

A FETAL FATTY-ACID OXIDATION DISORDER AS A CAUSE OF LIVER DISEASE IN PREGNANT WOMEN

JAMAL A. IBDAH, M.D., PH.D., MICHAEL J. BENNETT, PH.D., PIERO RINALDO, M.D., PH.D., YIWEN ZHAO, B.S., BEVERLY GIBSON, B.S., HAROLD F. SIMS, B.A., AND ARNOLD W. STRAUSS, M.D.

ABSTRACT

Background Acute fatty liver of pregnancy and the HELLP syndrome (hemolysis, elevated liver-enzyme levels, and a low platelet count) are serious hepatic disorders that may occur during pregnancy in women whose fetuses are later found to have a deficiency of long-chain 3-hydroxyacyl-coenzyme A (CoA) dehydrogenase. This enzyme resides in the mitochondrial trifunctional protein, which also contains the active site of long-chain 2,3-enoyl-CoA hydratase and long-chain 3-ketoacyl-CoA thiolase. We undertook this study to determine the relation between mutations in the trifunctional protein in infants with defects in fatty-acid oxidation and acute liver disease during pregnancy in their mothers.

Methods In 24 children with 3-hydroxyacyl-CoA dehydrogenase deficiency, we used DNA amplification and nucleotide-sequence analyses to identify mutations in the α subunit of the trifunctional protein. We then correlated the results with the presence of liver disease during pregnancy in the mothers.

Results Nineteen children had a deficiency only of long-chain 3-hydroxyacyl-CoA dehydrogenase and presented with hypoketotic hypoglycemia and fatty liver. In eight children, we identified a homozygous mutation in which glutamic acid at residue 474 was changed to glutamine. Eleven other children were compound heterozygotes, with this mutation in one allele of the α -subunit gene and a different mutation in the other allele. While carrying fetuses with the Glu474Gln mutation, 79 percent of the heterozygous mothers had fatty liver of pregnancy or the HELLP syndrome. Five other children, who presented with neonatal dilated cardiomyopathy or progressive neuromyopathy, had complete deficiency of the trifunctional protein (loss of activity of all three enzymes). None had the Glu474Gln mutation, and none of their mothers had liver disease during pregnancy.

Conclusions Women with acute liver disease during pregnancy may have a Glu474Gln mutation in long-chain hydroxyacyl-CoA dehydrogenase. Their infants are at risk for hypoketotic hypoglycemia and fatty liver. (N Engl J Med 1999;340:1723-31.)

©1999, Massachusetts Medical Society.

ACUTE fatty liver of pregnancy is a devastating disorder associated with substantial maternal and neonatal morbidity and mortality.¹⁻⁴ The HELLP syndrome (hemolysis, elevated liver-enzyme levels, and a low platelet count) is a more common maternal illness of late pregnancy and is associated with a better prognosis.^{2,5} The clinical and biochemical features of these two disorders overlap, suggesting that their underlying pathophysiologic mechanisms may be similar.

Long-chain 3-hydroxyacyl-coenzyme A (CoA) dehydrogenase catalyzes the third step in the β -oxidation of fatty acids in mitochondria (Fig. 1). The active site of this enzyme is located in the C-terminal domain of each of the four α subunits of the trifunctional protein that is associated with the inner mitochondrial membrane.^{6,7} The N-terminal domain of each of the α subunits is the site of long-chain 2,3-enoyl-CoA hydratase activity. The active site of long-chain 3-ketoacyl-CoA thiolase is in the four β subunits of the protein; this enzyme catalyzes the last step in the β -oxidation of fatty acids. The formation of a stable trifunctional-protein complex and the expression of all three enzymes require the presence of intact α and β subunits. The genes that encode the α and β subunits are closely linked on chromosome 2.⁸⁻¹⁰

Patients with either an isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or complete deficiency of the trifunctional protein (a loss of activity of all three enzymes) have been described.^{7,11} Several months after birth, children with these recessively inherited disorders present with nonketotic hypoglycemia during fasting and hepatic encephalopathy, which may progress to coma and death; cardiomyopathy; slowly progressive peripheral neuropathy; skeletal myopathy; or sudden, unexpected death.¹² Deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase has been found in the children of women who had acute fatty liver of pregnancy or

From the Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, N.C. (J.A.I., Y.Z.); the Departments of Pathology and Pediatrics, University of Texas Southwestern Medical Center, Dallas (M.J.B.); the Departments of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minn. (P.R.); and the Departments of Pediatrics (B.G., H.E.S., A.W.S.) and Molecular Biology and Pharmacology (A.W.S.), Washington University School of Medicine, St. Louis. Address reprint requests to Dr. Strauss at St. Louis Children's Hospital, 1 Children's Pl., St. Louis, MO 63110, or at strauss@kids.wustl.edu.

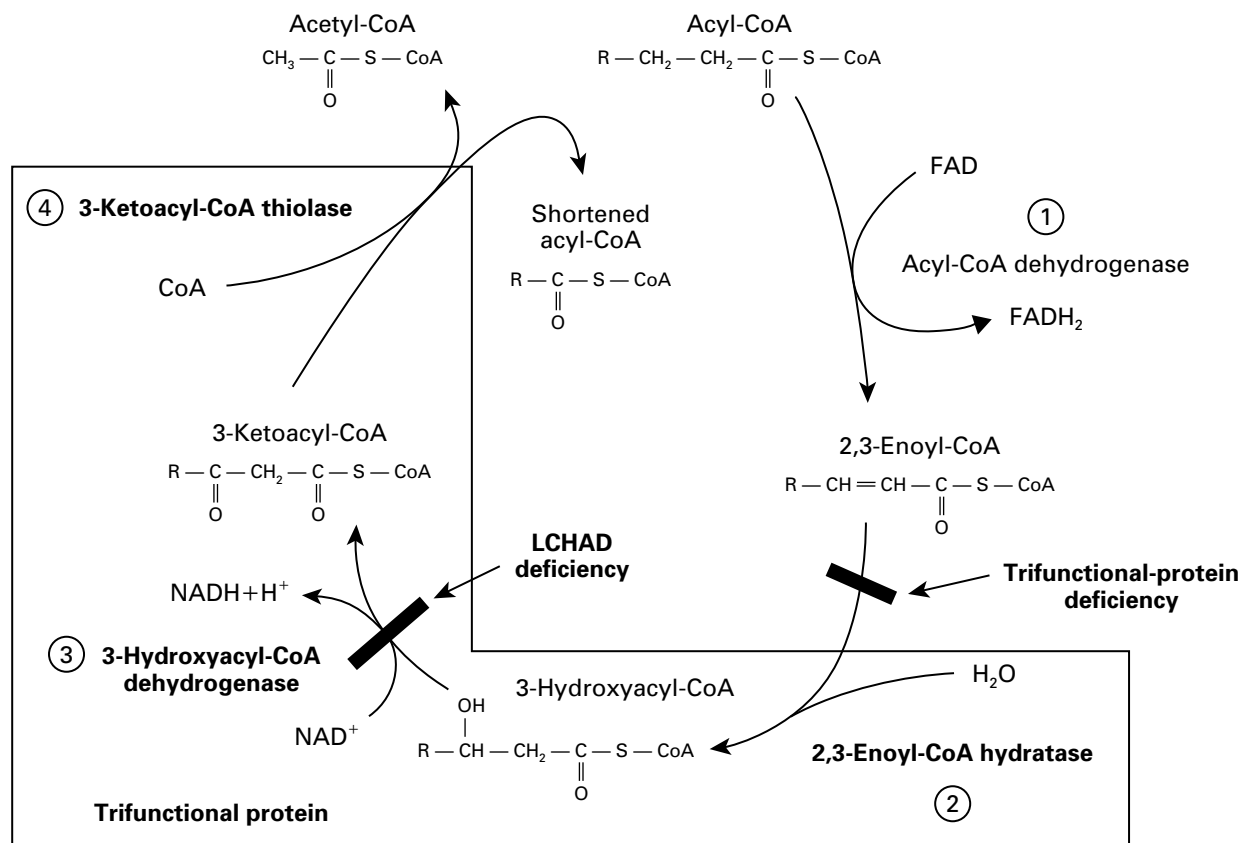


Figure 1. Biochemical Pathway of Mitochondrial Fatty-Acid Oxidation.

The four reactions of the pathway are shown in a clockwise loop. R represents the variable length of the fatty-acid chain; for example, palmitate, a typical long-chain substrate, contains 13 CH₂ groups. FAD denotes flavin adenine dinucleotide, FADH₂ the reduced form of FAD, and NAD⁺ nicotinamide adenine dinucleotide. Bars indicate the blockage of reactions as a result of isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) or complete deficiency of the trifunctional protein. In the oxidation of long-chain fatty acyl-CoA substrates that are 10 to 18 carbon residues in length, the trifunctional protein catalyzes the second, third, and fourth reactions (enclosed in the box) in the pathway. The second and third reactions are catalyzed by long-chain 2,3-enoyl-CoA hydratase and long-chain 3-hydroxyacyl-CoA dehydrogenase, respectively, which reside in the α subunit of the trifunctional protein. The fourth step, catalyzed by long-chain 3-ketoacyl-CoA thiolase, occurs on the β subunit of the trifunctional protein. The products of one cycle through the pathway are NADH, FADH₂, acetyl-CoA, and a fatty acyl-CoA shortened by two carbon residues. NADH and FADH₂ carry electrons to the respiratory chain (mitochondrial oxidative phosphorylation complexes) to generate ATP, and acetyl-CoA enters the citric-acid cycle. The shortened fatty acid reenters the fatty-acid oxidation spiral. Thus, in the oxidation of palmitate, seven rounds of the cycle produce eight acetyl-CoA molecules.

the HELLP syndrome during pregnancy.¹³⁻¹⁸ We have identified a mutation involving a change from glutamic acid to glutamine at amino acid residue 474 (Glu474Gln) of the α subunit of the trifunctional protein in three unrelated children whose mothers had acute fatty liver of pregnancy or the HELLP syndrome during pregnancy.⁸

However, not all women carrying fetuses with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency or trifunctional-protein deficiency have liver disease.¹⁹⁻²¹ Furthermore, liver disease in pregnant women occurs most often when the deficiency of enzymatic activity in the fetus is severe, although acute fatty liver of pregnancy has been reported in

two women who subsequently had healthy children with intermediate levels of long-chain 3-hydroxyacyl-CoA dehydrogenase, as determined by enzyme assay.¹⁸ The incidence of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency among the fetuses of women with acute fatty liver of pregnancy is unknown; some reports suggest that the association is rare.^{20,21} A recent review of liver disease during pregnancy² did not mention the relation between severe liver diseases in pregnant women and this fatty-acid oxidation disorder in their children.

Because the association between these two disorders is not well recognized^{2,3} and because it has important implications for mothers and their chil-

TABLE 1. CLINICAL MANIFESTATIONS OF A DEFICIENCY OF LONG-CHAIN 3-HYDROXYACYL-CoA DEHYDROGENASE OR TRIFUNCTIONAL PROTEIN IN 24 CHILDREN.

CLINICAL MANIFESTATION	NO. OF CHILDREN (%)	AGE AT PRESENTATION*	LCHAD DEFICIENCY†	TRIFUNCTIONAL-PROTEIN DEFICIENCY	DEATH
		mo		no. of children (%)	
Acute hepatic dysfunction	16 (67)	4.6±1.5	16	0	5
Acute cardiac dysfunction	3 (12)	0.4±0.2	0	3	2
Neuromuscular dysfunction	2 (8)	18±2.8	0	2	0
Multiple-organ dysfunction‡	3 (12)	24±27	3	0	1
Total	24 (100)	7.6±9.6	19 (79)	5 (21)	8 (33)

*Plus-minus values are means ±SD. Age at presentation is the age at the time of onset of symptoms or evaluation. In many children, the fatty-acid oxidation disorder was diagnosed months or years after onset.

†LCHAD denotes long-chain 3-hydroxyacyl-CoA dehydrogenase.

‡One of these three children became ill as molecular analysis, prompted by acute fatty liver of pregnancy in her mother, was under way.

dren,^{8,15,16} we examined the association between a deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or the trifunctional protein in children and severe liver disease in their mothers during pregnancy. We studied the frequency and molecular basis of this association in 24 families to correlate various genotypes to the clinical manifestations in the mothers and children.

METHODS

Subjects

We studied 24 families in which one child had clinical features suggestive of defects in fatty-acid oxidation, such as hypoketotic hypoglycemia; sudden, unexplained death with fatty liver at autopsy; or cardiomyopathy and abnormal biochemical or enzymatic findings^{7,10,12-16,22} consistent with an isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or complete deficiency of the trifunctional protein. Acute fatty liver of pregnancy or the HELLP syndrome was diagnosed by referring obstetricians according to standard criteria.¹⁻⁵ This study was approved by the institutional review boards of Washington University and Wake Forest University, and informed consent was obtained from the adult subjects and parents of the children.

Biochemical Studies

The activities of long-chain 3-hydroxyacyl-CoA dehydrogenase and long-chain 3-ketoacyl-CoA thiolase were measured in crude extracts of cultured skin fibroblasts or liver tissue, as previously described.^{8,19,23} Because the active sites of these two enzymes are in the α and β subunits, respectively, of the trifunctional protein, a deficiency of both activities is always associated with a lack of activity of long-chain 2,3-enoyl-CoA hydratase, whose active site is in the α subunit.^{7,9,23} A marked reduction in long-chain 3-ketoacyl-CoA thiolase activity, which is contained in the β subunit of the trifunctional protein, distinguishes the biochemical phenotypes of isolated long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and complete trifunctional-protein deficiency.^{8,23} Thiolase activity is very low (0 to 20 percent of normal levels) in patients with trifunctional-protein deficiency but is relatively preserved in those with isolated long-

chain 3-hydroxyacyl-CoA dehydrogenase deficiency (40 to 80 percent of normal levels).

Molecular Studies

DNA was isolated from cultured skin fibroblasts, frozen tissue, or peripheral-blood leukocytes from 24 children and various family members by means of alkaline lysis and protease digestion.²⁴ RNA was isolated from cultured skin fibroblasts or homogenized tissue according to a standard protocol.¹⁹ Polymerase-chain-reaction (PCR) amplification of the 20 exons of the gene encoding the α subunit and the 50 to 100 bp of flanking sequence was performed⁸ with [³²P]deoxycytosine triphosphate and 27-to-33-bp intronic primers designed on the basis of the previously identified genomic sequence⁸ (and unpublished data). The amplified fragments were then analyzed for single-strand conformation polymorphisms.²⁵

The Glu474Gln mutation was identified by *Pst*I digestion^{8,10,26} of the PCR-amplified product of exon 15. Nucleotide sequences were determined by the dideoxy chain-termination method, either manually with sulfur-35 radiolabeling or with an automated sequencer (model 373A, Applied Biosystems, Foster City, Calif.), with amplified exonic fragments by direct sequencing or after subcloning.

Dietary Treatment

Children were treated with frequent feedings of a low-fat diet in which the fats were medium-chain triglycerides. This regimen often prevents hypoketotic hypoglycemic liver dysfunction in patients with fatty-acid oxidation disorders.¹²

RESULTS

The clinical abnormalities and molecular mutations in 23 children who had isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or complete deficiency of the trifunctional protein and their mothers are shown in Tables 1 and 2. In Family 9, diagnosis of acute fatty liver of pregnancy in the mother prompted the analysis of the family members; the child subsequently had severe hepatic and cardiac dysfunction, and deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase was documented.

TABLE 2. MUTATIONS IN THE α SUBUNIT OF THE TRIFUNCTIONAL PROTEIN AND CLINICAL ABNORMALITIES IN THE 24 CHILDREN AND THEIR MOTHERS.*

FAMILY No.	CHILD			MOTHER	
	GENOTYPE	α SUBUNIT EXON	CLINICAL ABNORMALITIES	GENOTYPE†	CLINICAL ABNORMALITIES
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency					
1	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	AFLP
2	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	HELLP → AFLP‡
3	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	AFLP
4	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	HELLP
5	<i>Glu474Gln/Glu474Gln</i>	15/15	Mixed§	<i>Glu474Gln</i>	HELLP
6	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	AFLP
7	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	AFLP
8	<i>Glu474Gln/Glu474Gln</i>	15/15	Hepatic	<i>Glu474Gln</i>	None
9	<i>Glu474Gln/Asp67ter</i> ($\Delta 271-275$)	15/4	Mixed§	<i>Glu474Gln</i>	AFLP
10	<i>Glu474Gln/Asp67ter</i> ($\Delta 271-275$)	15/4	Hepatic	<i>Asp67ter</i>	AFLP
11	<i>Glu474Gln/A-2T</i>	15/5	Hepatic¶	<i>A-2T</i>	HELLP → AFLP‡
12	<i>Glu474Gln/Ala125ter</i> (TACC479-482AATA)	15/6	Hepatic	<i>Ala125ter</i> TACC479-482AATA	HELLP → AFLP‡
13	<i>Glu474Gln/Arg255ter</i>	15/9	Mixed	<i>Glu474Gln</i>	None
14	<i>Glu474Gln/Gln342ter</i>	15/12	Hepatic	<i>Glu474Gln</i>	HELLP
15	<i>Glu474Gln/$\Delta -4 \rightarrow 091$</i>	15/12	Hepatic	<i>Glu474Gln</i>	None
16	<i>Glu474Gln/$\Delta 1617 \rightarrow +1$</i>	15/15	Hepatic	<i>Glu474Gln</i>	AFLP
17	<i>Glu474Gln/Arg524ter</i>	15/16	Hepatic	<i>Arg524ter</i>	AFLP
18	<i>Glu474Gln/Leu620ter</i> ($\Delta T1964$)	15/18	Hepatic	<i>Leu620ter</i> ($\Delta T1964$)	None
19	<i>Glu474Gln/?</i>	15/?	Hepatic	?	AFLP
Complete trifunctional-protein deficiency					
20	<i>G+1A/A+3G</i>	3/3	Cardiac	<i>A+3G</i>	None
21	<i>A-2G/A-2G</i>	7/7	Cardiac	<i>A-2G</i>	None
22	<i>Arg640Cys/Arg640His</i>	19/19	Cardiac	<i>Arg640Cys</i>	None
23	<i>Val246Asp/Val246Asp</i>	9/9	Neuromuscular	<i>Val246Asp</i>	None
24	<i>Ile269Asn/Arg255ter</i>	9/9	Neuromuscular	<i>Ile269Asn</i>	None

*AFLP denotes acute fatty liver of pregnancy; HELLP the syndrome of hemolysis, elevated liver enzymes, and a low platelet count; *ter* a premature termination codon or nonsense mutation, and Δ a deletion. A question mark indicates that the data are unknown.

†Only the mutant allele is shown. In all the mothers, the second allele was normal.

‡The initial diagnosis of the HELLP syndrome was changed to acute fatty liver of pregnancy during the course of the illness.

§This child had combined hepatic and cardiac dysfunction.

¶This child also had mild cardiomyopathy.

||This child had hepatic abnormalities with peripheral neuropathy and myopathy.

Clinical Abnormalities

The 24 children with a deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or the trifunctional protein presented at a mean age of 7.6 months (range, birth to 60 months) with predominantly hepatic, cardiac, or neuromuscular abnormalities^{7,8,10-19,27,28} (Table 1). Seven of the children died very soon after presentation, and one died 18 months later despite treatment. The remaining 16 children were being treated with a modified diet at

the most recent follow-up visit.¹² At that time, eight of the surviving children were older than five years, and retinitis pigmentosa had developed in six.²⁹ Examination of the family histories revealed unexplained deaths in six siblings of the 24 children.

Nineteen children (79 percent) presented with acute hepatic dysfunction: 18 had hypoketotic hypoglycemia, hypotonia, hepatomegaly, hepatic encephalopathy, and high serum aminotransferase concentrations, and 1 had cholestasis of unknown cause and

hypocalcemia due to vitamin D deficiency; this child later died unexpectedly. The acute hepatic dysfunction progressed to coma and death in five children. Autopsies in two of these five children revealed massive hepatic steatosis, with necrosis, regenerative changes, and early nodules in one child. Two of the 19 children with acute hepatic dysfunction also had severe cardiomyopathy, and 1 had chronic myopathy. Four of these children have been described previously.^{8,17}

Three of the 24 children presented primarily with dilated cardiomyopathy in the first month of life. One, in whom complete deficiency of the trifunctional protein was diagnosed, had dramatic improvement when an appropriate diet and treatment for congestive heart failure were given but died suddenly at the age of 18 months during an episode of mild gastroenteritis.¹⁹ The second child died at the time of presentation; the third recovered completely and had normal cardiac function at the age of 10 years.

Two previously described^{27,28} children who had complete trifunctional-protein deficiency were first seen at the age of 18 months with hypotonia but no liver or cardiac involvement. Each child had slowly progressive peripheral neuropathy and myopathy.

Obstetrical and Family Histories

During their pregnancies with affected fetuses, 15 of the 24 women (62 percent) had acute fatty liver of pregnancy or the HELLP syndrome (Table 2); the other 9 women had normal pregnancies. In all 15 women with liver disease during pregnancy, the disorder developed during the third trimester, at a mean of 33.5 weeks of gestation. The HELLP syndrome was the initial diagnosis in three women. Severe acute fatty liver of pregnancy was the initial diagnosis in nine women and was proved by liver biopsy in two of them. Three other women presented with signs of the HELLP syndrome, but the diagnosis was later changed to acute fatty liver of pregnancy. In one woman with acute fatty liver of pregnancy, anoxic brain damage developed, which progressed to coma, a chronic vegetative state, and death. The other 14 women recovered quickly after emergency delivery.

Five of the 15 women with acute fatty liver of pregnancy or the HELLP syndrome (33 percent) were affected during their first pregnancy. In the other 10 women, liver disease was diagnosed during a second or later pregnancy, and their fetuses were found to have long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. These 10 women had had a total of 15 previous pregnancies, 3 of which had been complicated by acute fatty liver of pregnancy, and in all 3 cases the child had died suddenly and without explanation during infancy. One woman had had a miscarriage during the first trimester. The remaining 11 pregnancies had been normal, and the offspring were healthy.

Biochemical Abnormalities in the Children

The mean (\pm SE) 3-hydroxyacyl-CoA dehydrogenase activity measured with palmitoyl-CoA substrate in the 24 children was 26 ± 9 percent of normal levels, a finding similar to that reported for other children with these deficiencies.^{7-11,20,23} All 19 children with acute hepatic dysfunction had isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase and only partial loss of long-chain 3-ketoacyl-CoA thiolase activity (40 to 70 percent of normal). The children with cardiomyopathy or peripheral neuropathy and skeletal myopathy had complete deficiency of the trifunctional protein, with marked reduction of long-chain 3-ketoacyl-CoA thiolase activity (mean level of activity, 4 percent of normal).

Molecular Abnormalities

We used PCR amplification of all 20 exons of the α subunit gene and single-strand conformation polymorphism analysis to screen the 24 children for differences in genetic sequences.^{8,25} Mutations were detected on 47 of 48 alleles. Results in Families 5 and 9 (Fig. 2 and 3) illustrate this approach. The child in Family 5 presented at the age of eight months with severe myocardial dysfunction, left ventricular hypertrophy, hypoketotic hypoglycemia, and metabolic acidosis. His mother had had the HELLP syndrome during the pregnancy. The child was treated with a modified diet and inotropic drugs and was asymptomatic at the most recent follow-up visit, at the age of five years. These findings were consistent with homozygosity for the Glu474Gln mutation,^{8,26} which was confirmed by sequence analysis (data not shown).

Family 9 was tested solely because the mother had acute fatty liver of pregnancy with hepatic coma and renal failure. After emergency delivery and despite slight asphyxia during birth, the infant recovered. At the age of nine weeks, while molecular studies were under way, the infant had acute hypoketotic hypoglycemia and cardiogenic shock. In both the child and the father, exon 4 of the α subunit was found to be abnormal on single-strand conformation polymorphism analysis (Fig. 3A), a finding consistent with heterozygosity. This mutation (Fig. 3B) is a 5-bp deletion (Δ 271 through 275) that causes a frame shift and generates a premature termination codon at position 67 (aspartic acid) in the mature α subunit. The mother was heterozygous for the Glu474Gln mutation in exon 15.

Among the 24 children with a deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or of the trifunctional protein, we identified 17 different mutations involving 11 exons and affecting all domains of the α subunit gene (Table 2). Only 27 of the 47 abnormal alleles (57 percent) carried the Glu474Gln mutation; the other 20 alleles carried 16 different mutations. Thus, there was substantial heterogeneity

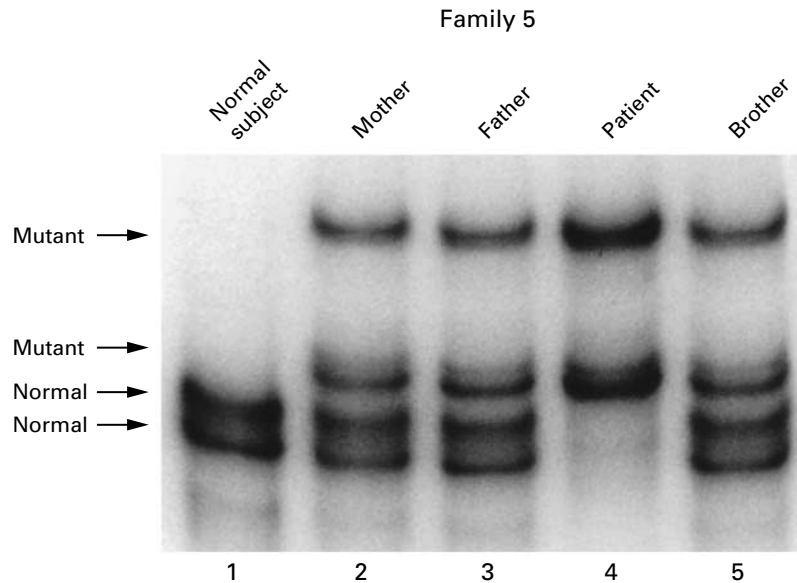


Figure 2. Results of Single-Strand Conformation Polymorphism Analysis in Family 5 and a Normal Subject.

Genomic DNA from exon 15 was extracted from blood leukocytes of the affected child, his parents, an apparently healthy sibling, and an unrelated normal subject, amplified,⁸ and analyzed by single-strand conformation polymorphism analysis as described previously.²⁵ In lane 1, the two normal bands represent the normal sequence. Lanes 2, 3, and 5 show two normal bands and two aberrantly migrating bands, representing DNA subsequently proved by sequence analysis to contain the Glu474Gln mutation. The parents and brother were therefore heterozygous for this mutation. Lane 4 shows only the two aberrant bands, and sequence analysis confirmed that the child was homozygous for the Glu474Gln mutation. His mother had had the HELLP syndrome during two pregnancies, one with the affected child and one with an older brother, who died unexpectedly at the age of five months. Her pregnancy with the healthy sibling was uncomplicated. No DNA from the presumably affected brother was available for analysis.

among the α subunit mutations, in contrast to results reported previously.^{10,16,20}

Correlation of the Children's Genotypes with Liver Disease in Their Mothers

All 19 children with an isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase had the Glu474Gln mutation on at least one allele. Eight unrelated children were homozygous for this mutation. The other 11 children were compound heterozygotes, with nine other known mutations, affecting exons 4, 5, 6, 9, 12, 15, 16, and 18. Of these mutations, three destroy a consensus splice site at an intron-exon junction and probably cause missplicing; four introduce a premature termination codon within an exon; and two, identified in three children, are deletions that alter the reading frame and introduce premature termination codons downstream.

All 15 mothers in whom acute fatty liver of pregnancy or the HELLP syndrome developed carried fetuses that were either homozygous for the Glu474Gln mutation or compound heterozygotes, with one allele carrying this mutation and a premature termination

codon or splice-site mutation on the other allele. Messenger RNA containing the Glu474Gln mutation is stably expressed,^{8,10} and in the resulting mutant protein the activity of long-chain 3-hydroxyacyl-CoA dehydrogenase is markedly reduced. In contrast, mutations introducing premature termination codons and splice-site mutations often result in low cytoplasmic concentrations of the corresponding messenger RNA.^{8,17} Of the 15 women who had liver disease during pregnancies with the affected children, only 10 had the Glu474Gln mutation; the other mothers had mutations causing premature termination codons (Families 10, 11, 12, and 17) or an unknown mutation (Family 19). Thus, the maternal genotype does not correlate with the development of acute fatty liver of pregnancy or the HELLP syndrome (Table 2).

Mutation analysis in six older siblings who were born after uncomplicated pregnancies revealed heterozygosity for the Glu474Gln mutation in four and a normal genotype in two. This result suggests that heterozygosity in the fetus is not associated with maternal liver disease during pregnancy.

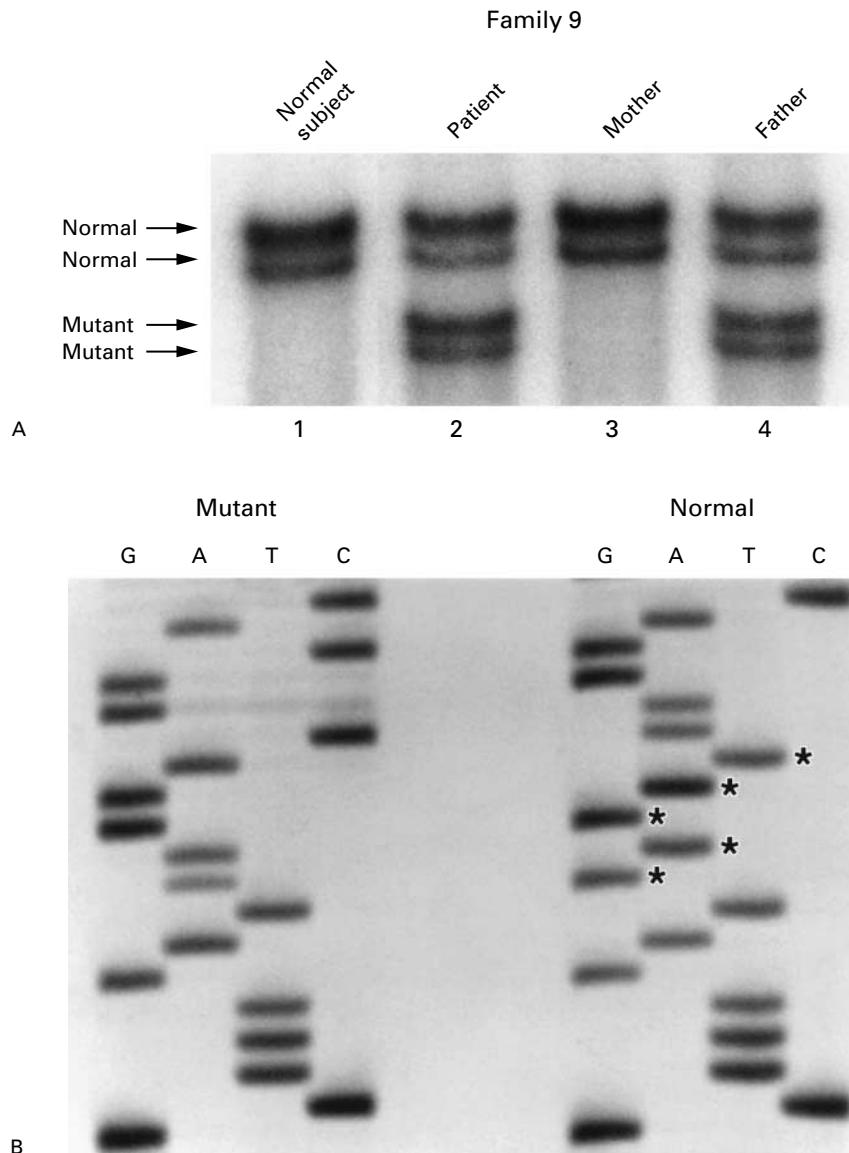


Figure 3. Results of Single-Strand Conformation Polymorphism Analysis in Family 9 (Panel A) and Sequence Analysis in the Affected Child (Panel B).

Genomic DNA from the affected child, her parents, and an unrelated normal subject was amplified with oligonucleotides flanking exon 4 and analyzed by single-strand conformation polymorphism analysis²⁵ (Panel A). Lane 1 shows the normal bands, a pattern also found in DNA from the child's mother (lane 3). The patterns for the affected child (lane 2) and her father (lane 4) reveal the two normal bands and two additional, aberrantly migrating bands, consistent with heterozygosity for a sequence mutation. While carrying the affected child, the mother had acute fatty liver of pregnancy. Sequence analysis (Panel B) revealed a 5-bp deletion ($\Delta 271-275$), which results in a premature termination codon at position 67 (Asp) of the mature α subunit. Amplified exon 4 genomic DNA from the affected child was subcloned, and eight clones were sequenced. The antisense sequences are shown. The five nucleotides marked by asterisks at normal positions 271 through 275 are deleted in the mutant allele. Heterozygosity for this mutation was confirmed by similar sequence studies in the father.

Among the five children with complete deficiency of the trifunctional protein (Table 2), three splice-site mutations, four missense mutations, and one termination-codon mutation were found, revealing the heterogeneity of genotype associated with this deficiency. None of these children had the Glu474Gln mutation. Two children (from Families 21 and 23), whose parents were consanguineous, were homozygous. One had an alteration in the consensus splice site preceding exon 7, in which the adenine at sequence position -2 was changed to guanine. The other had a missense mutation, a change from valine to aspartic acid at position 246, in exon 9. The other three children were compound heterozygotes. The first (from Family 20) had two different consensus splice-site mutations, one in exon 3 of each allele. The second (from Family 22) had two different mutations in the codon for arginine at residue 640. The third (from Family 24) had a missense mutation, a change from isoleucine at residue 269 to asparagine, and a termination mutation, both in exon 9. None of the mothers of these five children had liver disease while pregnant with the affected children. All five children with complete trifunctional-protein deficiency had cardiomyopathy or neuromuscular abnormalities; none had hepatic dysfunction.

DISCUSSION

Our results provide strong evidence that a fetal-maternal interaction can cause life-threatening hepatic disease in a woman who is carrying a fetus with isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase, a recessively inherited disorder of mitochondrial fatty-acid oxidation. Specifically, a woman whose affected fetus has the Glu474Gln mutation on one or both alleles of the α subunit of the trifunctional protein is likely to have acute fatty liver of pregnancy or the HELLP syndrome. In our study, none of the mothers had either of these disorders while carrying a fetus with one or two wild-type alleles of the α subunit or with complete trifunctional-protein deficiency.

Little is known about the mechanism of the association between isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase in a fetus with the Glu474Gln mutation on at least one allele and liver disease in the mother during the pregnancy. We hypothesize that in the presence of the Glu474Gln mutation, long-chain 3-hydroxyacyl metabolites produced by the fetus or placenta accumulate in the mother and are highly toxic to the liver; this reaction is perhaps exaggerated by the decreased metabolic utilization of fatty acids during pregnancy.³⁰

In contrast to previous reports^{10,16,20} indicating that 90 to 100 percent of abnormal alleles of the gene encoding the α subunit of the trifunctional protein had the Glu474Gln mutation, we found 17 different mutations among the 24 children in our

study. In the 19 children with isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase, 71 percent of alleles had the Glu474Gln mutation, and none of the 10 alleles (all of which were abnormal) in the 5 children with trifunctional-protein deficiency had this mutation. Clearly, mutations in the genes encoding trifunctional protein are heterogeneous.

In a molecular-screening study⁸ of 351 normal subjects, we found that 2 were heterozygous for the Glu474Gln mutation. If this group of subjects is representative of the general population, then isolated deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase would occur once in every 62,000 pregnancies, and either trifunctional-protein or long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency would occur once in 38,000 pregnancies.

The morbidity and mortality associated with liver disease in pregnant women are substantial.^{1-5,16} Our results indicate that fetal deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase is one cause of these maternal diseases. Because this disorder is recessive, one in four fetuses will be affected, and the rate of recurrence of maternal liver disease will be 15 to 25 percent¹⁶ (and unpublished data). Prenatal diagnosis by enzyme assay of amniocytes has been described,³¹ and we have performed molecular diagnoses by analyzing DNA from chorionic-villus samples (unpublished data).

The mortality rate among children with a deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase or complete deficiency of the trifunctional protein has been reported to be 75 to 90 percent.^{9,10,16,20} In contrast, 67 percent of the affected children in our study were alive and receiving dietary treatment at the most recent follow-up, and most were able to attend school. Dietary treatment of children with fatty-acid oxidation disorders dramatically reduces morbidity and mortality.¹² In Family 9, examination and prospective diagnosis of fetal long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency were prompted solely by the presence of acute fatty liver of pregnancy in the mother.

These results strongly suggest that all women with acute fatty liver of pregnancy or the HELLP syndrome, as well as their partners and children, should undergo molecular diagnostic testing. Because these women may have one of a variety of mutations, testing only for the Glu474Gln mutation in women with acute fatty liver of pregnancy²¹ is not sufficient to rule out long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency in the fetus or other family members. However, because their fetuses always have at least one allele with the Glu474Gln mutation, testing of both the mother and the father or of the newborn infant is sufficient to diagnose the deficiency.

In summary, we have determined the molecular basis of a fetal-maternal interaction that causes liver disease in pregnant women whose fetus has a defi-

ciency of long-chain 3-hydroxyacyl-CoA dehydrogenase. Deficiency of this enzyme creates a life-threatening situation for both fetus and mother. Women with acute fatty liver of pregnancy or the HELLP syndrome, as well as their partners and children, should undergo molecular testing for the Glu474Gln mutation of the α subunit of the trifunctional protein. Prospective diagnosis of this deficiency should allow proper genetic counseling about the risk of occurrence of severe liver disease in the mother and lifesaving therapy for the affected child.

Supported by grants (AM-20407 and DK-02574) from the National Institutes of Health (NIH), by an institutional training grant (5T32 DK-07130, to Dr. Ibdah) from the NIH, and by an Innovative Seed Grant in Clinical Research in Liver Diseases from the American Digestive Health Foundation.

REFERENCES

- Riely CA, Latham PS, Romero R, Duffy TP. Acute fatty liver of pregnancy: a reassessment based on observations in nine patients. *Ann Intern Med* 1987;106:703-6.
- Knox TA, Olans LB. Liver disease in pregnancy. *N Engl J Med* 1996; 335:569-76.
- Reyes H, Sandoval L, Wainstein A, et al. Acute fatty liver of pregnancy: a clinical study of 12 episodes in 11 patients. *Gut* 1994;35:101-6.
- Usta IM, Barton JR, Amon EA, Gonzales A, Sibai BM. Acute fatty liver of pregnancy: an experience in the diagnosis and management of fourteen cases. *Am J Obstet Gynecol* 1994;171:1342-7.
- Sibai BM, Ramadan MK, Usta I, Salama M, Mercer BM, Friedman SA. Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes, and low platelets. *Am J Obstet Gynecol* 1993;169: 1000-6.
- Uchida Y, Izai K, Orii T, Hashimoto T. Novel fatty acid β -oxidation enzymes in rat liver mitochondria. II. Purification and properties of enoyl-coenzyme A (CoA) hydratase/3-hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase trifunctional protein. *J Biol Chem* 1992;267:1034-41.
- Jackson S, Kler RS, Bartlett K, et al. Combined enzyme defect of mitochondrial fatty acid oxidation. *J Clin Invest* 1992;90:1219-25.
- Sims HF, Brackett JC, Powell CK, et al. The molecular basis of pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with maternal acute fatty liver of pregnancy. *Proc Natl Acad Sci U S A* 1995; 92:841-5.
- Ushikubo S, Aoyama T, Kamijo T, et al. Molecular characterization of mitochondrial trifunctional protein deficiency: formation of the enzyme complex is important for stabilization of both alpha- and beta-subunits. *Am J Hum Genet* 1996;58:979-88.
- Ijlst L, Ruiten JPN, Hoovers JMN, Jakobs ME, Wanders RJA. Common missense mutation G1528C in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Clin Invest* 1996;98:1028-33.
- Wanders RJA, Duran M, Ijlst L, et al. Sudden infant death and long-chain 3-hydroxyacyl-CoA dehydrogenase. *Lancet* 1989;2:52-3.
- Rinaldo P, Raymond K, al-Odaib A, Bennett MJ. Clinical and biochemical features of fatty acid oxidation disorders. *Curr Opin Pediatr* 1998; 10:615-21.
- Schoeman MN, Batey RG, Wilcken B. Recurrent acute fatty liver of pregnancy associated with a fatty-acid oxidation defect in the offspring. *Gastroenterology* 1991;100:544-8.
- Jackson S, Bartlett K, Land J, et al. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Res* 1991;29:406-11.
- Wilcken B, Leung K-C, Hammond J, Kamath R, Leonard JV. Pregnancy and fetal long-chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency. *Lancet* 1993;341:407-8.
- Tyni T, Ekholm E, Pihko H. Pregnancy complications are frequent in long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Am J Obstet Gynecol* 1998;178:603-8.
- Isaacs JD, Sims HF, Powell CK, et al. Maternal acute fatty liver of pregnancy associated with fetal trifunctional protein deficiency: molecular characterization of a novel maternal mutant allele. *Pediatr Res* 1996;40:393-8.
- Treem W, Shoup ME, Hale DE, et al. Acute fatty liver of pregnancy, hemolysis, elevated liver enzymes, and low platelets syndrome, and long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Am J Gastroenterol* 1996;91:2293-300.
- Brackett JC, Sims HF, Rinaldo P, et al. Two α subunit donor splice site mutations cause human trifunctional protein deficiency. *J Clin Invest* 1995;95:2076-82.
- Tyni T, Palotie A, Viinikka L, et al. Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency with the G1528C mutation: clinical presentation in 13 patients. *J Pediatr* 1997;130:67-76.
- Mansouri A, Fromenty B, Durand F, Degott C, Bernuau J, Pessayre D. Assessment of the prevalence of genetic metabolic defects in acute fatty liver of pregnancy. *J Hepatol* 1996;25:781.
- Bennett MJ. The laboratory diagnosis of inborn errors of mitochondrial fatty acid oxidation. *Ann Clin Biochem* 1990;27:519-31.
- Wanders RJA, Ijlst L, Poggi F, et al. Human trifunctional protein deficiency: a new disorder of mitochondrial fatty acid beta-oxidation. *Biochem Biophys Res Commun* 1992;188:1139-45.
- Ausubel FM, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. *Current protocols in molecular biology*. New York: John Wiley, 1987: 1.71-1.715.
- Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A* 1989;86:2766-70.
- Ijlst L, Wanders RJA, Ushikubo S, Kamijo T, Hashimoto T. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation in the alpha-subunit of the mitochondrial trifunctional protein. *Biochim Biophys Acta* 1994;1215: 347-50.
- Tein I, Donner EJ, Hale DE, Murphy EG. Clinical and neurophysiologic response of myopathy and neuropathy in long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency to oral prednisone. *Pediatr Neurol* 1995;12: 68-76.
- Vici CD, Burlina AB, Bertini E, et al. Progressive neuropathy and recurrent myoglobinuria in a child with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1991;118:744-6.
- Tyni T, Kivelä T, Lappi M, Summanen P, Nikoskelainen E, Pihko H. Ophthalmologic findings in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency caused by the G1528C mutation: a new type of hereditary metabolic chorioretinopathy. *Ophthalmology* 1998;105:810-24.
- Grimbert S, Fromenty B, Fisch C, et al. Decreased mitochondrial oxidation of fatty acids in pregnant mice: possible relevance to development of acute fatty liver of pregnancy. *Hepatology* 1993;17:628-37.
- Perez-Cerda C, Merinero B, Jimenez A, et al. First report of prenatal diagnosis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency in a pregnancy at risk. *Prenat Diagn* 1993;13:529-33.