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Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency with the G1528C mutation: Clinical presentation of thirteen patients

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Long-chain 3-hydroxyacyl—coenzyme A (CoA) dehydrogenase is one of three enzyme activities of the mitochondrial trifunctional protein. We report the clinical findings of 13 patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. At presentation the patients had had hypoglycemia, cardiomyopathy, muscle hypotonia, and hepatomegaly during the first 2 years of life. Seven patients had recurrent metabolic crises, and six patients had a steadily progressive course. Two patients had cholestatic liver disease, which is uncommon in β -oxidation defects. One patient had peripheral neuropathy, and six patients had retinopathy with focal pigmentary aggregations or retinal hypopigmentation. All patients were homozygous for the common mutation G1528C. However, the enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activities of the mitochondrial trifunctional protein were variably decreased in skin fibroblasts. Dicarboxylic aciduria was detected in 9 of 10 patients, and most patients had lactic acidosis, increased serum creatine kinase activities, and low serum carnitine concentration. Neuro-radiologically there was bilateral periventricular or focal cortical lesions in three patients, and brain atrophy in one. Only one patient, who has had dietary treatment for 9 years, is alive at the age of 14 years; all others died before they were 2 years of age. Recognition of the clinical features of long-chain 3-hydroxyacyl-CoA deficiency is important for the early institution of dietary management, which may alter the otherwise invariably poor prognosis. (J Pediatr 1997;130:67-76)

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Beta-oxidation of fatty acids in the mitochondria is an important source of energy, especially in fasting subjects and during metabolic stress. During the past 20 years an increasing number of disorders affecting fatty acid oxidation in the mitochondria have been recognized. Currently, eight defects of the β -oxidation spiral and four defects of the carnitine cycle are known. Patients with these disorders have hy-

CoA	Coenzyme A
LCHAD	Long-chain 3-hydroxyacyl-CoA dehydrogenase
MRI	Magnetic resonance imaging
PCR	Polymerase chain reaction
RT	Reverse transcriptase

poglycemia and metabolic crises, which are often provoked by infections.

Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency was first described in 1989,¹ after which about 20 patients with this disorder have been reported. It has recently been discovered that the LCHAD activity resides in a mitochondrial trifunctional protein. This protein harbors the activities of two other enzymes involved in β -oxidation of long-chain fatty acids: enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase.^{2,3} The trifunctional protein has eight subunits: four α -subunits that have LCHAD and long-chain enoyl-CoA hydratase activity, and four β -subunits that have long-chain 3-ketoacyl-CoA thiolase activity.⁴ Four patients have been described in the literature who have had a combined deficiency with a marked reduction of all three enzyme activities of the trifunctional protein.⁵⁻⁷ Still, the exact frequency of the combined deficiency is unknown because most reports on LCHAD deficiency lack data on the activities of enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase. Conversely, there are some reports of patients who have had LCHAD deficiency, together with impaired activity of the other two enzymes.^{8,9} In general, it appears that most of the patients with LCHAD deficiency have either normal or only slightly decreased activities of enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase.^{7,10}

Two different mutations of the α -subunit donor splice site have recently been described in a patient with trifunctional protein deficiency in addition to the single common mutation, G1528C, of the dehydrogenase coding part of the α -subunit known in LCHAD deficiency.^{5,11} Ilst et al.¹² reported a high frequency (24/26) of the G1528C mutation in LCHAD deficiency. Prenatal diagnosis is possible either by mutation analysis or by determination of the enzyme activities in chorionic villi.^{13,14}

Patients with LCHAD deficiency are often characterized by the following clinical features: cardiomyopathy, hepatopathy, hypoketotic hypoglycemia, retinopathy, hypotonia, and peripheral neuropathy.^{8,9,15-27} However, two patients with the combined defect of the trifunctional protein had a surprisingly mild clinical course. Their symptoms were dominated by conspicuous and progressive muscle weakness, and neither of the patients had hypoglycemia, clinical hepatopathy, or cardiomyopathy.²⁸

There is evidence that early institution of the appropriate dietary treatment could improve the prognosis of LCHAD deficiency and other defects of β -oxidation.^{15,18,22,29,30}

However, the diagnosis of β -oxidation defects of fatty acids is often complicated by the unremarkable findings in biochemical screening tests, especially between metabolic crises. Therefore an essential step to diagnosis would be recognition of the deficiency on clinical presentation. We report the clinical findings of 13 patients with LCHAD deficiency and the G1528C mutation, the largest single series reported thus far.

METHODS

Patients. The diagnosis of LCHAD deficiency was made in 13 patients during the period from 1991 to 1995, in 12 cases post mortem. The diagnosis relied on the measurement of the activities of 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase, and 3-ketoacyl-CoA thiolase for long-chain fatty acids in cultured skin fibroblasts (nine patients) or on typical clinical features in a sibling with a verified diagnosis of the disease (four patients).

Enzyme analysis. The enzyme activities of the trifunctional protein were determined by Dr. Venizelos (patients 1, 2, 6, 7, 12, and 13¹⁰), by Dr. Jackson (patients 4 and 9⁹), and by Dr Wanders (patient 10¹¹).

Molecular genetic analyses.

Ribonucleic acid extraction and reverse transcriptase reaction. Total ribonucleic acid was extracted from cultured skin fibroblasts with the RNazol kit (Tel-Tec, Frierwood, Tex.). The reverse transcriptase reaction was performed with random hexanucleotide primers as described earlier.³¹

Identification of the mutation. The G1528C mutation in the LCHAD α -subunit gene was identified from the patient fibroblast ribonucleic acid after RT-PCR with the solid-phase minisequencing technique,³² essentially as described earlier.³¹ The complementary DNA-PCR was carried out with the following primers: 5'-GACCTGAGAAGGTGAT-TGGC (sense) and 5'-ACCTCCCCCTGCTTGAGACC (biotinylated, antisense). PCR amplification of the RT-reaction product was performed with primers, one of which was biotinylated at its 5' end. The biotinylated PCR product was captured into streptavidin-coated microtiter wells, and the unbiotinylated strand was removed. In the minisequencing reaction a detection primer was hybridized immediately adjacent to the mutation site. Either type of the two test nucleotides (tritiated deoxycytidine triphosphate or tritiated deoxyguanosine triphosphate) was introduced into each microtiter well and incorporated by a DNA polymerase if it was complementary to the nucleotide at the mutation site. In practice, the minisequencing reaction was performed with 20 pmol of a primer harboring the mutation (5'-TGGACAAGAT-GCAGCTGCTG), 0.2 U Dynazyme DNA polymerase (Finnzymes, Espoo, Finland) and 2 pmol of tritiated nucleotide (Amersham, United Kingdom), corresponding to the mutation site (G for the wild type and C for the mutant). After the reac-

tion, the amount of incorporated ^3H -labeled test nucleotide was measured by a liquid scintillation counter. The ratio of mutated versus wild-type messenger ribonucleic acid was determined by the ratio (R-value) of the counts from the two test nucleotides (C/G) corrected for differences of specific activity. An R-value of less than 0.1 indicated a normal homozygote, and an R-value of more than 10 indicated a mutant expression phenotype.

Organic acid analysis. The organic acids in the urine were determined as follows: Urine (at an amount corresponding to 2 μmol of creatinine) was diluted to 2 ml with distilled water, and chlorobenzoic acid was added as an internal standard. The ketoacids were oximized, the sample was acidified, and the organic acids were extracted with ethyl acetate and silylated with 100 μl of a mixture of 94% *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co., Rockford, Ill.), 5% pyridine (Pierce), and 1% trimethylchlorosilane (Fluka, Buchs, Switzerland) for 30 minutes at 60° C. For gas-liquid chromatography we used a two-channel gas chromatograph with 25 m capillary columns containing either 100% methyl silicone or 86% methyl, 7% phenyl, and 7% cyanopropyl silicone. The chromatography peaks were identified by a computed chromatography program.³³ Mass spectrometry identification of the peaks was carried out in two instances (patients 12 and 13). The urine samples of two patients (Nos. 10 and 11) were analyzed in another hospital laboratory, where the method differed somewhat from that described above.

RESULTS

Clinical presentation

Family history. The clinical features of the patients are shown in Table I. The patients were born to unrelated parents in nine families. Except for the patients reported here, all the parents and siblings were healthy. Two relatives of patient 12 had died suddenly in infancy, and one had Leigh disease but normal enzyme activities of the trifunctional protein in fibroblasts. No other affected relatives were known.

Pregnancy and delivery. Hepatosis occurred in two pregnancies, but neither HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome nor acute fatty liver was diagnosed. One mother with severe hepatosis had mild preeclampsia, and fulminant bleeding after cesarean delivery. Her platelet count, thromboplastin time, and fibrinogen concentration were low (136×10^9 gm/L, <30%, 0.1 gm/L, respectively). An emergency cesarean delivery was performed because of the elevated serum transaminase values and decreased variability in cardiotocography recording. Patient 6 is a twin to a healthy brother. She was born asphyxiated (Apgar score 4/10) after delivery by vacuum extraction.

Clinical features. The first symptoms occurred between

the age of 2 days and 1 year 9 months (mean, 6.1 months, when transient neonatal symptoms were not included). Four patients had transient neonatal hypoglycemia, but two were born prematurely. Patient 10, whose elder brother had died of an undetermined β -oxidation defect, had dicarboxylic aciduria in the neonatal period. Later in infancy the typical first symptoms were vomiting, somnolence, and poor feeding, often associated with an infection. The predominant sign was hepatopathy in two, and cardiomyopathy in three patients. One patient was profoundly hypotonic. Patient 5 had recurrent infections and died suddenly at the age of 1 year 9 months. Two patients had gastrointestinal bleeding from gastritis.

HYPOGLYCEMIA. Eleven patients had intermittent hypoglycemia and required intravenous glucose infusion. Two had hypoglycemia only neonatally. The blood glucose concentration of patient 5 was not recorded. Three patients had seizures during hypoglycemic episodes, and epilepsy developed in one.

CARDIOMYOPATHY. All patients who underwent cardiac examination had radiographically demonstrated cardiomegaly, and the left ventricle contracted poorly on ultrasonography during the acute phase of the disease. Four patients required treatment for heart failure.

HEPATIC INVOLVEMENT. The liver was clinically enlarged in 11 of 12 patients at presentation to a physician. Later, hepatomegaly was found in all patients. Three patients had jaundice caused by cholestasis (>80% of serum bilirubin in conjugated form), which was a presenting sign in two.

OPHTHALMOLOGIC FINDINGS. An ophthalmologic examination was performed in 11 patients; six had pigmentary retinopathy. Five patients had focal pigmentary aggregations or granular pigmentation in what otherwise was a hypopigmented retina. Only one patient had a hypopigmented retina without pigment aggregations, and another had granular pigments without hypopigmentation. The retinopathy was first detected at the age of 4 to 17 months. Four patients had a normal retina when examined at the age of 3 days to 6 months. The retina of one of the patients appeared normal at the age of 4 months, but 1 month later the pigment changes were clear.

The patient, still alive, has had amblyopia since the age of 9 years because of retinopathy. At the age of 14 years she had myopia (−7.0 D) and severe retinal atrophy. The visual acuity of her right eye was 0.2 (examined with a Snellen E chart), the visual field of her left eye was restricted by a large central scotoma, and visual acuity was poor (finger counting at 20 cm). The electroretinogram and the visually evoked potentials were normal at the age of 1½ years. Thereafter, both responses were gradually attenuated, first the visually evoked potentials and then the electroretinogram. By fluorescein angiography, the retina appeared hypopigmented and

Table I. Clinical features of 13 patients with LCHAD deficiency

	Patient No. (sex)					
	1 (F)	2 (M)	3 (F)	4 (M)	5 (M)	6 (F)
Age at onset	2 mo	3 mo	(3 days) 4 mo	(2 days) 1 mo	1 yr 9 mo	1 yr 5 mo
Age at death	3 mo	4 mo	9 mo	3 mo	1 yr 9 mo	14 yr (alive)
Pregnancy and delivery	GWk 40 + 4 BW 3000 gm	GWk 30 + 3 BW 1370 gm Preeclampsia, cesarean delivery	GWk 38 + 1 BW 3130 gm	GWk 36 + 5 BW 3140 gm	Normal	GWk 38 BW 2930 gm Geminus, preeclampsia
Neonatal period	Vomiting	Hypoglycemia, vomiting	Hypoglycemia, elevated transaminase values	Hypoglycemia, acidosis	No problems	Asphyxia, A 4/10
First presentation	Failure to thrive, jaundice, metabolic crisis	Failure to thrive, jaundice, hepatomegaly	Hypoglycemia metabolic crisis	Failure to thrive, hypotonia	Recurrent infections, cardiorespiratory arrest	Hypoglycemia, metabolic crisis
Hypoglycemia*	+	Neonatal	+	+	ND	+
Hepatopathy	++	++	+	+	+	+
Hypotonia	+	+	+	+	ND	+
Cardiomyopathy	+	+	+	+	ND	++
Retinopathy	-	+	+	-	ND	+
Seizures	-	-	Infantile spasms	-	-	+
Neuropathy	ND	ND	ND	ND	ND	+
Retardation	+	+	++	+	ND	+
Cause of death	Hepatic failure, GI hemorrhage	Hepatic failure cardiorespiratory failure	Pneumonia, cardiorespiratory failure	Pneumonia, cardiac arrest	Pneumonia, SIDS-like	Alive
EEG	Abnormal background	ND	Hypsarrhythmia	ND	ND	Abnormal background
Neuroradiology	CT: atrophy	CT: atrophy, white matter hypodensities	CT normal	ND	ND	MRI: parietooccipital lesions

The following are siblings: patients 3 and 4, patients 5 and 6, patients 7 and 8, and patients 10 and 11.

GWk, Gestational week; A, Apgar scores; BW, birth weight; SGA, small for gestational age; ND, not determined; GI, gastrointestinal; SIDS, sudden infant death syndrome; CT, computed tomography; MRI, magnetic resonance imaging; EEG, electroencephalogram.

*Range of blood glucose values: 0.4 to 1.9 mmol/L. Criteria sufficient for hepatopathy were hepatomegaly and elevated serum transaminase values.

Table II. Enzyme activities in fibroblast homogenates of the patients

Patient No.	LCHAD (C 16)	Controls ($\pm$ SD)	LCEH (C 12)	Controls ($\pm$ SD)	LCKAT (C 16)	Controls ($\pm$ SD)
1	12.8	54.6 $\pm$ 6	42.6	64 $\pm$ 12	9.8	11.3 $\pm$ 1.4
2	12.3	54.6 $\pm$ 6	40.8	64 $\pm$ 12	10.5	11.3 $\pm$ 1.4
6	15.6	54.6 $\pm$ 6	34	64 $\pm$ 14	5.8	11.3 $\pm$ 1.4
7	5.9	54.6 $\pm$ 6	42	64 $\pm$ 12	5.9	11.3 $\pm$ 1.4
12	12	54.6 $\pm$ 6	42.8	64 $\pm$ 12	10.9	11.3 $\pm$ 1.4
13	15.1	54.6 $\pm$ 6	35.1	64 $\pm$ 12	5.5	11.3 $\pm$ 1.4
4	13.5	39.2 $\pm$ 7.6	80.5*	65.1 $\pm$ 5.5	24.3	24.2 $\pm$ 4.6
9	10.27	39.2 $\pm$ 7.6	43.6*	65.1 $\pm$ 5.5	15.7	24.2 $\pm$ 4.6
10	26	86 $\pm$ 3	69.0	84 $\pm$ 29	12.4	23.6 $\pm$ 5.3

The activities are expressed as nanomoles per minute per milligram protein.

Different control values are presented because the specimens were analyzed in three different laboratories.

C16/C12, Carbon chain length of the substrate used; LCKAT, long-chain 3-ketoacyl-CoA thiolase; LCEH, long-chain enoyl-CoA hydratase.

*Carbon chain length of the substrate used C14.

Patient No. (sex)—cont'd						
7 (M)	8 (M)	9 (F)	10 (F)	11 (M)	12 (M)	13 (M)
8-10 mo 13 mo Born at term BW 2800 gm	4 mo 13 mo GWk 38 BW 2660 gm	1 wk 2½ wk GWk 41 + 2 BW 3260 gm	8 mo 9 mo GWk 36 + 6 BW 2530 gm	(2 days) 2 mo 8 mo GWk 37 + 2 SGA(BW 2230 gm), hepatosis, cesarean delivery	2 mo 6 mo GWk 35 + 4 BW 2010 gm Hepatosi, preeclampsia, cesarean delivery	6-10 mo 10 mo GWk 39 + 5 BW 3870 gm
No problems	No problems	No problems	Urinary protein +, dicarboxyl aciduria	Failure to thrive	Asphyxia, A 6/8, hypoglycemia	No problems
Hypotonia, hypoglycemia, hepatomegaly,	Somnolence, hepatomegaly	Metabolic crisis	Recurrent infections, failure to thrive	Failure to thrive, vomiting, hypotonia	Failure to thrive, hypotonia	Motor retardation, metabolic crisis
+	+	+	+	—	Neonatal	+
+	+	+	+	+	+	+
+	++	+	+	++	+	+
+	+	++	++	++	+	+
—	—	ND	+	—	+	+
—	—	+	—	—	—	+
ND	—	ND	ND	—	—	—
+	+	ND	+	+	+	+
Cardiac arrest	Pneumonia, cardiac arrest	Vomiting, cardiorespiratory failure	Respiratory failure	Respiratory failure	Diarrhea, cardiac arrest	Cardiorespiratory failure
Abnormal background ND	Abnormal background CT normal	Discharges ND	ND ND	ND ND	Normal MRI: punctate white matter lesions	Abnormal background MRI: normal

lacked pigment in the pigment epithelium, the choroidal vessels were atrophic, and pepperlike pigment aggregations were present.

NEUROLOGIC FINDINGS. One patient was motorically delayed for at least 4 months before the first acute episode. The early psychomotor development was normal in all other patients, but the patients lost what skills they had acquired at the time of the metabolic crises. Then they became hypotonic and had diminished or absent tendon reflexes. During the stable phase of the disease, the infants acquired new skills but the muscle hypotonia usually persisted. In one patient the hypotonia was so severe that spinal muscular atrophy was suspected. One patient had spasticity of the lower extremities at the age of 6 months. Two patients with epilepsy were mentally retarded. One of them had infantile spasms and microcephaly. All other patients appeared to be mentally alert despite slow motor development. None of the patients had evident symptoms of rhabdomyolysis.

The only surviving patient of this cohort has mild mental retardation and has occasional seizures of a short duration despite clonazepam and oxcarbazepine treatment. She also has peripheral neuropathy with absent tendon reflexes and abnormal findings on electroneurography (sensory responses could not be detected from the sural and superficial peroneal nerve). The tendon reflexes have been absent for many years, but the nerve conduction velocities remained normal until the age of 13 years.

Follow-up and treatment. Seven patients had a relapsing-remitting disease, and they died after two or three deterioration phases. Between exacerbations the patients were more or less free of symptoms. In the remaining six patients the disease was progressive. Only one patient is alive at the age of 14 years. The other patients died between the ages of 2 weeks and 21 months (mean, 7.7 months). Three patients had unexpected cardiac or respiratory arrest when already recovering at the hospital, and they died despite resuscitation

Table III. Laboratory results of 13 patients with LCHAD deficiency

Laboratory test	Reference values	Patient No.				
		1	2	3	4	5
Creatine kinase (S)	<270 IU/L	ND	148	174	584	ND
Transaminase (S) (AST/ALT)	<40 IU/L	320	435	392	153	ND
Bilirubin (S)	<20 µmol/L	325	380	168	163	ND
Thromboplastin time (P)	70-130%	11	15	43	62	ND
Lactate (B/SF)	0.7-1.8 mmol/L	6.4	11.7/6.0	3.5	3.6	ND
	0.6-2.7 mmol/L					
Ammonia (S)	10-60 µmol/L	216	99	98	110	ND
Carnitine (S; total/free)	Total 35-70 mmol/L	<10/<10	30.7/30.7	30.2/30.1	32.4/32.4	ND
	Free 25-60 mmol/L					
Acidosis (BE/HCO ₃)		-9.1/17	-14.5/13	-9.4/17	-23.2/14	ND
Hemoglobin (B)	gm/L	59	77	86	88	ND
Platelets (B)	150-400 E9/L	32	27	116	211	ND
Organic acids (U)						ND
3-Hydroxyaciduria		-	-	?	?	
Dicarboxylic aciduria		+	+	-	+	
Tyrosine metabolites		++	++	+	++	

Laboratory values shown are the most aberrant ones.

-, Excretion not increased; +, excretion moderately increased; ++, excretion greatly increased; ?, detection unreliable; BE, base excess; U, urinary; B, blood; S, serum; SF, spinal fluid; AST, aspartate transaminase; ALT, alanine transaminase; ND, not determined.

*Specimen taken after starting the carnitine therapy; dicarboxylic aciduria (adipic, suberic and sebacic acids); tyrosine metabolites (4-hydroxyphenyllactate and 4-hydroxyphenylpyruvate).

†During the initial phase of the disease; later during the low fat diet the excretion of urinary organic acids was normal.

and intensive care (Table I). In two of them the blood glucose concentration was normal, and in one it was not measured. The heart rate was not monitored at the time of death in any of these three patients. All but one of the patients who died were hospital inpatients at the time of death, and six of them were treated in the intensive care unit. One patient was found unresponsive at home and did not respond to resuscitation. At autopsy, pneumonia was detected in four patients; otherwise the autopsies were uninformative.

Eight patients (Nos. 1 to 4, 9 to 11, and 13) received carnitine substitution (100 mg/kg per day) during infancy. Intravenous glucose infusion was given instead of enteral feedings during the metabolic crises to all but patient 5, who died suddenly at home. Four patients (Nos. 3, 9, 10, and 13) had a relatively low-fat, high-carbohydrate diet or received infusions devoid of lipids during infancy. The only patient still alive has received carnitine supplementation with a low-fat, high-carbohydrate diet since the age of 4 years. In her diet, carbohydrates provide 50% to 60%, protein 20% to 25% and fat 20% of the total energy. She has received medium-chain fatty acid supplementation (50% of the fat) and frequent feedings, also nocturnally, for 2 years. Before the institution of the low-fat diet, she had recurrent metabolic crises with hypoglycemia and cardiomyopathy with heart failure. Since she has been on the diet, hypoglycemia and acute deteriorations have subsided but serum creatine kinase values have been intermittently elevated (up to 6000 U/L); there have been no symptoms of rhabdomyolysis. Increased

creatinine kinase values could not be connected with infections or prolonged exercise. The patient's heart and liver are no longer enlarged, and she is cardially compensated without therapy. She had severe epilepsy with electroencephalographic features of the Lennox syndrome, which has evolved into occasional tonic-clonic seizures; interictally, only mildly abnormal background tracings are present on the electroencephalogram.

Laboratory findings. Results of the enzyme analysis are shown in Table II. There was a severe LCHAD defect in the fibroblasts in all patients studied, and some had the activities of long-chain enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase partially reduced. In all nine patients who were analyzed (affected siblings were not analyzed), a homozygous C-for-G substitution at nucleotide 1528 was unambiguously detected.

The laboratory results of the patients are shown in Table III. All patients had intermittent metabolic acidosis. Plasma ketones were not determined systematically during the hypoglycemic episodes. Ketones were not detected in any urine specimens taken either during hypoglycemia or normoglycemia. On the first admission, several patients had anemia and thrombocytopenia, and they required blood transfusions. Bone marrow aspiration, performed in three patients, showed vacuolization of the proerythroblasts in one of them. Otherwise, the bone marrow findings were normal.

Analyses of the amino acids of the plasma were performed on samples from five patients (13 different analyses). In three

Patient No.—cont'd							
6	7	8	9	10	11	12	13
6848	77	ND	56750	1270	953	136	864
189	608	350	1780	237	257	191	230
8	5	15	ND	44	27	12	28
52	ND	22	11	49	63	81	33
3.2	2.3	5.6	19.8/15.5	3.2	2.9	1.9/2.4	2.1
ND	87	92	153	217	71	75	76
10.8/4.3	ND	ND	63/36.6*	<10/ND	12.9/12.9	<10/<10	<10/<10
-10.4/16	-10.7/16	-9.6/17	-21.9/?	-12.2/13.4	-12.8/17.7	-7.3/15	-8.3/18
78	103	84	112	77	69	81	84
285	435	230	62	54	372	205	96
	ND	ND					
?			-	+	?	++	+
+†			+	+	+	++	++
-			++	-	-	-	-

patients, moderate hypertyrosinemia with or without hypermethionemia was interpreted as evidence of nonspecific liver disturbance. The concentrations of branched-chain amino acids were decreased in two patients, and one patient had raised concentrations of several amino acids. Thus the findings of plasma amino acid analyses did not follow any characteristic pattern.

Nine of ten patients studied had increased urinary adipic, suberic, and sebacic acids, and the amounts of these organic acids in urine varied from close to normal, to 10-fold to 100-fold increases. Three patients had abnormal excretion of 3-hydroxylated sebacic, dodecanedioic, and tetradecanedioic acids. Five patients had an increased amount of tyrosine metabolites (4-hydroxyphenyllactate and 4-hydroxyphenylpyruvate) in the urine during phases of deterioration.

Neuroradiologic findings. The neuroradiologic findings are shown in Table I. The age of the patients at the time of the study varied from 4 to 9 months, with the exception of patient 6, who had her first brain magnetic resonance imaging at the age of 11 years and the second at 14 years. One patient had marked cortical and mild central atrophy without focal parenchymal changes. Mild central atrophy was also present in patient 2, who had symmetric periventricular white matter hypodensities in the frontal and peritrigonal regions by computed tomography. An MRI study of patient 12 showed small punctate periventricular lesions bilaterally, hyperintense both in T2-weighted and proton density-weighted images. Myelination was normal. At the age of 11 years, patient 6 had bilateral parieto-occipital infarctlike lesions within the boundary zones between the posterior and middle cerebral arteries; these changes involved the deep

white matter and overlying cortex. Three years later there was, additionally, mild local atrophy.

DISCUSSION

The discovery that LCHAD activity resides in the mitochondrial trifunctional protein, which simultaneously contains two other enzyme activities, has caused some nosologic confusion, and the clinical presentation in the distinct forms of these disorders is not clearly characterized. Our series of 13 patients with LCHAD deficiency caused by the homozygous G1528C mutation allows a more precise delineation of the clinical features of one entity of the trifunctional protein deficiency.

The overall clinical presentation of our patients was uniform: there are hepatopathy, hypoglycemia, muscle hypotonia, cardiomyopathy, and retinopathy, in accordance with previous reports on LCHAD deficiency. However, even though the mutation was the same in each case, there was considerable interindividual variation in the clinical course: patient 9 died after fulminant disease at the age of 2 weeks despite intensive treatment, whereas patient 7, alive at 14 years of age, has survived several hypoglycemic episodes. It is also noteworthy that two patients had progressive cholestatic hepatopathy, in contrast to the recurrent episodes of metabolic decompensation of the other patients. The clinical presentation varied even between siblings: the symptoms of patient 11 were dominated by severe muscle weakness, whereas his sister had cardiomyopathy. Likewise, IJlst et al.¹² reported a different phenotypic presentation in their two patients with LCHAD deficiency and the G1528C mutation. Infections, fasting, and dietary or treatment differences ap-

parently modify the clinical course and account for the clinical variation of patients with the same genotype.

In addition to the clinical variation, the enzyme activities of the trifunctional protein also varied in our patients, which is in accordance with the findings reported by Hagenfeldt et al.²⁷ Long-chain 3-ketoacyl-CoA thiolase and enoyl-CoA hydratase activities were decreased to a lesser extent than in patients with the combined defect of the trifunctional protein.^{6,7} However, the clinical course of patient 9 was as severe as that described in two patients with the combined defect,^{5,7} whereas two other patients with the combined deficiency exhibited only muscle symptoms and had a later presentation than our patients.⁶ Thus the reductions in the enzyme activities of cultured skin fibroblasts did not correlate with the severity of the clinical outcome.

The cholestatic hepatopathy seen in two of our patients is not typical of mitochondrial β -oxidation defects.²² Although peripheral neuropathy does occur more often in LCHAD deficiency than in other β -oxidation defects,^{8,15,21} neuropathy occurred only in the oldest patient in the present series. The neuropathy was not verified by electromyography until the patient was 13 years old, which could explain its absence in the younger patients. Although cardiomyopathy was not a major presenting feature in our patients, it could be detected by ultrasonography in most patients. Symptomatic cardiomyopathy appears to be more common in the defects involving β -oxidation of long-chain fatty acids.³⁴⁻³⁶ The HELLP syndrome, described previously in pregnant mothers of LCHAD-deficient babies, was not diagnosed in any of the mothers in this series.^{37,38}

Pigmentary retinopathy was a common finding even among young patients and was conspicuous, with pigment aggregations in what otherwise was a hypopigmented retina. Although pigmentary retinopathy has been detected in a few patients with LCHAD deficiency, we did not find reports of retinopathy in other fatty acid β -oxidation defects, including defects of long-chain fatty acid oxidation. Such retinal hypopigmentation seems to be an unusual feature, which we have not seen in other disorders—respiratory chain enzyme defects included.

Lactic acidosis, often mild, was almost invariably present in our patients during acute episodes. Lactic acidosis appears to be typical of LCHAD and trifunctional protein deficiencies but has also been detected in some neonatal patients with deficiency of short-chain or medium-chain acyl-CoA dehydrogenase.^{6,39,40} Anemia was present in many patients, and the bone marrow biopsy specimen from one patient showed vacuolization in proerythroblasts, which has not been reported earlier in patients with β -oxidation defects. The most common pathologic findings of urinary organic acids were nonketotic dicarboxylic aciduria and an increased excretion of tyrosine metabolites. The latter was so prominent in some

patients that tyrosinemia was suspected but could be excluded by the lack of succinylacetone in the urine. The excretion of 3-hydroxylated dicarboxylic acids was inconsistent, but the detection of these metabolites may have been unreliable in samples analyzed before the description of LCHAD deficiency. It is noteworthy that the laboratory abnormalities were often absent between acute episodes. On the basis of our series and earlier reports on LCHAD deficiency, the characteristic urinary organic acids excreted in LCHAD deficiency are 3-hydroxydicarboxylic acids of 6 to 14 carbons in length.^{10,41} Dicarboxylic acid chains of medium length are also often excreted by these patients, but this fact does not help to differentiate LCHAD deficiency from other β -oxidation defects.

After the dietary treatment was started, the symptoms of the only survivor, a 14-year-old girl, have become ameliorated and the urinary excretion pattern of organic acids has become normal. Although this patient is mentally retarded and has epilepsy, there are reports of patients with LCHAD deficiency with a normal development after appropriate dietary treatment.^{15,18,22,30} Still, the dietary treatment does not seem to prevent the pigmentary retinopathy, because our eldest patient has progressive retinopathy and the 14-year-old patient recently reported by IJlst et al.¹² had signs of visual deterioration.

On the basis of this small series of patients treated somewhat variably, it is difficult to give any definitive treatment recommendations. Variability in the clinical course of our patients can hardly be explained solely by the differences in therapy, because treatment of our oldest patient did not essentially differ from that of others during infancy. However, the patients who did not receive carnitine survived longer or at least as long as those treated with it during infancy, which suggests that carnitine therapy is not beneficial. Taken together, our series and previous reports on LCHAD deficiency indicate that although long-term dietary therapy has favorable effects, intravenous glucose infusions and avoidance of lipids during periods of acute symptoms do not prevent death in all cases. Whenever LCHAD or trifunctional protein deficiency is suspected, immediate institution of a low-fat, high-carbohydrate diet and avoidance of fasting seem prudent. The role of carnitine and medium-chain triglyceride supplementation in the diet warrants further trials, although the findings of Hagenfeldt et al.²⁷ suggest that patients with LCHAD deficiency are able to metabolize medium-chain triglycerides.²⁷

Although the clinical presentation of LCHAD deficiency is typical, it has many common features both with other β -oxidation defects and with respiratory chain defects. It is often helpful to analyze urinary organic acids when one wants to differentiate between these two groups of disorders, but the analysis may be complicated by the intermittent na-

ture of the abnormal excretion of the organic acids and by the use of analytically nonsatisfactory methods. The presence of retinopathy, neuropathy, and 3-hydroxydicarboxylic aciduria in LCHAD deficiency helps to differentiate this disorder from other β -oxidation defects. Low serum concentrations of carnitine and hypoglycemia are not characteristic of respiratory-chain enzyme defects.

Because there are an increasing number of reports on LCHAD deficiency, it seems that this disorder is much more common than was previously thought, a possibility that should be kept in mind when patients with symptoms of β -oxidation defects are investigated. An apparently high frequency of the G1528C mutation in LCHAD deficiency facilitates the diagnosis and the detection of the mutation frequency in various populations. In the future, the etiology of the distinct forms of trifunctional protein deficiency can be further penetrated because more patients will be studied.

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