SELF-PERCEIVED VISUAL FUNCTION OF PEOPLE WITH LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE AND TRIFUNCTIONAL PROTEIN DEFICIENCIES AND FACTORS RELATED TO RETINOPATHY PROGRESSION

By

Lindsay LeBrun

A Thesis

Presented to the Graduate Programs in Human Nutrition and the
Oregon Health & Science University
School of Medicine
in partial fulfillment of
the requirements for the degree of

Master of Science in Clinical Nutrition

November 2018

School of Medicine

Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's Thesis of

Lindsay A. LeBrun

"Self-perceived Visual Function of People with Long-Chain 3-Hydrdoxyacyl CoA Dehydrogenase and Trifunctional Protein Deficiencies and Factors Related to Retinopathy Progression"

Has been approved

_____

Thesis Advisor

_____

Committee Member

_____

Committee Member

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

CACT	carnitine-acylcarnitine translocase
CI	confidence interval
CK	creatine kinase
CPT1	carnitine palmitoyl transferase 1
CPT2	carnitine palmitoyl transferase 2
DHA	docosahexaenoic acid
ERG	electroretinography
FAOD	fatty acid oxidation disorder
HSL	hormone-sensitive lipase
IRBP	interphotoreceptor retinoid-binding protein
LCFA	long-chain fatty acid
LCHAD(D)	long-chain 3-hydroxyacyl-CoA dehydrogenase (deficiency)
LHYD	long-chain enoyl-CoA hydratase
LKAT	long-chain 3-ketoacyl-CoA thiolase
MCFA	medium-chain fatty acid
MCT	medium-chain triglyceride
MS/MS	tandem mass spectrometry
MTP	mitochondrial trifunctional protein
NBS	newborn screening
NEI	National Eye Institute
OH-AC	hydroxyacylcarnitine
OS	outer segments
RPE	retinal pigment epithelium
TAG	triacylglycerol
TFP(D)	trifunctional protein (deficiency)
VFQ-25	25-item Visual Function Questionnaire
VLCAD	very-long-chain acyl-CoA dehydrogenase
3-OH-AC	3-hydroxy acylcarnitine

Acknowledgments

I would like to express my gratitude to Dr. Melanie Gillingham, my thesis mentor, for her thoughtful guidance and encouragement throughout the research process. I would also like to thank all the faculty and staff of the Graduate Programs in Human Nutrition for their sustained personal and academic support.

This thesis is dedicated to my mom and dad, whose many sacrifices have allowed me to pursue my dreams.

Abstract

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and trifunctional protein (TFP) deficiencies are inherited disorders of long-chain fatty acid metabolism caused by mutations to the genes encoding for the mitochondrial trifunctional protein. A progressive retinopathy occurs in both disorders and researchers have observed a faster progression of retinopathy in patients with LCHAD deficiency compared to TFP deficiency. This study used a modified version of the National Eye Institute 25-item Visual Function Questionnaire to assess the areas of vision that are affected in these patients and compared survey scores to evaluate whether the progression of retinopathy differed by genotype. We also examined factors thought to be related to retinopathy progression and their relationship with survey scores of visual functions. This study provides insight into the aspects of visual function and perceived effects of vision impairment on vision and socioemotional outcomes in subjects with LCHAD and TFP deficiencies.

Purpose

The cause of retinopathy in isolated long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is not fully understood but one proposed mechanism is the toxicity of accumulating 3-hydroxy acylcarnitines (3-OH-ACs) due to impaired fatty acid β-oxidation. LCHAD deficiency (LCHADD) is caused by the presence of the c.1528G→C common mutation which is observed in roughly 80% of mutated alleles in US people with mitochondrial trifunctional protein deficiencies (TFPD).[1] A more severe progression of retinopathy has been noted in patients who harbor at least one copy of the common mutation. There is a genotype-phenotype correlation between the presence of the common mutation and severity of retinopathy progression.

The overall goal of this project was to examine this genotype-phenotype correlation with visual impairment using responses to the National Eye Institute 25-Item Visual Function Questionnaire (VFQ-25). This survey measures outcomes of self-reported visual function which were then categorized by genotype. Outcomes assessed included near and distance vision, role limitations due to vision, limits to social functioning due to vision, and other outcomes regarding vision-related quality of life. Finally, we examined the relationship between the severity of retinopathy and several patient characteristics. We hypothesized that advanced retinopathy, as assessed by poorer self-reported visual function, is associated with carrying one copy of the c.1528G→C common mutation, older age at diagnosis, increased frequency of hospitalization due to metabolic crisis, higher plasma 3-OH-ACs, and older age at the time of the questionnaire. Understanding the factors contributing to retinopathy in LCHADD could help identify optimal therapy procedures to prevent or slow the progression of retinopathy and/or underscore the importance of early intervention.

Aims & Hypothesis

Responses to the questionnaire and data obtained from the medical records of individuals with LCHADD/TFPD were used to examine to what extent the above described factors impact the progression of vision loss. Data from the questionnaire was used to describe the impact of vision loss on self-reported vision-related health status and vision-related quality of life for individuals with LCHADD/TFPD. The specific aims of this project were:

- Aim 1: To investigate the genotype-phenotype correlation between the presence of one or two copies of the *HADHA* c.1528G→C common mutation and retinopathy severity in LCHADD.
    - Hypothesis: The presence of at least a single copy of the common mutation is correlated with advanced retinopathy as defined by poorer self-reported vision-related health in comparison to other genotypes.
- Aim 2: To examine the relationship between relevant patient characteristics and the severity of retinopathy.
    - Hypothesis: We hypothesize that advanced retinopathy is correlated with increased frequency of metabolic decompensations, higher plasma 3-OH-ACs, older age at diagnosis, and older age at the time of the completion of the questionnaire.

To our knowledge, no study has described the self-reported vision-related health of individuals with LCHADD/TFPD. This study may help us to better understand how these factors are related to the progression of retinopathy in these diseases and help us to further develop treatment guidelines that slow or prevent vision loss.

Background

Fatty Acid Oxidation

LCHADD and TFPD are two of several metabolic disorders that impair normal fatty acid β-oxidation. This pathway is used to meet energy demand during periods of fasting or prolonged physical exertion. Fatty acids in adipose tissue make up the body's largest compartment of energy reserves and are stored in the form of triacyclglycerol (TAG). Each TAG molecule contains three fatty acids, typically of varying chain length, attached to a glyceride backbone. During increased energy demand, the stored TAG is hydrolyzed to its fatty acid components by the enzyme hormone-sensitive lipase (HSL). This enzyme is widely distributed in the major-lipid oxidizing tissues such as adipose, cardiac, and skeletal muscle tissues. HSL preferentially cleaves the fatty acids at the sn-1 and sn-3 positions on the TAG to yield two fatty acids and a monoacylglycerol, with the remaining fatty acid cleaved by the action of monoacylglycerol lipase. HSL is considered the rate-limiting step of lipolysis. The rate of hydrolysis by HSL can be increased many fold to meet increased energy demands and is controlled by multiple hormonal and neural signaling mechanisms. In the fasting state, glucagon and catecholamines initiate cAMP and protein kinase A phosphorylation pathways to activate HSL and release fatty acids for oxidation.[2] Conversely, in the fed state, the binding of insulin to adipocyte insulin receptors stimulates phosphodiesterase-3B, promoting cAMP degradation and deactivation of HSL.[2] Sympathetic and sensory nerves innervating adipose tissue also play a role in facilitating lipolysis.[2]

Fatty acids hydrolyzed from adipocytes can then be mobilized to peripheral tissues. After traveling through the circulation bound to albumin, the fatty acid dissociates from albumin and crosses the plasma membrane by many specific fatty acid transporters. Fatty acids in skeletal and cardiac muscle are typically metabolized, and those in the liver are metabolized or esterified and packaged into very low-density

lipoproteins. A separate enzyme, lipoprotein lipase, hydrolyzes fatty acids found in the TAGs of circulating lipoproteins before they are taken up by the cell. Fatty acids that are not used may be taken up by adipocytes to be esterified back into TAG. In conditions with normal fatty acid oxidation, the rate of fatty acid β-oxidation is proportional to the plasma free fatty acid concentration.

Once in the cytosol, fatty acids are rapidly converted to their respective acyl-CoA derivatives by attachment of a CoA molecule. The fatty acyl-CoA is then mobilized to the mitochondria where the entire process of β-oxidation is contained. The pathway into the mitochondria for β-oxidation differs for fatty acyl-CoAs of varying chain length. Medium (C6-C12) and short-chain fatty acyl-CoAs (C6 or shorter) may freely diffuse across the mitochondrial membrane. Long-chain fatty acyl-CoAs (C14 or longer) are impermeable to the inner membrane and must enter through the carnitine shuttle.[3] This process begins with the exchange of a carnitine for the CoA by the enzyme carnitine palmitoyl transferase 1 (CPT1) to form a long-chain fatty acylcarnitine ester. Carnitine-acylcarnitine translocase (CACT) then binds to the acylcarnitine ester and transfers it across the inner mitochondrial membrane. Lastly, carnitine palmitoyl transferase 2 (CPT2) reverses the action of CPT1 by exchanging the carnitine with another CoA. The long-chain fatty acyl-CoA is now available for β-oxidation within the inner mitochondrial compartment.

The four-step process of β-oxidation proceeds by sequentially cleaving two carbons from the carboxyl end of the fatty acyl-CoA to yield acetyl-CoA and a shortened fatty acyl-CoA. Separate enzyme systems are specific to the oxidation of fatty acyl-CoAs of short, medium, and long-chain length. The first step in long-chain fatty acid β-oxidation is a dehydrogenation reaction by very long-chain acyl-CoA dehydrogenase that forms a double bond between the C-2 and C-3 carbon, yielding trans-Δ^2-enoyl-CoA and

using FAD as an electron acceptor. This bond is hydrated in the second step by long-chain enoyl-CoA hydratase (LHYD) to form an L-3-hydroxyacyl-CoA. Long-chain 3-hydroxyacyl-CoA (LCHAD) catalyzes the third step using NAD+ as an electron acceptor. This step converts the hydroxyl group into a keto group to yield a 3-ketoacyl-CoA. Lastly, long-chain 3-ketoacyl-CoA thiolase (LKAT) removes the β-ketoacyl-CoA by the thiol group of an additional CoA molecule. The formation of the acetyl-CoA and shortened acyl-CoA marks the end of one round of β-oxidation. The process proceeds until the fatty-acyl CoA is entirely cleaved into acetyl-CoA units. For fatty acyl-CoAs with odd-numbered chains, the product is propionyl-CoA which is converted to succinyl-CoA. The oxidation of unsaturated fatty acids proceeds in the same way as for saturated fatty acids until a double bond is reached, after which an isomerase or reductase enzyme must convert the trans-bond to its cis configuration before another round of β-oxidation can continue.[4] In LCHADD, the block at the third step of long-chain fatty acid (LCFA) β-oxidation ultimately reduces the supply of NADH for oxidative phosphorylation and the

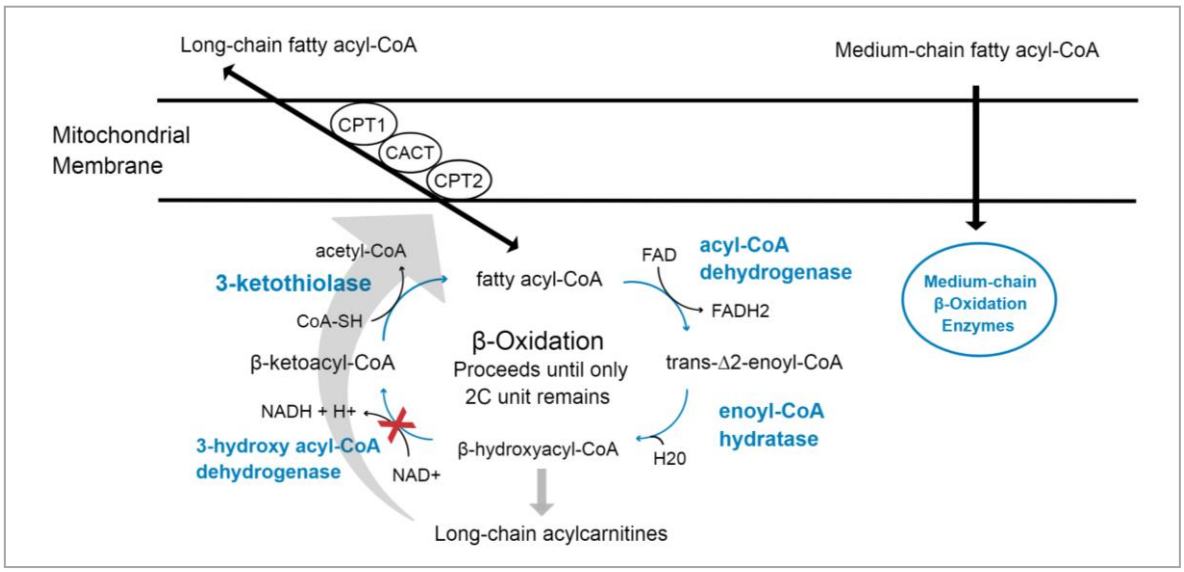


Fig. 1: Fatty acid β-oxidation in LCHAD deficiency

acetyl-CoA product for its respective pathways of energy production through the citric acid cycle and ketogenesis. Decreased energy availability damages body tissues in parallel to their level of dependence on β-oxidation for energy production and expression of the LCHAD enzyme. Additionally, there is a build-up of the upstream product, the L-3-hydroxyl-CoA. As the concentration increases, these molecules may be reattached to carnitine and transported back across the inner membrane by CACT to the intermembrane space. At high enough concentrations the hydroxyacylcarnitines (OH-ACs) will diffuse from the cell causing the increased OH-AC plasma concentrations seen in LCHADD/TFPD patients with acute metabolic decompensations.[3,5]

Genetics

The enzymes acting in the final three steps of LCFA β-oxidation are all housed within the mitochondrial trifunctional protein (MTP), with the action of LHYD and LCHAD within the α-subunit and LKAT within the β-subunit. The *HADHA* and *HADHB* genes located on chromosome 2p24 encode for the α- and β-subunits, respectively.[6] Isolated LCHAD deficiency is the most common MTP disorder and is defined by the presence of the c.1528G→C common point mutation in the *HADHA* gene encoding for the α-subunit. This mutation causes a guanine-to-cytosine change which results in the transition of a glutamate to a glutamine. Various other mutations in the *HADHA* and *HADHB* genes cause TFPD which reduces MTP protein expression and more universally decreases the activity of all three enzymes. LCHAD deficiency results in intact mutant proteins with significantly reduced LCHAD activity while the activities of the LHYD and LKAT enzymes usually remain at least 60% of the normal.[7] Very-long-chain acyl-CoA dehydrogenase (VLCAD) and LCHAD mRNA expression and enzymatic activity of VLCAD and LCHAD are most highly expressed in the liver, heart, the neural retina, and the central nervous system. This is in line with the spectrum of clinical symptoms seen in LCHADD/TFPD.[8] A genotype-phenotype correlation between a more aggressive progression of retinopathy

and presence of the common mutation has been proposed.[9] In comparison to other mutations, the common mutation may also be related to a more severe hepatic phenotype and greater incidence of sudden unexpected death.[10]

Prevalence

Among inherited metabolic diseases, MTP deficiencies are relatively rare. A handful of studies have attempted to estimate the prevalence in various populations. A systematic review estimated the prevalence in Western populations based on reports from 16 studies using mass spectrometry-based (MS/MS) screening programs and 5 studies reporting rates from clinical detection. However, of the 21 total studies, only two differentiated between isolated LCHAD and TFP deficiencies in their reporting. Based on MS/MS screening, the estimated prevalence for TFPD for Western populations is 0.65 per 100,000 live births [95% confidence interval (CI) of 0.46-0.91 per 100,00]. The estimated prevalence based on clinical detection methods for Western populations is less and estimated to be 0.41 per 100,000 live births (95% CI 0.19-0.90].[11] Data included in this estimation came largely from the United States, Australia, Germany and from smaller studies in Korea and Kuwait.

High prevalence of LCHADD in one part of Poland suggests a possible region of origin for the common mutation. Polish researchers identified the distribution of LCHADD using neonate blood spots among different regions in Poland and noted a high carrier frequency of 1:73 in the northern Pomeranian province in comparison to the average frequency of 1:217 in the other regions.[12] These carrier frequencies are also higher than those of other European, Asian, or North American countries. The researchers suggested that the Kashubian ethnic group that has inhabited the Pomeranian region may be the origin of the c.1528G→C common mutation. This is supported by a second study examining the frequency of the common mutation among 1023 adults of Kashubian descent which found a prevalence of 1:57.[13]

More studies are warranted in determining the prevalence of MTP deficiencies, particularly studies that distinguish the rates of isolated LCHAD deficiency and TFP deficiency. As newborn screening becomes more commonly practiced throughout the world, the feasibility of such studies becomes more of a reality.

Newborn Screening

In the 1960s, newborn screening (NBS) programs began to test for phenylketonuria so that early intervention could prevent or mitigate disease-related mental retardation. The advent of tandem MS/MS in the 1980s allowed for measurements of >40 analytes within a few minutes using a single assay.[14] This greatly enhanced the ability to test for a wide range of fatty acid oxidation disorders (FAODs), amino acid disorders, and organic acid disorders. All US states offer NBS programs though the panel of diseases screened for varies among states (all test for MTP deficiencies). A positive screen for MTP deficiency requires follow up tests such as plasma acylcarnitine profiling and direct molecular DNA testing to confirm the diagnosis.[14] The screening test may not identify all patients; some patients may not exhibit the characteristic elevations in OH-ACs to flag the sample as a potential positive for LCHADD/TFPD. These affected individuals may present symptomatically later in infancy. Among patients identified by NBS or those who present symptomatically, an elevation in plasma long-chain OH-ACs is indicative of LCHADD/TFPD. Plasma OH-AC concentrations are used as a biochemical marker to monitor metabolic control.

Early identification of an LCHADD/TFPD allows for early intervention to prevent or mitigate the life-threatening complications that can occur within the first weeks or even days of life.[15] Individuals with LCHADD/TFPD who were born after 2000 are more likely to have been screened for the disease at birth and therefore more likely to have been diagnosed earlier in life than are individuals born before the 2000s. It is unknown to what degree the age of diagnosis contributes to the degree of retinopathy progression, but we

suspect that lower exposure to OH-ACs through earlier intervention may preserve visual function.

Clinical Features of LCHADD

Acute Metabolic Decompensation

Several phenotypes of LCHADD/TFPD have been described including those with retinopathy, cardiomyopathy/myopathy, liver, and neurological presentations.[16,17] One or more of these different complications may be present but common to most LCHADD/TFPD patients are the episodes of acute metabolic decompensation that occur during periods of negative energy balance, similar to patients with VLCAD or CPT2 deficiency. These episodes may be provoked by illness, exercise, or prolonged fasting.[7] Younger patients can quickly develop secondary hypoglycemia due to the inability to spare glucose by using fatty acids and ketones during periods of low glucose supply.[4] Fatty acid β-oxidation is the major source of energy for skeletal muscle and cardiac tissue at rest and during prolonged exercise, while the liver oxidizes fatty acids primarily under the conditions of prolonged fasting, illness, and increased muscular activity. Incomplete LCFA β-oxidation leads to the accumulation of acylcarnitine intermediates in the muscle, liver, retina, and nervous tissues and increased plasma concentrations of OH-ACs.[8]

Rhabdomyolysis

Metabolic decompensations often present as rhabdomyolysis, the lysis of myocytes that releases intracellular contents into circulation among school age and older patients with LCHADD/TFPD. Local effects include pain, tenderness, and edema in the affected muscle area. The most concerning systemic effect is acute kidney failure due to the buildup of myoglobin in the renal tubules which are damaged by increased vasoconstriction, free radical generation due to excess iron, and direct heme-protein cytotoxicity.[19] Myoglobinuria occurs when plasma concentration exceeds 1.5 mg/dL and

the urine appears a dark-colored when the urine concentration exceeds 100 mg/dL. Elevation of creatine kinase (CK) is a sensitive marker, with CK>5000 U/l indicative of serious muscle injury. The rise in CK occurs within 12 hours of onset and peaks on days 1-3.[20] Myocyte injury can cause hyperkalemia, possibly leading to fatal cardiac arrhythmias. Local increases in extracellular calcium may also activate proteases leading to further muscle fiber necrosis.[20] Factors contributing to rhabdomyolysis may be related to the overall energy deficit and the accumulation of toxic by-products from partial fatty acid β-oxidation. Treatment is focused on the administration of fluids to flush the kidneys of myoglobin. Dextrose in saline is administered in hospitalized patients intravenously for rhabdomyolysis, but less severe cases may be treated with rest and administration of fluids at home. Acute metabolic acidosis may occur as a complication with release of intracellular uric acid and lactate, which is commonly treated with bicarbonate. Mannitol has been administered to promote diuresis and clearance of toxins but is not routinely administered in most centers.[21]

Biochemical Findings

Biochemical markers in the plasma and urine may be obtained for initial general diagnosis of a fatty acid oxidation disorder. Markers may not be specific to one disease, however, and further genetic testing is needed to differentiate the diagnosis. In LCHADD/TFPD, plasma acylcarnitines and plasma acylcarnitine/free carnitine ratio are typically elevated during periods of acute metabolic decompensations.[5] Urine analysis during decompensation may reveal elevated concentrations of organic acids, typically C6-C14 3-hydroxy dicarboxylic aciduria. ω-oxidation which normally plays a small role in overall fatty-acid oxidation is upregulated in LCHADD, causing an increase in dicarboxylic acids which are excreted in the urine. It is important that plasma and urine specimens are obtained at the earliest possible stage of acute decompensations as elevated levels of acylcarnitines and organic acids are typically transient. Levels of

plasma C8-C18 free fatty acids and their 3-hydroxy fatty acid analogues may also be elevated. These measures may be particularly reliable because quantitatively small yet significant amounts (<5 μmol/L) may persist even when asymptomatic and eating a low-fat diet.[5] The long-term value of acylcarnitine measurements is difficult to determine as some studies report high acylcarnitine levels in patients who present no obvious clinical signs or symptoms.[22] However, chronic elevations of OH-ACs have been associated with progression of retinopathy.[23]

Peripheral Neuropathy

Peripheral neuropathy is a long-term complication of LCHADD that mainly affects the lower extremities.[24] Early clinical findings may include attenuation of distal tendon reflexes in the lower extremities and difficulty walking on heels. The neuropathy is of a sensory axonal type with a marked loss of myelinated fibers and impairment of sensory conduction velocity. Electroneurography may show signs of denervation associated with reduced compound muscular action potentials.[24] Signs of neuropathy may begin as early as 6-12 years of age with abnormal motor responses in the lower limbs and decreased sensory perception extending to the upper limbs.[25] These findings were noted despite compliance with the current standard therapeutic diet.[25]

Growth

The assessment of growth is important in determining if patients with LCHADD/TFPD are receiving adequate energy and nutrients. Clinical evaluation and monitoring of patients should include an intake of height, weight, and assessment of psychomotor development at every follow up.[22] Growth may be altered in infants and children with FAODs due to either the disruption of energy production caused by the disease or the dietary intervention. The low-fat diet prescribed for LCHADD/TFPD is in sharp contrast to general nutrition recommendations for children and may impact the rate of growth and/or final height.

A retrospective study evaluating growth in 10 Swedish children with LCHADD reported a period of accelerated growth following diagnosis and initiation of treatment which then leveled off to a period of stable or decelerated growth.[26] Seven of the children were homozygous and three compound heterozygous for the common mutation. The age of diagnosis ranged between 2 days and 13 months. The children followed a low-fat diet; a series of 3-day food diaries indicated intake of 13-24% calories from fat distributed as 8-19% medium-chain triglycerides (MCT) and 4-5% long-chain triglycerides. The children met the recommended amount of fatty acids according to Swedish dietary guidelines. Fasting was limited to 3-4 hours depending on age. Three of five patients reached their final height within their target height. The authors suggested that neither the disease itself nor the dietary treatment effects final height negatively.[26]

The Swedish cohort also demonstrated a higher incidence of overweight and obesity which is consistent with reports from other cohorts.[23] Eight of the ten Swedish children developed overweight before the age of six. Growth rates were comparable to those of normal children with overweight or obesity.[26] The causes of overweight in LCHADD/TFPD are multifactorial. Increased weight gain may be related to high carbohydrate intake, short fasting periods, and continuous night feeds. Rapid weight gain may follow diagnosis as cautious parents may overfeed while initially learning to manage their child's condition. Children may also be less physically active due to muscular weakness or pain and/or neurological deficits.

Retinopathy

Pathophysiology

Patients with LCHADD begin developing long-term progressive retinopathy at an early age leading to significant visual impairment. In contrast, the rate of retinopathy progression in patients with TFPD is attenuated and impairments to function are generally noted much later in life.[27] Changes in the retina are evident by age 2 in roughly

50% of LCHADD cases, initially presenting as a peppery clumping of pigments in the macula.[9] If diagnosis and therapy are delayed, it may progress to atrophy of the central part of the choroid and retina, low vision, visual field defects, night blindness, nuclear cataracts, color vision defects and nearsightedness. Visual acuity is normal in the early course of the disease, but patients begin to lose color and night vision followed by loss of central vision which leads to legal blindness. The electroretinogram (ERG) of individuals with LCHADD progressively worsens over the lifespan. Tyni and colleagues proposed four stages of LCHAD retinopathy.[28] Stage 1 is characterized by normal retinal function and a hypopigmented fundus. Stage 2 is defined by the appearance of pigment clumping in the fovea as well as progressive retinal dysfunction as measured by electroretinography (ERG) while 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 towards the periphery. Patients report losing night vision followed by loss of color vision in stage 3. During stage 4, the posterior pole has lost all photoreceptors, most of the choroidal vessels, and central vision is lost.[9]

This study aims to expand upon the current understanding of factors related to metabolic control that are associated with preserved visual function. Studies have shown that preservation of vision is positively correlated with early diagnosis, treatment, and decreasing number of acute metabolic crisis.[29,30] Preserved retinal function as measured by ERG is correlated with lower plasma concentrations of 3-OH-ACs, which supports adherence to the low-fat diet and MCT supplementation that has been shown to reduce plasma 3-OH-ACs.[29]

Etiological Mechanisms

The pathology underlying the clinical manifestations of FAODs likely originates from two primary disturbances: energy deprivation and intoxication.[31] Impairments due to

energy deprivation result when the limited availability of reaction products disrupts cell function. Intoxication by substrate accumulation causes cell membrane disruption and inhibition of cellular enzymes. Both energy deprivation and intoxication are two of the proposed mechanisms by which the retinopathy in LCHADD/TFPD is hypothesized to occur, but it is important to note that retinopathy with vision loss is observed only in patients with LCHADD/TFPD and not among any of the other FAODs.

Energy deprivation in LCHADD/TFPD is due to the reduced synthesis of ATP and shortage of acetyl-CoA and reducing equivalents that impairs hepatic ketogenesis. This energy deprivation is one proposed etiology for LCHADD retinopathy. The retina has a high energy demand but until recently has been thought to be primarily glycolytic. Further disruptions in energy homeostasis have been found to occur with accumulating OH-ACs that act as uncouplers of oxidative phosphorylation.[31-33] Though energy deprivation occurs in other FAODs such as VLCAD or CPT2, these conditions do not have a retinal phenotype. Thus, it is more likely that the intoxication of metabolites is the cause of retinopathy in LCHADD due to the unique intoxicating 3-hydroxy acylcarnitine species occurring in this disorder that are not observed in other FAODs.

Several lines of evidence support the hypothesis that 3-OH-ACs intoxication is part of the pathology of LCHADD retinopathy. Gillingham and colleagues observed that low serum 3-OH-ACs and fatty acid byproducts were associated with a milder ophthalmic phenotype, even among patients of the same genotype.[34] In a study of 14 subjects with LCHADD/TFPD, high plasma OH-ACs were negatively correlated with maximum ERG amplitude. Visual acuity evoked potential was also measured but no difference was found between subjects with low OH-ACs compared to subjects with high OH-ACs.[29]

The presence of the mutated protein itself is hypothesized to be another mechanism. Normal protein expression of the MTP complex with selective decreased

LCHAD activity but relatively preserved hydratase and thiolase activity is observed in isolated LCHADD. In contrast, other mutations in the *HADHA* and *HADHB* genes result in unstable mRNA transcripts and a reduced MTP protein expression. A dominant negative effect of a pathogenic α-subunit protein caused by the common mutation has been suggested as a potential mechanism that induces retinal cell death.[9,35] However, no current molecular evidence exists to support this theory. This theory is less likely as it suggests that the mutated protein effects retinal cells differently than other lipid-oxidizing tissues.

Whether energy deprivation, accumulation of toxic metabolites, or presence of the mutated protein is the cause of retinopathy in LCHADD remains to be determined. Understanding the pathophysiology of LCHADD retinopathy is important to guide the development of treatments that can slow or prevent vision loss in patients with this disorder. If lower OH-AC concentrations preserve visual function, this supports the use of NBS and early initiation of dietary therapy that can reduce plasma OH-AC levels.

RPE Histology and Functions

The loss of vision in individuals with LCHADD is hypothesized to occur because of cell death in the retinal pigment epithelium (RPE). The physiological role of the RPE is to provide metabolic support for the rods and cones which is essential for the survival of the photoreceptor cells (PRs). The histology of this epithelial tissue will now be described and related to its metabolic functions, meanwhile showing how these functions support the photoreceptors and therefore the preservation of visual function. The main functions of the RPE include the recycling of rod outer segments, participation in the visual cycle through regeneration of 11-cis-retinal, prevention of oxidative stress, and the transport of nutrients, ions, and water across the blood-retinal barrier.[36]

Formation of the RPE begins early in development with RPE cells undergoing terminal differentiation at 4-6 weeks gestation.[37] The RPE cells then enter a dormant

state whereby proliferation of cells is infrequent except in certain disease states. Thus, the number of RPE cells in the human eye (approximately 3.5 x 10^6 cells) remains relatively constant throughout early adulthood.[37] Natural cell loss occurs with increasing age at the rate of roughly 2.3% of total RPE cells per decade of life.[38] The RPE layer extends from the optic nerve to the ora serrata, with the highest density of cells in the foveal area. The RPE is a simple cuboidal epithelial tissue lying between the blood-rich choroid and the neurosensory rods and cones. At its apical side, each RPE cell is in contact with 30 to 45 photoreceptors.[37] Microvilli covering the apical surface of the RPE envelop the outer segments of rods and cones and expand the surface area of the RPE 30-fold.[37,38] Adhesion between the RPE and photoreceptors is enhanced by expression of neural adhesion molecule on the apical surface of the RPE and structural support from the extracellular matrix.[37]

Rod/Cone Phagocytosis

The interdigitation of the photoreceptors with the RPE allows the RPE to participate in the phagocytosis of the photoreceptor outer segments (OS). In all vertebrates, the cyclical digestion of OS maximally occurs within the first few hours of waking. This multistep process begins with the recognition of the rod and cone outer segments (by the RPE followed by attachment via receptor-ligand interactions and then completed with the internalization and degradation of the OS.[37] Recognition of the OS occurs by binding of an opsonin on the OS surface to αVβ5 integrin while other soluble molecules act as bridging elements. The OS is then internalized into a phagosome and transported basally, at which point it fuses with a lysosome. Enzymes within the lysosome hydrolyze the captured OS into smaller molecules which can then be reused by the RPE or diffuse out of the cell.[39] Over a lifetime, each RPE cell will degrade approximately 200 million OS discs.[37]

Visual Cycle

The RPE also plays an integral part in the visual cycle by recycling 11-cis-retinal after it reacts with a photon. The 11-cis-retinal chromophores are light-sensitive pigments within the rhodopsin and cone opsin in rods and cones, respectively. Upon reaction with a photon, the 11-cis-retinal molecule undergoes a conformational change to all-trans-retinal, releases from the opsin, and then is reduced to all-trans-retinol. As it travels through the interphotoreceptor matrix (IPM), all-trans-retinol binds to interphotoreceptor retinoid-binding protein (IRBP) which is secreted from photoreceptors to aid in the translocation of the all-trans-retinol to the RPE.[37] Within the RPE, all-trans retinol is esterified to fatty acids by lecithin retinol acyltransferase to yield all-trans-retinol esters. This substrate is isomerized by RPE65 isomerase to 11-cis-retinol. The 11-cis-retinol is dehydrogenated to 11-cis-retinal, bound once again to IRBP, and transferred back to the PR cells where it can bind to opsin to be used in another visual cycle.[37]

Prevention of Oxidative Stress

The long-term exposure to light generates photooxidative stress and free radical generation within the retinal tissue. Other sources of oxidative stress to the RPE include the high consumption of oxygen by the retina and the high metabolism of polyunsaturated fatty acids by the RPE monolayer. The RPE exhibits several defenses to counteract the persistent stress. The pigment of the retinal pigment epithelium comes from the melanin granules that stain them various shades of brown depending on the quantity. The melanin absorbs photons that pass the photoreceptors, minimizing light scatter and protecting the retina from excessive light.[37] The antioxidant defense system of the RPE is extensive to counteract the high-oxidative stress environment and includes glutathione, glutathione peroxidase, catalase, thioredoxin, glutaredoxins, and superoxide dismutase.[37] The RPE also secretes neuroprotectin D1 derived from docosahexaenoic

acid (DHA) which downregulates the expression of proinflammatory genes and upregulates expression of antiapoptotic signaling molecules.[37,40]

Blood Retinal Barrier & Transport Functions

The basolateral membrane of the RPE is firmly attached to Bruch's membrane which separates the RPE from the choroid, the eye's blood supply. Both the choroid and the RPE contribute to the production of collagen that makes up Bruch's membrane. At the lateral sides of RPE cells, ultrastructural features such as tight junctions and junctional complexes assist in maintenance of the blood-retinal barrier. Tight junctions prevent paracellular diffusion between the retina and the choroid while junctional complexes allow for communication between cells of the RPE. When viewed *en face*, RPE cells are arranged in a hexagonal shape achieved by the tight junctions.[37]

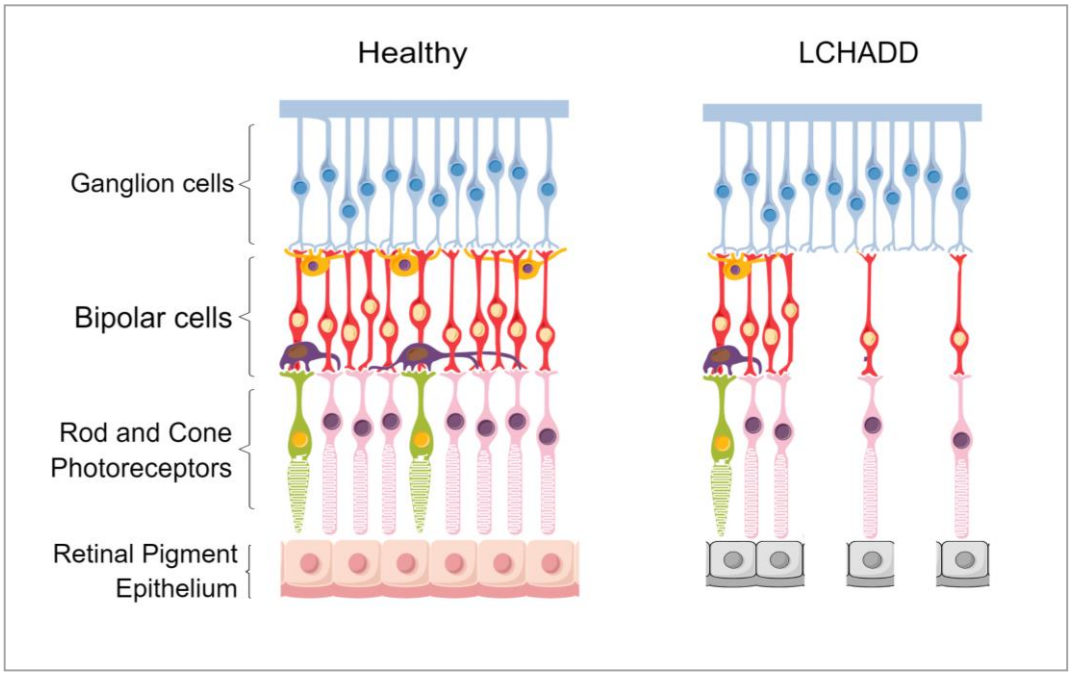


Fig. 2: Retinopathy in LCHAD deficiency

Cells of healthy vs. LCHADD retina. The RPE forms the barrier between the capillary-rich choroid and the photoreceptor cells. Death of the RPE cells in LCHAD deficiency leads to subsequent photoreceptor cell death.

Active transporters in the basolateral membrane are required to move nutrients and ions between the retina and choroid. RPE cells express high levels of glucose transporters GLUT1 and GLUT3 to support the high demand for glucose in the retina. The high metabolic activity of the retina results in accumulation of water which is removed through the RPE by Aquaporin-1 channels. In the RPE, the Na+/K+ ATPase transporter is located on the apical side (in contrast to most epithelia) in order to pump Na+ ions into the IPM to allow for PR dark currents.[37]

The RPE is hypothesized to be the initial layer of retinal tissue affected in LCHADD retinopathy.[39] Normal RPE cells demonstrate a high expression of MTP and metabolism of fatty acids, and death of these tissues may be caused by accumulating toxic OH-ACs in LCHADD/TFPD. Loss of the supportive functions of the RPE secondarily compromises the functioning of PR cells and leads to visual impairment. This is supported by the retinal autopsy report in a young child who died with LCHADD retinopathy and recent OCT images of patients showing loss of the RPE layer with photoreceptor tubulations.[41] Another study showed RPE cells in LCHADD/TFPD were

rounded in shape in comparison to the hexagonal shape of the control RPE cells, and thus disruption to the integrity of the barrier may play a role in retinopathy.[42]

Medical Nutrition Therapy

Treatment of mitochondrial LCFA β-oxidation disorders is focused on providing enough energy for growth and development while also preventing the adverse effects occurring with metabolic decompensations. One important goal of dietary therapy for LCHADD/TFPD is to limit LCFA β-oxidation to attenuate the production of toxic acylcarnitine species. This is significant regarding LCHADD/TFPD retinopathy because lower plasma 3-hydroxyacylarnitines are associated with retention of retinal function and visual acuity in children with the disease.[29]

Suppression of LCFA β-oxidation is achieved through several means including limiting dietary intake of LCFAs, supplementation of MCT, and shortened periods of time between meals. One study showed a diet providing <10% of energy from LCFA and 10-20% of energy from MCT was associated with lower plasma 3-OH-ACs.[23] Medium-chain fatty acids (MCFAs) provided by MCT supplementation are a rapidly metabolized energy source relative to that of LCFAs. This is because some MCFAs diffuse from the GI tract to the portal system as opposed to being absorbed by the lymphatic system like longer fatty acids. MCFAs are an appropriate energy source in LCHADD/TFPD as they are metabolized through an enzyme system distinct to that of LCFAs. Some researchers recommend an intake of MCT upwards to 30% of total energy in LCHADD/TFPD patients.[7] Supplementation of MCT is recommended prior to exercise to improve metabolic control and prevent the occurrence of rhabdomyolysis.[43] Periods of fasting are limited as to prevent hypoglycemia and mobilization of fatty acids for β-oxidation. Shortened fasting is important because increased lipolysis and accumulation of acylcarnitines occurs after 4 hours of fasting in LCHADD/TFPD children.[44] Tolerance to longer periods of fasting generally increases with age.

Both infants who present symptomatically and those who are asymptomatic and identified through newborn screening should cease breastfeeding and switch to an MCT-containing infant formula. Earlier treatment initiation limits acylcarnitine production which may help preserve visual function. For children, some of the energy provided as LCFAs should come from vegetable oil to prevent essential fatty acid deficiency. A daily multivitamin with all fat-soluble vitamins is also recommended to prevent vitamin deficiencies associated with low dietary fat intake. Despite such dietary treatment, morbidity of this condition is high with recurrent episodes of metabolic decompensation, potentially because the production of long-chain acyl-CoA esters can continue despite treatment.[22,45]

Biochemical DHA deficiency has been reported among patients with LCHADD. The reason for low plasma concentrations of DHA could be due to the LCFA restricted diet or due to an inability to endogenously synthesize DHA from the parent fatty acid, linolenic acid. Studies in cultured patient fibroblasts demonstrated that LCHAD-deficient cells had the ability to make DHA from LNA but that fibroblasts with a peroxisomal defect could not.[46,47] It is presumed that low plasma DHA in patients with LCHADD is related to a low-fat diet and supplementation with an algae DHA source normalizes plasma DHA. Normal or high normal plasma DHA does not slow or halt progression of LCHADD retinopathy but does promote nonspecific improvement in visual acuity.[48] DHA supplementation of 60-130 mg/day in LCHADD/TFPD is recommended.[29]

Carnitine is necessary for the transport of LCFAs through the inner mitochondrial membrane and may become depleted in LCHADD/TFPD. Carnitine supplementation has been proposed for long-chain FAODs as a means of normalizing plasma free carnitine and tissue carnitine. However, there is no evidence that carnitine supplementation normalizes these values or decreases the frequency of metabolic decompensations in

these disorders.[10,23,49] Improved metabolic control is more likely related to reduced intake of LCFAs and supplementation of MCT.[50]

Commercially available MCT preparations are predominantly a mixture of the even-chain fatty acids octanoate and decanoate. Several studies have investigated the use of odd-chain medium-chain fatty acids such as heptanoate (C7) in patients with LCHADD/TFPD. The rationale for the use of odd-chain MCFAs is that the metabolic end products of their oxidation are acetyl-CoA and propionyl-CoA. Propionyl-CoA is an anaplerotic molecule that can replace deficient odd-chain TCA cycle intermediates through conversion to succinyl-CoA. Thus, supplementation with odd-chain MCFAs may improve energy production by restoring TCA cycle intermediates. One study suggested that the poor response to even-chain MCT may be due to TCA intermediates leaking out of muscle and heart mitochondria in these patients.[51] This study noted improvement in cardiomyopathy, rhabdomyolysis, and muscle weakness with up to 26 months of odd-chain MCT supplementation. In a study of 29 patients with long-chain FAODs, triheptanoin supplementation of 30% of daily caloric intake was associated with improved exercise tolerance but also caused moderate gastrointestinal distress in most subjects.[52] A randomized double-blind study reported improved cardiac function and cardiorespiratory fitness in subjects randomized to C7 compared to those randomized to C8 but no difference in rhabdomyolysis or myopathy.[53]

Conclusion

Retinopathy is a clinical feature that is unique to LCHADD and TFPD among FAODs. The etiology of retinopathy remains unknown, but several hypotheses exist including the accumulation of toxic 3-OH-AC species, energy deficit, and presence of the mutated protein itself. It is also hypothesized that the first cell layer to be affected in LCHADD/TFPD retinopathy is the RPE, whose many functions are essential to the survival of PR cells and thus visual preservation. This study aimed to explore and

identify factors that are associated with preservation of visual function in LCHADD/TFPD which in turn may help guide the development of treatment protocols that attenuate vision loss. Visual impairment significantly impacts the lives of children and adults with LCHADD/TFPD as well as their families. Loss of vision can affect the capacity of an individual to perform fundamental tasks like reading and writing, leisure activities and socialization, cause difficulties at work, and create psychological distress and anxiety.[54] In children, low vision is associated with a delay in the development of motor function and poorer mathematical, social, and intermediate problem-solving skills.[55] Thus, to optimize the health and wellness of those living with LCHADD/TFPD, it is important to identify therapies that delay and/or prevent the progression of retinopathy.

Methods

Participants

We estimated that 150-250 individuals were living in the United States with LCHADD/TFPD and we aimed to survey as many as possible. Subjects for this study were recruited through the Fatty Acid Oxidation patient and family support network (www.FODsupport.org). Genetic metabolic physicians and dietitians were contacted through emailing lists such as metabL (http://www.daneel.franken.de/metab-l/) and asked to recruit patients in their care. A description and instructions for the study were sent by email to patients and clinicians within the online support network and the metabL email list. Subjects for the study participated remotely using a REDCap web-based survey. Participation in this study was voluntary and consent for the study was obtained through an online consenting process. Subjects or their guardians provided information through the survey regarding medical history, diagnosis, metabolic complications, and genotyping if known. At the end of the survey, subjects were asked for permission to access pertinent medical records. Criteria for inclusion in the study was a diagnosis of TFPD or LCHADD. Subjects of all ages were invited to participate in this study.

Survey Instrument

Subject information was collected by a REDCap web-based survey taken by either the subject or their guardian. The survey totaled between 45-53 questions depending on participant answers. Information collected included medical history, diagnosis, metabolic complications, and genotyping information if known. Questions were included regarding dietary intake of MCT and DHA intake and the maximum time interval fasting between meals, the frequency of visits to ophthalmologists and dietitians, the frequency of metabolic decompensations at home and decompensations requiring hospitalization, and LCHADD/TFPD diagnosis among familial relatives.

To assess patient perception of visual function, subjects completed 30-35 questions from the National Eye Institute Visual Function Questionnaire (VFQ-25). This survey was developed to measure the influence of visual disability and visual symptoms on generic health domains such as emotional wellbeing and social functioning in addition to task-oriented domains related to daily visual functioning. Questions presented in the VFQ-25 represent content identified during condition-specific focus groups in patients with age-related cataracts, glaucoma, age-related macular degeneration, diabetic retinopathy, or CMV retinitis. The longer NEI-VFQ has been shown to reliably assess perceived visual impairment among these groups, and the shorter VFQ-25 predicts 92% of the variance between the 51-item NEI-VFQ.[56] To our knowledge, this survey has not been used in a population with retinopathy related to LCHADD/TFPD.

Subjects who began the survey could pause and restart answering questions as needed. Subjects who signed up for the survey but did not finish were prompted via email to complete the remaining questions. The questions are divided into 10 related subscales and are combined to generate a score for that subscale construct. The subscales included:

1. Global vision rating
2. Near vision
3. Distance vision
4. Social functioning
5. Role limitations
6. Dependency on others
7. Mental health
8. Driving difficulties
9. Peripheral vision
10. Color vision

Nine of the ten subscales (all those except driving) were used to create an overall composite score reflecting the subject's vision. Subscale scores and the composite score were calculated as outlined by the NEI-VFQ instructions. Subjects that did not complete the full survey but fully completed at least 1 subscale were included in the analysis of the subscale(s) that they did complete. Optional questions were added to the near activities and distance activities subscales and were included in calculating the composite score. These questions were asked in a 5-item Likert-type format ranging from visual function causing "no difficulty" in completing the task to "stopped doing this because of your eyesight." Task-oriented questions assumed that the subject was wearing prescription glasses or contacts if they had them. Two additional items asked subjects to rank their health and vision on a scale from 1 to 100. The global health and global vision rating were used as outcome variables.

Medical Information

At the end of the survey, subjects were asked to release their medical records for the study. From the medical record, we gathered all acylcarnitine reports, genotyping reports, and information about the events leading to diagnosis. For subjects who provided consent to obtain medical records, a diagnosis of TFPD or LCHADD was confirmed by reviewing the medical records.

The Institutional Review Board at Oregon Health and Science University approved the study protocol (eIRB#7401). Consent for the study was obtained online when the subject initiated the survey.

Statistical Analysis

For the first aim, a Mann-Whitney U rank sum test was used to determine if a significant difference exists in the composite score, the vision rating, or any of the subscale scores between the LCHADD and TFPD groups. A Fisher's exact test was run to compare the distribution of those reporting visual impairment (composite score <100)

and those not reporting visual impairment (composite score =100) among those with and without the common mutation.

For the second aim, we performed multiple linear regression analysis to examine the relationships between four subject characteristics and the composite score, global vision rating, and global health rating. The subject characteristics included were age, acute hospitalized decompensations per year, diagnostic event, and an acylcarnitine composite. Age and the number of acute metabolic decompensations per year were obtained from the REDCap survey. The diagnostic event was a categorical variable based on the age of the subject at diagnosis and whether an acute metabolic decompensation preceded diagnosis. Subjects who experienced a metabolic decompensation in the newborn period (0-2 months) prior to diagnosis were considered the most severe, followed by infants (2-12 months), and juveniles or older (12+ months). Subjects who were diagnosed by newborn screening before a metabolic decompensation occurred were categorized separately as such. The acylcarnitine score was a composite of four acylcarnitine species: C14-OH, C16:1-OH, C16-OH, and C18:1-OH. Different diagnostic labs report varying number of OH-ACs. Only the species which are commonly reported across all labs were used in the analysis. These 4 species were chosen as all of them were reported at least once for the subjects with acylcarnitine data. The highest value for each species were added to create a composite for each subject.

Because acylcarnitine data was missing for 5 of the subjects, regression models were also constructed for all 14 subjects by eliminating the acylcarnitine composite variable. We ran additional models that included the variable acylcarnitine composite for the 9 subjects on which we had this data available. A stepwise regression was run to determine the model of best fit. A p-value of 0.05 or less was considered statistically significant.

Table 1: Overview of statistical analysis

Specific Aim	Hypothesis	Statistical Test
To investigate the genotype-phenotype correlation between the presence of one or two copies of the *HADHA* c.1528G→C common mutation and retinopathy severity in LCHADD.	The presence of at least a single copy of the common mutation is correlated with advanced retinopathy as defined by poorer self-reported vision-related health in comparison to other genotypes.	A rank sum test was used to determine if a statistical difference exists between the composite score, vision rating, and subscale scores of the LCHAD and TFPD groups. A Fisher's exact test was used to compare the odds of visual impairment or no visual impairment among those with either LCHADD or TFPD.
To examine the relationship between relevant outcomes from the medical record and the severity of retinopathy.	Advanced retinopathy is correlated with increased frequency of metabolic decompensations, higher plasma 3-OH-ACs, older age at diagnosis, and older age.	Multiple linear regression was used to examine the relationship between subject characteristics and the composite score, health rating, and vision rating. Subject characteristics entered into the models included age, hospitalized decompensations per year, diagnostic event, and acylcarnitine composite.

Results

All statistical analysis was run using IBM SPSS Statistics Processor v25 PC.

Subject Characteristics

Twenty people responded to our call for participation in this study. Of these, 13 people fully completed the VFQ-25 survey. One individual partially completed the survey and the scores for the sections that they did complete were included in the analysis. A total of 14 were included in the analysis, and medical records with acylcarnitine data were obtained from 9 of the 14 subjects. The study population included 11 subjects diagnosed with LCHADD and 3 with TFPD. The age of subjects ranged from 18 months to 26 years. Patient characteristics are in Table 2 including age and genotype if reported.

Table 2: Patient characteristics of 14 subjects with LCHADD or TFPD

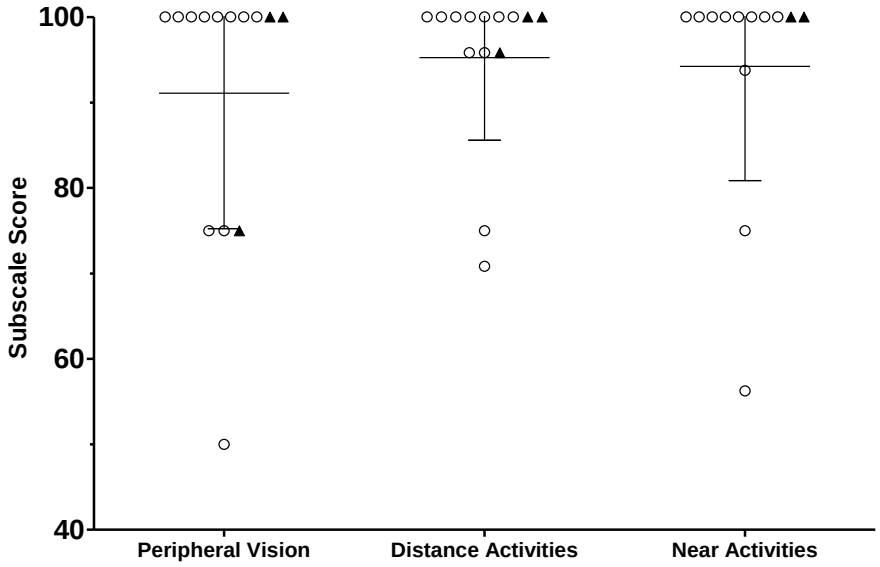
No.	Age (years)	Diagnostic event	Diagnosis	Genotype
1	9	Juvenile	TFP	
2	13	Newborn symptomatic	LCHAD	
3	4	Newborn screening	LCHAD	HADHA homozygous c.1528G→C
4	9	Newborn screening	LCHAD	HADHA homozygous c.1528G→C
5	4	Newborn screening	TFP	HADHB P172S, IVS82A→C
6	3	Newborn screening	LCHAD	HADHA c.274_278delTC, c.1528G→C
7	9	Newborn symptomatic	LCHAD	HADHA homozygous c.1528G→C
8	21	Infant	LCHAD	
9	6	Newborn screening	LCHAD	HADHA homozygous c1528G→C
10	10	Infant	TFP	
11	4	Newborn screening	LCHAD	
12	27	Infant	LCHAD	
13	19	Newborn symptomatic	LCHAD	
14	2	Infant	LCHAD	

Aim 1

The first aim of our study was to examine the genotype-phenotype correlation of the c.1428G→C common mutation and retinopathy. We performed a two-sided Fisher's exact test to see if there was a difference in the proportion of those with visual impairment or no visual impairment between the LCHADD and TFPD groups. Visual impairment was defined as having a composite score less than 100. Seven of the 11 subjects with LCHADD had visual impairment compared to 2 of 3 subjects with TFPD who were visually impaired. The proportion was relatively equal between groups (64% vs 66%) and there was no significant difference between groups (p=1.00).

The areas that subjects felt their vision impacted the most, based on mean subscales scores, were peripheral vision (91.1±15.2), near vision (94.2±12.9), and distance vision (95.2±9.3). Subjects also felt that their vision affected their mental health (95.7±7.5), functioning in roles (96.5±7.0), and dependency on others (99.0±1.6). However, for any given subscale, less than half of the subjects reported that their vision affected that area. None of the subjects felt that their vision affected their color vision or social functioning. A Mann-Whitney U test was used to compare the composite score, subscale scores, and vision rating between the LCHADD and TFPD groups. There was no score that significantly differed between groups.

A



B

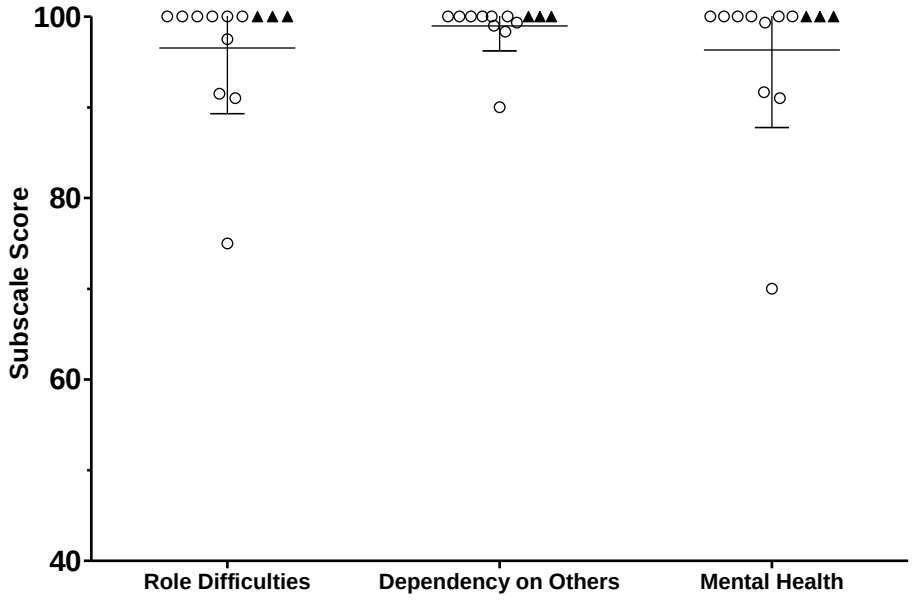


Fig. 3: Subscale scores

(A) Vision-related subscale scores from 14 subjects with LCHAD and TFP deficiency. (B) Socioemotional subscale scores from 14 subjects with LCHAD and TFP deficiency.

Table 3: Mann-Whitney U test of vision-related survey scores

	LCHAD	TFP	U	P value
Composite (n_1=11) (n_2=3)	95.14 ± 7.25	97.33 ± 3.35	16.5	1.00
Global vision rating (n_1=11) (n_2=3)	88.45 ± 13.46	90.00 ± 10.00	16.0	0.94
Peripheral vision (n_1=11) (n_2=3)	90.91 ± 16.86	91.67 ± 14.43	16.0	0.92
Distance vision (n_1=11) (n_2=3)	94.32 ± 10.75	98.61 ± 2.41	15.0	0.78
Role limitations (n_1=10) (n_2=3)	95.50 ± 8.04	1	9.0	0.22
Dependency on others (n_1=10) (n_2=3)	98.67 ± 3.10	1	9.0	0.22
Mental health (n_1=10) (n_2=3)	95.20 ± 9.55	1	9.0	0.22
Near vision (n_1=11) (n_2=2)	93.18 ± 14.38	1	8.0	0.42
Color vision (n_1=11) (n_2=2)	1	1	11.0	1.00
Social function (n_1=11) (n_2=3)	1	1	16.5	1.00

Data are mean ± SD. n_1=LCHAD group. n_2=TFP group. Subjects who did not complete all questions within a subscale were removed from the analysis of said subscale.

Aim 2

Composite Score

Older subjects tended to have a lower composite score. Age was the only variable that significantly predicted composite score in both models with and without the acylcarnitine composite variable (β= -0.73, p=0.02; β= -0.75, p>0.01). Age explained 36% and 52% of the variance in the models, respectively.

There was a strong negative correlation between age and composite score for all subjects (r= -0.747, p<0.01). Diagnostic event was positively correlated with composite score (r= 0.610, p=0.01). This suggests that patients for whom treatment is initiated prior

to an acute decompensation tend have better visual function. Age at the first metabolic decompensation is often thought to be related to residual enzyme activity. Patients presenting in acute crisis in the newborn period are thought to be more severe than patients who present later in life such as during childhood.[17] Diagnostic event may be a proxy for disease severity.

Global Vision Rating

Older subjects tended to report a lower vision rating. Age remained the only significant predictor for vision rating in the model without the acylcarnitine variable (β= -0.64, p= 0.01). There were no variables found to be significant in the model that included the acylcarnitine composite variable. Age explained 42% of the variance in vision rating in the first model. Age was also negatively correlated with vision rating (r= -0.637, p<0.01) and diagnostic event was positively correlated (r= 0.600, p<0.01).

Global Health Rating

None of the variables entered into either regression models significantly predicted health rating. However, there was a strong negative correlation between hospitalized decompensations per year and health rating in the model with 9 subjects (r= -0.661, p=0.02).

Table 4: Age as a significant predictor of composite score and vision rating (n=14)

	B	SE	β	p	Adjusted R^2
Composite score	-0.002	<0.001	-0.747	<0.01	0.521
Vision rating	-0.003	0.001	-0.637	0.014	0.356

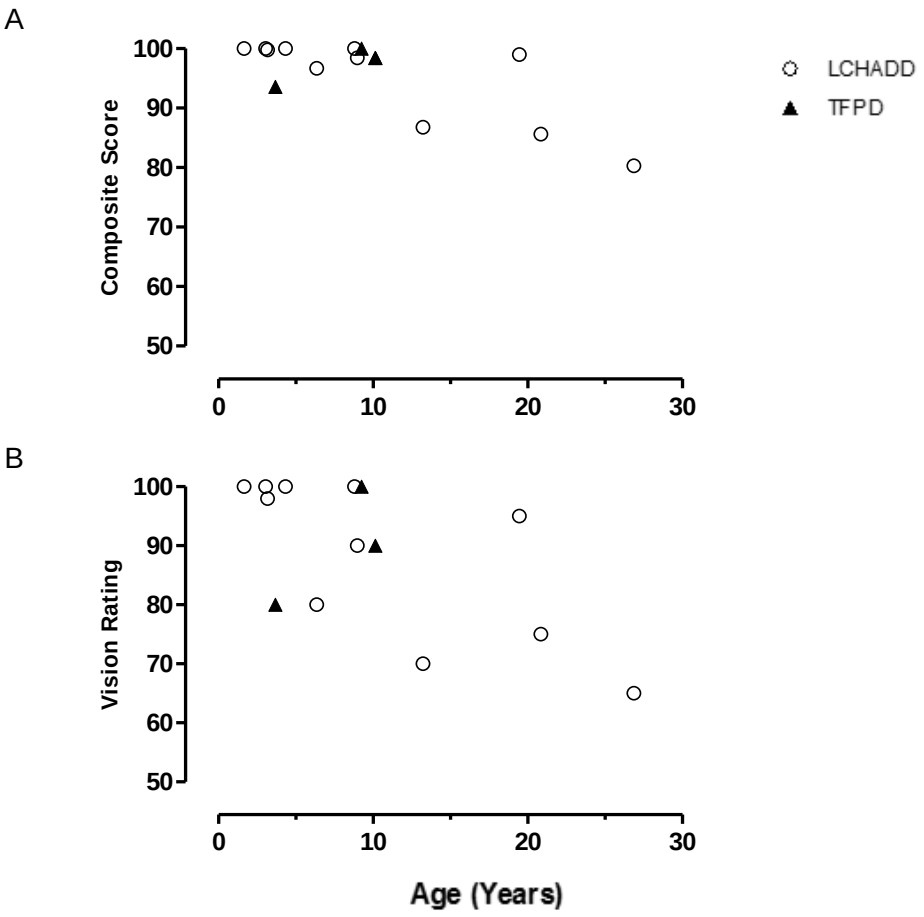


Fig. 4: Composite score and vision rating by age

(A) There is a negative correlation between age and composite score in 14 subjects with LCHAD or TFP deficiency (r= -0.747, p=0.001). (B) There is a negative correlation between age and vision rating in 14 subjects with LCHAD or TFP deficiency. (r= -0.637, p=0.007). Age was not significantly correlated with health rating.

Table 5: Significant correlation coefficients from regression models

	Composite score (n=14)		Vision rating (n=14)		Health rating (n=9)	
	r	p	r	p	r	p
Age	-0.747*	<0.01	-0.637*	<0.01		
Diagnostic event	0.610*	0.01	0.600*	0.01		
Decompensations/ year					-0.661	0.02

Discussion

Aim 1

There was no significant difference between the LCHADD and TFPD groups in the proportion of people with visual impairment. Likewise, there was no significant difference in the severity of retinopathy between the LCHADD and TFPD groups as measured by the subscale scores, composite score, and vision rating. Self-perception of all the measured vision-related functions were also not found to significantly differ between the two groups. Previous studies have reported that patients with TFPD tend to retain visual function for longer than patients with LCHADD. One study of 21 cases with an average follow-up of 7 years noted that TFPD subjects maintained visual acuity through follow-up while LCHAD subjects demonstrated a steady decline despite medical and dietary treatment.[27] Because of the many factors that can influence retinopathy progression, disease severity is highly variable even among patients of the same genotype. It is likely that our sample sizes were not large enough to detect a difference if it did exist. Further studies are needed to confirm other observations that the presence of the common mutation associated with LCHADD is associated with a more progressive retinopathy.

Several limitations may have contributed to why we did not find a difference in severity of retinopathy between the groups. In addition to our small sample size, our study population was relatively young so many subjects reported no or very little change in visual function. If a difference in retinopathy progression existed, it would be easier to detect with an adolescent or adult population. Lastly, it is difficult to determine what part of visual impairment is related to the disorder or other inherited changes in vision such as myopia. The prevalence of mild visual impairment among healthy populations is common, with an estimated 7.25 million people in North America and 9.47 million in Western Europe having some degree of visual impairment.[57]

Peripheral vision had the lowest absolute mean score of all the subscales. This subscale was assessed by a question about the level of difficulty faced in noticing objects to the side, to which 3 patients reported having “a little difficulty” and 1 reported “moderate.” In LCHADD and TFPD retinopathy, pathological changes typically originate in the central fundus. This may be explained by the active metabolism of this area, exposure to light damage, or differences in the choriocapillaris network and RPE in the posterior pole.[41] As retinopathy progresses, the degradation of the RPE and subsequent loss of rods and cones spread to the peripheral fundus.[27,58,59] Peripheral vision is therefore usually not affected until the later stages of the disease. In our cohort, the 4 subjects that perceived impaired peripheral vision were the oldest in our study population (10-26 years).

Four subjects with LCHADD and one subject with TFPD reported their distance vision was affected. The six questions in the distance subscale asked subjects about the level of difficulty experienced when reading street signs or store names, going down steps or curbs in dim light, recognizing familiar people from across a room, taking part in sports and outdoor activities, watching TV, and attending movies, plays, and sports events. One subject with TFPD was similar to the report of 2 of the LCHAD subjects in this subscale. They each reported “a little difficulty” with a different question; one in going down steps/curbs in dim light, one in reading street signs or store names, and one in participating in outdoor activities. This highlights that the activities affected by vision loss are individualized for any given person. The two oldest subjects in our study, ages 20 and 26, perceived the most impairment to their distance vision which is consistent with several studies that noted myopia only in the oldest subjects.[27,41,59] Progressive myopia is indicative of the 3rd stage of retinopathy proposed by Tyni et al. The 20-year old subject had the lowest score (70.8) and reported being affected in all six question areas while the 26-year old subject reported “extreme difficulty” in going down steps or

curbs in dim light. More subjects in our study reported change to their distance vision (36%) compared to any other subscale.

Three subjects with LCHADD reported their ability to perform near activities to be affected by their vision. This subscale was assessed with four questions regarding the difficulty subjects faced in reading ordinary print in newspapers, reading small print such as on a medicine bottle while wearing glasses, finding something on a crowded shelf, and doing work or hobbies that require you to see well close up such as cooking, sewing, or fixing things around the house. The oldest subject had the lowest score in this subscale, reported being affected in all four of these areas, and noted “extreme difficulty” in being able to find an item on a crowded shelf.

Subjects did not perceive their color vision to be impacted as indicated by all subjects scoring 100 in the color vision subscale. This subscale was assessed with a single 5-scale Likert question that asked about the amount of difficulty they experienced in picking out matching clothes because of their eyesight. In the case of older subjects, it is interesting that none of them reported deficit in color vision. Tyni et al. reported severe color vision deficit in two adolescent patients with LCHAD, ages 14 and 16.[41] The 14-year old patient was noted to have roughly normal color vision in the right eye and beginning tritanomaly (limited blue cone cells) in the left eye. A decline in color vision in both eyes were noted at four and six years follow up. Gillingham et al. similarly reported several adolescent patients with decreased color vision.[29] A case study of a 4-year old LCHAD patient found normal color vision when assessed by Ishihara plates, suggesting that color vision may be maintained in early age.[60] The question in the VFQ-25 regarding the ability to pick out matching clothes did not fully capture the subjects’ perceptions of their color vision. One study also found that this question was rated relatively easier by women than men of similar visual function.[61] Other problems that people with color vision loss may face include the ability to distinguish colors in a traffic stoplight or being

able to tell the difference between ripe and unripe produce. Additional questions reflecting these and other areas may be needed to better capture the perception of color vision in patients with LCHADD and TFPD.

As with color vision, no subjects perceived that their vision posed any difficulties for their social functioning. The social functioning subscale was assessed by two questions that asked about the amount of difficulty subjects faced in seeing how people react to things they said and the difficulty in visiting with people in their homes, at parties, or in restaurants. In contrast, visually impaired children and adults in numerous studies have reported an impact of their vision on social functioning such as decreased integration into school and work environments and hinderances to starting and sustaining social and romantic relationships.[55,62,63] This subscale's questions have less relevance to our younger subjects and they may not be adequate in scope to assess difficulties in social functioning experienced in other areas by people with LCHADD/TFPD.

Four subjects with LCHADD perceived role difficulties with regards to their vision. The role difficulties subscale was assessed by two questions that asked how often they accomplished less than they would like because of their vision and how often they are limited in how long they can do work or other activities due to their vision. Subjects reported their answer with a sliding scale from 1 to 100, with 1 representing "all of the time" and 100 representing "none of the time." A 13-year-old subject had the lowest score in this subscale, followed by the two oldest subjects. The fourth affected subject was a 1-year-old subject who reported being modestly affected in both areas.

Four subjects with LCHADD perceived that their vision affected their dependency on others. The dependency subscale was measured by three statements scaled from 1 to 100, with 1 representing "definitely true" and 100 representing "definitely false." The statements included "I stay home most of the time because of my eyesight," "I need a lot

of help from others because of my eyesight," and "because of my eyesight, I have to rely too much on what other people tell me." Besides color vision and social functioning, dependency on others was perceived to be the least affected subscale area among subjects. Only one subject reported that all three statement domains were affected by their vision. This was the oldest subject in our study population and the subject with the lowest dependency subscale score.

Four subjects with LCHADD perceived that their vision affected their mental health. Subjects rated four statements on a sliding scale of truth from 1 to 100 to determine the mental health subscale. The statements were "I feel frustrated a lot of the time because of my eyesight," "I have less control over what I do because of my eyesight," and "I worry about doing things that will embarrass others or myself because of my eyesight." The oldest subject had the lowest overall score and expressed experiencing a high amount of frustration due to their eyesight, giving the frustration statement a score of 20. Two other subjects felt the greatest agreement with the statement on frustration, while the fourth subject only felt agreement with the statement regarding embarrassment.

Aim 2

In our regression models with 14 subjects, age was significantly associated with composite score and global vision rating. For every one-year increase in age, there was a 0.73 decrease in composite score and a 1.1 decrease for vision rating. Prospective studies using fundus photography, visual evoked potentials, and electroretinography have demonstrated that the retinopathy of those with LCHADD tends to worsen over time.[27] Our finding that composite score and vision rating significantly decreases with age builds upon previous studies by confirming that patients perceive their vision getting worse as they age.

None of the patient characteristics significantly predicted health rating. In evaluating perceived health, a patient may consider the severity of their disease complications, other comorbid conditions, their health's impact on daily functioning, and their relative current health state as well as many other things. These features of health may overshadow any relationship between the variables that were captured in our study. Age may not be a good predictor of health rating as the severity of disease presentation can wary widely between patients of the same age and genotype.

Although early diagnosis and treatment is believed to delay the progression of retinopathy, patients who are diagnosed at an older age may have a milder phenotype and therefore a slower rate of vision loss. This was the case in our study population; for subjects diagnosed following a metabolic decompensation, the mean composite scores and vision ratings were higher for the infant group (n=3) than the newborn group (n=3) and highest in the juvenile group (n=1). Therefore, in general, patients who present symptomatically earlier in life may require more frequent ophthalmologic evaluation. As newborn screening for genetic MTP diseases is now standard practice, it is hoped that most patients will be diagnosed as newborns through screening before metabolic decompensation, though cases of delayed diagnosis despite flagged newborn screening panels in two European countries have been reported.[64]

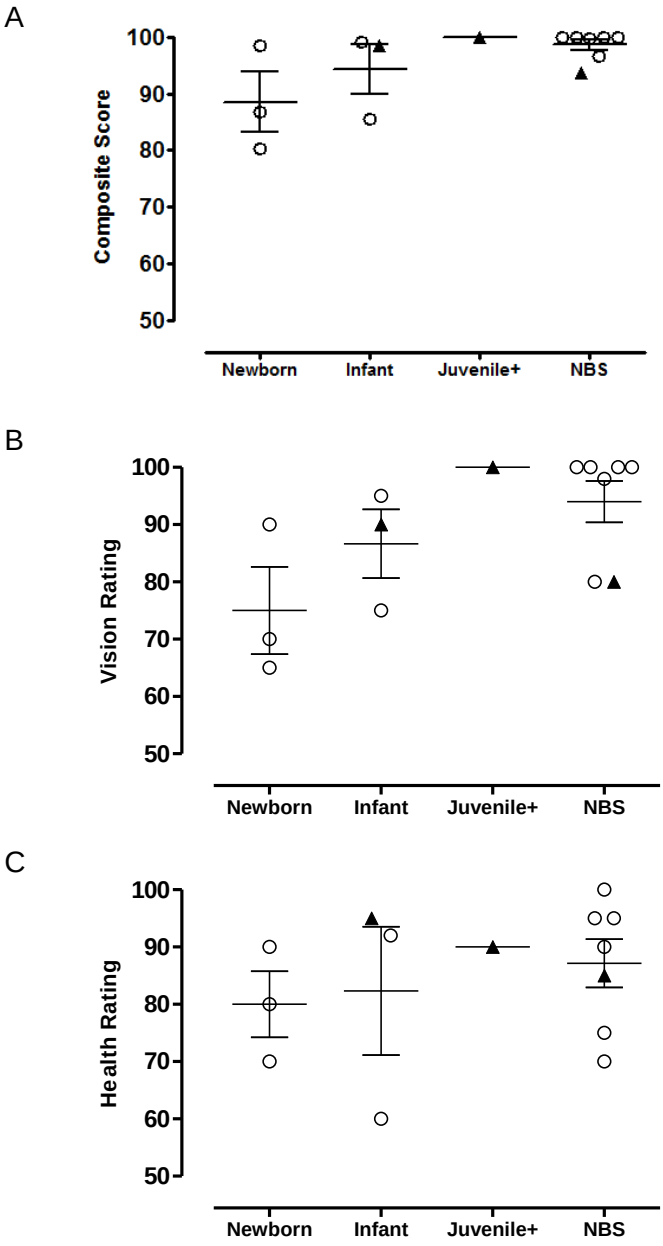


Fig. 5: Survey scores by diagnostic event

Regression outcome variables categorized by age at initial metabolic decompensation. Age ranges are defined as newborn: birth to 2 months, infant: 2 to 12 months, juvenile+: 12 months or older. Infants who were diagnosed by newborn screening before decompensation occurred were categorized as newborn screening (NBS). (A) There is a positive correlation between age at initial decompensation and composite score in 14 subjects with LCHAD or TFP deficiency (r=0.610, p=0.01). (B) There is a negative correlation between age at initial decompensation and vision rating in 14 subjects with LCHAD or TFP deficiency (r=0.600, p=0.012). (C) A positive correlation between age at initial decompensation and health rating was not significant. (r=0.328, p=0.19).

We hypothesized that earlier symptomatic presentation is associated with a more severe phenotype and therefore worse outcomes for visual function. The diagnostic event variable captured this concept and categorized subjects by age and symptoms at diagnosis. In our study population, 3 subjects were diagnosed after symptomatic presentation as newborns (0-2 months) and 3 after symptomatic presentation as infants (2-12 months). The single subject in the juvenile and older category was diagnosed with TFPD at 3 years of age after a younger sibling was diagnosed through newborn screening. This subject did not have any symptomatic presentation prior to their diagnosis. Seven subjects were asymptomatic when diagnosed by newborn screening. In our regression models, the diagnostic event was not a significant predictor of either composite score, vision rating, or health rating. However, subjects diagnosed by newborn screening had a higher mean score for all three areas than the groups diagnosed symptomatically as newborns or infants. The single juvenile subject who was asymptomatic at diagnosis also had higher scores than those of the symptomatic newborns and infants. Metabolic decompensations occurring in proximity to birth may impact the progression of retinopathy. Several studies have noted more pronounced pathological ocular changes and vision loss in patients with early and frequent metabolic decompensation and/or delayed diagnosis and initiation of therapy.[15,30,41,59] These findings highlight the importance of newborn screening as treatment initiated at an early age can delay the progression of retinopathy, particularly when the frequency and severity of metabolic decompensations are minimized.

The frequency and severity of decompensations are thought to be prognostic factors for the progression of LCHADD retinopathy as researchers have noted that patients who experience more decompensations tend to have worse visual function.[30,59] Damage to retinal tissue due to energy deprivation or the increased levels of circulating acylcarnitines during these episodes may explain these observations. In our survey, the

frequency of acute decompensations was gauged by the question “since being born, you have been hospitalized for low blood sugar episodes or other metabolic complications such as rhabdomyolysis a total of _ times.” The number of acute metabolic decompensations per year ranged from 0 to 6.3 decompensations per year, with a mean number of 1.7 hospitalizations per year. Acute hospitalized decompensations per year did not predict composite score, vision rating, or, health rating. The lack of a relationship between acute decompensations and perceived visual function may be explained by several reasons. First, decompensations were reported by subjects and therefore subject to recall bias, especially for older subjects or subjects with frequent decompensation episodes. Second, this variable did not account for mild decompensations treated at home that may also contribute to retinopathy progression. Third, the severity of a hospitalized decompensation can vary between episodes and between patients. Using the total number of days hospitalized per year due to metabolic decompensation may demonstrate a stronger relationship with visual function. Finally, this variable is dependent upon age which may be a covariate for hospitalizations per year. Despite acute decompensations per year not being a predictor, this variable had a direct negative correlation with global health rating (Table 5).

If circulating acylcarnitines contribute to LCHADD retinopathy, limiting exposure to these metabolites should theoretically preserve vision in these patients. High plasma acylcarnitines have been correlated to lower maximum ERG amplitude in patients with LCHADD.[29] In our study, the acylcarnitine composite did not contribute to prediction of self-perceived visual function. There are several explanations for why we may not have seen a relationship. Our sample size for this analysis was limited by only having acylcarnitine data for 9 of our 14 subjects. The acylcarnitine values used to create the composites were retrieved from the medical records without discerning whether the they were collected during a routine follow-up or during a decompensation. Even during

decompensation, the increase in circulating acylcarnitines is transient and dependent upon when the blood sample was drawn. It is also plausible that chronic lifetime exposure to acylcarnitines, which is not captured with this variable, is more important to the development of retinopathy. This notion is supported by the finding that patients who sustained low plasma acylcarnitines maintained a higher maximum ERG amplitude than those with chronically high levels.[29] Finally, it is possible that acylcarnitines do not in fact contribute to the development of retinopathy. Given that each eye is exposed to the same level of these circulating metabolites, both eyes should be relatively similarly diseased. However, Boese et al. noted a substantial difference in visual acuity between the left and right eyes of patients with LCHADD which grew more disparate over time.[27]

Our study has several limitations, most notably our small sample size and the size discrepancy between the LCHADD and TFPD groups. Recruiting subjects and administering the survey online may have been a barrier for potential subjects with severe visual impairment. Additionally, acylcarnitine data was available for only 9 of the 14 subjects and the scenario in which these acylcarnitine values were obtained was not contextualized. Our study population was relatively young with over half of all subjects under the age of 5 years. The true visual function of these young patients may be misinterpreted by the parents and guardians who answered the survey questions for them. Finally, our analysis of the events and characteristics that contribute to retinopathy progression is limited in very young subjects who still maintain normal or near normal visual function.

To our knowledge, no study to date has evaluated the personal assessment of visual impairment in a population with LCHADD/TFPD and related these outcomes to factors thought to influence retinopathy progression. With treatment, many patients with LCHADD and TFPD can lead a relatively normal vision-related quality of life, as most of our subjects reported little or no visual impairment. Clinicians should explain that

adherence to dietary therapy is the currently the best way to maintain visual function while also preparing patients and their families that visual impairment is part of the natural course of disease progression. Currently, gene therapy with the goal of preventing vision loss is being investigated in both cell culture and animal models out of studies at Oregon Health and Science University. Our findings on the psycho-social outcomes for patients with LCHADD/TFPD gives insight into the patient experience of vision loss due to a rare genetic disease with no known cure. Higher rates of depression have been reported in visually impaired populations compared to the general population.[65] Many rare disease patients also report mental health comorbidities with 75% and 86% experiencing depression and anxiety, respectively.[66] All patients with LCHADD and TFPD should be made aware of appropriate counseling and support services. Patients experiencing undue stress related to their vision should be referred to a therapist specialized in counseling patients with rare diseases or chronic illnesses. For patients experiencing night blindness, patients may prefer to make appointments that allow them to travel only during daylight hours. Healthcare related accommodations for low vision may include audible prescription bottles. The experience of vision-related activities and the level of perceived difficulty was unique to each subject and underlies the importance of treating and accommodating each patient on a case by case basis. For example, subjects who reported difficulty in taking part of outdoor recreational activities may benefit from participation in camps and programs that are designed specifically for the visually impaired.

Although the VFQ-25 is intended to measure self-reported vision-targeted health in those with chronic eye diseases, the questions are not specific to the retinopathy occurring in patients with LCHADD/TFPD. Content of questions were generated by focus groups of patients with age-related cataracts, glaucoma, age-related macular degeneration, diabetic retinopathy, or CMV retinitis. LCHAD/TFPD retinopathy may differ

from these conditions in the early onset of visual dysfunction, the occurrence of myopia and night blindness, and the limited treatment modalities available. In our study, all subjects reported no dysfunction in the areas of color vision, peripheral vision, and issues with social functioning due to vision. The subscale of driving that was removed from our scoring also did not pertain to many subjects as most were not of legal driving age. The utility of the VFQ-25 in this population may therefore be limited. Development of tools measuring self-reported visual function specific to LCHADD/TFPD patients may provide better insight. Specifically, questions targeted to assess myopia and night blindness would be of relevance. Questions often referred to activities participated in by adults rather than children (i.e. reading the newspaper), so more age-appropriate questions should be used. Because a difference in function between the left and right eye of patients has been noted, it would be relevant to include questions asking if patients notice being able to see better out of one eye than the other. The use of all subscales to create the composite score may not be a true reflection of vision as it demonstrates multidimensionality. Pesudovs et al. noted that the subscales should be separated into visual functioning and socioemotional constructs.[61]

Our study captured the self-perception of visual function and health in subjects with LCHADD and TFPD. For the combined group of LCHADD and TFPD subjects, older subjects perceived that their vision, ability to do vision-related tasks, and other vision-related health measures were worse than that of younger subjects. We did not find that the prevalence or severity of retinopathy was different between the LCHADD and TFPD groups, but more larger studies are needed to confirm or dispute this finding. Finally, we found that the main areas that vision affected were distance vision, peripheral vision, and near vision. Vision secondarily affected their mental health, role functioning, and dependency on others. Vision did not affect color vision or social functioning, but an improved questionnaire might better capture the perception of these and other areas.

References

1. Gregersen N, Andresen BS, Bross P. Prevalent mutations in fatty acid oxidation disorders: diagnostic considerations. *Eur J Pediatr.* 2000;159 Suppl 3:S213-218.
2. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. *Prog Lipid Res.* 2009;48(5):275-297.
3. Vockley J, Bennett MJ, Gillingham MB. Mitochondrial Fatty Acid Oxidation Disorders. In: Beaudet AL, Vogelstein B, Kinzler KW, et al., eds. *The Online Metabolic and Molecular Bases of Inherited Disease.* New York, NY: The McGraw-Hill Companies, Inc.; 2014.
4. Eaton S, Bartlett K, Pourfarzam M. Mammalian mitochondrial beta-oxidation. *Biochem J.* 1996;320(2):345-357.
5. Rimoin D, Connor J, Pyeritz R, Korf B. *Principles and Practice of Medical Genetics.* 6th ed. Edinburgh: Churchill Livingstone; 2007.
6. IJlst L, Wanders RJ, Ushikubo S, Kamijo T, Hashimoto T. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation in the alpha-subunit of the mitochondrial trifunctional protein. *Biochim Biophys Acta.* 1994;1215(3):347-350.
7. Petra E, Tiina T. LCHAD and MTP deficiencies - Two disorders of mitochondrial fatty acid β-oxidation with unusual features. *Curr Pediatr Rev.* 2007;3(1):53-59.
8. Oey NA, den Boer ME, Wijburg FA, et al. Long-chain fatty acid oxidation during early human development. *Pediatr Res.* 2005;57(6):755-759.
9. Fletcher AL, Pennesi ME, Harding CO, Weleber RG, Gillingham MB. Observations regarding retinopathy in mitochondrial trifunctional protein deficiencies. *Mol Genet Metab.* 2012;106(1):18-24.
10. den Boer ME, Wanders RJ, Morris AA, IJlst L, Heymans HS, Wijburg FA. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Clinical presentation and follow-up of 50 patients. *Pediatrics.* 2002;109(1):99-104.
11. Moorthie S, Cameron L, Sagoo GS, Bonham JR, Burton H. Systematic review and meta-analysis to estimate the birth prevalence of five inherited metabolic diseases. *J Inherit Metab Dis.* 2014;37(6):889-898.
12. Piekutowska-Abramczuk D, Olsen RK, Wierzba J, et al. A comprehensive HADHA c.1528G>C frequency study reveals high prevalence of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency in Poland. *J Inherit Metab Dis.* 2010;33 Suppl 3:S373-377.
13. Nedoszytko B, Siemińska A, Strapagiel D, et al. High prevalence of carriers of variant c.1528G>C of HADHA gene causing long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) in the population of adult Kashubians from North Poland. *PLoS ONE.* 2017;12(11).
14. Garg U, Dasouki M. Expanded newborn screening of inherited metabolic disorders by tandem mass spectrometry: Clinical and laboratory aspects. *Clin Biochem.* 2006;39(4):315-332.

15. Hintz SR, Matern D, Strauss A, et al. Early neonatal diagnosis of long-chain 3-hydroxyacyl coenzyme A dehydrogenase and mitochondrial trifunctional protein deficiencies. *Mol Genet Metab*. 2007;75(2):120-127.

16. Tyni T, Rapola J, Paetau A, Palotie A, Pihko H. Pathology of long-chain 3-hydroxyacyl-Coa dehydrogenase deficiency caused by the G1528C mutation. *Pediatr Pathol Lab Med.* 1997;17(3):427-447.

17. Spiekerkoetter U. Mitochondrial fatty acid oxidation disorders: clinical presentation of long-chain fatty acid oxidation defects before and after newborn screening. *J Inherit Metab Dis.* 2010;33(5):527-532.

18. Olpin SE, Clark S, Andresen BS, et al. Biochemical, clinical and molecular findings in LCHAD and general mitochondrial trifunctional protein deficiency. *J Inherit Metab Dis.* 2005;28(4):533-544.

19. Visweswaran P, Guntupalli J. Rhabdomyolysis. *Crit Care Clin.* 1999;15(2):415-428.

20. Huerta-Alardin AL, Varon J, Marik PE. Bench-to-bedside review: Rhabdomyolysis - an overview for clinicians. *Crit Care.* 2005;9(2):158-169.

21. Efstratiadis G, Voulgaridou A, Nikiforou D, Kyventidis A, Kourkouni E, Vergoulas G. Rhabdomyolysis updated. *Hippokratia.* 2007;11(3):129-137.

22. Lund AM, Skovby F, Vestergaard H, Christensen M, Christensen E. Clinical and biochemical monitoring of patients with fatty acid oxidation disorders. *J Inherit Metab Dis.* 2010;33(5):495-500.

23. Gillingham MB, Connor WE, Matern D, et al. Optimal dietary therapy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Mol Genet Metab.* 2003;79(2):114-123.

24. Bertini E, Dionisi-Vici C, Garavaglia B, et al. Peripheral sensory-motor polyneuropathy, pigmentary retinopathy, and fatal cardiomyopathy in long-chain 3-hydroxy-acyl-CoA dehydrogenase deficiency. *Eur J Pediatr.* 1992;151(2):121-126.

25. Tuuli I, Emilia A, Jussi T, Risto L, Tiina T, Leena L. Peripheral neuropathy in patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency - A follow-up EMG study of 12 patients. *Eur J Paediatr Neurol.* 2016;20(1):38-44.

26. Haglind CB, Stenlid MH, Ask S, et al. Growth in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *JIMD Rep.* 2013;8:81-90.

27. Boese EA, Jain N, Jia Y, et al. Characterization of chorioretinopathy associated with mitochondrial trifunctional protein disorders: Long-term follow-up of 21 cases. *Ophthalmology.* 2016;123(10):2183-2195.

28. Tyni T, Immonen T, Lindahl P, Majander A, Kivelä T. Refined staging for chorioretinopathy in long-chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency. *Ophthalmic Res.* 2012;48(2):75-81.

29. Gillingham MB, Weleber RG, Neuringer M, et al. Effect of optimal dietary therapy upon visual function in children with long-chain 3-hydroxyacyl CoA dehydrogenase and trifunctional protein deficiency. *Mol Genet Metab.* 2005;86(1):124-133.

30. Fahnehjelm KT, Liu Y, Olsson D, et al. Most patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency develop pathological or subnormal retinal function. *Acta Paediatr.* 2016;105(12):1451-1460.

31. Ventura FV, Ruiter JP, IJlst L, de Almeida IT, Wanders RJ. Inhibitory effect of 3-hydroxyacyl-CoAs and other long-chain fatty acid β-oxidation intermediates on mitochondrial oxidative phosphorylation. *J Inherit Metab Dis.* 1996;19(2):161-164.

32. Cecatto C, Godoy KDS, da Silva JC, Amaral AU, Wajner M. Disturbance of mitochondrial functions provoked by the major long-chain 3-hydroxylated fatty acids accumulating in MTP and LCHAD deficiencies in skeletal muscle. *Toxicol In Vitro.* 2016;36:1-9.

33. Tonin AM, Ferreira GC, Grings M, et al. Disturbance of mitochondrial energy homeostasis caused by the metabolites accumulating in LCHAD and MTP deficiencies in rat brain. *Life Sci.* 2010;86(21-22):825-831.

34. Gillingham MB, Purnell JQ, Jordan J, Stadler D, Haqq AM, Harding CO. Effects of higher dietary protein intake on energy balance and metabolic control in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. *Mol Genet Metab.* 2006;90(1):64-69.

35. Spiekerkoetter U, Khuchua Z, Yue Z, Bennett MJ, Strauss AW. General mitochondrial trifunctional protein (TFP) deficiency as a result of either alpha- or beta-subunit mutations exhibits similar phenotypes because mutations in either subunit alter TFP complex expression and subunit turnover. *Pediatr Res.* 2004;55(2):190-196.

36. Strauss O. The retinal pigment epithelium in visual function. *Physiol Rev.* 2005;85(3):845-881.

37. Ryan S, Schachat A, Wilkinson C, Hinton D, Sadda S, Wiedemann P. Cell Biology of the Retinal Pigment Epithelium. In: *Retina.* 5th ed. Elselvier; 2012.

38. Sparrow JR, Hicks D, Hamel CP. The retinal pigment epithelium in health and disease. *Curr Mol Med.* 2010;10(9):802-823.

39. Kevany BM, Palczewski K. Phagocytosis of retinal rod and cone photoreceptors. *Physiology (Bethesda).* 2010;25(1):8-15.

40. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc Natl Acad Sci U S A.* 2004;101(22):8491-8496.

41. Tyni T, Kivela T, Lappi M, Summanen P, Nikoskelainen E, Pihko H. Ophthalmologic findings in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency caused by the G1528C mutation: a new type of hereditary metabolic chorioretinopathy. *Ophthalmology.* 1998;105(5):810-824.

42. Polinati PP, Ilmarinen T, Trokovic R, et al. Patient-specific induced pluripotent stem cell-derived RPE cells: Understanding the pathogenesis of retinopathy in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Invest Ophthalmol Vis Sci.* 2015;56(5):3371-3382.

43. Gillingham MB, Scott B, Elliott D, Harding CO. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-

hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. *Mol Genet Metab.* 2006;89(1–2):58-63.

44. Haglind CB, Nordenström A, Ask S, von Döbeln U, Gustafsson J, Stenlid MH. Increased and early lipolysis in children with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency during fast. *J Inherit Metab Dis.* 2015;38(2):315-322.

45. Gillingham M, Van Calcar S, Ney D, Wolff J, Harding C. Dietary management of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). A case report and survey. *J Inherit Metab Dis.* 1999;22(2):123-131.

46. Wanders RJ, IJlst L, van Gennip AH, et al. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Identification of a new inborn error of mitochondrial fatty acid β-oxidation. *J Inherit Metab Dis.* 1990;13(3):311-314.

47. Moser AB, Jones DS, Raymond GV, Moser HW. Plasma and red blood cell fatty acids in peroxisomal disorders. *Neurochem Res.* 1999;24(2):187-197.

48. Harding CO, Gillingham MB, van Calcar SC, Wolff JA, Verhoeve JN, Mills MD. Docosahexaenoic acid and retinal function in children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis.* 1999;22(3):276-280.

49. Primassin S, Ter Veld F, Mayatepek E, Spiekerkoetter U. Carnitine supplementation induces acylcarnitine production in tissues of very long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine. *Pediatr Res.* 2008;63(6):632-637.

50. Spiekerkoetter U, Bastin J, Gillingham M, Morris A, Wijburg F, Wilcken B. Current issues regarding treatment of mitochondrial fatty acid oxidation disorders. *J Inherit Metab Dis.* 2010;33(5):555-561.

51. Roe CR, Sweetman L, Roe DS, David F, Brunengraber H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. *J Clin Invest.* 2002;110(2):259-269.

52. Vockley J, Burton B, Berry GT, et al. UX007 for the treatment of long chain-fatty acid oxidation disorders: Safety and efficacy in children and adults following 24weeks of treatment. *Mol Genet Metab.* 2017;120(4):370-377.

53. Gillingham MB, Heitner SB, Martin J, et al. Triheptanoin versus trioctanoin for long-chain fatty acid oxidation disorders: a double blinded, randomized controlled trial. *J Inherit Metab Dis.* 2017;40(6):831-843.

54. Lindo G, Nordholm L. Adaptation strategies, well-being, and activities of daily living among people with low vision. *J Vis Impair Blind.* 1999;93(07):434-446.

55. Rainey L, Elsman EB, van Nispen RM, van Leeuwen LM, van Rens GH. Comprehending the impact of low vision on the lives of children and adolescents: a qualitative approach. *Qual Life Res.* 2016;25(10):2633-2643.

56. Mangione CM, Lee PP, Gutierrez PR, et al. Development of the 25-list-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol.* 2001;119(7):1050-1058.

57. Bourne RRA, Jonas JB, Bron AM, et al. Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe in 2015: magnitude, temporal trends and projections. *Br J Ophthalmol.* 2018;102(5):575-585.

58. Pons R, Roig M, Riudor E, et al. The clinical spectrum of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Neurol.* 1996;14(3):236-243.

59. Fahnehjelm KT, Holmström G, Ying L, et al. Ocular characteristics in 10 children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: A cross-sectional study with long-term follow-up. *Acta Ophthalmol.* 2008;86(3):329-337.

60. Lawlor DP, Kalina RE. Pigmentary retinopathy in long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Am J Ophthalmol.* 1997;123(6):846-848.

61. Pesudovs K, Gothwal VK, Wright T, Lamoureux EL. Remediating serious flaws in the National Eye Institute Visual Function Questionnaire. *J Cataract Refract Surg.* 2010;36(5):718-732.

62. Naraine MD, Lindsay PH. Social inclusion of employees who are blind or low vision. *Disabil Soc.* 2011;26(4):389-403.

63. Kef S, Bos H. Is Love Blind? Sexual Behavior and Psychological adjustment of adolescents with blindness. *Sex Disabil.* 2006;24(2):89-100.

64. Lotz-Havla AS, Roschinger W, Schiergens K, et al. Fatal pitfalls in newborn screening for mitochondrial trifunctional protein (MTP)/long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. *Orphanet J Rare Dis.* 2018;13(1):122.

65. Choi HG, Lee MJ, Lee SM. Visual impairment and risk of depression: A longitudinal follow-up study using a national sample cohort. *Sci Rep.* 2018;8(1).

66. Shire. *Rare diseases impact report: Insights from patients and the medical community*. *J Rare Disord*. 2014:1-34.

Appendix 1: Study Survey Questions

	Date of Birth:	
	Gender:	**Male** **Female**
	You were diagnosed because (please check ALL that apply):	**Your newborn screen result was abnormal (heel stick after birth)** **You were hospitalized for low blood sugar episode(s)** **Your enzyme activity was abnormal (skin biopsy -thigh punch)** **A change in your DNA sequence was discovered by genetic analysis (blood draw)** **Enzyme byproducts (acylcarnitines) were detected in your blood** **You had an eye exam with abnormal results**
	You were diagnosed with:	**LCHADD** **TFPD** **It was either LCHADD or TFPD but you are unsure which** **Other diagnosis** **You don't know**
	When were you diagnosed? (If known, please enter the full date):	
	Month of diagnosis	**JanuaryFebruaryMarchAprilMayJune JulyAugustSeptem berOctoberNovemberDecember**
	Day/date of diagnosis	**1234567891011121314151617181920212223242526 2728293031**
	Year of diagnosis	**19851986198719881989199019911992 19931994199 5199619971998199920002001200220032004 2005 2006200720082009201020 11**
	Your current weight:	
	pounds	
	ounces (if applicable):	
	Your current height:	
	feet	
	inches	
	History and treatment of low blood sugar episodes (also called metabolic decompensations)	
	Since being born, you have been hospitalized for low blood sugar episodes or other metabolic complications such as	

	rhabdomyolysis a total of _____ times (please enter a number, even if the answer is 0).	
	In the last year, you have been hospitalized for low blood sugar episodes or other metabolic complications such as rhabdomyolysis _____ times (please enter a number, even if the answer is 0).	
	Since being born, you have had a total of ____ (please enter a number, even if the answer is 0) low blood sugar episodes or other metabolic complications such as rhabdomyolysis that were treated at home.	
	In the last year, you have had ____ (please enter a number, even if the answer is 0) low blood sugar episodes or metabolic complications such as rhabdomyolysis that were treated at home.	
	Please describe how you treat metabolic complications at home.	
	Diet and supplements If you do not know the amount of fat that you eat, or doses of the supplements you take, please give an estimate.	
	Assuming that you calculate the amount of fat you eat daily, do you calculate fat intake in grams or percent calories?	Grams Percent calories
	Total amount of fat (number only):	
	Do you take medium chain triglyceride supplements?	yes no
Only seen if "mct" = yes	Do you calculate MCT intake in milliliters or tablespoons?	Milliliters Tablespoons
Only seen if "mct" = yes	How much MCT do you take per day (number)?	
	Do you take carnitine supplements?	yes no
Only seen if "carnitine" = yes	How much carnitine do you take per day (number of milligrams)?	
	Do you take docosahexanoic acid (DHA) supplements?	yes no
Only seen if "dha" = yes	How much DHA do you take per day (number of milligrams)?	
	What is the maximum amount of time that you go without eating or drinking something with calories (number of hours)?	

Medical treatment history		
	Do you see a genetic metabolism doctor?	yes no
Only seen if "geneticmetabdoc" = yes	How often do you see a genetic metabolism doctor?	less than every other year every other year once per year twice per year more than twice per year
Only seen if "geneticmetabdoc" = yes	The last time you saw a genetic metabolism doctor, they said your test results were:	normal mildly abnormal very abnormal
	Do you see an eye doctor?	yes no
Only seen if "eyedoc" = yes	How often do you see an eye doctor?	less than every other year every other year once per year twice per year more than twice per year
Only seen if "eyedoc" = yes	The last time you saw an eye doctor, they said your test results were:	normal mildly abnormal very abnormal
Only seen if "eyedoc_test" = mildly abnormal or very abnormal	At what age (in years) did your eye doctor first indicate that your eye exam was abnormal?	
	Do you have any trouble seeing in the dark?	yes no
Only seen if "nightvisionloss" = yes	At what age (in years) did you first notice trouble seeing in the dark?	
	Do you have any trouble seeing in daylight?	yes no
Only seen if "dayvisionloss" = yes	At what age (in years) did you first notice trouble seeing in daylight?	
	Do you see a dietitian?	yes no
Only seen if "dietician" = yes	How often do you see a dietitian?	less than every other year every other year once per year twice per year more than twice per year
Only seen if "dietician"	Does your dietitian instruct you to calculate fat intake in grams or percent calories?	Grams Percent calories

= yes		
Only seen if "dietician" = yes	How much fat does your dietitian recommend that you eat daily (number only)?	
Only seen if "dietician" = yes	What else does your dietitian recommend that you do to help prevent complications?	
	Has any doctor mentioned that you have any loss of reflexes in your legs?	yes no I don't know
	Do you have a "foot drop" or any difficulty walking (not related to an injury)?	yes no I don't know I am confined to a wheelchair
Only seen if "diffwalk"= yes or wheelchair	At what age did you first notice trouble with walking?	
Only seen if "diffwalk"= yes	Do you use a cane or walker to assist with walking?	yes no
Only seen if "diffwalk" = wheelchair	At what age were you no longer able to walk?	
	Was your DNA tested to determine the changes* in the genes that code for trifunctional protein? *For examples, please refer to the article "What causes retinopathy in long-chain 3-hydroxyacylCoA dehydrogenase deficiency?" on page 12 of the January 2011 FODSupport newsletter at http://www.fodsupport.org/documents/Jan2011issue.pdf	yes no I don't know
Only seen if "test" = yes	Do you know if a change in your DNA was identified?	yes no
Only seen if "test" = yes AND "change" = yes	Did your doctor tell you the specific change(s) that were found in your DNA?	yes, and I can tell you at least one of the (two) changes yes, and the doctor said something about "the common" change (or mutation) but that's all I know. yes, but I don't remember what the changes were or

		have a copy of the report no, the doctor never told me
Only seen if "test" = yes AND "change" = yes AND "specific_change" = Icantellyou	What is (are) the specific change(s)* in your DNA that were found? *For examples, please refer to the article "What causes retinopathy in long-chain 3-hydroxyacylCoA dehydrogenase deficiency?" on page 12 of the January 2011 FODSupport newsletter at http://www.fodsupport.org/documents/Jan2011issue.pdf	
	Were any of your family members tested for changes* in the genes that code for trifunctional protein? *For examples, please refer to the article "What causes retinopathy in long-chain 3-hydroxyacylCoA dehydrogenase deficiency?" on page 12 in the January 2011 FODSupport newsletter at http://www.fodsupport.org/documents/Jan2011issue.pdf	yes no I don't know
Only seen if "fam_test" = yes	Which family members were tested for changes in the DNA? If changes were found, please identify them if possible.	
	How many siblings do you have total?	
	Do you have any siblings that suffer from LCHADD or TFPD?	yes no
Only seen if "sib_TFPLCHADD" = yes	How many siblings do you have that suffer from LCHADD or TFPD?	
The remaining questions have been adapted from the National Eye Institute Visual Function Questionnaire, version 2000. © R 1996 **General health and eyesight**		
	How would you rate your overall health, on a scale where zero is as bad as death and 100 is the best possible health?	0-Worst 50 100-Best
	How would you rate your eyesight now (with glasses or contact lenses on, if you wear them), on a scale of from 0 to 100, where zero means completely blind, and 100 means the best possible eyesight?	0-Worst 50 100-Best
The next questions are about how much difficulty, if any, you have doing certain activities wearing your glasses or contact lenses if you use them for that activity.		
	How much difficulty do you have reading ordinary print in newspapers?	No difficulty at all A little difficulty Moderate difficulty

		Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	How much difficulty do you have doing work or hobbies that require you to see well up close, such as cooking, sewing, fixing things around the house, or using hand tools? (Please also consider things like making jewelry, or other crafts in which you may participate)	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have finding something on a crowded shelf?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	How much difficulty do you have reading street signs or the names of stores?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have going down steps, stairs, or curbs in dim light or at night?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have noticing objects off to the side while you are walking along?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have seeing how people react to things you say?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your

		eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have picking out and matching your own clothes?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have visiting with people in their homes, at parties, or in restaurants?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Because of your eyesight, how much difficulty do you have going out to see movies, plays, or sports events?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	Wearing glasses, how much difficulty do you have reading the small print in a book, on a medicine bottle, or on legal forms?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	How much difficulty do you have seeing and enjoying programs on TV?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	How much difficulty do you have recognizing people you know from	No difficulty at all A little difficulty

	across a room?	Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
	How much difficulty do you have taking part in active sports or other outdoor activities that you enjoy because of your vision (like golf, bowling, jogging, or walking)?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
The next questions are about any difficulty you may have with driving, if you drive.		
"drive"	Are you currently driving, at least once in a while?	yes no
"whynodrive" Only seen if "drive" = no	Have you never driven a car or have you given up driving?	Never drove (or too young to drive) Gave up driving
Only seen if "drive" = no AND "whynodrive" = gave up driving	Did you give up driving because of your eyesight, mainly for some other reason, or because of both your eyesight and other reasons?	Mainly eyesight Mainly other reasons Both eyesight and other reasons
Only seen if "drive" = yes	How much difficulty do you have driving during the daytime in familiar places?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
Only seen if "drive" = yes	How much difficulty do you have driving at night?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or not interested in doing this
Only seen if "drive" = yes	How much difficulty do you have driving in difficult conditions, such as in bad weather, during rush hour, on the freeway, or in city traffic?	No difficulty at all A little difficulty Moderate difficulty Extreme difficulty Stopped doing this because of your eyesight Stopped doing this for other reasons or

		not interested in doing this
The next questions are about how things you do may be affected by your vision.		
	Do you accomplish less than you would like because of your vision?	1 - All of the time 50 100 - none of the time
	Are you limited in how long you can work or do other activities because of your vision?	1 - All of the time 50 100 - none of the time
	I stay home most of the time because of my eyesight.	1- Definitely true 50 100 - Definitely false
	I feel frustrated a lot of the time because of my eyesight.	1- Definitely true 50 100 - Definitely false
	I have much less control over what I do, because of my eyesight.	1- Definitely true 50 100 - Definitely false
	Because of my eyesight, I have to rely too much on what other people tell me	1- Definitely true 50 100 - Definitely false
	I need a lot of help from others because of my eyesight.	1- Definitely true 50 100 - Definitely false
	I worry about doing things that will embarrass others or myself because of my eyesight.	1- Definitely true 50 100 - Definitely false
Thank you for completing the questionnaire portion of this study.		
	Are you willing to release your medical records to study personnel for review?	yes no