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Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency in Finland

– earlier diagnosis and strict diets improve the survival rate and clinical course

Tuuli Immonen

ACADEMIC DISSERTATION

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Contents

1. TIIVISTELMÄ

Pitkäketjuisten rasvahappojen 3-hydroksiasyyli-CoA-dehydrogenaasin puutos (LCHADD) on vakava pitkäketjuisten rasvahappojen aineenvaihdunnan sairaus, joka yleensä johtaa kuolemaan ilman hoitoa. Suomessa LCHADD on tavallisin pitkäketjuisten rasvahappojen beetaoksidaatiohäiriö. LCHADD ilmenee usein ensimmäisen ikävuoden aikana äkillisinä aineenvaihduntakriiseinä, hypoketoottinen hypoglykemiana, hepatopatiana, kardiomyopatiana, ja kasvuhäiriönä. Pitkäaikaisongelmina ovat retino- ja neuropatia. Varhain aloitettu niukkarasvainen ja runsashiilihydraattinen ruokavalio ja paaston välttäminen lievittävät oleellisesti taudin vaikeaa luonnollista kulkua. Sairauden ennuste on parantunut viime vuosikymmeninä merkittävästi parantuneen ruokavaliohoidon ja vastasyntyneiden seulonnan ansiosta. Monet maat ovatkin liittäneet LCHADD- sairauden seulonnan osaksi vastasyntyneiden seulontaa. Tutkimustietoa potilaiden taudinkulkuun vaikuttavista tekijöistä, kuten ruokavaliohoidon koostumuksesta ja pitkäaikaisongelmien, retino-ja neuropatian esiintyvyydestä on vähän.

Tämän väitöskirjatutkimuksen tarkoituksena oli arvioida Suomessa hoidettujen LCHADD-potilaiden taudin kliinistä kulkua, ennustetta ja kehittää LCHADD-potilaiden seurantaa ja hoitoa.

Tutkimus käsitti 47 Suomessa vuosina 1976–2014 diagnosoitua potilasta, joiden diagnoosi oli geneettisesti varmennettu. Kaikilla potilailla oli LCHADD- taudin homotsygootti mutaatio c.1528G>C (E510Q). Tutkimuksessa vuosina 1997–2010 (Study II) syntyneiden LCHADD potilaiden taudin kliinistä kuvaa ja ennusteta verrattiin aiempaan suomalaiseen tutkimukseen, joka käsitti vuosina 1976–1996 syntyneet LCHADD-potilaat. Vuosina 1976–1996 (N=28) diagnosoitujen potilaiden kymmenen vuoden eloonjäämisennuste oli 14.3 %. 1997–2010 syntyneistä LCHADD-potilaista (N=16) 62.5 % oli elossa tutkimuksen päättyessä. 1997–2010 syntyneiden potilaiden oireet alkoivat 0-5 kuukauden iässä. Tavallisimmat ensioireet olivat hypoketoottinen hypoglykemia, kasvuhäiriö, hepatopatia, metabolinen asidoosi ja kardiomyopatia. Ruokavaliohoito aloitettiin 0-30 päivää diagnoosin jälkeen. Ensimmäisten ikävuosien aikana riittävä ravitsemus turvattiin yöaikaisella infuusiolla nenämahaletkuun tai gastrostoomaan. Valtaosa eloonjääneistä potilaista voi hyvin ja heidän älykkyysosamääränsä oli yhtä potilasta lukuunottamatta normaali.

Polyneuropatian kehittymistä tutkittiin elektroneurofysiologisilla menetelmillä Suomessa 1976–2014 diagnosoiduilla potilailla (Study III). Kahdelletoista potilaalle tehtiin elektroneuromyografia (ENMG) 1-12 kertaa. Polyneuropatia todettiin 6-12 vuoden iässä. Ensimmäinen poikkeava löydös oli nervus suraliksen sensorisien amplitudien lasku. Seurannassa osalla potilaista polyneuropatia progredioi yläraajoihin. Ruokavaliohoidosta huolimatta osalle nuoremmista potilaista (6/10) kehittyi polyneuropatia, mutta taudinkuva oli aiempaa raportoitua lievempi.

LCHADD-potilaiden silmämuutosten seurannan kehittämiseksi luotiin tarkennettu silmänpohjamuutosten luokittelujärjestelmä (Study I). Tutkimuksessa luokiteltiin seitsemän potilaan silmänpohjakuvat, jotka edustivat luokan 2 retinopatiaa. Aiemman luokituksen mukaisesti jaettiin pigmentti muutokset (P1-P3) ja retinan pigmenttiepiteeli atrofia (RPE) (A1-A3) kolmeen luokkaan. Kolmen arvioitsijan (silmälääkärin) arviot olivat melko yhdenmukaiset pigmentti muutoksien luokasta (yhdistetty K-arvo 0.38), sen sijaan RPE atrofian luokituksesta arviot eivät olleet yhdenmukaiset (yhdistetty K-arvo 0.018). Tutkimuksen rajoituksena oli kuvausmenetelmien vaihtelevuus. Tästä huolimatta silmänpohjan pigmenttimuutosten arvioitsijoiden yhdenmukaisuus vastasi aiemmin raportoitua keskosina syntyneiden retinopatian luokituksen yhdenmukaisuutta. Tämän tutkimuksen mukaan LCHADD-potilaiden silmänpohjamuutosten seuranta silmänpohjakuvauksin ja retinan pigmenttiepiteelimuutosten luokittelu ja vertailu referenssivalokuviin on suositeltavaa LCHADD-potilaiden seurannassa.

Vuosina 2000–2014 11 ruokavaliohoidolla hoidettua LCHADD-potilasta seurattiin kontrollikäyntien yhteydessä (Study IV). Välttämättömien rasvahappojen saanti oli riittävää. Pitkäketjuisten rasvahappojen saanti oli 5-9 energiaprosenttia, mikä vastaa LCHADD-potilaiden ruokavaliosuosituksia. Ruokavaliohoito ei ole kuitenkaan optimaalinen, koska toksiset aineenvaihduntatuotteet, asyylikarnitiinit, olivat lievästi kohonneet kaikilla potilailla hyvästä hoitomyöntyvyydestä huolimatta. Tämä tutkimus osoittaa, että LCHADD-potilaiden rasvahappojen, erityisesti välttämättömien, saantia on syytä monitoroida tarkasti, rekisteröimällä ruokapäiväkirjoja ja mittaamalla rasvahappo profiileita, jotta ruokavalion pitkäketjuisten rasvahappojen määrä pysyisi niin matalana kuin mahdollista.

Yhteenvetona todetaan, että LCHADD-taudin ennuste on parantunut. Vastasyntyneiden seulonnan ansioista ennuste paranee todennäköisesti edelleen. Potilaat tarvitsevat edelleen ruokavaliohoidon toteutumisen tiivistä seurantaa taudin pitkäaikaisongelmien, retino-ja neuropatian sekä taudin huononemisvaiheiden ennaltaehkäisemiseksi.

2. ABSTRACT

Long-chain acyl-CoA dehydrogenase deficiency (LCHADD), a severe long-chain β-oxidation disorder which, without treatment, usually leads to death, is the most frequent β-oxidation disorder in Finland. Its typical manifestations are hypoketotic hypoglycemia, hepatopathy, failure to thrive, cardiomyopathy, and metabolic crisis during the first year of life. The long-term complications are retinopathy and polyneuropathy. In recent years, diagnostics and treatment of LCHADD have produced major advantages. Many countries have implemented LCHADD in their newborn screening programs. Treatment with a low-fat, high-carbohydrate diet with avoidance of fasting is effective. Few follow-up studies, ones on the outcome of LCHADD and whether the current dietary treatment prevents the long term complications retinopathy and peripheral neuropathy, exist.

The aims of the study were to evaluate the clinical course and outcome of LCHADD patients in Finland who have the homozygous c.1528G>C (E510Q) mutation, with strict dietary treatment and develop further their treatment and follow-up strategies.

A total of 47 patients with LCHADD caused by a homozygous c.1528G>C mutation were diagnosed in Finland from 1976 through 2014. In our study, the outcome and course of the disease of the LCHADD patients born between 1997 and 2010 (Study II), were compared with an earlier Finnish study of LCHADD patients born 1976 to 1996. In 1976-1996 (N=28) the ten-year survival rate was 14.3%, while in 1997-2010 (N=16) at the end of the study 62.5% were alive. Patients born between 1997 and 2010 presented at the age of 0 to 5 months with hypoketotic hypoglycemia, failure to thrive, hypotonia, hepatomegaly, metabolic acidosis, and cardiomyopathy. The therapy was started 0 to 30 days after diagnosis. Gastrostoma prove beneficial during infancy, ensuring continuous night-time feeding. Most long-term survivors were in good overall condition. All except one, who had metabolic crises leading to resuscitation before diagnosis, had a normal intelligence quotient (IQ).

We studied the development of polyneuropathy (PNP) by clinical neurophysiological methods in the LCHADD patients diagnosed between1965 and 2014 (Study III). Electroneurography (ENG) was performed 1 to 12 times for 12 patients. The first abnormality was reduction in the sensory amplitudes of the sural nerves. During follow-up, progression extended to the upper limbs. Despite good compliance with the diet, of the 10 younger patients, 6 developed polyneuropathy but in a milder form than reported earlier. Their polyneuropathy had been detected at the ages of 6-12 years.

To improve the ophthalmological follow-up, we rated the fundus images from seven children in stage 2 retinopathy to create a grading system to monitor retinopathy development (Study I). According to this rating the original staging was divided into three substages of pigmentary deposits (P1-P3) and retinal pigment epithelial (RPE) atrophy (A1-A3). Three ophthalmologists expressed moderate agreement in the assessment of pigmentary deposits

(combined weighted K statistic, 0.38), whereas the assessment of RPE atrophy showed poor agreement (combined K statistic 0.018). The visual assessment of fundus photographs based on reference images showed agreement identical to that reported for grading of retinopathy of prematurity, so fundus photography is the suggestion for ophthalmological follow-up of LCHADD retinopathy.

We followed up 11 LCHADD patients treated with the current dietary regimen in Helsinki University Central Hospital between 2000-2014 during routine visits (Study IV). Their intake of essential fatty acids (EFAs) was within normal limits. The amount of long-chain triglyceride (LCT) was 5 to 9 percent of total energy intake (E%), consistent with dietary recommendations of LCHADD. We detected, surprisingly, that the patients received one-third of their linoleic acid (LA) and α-linolenic (ALA) from their diet and two-thirds from the supplements. Acylcarnitine levels remain elevated, despite good compliance with the diet, which indicated that the diet was not optimal. This demonstrates that in order to keep LCTs as low as possible, we should monitor EFA intake carefully by dietary regimen and by measuring fatty acid profiles.

To conclude, the outcome of LCHADD has improved, but the patients still need careful monitoring for dietary compliance in order to prevent retinopathy and neuropathy and especially during infections to prevent hypoglycemia and metabolic decompensation. Future challenges are to adjust the therapy to be lifelong and to find new treatment strategies.

3. LIST OF ORIGINAL PUBLICATIONS:

I. Tyni T, Immonen T, Lindahl P, Majander A, Kivelä T. Refined staging for chorionretinopathy in long-chain 3-hydroxyacyl Coenzyme A dehydrogenase deficiency. Ophthalmic Res 2012; 48: 75-81.

II. Immonen T, Turanlahti M, Paganus A, Keskinen P, Tyni T, Lapatto R. Earlier diagnosis and strict diets improve the survival and clinical course of longchain 3-hydroxyacyl-CoA dehydrogenase deficiency. Acta Paediatr 2015 Dec 16 (Epub ahead of print)

III. Immonen T, Ahola E, Toppila J, Lapatto R, Tyni T, Lauronen L. Peripheral neuropathy in patients with longchain 3-hydroxyacyl-CoA dehydrogenase deficiency-A follow-up EMG study of 12 patients. Eur J Paediatr Neurol 2016 Jan; 20(1): 38-44.

IV. Immonen T, Tuokkola J, Lapatto R. Essential fatty acids in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency care.

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4. ABBREVATIONS

AA arachidonic acid
AFLP acute fatty liver of pregnancy
ALA α-linolenic acid
ALT serum alanine aminotransferase
CACT carnitine acylcarnitine translocase
CHO carbohydrate
CK, serum creatine kinase
CPT1, carnitine palmitoyl-coenzyme A transferase 1
CPT2, carnitine palmitoyl-coenzyme A transferase 2
DCM dilatating cardiomyopathy
DHA docosahexanoic acid
EFA essential fatty acids
ER emergency room
FADH flavin adenine dinucleotide
FAO mitochondrial fatty acid β-oxidation
FFA serum free fatty acids
%E percent of total energy intake
ECG electrocardiogram
ECHO echocardiogram
ENG electroneurography
ERG electroretinography
HELLP syndrome of hemolysis, elevated liver enzymes, and low platelets counts
HCM hyperthopic cardiomyopathy
IQ intelligence quotient
LA linoleic acid
LCHAD long-chain 3-hydroxyacyl-CoA dehydrogenase
LCHADD long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency
LCT long-chain triglyceride
LHYD long-chain 2,3-enoyl-CoA
LKAT long-chain 3 ketoacyl-CoA thiolase
LCPUFA long-chain polysaturated fatty acids
MCT medium-chain triglyceride
MCAD medium-chain acyl-coenzyme A dehydrogenase
MTP Mitochondrial trifunctional protein
NADH Nicotinamide adenine dinucleotide
NBS newborn screening
PUFA polysaturated fatty acid
OCTN2 organic cation transporter 2
PNP polyneuropathy
RPE retinal pigment epithelium
TFP trifunctional protein

TMS tandem mass spectrometry
VEP visual evoked potentials
VLCAD very long-chain acylcoenzyme A dehydrogenase

5. INTRODUCTION

Awareness of treatable inborn errors of metabolism has over recent decades greatly improved. These disorders can present at birth, in the neonatal period, or after a symptom- free period with acute symptoms, or even as sudden death. Immediate diagnosis and treatment of these disorders enhances the prognosis and may even prevent serious complications including neurological manifestations or fatal outcome. Therefore many countries have implemented newborn screening for inborn errors of metabolism.

As a group, mitochondrial fatty acid β-oxidation (FAO) defects are among the commonest inborn errors of metabolism. The course of FAO disorders is severe because they provide most of the energy during fasting and exercise for the heart, skeletal muscles, kidneys, liver, and even the brain, when carbohydrate sources become depleted. Breakdown of this main metabolic pathway in FAO disorders endangers the energy supply of these tissues (Wanders et al. 1999, Olpin 2005). These disorders can manifest with hypoketotic hypoglycemia, failure to thrive, hepatopathy, cardiomyopathy, and even sudden death in infancy (Wanders 1989).

Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCAHDD) is the most frequent mitochondrial fatty acid β-oxidation defect in Finland, while medium-chain acyl-CoA dehydrogenase deficiency (MCAD) is most frequent worldwide (Pastinen et al 2001).

LCHADD usually presents during the first year of life with hypoketotic hypoglycemia, failure to thrive, hepatopathy, cardiomyopathy, and often with acute metabolic crisis. The long-term-complications are retinopathy and peripheral neuropathy. The etiology of these long-term complications of LCHADD is unknown. Debated as the possible etiology are the accumulation of toxic metabolites such as long-chain hydrocylated acylcarnitines and long chain fatty acids, and a deficiency of essential fatty acids.

Prompt diagnosis, as well as treatment with a low-fat, high-carbohydrate diet proves effective and improves clinical outcome of LCHADD. The intention of this diet is to bypass the metabolic block by providing medium-chain fatty acids instead of long-chain fatty acids. The frequent high carbohydrate meals ensure a constant energy supply and satisfy the need for essential fatty acids and fatsoluble vitamins (Gillingham et al 2003, Spiekerkoetter et al 2009). Despite the diet, especially during infections, the patients still suffer serious metabolic decompensations. Few data exist on the long-term outcome or on complications: retinopathy (Tyni et al 1997, Gillingham et al 2005, Fahnehjelm et al 2008) and polyneuropathy (Poll et al 1988, Bertini et al 1991).

This unique study with relative large patient's series, where all the patients have been treated and followed-up in the same way, aims to show how the current treatment procedures of LCHADD influence the outcome and long-term complications: retinopathy and polyneuropathy, and how we can create new follow-up strategies.

6. REVIEW OF LITERATURE

6.1 Fatty acid oxidation

Fat, the significant reservoir of energy in the body, is stored in adipose tissue as triglycerides. Especially during fasting and prolonged exercise, when carbohydrate stores become short, they are released and transported to other tissues for oxidation, as lipoproteins or bind to albumin, to produce energy. Most of the fatty acids are oxidized through β-oxidation in mitochondria in all cells except mature erythrocytes.

Mitochondrial β-oxidation consists of several repeated enzyme cycles, where long-chain fatty acids flow through β-oxidation in the inner membrane of mitochondria, producing acyl-CoA.

The mitochondrial β-oxidation process requires long-chain fatty acids to be moved through the cell membrane by the organic cation transporter 2 (OCTN2), and activated to CoA esters by acyl-CoA synthase and further move through mitochondrial membranes via the carnitine cycle: carnitine palmitoyltranferase I (CPT1) and II (CPT2), carnitine acylcarnitine translocase (CATR). The next step of β-oxidation includes four steps of the reaction, which are repeated until the acyl-CoA is degraded to 2-carbon length. One β-oxidation cycle shortens the acyl-CoA by two carbon atoms. The first enzyme activity is very long-chain acyl-CoA dehydrogenase (VLCAD) producing 2,3-enoylCoA. The second enzyme activity is long-chain 2,3-enoyl-CoA (LHYD) which hydrate the 2,3-enoyl-CoA, leading to L-3-hydroxyacyl-CoA. The third enzyme activity is long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) producing 3-ketoacyl-CoA. The last step includes thiolytic cleavage of 3-ketoacyl-CoA, producing acetyl-CoA and its acyl-CoA esters, is catalyzed by long-chain 3 ketoacyl-CoA thiolase (LKAT). The FADH and NADH are produced by the first and third step of β-oxidation and are transferred to the respiratory chain producing ATP.

The end product of β-oxidation, acetyl-CoA, serves as the source of energy for the citricacid cycle (skeletal, cardiac muscle), or is converted to ketone bodies, acetoacetate, and β-hydroxybutyrate in the liver. Ketone bodies serve as fuel during exercise and fasting for the muscles, kidneys, hearts, liver, and even the brain.

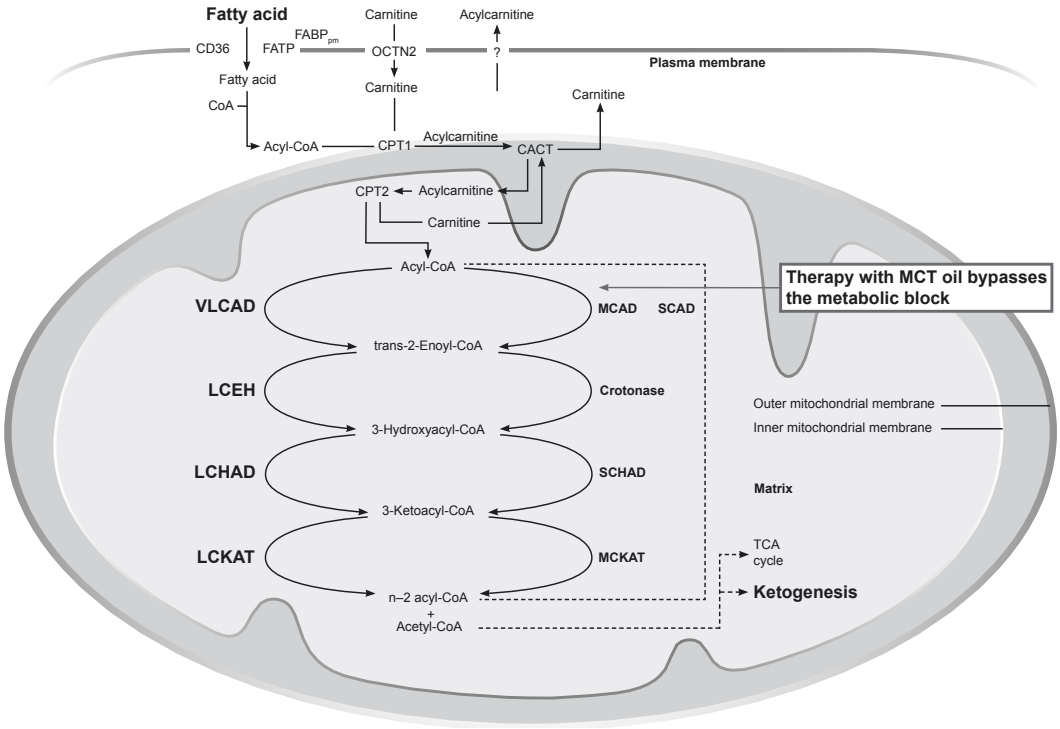

Figure 1. Mitochondrial long-chain fatty acid β-oxidation. See text for more details.
(Diagram by Miia Kerimaa)

6.2 Mitochondrial β-oxidation disorders

Mitochondrial β-oxidation disorders are inherited metabolic disorders which manifest usually during early infancy or childhood. They are inherited autosomally recessively. These disorders markedly impair supply of energy of many tissues: brain, heart, kidney, liver, and muscle and typically manifest with hypoketotic hypoglycemia after fasting, hepatopathy, rhabdomyolyses, and cardiomyopathy or even sudden death. However, some patients show chronic symptoms, hypotonia, failure to thrive, and feeding difficulties. Prompt diagnosis and therapy is therefore critical.

These disorders became known in the 1980s as medium-chain acyl-CoA dehydrogenase deficiency (MCAD) (Wanders et al. 1999), and ever since then, multiple enzymes participating either in β-oxidation or in carnitine-dependent transport to mitochondria have emerged (Table 1).

Table 1. The most common β-oxidation disorders

protein	blood acylcarnitine profile	cardiomyopathy	hepatopathy	myopathy
OTCN2	N	yes	yes	yes
CPT1	N	no	yes	no
CACT	↑C18,C16	yes		
CPTII	↑C16,C18	yes	yes	yes
VLCAD	↑C14:1-C16	yes	yes	yes
TFP	C18(OH) C18:1(OH)	yes	yes	yes
LCHAD	C16(OH) C18(OH) C18:1(OH)	yes	yes	yes
MCAD	↑C8,C8/C10	no	yes	yes/no
SCAD	↑C4	?	no	yes

The long-chain fatty acid oxidation disorders include 1. Defects of the carnitine cycle, causing impaired entry into mitochondria of long-chain fatty acids: carnitine palmitoyl transferases I and II (CPT I, II) defects, and carnitine acylcarnitine translocase (CACT) defect. 2. Defect of carnitine uptake through the cell membrane: organic cation carnitine transporter (OTCN2) defect. 3. Defects of longchain fatty acid oxidation (VLCAD, TFP, LCHAD, LKAT, and ACDD9 deficiencies) and multiple acyl-CoA dehydrogenase deficiency (MAD) (Das et al 2006, Spiekerkoetter et al 2009) (Figure 1). This review is focused on the isolated long-chain fatty acid β-oxidation defect LCHADD.

6.2.1 Incidence

The most frequent β-oxidation defect in Finland, with a carrier frequency of 1:132 to 1:365, is long-chain acyl-CoA dehydrogenase deficiency (LCHADD) (Pastinen et al 2001). Boer et al (2000) reported a carrier frequency of 1:680 in the Netherlands, and Joost et al (2012) of 1:173 in Estonia, where the estimated prevalence of LCHADD was 1:91 700 (den Boer et al 2000, Joost et al 2012). Moorthie et al (2014) estimated the birth prevalence of LCHADD for western populations 0.41/100 000 (based on clinical diagnosis) and 0.65/100 000 (NBS). The most frequent β-oxidation disorder worldwide is MCAD, with a carrier frequency of 1:58-86; an incidence of 1:13 000-30 000 (Gregersen et al 2001). Recent NBS has revealed an in-creased incidence (Rhead et al 2006). Arnold et al (2009) reported a prevalence of 1:31 500 for the most frequent long-chain disorder, very long-chain acyl-CoA deficiency (VLCADD).

6.2.2 Presentation

LCHADD was first described by Wanders and colleagues (1989, 1990). The patients usually present during the first year of life with hypoketotic hypoglycemia, hepatopathy, cardiomyopathy, failure to thrive, rhabdomyolyses, and metabolic crises even leading to

death. All patients become symptomatic over time. (Tyni et al 1996, den Boer et al 2002, Wilcken 2010, Baruteau et al 2013). Trifunctional protein (TFP) deficiency presents with cardiomyopathy, liver disease, and early mortality despite treatment (Wanders et al 1992, Boer et al 2003), a milder form is neuromyopathic phenotype (Spiekerkoetter et al 2004). In another long-chain defect, very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), three forms of disease are identifiable: neonatal onset, cardiomyopathy with high mortality, a milder form with late onset with hypoketotic hypoglycemia, hepatopathy, and a lateonset myopathic form with rhabdomyolysis. (Bertrand et al 1993, Smelt et al 1998, Spiekerkoetter et al 2003, Laforet et al 2009). Asymptomatic milder genotypes have emerged since initiation of NBS (Touma et al 2001). MCAD usually manifests as hypoketotic hypoglycemia, although some patients remain asymptomatic. After the start of therapy, mortality is low. (Iafolla et al 1994, Wanders et al 1999, Olpin 2005, Andresen et al 2001, Grosse et al 2006, Spiekerkoetter 2010, Wilcken et al 1994, 2007, 2010).

In long-chain disorders, different phenotypes occur, but with no definitive genotype phenotype correlation recognized (Spiekerkoetter et al 2003, 2010). While a common mutation (c.1528G>C) in LCHADD induces clinical manifestations of variable severity (Ijlst et al 1996, Gregersen 2001), other factors, such as environmental ones are presumably involved. In MCAD and VLCADD, after implementation of newborn screening, milder phenotypes have emerged, and a correlation between milder phenotypes and in some cases residual enzyme activities has been detectable (Spiekerkoetter et al 2003, Liebig et al 2006, ter Veldt et al 2009).

Pregnancies and LCHADD

The pregnancy complications of carriers of LCHADD occur frequently with a LCHADD-affected fetus, ones such as pre-eclampsia, the syndrome of hemolysis, elevated liver enzymes, and low platelets counts (the HELLP syndrome), pregnancy-induced hypertension, and acute fatty liver of pregnancy (AFLP), as well as prematurity, asphyxia, and low birth weight for gestational age (Wilcken et al 1993, Tyni et al 1997, Strauss et al 1999, Gutierrez Junguera et al 2009, Karall et al 2015). This has not been detectable in other long-chain β-oxidation defects. Ibdah and colleagues (2000) reported through prospective analysis of LCHADD-affected pregnancies, AFLP in 15-20%, and in 2%, the HELLP syndrome. The intermediates from abnormal β-oxidation of fatty acids may possibly cause pregnancy complications. (Wilcken et al 1993). Eskelin et al reported in 2010 elevated hydroxycarnitine levels in an AFLP-complicated pregnancy of a LCHADD carrier with a fetus having LCHADD.

6.2.3 Molecular genetics and pathophysiology of LCHADD

The mitochondrial trifunctional protein catalyses the last three steps in β-oxidation of long- chain fatty acids. This complex consists of an α-subunit, which shows long-chain enoyl-CoA hydratase and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activities, and the β-subunit, which shows long-chain thiolase activity. Disorders of this complex are two: first, an isolated LCHAD deficiency (OMIM 143450) caused by a common

c.1528G>C (E510Q) mutation resulting in a guanine-to-cytosine change and thus reduced LCHAD enzyme activity, and second, TFP deficiency (OMIM 6008890) with reduced protein expression and combined enzyme deficiency.

The symptoms of LCHADD, as in most long-chain defects, result from 1. Shortage of end-products, causing dysfunction of the heart, kidneys, and brain, all of which are dependent on ketone bodies as fuel. This contrasts with MCAD, where degraded medium-chain fatty-acid oxidation still supplies adequate energy for the heart and muscles. 2. Accumulation of intermediates proximal to the metabolic block. This accumulation of intermediates are transferred out of the mitochondria bound to carnitine as acylcarnitines, and thereby the free carnitine is reduced in plasma, and elevated acylcarnitine levels occur in plasma and urine. The abundant fatty acids are also converted to triacylglycerols, leading, in organs, to accumulation of fats. As a result of incomplete oxidation of fatty acids, the dicarboxylacids become detectable in urine.

6.2.4 Diagnosis

The fatty acid disorders should be suspected in any patient presenting with hypoketotic hypoglycemia, acute metabolic derangement, and manifestations of tissues dependent from β-oxidation as fuel (cardiac, skeletal muscle, hepatic). Typical laboratory findings for LCHADD are hypoketotic hypoglycemia, elevated transaminases, and acidosis, and elevated creatine kinase, and also elevated hydroxycompounds of C14-OH, C16-OH, C18-OH, C18: 1-OH, in acylcarnitine profiles, and C6-14 hydroxydicaeboxylic acids in urine.

The clinical diagnosis can be confirmed by either measuring the enzyme activities of TFP in fibroblasts or by blood acylcarnitine analysis allowing by chain length identification of different enzyme defiencies and confirmation of this diagnosis by mutation analysis. The most common mutation for isolated LCHADD worldwide, and the only mutation identified in Finland, is the homozygous mutation1528G>C (E510Q), which is located in the α-subunit of the trifunctional protein. (IJlst et al 1994, 1996 Tyni et al 1997, den Boer 2002, Spiekerkoetter et al 2009, Joost et al 2012, Karall et al 2015)

Newborn screening

Many countries have included tandem mass spectrometry (TMS) in their routine newborn screening program, which enables early detection and treatment of fatty acid oxidation disorders (FAO). During the first days of life, blood samples are collected by a heelprick puncture for bloodspot samples. To detect any abnormal levels of acylcarnitines, the signals of acylcarnitines are compared with the known standards D3C14, D3C16, and D3C18. In Finland, the newborn screening implemented in 2015 included screening for LCHADD.

6.2.5 Treatment

The aim is to maintain the energy supply to the tissues by frequent meals and avoidance of fasting. The basis of LCHADD treatment is twofold: to bypass the metabolic block by, instead of long-chain fatty acids, providing medium-chain fatty acids (Figure 1), thereby preventing accumulation of toxic metabolites proximal to the defect, and avoiding mobilization of endogenous fat by supplying sufficient energy with frequent highcarbohydrate meals.

Dietary therapy in LCHADD

Treatment consists of a low-fat, high-carbohydrate diet, approx. 5 percent of total energy intake (E%) coming from long-chain triglycerides (LCT), plus medium-chain triglyceride supplementation (MCT) (15-20% of the total energy intake) (Spiekerkoetter et al 2009, 2010). Because patients with LCT-restricted diets are at risk for EFA deficiency, additional supplementation of essential fatty acids (EFA) is therefore mandatory, usually walnut oil and omega-3 oil, including docosahexanoic acid (DHA) for its favorable effect on vision (Gillingham et al 2005). Hydrolyzed cornstarch serves as carbohydrate supplementation (Spiekerkoetter et al 2009, 2010). Because these patients are sensitive to exercise-induced rhabdomyolysis, extra energy as carbohydrate (CHO) or MCT oil is recommended before exercise (Gillingham et al 2006). Supplementation with L-carnitine is no longer suggested. (Spiekerkoetter et al 2009, 2010) An observation by Bridget Wilcken is that, with strict dietary treatment, carnitine levels normalize 2 to 4 weeks after decompensation, without supplementation, quoted in Spiekerkoetter and colleagues (2010). Supplementation with carnitine leads to excess amounts of circulating acylcarnitines, which may provoke cardiac arrhythmias (Corr et al 1989).

During febrile and gastroenteric infections and metabolic decompensations, patients should usually be hospitalized and treated according to an emergency protocol: intravenous 10% glucose infusion. A few case reports concern with the anaplerotic substance, heptanoate, to have a more favorable outcome (Roe 2002, Karall et al 2014, 2015).

The human body cannot produce essential fatty acids (EFAs), linoleic acid (18:2n-6, LA, or α-linolenic acid (18:3n-3, ALA), which therefore must come from the diet. Dietary recommendations advise that diets contain n-6 polysaturated fatty acids (PUFAs): arachidonic acid (AA) and linoleic acid (LA), and n-3 PUFAS: alpha linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). The long-chain polysaturated fatty acids LCPUFA (DHA, AA) can be produced from LA and ALA, which, however, do not fully cover daily dietary needs.(Fatty Acids in Human Nutrition 2010). Infant formulas should therefore contain DHA and AA, because EFAs are important for growth and development (Innis et al 2008, Hoffman et al 2009). DHA has a favorable effect on visual and cognitive development in breastfed infants. (Mitmesser and Jensen 2007).

Recent studies point out the importance of avoidance of fasting periods, Haglind et al (2015) reported increased levels of long-chain acylcarnitines after a 4-hour fast. Karall et al (2015) debated less restriction of dietary fat content of the diet.

6.2.6 Monitoring the disease

Patients with LCHADD should be monitored as to diet, and clinically monitored for growth and development, and for cardiac function by electrocardiograms (ECGs) and echocardiograms. To detect retinopathy means ophthalmic examination, electroretinography (ERG), visual evoked potentials (VEP), fundus photography; to discover neuropathy requires electroneurography (ENG). The role of a dietician is critical (collection of dietary history and dietary regimen). Frequent dental care is crucial, because the high carbohydrate diet predisposes patients to dental caries (Blake et al 2007).

Biochemical monitoring includes plasma glucose, free fatty acids (FFA), acyl-carnitineprofiles, free carnitine, plasma transaminases, creatine kinase (CK), and fatty acid profiles to secure a sufficient supply of EFAs. CK is a good indicator of decompensation (Spiekerkoetter 2009, Lund et al 2010, Karall et al 2015).

6.2.7 Long-term outcome of LCHADD

Since the time that newborn screening was implemented for diagnosis of LCHADD and enabled the early start of strict diets, the outcome has improved markedly (Wilcken et al 2010). The two-year survival of seven neonatally screened patients was 100% (Spiekerkoetter et al. 2009). Survival rates in recent studies of nonscreened patients of LCHADD ranged from 62 to 76.9%, this is less than for MCAD, where risk of death after diagnosis is low (Boer et al 2002, Spiekerkoetter 2009 et al, Sukyt- Cegielska et al 2011). Data on the long-term outcome of these patients are still scarce.

Growth

Typically, LCHADD patients manifest with failure to thrive and feeding difficulties. Their growth normalizes after the start of therapy (Haglind et al 2013). This group has stressed the importance of weight monitoring, because a diet high in carbohydrates leads to risk for excessive weight gain; one report was that 30% of the patients were overweight. (Haglind et al 2014, Gillingham 2007).

Developmental outcome

Strandqvist et al in 2015 reported a series of eight nonscreened LCHADD patients, five having a normal cognitive pattern with a specific cognitive profile (weakness in verbal working memory and in adaptive and executive functions) and three having an intellectual disability. Karall et al (2015) reported a normal IQ in 12 of their 14 patients.

Hepatopathy

Hepatic dysfunction is common finding of β-oxidation disorders. Baruteau et al in 2013 found that in their series of 158 patients with β-oxidation defects, 144 had hepatic manifestations. Saudubray et al in 1999 reported that 73% of their patients with β-oxidation defects had hepatic findings, however, hepatic failure appeared in only one. In their study with 13 LCHADD patients, Tyni et al (1997) detected hepatomegaly in 92% at presentation; three of them had jaundice and cholestasis. At follow-up, all their patients had liver

involvement. Den Boer et al (2002) reported hepatomegaly in 60 to 78% and hepatic dysfunction in 79 to 80% of their patients at diagnosis. Karall et al (2015) reported hepatopathy in 36% of their 14 patients during follow-up. Liver function normalizes after initiation of dietary therapy. During the metabolic derangements, elevated aminotransferases appear.

Cardiomyopathy

The mechanism of cardiomyopathy in β-oxidation disorders is unknown. The toxic effects of acylcarnitines may cause cardiac complications. Since accumulation of proarrhythmic acylcarnitines in fatty acid oxidation disorders simulate electrophysiological findings in myocardiac infarction. Arrhythmias did not occur in β-oxidation disorders where accumulation of acylcarnitines did not exist and where the metabolic pathway did not use any carnitine shuttle (Bonnet et al 1999). In FAO disorders, cardiac manifestations were reported in 55 to 51%, of whom cardiomyopathy occurred in 67 to 85% and arrhythmias in 44 to 55% (Baruteau et al 2013, Bonnet et al 1999, Saudubray et al 1999).

Cardiomyopathy is a typical clinical finding of LCHADD and usually presents as hypertrophic cardiomyopathy (HCM) or dilatating cardiomyopathy (DCM). Den Boer et al reported in 2002 a cohort of 50 patients, of which 42% had cardiomyopathy, and Tyni and colleagues in 1997, 13 patients, of whom 92% had cardiomyopathy; they did not report any detailed data on cardiomyopathies. Spiekerkoetter et al reported in 2009 cardiomyopathy in 25% of neonatally screened patients, whereas 54% of their clinically diagnosed patients had it. Dyke et al reported in 2009 that a LCHADD patient with poor compliance with therapy developed dilatating cardiomyopathy in only a week and died later in arrhythmia. Baruteau et al (2013) reported cardiomyopathy at diagnosis in 28 of their 40 LCHADD patients, of which 13 were hypertrophic and 15 dilatating.

Retinopathy

Progressive peripheral neuropathy and retinopathy are the main long-term complications of LCHADD, but are absent from other β-oxidation defects. An accumulation and toxicity of hydroxylacylcarnitines and long-chain fatty acid metabolites or deficiency of essential fatty acids, especially (DHA) docosahexanoic acid, may cause these complications (Gillingham et al 2003).

In recent studies, the retinopathy has been evident in 13 to 100% of LCHADD patients (Tyni et al 1998, Boer et al 2002, Fahnehjelm et al 2008, Baruteau et al 2013).

The retina uses mainly glucose for its fuel. Tyni and colleagues (2002, 2004) showed that TFP is expressed in retinal pigment epithelium (RPE), and Polinati and colleagues (2015) demonstrated in a LCHADD-patient RPE cell-model that the triglycerides (TGs) were accumulated in patient's RPEs. The cells were small and irregular, with hypopigmentation, few melanosomes, an excess amount of melanosomes, and disorganized cell-cell junctions (Polinati et al 2015).

The chorionretinopathy in LCHADD starts with dispersion and aggregation of pigment of the macular region from the age of 4 months to 5 years. (Poll et all 1988, Bertini et al 1992, Tyni et al 1998, 1998) The fundus changes can be staged as follows:

Stage 1: In early infancy with pallor of the fundus. Stage 2: Clumping of the RPE especially in the posterior pole. Stage 3: Progression may lead to circumscribed chorioretinal atrophy with sparing of the foveolar area and peripheral fundus. Stage 4 posterior staphylomas with a central scotoma may develop (Tyni et al 1998) (Table 2).

Table 2. Clinical stages in chorionretinopathy of LCHADD

stage	1	2	3	4
fundus	normal or pale	hypopigmentation, pigment clumping particularly in the macula	progressive chorioretinal atrophy in the posterior pole, relative sparing of the central macula	total atrophy of the posterior pole, posterior staphyloma, sparing of the peripheral fundus
vision	normal	normal	paracentral scotoma, progressive myopia, deteriorated color vision	central scotoma
ERG/VEP	normal	progressively low ERG, normal VEP	unrecordable ERG	unrecordable ERG, relatively normal VEP

ERG electroretinography, VEP visual evoked potentials

Gillingham and colleagues in 2005 reported that patients with dietary therapy and with fewer decompensations and lower 3-OHAC have their retinopathy progress more slowly. Fahnehjalm and colleagues in 2008 suggested that neonatal hypoglycemia, late diagnosis with severe symptoms, and high number of decompensations may lead retinopathy to progress.

Peripheral neuropathy

Peripheral neuropathy is specific to LCHADD and TFP deficiency (Poll et all 1988, Dionici et al 1991, Bertini et al 1992, Tein et al 1995, 1999, Tyni et al 1997, Saudubray et al 1999, den Boer et al 2002, 2003, Spiekerkoetter et al 2009a, 2009b, Karall et al 2015). The neuropathy in LCHADD appeared in recent studies to be quite severe, predominantly axonal with possible secondary demyelination and being more prone to occur in the lower limbs (Bertini et al 1992, Tein et al 1995, Tyni et al 1997).

Peripheral neuropathy was observable in 4 (10%) patients (Baruteau et al 2013), and among their 14, in only one patient (Karall 2015); these studies neglected, however, to report the character of the peripheral neuropathy.

Rhabdomyolysis

Rhabdomyolysis or increase in CK is provoked in LCHADD patients by exercise and infections when the supply of energy is insufficient. The muscle symptoms in LCHADD decrease or even cease by consumption of MCT oil or carbohydrates before exercise (Gillingham et al 2006). Karall and colleagues (2014) reported that heptanoate treatment had a favorable effect.

7. AIMS OF THE STUDY

The aims of this study were to evaluate the medical history and clinical status of the current LCHADD patients in Finland and to further develop their treatment and follow-up, and to

1. study the effect of strict dietary therapy started early on clinical course and long-term complications, such as progressive pigmentary retinopathy and peripheral neuropathy.

2. refine the staging of pigmentary chorionretinopathy and thus improve monitoring and comparability of patients.

3. develop a method to assess the compliance and outcome of dietary therapy.

8. PATIENTS AND METHODS

8.1 Patients

In Finland, 47 patients with LCHADD were diagnosed in 1976-2014. All these patients had the homozygous common mutation c.G1528C and were treated in the University Hospitals of Helsinki, Oulu, Tampere, and Turku, Finland. For 47 patients, the diagnosis was for 27 post mortem by mutation analysis from blood or tissue samples. One of the patients was diagnosed prenatally because of a family history of LCHADD, none by NBS. Tyni and colleagues (1997, 1998) reported on the LCHADD patients born 1976-1996 in Finland, and these patients were included in this study (Table 3).

Study I

Of 10 surviving patients diagnosed 1976-2006 with LCHADD caused by homozygous G1528C mutation and treated and followed up at Helsinki University Hospital during 1997–2006, 7 had stage II retinopathy and were included in this study.

Study II

From 1976 through 2010, 44 patients with LCHADD were diagnosed in Finland. The 15 patients who surviving for six months after their first decompensation were treated with a low-fat, high-carbohydrate diet and followed up for 1 to 45 years (mean 11.2 years) at Helsinki University Hospital, Helsinki, Finland.

Study III

A total of 47 patients with LCHADD were diagnosed in Finland from 1976 through 2014. All of the 12 patients who had electroneurograhies (ENGs) were included in this study.

Study IV

This study included 11 LCHADD patients born 1999-2013 and treated with the current dietary regimen in Helsinki University Hospital during 2000-2014.

Table 3. Patients with LCHADD diagnosed in 1976-2014 in Finland. Patient numbering 1-28 originate from the study of Tiina Tyni et al (1997, 1998). From number 29, patients are numbered by year of birth.

Patient no	Year of birth	Current age in years or age at death in survived patients	Study I	II	III	IV
1	1991	† 3 months		X		
2	1991	† 4 months		X		
3	1987	† 9 months		X		
4	1989	† 3 months		X		
5	1978	† 1 year 9 months		X		
6	1980	† 30 years		X	X	
7	1976	†13 months		X		
8	1979	†13 months		X		
9	1991	† 0.5 months		X		
10	1992	† 9 months		X		
11	1988	† 8 months		X		
12	1994	† 6 months		X		
13	1994	† 10 months		X		
14	1984	† 10 months		X		
15	1984	† 10 months		X		
16	1979	† 6 months		X		
17	1981	† 1 year 2 months		X		
18	1989	† 6 months		X		
19	1965	49 years		X	X	
20	1980	†8 moths		X		
21	1979	† 13 days		X		
22	1990	24 years	X	X	X	
23	1981	† 6 months		X		
24	1985	† 2 days		X		
25	1991	23 years	X	X	X	
26	1989	† 4 months		X		
27	1987	† 10 months		X		
28	1996	† 3 months		X		
29	1998	† 7 months		X		
30	1998	† 3 months		X		
31	1999	15 years	X	X	X	X
32	2000	14 years	X	X	X	
33	2000	†2 years	X	X		
34	2001	† 3 months		X		
35	2001	13 years	X	X	X	X
36	2001	13 years	X	X	X	X
37	2006	8 years		X	X	X
38	2006	† 5 months		X		
39	2007	7 years		X	X	X
40	2008	6 years		X	X	X
41	2008	6 years		X	X	X
42	2008	† *		X		
43	2009	5 years		X		X
44	2010	4 years		X		X
45	2013	1 years				X
46	2013	1 years				X
47	2014	*				

† age of death *data for this patient are missing

8.2 Methods

8.2.1 Dietary regimen

In infancy and childhood, fasting longer than 3 to 4 hours was avoided. A nasogastric tube or gastrostomy was used to allow prolonged nighttime feeding. Uncooked corn starch was started during the teenage years to allow the patients a prolonged nighttime fasting up to nine hours. In the low-fat high-carbohydrate diet, a maximum of 5% of total energy intake came from long-chain triglycerides (LCTs), including important fatty acids such as docosahexanoic, linoleic acid and α-linolenic acid. To provide more calories from lipids, medium-chain triglyceride (MCT) supplementation (15-20% of total energy intake) was provided either as Monogen®-formula (Nutricia Clinical) or MCT-oil® (Nutricia Clinical). Essential fatty acids (EFA) came from walnut oil and omega-3 oil, and the need for fat-soluble vitamins was met with a fat-soluble vitamin preparation Sanasol® (Nycomed Pharma AS) or vitamin A or E preparations sold by a local pharmacy. Hydrolyzed corn starch, Fantomalt®, (Nutricia Clinical) served as a carbohydrate supplementation. Before physical exercise, the patients were advised to take extra Fantomalt® or MCT oil. Carnitine supplementation was no longer used because recent reports suggest that this may not be beneficial (Spiekerkoetter et al 2009, Spiekerkoetter et al 2010). During febrile illness and gastroenteritis infections or metabolic decompensations, the patients were usually hospitalized and treated according to an emergency protocol, which included covering daily energy needs and losses orally, via gastrostomy or with an intravenous glucose infusion.

The dietitian collected detailed dietary records, and families completed a 3-day estimated (weighed or household measures) dietary record for 3 consecutive days, including a Saturday or Sunday. The dietary records were analyzed using AivoDiet® software.

8.2.2 Clinical and laboratory follow-up

Survival and all clinical data on the deceased patients were collected retrospectively from hospital records. Their clinical course and response to the dietary therapy were reviewed in all the surviving patients. Weight, height, and head circumference and the clinical follow-up data including number of 1) decompensations, 2) hypoglycemic episodes, 3) signs of liver involvement, 3) rhabdomyolysis, and 4) generalized multiorgan involvement were recorded at every visit. Additional laboratory results collected included fasting blood glucose, plasma ketones, serum alanine aminotransferases (ALAT), serum creatine kinase (CK), serum free fatty acids (FFA), plasma fatty acid profiles, serum total and free carnitine, serum fat-soluble vitamins, and blood acylcarnitine profiles. The acylcarinitines were analyzed in Newcastle upon Tyne, UK.

The dietitian offered dietary recommendations every 3 months to 1 to 2 years depending on patient age. The patients visited every 6 months to 2 years an ophthalmologist, pediatric neurologist, and dentist. (Study II-IV)

8.2.3 Cardiac follow-up

Electrocardiograms (ECGs) and echocardiograms were performed every 3 months to 5 years depending on patient age. The echocardiographic criteria for cardiomyopathy were impaired systolic function of the left ventricle (fraction shortening less than 27%) with or without an enlarged left ventricle or thickening of the ventricular septum compared with reference values of the same age (over +2 SD) or signs of noncompaction of the left ventricle wall (Study II).

8.2.4 Electroneurophysiological follow-up

The ENGs were performed in 12 patients in the laboratory of clinical neurophysiology at the Hospital for Children and Adolescents in Helsinki during 1982-2014. (Study III)

8.2.5 Staging of fundus photographs

In 1997–2006, sequential fundus photography for stage 2 chorioretinopathy was done for seven children with LCHAD deficiency caused by a homozygous G1528C mutation during routine visits. This was performed under general anesthesia and full papillary dilatation until the age of 3 to 4 years, and without anesthesia thereafter. The fundus photographs were printed on photoquality paper. Then the 21 pairs of fundus photographs were ranked according to severity of the fundus changes, and the best images representing three different grades of pigmentary deposits (P1–P3) and retinal pigment epithelial (RPE) atrophy (A1–A3) were designated as reference photographs. For evaluation of the substaging, 29 photographs were rated according to these reference photographs. (Study I)

8.2.6 Statistical methods

Agreement between raters was analyzed with a weighted K statistic (standard weighting, STATA 11, StatCorp, College Station, TX, USA) (Study I). The SPSS served for data analysis (Study IV).

9. RESULTS

All of the 47 patients diagnosed with LCHADD in Finland had the homozygous common mutation 1528G>C, and they originated from 34 families (Figure 2).

Artwork: Pilvi Putkonen

Figure 2. Birthplaces of grandparents of LCHADD patients in Finland born 1976-2001. Data available for 38 patients. Attached map represents Finnish territory prior to 1944; the small area in the southeast is now part of Russia.

The most frequent first presentations of LCHADD were failure to thrive, hepatopathy, hypotonia, lethargy, and vomiting (Table 4).

Table 4. The first presentation of 44 patients (diagnosed 1976-2010) with LCHADD, number of patients (%).

hypotonia	41 (93)
lethargy,vomiting	24 (54)
hypoglycemia	19 (43)
liver dysfunction	17 (39)
hepatopathy	43 (98)
failure to thrive	43 (98)
lowered consciousness	10 (23)
metabolic decompensation	9 (20)
cardiac arrest	6 (14)
hypoparathyroidism	3 (7)
steatorrhea	3 (7)
sudden death	2 (5)
portal vein thrombosis	1 (2)
intrahepatic cholestasis	1 (2)
(data for one patient missing)	

9.1 Clinical and laboratory follow-up (Study II)

From 1976 through 2010, a total of 44 patients with LCHADD have been treated in the University Hospitals of Helsinki, Oulu, Tampere, and Turku, Finland. The age at the first presentation was from birth to 1 year 9 months. (Mean 0.38 years).

All 15 patients who survived more than 6 months after the first decompensation episode had relatively low blood glucose, and elevated transaminases and acyl-carnitines at diagnosis (Table 5).

Table 5. Laboratory findings at diagnosis for 15 patients who survived more than 6 months after their first decompensation episode.

	median	SD**	normal
glucose	3.34 mmol/l	1.9 mmol/l	4-6 mmol/l
ALT	114 U/l	424 U/l	<40 U/l
CK	229 U/l	276 U/l	50-270 U/l
acylcarnitine*	0.497	0.53	0.017

**Standard deviation

*([C16OH+C18:10H+C18OH]/C2) concentration ratio

Serum alanine aminotransferase (ALT), creatine kinase (CK). (data of the four oldest patients incomplete)

In 1976–1996, for 28, the survival rate was 14%, while in 1997 to 2010, for 16, the survival rate was 63%.

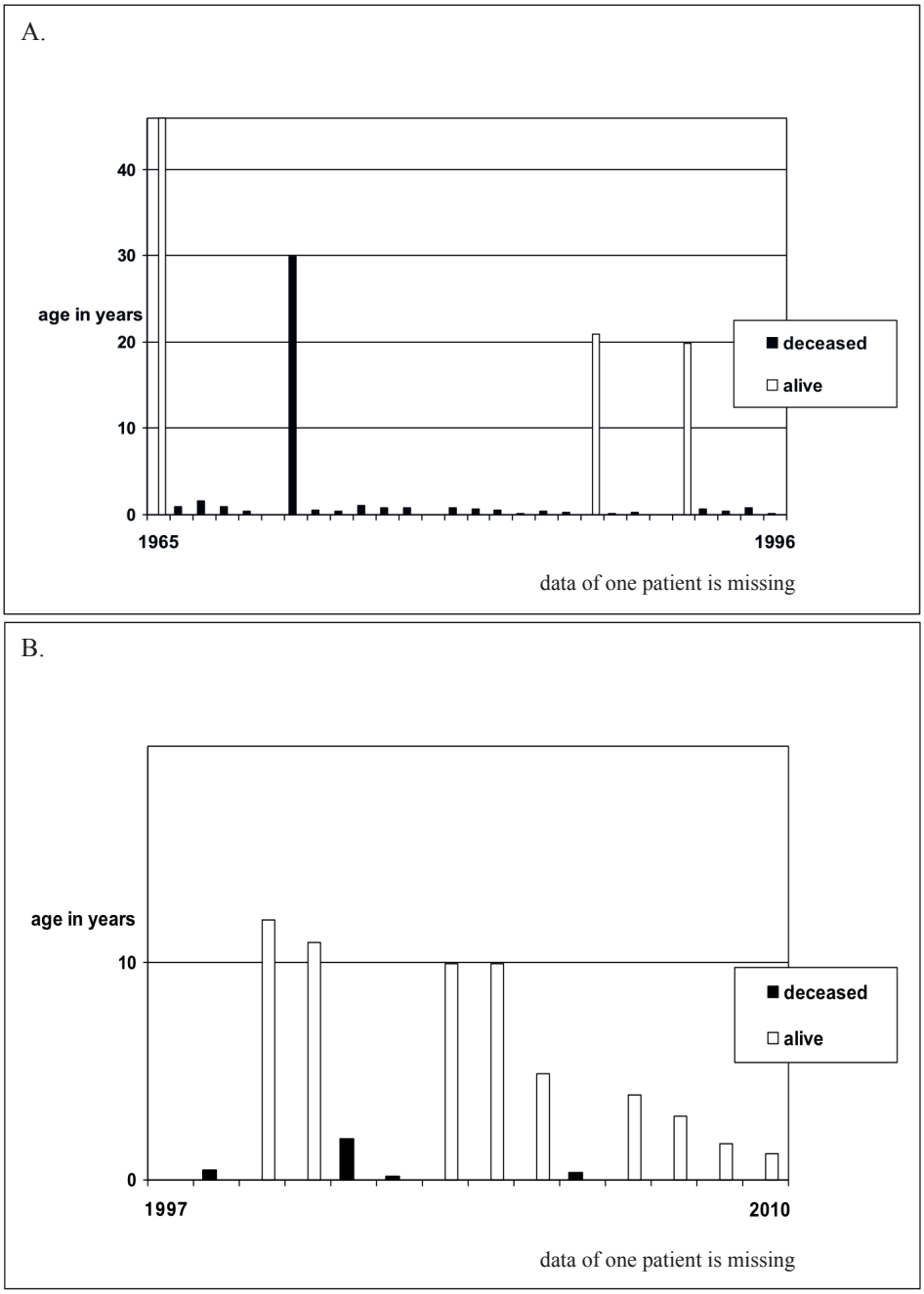

Figure 3. Survival improvement during 1965-2010. Each bar represents one patient. A. Survival of patients born 1965-1996. B. Survival of patients born 1997-2010.

Diet initiation
Start of dietary therapy was delayed in patients born 1965 to 1997, and some were not provided diet therapy at all. For patients diagnosed between 1997 and 2010, dietary therapy started within 1 to 30 days of diagnosis.

Pregnancies
A total of 44 patients were diagnosed with LCHAD deficiency from 1976 to 2010. We have earlier reported the pregnancy complications in 18 carriers of LCHAD deficiency (Tyni et al 1998). Most of the pregnancies with a LCHAD-deficient fetus were complicated; of 44 pregnancies, only 17 were normal. Pregnancy complications included pregnancyinduced hypertension in 4, preeclampsia in 7, hemolysis, elevated liver enzymes and low-platelets (HELLP) syndrome in 4, acute fatty liver of pregnancy in 2, and intrahepatic cholestasis of pregnancy in 4. Patients with LCHADD were often born premature (19 patients) and were small for gestational age (SGA) (14) (Unpublished data).

Follow-up
The two oldest long-term survivors: for 30 years (patient 6) and 45 years (patient 1) with very delayed initiation of the dietary therapy showed a constantly fluctuating clinical course during their first 2 to 5 years of life, interrupted by numerous moderate to severe decompensations that led to hospitalizations due to a multiorgan failure (approximately 6 - 11 decompensations per patient). One had mild mental retardation and chronic epilepsy from the age of 1 year 4 months, and developed a visual handicap and neuropathy later in life. This patient died at the age of 30 years from severe heart dysfunction due to restrictive cardiomyopathy. The other patient has visual handicap, neuropathy and moderate lower extremity muscle weakness. Two younger patients (both aged 20) who were treated with a low-fat, high-carbohydrate diet from the age of 4 months and 3 months without specific diagnosis had only 0 to two registered metabolic decompensations each patient during first years of life. As adults, these patients had few metabolic decompensations, occasional muscle pains and one rhabdomyolysis (CK > 5000 UI/l) each. At present, they have no severe chronic complications and have normal intelligence quotient (IQ).

Among 16 patients diagnosed after 1997, the first decompensation led to death in 3, and another 3 patients later died of a severe metabolic crisis. Most long-term survivors are doing fine, and all except one, who had resuscitation and metabolic crises before diagnosis, have a normal intelligence quotient (IQ). Liver function normalized after start of the diet; hepatic findings ranged at diagnosis from hepatomegaly to cholestasis icterus and portalvein thrombosis. During follow-up viral gastroen-teritis, respiratory infections, and prolonged exercise, particularly in cold weather, provoked between one and three episodes of rhabdomyolysis (CK>5000 U/l) in each of surviving 10 patients diagnosed after 1997. Hypoglycemia was detectable only in one of the patients during infection.

The laboratory findings remained mainly stable during follow-up in 15 patients who survived more than six months after their first decompensation episode. Acylcarnitines were

elevated during follow-up, and single elevations of transaminases and CK were detectable especially during infections (Table 6).

Table 6. Laboratory findings during follow-up in 15 patients who survived more than 6 months after their first decompensation episode.

	range	median	normal
glucose	0.5-12.4 mmol/l	5 mmol/l	4-6 mmol/l
ALT	4-1504 U/l	48.5 U/l	<40 U/l
CK	7-45 600 IU/l	206 IU/l	50-270 IU/l
acylcarnitine*	0.02-0.77	0.09	<0.017
plasma vitamin A	0.2-3.4 µmol/l	1.6 µmol/l	1-3 µmol/l
FFA	0.05-2.68 mmol/l	0.41 mmol/l	0.08-0.7
serum carnitine	2-199 µmol/l	18 µmol/l	20-60 µmol/l

*([C16OH+C18:10H+C18OH]/C2) concentration ratio

Data on the four oldest patients were incomplete. Serum alanine aminotransferase (ALT), creatine kinase (CK), serum free fatty acids (FFA).

Of 15 patients diagnosed, 7 of these fulfilled the criteria for cardiomyopathy. During follow-up in four patients, the cardiac findings normalized, and two developed cardiomyopathy (Table 7).

Table 7. Cardiac findings for 15 patients with LCHADD who survived more than 6 months after their first decompensation episode, at diagnosis and during follow-up.

clinical findings of cardiomyopathy				
patient no.	**age***	**gender**	**at diagnosis**	**at end of study period**
1	45	M	normal	normal
6	†30	F	severe dysfunction of the heart	chronic heart failure with restricted left ventricle
22	21	M	normal	normal
25	20	M	normal	normal
31	11	M	normal	hypertrophic left ventricle and septum (+1sd)
10	10	F	resuscitation, dilatated left ventricle, inotrophic medication	normal
33	†2	F	hypertrophic left ventricle and septum (+2sd)	normal
35	9	F	hypertrophic left ventricle and septum (+2sd)	normal
36	9	F	normal	hypertrophic left ventricle and septum (+1sd)
37	4	F	hypertrophic left ventricle and septum (+2sd)	normal
39	3	F	normal	normal
40	2	M	hypertrophic left ventricle and septum (+2sd)	hypertrophic left ventricle (+2sd)
41	2	F	normal	normal
43	1	F	acute pericardial tamponation, drainage, inotrophic medication	fluctuating pericardial effusion
44	1	M	normal	normal

†deceased,* current or at death, SD= standard deviation

9.2 Electroneurophysiological follow-up (Study III)

LCHADD patients under strict dietary therapy, numbering 12, were included in this study, and 61 ENGs performed. Polyneuropathy (PNP) was diagnosed in 8 of the 12 patients (Figure 4).

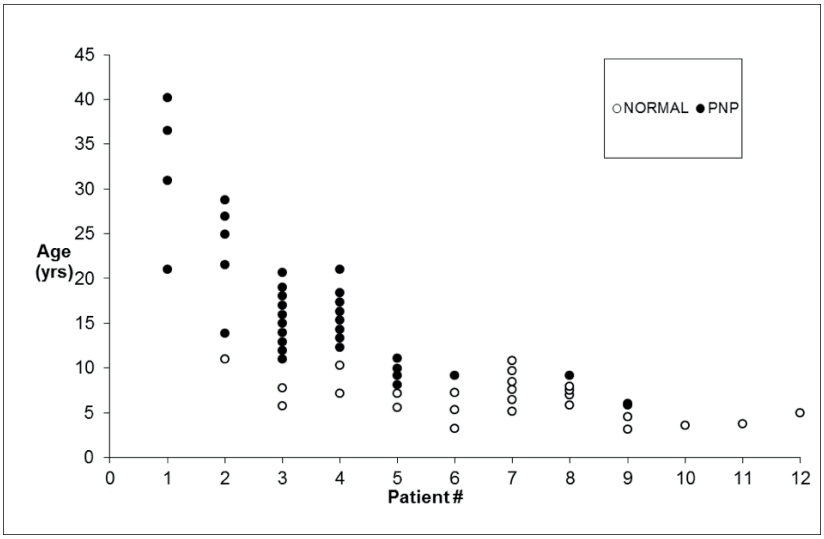

Figure 4. Evolution of polyneuropathy (PNP) in patients with LCHADD. All ENG measurements are shown at corresponding ages, patient number on the y-axis.

The clinical signs of PNP were absent tendon reflexes in the lower extremities and difficulty of walking on the heels; these were detectable as the first signs of polyneuropathy. During follow-up, physical performance was good in most of the patients, but tightness in muscles and the Achilles tendon caused limitation in joint movement in the ankle.

The first finding of PNP was reduction in sensory amplitudes of the sural nerves. In the upper limbs' ulnar nerve, sensory amplitudes started to decline during follow-up; in six patients they become abnormally low. In the majority of the patients, motor amplitudes and conduction velocities remained normal; in only one patient, who was severely neurologically handicapped with mental retardation after resuscitation at the age of 1 month (patient 6), the motor amplitudes in the peroneal nerve were abnormally low at the age of 9 years. Motor conduction velocities were within normal limits, except for the one with a neurological handicap. Of 12 patients in this ENG study, in 4 among the youngest patients, when examined at ages 3 to 10 years, had no PNP findings in ENG. No signs of involvement of cranial nerve were detectable.

9.3 Staging of fundus photographs (Study I)

In seven children with LCHADD deficiency and stage 2 chorionretinopathy, sequential fundus photography was performed from 1997 to 2006. The technically adequate and representative 21 pairs of fundus photographs were scored twice, and the median of these scores were calculated per rater. The median scores by two pediatric ophthalmologists were 3.5 and 3.0 and by two pediatric neurologists 4.5 and 3.0. The four raters also classified the photographs independently in six groups. There were no discrepancies between raters indicating that the severity of fundus changes could be divided in substages. Mean score substage 1 (1.0 to 2.0), substage 2 (3.0 to 4.0), substage 3 (4.5 to 6). The two opthalmologists agreed on 13 of 21 pairs (agreement 76%, K 0.46), and the two experienced graders had agreement of 71%, (weighed K 0.28).

Images best illustrating the three different grades of pigmentary deposits (P1 to P3) and RPE (A1 to A3) atrophy were chosen to reference photographs by two experienced graders (a pediatric ophthalmologist and a neurologist).

Three pediatric opthalmologists verified the system by regrading the 29 photographs in system of reference photographs. Moderate agreement was achieved (combined K statistic 0.38) in the assessment of pigmentary deposits. Agreement was poor in exploring RPE atrophy (combined K statistics 0.018). Pairs of raters were formed, and the best agreement (fair and moderate) was from the most experienced raters of LCHADD patients.

During follow-up, the grading of pigment deposits can progress or regress. The progression or regression of substages of pigmentary deposits were detectable in two of six, and in two patients, inconsistencies of the grading were detectable. In one patient, the grading regressed.

RPE changes can progress or remain stable. Logical progression was noted in two of the six patients, and one patient regressed unexpectedly, in three the grading were chronologically inconsistent.

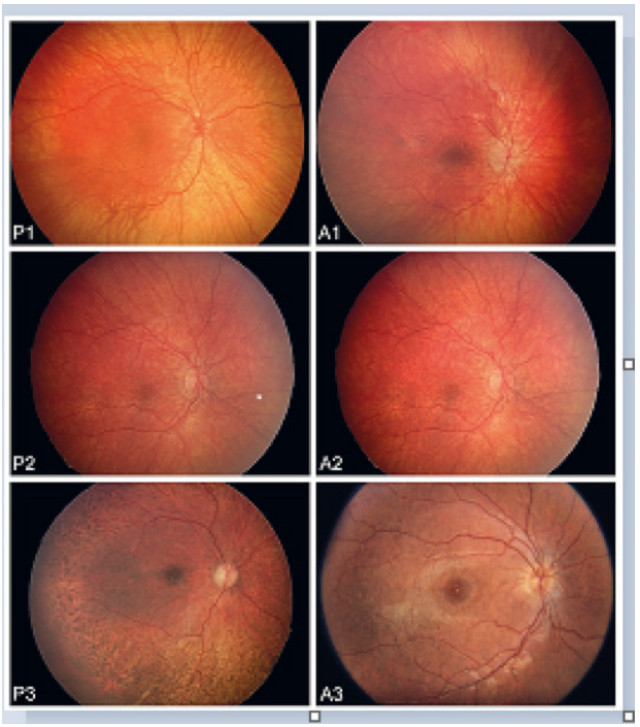

Figure 5. Reference photographs representing advancing grades of pigmentary deposits (P1 to P3) and RPE atrophy (A1 to A3) in chorioretinopathy associated with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD). Reprinted with permission of Karger Publishers, by Tyni T, Immonen T, Lindahl P, Majander A, Kivelä T. Refined staging for chorionretinopathy in long-chain 3-hydroxyacyl Coen-zyme A dehydrogenase deficiency. Ophthalmic Res 2012;48: 75-81.(Study I)

9.4 Dietary follow-up (Study IV)

During 2000-2014, 11 surviving LCHADD pediatric patients were followed up under current dietary therapy at Helsinki University Hospital. All of them are alive. Their ages ranged from 1 to 15 years during follow-up. The detailed dietary regimens were collected by a dietitian, and compliance was monitored by the dietary records and laboratory values.

Compliance with the diet was good in 9 of 11 patients. Physical performance was good in all of the patients, and they had normal IQ, and were attending normal school. These patients had suffered annually 0 to 3 episodes of rhabdomyolysis or decompensations, typically provoked by infections. Elevated CK and ALT were seen during these episodes and which normalized soon after the clinical condition stabilized. Only one patient had hypoglycemic episode during infection. Acylcarnitine levels were elevated despite strict and good compliance with the diet.

To secure continuous night-time feeding during the first years of life, all the children were provided nasogastric tubes and later on most had a gastrostoma. For teenagers, uncooked corn starch was given in the evening to compensate for their pro-longed night-time fast.

The amount of LCT was between 5 to 9 %E. None of the patients had an EFA deficiency. Surprisingly, 26.0 to 35.4% of the ALA and 12.7 to 42.0% of the LA came from the diet. Dietary intake of DHA was smaller. However, AA levels were elevated in patients provided with Lipistart® formula, which is supplemented with AA (Table 7).

Table 7. Examples of mean daily dietary intakes of macronutrients and essential fatty acids at different ages in children with LCHADD.

Age in years		13 (pat no.3)	7 (pat no. 5)	5 (pat no. 8)	1 (pat no. 11)
Energy kcal, (kcal/kg)		1984.4 (41,95)	1640.8 (57,67)	1254.5 (69,28)	706.7 (65,16)
Carbohydrate g, (E%)		280.0 (57,85)	260.3 (65,17)	210.5 (68,81)	109.6 (63,61)
Protein g (E%; g/kg)		74.6 (15.4; 1,6)	57.8 (14.3; 1,9)	32.0 (10.4; 1,8)	23.7 (13.8; 2,2)
LCT g, (E%)		20.4 (9,06)	14.3 (7,58)	7.1 (4,99)	4.6 (5,75)
MCT g (E%)		38 (16,85)	23.8 (12,85)	22.6 (15,88)	14.4 (17,98)
Linoleic acid	total mg (E%)	2995.8 (1.33)	2008.2 (1.05)	1055.8 (0.74)	1732.5 (2.2)
	from food: mg (%)	1444.3 (38.2)	884.4 (42.0)	261.7 (24.8)	220.5 (12.7)
	from supplements and infant formulas: mg (%)	1851.5 (61.8)	1163.8 (58.0)	794.1 (75.2)	1512.0 (87.3)
α-linolenic acid	total: mg (E%)	563.8 (0.25)	317.9 (0.17)	291.3 (0.16)	286.1 (0.36)
	from food: mg (%)	199.8 (35.4)	88.2 (28.0)	135.3 (28.8)	74.3 (26.0)
	from supplements and infant formulas: mg (%)	364.0 (64.6)	228.8 (72.0)	156.0 (71.2)	211.8 (74.0)
DHA	total mg (E%)	328.0 (0.15)	162.1 (0.09)	116.3 (0.08)	89.7 (0.11)
	from food: mg (%)	6 (1.8)	14.9 (9.2)	5.9 (5.0)	1.3 (1.5)
	from supplements and infant formulas: mg (%)	322 (98.2)	147.2 (90.8)	110.4 (95.0)	88.4 (98.5)

Reference values: Energy as LCTs 5-10% (E%), α-linolenic acid 0.2 E%, linoleic acid 1 E%, DHA 60-120mg/d (9)

Growth

Most of the patients presented with a progressively declining weight gain and poor height gain prior to their diagnosis. Soon after initiating the dietary therapy, the patients' growth normalized. During follow-up, only one patient was obese.

10. DISCUSSION

The severe forms of mitochondrial long-chain β-oxidation disorders usually present during the first year of life, endangering tissue energy supply; they manifest with hypoketotic hypoglycemia, failure to thrive, hepatopathy, cardiomyopathy, and rhabdomyolysis, or even sudden death. LCHADD is the most frequent β-oxidation disorder in Finland, with a carrier frequency of 1:132 to 1:365, and the MCAD is the most frequent worldwide with carrier frequency of 1:58 to 86 (Pastinen et al 2001, Gregersen et al 2001). Specific characteristics of the long-chain β-oxidation disorders-LCHADD and TFP deficiency--are peripheral neuropathy and retinopathy, whose pathophysiology is not yet fully known. Therapy with frequent meals, avoidance of fasting, and bypassing the metabolic block by providing medium-chain fats and carbohydrate and restricting LCTs is effective. Therapy for LCHADD patients is, however, based mainly on expert opinion (Spiekerkoetter et al 2009).

The aim of this study was to evaluate the current status of the LCHADD patients in Finland and develop further their treatment and our follow-up strategies.
This unique series of LCHADD patients followed up and treated by the same procedures, shows how in recent years diagnostics and treatment of LCHADD has exhibited major advantages. For this reason, survival of nonscreened LCHADD patients in Finland with the homozygous c.1528G>C mutation has greatly improved during 1976 to 2010 from 14.3% to 62.5%. This is uniform across recent reports, which showed survival rates of 54.5% to 62% of nonscreened patients (den Boer et al 2002, Spiekerkoetter et al 2009, Sykut-Cegielska et al 2011). The survival of seven screened patients was reportedly 100% in a small series of patients Spiekerkoetter 2009) Finland started screening for LCHADD as a part of newborn screening in 2015; this may improve surveillance even further.
In this study, patients presented, from birth to 1 year and 9 months (mean 0.38 years), most frequently with failure to thrive, lethargy, vomiting, hypotonia, hepatopathy, and metabolic decompensation. The acute symptoms and growth normalized after initiation of the dietary therapy as reported by us and earlier by others (Karall et al 2015).

Hepatopathy
Hepatic manifestations ranging from hepatomegaly and mild fatty liver to cholestatic icterus were detectable at diagnosis. During follow-up, hepatic manifestations resolved in all patients after initiation of therapy.

Cardiomyopathy
Our study detected cardiomyopathy in 61.5% of the patients at diagnosis, compared with 36 to 70% reported earlier among nonscreened LCHADD patients (Saudubray et al 1999, den Boer et al 2002, Dyke et al 2009, Baruteau 2013). Of our study patients, 83.3% had hypertrophic and 16.7% had dilatating cardiomyopathy. Baruteau (2013) reported that of his 40 patients, 28 had cardiomyopathy, 46% hypertrophic and 54% dilatating. During follow-up of six patients, in four, the cardiac findings normalized. Only one patient developed cardiomyopathy during a 10-year follow-up.

Rhabdomyolysis

As reported here and by others (Den Boer et al 2002, Karall et al 2015), patients had rhabdomyolysis usually provoked by exercise and infections, and it therefore seems crucial to provide for such patients MCT or carbohydrate before exercise, which reduces muscle symptoms (Gillingham et al 2006). Some small case reports state LCHADD patients treated with heptanoate have a better outcome and fewer rhabdomyolysis events. (Karall et al 2015) However, the study of Karall and colleagues (2015) showed no obvious difference between patients with or without heptanoate. Heptanoate is unavailable in Finland.

Decompensations

Despite early diagnosis, start of therapy within 30 days after diagnosis, and good compliance, our patients have had lifethreatening metabolic decompensations, especially during infections. One patient died at the age of 2 years from hypoglycemia and cardiac arrest because of gastroenteritis. One case report involves a 3-year-old patient who died from dilatating cardiomyopathy and arrhythmia after having brief poor compliance with dietary therapy (Dyke et al 2009). Corr and colleagues (1998) reported also that poor compliance may even predispose to lethal heart arrhythmias. Patients therefore require careful monitoring of their compliance and a balanced diet. Especially teenagers should be sufficiently guided in selfmanagement. Acute care, such as supplying sufficient energy, is challenging for parents, and even for emergency room (ER) personnel. The urge exists for creating uniform emergency protocols worldwide that patients and their caretakers could carry with them at all times, providing instantcare instructions for ER units not familiar with LCHADD.

Long-term complications

In LCHADD, the major long-term complications are pigment retinopathy and polyneuropathy. The etiology and mechanism of development of pigmentary retinopathy and polyneuropathy is unknown, although some factors considered have been accumulation of toxic metabolites such as hydroxylated acylcarnitines and long-chain fatty acids and deficiency of essential fatty acids (Gillingham et al 2003).

Pigmentary retinopathy

Pigmentary retinopathy is one of LCHADD`s earliest manifestations (Tyni et al 1997). This was consistent in our findings, showing the signs of retinopathy as being detectable during the first years of life. Although the current dietary regimen halts the progression of chorionretinopathy, possibly preventing the visual handicap (Fahnehjelm et al 2008), we recommend additional ophthalmological follow-up, with documentation by fundus photography and substaging of retinopathy based on reference photography for two reasons: 1. In infants the ophthalmological examination has its challenges, as to ensure good-quality comprehensive fundus photographs the infant needs to be sedated. 2. Assessments of fundus photographs varies between experts, as reported by Chiang et al (2007). Fundus photographic documentation and refined staging of retinopathy based on reference photography by an ophthalmologist is a good tool for following the course of the disease and adjusting the therapeutic interventions.

Peripheral neuropathy

The first signs of peripheral neuropathy were detected at 6 to 9 years of age in 67.5% of the patients. This is much more than reported by Karall et al 2015, and of their 14, only 1 patient had neuropathy, they did not, however, report how they diagnosed neuropathy (Karall et all 2015). In our relative large cohort of patients, repeated clinical evaluations by a child neurologist and performance of 61 ENGs allowed diagnosis of neuropathy, if present. Polyneuropathy seems to develop under the current treatment regimen, however, in a milder form than previously reported. Our study points out the importance of 1) ENG studies, around age 10 to detect polyneuropathy, 2) good compliance with the therapy, which seems to slow down the evolution of the polyneuropathy 3) physiotherapy of the affected patients to prevent contractures in the lower extremities.

Pregnancies in LCHADD

As reported earlier (Wilcken et al 1993, Tyni et al 1997, Strauss et al 1999, Ibdah et al 2000, Gutierrez Junguera et al 2009, Karall et al 2015) and by this study, most of the pregnancies with a LCHAD deficientfetus were complicated, only 38.6% being normal; 43.2% of the LCHADD patients were born premature and 31.8% were small for gestational age (SGA). Pregnancy complications comprised pregnancyinduced hypertension in 9.1%, preeclampsia in 16%, hemolysis, elevated liver enzymes, or lowplatelet (HELLP) syndrome in 9.1%, acute fatty liver of pregnancy in 4.5%, and intrahepatic cholestasis of pregnancy in 9.1%, patients.

Dietary therapy

The therapy consisted of avoidance of fasting: during infancy and childhood, fasting was restricted to 3 or 4 hours. In this study, a nasogastric tube and gastrostomy were in more frequent use than in recent studies, this allowed night-time feeding and ensuring feeding during infections (Sander et al 2005, Spiekerkoetter 2009, Karall et al 2015). Uncooked corn starch facilitated a prolonged night-time fast after age 9 years. The patients followed a low-fat high-carbohydrate diet, where a maximum 5 E% came from LCTs, including essential fatty acids. At follow-up, the essential fatty acids were within normal limits, including DHA, ALA, and LA in all the patients. Surprisingly, the diet covered the needs of 26 to 35% of the ALA and 13 to 42% of the LA. Supplemental DHA may, as reported by Gillingham et al (2003), in “moderate amounts (60-130 mg/day) improve the visual acuity in children with LCHAD deficiency regardless of age and metabolic control”. AA levels were elevated in patients fed infant formula supplemented (Lipistart®) with AA. The effects of moderately high concentrations of AA are unknown.

Modification of the diet, in which fat content is of less importance, has been under debate in recent studies (Karall et al 2015). We still recommend strict metabolic control of the diet, including restriction of LCTs and follow-up of fatty-acid fractions and acylcarnitines because the exact mechanism of the long-term complications is unknown. As reported by this study and the others, the acylcarnitines remain elevated, showing that the diet is not yet optimal. Based on this study our recommendations for the follow-up protocol and the

treatment recommendations with concurrent illness are summarized in Tables 9 and 10.

Table 9. Follow-up protocol for LCHADD patients in the Helsinki University Hospital. Actual interval depends on the age and clinical condition of the patient.

Medical, every 3 mo-1 y	**History of** 1. decompensations 2. hypoglycemic episodes 3. rhabdomyolysis **Current status of** 1. weight, height, and head circumference 2. signs of liver involvement 3. signs of other organ involvement **Laboratory tests** fasting blood glucose, plasma ketones, ALT, GT, AFOS,TT, CK, FFA, plasma fatty acid profiles, serum total and free carnitine, serum fat-soluble vitamins, and blood acylcarnitine profiles, cholesterol, Mg, phosfate, HbA1c, folate, blood coagulation panel and other tests as needed
Dietitian, every 1-6 mo	Dietary records and dietary recomendations
Neurological, every 3 mo-1 y	Clinical evalutation by (child) neurologist ENMG studies by age 10 years to detect polyneuropathy Physiotherapy of affected patients to prevent contractures in lower extremities.
Opthalmogical, every 6 mo-2 y	Fundus photography (substaging the changes), visual aquity, ERG, VEP
Cardiac, every 3 mo-5 y	ECG and echocardiograms
Dental, every 3-6 mo	Fluoride-containing toothpaste Prophylactic measures: topical application of fluoride varnish

Alkaline phosfatase (AFOS), serum alanine aminotransferases (ALT), serum creatine kinase (CK), electrocardiograms (ECG), electroretinography (ERG), serum free fatty acids (FFA), glutamyl transferase (GT), glycosylated hemoglobin (HbA1c), magnesium (Mg), tromboplastin time (TT), visual evoked potentials.

Table 10.Treatment recommendations for LCHADD patients with concurrent illness

	At home	In the emergency room	On the ward
Respiratory infection	**1.** Oral emergency regimen with glucose polymer and MCT, providing the expected age- and weight-specific energy and fluid need	**1.** Intravenous glucose infusion (10%) 5 mg/kg/min **2.** Laboratory tests**:** glucose, electrolytes, CK, myoglobin, ALT, CBC, CRP, creatinine, blood ammonia, TT blood gas for pH, and other tests as needed. **3.** ECG, chest X-ray and echocardiography as needed	**1.** Intravenous glucose infusion (10%) 5 mg/kg/min MCT- oil to avoid energy deficit. **2.** If possible- give at least part of the normal diet orally/via tube. **3.** Specific treatment of the infection. **4.** Monitor: Blood glucose monitoring (target range >5 mmol/l), CK, ALT. **5.** Consult dietitian
Gastroenteritis	Admit always	**1.** Intravenous glucose infusion (10%) 5 mg/kg/min **2.** Laboratory tests: glucose, electrolytes, CK, myoglobin, ALT, creatinine, blood ammonia, CBC, TT, blood gas for pH, and urine for myoglobin and other tests as needed **3.** ECG,chest X-ray and echocardiography as needed	**1.** Intravenous glucose infusion (10%) 5 mg/kg/min Ringer solution to compensate for extra fluid losses MCT-oil to avoid an energy deficit **2.** If possible- give at least part of the normal diet orally/via tube. **3.** Specific treatment of the infection **4.** Monitor: Blood glucose (target range >5 mmol/l) CK, ALT. **5.** Consult dietitian
Rhabdomyolysis Patients with CK elevation in excess of 2-3 times the reference range	Admit always	**1.** Intravenous glucose infusion (10%) 5 mg/kg/min **2.** Remaining fluid as isotonic saline or Ringer solution **3.** Laboratory tests: glucose, electrolytes, CK, myoglobin, ALT, creatinine, TT, blood ammonia, CBC, blood gas for pH, and and other tests as needed **4.** ECG, chest X-ray and echocardiography as needed	**1.** Hydration therapy (1,5-2 x normal) as glucose infusion (10%) 5 mg/kg/min + Ringer solution until CK level begins to decrease significantly, creatinine normalizes, and gross hematuria has resolved **2.** MCT- oil to avoid an energy deficit **3.** If possible- give at least part of the normal diet orally/via tube **4.** Specific treatment of the infection **5.** Monitor: Blood glucose (target range >5 mmol/l), CK, renal function **6.** Consult dietitian

Serum alanine aminotransferases (ALT), complete blood count (CBC), serum creatine kinase (CK), electrocardiogrram (ECG), tromboplastin time (TT)

Limitations and strengths of the study

The patient cohort, although comprising all Finnish LCHADD patients, was too small for statistical analysis, as is the case in most studies on rare diseases. The cohort, however, was homogeneous both genetically and clinically, and it was the largest LCHADD cohort ever reported. The different FAOs yield different clinical phenotypes, and thus it is important not to generalize to other disorders. The variation observed in this study between patients was partly due to differences in treatment, but there are as yet unknown genetic determinants influencing the outcome as well. These patients had the common Finnish LCHAD mutation. Other patients with other mutations may have different clinical pictures.

The study patients were followed up for a variable time period. Most are still children. Therefore, one has to be careful about conclusions regarding long-term out-come. Nevertheless, the current patients are doing much better than their peers two decades ago.

This study aimed at developing an improved treatment and follow-up protocol. This was partially accomplished. Laboratory tests indicated changes in diet and compliance. The development of long-term complications was slowed.

Future challenges

The main challenge is adjusting the treatment and follow-up lifelong, not just keeping the patients alive, but also finding new treatment strategies, because the patients treated with strict diet are reaching adulthood. Some of them are even having or planning their own families. No reports yet exists of pregnancies of LCHADD patients. The pregnancy of an LCHADD patient presumably constitutes a major risk for the patient and the fetus. Genetic counseling must be available for all the patients and their families.

Benzafibrate has been reported to restore and correct the FAO activities in patient cells in CPT2 deficiency (Bastin et al 2014), but the use of bezafibrate as pharmacological up-regulator of MTP remains controversial (Orngreen et al 2014, Djouadi et al 2016).

The future aspects of treatment involve individualizing patients' therapies. Whereas in LCHADD no genotype-phenotype correlation has been detectable, prediction of patient outcome is challenging. We therefore need still more data on these rare diseases to be able to find targeted pharmacological treatment or find modifying genes that could up-regulate substrate levels modifying disease severity and thereby improving clinical care.

Conclusions:

1. This study shows how the survival and outcome of LCHADD patients has greatly improved during the current strict dietary regimen. Outcomes will improve even further after implementation of neonatal screening. Patients, however, still suffer decompensation during infections, which constitutes an emergency situation in which they require careful management. Long-term complications: retinopathy and neuropathy, still occur, but their progression seem to slow under current dietary management.
2. Staging of retinopathy by fundus photography offers a good tool for clinicians to follow the course of the disease.
3. Assessment of fatty acid profiles and dietary intake as part of the follow-up regimen seems inevitable in order to enable LCT restriction of the diet to be as close to optimal as possible; it will also ensure the intake of essential fatty acids.

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