PHOSPHOLIPASE A2 GROUP V IN BENIGN FAMILIAL FLECK RETINA IN A SET OF TRIPLETS

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Abstract

Purpose: To evaluate the association of phospholipase A2, Group V (PLA2G5), with benign familial fleck retina in a consanguineous family with triplets.

Methods: Clinical eye examination, including fundus examination and spectral domain optical coherence tomography, was performed for all the family members. After blood sample collection and DNA extraction, polymerase chain reaction was performed to amplify regions spanning Exons 2, 3, 4, and 5 of PLA2G5. The amplified products were sequenced to observe the presence of any mutations.

Results: Fundus examination in two of the triplets revealed discrete yellow-white flecks and both had good vision and absence of night blindness, consistent with benign familial fleck retina. The flecks were hyperautofluorescent. Furthermore, spectral domain optical coherence tomography showed focal thickening of the retinal pigment epithelium because of the presence of these flecks. Molecular investigations showed that PLA2G5 Exons 2, 4, and 5 harbored no misalignments among all family members. However, PLA2G5 Exon 3 showed a p.Gly45Cys mutation for the father and the third triplet who was affected.

Conclusion: The clinical findings in this family suggest a diagnosis of benign familial fleck retina with excellent prognosis, in which the PLA2G5 gene may play a role.

Benign familial fleck retina is an ocular condition in which affected individuals are considered asymptomatic, because they have normal visual acuity. However, on fundus examination, distinctive diffuse yellow-white flecklike lesions can be seen. These flecks extend from the parafoveal region to the far periphery of the retina but spare the central macula region.1 Benign familial fleck retina is one of the types of fleck retina syndromes.2 Very few cases pertaining to this retina entity have been reported in the literature. Benign familial fleck retina has been described in patients during their first decade of life. It is classically inherited as an autosomal recessive condition and is strongly associated with consanguinity.

The genetic basis of benign familial fleck retina has not been fully elucidated to date. However, ocular genetic research related to this condition has been using whole-exome sequencing and next-generation sequencing to identify the disease-causing mutation.3 Phospholipase A2, Group V (PLA2G5), located at 1p36-p34 in humans, was identified as a candidate gene in this retinal disorder. The gene encodes for a calcium-dependent low-molecular weight (15 kD) phospholipase A2. The human Group V phospholipase A2 contains 12 cysteines.4 Phospholipase A2 forms a superfamily of enzymes that is generally involved in phospholipid metabolism, host defense, membrane repair and remodeling, and signal transduction.5 This superfamily of enzymes has the ability to hydrolyze the middle ester bonds of glycerophospholipids, releasing bioactive lipids and free fatty acids.6 PLA2G5 is expressed in the heart and eyes, and to a lesser extent in the placenta, lungs, and brain. The enzyme has thus been implicated in cardiovascular disease 7 and benign fleck retina.8
The objective of this study was to evaluate the association of PLA2G5 with benign familial fleck retina in a consanguineous Malaysian family with triplets.

Materials and Methods

Subjects

The propositus was a 12-year-old Tamil boy, who presented with the left upper eyelid swelling typical of chalazion. He had no other ocular and systemic symptoms. He was the youngest of a set of triplets. There was no family history of night blindness or ocular diseases. In view of the incidental fundal findings in this patient, all seven members of the family were studied. A family pedigree was constructed based on interviews and fundus examination. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Medical Ethics Committee, University of Malaya Medical Center. Informed consent was obtained from all participating individuals.

Clinical Examination

Fundus examination was performed on the subjects. Tropicamide 1% (Mydriacyl; Alcon, Fort Worth, TX) and phenylephrine 2.5% (Mydfrin; Alcon) were used to dilate the pupils. Beginning with posterior landmarks, the disk and the macula, the four quadrants were systematically examined by following each of the major vessel groups to the periphery. Ophthalmoscopy was performed using a slit lamp with a 90-diopter lens. Fundus images were captured by using a mydriatic fundus camera under the color and autofluorescent photographing mode (Topcon TRC-NW8; Topcon, Tokyo, Japan). This was followed by in vivo cross-sectional imaging through spectral domain optical coherence tomography (Carl Zeiss Cirrus High Definition Optical Coherence Tomography; Carl Zeiss, Oberkochen, Germany). The signal strength in all scans was more than six.

Molecular Analysis

DNA was extracted from whole blood using conventional phenol-chloroform method. Polymerase chain reaction was performed using a previously described protocol. Four exons, namely Exons 2, 3, 4, and 5 of PLA2G5 were amplified. The respective polymerase chain reaction products were subjected to direct Sanger sequencing (Applied Biosystems 3730xl DNA Analyzer, Foster, CA) and were aligned to the PLA2G5 human reference sequence (NCBI accession number NM_000929) using BLAST and Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI). Taken together, the diagnosis of benign familial fleck retina was made on studying the fundus color photographs and correlating the spectral domain optical coherence tomography findings with the genetic and phenotypic presentation of each subject (Table 1).
A family comprising seven members of South Indian ethnicity formed the basis of this study. The propositus was the youngest in a set of triplets (Figure 1, Subject IV-5). His parents are first cousins.

On examination, the visual acuities of the propositus were 6/6 in both eyes. The anterior segments of both eyes were normal. Fundus examination revealed small, round discrete yellow-white flecks of varying sizes scattered from the midperipheral to the far peripheral retina, sparing the posterior pole (Figure 2). The flecks located nearer to the posterior pole appeared to be more round and sparse compared with the equatorial and far peripheral ones that were slightly elongated and irregular in shape, with some appearing to be coalesced. There were no spared areas in the periphery (Figure 3). None of the lesions were hyperpigmented, and no other changes such as calcification or choroidal neovascularization were observed. Fundus autofluorescence demonstrated the
areas of hyperautofluorescence, representing an accumulation of lipofuscin mainly in the peripheral area in both eyes (Figure 4).

Fig. 1. Family pedigree of the propositus with benign familial fleck retina.

Spectral domain optical coherence topography across the superotemporal vascular arcade of the two triplets with the retinal flecks showed discrete deposit accumulation located posterior to the photoreceptor inner/outer segment junction (Figure 5, arrows). These discrete deposits represent the flecks that are located at the retinal pigment epithelium (RPE) layer, giving rise to the focal thickening of the RPE. Two of the triplets had similar retinal findings (Figure 5, A and B) with the inner segment/outer segment junction (the ellipsoid layer) apparently intact. The remaining triplet (Figure 5C), older siblings, and parents had no abnormalities, with normal fundal appearances. Although the flecks were present, the two affected triplets did have normal vision.

Fig. 2. Fundus examination showing yellow-white flecks throughout both fundi, sparing the posterior pole in Subject IV-5 (propositus).

Fig. 3. Fundus examination showing denser retinal flecks at the periphery in Subject IV-4.
PLA2G5 Exons 2, 3, 4, and 5 from all 7 members of the family were amplified and sequenced. After sequence confirmation and alignment, no mismatches were identified in Exons 2, 4, and 5 of all family members. However, Exon 3 alignment revealed a single base mismatch for the father (III-14) and the propositus (IV-5) (Figure 6).

The term flecked retina syndrome was used to describe a heterogeneous group of disorders with the characteristics of discrete white or yellow dots, with or without night blindness. Before the genomic period, the different forms of the syndrome were differentiated based on clinical examinations, functional abnormalities, and electrophysiologic studies.
Benign familial fleck retina is a very rare inherited retinal disorder. Individuals affected are asymptomatic and hence are usually discovered by chance. This disorder has a similar distribution of the yellow-white flecks as fundus albinopunctatus, with variable sizes and shapes extending to the far periphery and with normal visual function.

Such similar, characteristic fundal appearances with night blindness have been reported, and it was concluded that the distribution pattern and the fundal fluorescein angiography of both benign familial fleck retina and fundus albinopunctatus had similarities.\(^9\) Subsequently, Hayashi et al\(^9\) hypothesized that benign fleck retina with night blindness could be a retinal disorder of variable expressivity of fundus albinopunctatus. However, benign familial fleck retina shows more extensive involvement of the fundus compared with the fleck retina of Kandori, with more heterogeneous patterns of flecks. In addition, the increased autofluorescence of the flecks was reported, suggesting that the flecks may be of lipofuscin material at the RPE layer, which does not lead to functional disturbances. Fluorescein angiography reveals irregular hyperfluorescence that does not correspond with the distribution of flecks, suggesting a diffuse abnormality of the RPE. In this study, clinical examination revealed that the two affected subjects had extensive yellow-white flecks, which were observed to be subretinal stereomicroscopically, in keeping with the diagnosis of benign familial fleck retina. Unlike other forms of flecked retina syndrome, the two had good vision and absence of night blindness, justifying the condition as benign with excellent prognosis. Benign familial fleck retina has also been reported in conjunction with neuroretinitis.\(^10\)

Benign familial fleck retina was previously believed to be inherited as an autosomal recessive disorder. Molecular genetic studies have linked it to the homozygous or compound heterozygous mutation of \(\text{PLA2G5}\) gene.\(^8\) Being expressed in the heart and eyes,\(^8\) this enzyme may play a role in cardiovascular disease and benign fleck retina, respectively. From the outset, Sergouniotis et al used whole-exome sequencing to identify all coding variants in two consanguineous families with familial fleck retina. \(\text{PLA2G5}\) emerged as a candidate gene. \(\text{PLA2G5}\) had in fact been implicated in retinal dystrophies and age-related macular degeneration in several studies.\(^11\)

A genetic investigation was carried out to gain insight on this rare retinal condition. Our investigations revealed that \(\text{PLA2G5}\) Exons 2, 4, and 5 harbored no misalignments among family members. Although the absence of mutation on Exons 2 and 4 was in agreement with a previous report, a mutation-free Exon 5 was not in agreement with the same report, which showed mismatch mutations in both Exons 3 and 5.\(^8\) However, Exon 3 direct Sanger sequencing of the family showed a mismatch for only the father (III-14) and the third triplet (IV-5). The mutation was seen at Position 45 (p.Gly45Cys) in \(\text{PLA2G5}\) in both the father and third triplet, which would result in an amino acid change at Position 45 of the \(\text{PLA2G5}\) protein (a cysteine instead of glycine). This finding was in agreement with the study by Sergouniotis et al, in which Sanger sequencing of fleck retina patients revealed a c.133G>T (p.Gly45Cys) mutation in Exon 3. They detected 3 homozygous rare missense changes that included c.133G>T (p.Gly45Cys) in \(\text{PLA2G5}\), and 1 compound heterozygote but did not detect any loss-of-function variants. These mutations were thus implicated in benign familial fleck retina syndrome.

Of note was the finding that the triplets in this study showed different genotype-phenotype manifestations. The first triplet did not have any mutation in Exon 3 as expected, because he did not present with benign familial fleck retina. However, the second triplet did not have any mutation either, despite presenting with signs of familial fleck retina. In addition, the third triplet had both the mutation and signs of the retinal condition. Furthermore, the father had the mutation but without the clinical signs. These molecular findings complicate the picture, because no clear mode of inheritance was seen. One consideration is that the polymorphism that is present in the father is not expressed. Of the triplets, the different genotypic-phenotypic patterns seen could also be suggestive of the degrees of penetrance and expressivity.

However, genetic diversity does support the prevailing view that benign familial fleck retina may be a heterogeneous condition and that other genes may also contribute to its pathogenicity. The presence or absence of this mutation alone does not define the disease. In fact, the mutation at Position 45 of \(\text{PLA2G5}\) was only 1 of several mutations that were associated with this disorder. There were other mutations at Positions 49, 53, and 62 of the \(\text{PLA2G5}\) gene, and there was also absence of mutations in patients with benign fleck retina based on the study by Sergouniotis et al.\(^8\) The difference was that the other mutations (at Positions 49, 53, and 62) were not present in this study's family, whereas the absence of mutation in 1 of their patients was in agreement with our findings.
The human PLA2G5 contains 12 cysteines, the thiol groups of which are oxidized to form 6 disulfide bridges and thus cystines 4; a mutation at Position 45 of PLA2G5 gives rise to an additional cysteine (from 12 to 13). In general, cystines serve an important structural role in many proteins. In the retina, PLA2G5 was implicated in the phagocytosis of photoreceptor outer segment disks by the RPE.8 The phospholipase enzyme catalyzes the hydrolysis of membrane phospholipids to generate lysophospholipids and free fatty acids. The presence of lipofuscin material in the retina of the two subjects with fleck retina in this study indicates the product of oxidation of unsaturated fatty acids, and may be symptomatic of membrane damage, or damage to mitochondria and lysosomes. At this stage, it is not clear as to the effect the additional cysteine may have on this type of fleck retina.

Incidentally, qualitative observation of the electrophoresed polymerase chain reaction products on agarose gels consistently showed that amplicons from the two affected triplets were thinner compared with the other family members (data not shown). The implications of this are as yet not known.

Previous reports have included patients from various backgrounds: South Asian,8,12 Arab Palestinian,1 Japanese,9 and mixed Australian aboriginal and white descent.13 The family in this study was of South Indian heritage. It would seem that benign familial fleck retina is not exclusively associated with a particular ethnic group. However, consanguinity was a trait that those affected families shared, a trait that is similar to the family studied here.

In conclusion, the PLA2G5 gene may have a role in benign familial fleck retina as analyzed in this study. A long-term follow-up of this family will provide further information in understanding the possible progression of the disease entity, especially in relation to the molecular finding.

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References


Key words: consanguineous; familial fleck retina; Malaysian; phospholipase A2 Group V; South Indian ethnicity; spectral domain optical coherence tomography

IMAGE GALLERY

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Fig. 6

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