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Observations regarding retinopathy in mitochondrial trifunctional protein deficiencies

Autumn L. Fletcher^{a,*}, Mark E. Pennesi^{b,c}, Cary O. Harding^{a,d}, Richard G. Weleber^{b,c}, and Melanie B. Gillingham^{a,d,e}

^aDepartment of Molecular & Medical Genetics, School of Medicine, Oregon Health & Science University, Mail Code L-103, 3181 SW Sam Jackson Park Rd Portland, OR 97239, USA

^bThe Casey Eye Institute, 3375 SW Terwilliger Blvd., 3181 SW Sam Jackson Park Rd Portland, OR 97239, USA

^cDepartment of Ophthalmology, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd Portland, OR 97239, USA

^dDepartment of Pediatrics, Oregon Health & Science University, Mail Code CDRC-P, 3181 SW Sam Jackson Park Rd Portland, OR 97239, USA

^eGraduate Programs in Human Nutrition, School of Medicine, Oregon Health & Science University, Mail Code: GH207, 3181 SW Sam Jackson Park Rd Portland, OR 97239, USA

Abstract

Although the retina is thought to primarily rely on glucose for fuel, inherited deficiency of one or more activities of mitochondrial trifunctional protein results in a pigmentary retinopathy leading to vision loss. Many other enzymatic deficiencies in fatty acid oxidation pathways have been described, none of which results in retinal complications. The etiology of retinopathy among patients with defects in trifunctional protein is unknown. Trifunctional protein is a heterooctomer; two genes encode the alpha and beta subunits of TFP respectively, *HADHA* and *HADHB*. A common mutation in *HADHA*, c.1528 G>C, leads to a single amino acid substitution, p.Glu474Gln, and impairs primarily long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity leading to LCHAD deficiency (LCHADD). Other mutations in *HADHA* or *HADHB* often lead to significant reduction in all three enzymatic activities and result in trifunctional protein deficiency (TFPD). Despite many similarities in clinical presentation and phenotype, there is growing evidence that they can result in different chronic complications. This review will outline the clinical similarities and differences between LCHADD and TFPD, describe the course of the associated retinopathy, propose a genotype/phenotype correlation with the severity of retinopathy, and discuss the current theories about the etiology of the retinopathy.

Keywords

Long-chain 3-hydroxyacyl-CoA; dehydrogenase deficiency; Mitochondrial trifunctional protein deficiency; Fatty acid oxidation; Inborn errors of metabolism; Hydroxyacylcarnitines; Retinopathy

1. Introduction

The retina has traditionally been thought to be glycolytic, oxidizing glucose for fuel rather than relying on β -oxidation of fatty acids. However, in 2002 Tyni et al. convincingly demonstrated that mitochondrial trifunctional protein (TFP) is expressed in normal retina [1]. The expression of β -oxidation proteins such as TFP in the retina [1], and the progressive retinopathy that occurs with inherited deficiencies in one or more enzymatic activities of this protein [2] suggest that β -oxidation is important in retinal metabolism, although the role of fatty acid oxidation in retinal metabolism has not been defined [3].

TFP is a protein complex bound to the inner mitochondrial membrane that catalyzes three distinct steps in the beta-oxidation of dietary long chain fatty acids (Fig. 1a). It is a heterooctamer made up of four alpha subunits and four beta subunits and contains three distinct functional domains. The four alpha subunits mediate the enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities while the four beta subunits contain the beta-ketothiolase activity (Fig. 1b). These subunits are encoded by the *HADHA* (OMIM # 600890) and *HADHB* (OMIM # 143450) genes, respectively [4]. Although inherited deficiencies are described for many different enzymes in the mitochondrial fatty acid β -oxidation pathway, only deficiency in one or more activities of TFP is associated with progressive retinopathy resulting in vision loss [5].

Mitochondrial trifunctional protein (TFP) deficiency (TFPD, OMIM #609015) and long-chain 3-hydroxyacyl-CoA Dehydrogenase Deficiency (LCHADD, OMIM # 609016) are caused by different mutations in the same protein, mitochondrial trifunctional protein. Both of these fatty acid oxidation (FAO) deficiencies can result in hypoketotic hypoglycemia during times of fasting, illness, and/or prolonged exercise. However, there are some distinct differences in pathology between LCHADD and TFPD when compared to each other (Table 1), making them two related but unique disorders of mitochondrial FAO [5].

Among all of the fatty acid oxidation disorders (FAODs), LCHADD is associated with a greater number of different complications. Some of the first cases of isolated LCHADD diagnosed presented with severe liver pathology; including acute liver failure in newborns and chronic liver failure in infancy, sometimes progressing to cirrhosis. Other complications reported during acute decompensation include cardiomyopathy, rhabdomyolysis and/or myoglobinuria and sudden death [5]. In 30 to greater than 50% of cases of isolated LCHAD deficiency an irreversible retinopathy is observed, and about 5–10% of cases result in an irreversible peripheral neuropathy [6]. The literature suggests pigment changes in the retina are evident by the age of 2 years in around 50% of individuals with LCHADD [7].

In contrast, trifunctional protein deficiency (TFPD), when identified due to metabolic crisis and hypoketotic hypoglycemia, typically presents with cardiomyopathy or skeletal muscle myopathy, but rarely with liver pathology [5]. Also, up to 80% of TFPD cases have some degree of peripheral neuropathy at long-term follow up, while retinopathy with vision loss occurs in only 5–13% [6]. We have observed that the progression of retinopathy tends to be much slower with less functional vision loss in patients with TFP deficiency as compared to patients with selective loss of the LCHAD activity [2].

All 50 states now perform newborn screening for LCHADD/TFPD through acylcarnitine profiling in dried blood spots as part of a newborn screening program (<http://genes-r-us.uthscsa.edu/nbsdisorders.htm>). This is a screening test and screening may not identify some patients. These affected individuals may present symptomatically and an abnormal acylcarnitine profile at that time would also suggest the LCHADD/TFPD diagnosis. Confirmation of the diagnosis suggested by an abnormal acylcarnitine profile involves enzyme assays, or fatty acid oxidation probe studies in cultured patient fibroblasts obtained

through full-thickness skin biopsy, or gene sequencing and mutation identification. There are problems with all of these confirmatory tests; the presence of two of these three indicators (abnormal acylcarnitines, low enzyme activity and/or identified mutations) is conventionally sufficient for an LCHADD or TFPD diagnosis [8].

In 2009 the American College of Medical Genetics published guidelines for making a diagnosis of LCHADD/TFPD. However, there are no established guidelines for distinguishing between these two disorders except by optional confirmatory genetic testing and/or enzyme activity assays in patient fibroblasts (http://www.acmg.net/StaticContent/ACT/Algorithms/Visio-C16-OH_+---C18-1-OH.pdf). The most common mutation in TFPD is a c.1528 G>C single nucleotide substitution leading to a point mutation, p. Glu474Gln, in *HADHA* that causes LCHAD deficiency [9,10]. This mutation also reduces the hydroxylase and ketothiolase activities, but reduces the LCHAD activity of TFPD more substantially than the other two activities. LCHADD is assumed if one copy of this common mutation is found. If the common mutation is not found, it is suggested that both *HADHA* and *HADHB* genes be sequenced. It is believed that all other mutations in *HADHA* or *HADHB*, other than the common mutation, result in TFPD [6], although it is biologically plausible that point mutations in the active sites of the other two enzymatic activities may exist [5]. The guidelines also mention that LCHADD leads to higher hydroxylated fatty acid or 3-hydroxyacylcarnitine levels in patient plasma than does TFPD (also illustrated in Fig. 1a), but no concentration threshold is suggested, leaving it up to each individual physician whether further confirmation of a diagnosis of LCHADD versus TFPD is warranted (<http://www.medicalhomeportal.org/diagnoses-and-conditions/lchadd-tfp-deficiency/initial-diagnosis#PracticeGuidelinesd32534e205>). Some reports have suggested it is unimportant to distinguish between TFPD and LCHADD [11], but there is increasing evidence that these two disorders result in unique sets of complications which may require unique clinical management and treatment [5,12–18].

The focus of this review will be to discuss the etiology of the retinopathy observed primarily in LCHAD deficiency. We will briefly review the literature regarding the severity and characteristics of the retinopathy, as well as the *HADHA* and *HADHB* genotype variation seen within and between populations. These will be followed by the evidence for a genotype/phenotype association with progression of retinopathy in these disorders. Finally, we will describe the hypothesized mechanisms leading to this unique retinopathy and discuss current and future studies that aim to elucidate this mechanism.

2. LCHADD/TFPD retinopathy

LCHAD deficiency was initially described in 1989, and by 1992 at least 10 patients had been identified [19,20]. Existence of the trifunctional protein complex was proven in 1992; that same year two unrelated individuals with compound deficiency of all three enzymatic activities were described [4,21–24]. The first description of pigmentary retinopathy in association with LCHADD or TFPD appeared in 1996; its presence is now known to be pathognomonic for LCHADD or TFPD among individuals presenting with fasting induced hypoketotic hypoglycemia [25].

In a 1998 a case series of 15 individuals and a reexamination of 4 survivors that were homozygous for the common mutation associated with isolated LCHADD were performed [26]. Children with LCHADD were observed to develop granulation with pigment clumping in the macula of the retina visible as early as four months of age. Although many of these patients had died before 2 years of age, greater than 50% of them had abnormal fundus examinations prior to death. Tyni et al. proposed four stages of LCHADD retinopathy based on these data. Stage 1 is characterized by normal retinal function and a hypopigmented

fundus. Stage 2 is demarcated by the appearance of pigment clumping in the fovea as well as progressive retinal dysfunction as measured by electroretinogram. However, in spite of ERG decreases, age-appropriate performance and visual acuity remain intact. In stage 3, central pigmentation disappears as chorioretinal atrophy leads to notable macular pallor and pigmentary changes migrate toward the periphery. ERG readings continue to decline with markedly reduced amplitudes and prolonged implicit times, or become unrecordable. Patients often report loss of night vision in stage 3, followed by loss of color vision. Finally in stage 4, the posterior pole of the eye has lost all photoreceptors, most of the choroidal vessels, and macular (central) vision is lost. Morphology reminiscent of previous stages can be seen spreading outward to the peripheral retina as progression continues in stage 4 [26]. Retinal photos taken during our longitudinal study illustrate a normal retina compared to stages 2 and 4 retinopathy (Fig. 2).

Two other large case studies have been published. One study prospectively followed 14 subjects with LCHADD/TFPD for 5 years. Subjects in this study included five who were homozygous for c.1528 G>C, six who were compound heterozygous and three who did not carry the common mutation [2]. The second case series included a retrospective review of 10 subjects who were all homozygous for the common mutation [27]. There has been no report examining TFPD retinopathy specifically.

Histologic examination of this pigmentary retinopathy has been reported for two pairs of eyes from children with LCHADD who were 7 months and 14 months of age at death. The examination revealed macrophage infiltration of the retinal pigment epithelial (RPE) layer and evidence of RPE cell death [28]. RPE cells are a supporting cell type that is present in a monolayer in the retina directly contacting rod and cone photoreceptor cells [3]. The authors hypothesized the RPE cells die first leaving no support for the rods and cones during the progression of LCHADD retinopathy [28]. Loss of the supportive RPE layer would lead to death of the photoreceptors over time. This has not been confirmed in other models because there is no viable animal or cell culture model of LCHAD or TFPD deficiency. Based on the histopathologic evidence, we propose that initial death of the RPE results in macrophage infiltration of the RPE, pigment clumping in areas of macrophage infiltration and ultimately to the loss of the photoreceptors.

3. *HADHA* or *HADHB* mutations and retinopathy

Mutations in *HADHA* and *HADHB* have varying effects on protein folding, oligomerization, and the enzymatic activities of TFP [14]. Among the 28 subjects we have evaluated, approximately 80% of the mutations are the common c.1528 G>C single nucleotide substitution in *HADHA* that causes isolated LCHAD deficiency [9,10]. This mutation leads to a glutamine substituted for a glutamate at position 474 in the alpha subunit of TFP (p. Glu474Gln), which decreases only the dehydrogenase activity of the protein complex, leaving hydratase and thiolase activities relatively intact [5]. Mutations in these genes are being discovered at a rapid pace. According to the Human Gene Mutation Database, in February 2011 there were 32 discovered mutations in *HADHA* and 29 in *HADHB*. As of December 2011 the same database lists 58 mutations in *HADHA* and 43 in *HADHB*. These mutations include missense and nonsense mutations, splice site variations that can result in exon skipping during mRNA splicing, and small insertions and/or deletions that result in misfolded, incomplete or truncated protein (<http://www.hgmd.org>). Function of the enzyme complex requires folding and oligomerization of subunits to occur correctly, as shown by pulse-chase experiments in cultured fibroblasts [29]. Various mutations in both the α - and β -subunits have been reported to destabilize the protein complex, leading to a decrease in all three enzyme activities and lower total protein levels in patient cells carrying

these mutations. The common mutation does not have this effect; levels of mutant protein in cells homozygous for the common mutation are comparable to levels in control cells [14].

The common mutation in TFP, c.1528 G>C in the *HADHA* gene, is very prevalent in patients of European descent, but is relatively absent in Asian populations (Table 2) [7,34,35,42]. In a recent Chinese study using 1200 cord blood samples from individuals of Han descent not one carrier of the c.1528 G>C allele was found [33]. Mutation analysis in patients in China, Japan and Korea reveals zero patients carrying this mutation [17,34,36–41]. In contrast, European countries report allele frequencies from as low as 1:680 in the Netherlands to a high of 1:79 in one region of Poland [30,32]. The carrier rate in the United States has never been investigated to our knowledge. Out of approximately 1.2 million children screened, five cases of LCHADD/TFPD have been diagnosed since beginning expanded newborn screening by the Northwest Regional Newborn Screening program that screens all children born in the states of Oregon, Idaho, Nevada, Alaska, and Hawaii. Based on these numbers, the minimal disease frequency for TFPD and LCHADD combined in this screening area is around 1/200,000. Genotype information was not obtained for all of these cases, so estimation of carrier frequency of the common mutation in this area is impossible.

Retinopathy has not been reported in any individuals of Asian descent with TFPD, yet it is reported in 30% to >50% of LCHADD and TFPD cases in Europe and the U.S. [6,17,43]. This observation suggests that the common mutation is more clearly associated with the development of retinal pathology in LCHADD/TFPD, although this hypothesis remains to be proven.

4. Hydroxyacylcarnitines (OHACs), hydroxy fatty acids (OHFAs) and vision loss

The mitochondrial long-chain fatty acid β -oxidation pathway is shown in Fig. 1a, with the blocked step in isolated LCHAD deficiency illustrated. When long-chain fatty acids are oxidized in patients with LCHADD, the hydratase creates a 3-hydroxyl fatty acid but further oxidation by the dehydrogenase is blocked resulting in the accumulation of long-chain 3-hydroxy fatty acids (3-OHFAs) in patient plasma. Esterification of these 3-OHFAs to carnitine leads to build up of 3-hydroxyacylcarnitines (3-OHACs) (Fig. 1a) [44].

Increased 3-OHFAs and 3-OHACs are seen in the plasma of patients with LCHAD deficiency and to a lesser extent in patients diagnosed with TFP deficiency [45,46]. These 3-OHFAs and 3-OHACs increase with metabolic crisis, prolonged fasting or prolonged exercise when the body attempts to utilize long-chain fatty acids for energy [47]. When patients with LCHAD or TFP deficiency are healthy, eat regular meals, and consume a low-fat diet these levels fall and the progression of the retinopathy is slowed [2]. In addition, early diagnosis, treatment, and decreasing the number of metabolic crises are associated with slower progression of retinopathy [27]. A direct mechanism connecting metabolites with retinopathy is not yet clear, but there is a strong negative correlation between 3-OHAC concentration in plasma and phototransduction as measured by electroretinogram (ERG) in a prospective study of patients with LCHAD and TFP deficiency followed over 5 years. This study concluded that lowering 3-OHAC byproducts slows the progression of retinopathy [2].

Over the course of our prospective study, we measured plasma acylcarnitines once per year. Even though this was a limited and inaccurate measure of overall hydroxyacylcarnitine and hydroxy fatty acid exposure during the follow up period, a correlation was noted. Table 3 is a reorganization of previously published data illustrating that LCHADD/TFPD genotype correlates with retinopathy progression and 3-OHAC levels [2]. The 3-OHAC levels reported here are the sum of the following species measured in 5 annual blood samples

(C14OH, C14:1OH, C16OH, C16:1OH, C18OH, C18:1OH, C18:2OH). This table illustrates a relationship between the common mutation and elevated 3-OHACs, as well as a correlation between 3-OHAC levels and retinopathy progression. Three individuals in this cohort have TFPD and do not carry the common mutation. These patients had 5.3 $\mu\text{mol/L}$ 3-OHACs over 5 years, and all three had no change over time in their retinal pathology. The remaining 11 patients carry one or more c.1528 G>C mutation and had 3.2 to 15.4 $\mu\text{mol/L}$ 3-OHACs over 5 years [44]. All but one of these eleven had mild to severe progression of their retinopathy. The exception in this group had no noted change in fundus appearance despite high levels of 3-OHACs at the time of testing (patient 13). This patient was examined by an ophthalmologist at another institution and was lost to further follow up. Subsequent changes in fundus appearance could not be confirmed. Thus within the group of patients that had significant change in fundus appearance and progression of retinopathy with loss of vision, annual measures of 3-OHACs strongly correlated with that progression. Also, progression trended toward greater severity in compound heterozygotes with one copy of the common mutation and a predicted null allele such as a deletion or frame shift mutation compared to patients who were homozygous for the c.1528 G>C mutation. To further illustrate the effect of 3-OHACs on retinal function we have included previously collected ERG data in Fig. 3. The waveforms from two age and test condition matched subjects: one patient with high 3-OHACs and one with low 3-OHACs are compared to a normal control [2]. ERG amplitude and implicit time was significantly lower in the subject with high cumulative 3-OHACs. The data is correlative only and no direct toxicology data has been published indicated 3-OHACs and or 3-OHFAs are specifically toxic to retinal cells.

5. Conclusions and future directions

The current evidence supports the theory that progression of retinopathy is associated with the presence of the common mutation, and therefore with LCHAD deficiency. Patients with at least one c.1528 G>C allele typically have high 3-OHACs during metabolic crisis, after prolonged fasting or exercise, and when they consume a diet with excess long-chain fat [44,47]. These patients also more commonly develop retinopathy progressing to stage 3 and functional vision loss. In contrast, patients with other mutations trend toward lower byproduct levels and do not as often progress to vision loss although some retinal pathology is often still evident.

Multiple theories have been put forward in the past twenty years regarding the cause of LCHADD retinopathy. One theory suggests that the number of metabolic decompensations and/or hospitalizations for hypoketotic hypoglycemia the patient experienced is positively correlated with vision loss [27]. We suggest that during metabolic crises levels of byproducts are likely high. Therefore the retinal pathology seen in patients that have undergone multiple hospitalizations could also be due to byproduct build up. Patients that carry at least one c.1528 G>C allele that follow the recommended diet and have fewer metabolic decompensations also have lower 3-OHACs and slower progression of their retinopathy [2]. There has not been a comparison of either the differences in 3-OHACs concentrations or retinopathy progression between subjects homozygous for the common mutation and compound heterozygotes with one common mutation in combination with another pathogenic allele.

It has also been suggested that the common mutation leads to production of an alpha subunit, p. Glu474Gln, that has a dominant negative effect that induces retinal cell death. There is no current molecular evidence to support this theory. The normal protein levels of TFP measured by western blot in patients with c.1528 G>C explain their more normal levels of hydratase activity compared to patients with truncating mutations or deletions that result

in no or significantly reduced protein levels and therefore very low hydratase activity [14]. The increased 3-OHACs seen in greater concentrations in LCHADD may be a marker of nothing more than the presence of a pathogenic protein encoded by the common mutation. There are many potential ways that the mutant TFP protein could affect the stability and/or activity of associated proteins [48]; there is a possibility that TFP with the p. Glu474Gln substitution interferes with the activity of other associated enzymes. Recent studies have demonstrated TFP forms a multi-enzyme complex with VLCAD and its redox partner ETF in the inner mitochondrial membrane, and immunoprecipitates with Complex I of the electron transport chain [49]. This arrangement is ideal for substrate and cofactor channeling which depends upon close association of specific amino acid residues in these enzymes. To our knowledge, the effects of a p. Glu474Gln alpha subunit on the stability or activity of this multi-enzyme complex have not been studied. While all tissues needing energy are affected in FAODs, the theory that the presence of this mutant TFP protein is involved in the development of retinopathy suggests that this protein affects the retina and the RPE cells differently than other cell types or tissues, such as skeletal muscle or liver. However, we can think of no reason that a mutant alpha subunit of TFP would uniquely affect RPE cells as opposed to other cell or tissue types.

Further research to determine if metabolic decompensations, 3-OHACs and/or 3-OHFAs, and/or the presence of the mutant α -subunit of the protein induce retinal cell death is needed before novel treatments can be formulated. In particular, if the presence of toxic byproducts induces RPE or photoreceptor apoptosis, retinal gene therapy that lowers 3-OHAC and 3-OHFA accumulation through gene replacement is an exciting potential treatment. If the presence of an α -subunit with the common mutation is in itself leading to retinal cell death, gene replacement will not be sufficient to halt retinopathy and additional therapeutic strategies such as gene silencing or gene repair may be required.

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Abbreviations

TFP	trifunctional protein
TFPD	trifunctional protein deficiency
LCHAD	long-chain 3-hydroxyacyl-CoA dehydrogenase
LCHADD	LCHAD deficiency
FAO	fatty acid oxidation
FAOD	fatty acid oxidation deficiency
HADHA	gene encoding trifunctional protein alpha subunit
HADHB	gene encoding trifunctional protein beta subunit
3-OHFA	3-hydroxy fatty acid
3-OHAC	3-hydroxyacylcarnitine

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Fig. 1.

a. Pathway of long-chain fatty acid oxidation with blocked activities of TFP and blocked dehydrogenase step and the respective metabolite build up illustrated. b. A cartoon illustration of TFP with subunits and activities labeled.

Fig. 2.
a) Normal retinal photograph; b) patient with stage 2 chorioretinopathy of LCHADD, c) patient with stage 4 chorioretinopathy of LCHADD; d) same eye as pictured in panel c illustrating peripheral progression of stage 4 chorioretinopathy.

Fig. 3. ERG waveforms of two age and test condition matched subjects compared to a normal control 30 Hz Flicker and photopic single flash = cone-driven response; scotopic Ops, scotopic single flash = rod-driven response. Patient 4 has lower amplitude and increased implicit time representing rod/cone dysfunction compared to patient 5 and the normal control.

Table 1

Complications in LCHADD and TFPD compared to each other and to VLCAD deficiency (VLCADD), a long-chain FAO enzyme upstream of TFP. (–) absent, (+) mild to moderate, (++) moderate to severe, (+++) severe to debilitating, (+/–) absent to mild, (++/–) absent to moderate.

Complication	Mitochondrial trifunctional protein deficiencies		Other FAO deficiency
	LCHADD	TFPD	VLCADD
Hypoketotic hypoglycemia	+	+	+
Rhabdomyolysis/myoglobinuria	+	++	+
Cardiomyopathy	+/-	++/-	+/-
Hepatic encephalopathy	++	–	+/-
Peripheral neuropathy	+/-	+++	–
Retinopathy	+++	+/-	–

Table 2

Reported frequency of the common mutation in HADHA (c.1528 G>C) by geographic area.

Geographic area	c.1528 G>C allele frequency	Reference
Netherlands	1:680	den Boer et al., 2000 [30]
Finland	1:240	Tyni et al., 1999 [31]
Poland	1:189	Piekutowska-Abramczuk et al., 2010 [32]
Beijing – Han	0:1200	Zhu et al., 2005 [33]
Beijing	0:90	Wang et al., 2006 [34]
Japan	0 reported alleles	Fukushima et al., 2004 Orie et al., 1997 Park et al., 2009; Purevsuren et al., 2008; Purevsuren et al., 2004 Tamaoki et al., 2000 Yamazaki et al., 2004 [17,35–40]
Korea	0 reported alleles	Choi et al., 2007 [41]

Table 3

Compiled published data showing correlation between genotype, 3-OHAC levels and progression of retinopathy [1]. OHACs = sum of C14OH, C14:1OH, C16OH, C16:1OH, C18OH, C18:1OH, C18:2OH measured once annually for 5 years.

Patient	Allele	Subunit	Cumulative OHACs (μmol/L)	Change in <i>fundus</i> appearance
1	c.901 G>A/?	β	0.386	No change with time
3	c.901 G>A/?	β	0.638	No change with time
6	c.725A>G/c.839 G>A	β	5.306	No change with time
5	c.1528 G>C/c.1528 G>C	α	3.674	Mild progression
10	c.1528 G>C/c.1528 G>C	α	5.319	Mild progression
9	c.1528 G>C/c.1528 G>C	α	8.197	Mild progression
14	c.1528 G>C/c.1678 C>T	α	3.282	Moderate progression
2	c.1528 G>C/c.1528 G>C	α	5.243	Moderate progression
11	c.1528 G>C/?	α	7.042	Moderate choriocapillaris atrophy
12	c.1528 G>C/5 bp del exon15/intron boundary	α	7.575	Moderate progression
8	c.1528 G>C/c.274_278del	α	11.892	Moderate choriocapillaris atrophy
7	c.1528 G>C/c.1528 G>C	α	8.198	Severe choriocapillaris atrophy
4	c.1528 G>C/c.274_278del	α	13.710	Severe choriocapillaris atrophy
13	c.1528 G>C/c.1132 C>T	α	15.4	No change with time ^a

^aFinal *fundus* exam per report of ophthalmologist at a different institution.