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Molecular genetics of cone-rod dystrophy in Chinese patients: New data from 61 probands and mutation overview of 163 probands

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ABSTRACT

Cone-rod dystrophy (CORD) is a common form of inherited retinal degeneration. Previously, we have conducted serial mutational analysis in probands with CORD either by Sanger sequencing or whole exome sequencing (WES). In the current study, variants in all genes from RetNet were selected from the whole exome sequencing data of 108 CORD probands (including 61 probands reported here for the first time) and were analyzed by multistep bioinformatics analysis, followed by Sanger sequencing and segregation validation. Data from the previous studies and new data from this study (163 probands in total) were summarized to provide an overview of the molecular genetics of CORD. The following potentially pathogenic mutations were identified in 93 of the 163 (57.1%) probands: *CNGA3* (32.5%), *ABCA4* (3.8%), *ALMS1* (3.1%), *GUCY2D* (3.1%), *CACNA1F* (2.5%), *CRX* (1.8%), *PDE6C* (1.8%), *CNGB3* (1.8%), *GUCA1A* (1.2%), *UNC119* (0.6%), *RPGRIP1* (1.2%), *RDH12* (0.6%), *KCNV2* (0.6%), *C21orf2* (0.6%), *CEP290* (0.6%), *USH2A* (0.6%) and *SNRNP200* (0.6%). The 17 genes with mutations included 12 known CORD genes and five genes (*ALMS1*, *RDH12*, *CEP290*, *USH2A*, and *SNRNP200*) associated with other forms of retinal degeneration. Mutations in *CNGA3* is most common in this cohort. This is a systematic molecular genetic analysis of Chinese patients with CORD.

1. Introduction

Cone-rod dystrophy (CORD, OMIM #120970), with a prevalence of 1/40 000, is one of the common forms of inherited retinal degeneration. The characteristics of CORD are the impairment of cone photoreceptors with or without dysfunction of rod photoreceptors. The clinical manifestations are photophobia, reduced visual acuity, color vision defects, and central scotomata. Recording on electroretinography (ERG) provides valuable tool in the diagnosis of CORD (Hamel, 2007).

At present, a total of 30 genes have been associated with CORD, including 10 genes related to autosomal dominant (AD) CORD (*AIPL1*, *CRX*, *GUCA1A*, *GUCY2D*, *PITPNM3*, *PROM1*, *PRPH2*, *RIMS1*, *SEMA4A*, *UNC119*), 18 genes related to autosomal recessive (AR) CORD (*ABCA4*, *ADAM9*, *C21orf2*, *C8orf37*, *CACNA2D4*, *CDHR1*, *CERKL*, *CNGA3*, *CNGB3*, *CNNM4*, *KCNV2*, *PDE6C*, *PDE6H*, *RAB28*, *RAX2*, *RDH5*, *RPGRIP1*, *TTLL5*), and two genes related to X-linked (XL) CORD (*CACNA1F*, *RPGR*) (RetNet: https://sph.uth.edu/retnet/sum-dis.htm). Mutation screenings of these CORD genes have been reported in several studies in different populations (Freund et al., 1997; Ito et al., 2004; Jiang et al., 2005; Littink et al., 2010), but mutations in all these genes are only responsible for a small fraction of families with CORD. Most of previous studies have focused on individual genes or, occasionally, a subset of genes, rather than on all of the known genes that are associated with retinal diseases (Michaelides et al., 2006). An overlap of genotype and phenotype has also been reported (Berger et al., 2010; den Hollander et al., 2010), which suggests a relationship between CORD and other forms of retinal degeneration. It will be curious to know if mutations in genes associated with other forms of retinal dystrophy plays a role in CORD and to what extent? Meanwhile, identification of the genetic defects is the prerequisite for precision medicine and gene therapy (Maguire et al., 2009).

A series of genetic analysis on known CORD genes have been performed on Chinese patients with CORD in our lab before (Huang et al., 2012, 2013a, 2013b, 2013c; Li et al., 2014). Here, a new cohort

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of 61 probands with CORD has been analyzed by whole exome sequencing (WES). Mutations in genes known associated with CORD as well as all those in genes listed in RetNet responsible for other forms of retinal degeneration have been systemically analyzed. Meanwhile, Mutations in genes for other forms of retinal degeneration have also been analyzed in the 47 probands previously analyzed by WES (Huang et al., 2013c). Then, we systemically summarized all mutations in these patients as well as in those patients with CORD previously reported by our lab. These may provide us an overview of mutations in 217 genes listed in RetNet in our cohorts with CORD as well as the spectrum and frequency of identified mutations in 93 of the 163 probands with CORD in a single institute.

2. Materials and methods

2.1. Patients

Our cohort consisted of 163 unrelated CORD probands (from 15 AD families, 33 AR families, and 115 sporadic families) who were recruited for the molecular genetic analysis from our Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Center from 1996 to 2014. Written informed consent was obtained from participating individuals or their guardians prior to the study. This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center. Genomic DNA was prepared from venous leukocytes using a method described previously (Wang et al., 2010). All research adhered to the tenets of the Declaration of Helsinki.

2.2. Molecular genetic analysis

The molecular genetics data for 163 Chinese CORD patients in our cohort were summarized, including new data from 61 probands. Previous studies was divided into groups chronologically starting from Apr 2011 (Table 1). Linkage was performed first in the family QT241 (Xiao et al., 2011), and Sanger sequencing was performed in all coding exons of *CRX*, *GUCA1A*, *GUCY2D*, *KCNV2*, *UNC119*, and *PRPH2*, and in the coding exons with reported mutations in the remaining CORD genes (Huang et al., 2013a, 2013b, 2012). WES of the first cohort with 47 probands whose mutations in 25 known CORD genes were analyzed (Huang et al., 2013c). All exons of *CNGA3* were sequenced in 138 CORD patients simultaneously (Li et al., 2014).

In addition to the summary of previous studies, a new cohort of CORD with 61 probands has been analyzed in the current study by WES. Detailed methods used for the sequencing process were published in a previous study (Li et al., 2015). Then, multistep bioinformatics analysis was used to identify pathogenic variants in the 61 probands for all 217 genes (Jan 2015, Table A.1) listed in RetNet (https://sph.uth.edu/retnet/, a web site that provides tables of genes and loci that cause inherited retinal diseases, including 30 genes that are responsible for CORD and 187 genes that are responsible for other forms of retinal degeneration. Genes associated with complex diseases as well as genes encoded by mitochondrial DNA were not included). Meanwhile, similar strategy was used to identify additional pathogenic mutations in the first cohorts (47 probands) with WES for the remaining 192 genes listed in RetNet (except for the 25 genes previously reported (Huang et al., 2013c). The process for bioinformatics analysis was the same as previously described (Xu et al., 2014).

The resulting candidate mutations were verified by Sanger sequencing. Segregation analysis was performed in the families with available family members. The WES data of 257 glaucoma patients (514 chromosomes) was used as in house controls, and the WES data from the Exome Aggregation Consortium (ExAC: http://exac.broadinstitute.org/) were used for reference. Novel mutations were confirmed in 96 normal controls (192 chromosomes). The online prediction tools Polyphen-2 (Adzhubei et al., 2010) (http://genetics.bwh.harvard.edu/pph2/), SIFT (Kumar et al., 2009) (http://sift.jcvi.org/), and Berkeley Drosophila Genome Project (Celniker et al., 2002) (BDGP: http://fruitfly.org/) were used to analyze the bioinformatics of these mutations. All of the mutations were named according to the guidance of the Human Genome Variation Society (HGVS: http://www.hgvs.org/mutnomen/) and were verified using the Mutalyzer program (http://www.lovd.nl/mutalyzer/).

2.3. Clinical examination

Clinical features, including the first symptom, the onset age, the motion of the eye, and fundus changes, were determined by experienced ophthalmologists at the Zhongshan Ophthalmic Center. Other ophthalmic examinations included fundus photography, color vision tests, best corrected visual acuity (BCVA), ERG, and optical coherence tomography (OCT).

Table 1
Workflow of the whole cohort.

Time	No. of samples	Methods	Analyzed genes	Reference
2007.10	1	Linkage and Sanger Sequencing	GUCY2D	Xiao et al., 2011
2011.11	130	Sanger sequencing	Genes known for CORD: (1) All coding exons of CRX, GUCY2D, GUCA1A, KCNV2, PRPH2, UNC119; (2) Totally 35 exons with reported mutations in the following 17 genes (AIPL1, PITPNM3, PROM1, PRPH2, RIMS1, SEMA4A, ABCA4, ADAM9, CACNA2D4, CDHR1, CERKL, CNGB3, CNNM4, PDE6C, RAX2, RPGRIP1, RPGR)	Huang et al., 2013a; Huang et al., 2013b; Huang et al., 2012
2012.8	138	Sanger sequencing	CNGA3	Li et al., 2014
2012.8	47	WES of the first cohort	25 genes known for CORD (AIPL1, CRX, GUCA1A, GUCY2D, PITPNM3, PROM1, PRPH2, RIMS1, SEMA4A, UNC119, ABCA4, ADAM9, C8ORF37, CACNA2D4, CDHR1, CERKL, CNGB3, CNNM4, KCNV2, PDE6C, RAX2, RDH5, RPGRIP1, CACNA1F, RPGR)	Huang et al., 2013c
2015.1	47	WES of the first cohort	192 RetNet genes except for the 25 known genes listed above	this study
2015.1	61	WES of the second cohort	217 RetNet genes[a]	this study

WES= Whole exome sequencing.
[a] The 217 genes represent all the genes listed in RetNet except for the genes associated with complex diseases as well as genes encoded by mitochondrial DNA.