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Analysis of *ELOVL4* and *PRPH2* genes in Turkish Stargardt disease patients

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ABSTRACT. Stargardt disease (STGD) is an inherited genetic eye condition involving bilateral macular dystrophy leading to progressive central vision loss. It is the most common form of autosomal recessive juvenile macular dystrophy. In this study, *ELOVL4* and *PRPH2* genes were analyzed in 30 STGD probands for genetic variations using next-generation sequencing. In the patient group, two genetic variants in exon 6 of *ELOVL4*, and three in exon 3 of *PRPH2* were detected. All sequence modifications in both *ELOVL4* and *PRPH2* were recorded, including

those of a non-pathogenic nature. In the control group, four different genetic variations were detected in *ELOVL4*, and five in *PRPH2*. STGD patients of different ethnicities may carry distinct *ELOVL4* and *PRPH2* sequence variants. We believe that the genetic variations identified in this study may be related to STGD etiopathogenesis.

Key words: *ELOVL4* gene; *PRPH2* gene; Stargardt disease

INTRODUCTION

Stargardt disease (STGD) is an inherited genetic eye condition comprising bilateral macular dystrophy leading to progressive central vision loss, and is the most common form of autosomal recessive juvenile macular dystrophy, with a reported prevalence of 1 in 10,000 (Weleber, 1994; Walia and Fishman, 2009). The majority of affected individuals, during their teenage years, show uncorrectable decreased visual acuity and legal blindness. STGD is characterized by premature accumulation of lipofuscin in the retinal pigment epithelium, degeneration of the neuroretina, cone-rod dysfunction, and subsequent central vision loss (Charbel Issa et al., 2015). Clinical findings include fundus flavimaculatus, bulls-eye maculopathy, macular flecks, and a beaten-bronze macular appearance (Franceschetti and François, 1965). To date, no approved intervention for STGD exists.

Mutations have been reported in the genes *ABCA4*, *ELOVL4*, *PROM1*, *PRPH2*, and *CRB1* in STGD patients (Zhang et al., 2001; September et al., 2004; Yang et al., 2008; Coco et al., 2010; Oldani et al., 2012; Strom et al., 2012). The *ELOVL4* gene, located on chromosome 6q14, contains six exons and encodes an integral membrane protein of approximately 37 kDa and 314 amino acids (Zhang et al., 2001). Mammalian ELOVL enzymes (ELOVL1-7) catalyze the elongation of fatty acids in the endoplasmic reticulum (Shanklin and Somerville, 1991; Suzuki et al., 1991; Shanklin et al., 1994). ELOVL4, as other ELOVL family members, has a histidine motif (HXXHH), an endoplasmic reticulum retention motif (KXKXX), and five transmembrane domains responsible for enzymatic function (Fox et al., 1994). The ELOVL4 protein, which localizes to the endoplasmic reticulum, is most highly expressed in the retina, but is also present in the testes, skin, and brain (Zhang et al., 2001; Mandal et al., 2004). The *ELOVL4* gene is strongly conserved across vertebrates (Lagali et al., 2003; Zhang et al., 2003). It is thought that the dominant inheritance pattern of Stargardt-like disease may be due to *ELOVL4* gene haploinsufficiency or the dominant negative effect of mutant protein (Raz-Prag et al., 2006; Li et al., 2007).

The *PRPH2* gene, located on chromosome 6p21.1, contains three exons encoding a protein of approximately 39 kDa and 346 amino acids. This gene has been associated with retinitis pigmentosa, macular dystrophy, and multifocal pattern dystrophy (Boon et al., 2007; Bocquet et al., 2013).

Genetic diagnosis of STGD patients is difficult owing to the large sizes of the genes involved. Therefore, we aimed to perform a mutational analysis of *ELOVL4* and *PRPH2* in clinically verified STGD patients using next-generation sequencing (NGS).

MATERIAL AND METHODS

Patient recruitment

The patient group consisted of 30 unrelated STGD patients, and the control group

consisted of 250 unrelated healthy subjects for whom exome sequencing data were available. The study was approved by the Local Ethics Committee of Suleyman Demirel University Faculty of Medicine. All participants signed informed consent forms.

DNA collection

Peripheral blood samples were obtained from 30 STGD probands. STGD diagnoses were based on clinical investigations. Genomic DNA was isolated from peripheral blood using a REALPURE Spin Kit (Real Laboratory, Valencia, Spain) following the manufacturer protocol.

Targeted NGS

Sequencing analysis of *ELOVL4* and *PRPH2* was performed using the MiSeq NGS platform (Illumina, Inc., San Diego, CA, USA). The concentration and purity of DNA samples was quantified using an ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). A concentration of 50 ng/µL was used for the analysis. Exons 1-6 and 1-3 of *ELOVL4* and *PRPH2*, respectively, and their flanking splice site junctions were amplified using polymerase chain reaction (PCR) primers designed with Primer Designer v.2.0 (Scientific & Educational Software, Cary, NC, USA). PCRs were validated using agarose gel electrophoresis. Amplification products for each individual were mixed to create PCR pools, which were purified and quantified. Purification was achieved with a NucleoFast 96 PCR kit (Macherey-Nagel GmbH, Düren, Germany), and quantification of purified amplicons was carried out using an ND-1000 spectrophotometer. The concentration of each PCR pool was then standardized to 0.2 ng/µL. Libraries were prepared with the NexteraXT DNA Library Preparation Kit (Illumina, Inc.) following the manufacturer protocol.

RESULTS

In the patient group, two genetic variations in exon 6 of *ELOVL4* and three in exon 3 of *PRPH2* were detected with this technique. However, all such variants in both genes were previously recorded, non-pathogenic modifications. In the control group, four different sequence changes in the *ELOVL4* gene and five in the *PRPH2* gene were identified (Tables 1 and 2).

Table 1. Genetic variations detected in the *ELOVL4* gene in case and control groups.

Group	Variation	Protein	dbSNP ID	MAF (1000 Genomes Project)	Allele frequency	Number of cases/controls	
						Homozygous	Heterozygous
Case	c.814G>C	E272Q	rs148919174	0.0012	0.033	-	2
	c.895A>G	M299V	rs3812153	0.2416	0.20	2	8
Control	c.351T>A	N117K	rs148018494	0.0004	0.002	-	1
	c.800T>C	I267T	rs148594713	0.0058	0.008	-	4
	c.895A>G	M299V	rs3812153	0.2416	0.156	10	58
	c.370-26T>A	Intronic variant	rs700483	-	0.532	133	-

MAF = minor allele frequency.

Table 2. Genetic variations detected in the *PRPH2* gene in case and control groups.

Group	Variation	Protein	dbSNP ID	MAF (1000 Genomes Project)	Allele frequency	Number of cases/controls	
						Homozygous	Heterozygous
Case	c.910C>G	Q304E	rs390659	0.2434	0.80	18	12
	c.929G>A	R310K	rs425876	0.0587	0.93	26	4
	c.1013A>G	D338G	rs434102	0.2426	0.80	18	12
Control	13C>T	3'-UTR variant	rs361524	0.257	0.16	8	64
	c.910C>G	Q304E	rs390659	0.2434	0.73	131	105
	c.929G>A	R310K	rs425876	0.0587	0.81	167	75
	c.1013A>G	D338G	rs434102	0.2426	0.67	120	99
	c.318T>C	V106=	rs7764439	0.4155	0.58	87	120

UTR = untranslated region, MAF = minor allele frequency.

DISCUSSION

To date, more than 800 distinct mutations have been reported in the *ABCA4* gene, which is implicated in STGD. Oldani et al. (2012) used PCR and direct DNA sequencing to study this gene in 12 STGD patients from 12 families. They identified two mutations present in 75% of the families tested (9/12), 17% of which (2/12) carried G1961E, the most frequently observed variant. Four novel sequence variations were also reported: Tyr1858Asp, Leu1195fsX1196, p.Tyr850Cys, and p.Thr959Ala. Nevertheless, the most frequent variant type was the missense mutation in the *ABCA4* gene.

Only a small number of studies have examined variations in the genes *ELOVL4* and *PRPH2*, which are associated with STGD and Stargardt-like disease phenotypes (Zhang et al., 2001; Strom et al., 2012; Yi et al., 2012). To the best of our knowledge, the present study is the first investigation of STGD in the Turkish population. We analyzed the genomic sequences of the *ELOVL4* and *PRPH2* genes in 30 STGD probands using NGS. The following three mutations located in exon 6 of *ELOVL4* responsible for the Stargardt-like disease phenotype have been defined: two deletions of 5 and 2 bp, resulting in the loss of the same 51 amino acids from the protein's C-terminus, and a nonsense mutation causing the deletion of 45 amino acids, respectively. These variants lead to the absence of almost the entire C-terminal hydrophilic region (Bernstein et al., 2001; Zhang et al., 2001; Maugeri et al., 2004; Agbaga et al., 2010). Several studies have reported that the endoplasmic reticulum retention signal is lost as a result of these three mutations. The wild-type ELOVL4 protein is correctly sited in this organelle, while the mutant form is mislocalized to the Golgi apparatus or aggresomes (Ambasudhan et al., 2004; Grayson and Molday, 2005; Vasireddy et al., 2005). In addition, it has been reported that when mutant ELOVL4 and the wild-type protein are expressed together, the former exerts a "dominant-negative effect", leading to mislocalization of the latter (Grayson and Molday, 2005; Karan et al., 2005; Vasireddy et al., 2005).

Studies employing animal models have revealed that *Elovl4* knockout mice die shortly after birth and exhibit serious skin barrier formation defects. The researchers involved suggested that this may be because the mutant ELOVL4 protein is enzymatically inactive (Vasireddy et al., 2006, 2007; McMahon et al., 2007). Aldahmesh et al. (2011) demonstrated that patients carrying recessive mutations in the *ELOVL4* gene have serious skin and brain dysfunctions.

The three STGD-associated mutations of *ELOVL4* were identified in large families exhibiting an autosomal dominant inheritance pattern. We may not have detected these three previously reported variants or any novel mutations because we examined probands with classical STGD in our study. However, we detected three known, non-pathogenic genetic

variants in exon 6 of *ELOVL4*. Yi et al. (2012) found five single nucleotide polymorphisms (SNPs) in *ELOVL4* and *PRPH2* in Chinese STGD patients; however, no pathogenic variants were discovered in either gene. Three of these five SNPs were also present in our study population. Similarly, Zernant et al. (2011) failed to find mutations in the *ELOVL4* and *PRPH2* genes of STGD patients in their study. Moreover, in a study of a family with autosomal dominant Stargardt-like macular dystrophy, Lai et al. (2005) detected no mutations in *ELOVL4* and *PRPH2* associated with the disease. Using exome sequencing, Strom et al. (2012) identified a *PRPH2* sequence variant in three STGD patients, and concluded that restoring ABCA4 function would not be sufficient as a treatment for patients harboring pathogenic mutations in other genes. Zaneveld et al. (2015) reported that 30% of STGD patients from different ethnic groups had no mutations in the *ABCA4* gene, and found no pathogenic variations in *ABCA4* copy number. These findings suggest that epigenetic mechanisms and genes not previously associated with STGD may contribute to its pathogenesis. STGD patients of different ethnicities may vary in terms of the *ELOVL4* and *PRPH2* variations that they carry. We believe that the genetic variations found in the present study may be relevant to the etiopathogenesis of STGD.

Conflicts of interest

The authors declare no conflict of interest.

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