

New recessive compound heterozygous variants of *RP1L1* in *RP1L1* maculopathy

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Received: 2023-06-02 Accepted: 2023-11-28

Abstract

• **AIM:** To identify a maculopathy patient caused by new recessive compound heterozygous variants in *RP1L1*.

• **METHODS:** Comprehensive retinal morphological and functional examinations were evaluated for the patient with *RP1L1* maculopathy. Targeted sequence capture array technique was used to screen potential pathologic variants. Polymerase chain reaction and Sanger sequencing were used to confirm the screening results.

• **RESULTS:** Fundus examination showed round macular lesions appeared in both eyes. Optical coherence tomography showed that the inner segment/outer segment continuity was disorganized and disruptive in the left eye, but it was uneven and slightly elevated in the right eye. Fundus autofluorescence showed patchy hyper-autofluorescence in the macula. Visual field examination indicates central defects in both eyes. Electroretinogram (ERG) and multifocal ERG showed no obvious abnormalities. Fundus fluorescein angiography in the macula showed obviously irregular hyper-fluorescence in the right eye and slightly hyper-fluorescence in the left eye. We found that the proband carried a missense variant (c.1972C>T) and a deletion variant (c.4717_4718del) of *RP1L1*, which were originated from the parents and formed compound heterozygous variants. Both variants are likely pathogenic according to the ACMG criteria. Multimodal imaging, ERG and detailed medical history are important diagnostic tools for differentiating between acquired and inherited retinal disorders.

• **CONCLUSION:** A maculopathy case with detailed retinal phenotype and new recessive compound heterozygous variants of *RP1L1* is identified in a Chinese family, which

expands the understanding of phenotype and genotype in *RP1L1* maculopathy.

• **KEYWORDS:** maculopathy; recessive; compound heterozygous variants; *RP1L1*

DOI:10.18240/ijo.2024.01.14

Citation: Cao WC, Chen QS, Gan R, Huang T, Yan XH. New recessive compound heterozygous variants of *RP1L1* in *RP1L1* maculopathy. *Int J Ophthalmol* 2024;17(1):107-112

INTRODUCTION

RP1L1 is located on chromosome 8 (8p23.1), which spans 50 kB genomic DNA and encodes a protein with 2400 amino acids^[1-3]. *RP1L1* is highly similar to *RP1* protein in structure, size and location^[4]. *RP1* is expressed in the retinal photoreceptor cell cilia and it contributes to the structural maintenance of junctional cilia^[5]. *RP1L1* is only expressed in retinal photoreceptors and it is a unique cilia component of photoreceptors^[6]. Studies have shown that knockout of *RP1L1* in mice can lead to shortening and progressive degeneration of the outer segment layer of photoreceptors^[7]. Therefore, *RP1L1* is important for the maintenance of the structure of outer segment layer of photoreceptor. Pathologic variants in *RP1L1* can lead to occult macular dystrophy (OMD), which is a hereditary macular dystrophy characterized by progressive visual loss but without apparent fundus abnormality^[8]. The disease was firstly reported in 1989 and regarded as autosomal dominant inheritance while some sporadic cases have been reported since then^[9-10]. In 2010, *RP1L1* was identified as the pathogenic gene of OMD^[11]. However, in almost 50% of OMD cases the causative remains unknown^[6]. In typical OMD patients, reduced peak amplitude occurs in the macula by multifocal electroretinogram (ERG), which reflects central cone dysfunction^[12-13]. However, other examinations such as fundus ophthalmoscopy, fundus fluorescein angiography and full-field ERG may be normal^[14-15]. Although the fundus appears normal, subtle macular change may be shown by optical coherence tomography (OCT) dependent on the severity and duration of the disease^[16]. The minor OCT features including macular ellipsoid zone (EZ) blurring and absence of the interphalangeal zone (IZ) can be detected in OMD^[17-18].

Recently, pathologic variants in *RP11* can also cause maculopathy with more pronounced macular phenotype^[6,19]. The identification of *RP11* pathologic variants can improve the accuracy of disease diagnosis and also help to improve the effectiveness of genetic counseling^[20]. Here we found an unusual recessive case of *RP11* maculopathy and identified new compound heterozygous *RP11* variants in the disease.

SUBJECTS AND METHODS

Ethical Approval With the approval of the Ethics Committee of Shenzhen Eye Hospital, the informed consents were obtained from the patient and her family members.

Comprehensive ophthalmic examinations were performed to evaluate the morphological and functional changes in the patient with *RP11* maculopathy, such as color fundus imaging, fundus autofluorescence, OCT, fundus fluorescein angiography, indocyanine green angiography, ERG, multifocal ERG and visual field examination. Genomic DNA was extracted from peripheral blood and targeted sequence capture array was used to screen potential pathologic genes. Briefly, 1 µg genomic DNA was extracted from 200 µL of human peripheral blood using Qiagen DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany). The 50 ng genomic DNA was broken into small fragments of about 200 bp by enzymology method, followed by end repair and an addition. Then DNA fragments were connected to the sequencing adaptor containing barcodes, and the fragments of about 320 bp were selected for polymerase chain reaction (PCR) amplification. The pre-library was obtained after that. The pre-library was captured by liquid phase hybridization according to the standard procedures according to the xGen Exome Research Panel V1.0 (Integrated DNA Technologies, San Diego, USA). After elution and recovery of the captured products, the exon library was obtained by PCR amplification and purification. The library was quantified by quantitative PCR, and the band size was tested by Agilent 2100. Finally, the exon library of the proband was sequenced with the read length of PE150 by the Illumina Novaseq 6000 sequencing system, and the original image was identified by CASAVA v1.82 software to produce the original sequencing data. A panel of 1690 genes which are related eye disease was further analyzed. PCR and Sanger sequencing were used to confirm the screening results.

RESULTS

The proband is an 18-year-old girl who came to the hospital due to visual deformation of her right eye for more than 4mo. The patient had no other systemic diseases, nor did other family members have similar eye diseases. The best corrected visual acuity was 0.5 (nearly 20/40) in the right eye and 1.0 (20/20) in the left eye. The intraocular pressure was normal in both eyes. Slit lamp examination showed that the cornea, the conjunctiva, the anterior chamber, the lens, and the pupil were

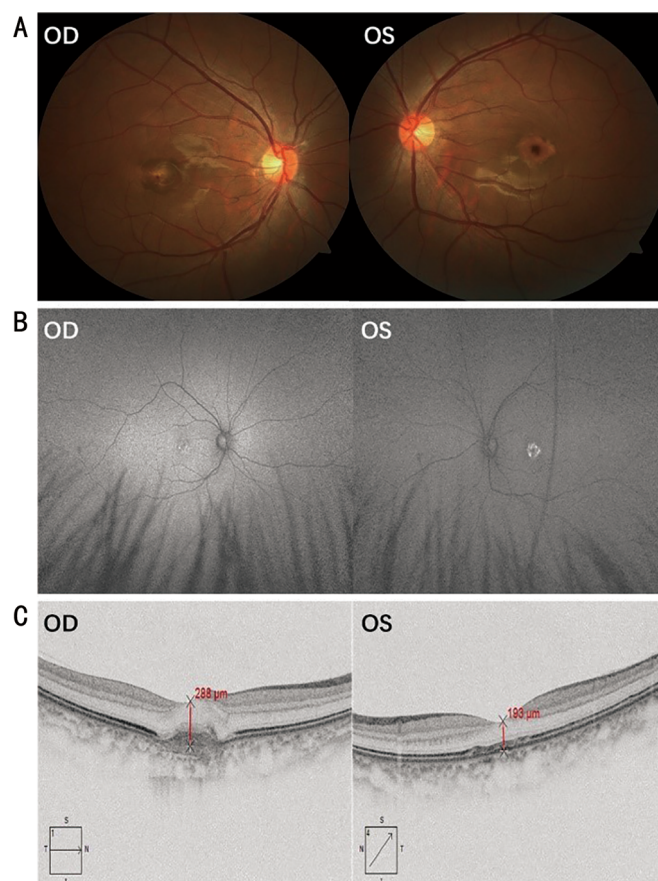


Figure 1 Multi-mode retinal imaging of both eyes in the proband
 A: Gray-white lesions can be seen in fundus photography; B: Patchy hyper-autofluorescence can be seen in the macula in fundus autofluorescence; C: OCT indicated that the inner segment/outer segment continuity was disorganized in the left eye, and was disruptive in the right eye. OCT: Optical coherence tomography.

normal. Color fundus photos showed round macular lesions appeared in both eyes (Figure 1A). Fundus autofluorescence showed patchy hyper-autofluorescence in the macula in both eyes, but more pronounced in the left eye (Figure 1B). OCT showed that the inner segment/outer segment continuity was disorganized and disruptive in the left eye, but it was uneven and slightly elevated in the right eye (Figure 1C). Fundus fluorescein angiography in the macula showed obviously irregular hyper-fluorescence in the right eye and slightly hyper-fluorescence in the left eye. Indocyanine green angiography showed dark fluorescence in the macula of both eyes but more pronounced in the right eye (Figure 2). The angiography showed a possible macular neovascularization in the right eye. ERG showed that it is generally normal in both eyes (Figure 3A). Multifocal ERG showed that the peak amplitude of macula was almost normal in both eyes (Figure 3B). Visual field examination showed slightly central defects in both eyes (Figure 3C).

To identify the underlying pathologic variant in this family, targeted sequence capture array technique was used to screen

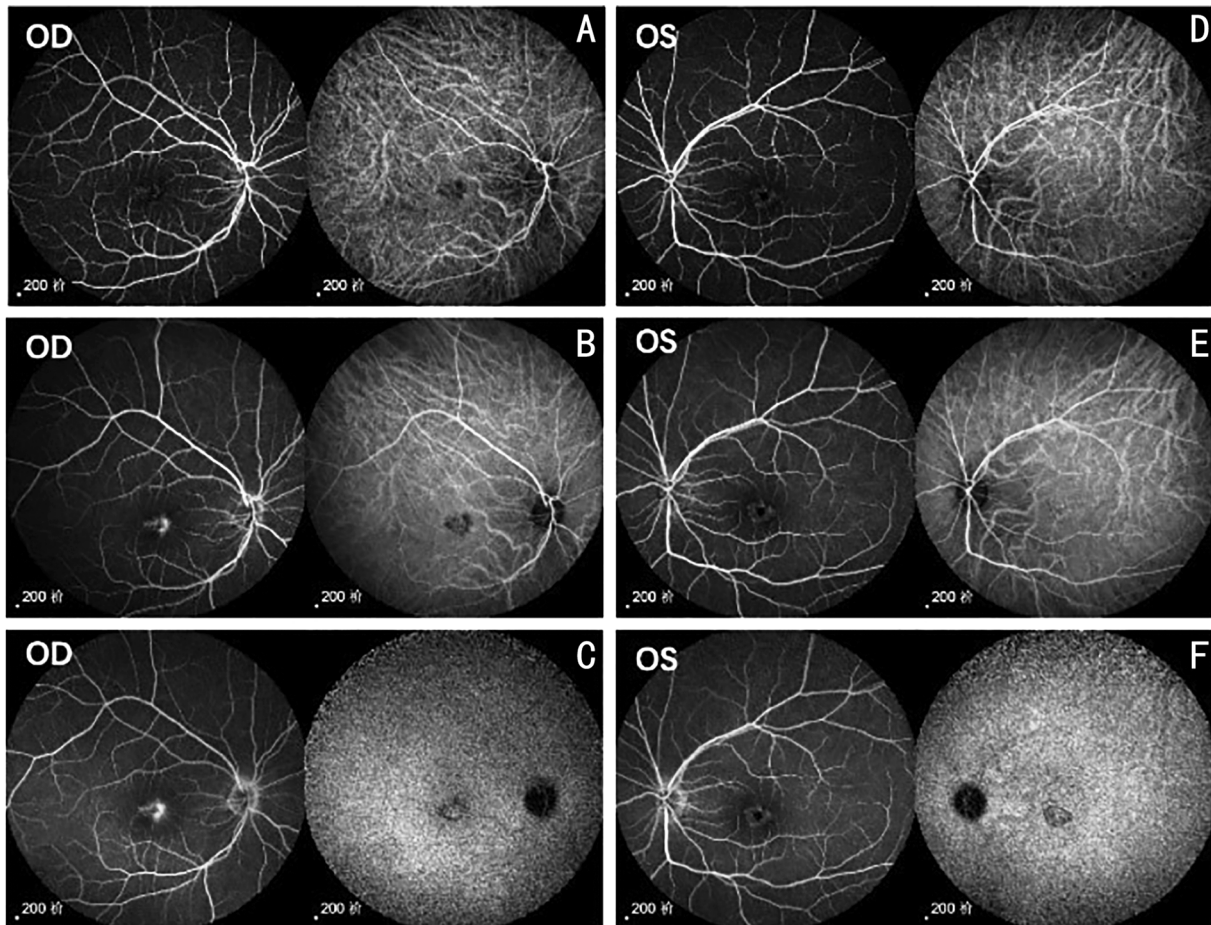


Figure 2 FFA and ICGA imaging in the proband Fundus fluorescein angiography indicated irregular hyper-fluorescence in the macular area. FFA: Fundus fluorescein angiography; ICGA: Indocyanine green angiography.

the potential variant of the proband. We found that the proband carried two heterozygous variants in *RP11L1*, including a variant c.1972C>T and a deletion variant c.4717_4718del. Her father carries the heterozygous c.4717_4718del variant, and her mother carries the heterozygous c.1972C>T variant (Figure 4). Therefore, the two variants were inherited from the father and mother, respectively. Both formed compound heterozygous variants in the proband.

DISCUSSION

In this study, target gene capture sequencing was performed to screen the potential pathogenic gene variants in the patient and we found two compound heterozygous *RP11L1* pathologic variants, including c.1972C>T (p.R658*) and c.4717_4718del (p.K1573Efs*12). Through family analysis, the genetic mode of the family is autosomal recessive inheritance. Both father and mother have normal retinal phenotypes. Either c.1972C>T (mother) or c.4717_4718del (father) does not cause eye manifestations. The *RP11L1* c.1972C>T variant (p.R658*) is a missense variant which forms a stop codon, leading to loss of function of *RP11L1*. Previously, it is reported that this variant is associated with retinitis pigmentosa^[6,21]. The other variant *RP11L1* c.4717_4718del (p.K1573Efs*12) is a deletion variant and it causes a frame shift after codon 1573, forming

a premature stop codon after 12 amino acids. This deletion is a loss-of-function variant, deleting the functionally important part of the protein. The c.4717_4718del variant has not been reported previously. Moreover, the variant c.4717_4718del occurs in the last exon of the gene and is not predicted to be nonsense-mediated mRNA decay, and the truncated region is the key functional domain of the protein (PVS1_Strong). This variant c.4717_4718del is not found in the Exome Aggregation Consortium or in the 1000G database, but the frequency of this variant is 0.000340663 in the Genome Aggregation Database (PM2). This variant is a compound heterozygosity with the other variant c.1972C>T (PM3). According to the ACMG guideline, the variant c.4717_4718del satisfies the evidence PVS1_Strong+PM2+PM3, which is determined to be a likely pathogenic variant. The variant c.1972C>T occurs in the last exon of the gene and is not predicted to be nonsense-mediated mRNA decay, and the truncated region is the key functional domain of the protein (PVS1_Strong). The frequency of this variant is 3.32883940014314e-05, 0.000199681 and 0.000319081 in ExAC, 1000G and gnomAD databases, respectively (PM2). This variant a compound heterozygosity with the variant c.4717_4718del (PM3). According to the ACMG guideline, the variant c.1972C>T satisfies the evidence

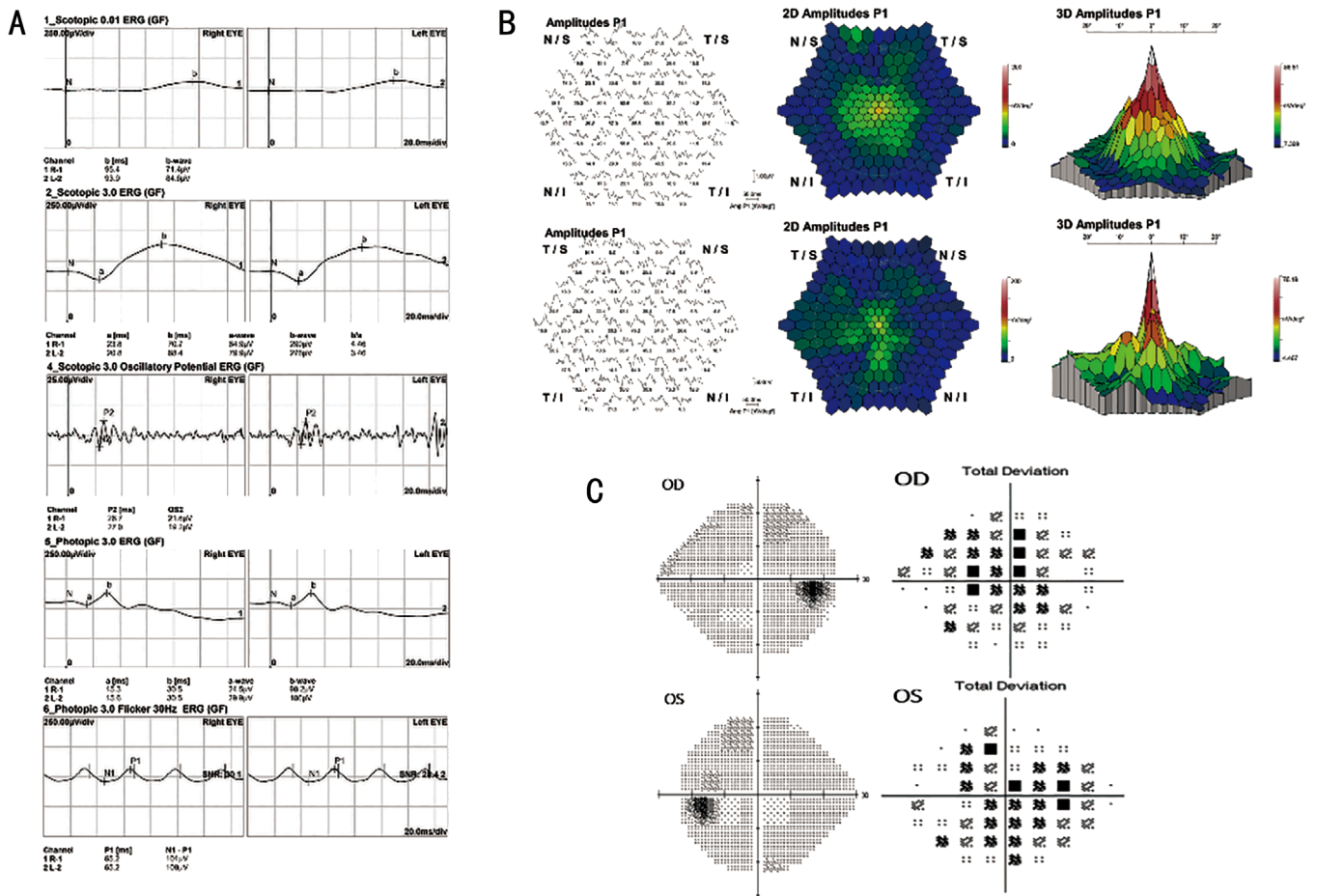


Figure 3 Retinal functional changes in the proband A: Electroretinogram indicated that it is generally normal in both eyes; B: Multifocal electroretinogram indicated no obvious abnormality; C: Visual field examination indicates central field defect.

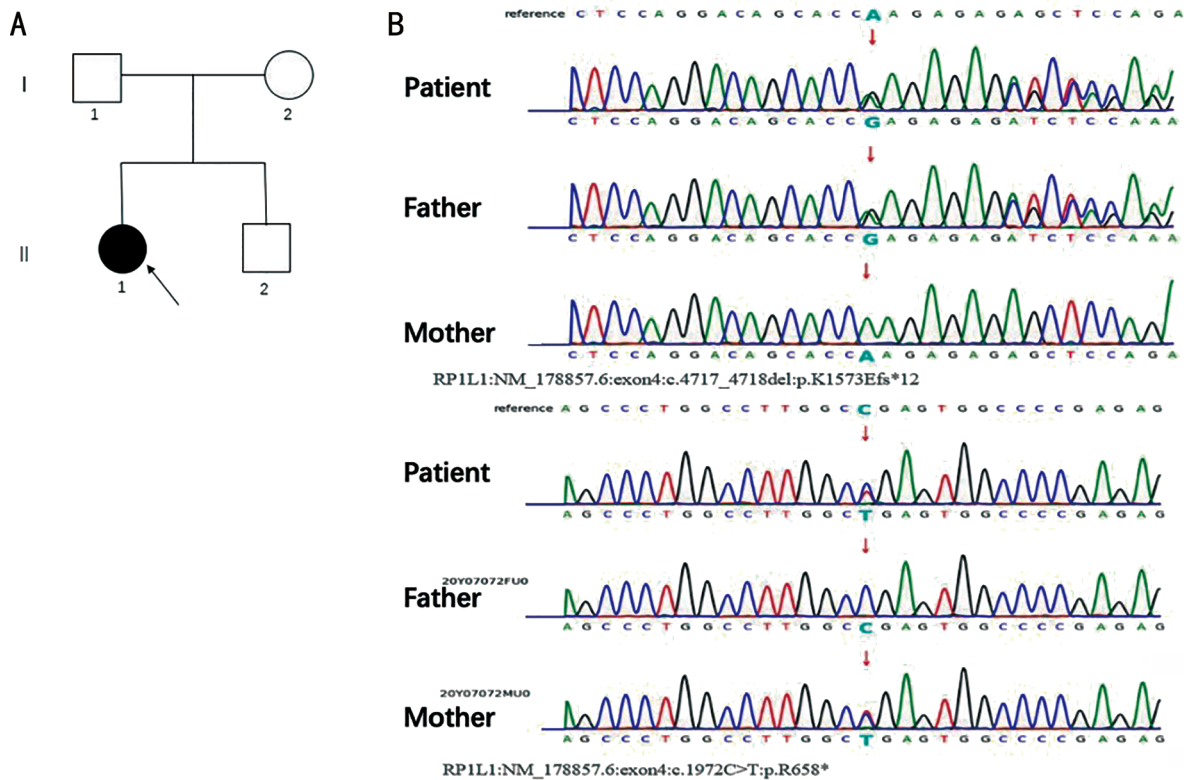


Figure 4 Pedigree of the family and genetic analysis A: Pedigree of the family with *RP11* maculopathy; B: Sanger sequencing of the patient and her parents confirmed compound heterozygous variants in *RP11*.

PVS1_Strong+PM2+PM3, which is determined to be a likely pathogenic variant. Therefore, both variants c.1972C>T and c.4717_4718del are likely pathogenic.

Our study indicated that c. 1972C>T variant not only causes RP, but also may lead to *RP1L1* maculopathy. In addition, c.4717_4718del variant has not been reported previously, which forms a frameshift stop codon. It is reported that either variant in *RP1L1* has not been reported in some patients with maculopathy, suggesting that the disease may be caused by both variants^[9]. *RP1L1* variants can also be found in RP, OMD or maculopathy^[6]. Due to pronounced phenotypic presentation in the OCT and appearance, we referred our case as *RP1L1* maculopathy. A previous study showed that the median onset age of OMD patients was 25y (range 6-51y) in China, and the median visual acuity was 0.20 (0.04-0.5)^[14]. Our patient's visual acuity was also in this range, but the exact time of onset is still unknown. In contrast, the retinal pigment epithelium (RPE) layer, which is the main source of autofluorescence, is intact in their OMD patients, while our patient has mild changes or even continuous interruption of the RPE layer in both eyes. It remains unclear whether OMD can develop to more obvious maculopathy like our case. Currently, the presence of p.Arg45Trp variant in *RP1L1* is the hot mutation in OMD^[22]. Other studies showed that the region between p.1194 and p.1201 is another hot spot of OMD^[23]. Based on OCT, patients with OMD can usually be divided into two phenotypic groups, a group with the classical SD-OCT findings in which there was blurring of the EZ and absence of the IZ^[24]. The second group was the nonclassical group with at least one of the two classical features lacking^[25]. In our case, the left eye of OCT finding was consistent with the characteristics of the typical group, especially EZ blurring was observed. In the study by Nakamura *et al*^[26], OMD can be clinically staged based on patients' clinical symptom and OCT image. They classified OMD into three stages by the presence of visual symptoms, IZ and EZ. However, the right eye of our patient does not seem to fit any of the described stages, further suggesting that this could be a novel phenotype or a case of OMD complicated by focal choroidal excavation. Patients with OMD may have progressive exacerbation of photoreceptor cell degeneration, which in turn develops into a macular phenotype visible on fundus examination. As mentioned by Noel and MacDonald^[6], at this point the patient no longer has an occult phenotype but can be called an *RP1L1* maculopathy. Since most patients with OMD may not present any symptoms, early screening with OCT is helpful for detecting the disease. Future study is needed for clarifying the pathological mechanism of OMD or *RP1L1* maculopathy.

In conclusion, we found a recessive maculopathy case and identified new compound heterozygous variants (c.1972C>T

and c.4717_4718del) in *RP1L1*, which expanded and updated our understanding of phenotype and genotype in *RP1L1* maculopathy.

ACKNOWLEDGEMENTS

Authors' contributions: Conceived and designed the experiments: Yan XH; Performed the experiments: Cao WC, Chen QS, Gan R, Huang T; Analyzed the data: Cao WC, Chen QS, Gan R, Yan XH; Contributed reagents/material/analysis tools: Chen QS and Huang T; Wrote the manuscript: Cao WC, and Yan XH. All authors reviewed the manuscript.

Foundations: Supported by Shenzhen Science and Technology Program, Shenzhen, China (No.JCYJ20200109145001814; No.SGD20211123120001001); the National Natural Science Foundation of China (No.81970790); Sanming Project of Medicine in Shenzhen (No.SZSM202011015).

Conflicts of Interest: Cao WC, None; Chen QS, None; Gan R, None; Huang T, None; Yan XH, None.

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