutations were identified in genes responsible for adCORD but none in genes for arCORD and xlCORD. The genetic cause for most families (the remaining 123 of 130 (94.62%) Chinese families) was still unknown. In order to identify the additional cause of most CORD and to disclose further the mutation spectrum and frequency of the 25 genes, whole exome sequencing was used to screen for mutations in 47 unrelated Chinese families with CORD.

**Materials and Methods**

**Patients**

Forty seven probands from unrelated families with CORD were recruited from the Eye Hospital of Zhongshan Ophthalmic Center, Sun Yat-sen University. Patients with identified mutation who were included in our previous study were excluded from this one. Written informed consents were obtained from the participants or their guardians before the study, which was conforms to the tenets of the Declaration of Helsinki and follows the Guidance of Sample Collection of Human Genetic Diseases (863-plan) by the Ministry of Public Health of China. This study was approved by the Institute Review Board of the Zhongshan Ophthalmic Center. Genomic DNA was prepared from the blood leukocytes as previously described [30].

**Exome Sequencing**

Exome sequencing was completed by using a commercial service from BGI Shenzhen (http://www.genomics.cn/index.php). The exome sequencing, genotype calling, and SNP calling were in the same way as the methods reported before [31]. In brief, exome capture was carried out by using a NimbleGen SeqCap EZ Exome (44 M) array. Exon-enriched DNA fragments were sequenced by the Illumina Genome Analyzer II. The average sequencing depth was set to 60-fold. SOAP aligner was used to set the sequencing reads to UCSC hg19 [32,33]. The likelihood of possible genotypes in the target regions was calculated using SOAPsnp [34]. Variants in all the 25 genes detected by exome sequencing were selected for validation. Exome sequencing dataset of the patients with identified mutations in this study have been deposited to NIH (http://www.ncbi.nlm.nih.gov/biosample: accession number SAMN01997562 to SAMN01997571).

**Sanger Sequencing**

Sanger sequencing was used to validate variants in the 25 genes that resulted from exome sequencing, including heterozygous variants in the adCORD genes, homozygous or compound heterozygous variants in the arCORD genes, or hemizygous variants in the xlCORD genes. Primers (Table S2) used to amplify the regions containing the variants were designed by primer design tool Primer3 (http://frodo.wi.mit.edu/primer3/) [35]. A touchdown polymerase chain reaction (PCR) was used to amplify the fragments with variants, as previously reported [36], and the amplicons were analyzed with an ABI BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA) on an ABI3100 Genetic Analyzer (Applied Biosystems). Sequencing results from patients and controls were compared using the SeqManII program of the Lasergene package (DNAStar Inc, Madison, WI). Detected variants were further sequenced in the available family members. Novel variants were further evaluated in 192 control individuals. The description of mutations was in accordance with the nomenclature for the description of sequence variants [37](HGVS: http://www.hgvs.org/mutnomen/). The conservation of a variation was evaluated by Phastcons_score (http://varianttools.sourceforge.net/Annotation/PhastCons) [38], the effect of a missense variation was analyzed by using SIFT [39] (http://sift.jcvi.org/) and Polyphen-2 [40] (http://genetics.bwh.harvard.edu/pph2/) online tools, and the effect of splicing site changes was predicted by Berkeley Drosophila Genome Project (BDGP) [41] (http://www.fruitfly.org/).

Considering that CORD-causing mutations are rare and the presence of the normal carriers of arCORD gene mutations, we assumed that the affected individuals were likely homozygous or
compound heterozygous, so variants absent in the dbSNP134, 1000Genome or with allelic frequencies ≤0.006 were considered to be potentially pathogenic (frequency of heterozygote carriers calculated based on a disease incidence of 1:40,000, under the hypothesis that a unique arCORD gene would explain the remaining 40% of cases [42]).

Results

Whole exome sequencing identified 14 potential pathogenic mutations in 10 of the 47 (21.28%) families with CORD (Table 1), including seven homozygous or compound heterozygous mutations in four (ABCA4, CNGB3, PDE6C, and RPGRIP1) of the 13 genes associated with arCORD, and three hemizygous mutations in the two genes (RPGR and CACNA1F) associated with xICORD. Of the 14 mutations, nine were novel. The 14 mutations in the 10 families involved six of the 25 CORD-associated genes, including CNGB3 (three families), PDE6C (two families), RPGR (two families), ABCA4 (one family), RPGRIP1 (one family), and CACNA1F (one family) (Figure 1), respectively. Sanger sequencing confirmed the 14 mutations in the 10 families (Figure S1). Segregation analysis was available for five of the 10 families in where the mutations co-segregated with the disease in the family (Figure 2). No potential pathogenic mutation was identified in the other 19 genes of the 47 families.

Clinical data of the 10 probands with potential pathogenic mutations are listed in Table 2. All probands with identified mutations had an early onset severe form of retinal dystrophy with predominantly cone involvement. Fundus changes were mainly in

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*Figure 2. Pedigrees of the 10 families with mutations.* The family numbers and their corresponding mutations were shown just above the pedigrees. doi:10.1371/journal.pone.0065546.g002
the macular regions, showing mild pigmentary changes and a loss of foveal reflex, as well as attenuated retinal arteries in rare cases.

**Discussion**

Based on an initial screening of exome sequencing and the subsequent confirmation of Sanger sequencing, potential pathogenic mutations were identified in 10 of the 47 (21.28%) families with CORD, involving 14 mutations in six of the 25 CORD-associated genes. For the 47 families in this study, the contributions of causative mutations in individual genes are as follows: CNGB3 (6.38%), PDE6C (4.26%), RPGR (4.26%), ABCA4 (2.13%), RPGRIP1 (2.13%), and CACNA1F (2.13%).

The frequency of mutations detected in this study is significantly higher than in our previous study, in which mutations were only detected in seven of 130 (5.38%) Chinese families with CORD through the process of sequencing the coding exons of five genes and all exons with reported mutations in 17 other genes [26,27,28,29]. In addition, in this study, mutations in seven of the 10 families were found in arCORD-associated genes but no mutations were detected in the adCORD-associated genes; in contrast, mutations in all seven families from our previous study were found in adCORD-associated genes. This difference may

**Table 1. Potential pathogenic mutations detected in 10 of the 47 families.**

| Family ID | Gene   | Variations | Status | Bioinformation analysis | Allele frequency | Reference |
|-----------|--------|------------|--------|-------------------------|-----------------|-----------|
|           | DNA    | Protein    | SIFT   | Polyphen-2 | Splice | Phastcons_score | patients | controls |               |
| Family 1  | ABCA4  | c.4604dup  | p.T1537Nfs*18 | hetero | – | – | 0.997 | 1/94 | 0/384 | novel |
| Family 1  | ABCA4  | c.1957C>T  | p.R653C | hetero | D | PD | – | 1.000 | 1/94 | NA | [38] |
| Family 2  | CNGB3  | c.1774dup  | p.E592fs*44 | homo | – | – | – | 1.000 | 2/94 | 0/384 | novel |
| Family 3  | CNGB3  | c.129+1G>A | –       | homo | – | – | DSA | 1.000 | 2/94 | 0/384 | novel |
| Family 4  | CNGB3  | c.2415A>C  | p.E805D | homo | D | PD | – | 1.000 | 1/94 | NA | rs186448979^2 |
| Family 4  | CNGB3  | c.1957G>A  | p.A653T | homo | tolerated | benign | – | 0.000 | 1/94 | 0/384 | novel |
| Family 5  | PDE6C  | c.1935+1del | –       | hetero | – | – | DSA | 1.000 | 1/94 | 0/384 | novel |
| Family 5  | PDE6C  | c.2518+5G>C | NA     | hetero | – | – | DSA | 0.112 | 1/94 | 0/384 | novel |
| Family 6  | PDE6C  | c.1004+1G>A | –       | homo | – | – | DSA | 1.000 | 2/94 | 0/384 | novel |
| Family 7  | RPGRIP1| c.2592T>G  | p.Y864* | hetero | – | – | – | 0.994 | 1/94 | 0/384 | novel |
| Family 7  | RPGRIP1| c.799C>T   | p.R267* | hetero | – | – | – | 1.000 | 1/94 | NA | [36] |
| Family 8  | CACNA1F| c.2542G>A  | p.G848S | heni | tolerated | benign | – | 1.000 | 1/94 | 0/384 | novel |
| Family 9  | RPGR   | c.785C>G   | p.A848S | heni | tolerated | benign | – | 0.002 | 1/94 | NA | [39] |
| Family 10 | RPGR   | c.2447_2461del | p.G816_E820del | heni | – | – | NA | 1/94 | NA | [40] |

Note: D = damaging; PD = probably damaging; DSA = donor site abolished.

^2The variation was found in 1000 Genomes database with the Global minor allele frequency (MAF) of G = 0.001/3 so that the pathogeneity of the variants in this family need to be clarified further.

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**Table 2. Clinical data of the probands with CORD and identified potential pathogenic mutations.**

| Family ID | Gene     | Nucleotide changes | Sex | Age (year) at onset | First symptom | Best visual acuity | Fundus changes | ERG responses from |
|-----------|----------|--------------------|-----|---------------------|---------------|-------------------|----------------|-------------------|
|           |          |                    |     |                     |               |                   |                | rod | cone |
| Family 1  | ABCA4    | c.[1957C>T];[4604dup] | M   | 7.0                | 6.0           | PV, NYS          | 0.20           | MA, TPOD, ARA | Normal | Reduced |
| Family 2  | CNGB3    | c.[1774dup];[1774dup] | M   | 6.5                | EC            | PV, NYS          | 0.20           | MA, TPOD, ARA | Normal | Reduced |
| Family 3  | CNGB3    | c.[129+1G>A];[129+1G>A] | F   | 5.0                | 0.3           | PV, NYS          | 0.20           | MA, TPOD, ARA | Normal | Extinguished |
| Family 4  | CNGB3    | c.[1957G>A];[2415A>C] | F   | 4.5                | 0.5           | PP, NYS          | 0.10           | 0.20          | ARA | Severely reduced |
| Family 5  | PDE6C    | c.[1935+1del];[2518+5G>C] | M   | 7.0                | EC            | NYS              | 0.05           | 0.05          | High myopic | Normal | Severe reduced |
| Family 6  | RPGR     | c.[2447_2461del];[1004+1G>A];[1004+1G>A] | M   | 2.3                | NAP, NYS      | NANA             | ARA            | Normal | Extinguished |

Note: F = female; M = male; EC = early childhood; FMB = first few months after birth; PV = poor vision; PP = photophobia; NYS = nystagmus; PO = pursuing object; NA = not available; MA = macular atrophy; TPOD = temporal pallor of optic disc; ARA = attenuated retinal arteries; CRD = carpet-like retinal degeneration.

*This patient did not have the "electronegative" ERG in the standard combined response.

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Exome Sequencing of 47 Families with CORD

Previously studied on an individual gene or a set of genes have revealed a different frequency of mutation in CORD-associated genes in different populations. Sanger sequencing of 10 adCORD-associated genes identified mutations in 25 of 52 (48%) German families with adCORD, and mutations were found in GUCA1A (24%), PRPH2 (12%), GUCA1A (8%), CRX (4%), and PROM1 (2%) [43]. In other studies for individual genes, mutations in CRX are responsible for a 4.76% proportion of CORD [26], GUCA1A for 11.11% of CORD in a Japanese population [44] and 9.09% in a Spanish one [45], GUCA1A for 16.67% of CORD in a German one [46], PRPH2 for 11% of CORD in another German population [43], AIPL1 for 1.82% of CORD in an American sample [1], PROM1 for 0.93% of CORD in a Dutch population [16], SEMAI4 for 8.00% of CORD in a Pakistani group [9], and UNCI119 for 5.00% of CORD in another American population [10].

For genes known to be associated with arCORD, their mutations might be responsible for nearly 40% of arCORD [42], whereas mutations in ABCA4 are most frequent in European and American populations, ranging from 16.13% to 65.00% [47,48,49,50]. However, ABCA4 mutations are very rare in Chinese families with CORD. The mutation frequency of CNGB3 [47,48,49] and CRX [43] in other populations are also rare, as with RAX2 in 0.62% of CORD [21], CERKL in 1.85% of Canadian CORD [16], or ADAM9 [12], RDH5 [22], CDHR1 [15], and CIBORF57 [13] in a few reports based on an analysis of a limited number of families.

Supporting Information

Table S1 Genomic information of the 25 genes referred in this study. This table listed the accession numbers of the genomic DNA, mRNA, and protein for each of the 25 genes. The information is based on human genome reference GRCh37.p10. (XLS)

Table S2 Sequences of primers used in this study. This table listed 52 primers used to amplify the genomic fragments with variants detected by exome sequencing. (XLS)

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Author Contributions

Conceived and designed the experiments: QJZ. Performed the experiments: LH QYZ LG JZ WS YY JW QJZ. Analyzed the data: LH QYZ LG JZ WS YG PW XG JW QJZ. Contributed reagents/materials/analysis tools: LH SL XX JZ XJ WS YG PW XG JW QJZ. Wrote the paper: LH QJZ.

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