

# Exome sequencing analysis identifies novel homozygous mutation in *ABCA4* in a Chinese family with Stargardt disease

Xiao-Dan Hao<sup>1</sup>, Ying Liu<sup>1</sup>, Bao-Wei Li<sup>1</sup>, Wei Wu<sup>1</sup>, Xiao-Wen Zhao<sup>2</sup>

<sup>1</sup>Institute for Translational Medicine, College of Medicine, Qingdao University, Qingdao 266021, Shandong Province, China

<sup>2</sup>State Key Laboratory Cultivation Base, Shandong Provincial Key Laboratory of Ophthalmology, Shandong Eye Institute, Shandong First Medical University & Shandong Academy of Medical Sciences, Qingdao 266071, Shandong Province, China

**Correspondence to:** Xiao-Dan Hao. Institute for Translational Medicine, College of Medicine, Qingdao University, Qingdao 266021, Shandong Province, China. haoxiaodan1987@163.com; Xiao-Wen Zhao. State Key Laboratory Cultivation Base, Shandong Provincial Key Laboratory of Ophthalmology, Shandong Eye Institute, Shandong First Medical University & Shandong Academy of Medical Sciences, Qingdao 266071, Shandong Province, China. xiaowenzhao126@126.com

Received: 2019-05-22 Accepted: 2019-08-14

## Abstract

• **AIM:** To identify the disease-associated mutations in a Chinese Stargardt disease (STGD) family, extend the existing spectrum of disease-causing mutations and further define the genotype-phenotype correlations.

• **METHODS:** A Chinese STGD family and 200 normal controls were collected. Whole exome sequencing (WES) and bioinformatics analysis were performed to find the pathogenic gene mutation. Physico-chemical parameters of mutant and wildtype proteins were computed by ProtParam tool. Domains analysis was performed by SMART online software. HOPE online software was used to analyze the structural effects of mutation. Immunofluorescence, quantitative real-time polymerase chain reaction and Western blotting were used for expression analysis.

• **RESULTS:** Using WES, a novel homozygous mutation (NM\_000350: c.G3190C, p.G1064R) in *ABCA4* gene was identified. This mutation showed co-segregation with phenotype in this family. It was not found in the 200 unrelated health controls and absent from any databases. It was considered “Deleterious” as predicted by five function prediction softwares, and was highly conserved during

evolution. *ABCA4* was expressed highly in the human eye and mouse retina. The p.G1064R was located in AAA domain, may force the local backbone into an incorrect conformation, disturb the local structure, and reduce the activity of ATPase resulting in the disease pathology.

• **CONCLUSION:** We define a novel pathogenic mutation (c.G3190C of *ABCA4*) of STGD. This extends the existing spectrum of disease-causing mutations and further defines the genotype-phenotype correlations.

• **KEYWORDS:** Stargardt disease; whole-exome sequencing; *ABCA4*; novel mutation; retina

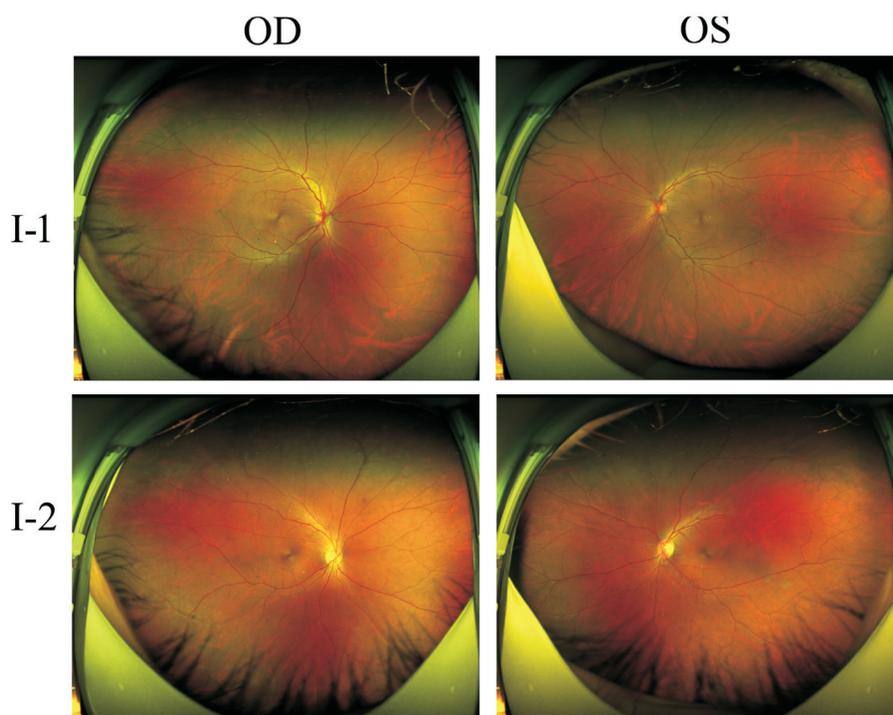
**DOI:10.18240/ijo.2020.04.22**

**Citation:** Hao XD, Liu Y, Li BW, Wu W, Zhao XW. Exome sequencing analysis identifies novel homozygous mutation in *ABCA4* in a Chinese family with Stargardt disease. *Int J Ophthalmol* 2020;13(4):671-676

## INTRODUCTION

Stargardt disease (STGD) is a kind of hereditary retinal dystrophies, characterized by adolescent-onset macular dystrophy associated with decreased central vision and atrophic changes in the bilateral macular area<sup>[1-2]</sup>. STGD is predominantly autosomal recessive inheritance, although a few are X-linked recessive inheritance or autosomal dominant inheritance<sup>[3]</sup>. Until now, mutations in six genes (*ABCA4*<sup>[4-5]</sup>, *ELOVL4*<sup>[3]</sup>, *PROM1*<sup>[6]</sup>, *BEST1*<sup>[7]</sup>, *PRPH2*<sup>[8]</sup> and *CRB1*<sup>[9]</sup>) have been reported to be associated with STGD or “Stargardt-like” disease. STGD1 is an autosomal recessive form of STGD cases, which is caused by homozygous or compound heterozygous mutation in the *ABCA4* gene (OMIM 601691) on chromosome 1p21-22<sup>[4-5]</sup>. So far, large number of (>900) sequence variants in *ABCA4* have been identified<sup>[10]</sup>. However, more than half mutations have been detected only once<sup>[11]</sup>.

In spite of the fact that a large number of mutations in the *ABCA4* gene have been reported worldwide, most of the mutations were not repeated in other studies<sup>[11]</sup>. The molecular basis for STGD remains idiopathic in some cases. Increasing evidence suggests that the identified variants may have different level of deleterious effects and lead to different onset



**Figure 1** Fundus photography results of the patients' parents OD: Right eye; OS: Left eye.

time. The STGD patient with earlier onset, tend to have worse prognosis, resulting in more serious vision loss in the children compared with adults<sup>[12]</sup>. In this paper, we present the results of a clinical and genetic analysis of a Chinese family with STGD, with the aims to extend the existing spectrum of disease-causing mutations and further define the genotype-phenotype correlations. These will be helpful for making an accurate diagnosis and classification for STGD patients with variable and atypical phenotypes.

#### SUBJECTS AND METHODS

**Ethical Approval** The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Qingdao University (Qingdao, China). Written informed consent was obtained from all participants (or guardians). The Clinical Trial Registry Number: ChiCTR1800020271.

**Subjects** Patients diagnosed with STGD were recruited from Qingdao Eye Hospital (Qingdao, China). The diagnosis of STGD was based on the results of fundus optical coherence tomography (OCT) and fundus photographs (neuro-epithelial layer thickness, high reflective slice and ellipsoid zone disruption in macular region). In total, a family of STGD and 200 unrelated healthy individuals of Chinese origin were collected.

**Whole Exome Sequencing and Data Analysis** The BBG-39M Exon Kit was used for exome capture. The Illumina X-10 platform (Illumina Inc., San Diego, CA, USA) was employed for the genomic DNA sequencing of the proband. Then conducted data analysis and filtering as the filtering strategy described in the previous study<sup>[13]</sup>. Sanger sequencing and

genotyping of the candidate gene detected by the whole exome sequencing (WES) were performed as our previous study<sup>[13]</sup>.

**Protein Structure and Function Prediction** Physicochemical parameters of proteins were computed by ProtParam tool (<http://web.expasy.org/protparam/>). SMART online software (<http://smart.embl-heidelberg.de/>) was used to do domains analysis. HOPE online software (<http://www.cmbi.umcn.nl/hope>) was used to analyze the structural effects of mutation<sup>[14]</sup>.

**Expression Detection** NCBI UniGene database (<http://www.ncbi.nlm.nih.gov/unigene/>) was used to count the expressed sequence tags (ESTs) obtained from complementary DNA clones of eye tissues. *ABCA4* expression in mouse retina and other tissues using immunofluorescence, quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting were performed as described in the previous study<sup>[15]</sup>.

#### RESULTS

**Clinical Features** Our group collected a Chinese pedigree with STGD. Clinical examination showed that the proband and her elder sister, aged 13 and 18 years old respectively, both had STGD, but their parents did not (Figures 1, 2). The pedigree suggested that the inheritance pattern of this family was autosomal recessive (Figure 3A). The proband had poor vision for 8y. The visual acuity of his left and right eye was 0.1 and 0.12 respectively. Both his eyes had macular neuro-epithelial layer atrophy, and the dioptric media was clear. Signs of fundus OCT showed neuro-epithelial layer thickness, high reflective slice and ellipsoid zone disruption in macular region (Figure 3B, 3C). The sister had poor vision for 10y. Visual acuity of her left and right eye was 0.08 and 0.1 respectively.

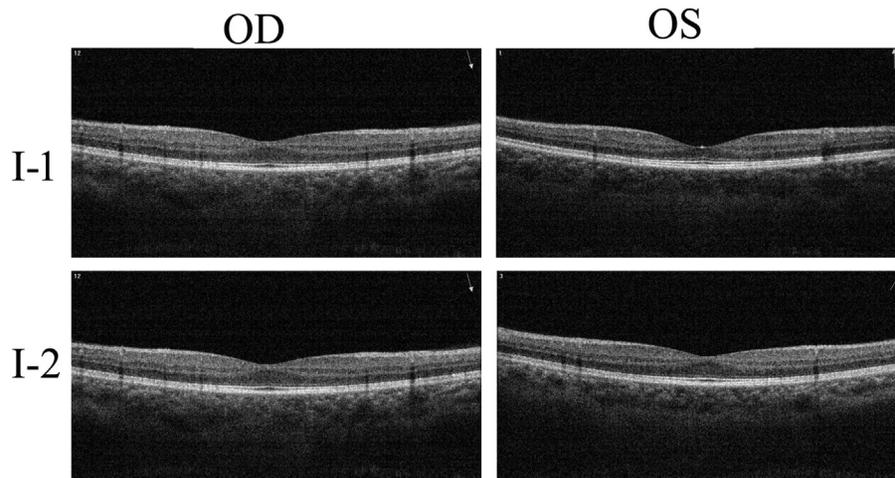


Figure 2 Fundus OCT results of the patients' parents OD: Right eye; OS: Left eye.

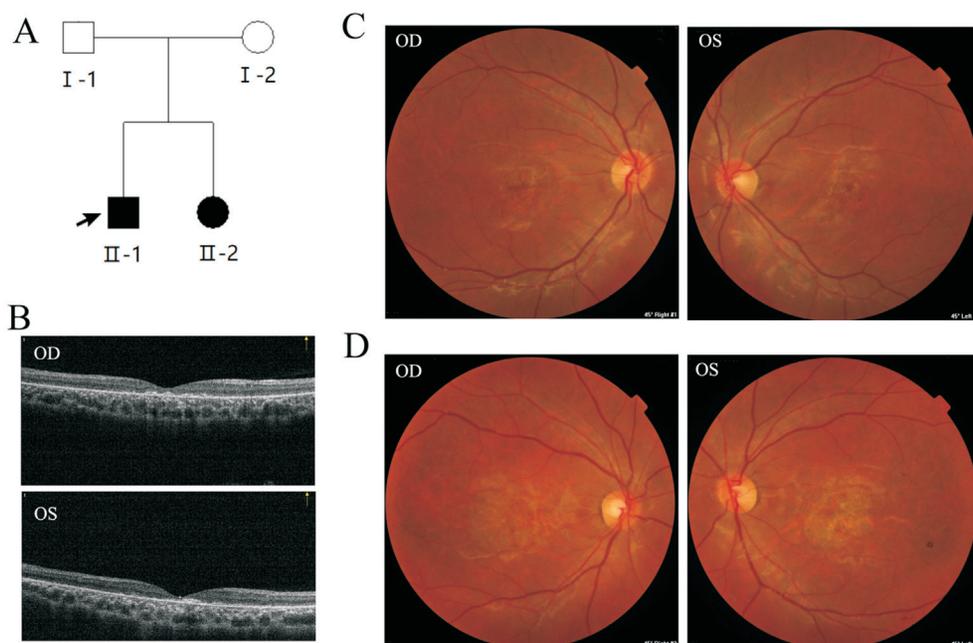


Figure 3 Pedigree and clinical characteristics of the family A: Pedigree of the family with STGD. The proband is indicated by an arrow. B: Fundus OCT results of the proband; C: Fundus photographs of the proband; D: Fundus photographs of the sister. OD: Right eye; OS: Left eye.

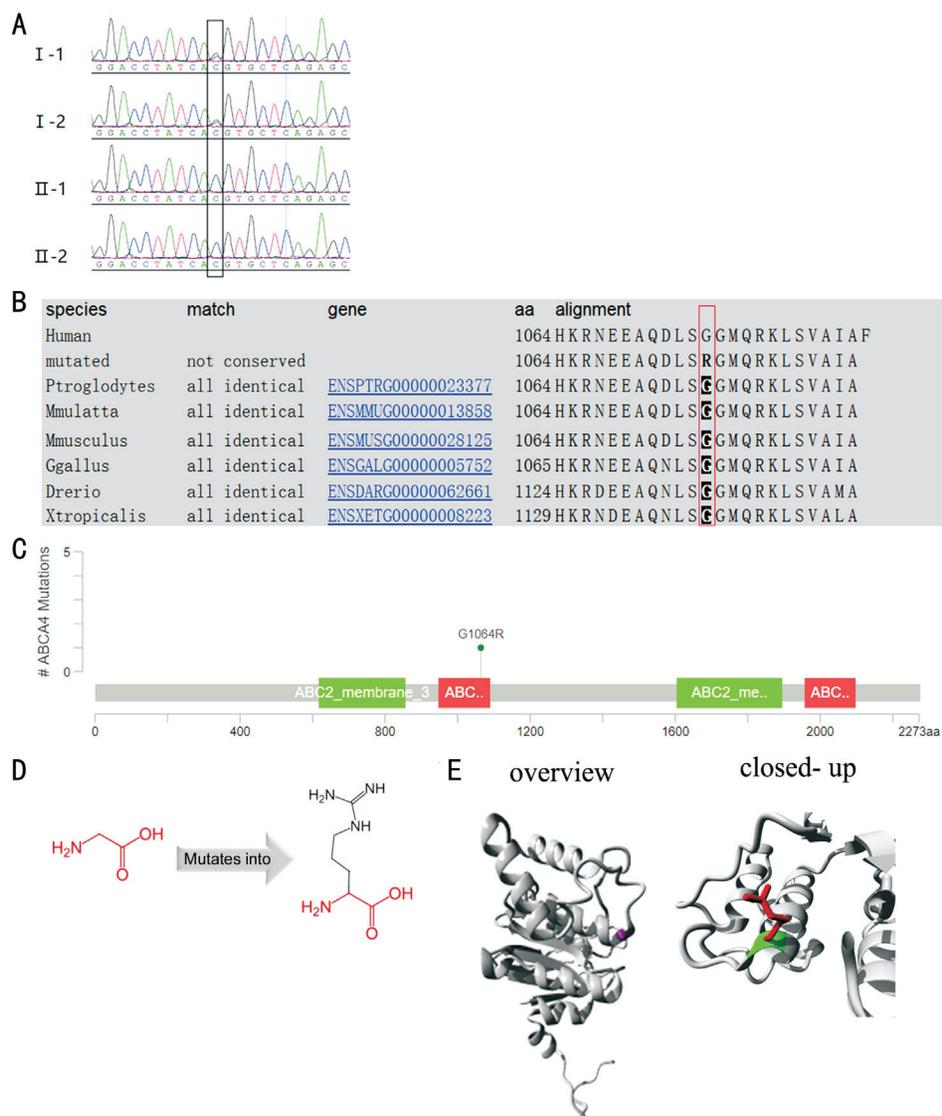
The sister's symptoms were more serious than him (Figure 3D). Her macula area region showed white yellow changes, beaten bronze appearance (Figure 3D).

**Exome Sequencing Analysis Detected a Novel Mutation of *ABCA4*** To find the pathogenic gene mutation of this family, we conducted WES on the proband. The average sequencing depth and coverage were 166× and 97.08%, respectively. Given the characteristics of the pedigree, homozygous, compound heterozygous variations were considered to be candidate causal variations. After raw data analysis, variants calling and filtering as described in the methods section, we filtered the remaining variations with the reported candidate genes and found a novel homozygous mutation in *ABCA4* (NM\_000350: c.G3190C, p.G1064R in *ABCA4*).

To confirm the results obtained by WES, we conducted Sanger

sequencing in all family numbers. The results showed that the affected siblings have the homozygous mutation of *ABCA4*, and the parents carry heterozygous mutation (Figure 4A). The homozygous novel mutation showed co-segregation with phenotype, conforming to the autosomal recessive inheritance pattern. The mutation c.G3190C of *ABCA4* was also genotyped in 200 unrelated healthy controls by high-resolution melt (HRM) analysis, and not found in anyone. The mutation was absent from the dbSNP, HapMap, 1000 Genomes, NHLBI Exome sequencing project databases, and ExAC database. It was considered "Deleterious" as predicted by Sorting Intolerant from SIFT, polyphen\_HDIV, polyphen\_HVAR, Mutation Taster, and CADD.

**Protein Structure and Function Prediction** The c.G3190C generated a missense mutation (p.G1064R). Multiple



**Figure 4 Conservative and tertiary structure prediction of mutant proteins** A: Sanger sequencing in all family numbers; B: Multiple alignments of Pro1064 of ABCA4 protein from different species; C: Domains analysis of Pro1064; D: The schematic structures of the original (Glycine) and the mutant (Arginine) amino acid; E: The overview and closed-up 3D-structure of mutant protein. The protein is colored grey, the side chains of both the wild-type and the mutant residue are shown and colored green and red respectively.

**Table 1 The physico-chemical parameters changes of mutant protein compared to wildtype**

Types	No. of amino acids	Molecular weight	Theoretical pI	Estimated half-life	Instability index	Aliphatic index	Grand average of hydrophaticity
Wildtype	2273	255943.83	5.89	30h	44.28 (unstable)	94.14	-0.055
c.3190 G>C	2273	256042.97	5.91	30h	44.27 (unstable)	94.14	-0.056

alignments of Pro1064 of the human *ABCA4* protein from different species revealed 100% identity, suggesting that it was highly conserved during evolution (Figure 4B). The ProtParam tool analysis result showed that there is no significant change of the physico-chemical parameters between mutant protein and wildtype (Table 1). Domains analysis results showed that the AAA ATPase core of ABCA4 is present from amino acid 955 to 1145, and the p.G1064R was located in AAA domain (Figure 4C), may reduce the activity of ATPase resulting in the disease pathology.

To visualize and understand this mutation, we used HOPE online software to analyze the structural effects of p.G1064R. The schematic structures of the original (Glycine) and the mutant (Arginine) amino acid were shown in Figure 4D. The overview and closed-up 3D-structure of mutant protein were shown in Figure 4E. Compared with the wild-type residue, the mutant residue is bigger, less hydrophobic and charge changes from NEUTRAL to POSITIVE (Figure 4D). The residue is located on the surface of the protein (Figure 4E). The torsion angles for this residue are unusual (Figure 4E).

**ABCA4 Expression Analysis** From the UniGene database, 60 ESTs in different parts of the eye out of about 135 matching the *ABCA4* gene were identified. The immunofluorescence microscopy result of mouse eye showed that *ABCA4* was highly expressed in the mouse retina, lens capsule and extraocular muscle (Figure 5A). The qRT-PCR and Western blotting results showed that *ABCA4* gene and protein were expressed in the mouse retina (Figure 5B, 5C).

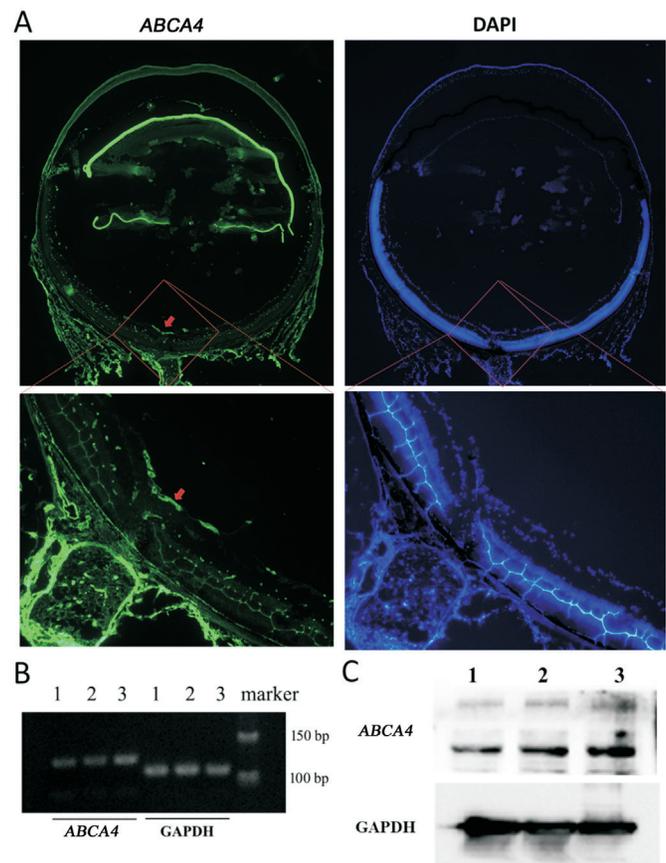
## DISCUSSION

STGD is a common retinal hereditary dystrophy which affects quality of life of young patients by causing a severe decline in central vision<sup>[1-2]</sup>. The molecular basis for STGD remains idiopathic in some cases. In this study, using WES of a Chinese STGD pedigree, we defined a homozygous mutation in *ABCA4* (c.G3190C, p.G1064R). According to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines, this novel mutation were “pathogenic”, supported by the following evidences: 1) located in the last position of the 22 exon, which was the donor site of splicing junction (PVS1); 2) located in AAA domain of *ABCA4* (PM1); 3) not found in the 200 unrelated health controls and absent from any databases (PM2); 4) co-segregation with phenotype in this family (PP1); 5) *ABCA4* is main responsible for autosomal recessive inherit STGD, and a large number of mutations in the *ABCA4* gene have been reported worldwide (PP2); 6) It was considered “Deleterious” as predicted by function prediction software, and was highly conserved during evolution (PP3); 7) this pedigree with typical genotype of STGD (PP4).

The c.G3190C generated a missense mutation (p.G1064R). The analysis result of HOPE showed that compared with the wild-type residue (Glycine), the mutant residue (Arginine) is bigger, less hydrophobic and charge changes from NEUTRAL to POSITIVE. The predicted 3D-structure of the protein showed that the torsion angles for this residue are unusual. Only glycine is flexible enough to make these torsion angles, mutation into another residue will force the local backbone into an incorrect conformation and will disturb the local structure. Mutation of this residue may disturb interactions with other molecules or other parts of the protein. The p.G1064R, which was located in AAA domain of *ABCA4*, may reduce the activity of ATPase resulting in the disease pathology.

On the other hand, this mutation located in the last position of the 22 exon, was the donor site of splicing junction. Mutations in these sequences may lead to retention of large segments of intronic DNA by the mRNA, or to entire exons being spliced out of the mRNA. These changes could result in production of a nonfunctional protein. Unfortunately, there is no eye tissue available from the patient’s family member for us to verify this hypothesis.

Form NCBI UniGene database, we found 60 ESTs of *ABCA4*



**Figure 5** The expression of *ABCA4* in different mouse tissue  
 A: Representative figures of immunofluorescence staining of *ABCA4* in mouse eye. The upper was panoramic image, and the downer was local image; B: mRNA expression of *ABCA4* in mouse retinas ( $n=3$ ); C: *ABCA4* expression analysis in mouse retinas ( $n=3$ ) by Western blotting.

from human eye tissues. This result suggested that *ABCA4* is expressed in human eye. *ABCA4* expression analysis in whole eye ball and retina of mouse also showed that *ABCA4* was highly expressed in the mouse retina. These results suggested that *ABCA4* is involved in normal function of retina.

In summary, we defined a novel pathogenic mutation (c.G3190C of *ABCA4*) of STGD. This extended the database of genotype-phenotype correlations and was helpful to make an accurate diagnosis and classification for patients with variable and atypical phenotypes. However, the contribution of this mutation to STGD needs to be further detected in a large number of patients. In the future, study the mechanism of this mutation is also needed, which may provide new insights into the molecular mechanisms underlying STGD, then provide theoretical basis for precision medicine.

## ACKNOWLEDGEMENTS

**Foundations:** Supported by the National Natural Science Foundation of China (No.81500763; No.81800805; No.81600721); Young and Middle-aged Scientists Research Awards Fund of Shandong Province (No.BS2015YY014); China Postdoctoral Science Foundation (No.2019M652311);

Medical and Health Science and Technology Development Project of Shandong Province (No.2017WS012).

**Conflicts of Interest:** Hao XD, None; Liu Y, None; Li BW, None; Wu W, None; Zhao XW, None.

**REFERENCES**

- 1 Noble KG, Carr RE. Stargardt's disease and fundus flavimaculatus. *Arch Ophthalmol* 1979;97(7):1281-1285.
- 2 Albert S, Garanto A, Sangermano R, Khan M, Bax NM, Hoyng CB, Zernant J, Lee W, Allikmets R, Collin RWJ, Cremers FPM. Identification and rescue of splice defects caused by two neighboring deep-intronic ABCA4 mutations underlying stargardt disease. *Am J Hum Genet* 2018;102(4):517-527.
- 3 Zhang K, Kniazeva M, Han M, Li W, Yu Z, Yang Z, Li Y, Metzker ML, Allikmets R, Zack DJ, Kakuk LE, Lagali PS, Wong PW, MacDonald IM, Sieving PA, Figueroa DJ, Austin CP, Gould RJ, Ayyagari R, Petrukhin K. A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet* 2001;27(1):89-93.
- 4 Nasonkin I, Illing M, Koehler MR, Schmid M, Molday RS, Weber BH. Mapping of the rod photoreceptor ABC transporter (ABCR) to 1p21-p22.1 and identification of novel mutations in Stargardt's disease. *Hum Genet* 1998;102(1):21-26.
- 5 Allikmets R. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 1997;17(1):122.
- 6 Yang Z, Chen Y, Lillo C, et al. Mutant prominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. *J Clin Invest* 2008;118(8):2908-2916.
- 7 Zaneveld J, Siddiqui S, Li H, et al. Comprehensive analysis of patients with Stargardt macular dystrophy reveals new genotype-phenotype correlations and unexpected diagnostic revisions. *Genet Med* 2015;17(4):262-270.
- 8 Coco RM, Tellería JJ, Sanabria MR, Rodríguez-Rúa E, García MT. PRPH2 (Peripherin/RDS) mutations associated with different macular dystrophies in a Spanish population: a new mutation. *Eur J Ophthalmol* 2010;20(4):724-732.
- 9 Strom SP, Gao YQ, Martinez A, Ortube C, Chen ZG, Nelson SF, Nusinowitz S, Farber DB, Gorin MB. Molecular diagnosis of putative Stargardt disease probands by exome sequencing. *BMC Med Genet* 2012;13:67.
- 10 Zernant J, Xie YA, Ayuso C, et al. Analysis of the ABCA4 genomic locus in Stargardt disease. *Hum Mol Genet* 2014;23(25):6797-6806.
- 11 Ran X, Cai WJ, Huang XF, Liu Q, Lu F, Qu J, Wu JY, Jin ZB. 'RetinoGenetics': a comprehensive mutation database for genes related to inherited retinal degeneration. *Database (Oxford)* 2014;2014.
- 12 Tanna P, Strauss RW, Fujinami K, Michaelides M. Stargardt disease: clinical features, molecular genetics, animal models and therapeutic options. *Br J Ophthalmol* 2017;101(1):25-30.
- 13 Hao XD, Chen P, Zhang YY, Li SX, Shi WY, Gao H. De novo mutations of TUBA3D are associated with keratoconus. *Sci Rep* 2017;7(1):13570.
- 14 Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics* 2010;11:548.
- 15 Chen P, Hao X, Li W, Zhao X, Huang Y. Mutations in the TMCO3 gene are associated with cornea guttata and anterior polar cataract. *Sci Rep* 2016;6:31021.