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Short Communication

Evaluation of the *ELOVL4* gene in a Chinese family with autosomal dominant STGD3-like macular dystrophy

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Abstract

Stargardt disease-3 (STGD3) is an autosomal dominant juvenile-onset macular dystrophy characterized by progressive decreasing visual acuity, bilateral atrophic changes in the macula and absence of characteristic dark choroids. We identified a STGD3-like macular dystrophy pedigree by clinical examination. To explore whether the STGD3-like phenotype in the kindred is linked to *ELOVL4* gene or associated with any other identified STGD gene, we extracted genomic DNA from leukocytes of peripheral blood from the available family members and 50 normal controls for mutation analysis. Then the exons of *ELOVL4*, *RDS* and the three exons of *ABCR* were amplified by polymerase chain reaction (PCR). All PCR products were screened for mutations by combination of denaturing high-performance liquid chromatography (DHPLC) analysis and DNA sequencing. No mutation was found in the exons of three candidate genes, but we obtained three non-pathogenic polymorphisms, IVS5-2533T→A in *ELOVL4*, 558C→T (Val106Val) and 1150G→C (Glu304Gln) in *RDS*. And IVS5-2533T→A is never shown in the previous references. These data suggested that there exist other unknown genes responsible for the STGD3-like phenotype in the pedigree.

Keywords: Stargardt disease-3-like macular dystrophy • autosomal dominant • *ELOVL4* gene • *RDS* gene • *ABCR* gene • mutation

Introduction

Stargardt disease-3 (STGD3, OMIM 600110) is an autosomal dominant juvenile-onset macular dystrophy characterized by progressive decreasing visual acuity, bilateral atrophic changes in the macula, degeneration of the underlying retinal pigment epithelium (RPE), the presence of prominent flecks in the posterior pole, absence of characteristic dark choroids on fundus fluorescein angiography (FFA) [1].

A gene responsible for STGD3, elongation of very long chain fatty acids-4 (*ELOVL4*), has been identified [2]. *ELOVL4* is composed of 6

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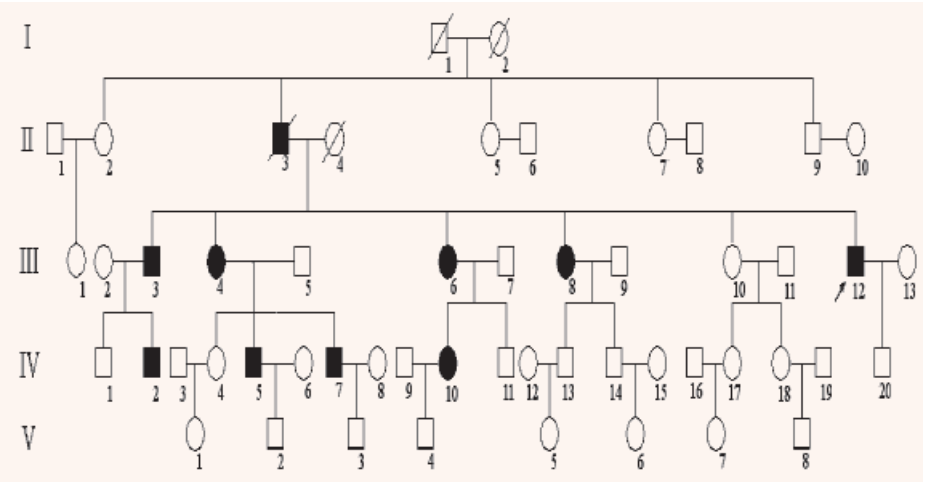

Fig. 1 Diagram of the pedigree. Circles represent females, and squares represent males. Diagonal lines through symbols indicate deceased individuals. Solid symbols indicate affected patients. Arrow indicates proband.

exons and encodes a 314-amino-acid protein with an estimated molecular weight of 37kDa [3]. There is little mutation analysis about *ELOVL4* (http://www.retina-international.org/sci-news/elovlmut.htm). Only three mutations, 790delT+794delT, 797-801delAACTT and 810C>G have been reported in exon 6 of *ELOVL4* [4, 5]. Interestingly, all three mutations occur around the same location and subsequent deletion of the C-terminus includes loss of the putative dilysine ER retention signal.

In current study, our pedigree showed an autosomal dominant pattern STGD3-like phenotype, so we investigated the potential involvement of *ELOVL4* gene variation in this pedigree.

Materials and methods

We identified one kindred from the clinic population of the Second Hospital of Ningbo, Ningbo, Zhejiang Province, China, with STGD3-like phenotype by complete eye examinations, including best corrected visual acuity, colour fundus photography, electroretinogram (ERG) and FFA, complying with the tenets of the Declaration of Helsinki. The pedigree is a five-generation family including 10 affected and 24 unaffected individuals (Fig. 1). 11 family members (III-1, III-4, III-5, III-6, III-10, III-12, III-13, IV-5, IV-7, IV-17, IV-20) from 18 to 70 years of age are available. Peripheral blood samples were obtained from the available family members and 50 unrelated normal control subjects without history of retinal degeneration. Genomic DNA was isolated for PCR amplification. All PCR products of *ELOVL4*, *RDS*, and partial *ABCR* gene were screened for mutations by combination of denaturing high-performance liquid chromatography (DHPLC) analysis and DNA sequencing. Informed consent was obtained from all subjects.

Results

Phenotypic evaluation of patients

In this pedigree, the onset of the disease was at the third or forth decade of life and visual acuity in both eyes were progressive decreasing but still preserved to levels measurable with conventional Snellen charts, the best-corrected visual acuity from 40/200 to 20/200. In fundus examinations, five affected members (III-4, III-6, III-12, IV-5, IV-7) exhibited varying degrees of maculopathy (Fig. 2), while the six unaffected individuals had completely normal results and no evidence of significant visual loss. Fluorescein angiography were also done in 5 affected subjects, all manifested typical STGD, but did not demonstrate the dark choroids effect (Fig. 3). III-4, IV-5, IV-7 also showed some symptoms of retinal pigmentosa (Fig. 2C). And the electroretinographic testing of III-4, III-6, III-12 revealed both abnormal cone-derived and rod-derived responses.

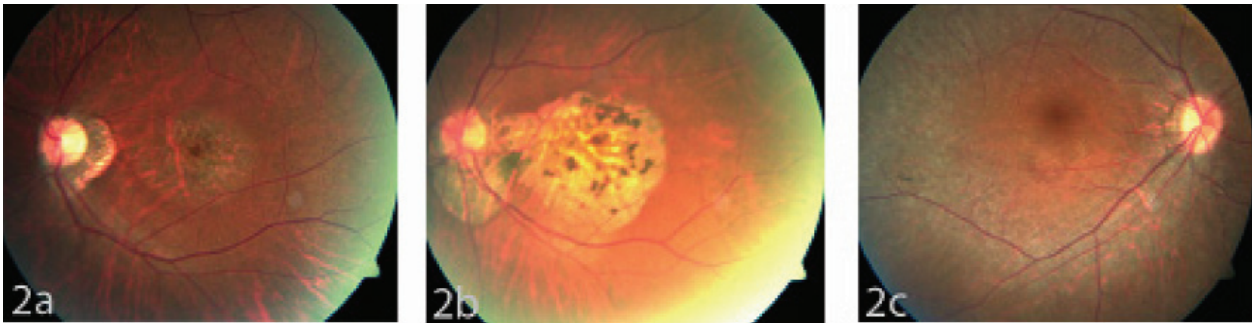

Fig. 2 Fundus photographs of the affected demonstrating the phenotypic variability. a: Typical "beaten bronze" appearance of fovea observed in left eye of a 50-year-old patient (proband, III-12). b: Central regions of chorioretinal atrophy measuring 3 disk diameters with prominent pigment deposits at the level of retinal pigment epithelium seen in left eye of a 66-year-old patient (III-6). c: Mild atrophic-appearing foveal lesion with periphery pigment deposits in RPE shown in right eye of a 43-year-old patient (IV-7).

Sequence variations in the *ELOVL4, RDS* and partial *ABCR* gene

We performed mutational analysis of *ELOVL4* by combination of DHPLC analysis and direct DNA sequencing. However, we did not detect any pathogenic variations, but only one non-pathogenic polymorphism, IVS5-2533T→A in *ELOVL4* (Fig. 4), which was identified only in the proband, not in the other patients and 50 controls. Some previous researches reported *RDS* gene mutations in patients with autosomal dominant macular dystrophy and retinitis pigmentosa, and pathogenic variations also existed fairly common in exon 17, 29, 42 of *ABCR* gene in STGD patients [6]. So, we further performed mutation analysis of *RDS* and exon 17, 29, 42 of *ABCR*. We still did not identify any genetic variations, but two non-pathogenic polymorphisms, 558C→T (Val106Val) and 1150G→C (Glu304Gln) in *RDS* (Fig. 4). Val106Val was observed in the affected III-12 and IV-5, Glu304Gln in the affected III-6 and III-12, and these two polymorphisms of *RDS* were also found in some normal controls (data not shown). Val106Val and Glu304Gln have been reported previously [6].

Discussion

STGD3 usually features progressive macular dystrophy, macular flecks, central macular atrophy, decreased visual acuity and central vision loss between 5 and 23 years of age. In our study, all available patients demonstrated the STGD3-like phenotypes. They also failed to show any charac-

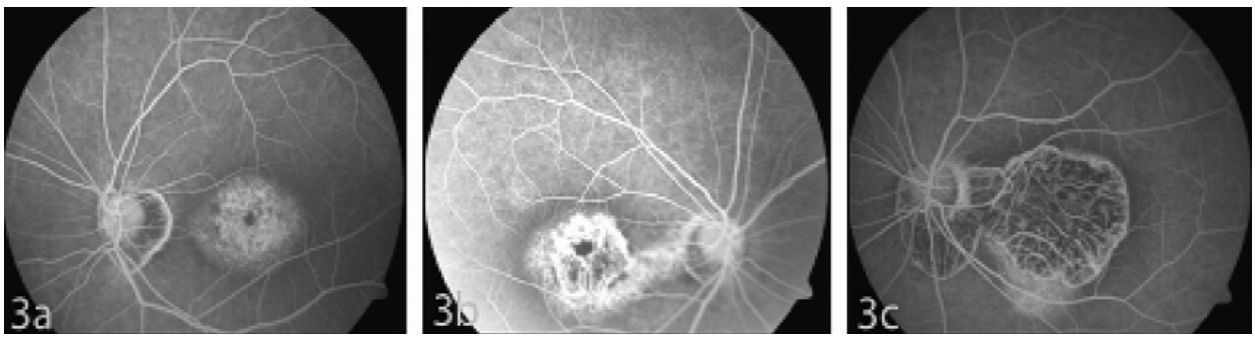

Fig. 3 Fluorescein angiography demonstrating absence of dark choroids. a and b: Foveal choroidal capillary and perifoveal transmitted hyperfluorescent dots shown in the left eye and right eye of III-12. c: severe atrophy in the fovea observed in left eye of III-6.

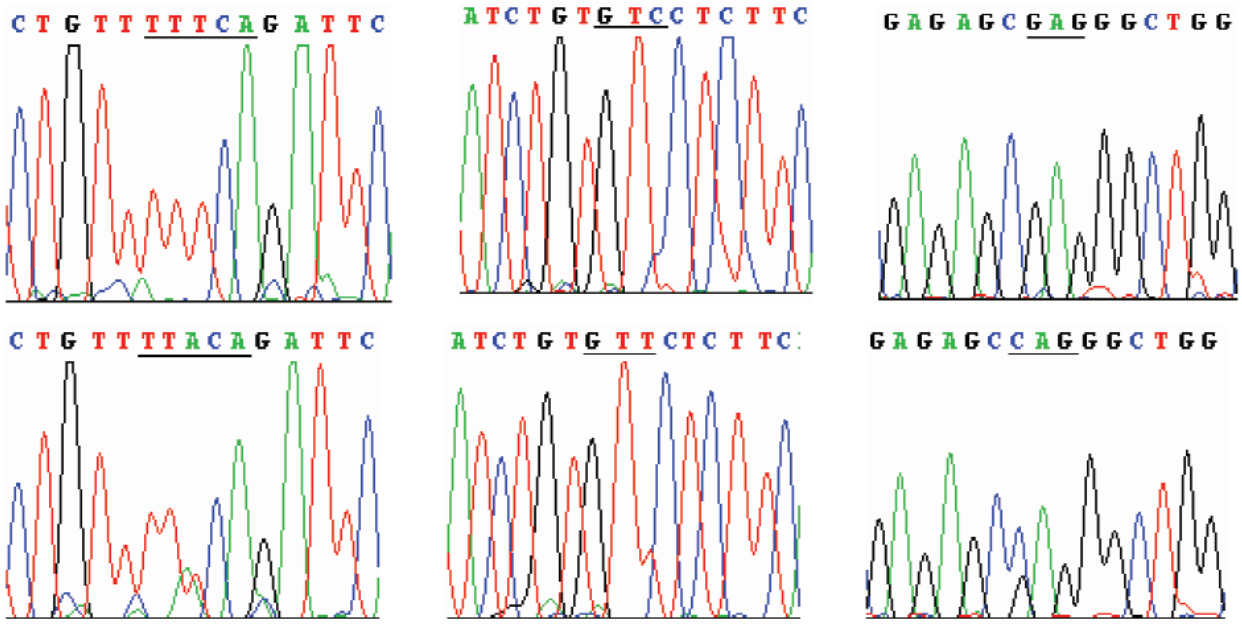

Fig. 4 DNA sequences showing wild sequence and polymorphism in *ELOVL4* and *RDS*. (Top left and Bottom left) IVS5-2533T→A in intron 5 of *ELOVL4*. (Top middle and Bottom middle) 558C→T (Val106Val) in exon 1a of *RDS*. (Top right and Bottom right) 1150G→C (Glu304Gln) in exon 3a of *RDS*.

teristic dark choroids, but the onset was comparatively later, between 30 to 40 years old.

We completed the mutation analysis of *ELOVL4, RDS* and partial *ABCR*, but we didn't find any mutations. Only three non-pathogenic polymorphisms, IVS5-2533T→A in *ELOVL4*, 558C→T (Val106Val) and 1150G→C (Glu304Gln) in *RDS* were observed. Up to date, just four polymorphisms (IVS1-24T→C, IVS2-99T→C, IVS3-18C→T, IVS4-32G→A) in *ELOVL4* have been found [2, 7, 8], and IVS5-2533T→A is never shown in the previous references. Thus we excluded *ELOVL4* and *RDS* as the genes responsible for the STGD3-like phenotype in our pedigree. No mutations were found in the three exons of *ABCR*, and *ABCR* is linked to autosomal recessive STGD, so in some way we may extrapolate *ABCR* is not a disease-causing gene in this family. According to the above, we preferably consider that there exist other unknown genes responsible for the STGD3-like phenotype. And by far, there is little information about autosomal dominant STGD. The further identification of the pathogenic genes in our pedigree will be significant, as well as further genetic and molecular study of autosomal dominant STGD, which will provide more opportunities to improve clinical diagnosis and to offer more-effective, targeted therapies for the patients [9].

Acknowledgements

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References

1. **Zhang XM, Yang ZL, Karan G, Hashimoto T, Baehr W, Yang XJ, Zhang K.** ELOVL4 mRNA distribution in the developing mouse retina and phylogenetic conservation of *ELOVL4* genes. *Mol Vis.* 2003; 9: 301–7.
2. **Zhang K, Jbuazeva M, Han M, Li W, Yu ZY, Yang ZL, 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: 89–93.

3. **Donoso LA, Edwards AO, Frost A, Vrabec T, Stone EM, Hageman GS, Perski T.** Autosomal dominant Stargardt-like macular dystrophy. *Surv Ophthalmol.* 2001; 46: 149–63.
4. **Vrabec TR, Tantri A, Edwards A, Frost A, Donoso LA.** Autosomal dominant Stargardt-like macular dystrophy: Identification of a new family with a mutation in the *ELOVL4* gene. *Am J Ophthalmol.* 2003; 136: 542–5.
5. **Maugeri A, Merie F, Hoyng CB, Vink C, Regemorter NV, Karan G, Yang ZL, Cremers FPM, Zhang K.** A novel mutation in the *ELOVL4* gene causes autosomal dominant Stargardt-like macular dystrophy. *Invest Ophthalmol Vis Sci.* 2004; 45: 4263–7.
6. **Fujiki K, Hotta Y, Hayakawa M, Fujimaki T, Takeda M, Isashiki Y, Ohba N, Kanai A.** Analysis of peripherin/RDS gene for Japanese retinal dystrophies. *Jpn J Ophthalmol.* 1998; 42: 186–92.
7. **Kakuk LE, Zhang K, Swaroop A, Yashar BM, Yang Z, Li Y, Petrukhin K, Sieving PA, Ayyagari R, Allikmets R.** Analysis of the *ELOVL4* gene in patients with AMD and dominant macular dystrophies. *Invest Ophthalmol Vis Sci.* 2001; 42: S638.
8. **Li Y, Marcos I, Borrego S, Yu ZY, Zhang K, Antinolo G.** Evaluation of the *ELOVL4* gene in families with retinitis pigmentosa linked to the RP25 locus. *J Med Genet.* 2001; 38: 478–80.
9. **Karan G, Lillo C, Yang Z, Cameron DJ, Locke KG, Zhao Y, Thirumalaichary S, Li C, Birch DG, Vollmer-Snarr HR, Williams DS, Zhang K.** Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant *ELOVL4* transgenic mice: a model for macular degeneration. *Proc Natl Acad Sci USA.* 2005; 102: 4164–9.