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The molecular basis of pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with maternal acute fatty liver of pregnancy

(mitochondrial/β-oxidation/fatty acids/sudden death)

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ABSTRACT **Mitochondrial long chain fatty acid β-oxidation provides the major source of energy in the heart. Deficiencies of human β-oxidation enzymes produce sudden, unexplained death in childhood, acute hepatic encephalopathy, skeletal myopathy, or cardiomyopathy. Long chain 3-hydroxyacyl-CoA dehydrogenase [LCHAD; long-chain-(*S*)-3-hydroxyacyl-CoA:NAD$^+$ oxidoreductase, EC 1.1.1.211] catalyzes the third step in β-oxidation, and this activity is present on the C-terminal portion of the α subunit of mitochondrial trifunctional protein. We used single-stranded conformation variance analysis of the exons of the human LCHAD (α subunit) gene to determine the molecular basis of LCHAD deficiency in three families with children presenting with sudden unexplained death or hypoglycemia and abnormal liver enzymes (Reye-like syndrome). In all families, the mothers had acute fatty liver and associated severe complications during pregnancies with the affected infants. The analysis in two affected children revealed a G to C mutation at position 1528 (G1528C) of the α subunit of the trifunctional protein on both alleles. This is in the LCHAD domain and substitutes glutamine for glutamic acid at position 474 of mature α subunit. The third child had this G1528C mutation on one allele and a different mutation (C1132T) creating a premature termination codon (residue 342) on the second allele. Our results demonstrate that mutations in the LCHAD domain of the trifunctional protein α subunit in affected offspring are associated with maternal acute fatty liver of pregnancy. This is the initial delineation of the molecular basis of isolated LCHAD deficiency.**

Long chain 3-hydroxyacyl-CoA dehydrogenase [LCHAD; long-chain-(*S*)-3-hydroxyacyl-CoA:NAD$^+$ oxidoreductase, EC 1.1.1.211] activity catalyzes the third step in the mitochondrial β-oxidation spiral. β-Oxidation provides the major source of energy in heart and skeletal muscle and is essential for intermediary metabolism in the liver. The β-oxidation of long chain fatty acids requires four sequential enzyme activities: the initial fatty acyl-CoA dehydrogenase, a 2,3-enoyl-CoA hydratase, a 3-hydroxyacyl-CoA dehydrogenation step, and a 3-ketoacyl-CoA thiolase. With each cycle through the spiral, the long chain fatty acid substrate is reduced by two carbons, producing acetyl CoA. Because of the range of fatty acid substrate lengths, each of these reactions is catalyzed by two to four separate enzymes encoded by individual nuclear genes and with differing substrate specificities.

Inherited deficiencies (1) of β-oxidation enzymes usually present in the neonatal period or in early childhood, frequently after a period of fasting or viral illness, which reduces normal caloric intake. The presenting manifestations include (*i*) a Reye-like syndrome onset with hypoglycemia, fatty liver, and coma, which may prove fatal; (*ii*) sudden, unexplained death; (*iii*) dilated or hypertrophic cardiomyopathy (2); or (*iv*) skeletal myopathy.

For long chain substrates, a trifunctional protein catalyzes the last three steps of β-oxidation (3). This protein is a heterocomplex of four α and four β subunits, which are encoded by two nuclear genes. The cloning and expression of rat trifunctional protein subunits (4) allowed assignment of the long chain 3-ketoacyl-CoA thiolase activity to the β subunit. The α subunit contains the long chain enoyl-CoA hydratase and LCHAD activities. Using immunologic and biochemical techniques, two groups (5, 6) proved that LCHAD deficiency has two different biochemical phenotypes. The first includes deficiency of all three enzyme activities of the trifunctional protein, with loss of both α and β subunits by immunoblotting studies. The second biochemical phenotype has isolated LCHAD deficiency with preservation of the long chain enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activities and normal amounts of α- and β-subunit proteins.

We (7) and others (8) recently noted that isolated LCHAD deficiency in children may be associated with severe maternal illness occurring during pregnancies with affected fetuses. These maternal illnesses include the acute fatty liver of pregnancy (AFLP) syndrome; the hypertension or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; and hyperemesis gravidum. The AFLP syndrome is characterized by anorexia, nausea, vomiting, abdominal pain, and jaundice in the third trimester. Fulminant liver failure and death may occur. HELLP syndrome is more common and may represent the severe end of the spectrum of preeclampsia. In both syndromes, microvesicular fatty infiltration of maternal liver occurs, a pathologic picture similar to that in children with fatty acid oxidation defects (9).

We used single-stranded conformation variance (SSCV) analysis of the exons encoding the α subunit of trifunctional protein to elucidate the molecular defects in three families with children with isolated LCHAD deficiency and mothers with AFLP or HELLP. We report two different mutations in the LCHAD portion of the α subunit as causes of this disease.

MATERIALS AND METHODS

Cell Lines, Enzyme Assays, DNA, and RNA Isolation. Skin fibroblasts were maintained in minimal essential medium

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Abbreviations: LCHAD, long chain 3-hydroxyacyl-CoA dehydrogenase; HELLP, hemolysis, elevated liver enzymes, and low platelets; AFLP, acute fatty liver of pregnancy; SSCV, single-stranded conformation variance; UTR, untranslated region.

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supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics. LCHAD (the reverse reaction) and long chain 3-ketoacyl-CoA thiolase activities were measured as described (10). RNA was extracted from fibroblasts with guanidine isothiocyanate. DNA was isolated from fibroblasts or blood by alkaline lysis and protease digestion (11).

α-Subunit cDNA and Gene Isolation and Characterization. Primers from the rat α-subunit cDNA (4) (sense, bp 1710–1737; antisense, bp 1933–1964) were used to amplify a segment of human heart mRNA encoding the α subunit. This fragment of the human cDNA was used to initially screen a human cardiac cDNA library (Stratagene). The complete nucleotide sequences of positive and purified cDNA clones were obtained by the dideoxynucleotide chain-termination method. This human α-subunit cDNA sequence was used to generate primers for PCR-based screening of a human genomic P1 plasmid library (12). A single genomic clone containing exon sequences from bp 677 through the 3′ end of the cDNA was isolated. Using this sequence, a second set of primers was designed, the P1 library was rescreened, and a clone with the first exon was isolated. Fragments containing all exons were subcloned, and the nucleotide sequences of each exon and 100–300 bp of flanking introns of this human α-subunit gene were determined.

SSCV Analysis of α-Subunit Exons. PCR amplification of genomic DNA was done with the following primer pairs containing artificially engineered *Hin*dIII restriction sites, as indicated by underlined nucleotides:

exon12, sense 5′-agataagcttaccgaaagtcatttcatccc-3′

antisense 5′-cattctaagcttgcaaagagagagagcaggc-3′;

exon15, sense 5′-tctgttgaagcttttggttccccttgccag-3′

antisense 5′-ctctgcaagcttctgttatacagccccttac-3′

The amplified products were analyzed for SSCV by nondenaturing gel electrophoresis according to the recommended protocol, except that separation was at 35 W for 4 h at 4°C (13). Amplified exonic DNA was placed into plasmid vectors, and complete nucleotide sequence analysis was obtained on 4–12 subclones from each individual. For restriction enzyme analysis of mutations, exon 15 was amplified with two different oligonucleotides (sense, 5′-cctagcagagaaggaagcttctcaggttcctc-3′ antisense, 5′-agtcttattagaagcttttcaaaaactctgc-3′) and exon 12 was amplified with those listed above. After amplification and restriction digestion with *Pst* I (exon 15) or *Dde* I (exon 12), the products were analyzed by electrophoresis on a 2% agarose gel.

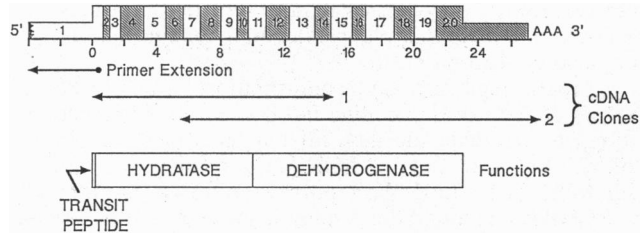

FIG. 1. Structure of human trifunctional protein α-subunit cDNA. α-Subunit cDNA clone 2 was isolated from a human heart library by probing with a 250-bp amplified fragment. Clone 1 was isolated by probing with a 5′ piece of clone 2. Complete sequence determination with comparison to the rat α-subunit cDNA (4) allowed delineation of the start codon, stop codon, and the functional regions illustrated as transit peptide, hydratase domain, and LCHAD domain. A poly(A) tail of 46 bp was present on clone 2. The transcription start site and size of the 5′ UTR remain unknown. UTRs are illustrated in smaller boxes. Determination of intron–exon boundaries in the human α-subunit gene allowed assignment of the exons, here drawn to scale in larger boxes.

Bacterial Expression of the LCHAD Domain. Synthetic sense primer (5′-AAAGGCCTTGCATATGGGACTCTACCATGGT-3′) containing an artificial *Nde* I site corresponding to amino acid 304 and an antisense oligonucleotide (5′-gactgagtcgacggcatgaggcctgctca-3′) from the 3′ untranslated region (UTR) with an engineered *Sal* I site were used to amplify DNA from bp 1008–2316 with α-subunit cDNA clone 2 plasmid DNA (Fig. 1) as the template. The amplified fragment was placed into the *Nde* I and *Sal* I sites of the bacterial expression plasmid pet21a (Novagen). The G1528C mutation was created by PCR-mediated, site-specific mutagenesis using the clone 2 cDNA plasmid as substrate and placed into pet21a. After transformation into *Escherichia coli* strain BL21(DE3) and induction with isopropyl β-D-thiogalactoside for 4 h, bacteria were lysed by sonication and subjected to centrifugation at 5000 × *g* for 10 min. The supernatants were assayed for LCHAD activity (10).

RESULTS

Isolation and Characterization of the α-Subunit cDNA, Including the LCHAD Domain. We used oligonucleotides from the rat α-subunit cDNA of trifunctional protein reported by Hashimoto and coworkers (4) to amplify part of this subunit with human heart mRNA as the template. The labeled 250-bp fragment was used to probe a human heart cDNA library. The longest human α-subunit cDNA clone (Fig. 1, clone 2) reacting with this probe was 2.2 kb long and contained a poly(A) tail. Because the human mRNA is ≈3 kb long, to obtain the entire coding region, we reprobed the library with a 5′ fragment of cDNA clone 2 and isolated an overlapping α-subunit cDNA of 1.6 kb (Fig. 1, clone 1), which proved to contain the translation start codon. Sequence analyses showed that these overlapping clones contained 6 bp of 5′ UTR, 2289 bp of coding region designating a 36-amino acid transit peptide and a 727-amino acid mature protein, 385 bp of 3′ UTR, and a poly(A) tail of 46 bp. As our work was in progress, Hashimoto and coworkers reported (14) the human α-subunit cDNA. Comparison of their reported sequence to ours reveals complete identity except for (*i*) the presence in our sequence of 9 additional bp in the 3′ UTR followed by the poly(A) tail, defining the 3′ end of the α-subunit cDNA, and (*ii*) a single difference in the coding region (bp 2081), which is a T in our sequence, changing the reported serine at amino acid 658 to a leucine. In our studies of genomic DNA from several individuals, this nucleotide is always a T, suggesting that this may be a polymorphism related to different racial backgrounds. The human α-subunit cDNA is 84% identical at the nucleotide level to the rat α-subunit cDNA in the coding region and 89% identical at the amino acid level. It is noteworthy that the NAD-binding consensus domain (GAGLMGAGIAQ) at amino acids 332–342 is absolutely conserved in both species. Surprisingly, the 3′ UTRs of rat (4) and human α-subunit mRNAs are highly homologous, with 68% nucleotide sequence identity over 350 bp. Such conservation across species is unusual. Using the human α-subunit cDNA, we characterized the gene, which contains 20 exons spanning >52 kb. All intron–exon boundaries conform to consensus splice site sequences.

Patient and Family Histories. The medical history of family A has been reported (7). The mother (AI-2) presented in late gestation with severe HELLP syndrome necessitating delivery by caesarean section. Severe maternal hemorrhage and hypovolemic shock resulted in maternal anoxic encephalopathy and irreversible coma. The female infant (AII-1) developed transient neonatal hypoglycemia but then did well until 4 months of age when severe liver failure, associated with biopsy-proven fatty infiltration, occurred. She recovered from coma and respiratory distress syndrome and is currently well. Biochemical and enzymatic assays (Table 1) revealed isolated LCHAD deficiency. The mother's two previous pregnancies were un-

Table 1. LCHAD activities in fibroblasts from members of three families

Individual	Activity vs. substrate, nmol·min^{-1}·mg^{-1}	
	Acetoacetyl CoA	3-Ketopalmitoyl CoA
AI-1 (father)	42	17
AI-2 (mother)	57	19
AII-1 (affected child)	26	8
BI-1 (father)	39	18
BI-2 (mother)	50	16
BII-1 (affected child)	50	10
CI-1 (mother)	28	11
CI-2 (father)	38	16
CII-1 (affected child)	32	10
Normal controls (n = 44)	44.4 ± 12.7	28.9 ± 6.5
LCHAD heterozygotes (n = 6)	40.4 ± 11.3	16.7 ± 2.6
LCHAD homozygotes (n = 9)	33.6 ± 9.2	9.1 ± 2.5

Activity measurements are averages of 5–10 different enzymatic assays. Numbers for controls at the bottom of the table do not include individuals within the three families.

complicated and resulted in healthy males. Enzymatic assays of the parents' fibroblasts revealed LCHAD activities in the heterozygous range (Table 1).

The mother of family B (BI-2) had HELLP and AFLP during her first pregnancy, which resulted in premature, caesarean delivery of a female infant. At 5 months of age, the infant developed lethargy, anorexia, profound hypoglycemia, and metabolic acidosis and died. The autopsy revealed fatty infiltration of the liver. At 28 weeks of her second pregnancy, the mother had preeclampsia and HELLP syndrome. The premature female infant (BII-1) recovered from hyaline membrane disease secondary to prematurity. Her fibroblasts were examined for β-oxidation defects because of the maternal and family history, and isolated LCHAD deficiency was documented. The parents' cells had heterozygous levels of LCHAD activity (Table 1). Both mother and infant are currently well.

The mother in family C presented at 30 weeks gestation with HELLP syndrome and was delivered by caesarean section. At 4 months of age, the female infant (CII-1) developed hypoglycemia and lethargy. An organic acid profile demonstrated 3-hydroxyacyldicarboxylic aciduria, and enzymatic assay (Table 1) confirmed isolated LCHAD deficiency. The child is now well. Assay of the father's (CI-2) cells revealed LCHAD activity in the heterozygous range. The mother's (CI-1) cells had quite low LCHAD activity, in the range of affected individuals.

Within these families, there is no history of consanguinity. All three families reside in the United States and are of northern European ancestry.

Thus, the three mothers presented late in pregnancy with AFLP or HELLP syndrome, and one remains in coma from complications of her pregnancy. The three surviving infants of these pregnancies are all affected with LCHAD deficiency. Although all three are now well children, one (AII-1) had life-threatening liver failure, the second (BII-1) had a sibling who died with hepatic steatosis consistent with LCHAD deficiency, and the third (CII-1) had symptomatic hypoglycemia. We conclude that isolated fetal LCHAD deficiency is associated with maternal AFLP and HELLP syndromes.

Delineation of the Molecular Defect in Exon 15 of the LCHAD Region of the α Subunit. With sequence data of the α-subunit gene, including 100–300 bp flanking both ends of all 20 exons (unpublished results), we designed oligonucleotides of 27–33 bp to amplify each exon. Genomic DNA from the affected children with LCHAD deficiency was amplified with all 20 sets of oligonucleotides and analyzed by SSCV simultaneously with amplified exons from a normal individual.

For all three LCHAD-deficient children (CII-1, BII-1, AII-1), SSCV analysis of exon 15 revealed differences from normal (Fig. 2*a*). Comparison of the conformers reveals that two of the affected children (CII-1 and AII-1) have patterns that are identical to each other, suggesting that they share a similar homozygous mutation. This was confirmed by sequence data (Fig. 2*b*). The third affected child (BII-1), both mothers (BI-2 and AI-2), and one of the fathers (AI-1) exhibit an identical pattern that shares both normal bands and mutant bands, suggesting heterozygosity for this mutation. One father's (BI-1) pattern is normal. The amplified exon 15 DNA was cloned into plasmid vectors and 4–12 subclones from each of these six individuals were sequenced. All clones from two affected children (CII-1 and AII-1) contained a single mutation, G to C, corresponding to nucleotide 1528 of the α-subunit mRNA (Fig. 2*b*). Subclones from the three parents and one

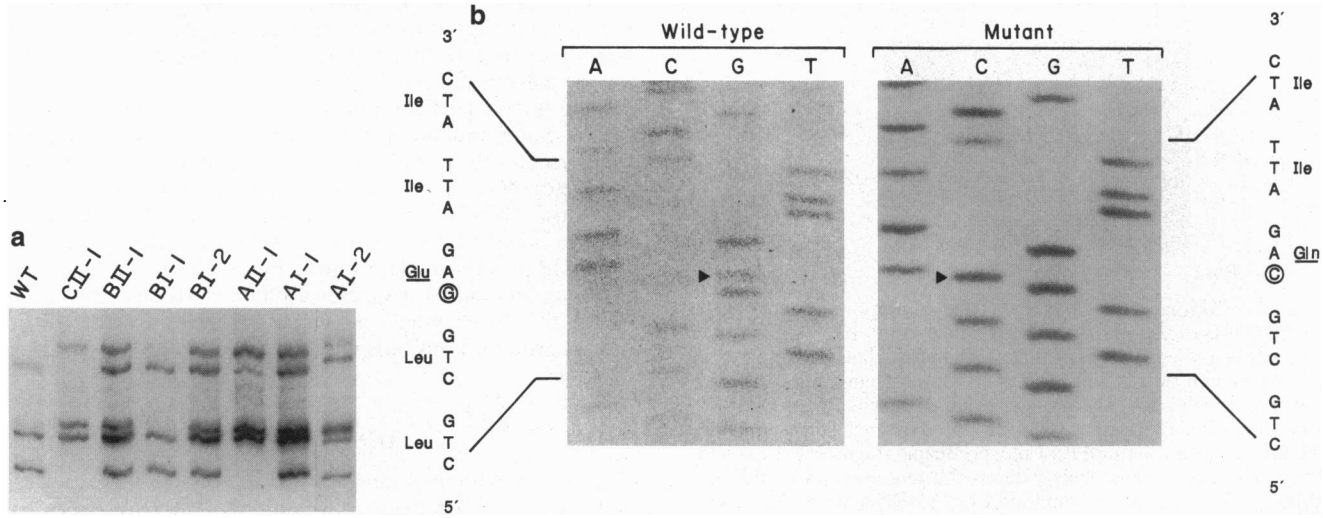

FIG. 2. Delineation of an exon 15 shared mutation in three families with LCHAD deficiency. (*a*) SSCV analysis. Amplification of exon 15 genomic DNA from members of three families and a normal (WT) individual was performed as described and analyzed by SSCV gel electrophoresis. Affected children are individuals CII-1, BII-1, and AII-1. Fathers are BI-1 and AI-1. Mothers are BI-2 and AI-2. (*b*) Exon 15 mutation is a G to C transversion at bp 1528 of the α-subunit coding region. Amplified exon 15 genomic DNA was subcloned in a plasmid vector and nucleotide sequences were determined from a normal (wild type) individual (*Left*) and from individual AII-1 (*Right*). Normal sequence at bp 1528 is G, the third base of a glutamic acid (Glu) codon. The mutation is a C, part of a glutamine (Gln) codon.

affected child (BII-1) predicted as heterozygous by SSCV equally shared this G1528C mutation and the normal sequence. Thus, the affected children in families A and C are homozygous for this mutation, which alters the glutamic acid at residue 474 of mature α subunit to glutamine. The affected child in family B, however, is heterozygous for this mutation and inherited the G1528C allele from her mother.

Confirmation of the G1528C Mutation by Restriction Analyses in All Three Families. The G1528C mutation changes the normal α-subunit cDNA sequence from CTGGAG to CTGCAG, creating a *Pst* I restriction site. To confirm the familial inheritance pattern of this mutation, we analyzed exon 15 DNA from members of these three families after digestion with *Pst* I (Fig. 3). This amplified fragment of 270 bp contains a natural *Pst* I site at bp 238. In two of the affected children (CII-1 and AII-1), complete digestion of the amplified band to smaller (121- and 117-bp doublet) fragments occurred, confirming that these individuals are homozygous for this mutation. After digestion of the amplified exon 15 genomic DNA of both parents of family A, some of the 238 bp remained intact, but the two smaller bands were also present, proving that these parents are heterozygous for the G1528C mutation. Similarly, the results show that the affected child of family B (BII-1) and her mother (BI-2) were heterozygous for this mutation. The lack of digestion of exon 15 genomic DNA in the father (BI-1) indicates that the G1528C mutation is absent. This restriction enzyme digestion thus allows a rapid screening for this shared mutation.

Delineation of a Heterozygous Mutation in Exon 12 of the LCHAD Domain of the α Subunit in Family B. SSCV analysis of exon 12 in family B (Fig. 4*a*) revealed an abnormal banding pattern for the affected child (BII-1) and her father (BI-1). The pattern shared some conformers with amplified exon 12 normal genomic DNA, suggesting that these two members of family B are heterozygous for a nucleotide difference. All other individuals from the three families had the normal banding pattern. Half of the sequence determinations of exon 12 subclones from the father (BI-1) and affected child (BII-1) (Fig. 4*b*) revealed a C to T mutation corresponding to nucleotide 1132 of the α-subunit mRNA. This mutation produces a termination codon at residue 342 (Q342Stop) of the mature

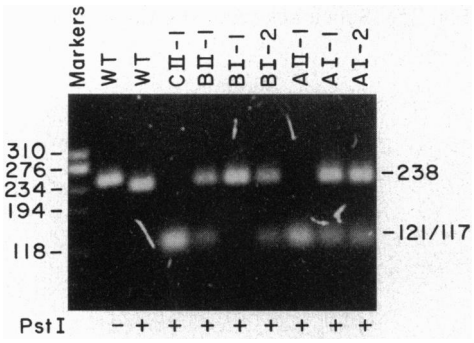

FIG. 3. Confirmation of the G1528C mutation by restriction enzyme (*Pst* I) digestion of exon 15 genomic DNA. Exon 15 genomic DNA was amplified from the same individuals as illustrated in Fig. 2 and treated with *Pst* I. Amplified exon 15 DNA from a normal (WT) individual, which is 270 bp long, was analyzed without digestion. This normal amplified fragment is slightly reduced in size by digestion with *Pst* I, which cuts at a natural *Pst* I site, producing fragments of 238 and 32 bp. DNA from individuals without the mutation (WT and one father, BI-1) is the same. Amplified exon 15 DNA from two of the affected children (CII-1 and AII-1) is completely digested to smaller fragments of 121, 117 (a blurred doublet), and 32 (not visualized on the gel) bp, indicating that both alleles contain the G1528C mutation. Digestion of heterozygote DNA derived from the father (AI-1) and mothers (BI-2 and AI-2) generates a mixture of all bands, the normal 238-bp band, and the smaller fragments of 121, 117, and 32 bp. Migration of marker DNA fragments is indicated on the left.

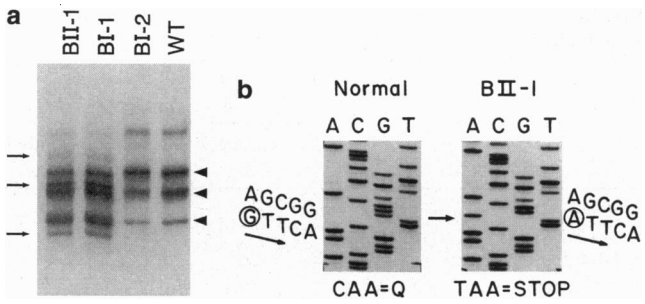

FIG. 4. Delineation of exon 12 mutation C1132T, Q342Stop in family B. (*a*) SSCV analysis of amplified exon 12 genomic DNA from members of family B. Amplification of DNA and SSCV analysis were performed as described. The affected child (BII-1) and father (BI-1) have a different conformer pattern, as indicated by bands with arrows, from the mother (BI-2) and a normal individual. However, some bands are shared among all individuals (arrowheads), demonstrating that the father and child are heterozygotes for a nucleotide difference. (*b*) Sequence analysis demonstrates the C1132T mutation, creating a termination codon at position 342 of the α-subunit cDNA. Exon 12 amplified genomic DNA from the affected child (BII-1) was subcloned and eight clones were sequenced. The antisense sequences are shown. Half of the clones contained the normal C at bp 1132, as shown by the circled G in the antisense sequence on the left, and half had the T mutation, as shown by the circled A in the antisense sequence on the right.

protein, within the NAD-binding consensus sequence of the LCHAD domain. Thus, the affected child is a compound heterozygote, who inherited this premature stop codon mutation (C1132T) from her father and the G1528C mutation from her mother.

Confirmation of the C1132T Stop Mutation by Restriction Enzyme Digestion. The C1132T mutation of the α subunit alters the sequence from CCAAG to CTAAG, which creates a *Dde* I site. Digestion of amplified exon 12 genomic DNA from family B members confirmed that the affected child and her father are heterozygous for this mutation and the restriction site and that her mother is normal (results not shown).

Expression of the G1528C/E474Q Mutation. The cDNA sequences corresponding to amino acids 304–727 of the normal α subunit and that containing the G1528C mutation were placed into bacterial expression plasmids. This region of the α subunit is homologous to the short chain 3-hydroxyacyl-CoA dehydrogenase (15). It contains the NAD consensus binding domain and, thus, is likely to encode LCHAD activity. Lysates were prepared from bacteria transformed with the vector alone, the normal partial (amino acids 304–727) α subunit cDNA, and the partial (amino acids 304–727) mutant (E474Q) cDNA. Supernatants ($n = 3$) from bacteria transformed with the normal cDNA produced LCHAD activities ≈2-fold above the vector controls, confirming that this region of the α subunit contains LCHAD enzymatic activity. Transformants containing the E474Q mutant cDNA produced similar amounts of LCHAD protein as determined by qualitative Western blot with human α-subunit-specific antibody (data not shown) but had <10% the enzymatic activity of LCHAD after subtraction of background. Thus, this mutation severely reduces LCHAD activity.

DISCUSSION

Our results demonstrate autosomal recessive inheritance of isolated LCHAD deficiency in children of three families whose mothers suffered serious complications, AFLP or HELLP syndrome, during their pregnancies with the affected fetuses. Molecular characterization of the α subunit of mitochondrial trifunctional protein in these families revealed point mutations in the LCHAD coding region. Two affected children were homozygous for a G1528C mutation, creating an E474Q

change in the LCHAD domain. The third affected child inherited this mutation from her mother, but the paternal mutant allele generated a termination codon (Q342Stop) near the N terminus of the LCHAD domain.

Our results allow some interesting conclusions. First, comparison between rat (4) and human α-subunit cDNA sequences (Fig. 1 and ref. 14) reveals striking homology of the 3′ UTR across species (68% over 350 bp). In the other mitochondrial β-oxidation enzyme mRNAs (16) and in most other mRNAs, this degree of 3′ UTR homology across species is not present. However, the 3′ UTR is highly conserved across species in a few mRNAs, including cytosolic B-creatine kinase (17), mitochondrial creatine kinases (18), α-actin (19), and selenoproteins (20). These conserved 3′ UTRs are essential for translational regulation, mRNA stability, or mRNA localization (21). The unusual homology for α subunit raises the possibility that this 3′ UTR may serve a similar role.

Second, expression of a partial cDNA clone of the α subunit in bacteria demonstrates that this region, amino acids 304–727, contains LCHAD activity. This is not surprising because the NAD cofactor-binding consensus sequence (residues 332–342) and some (15% identity, 20% similarity) homology to the short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) are present in this region. Structural characterization by crystallographic methods of SCHAD (22), which is a member of the family of oxidoreductases similar to LCHAD, demonstrates a double-domain structure with a bilobal appearance. In SCHAD, the N-terminal domain of 200 residues, which mediates NAD binding, consists of four parallel β-sheets and one α-helix. In the trifunctional protein α subunit, the homologous region includes residues 315–515. Our expression of the LCHAD domain may allow further structural characterization and comparison within this enzyme family.

The most intriguing aspect of our study is the molecular characterization of the mutations causing isolated LCHAD deficiency. Two forms of trifunctional protein deficiency have been described (5, 6). In the less common form, marked reduction in the amount of both α and β subunits is present by immunoanalysis and all three enzymatic activities are reduced. The more common form of deficiency is characterized by isolated reduction in LCHAD activity, with preservation of the long chain enoyl-CoA hydratase and β-ketoacyl-CoA thiolase activities. The molecular basis of this isolated LCHAD deficiency was not previously known. In the three families reported here, this form of LCHAD deficiency, which is associated with maternal AFLP and HELLP syndromes (7, 8), shares a common molecular basis. That is, five of the six mutant alleles in these families were identical, containing the G1528C mutation that alters the glutamic acid residue at position 474 of mature α subunit to glutamine. Expression in bacteria of a partial cDNA encoding this mutation demonstrated a marked reduction in LCHAD activity as compared to the normal, partial cDNA. The homology data suggest that this mutation is in the active site domain of LCHAD, and the loss of activity is consistent with interference with substrate binding or enzyme activity. The sixth mutant allele (C1332T) in these families created a premature termination codon (Q342Stop) within the cofactor NAD-binding domain. Any truncated α subunit produced by this mutation would not contain the LCHAD active site.

In summary, this report describes the molecular genetic defect in three families with isolated LCHAD deficiency. A shared mutation in the LCHAD domain of the trifunctional protein α subunit is associated with hypoglycemia, hepatic steatosis, or death in the affected children and with maternal AFLP or HELLP syndrome. This initial definition of the molecular basis of this deficiency in another β-oxidation enzyme adds to the growing evidence that defects in enzymes of this cycle cause multiple disease phenotypes in patients of all ages.

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