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**RESEARCH ARTICLE**

# Whole-exome sequencing identified a novel mutation in *CHM* of a Chinese family

HUI TANG[1,2], JUN MAO[1,2], JINGJING XIANG[1,2], MINJUAN LIU[1,2], HAIBO LI[3]* and TING WANG[1,2]*

[1]*Center for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu, People's Republic of China*
[2]*Center for Reproduction and Genetics, Suzhou Municipal Hospital, Suzhou, Jiangsu, People's Republic of China*
[3]*Ningbo Women & Children's Hospital, Ningbo, Zhejiang, People's Republic of China*
*For correspondence. E-mail: Haibo Li, lihaibo-775@163.com; Ting Wang, biowt@njmu.edu.cn.

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**Abstract.** Choroideraemia (CHM) is a rare X-linked progressive-inherited retinal disease. In this study, we diagnosed and explored the genetic cause in a Chinese pedigree exhibiting nyctalopia and decreased visual acuity in early life. Clinical data and peripheral blood samples were collected from available family members. Sanger sequencing of *RPGR* and *RP2* genes, and subsequently whole-exome sequencing was carried out to investigate the molecular cause. The proband was initially diagnosed as retinitis pigmentosa and experienced night blindness at an early age and decreased visual acuity in teens. The other affected males in this family suffered from the same problem. Direct sequencing failed to reveal the genetic cause and hence a novel hemizygous mutation c.861_862insGCTT was detected by WES in *CHM* gene resulting in a premature stop codon and a truncated protein. Subsequently, it was confirmed by Sanger sequencing and co-segregation analysis. We describe a novel mutation c.861_862insGCTT in *CHM* gene in a Chinese pedigree with choroideraemia. Our study emphasizes the utilization of next-generation sequencing in the diagnosis and genetic analysis of retinal diseases.

**Keywords.** choroideraemia; retinitis pigmentosa; whole-exome sequencing; *CHM* gene.

## Introduction

Vision impairment, especially blindness, is a wide public concern globally. According to a systematic study in 2015, 441.1 million people were involved including 36.0 million blind people (Bourne *et al.* 2017). In developed countries, one-third of people older than 75 years inherited retinal diseases like retinitis pigmentosa (RP), macular degeneration and choroideraemia (CHM) contribute to blindness (Tucker *et al.* 2014).

RP, the most common inherited disease of the retina refers to a group of disorders and progresses with a variety of clinical manifestations, specifically affecting the rod photoreceptors (Hartong *et al.* 2006). To date, 91 different genes have been found to be responsible for RP and inherited in autosomal dominant, autosomal recessive and X-linked recessive patterns according to RetNet (https://sph.uth.edu/retnet/). They account for ~60% RP patients (Hartong *et al.* 2006), among whom 5–15% exhibit X-linked recessive transmission mode (Anasagasti *et al.* 2012). Conversely, CHM is a rare X-linked progressive inherited retinal disease characterized by progressive chorioretinal atrophy and its incidence is estimated at 1: 50.000–100.000 (Cremers *et al.* 1990; van den Hurk *et al.* 1997). Notably, it presents several common clinical symptoms of RP such as initially night blindness, constriction of the visual field, gradually reduction in visual acuity, and retinal degeneration which probably makes it difficult to distinguish it from RP and make accurate diagnosis especially without typical fundus appearance. Hence, molecular diagnosis is of great importance.

In this study, we describe a male patient initially diagnosed as RP with nyctalopia and reduced visual acuity. The pedigree presented with X-linked recessive mode. Firstly, we directly sequenced *RPGR* and *RP2* genes that accounted for 70–90% and 10–20% of X-linked recessive RP, respectively (Vervoort *et al.* 2000). No pathogenic mutation was

Hui Tang and Jun Mao contributed equally to this work.

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revealed. Further, whole-exome sequencing was performed and a novel mutation c.861_862insGCTT in *CHM* gene leading to premature termination of protein was detected and confirmed by Sanger sequencing. Our research emphasizes the utilization of whole-exome sequencing and suggests it could benefit the diagnosis of retinal diseases with similar phenotypes like CHM and RP.

## Patients and methods

### *Family pedigrees and samples*

We collected 11 peripheral blood samples from the family including three affected males. Genomic DNA was extracted from venous leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to standard extraction methods.

### *Direct sequencing of* RPGR *and* RP2 *genes*

In consideration of the symptoms, we implemented direct sequencing for *RPGR* and *RP2* genes. The primers were selected from previous researches (Yang *et al.* 2002; Pomares *et al.* 2009; Jiang *et al.* 2017). All coding regions and intron–exon junctions of *RPGR* and *RP2* were amplified by the polymerase chain reaction (PCR). The products were verified by agarose gel and sequenced in both directions using ABI 3130 genetic analyzer.

### *Whole-exome sequencing and mutation confirmation by Sanger sequencing*

After quantification, a minimum of 3 $\mu$g DNA of the proband and his parents was used to create library enriched by SureSelect All Exon Target enrichment kit (Agilent, Carpinteria, USA) and sequenced by Illumina HiSeq. Sanger sequencing and cosegregation analysis of the family member samples were used to confirm the candidate variants. The sequence containing the mutation was amplified by PCR with the primers. The reagents used for a 20 $\mu$L reaction were as follows: 25 $\mu$L of 0.3 $\mu$L, 5 U/$\mu$L *Taq* DNA Polymerase (Thermo Fisher, Carlsbad, USA), 2 $\mu$L 10× PCR buffer, 2 $\mu$L 25 mM $Mg^{2+}$, 3 $\mu$L 2 mM dNTP, 0.5 $\mu$L each of 10 $\mu$M forward and reverse primers, 100 ng of genomic DNA template, and sterile $H_2O$. Amplification was performed as the following PCR protocol: 95°C for 5 min, 32 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and a final extension phase at 72°C for 7 min. Further, the products were stored at 4°C. PCR products were verified by agarose gel electrophoresis and subsequently sequenced in both directions using ABI 3130 Genetic Analyzer.

This study was approved by the institutional ethics committee of the Affiliated Suzhou Hospital of Nanjing Medical University. Informed consent was obtained from all individuals participated in this study.

## Results

### *Clinical features*

The pedigree of this family showed X-linked recessive transmission mode for 19 individuals (figure 1). We gathered samples from 11 members including three affected males and four female carriers. Three affected males presented with poor night vision and reduced visual acuity at different ages. The member III: 1 exhibited ocular symptoms at 13 years old and III: 2 at 20. The proband was initially diagnosed as RP, and experienced nyctalopia at an early age and decreased visual acuity in teens. When he came for consultation for *in vitro* fertilization at our centre at 29 years old in 2011, he had tunnel vision and terribly poor impaired vision. Unfortunately, all of the patients did not receive ophthalmologic examination hence we could not gather more clinical information about their ocular physiological condition.

### *Direct and whole-exome sequencing*

To expedite the genetic result, we conducted Sanger sequencings of *RPGR* and *RP2* genes in the light of phenotypes. However, we did not find the disease-causing mutations. Further whole-exome sequencing was carried out to explore molecular causes. It generated about 9.5 Gb of sequence data and 98.7% of the targeted region were sequenced at least 10× and 95.4% were sequenced 20×. The coverage of target region was 99.4% and the average sequencing depth was 131. Among all the nonsynonymous variants, we filtered the variants with minor allele frequencies $< 0.01$ in the 1000 Genome Project and The Exome Aggregation Consortium (ExAC). Several online websites were used to analyse and predict the influence and conservation such as MutationTaster and SIFT. In view of patients' clinical performance, a novel hemizygous mutation c.861_862insGCTT in *CHM* gene (NM_000390) came into sight.

### *Confirmation of candidate mutation and cosegregation analysis*

Sanger sequencing confirmed the mutation c.861_862insGCTT in the proband and the cosegregation analysis of other family members revealed that all the affected male patients III: 1 and III: 2 harboured this mutation while four female carriers were heterozygous for this insertion mutation (figure 2). In addition, other normal members did not carry the mutation. Consequently,

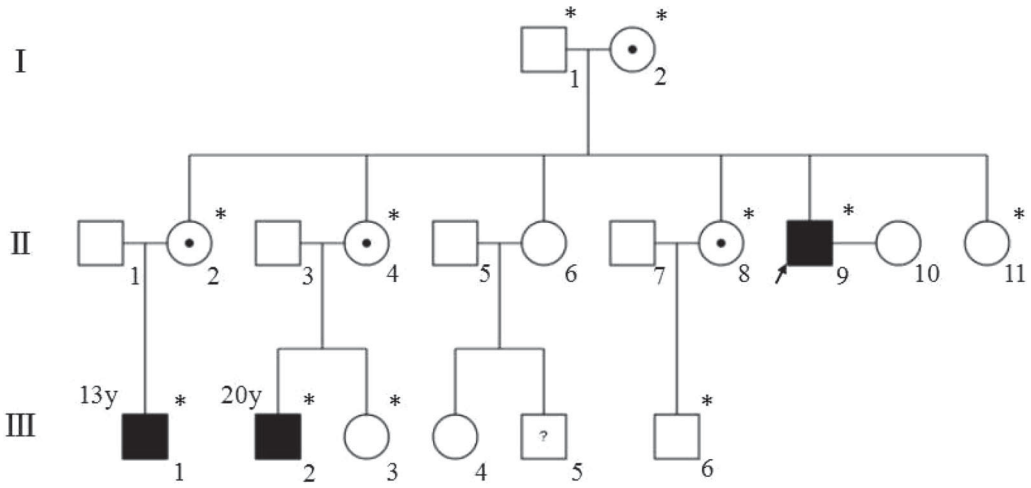


**Figure 1.** Segregation of the *CHM* mutation in the family. *Family members with collected blood samples. The onset age of decreased visual acuity in the patients is indicated by 13y and 20y.

c.861_862insGCTT in *CHM* satisfied the cosegregation situation in this family.

## Discussion

Choroideraemia is an X-linked recessive inherited chorioretinal dystrophy with progressive structural changes (Khan *et al.* 2016). Affected males experienced nyctalopia and progressive loss of peripheral vision in their first or second decade of life that lead to tunnel vision and eventual blindness (Coussa and Traboulsi 2012). Female carriers usually maintain a good vision throughout their life and demonstrated characteristic fundus changes. *CHM*, the gene responsible for choroideraemia, was cloned in 1990 (Cremers *et al.* 1990). It consists of 15 exons and encodes a protein of 653 amino acids: the Rab Escort protein 1 (REP-1) (van Bokhoven *et al.* 1994). REP1 is a prerequisite for activation of the Ras-associated binding (Rab) proteins (Seabra 1996; Seabra *et al.* 1993) which play an important role in several vesicles trafficking processes and signalling to other organelles (Corbeel and Freson 2008). REP-1 attracts Rab proteins in the cytosol, conduces them to GGTase-II heterodimeric complex for prenylation and delivers to donor membrane (Patricio *et al.* 2018). Pylypenko *et al.* (2003) and Rak *et al.* (2004) described two domains of REP-1 interacting with Rab-7 (Pylypenko *et al.* 2003; Rak *et al.* 2004). Based on a model of protein structure, the insertion mutation c.861_862insGCTT (p.Thr288Alafs*20) yielded a stop codon and eliminates 346 residues of the protein structure including the C-terminus, which may result in the loss of interaction with Rab-protein even disrupting rep-1 activity (Sergeev *et al.* 2009).

To date, 346 unique variants are reported in locus specific database list (LOVD). Nonsense, splicing mutations and frameshift mutations account for more than 90%. Meanwhile five missense mutations and a single-base substitution in promoter are described (Sergeev *et al.* 2009; Esposito *et al.* 2011; Ramsden *et al.* 2013; Torriano *et al.* 2017; Heon *et al.* 2016; Radziwon *et al.* 2017), and no clear genotype–phenotype correlation was determined (Simunovic *et al.* 2016). Interestingly, our proband exhibited decreased vision at 10 years of age which is much younger than reported age over 60. In addition, research suggested that a typically slow rate of VA loss and a good prognosis for central VA retention until the seventh decade (Roberts *et al.* 2002). We also searched articles in Chinese about choroideraemia from 2000 to 2017 and discovered that almost all the authors mentioned the signs of reduced visual acuity early in Chinese patients and some of research were without the description about the symptoms. One study observed damaged vision in patients at the age of 20 that was similar to our proband and others from 30 to 40 who were also younger than 60. The summaries about reduced visual acuity in Chinese patients are listed in table 1. Meanwhile, the mutation c.862dupA (p.Thr288Asnfs*19) was detected in Iberian population and resulted in a stop codon at residue 306. However, the patient's best corrected visual acuity was 1 OD (LogMAR) and 1 OS in 28 years old (Sanchez-Alcudia *et al.* 2016) suggesting variable phenotypes between Chinese patients and patients of other races and Chinese patients with CHM may experience severe vision loss at an earlier age than those of Caucasian origin (Zhou *et al.* 2012, 2017).

In conclusion, we detected a novel mutation c.861_862insGCTT in *CHM* gene in a Chinese pedigree presenting with night blindness at an early age and decreased visual acuity in teens by WES whose initial diagnosis was RP. Then the diagnosis was corrected to CHM. We emphasize the utilization of next-generation sequencing for the diagnosis and genetic analysis of retinal diseases and also found Chinese choroideraemia patients experienced reduced visual acuity early than previously reported patients from other races.

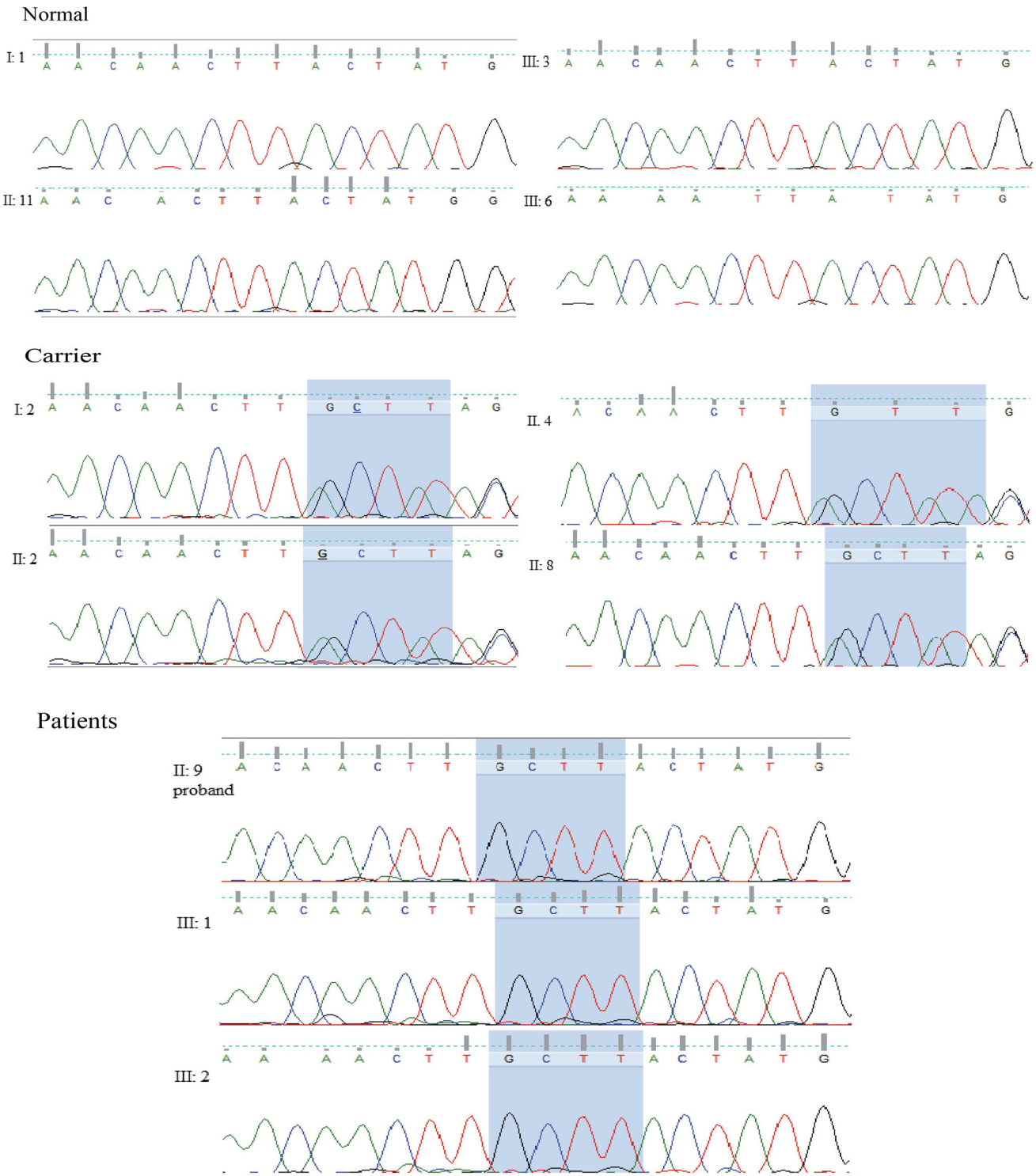


**Figure 2.** Sanger sequencing of 11 family members. The mutations are shaded.

**Table 1.** Decreased vision in Chinese choroideraemia patients.

| Subjects | Variations | Types of variations | Reduced visual acuity | Age of reduced visual acuity |
|---|---|---|---|---|
| Yip *et al.* (2007) | c.627dupA | Frameshift | NR | NR |
| | c.652_655delTCAC | Frameshift | NR | NR |
| | c.703-1G>C | Splicing | NR | NR |
| | c.1019C>A | Nonsense | NR | NR |
| | c.1584_1587delTGTT | Frameshift | NR | NR |
| Lin *et al.* (2011) | c.1488delGinsATAAC | Frameshift | Y | NR |
| | c.1703 C>G | Nonsense | Y | NR |
| Zhou *et al.* (2012) | c.1801–1G>A | Splicing | Y | 20s |
| | c.1130 T>A | Nonsense | Y | 30s-40s |
| | c.612delAG | Frameshift | Y | 20s |
| Li *et al.* (2012) | c.703-1G>A | Splicing | Y | Early age |
| | c.558_559delTT | Frameshift | Y | Before 30s |
| | c.1166+2T>G | Splicing | Y | Early age |
| | c.966delA | Frameshift | Y | Before 30s |
| | c.964G>T | Nonsense | Y | Early age |
| | c.1584_1587delTGTT | Frameshift | Y | Early age |
| Guo *et al.* (2015) | c.1475_1476insCA | Frameshift | Y | Early age |
| Cai *et al.* (2016) | c.227_232delinsTGTCATTTCA | Frameshift | Y | NR |
| | c.1584_1587del TGTT | Frameshift | Y | NR |
| | c.710dupA | Frameshift | Y | NR |
| Zhu *et al.* (2017) | c.C799T | Nonsense | Y | NR (60 yrs proband with extreme ametropia) |
| Zhou *et al.* (2017) | Exon1-15 deletion | Deletion | Y | About 40s |
| | Exon4 duplication | Duplication | Y | |
| | c.49+5G>C | Splicing | Y | |
| | Exon3-15 deletion | Deletion | Y | |
| | Exon1-15 deletion | Deletion | Y | |
| | Exon1-15 deletion | Deletion | Y | |

NR, not reported; Y, yes.

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