Molecular Heterogeneity in Very-Long-Chain Acyl-CoA Dehydrogenase Deficiency Causing Pediatric Cardiomyopathy and Sudden Death

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Background—Genetic defects are being increasingly recognized in the etiology of primary cardiomyopathy (CM). Very-long-chain acyl-CoA dehydrogenase (VLCAD) catalyzes the first step in the β-oxidation spiral of fatty acid metabolism, the crucial pathway for cardiac energy production.

Methods and Results—We studied 37 patients with CM, nonketotic hypoglycemia and hepatic dysfunction, skeletal myopathy, or sudden death in infancy with hepatic steatosis, features suggestive of fatty acid oxidation disorders. Single-stranded conformational variance was used to screen genomic DNA. DNA sequencing and mutational analysis revealed 21 different mutations on the VLCAD gene in 18 patients. Of the mutations, 80% were associated with CM. Severe CM in infancy was recognized in most patients (67%) at presentation. Hepatic dysfunction was common (33%). RNA blot analysis and VLCAD enzyme assays showed a severe reduction in VLCAD mRNA in patients with frame-shift or splice-site mutations and absent or severe reduction in enzyme activity in all.

Conclusions—Infantile CM is the most common clinical phenotype of VLCAD deficiency. Mutations in the human VLCAD gene are heterogeneous. Although mortality at presentation is high, both the metabolic disorder and cardiomyopathy are reversible. (Circulation. 1999;99:1337-1343.)

Key Words: cardiomyopathy n death, sudden n fatty acids n genetics n metabolism

Cardiomyopathy (CM) is an important cause of morbidity and mortality, with a prevalence in children estimated at 1 in 10,000.1 Despite medical therapy and the increasing use of cardiac transplants, CM remains among the leading cardiac causes of death in children, with mortality rates of 37% to 66%.1,2 Recent clinical studies reveal a familial cause in 20% to 30% of CM patients.1,3 This finding has focussed attention on single gene defects as causes of CM.4,5 Proven genetic defects in dominantly inherited hypertrophic CM include mutations in the cardiac myosin heavy chain, α-tropomyosin, cardiac troponins I and T, frataxin, essential and regulatory myosin light chains, and myosin binding protein genes.4,5 Dilated CM occurs in the X-linked muscular dystrophies and Barth’s syndrome with dystrophin or tafazzin mutations, respectively. At least 6 loci have been mapped in families with dominantly inherited dilated CM, but the specific gene defects are not yet known.6 Mutations in cardiac actin cause dilated CM.7

Prodigious energy generation is crucial to cardiac function, and genetic deficiencies of proteins essential for cardiac energetics also cause CM.4,8–10 After birth, mitochondrial fatty acid β-oxidation becomes the major source of myocardial energy. We and others have documented that defects in the transport, mitochondrial uptake, and β-oxidation of long-chain fatty acids cause CM in infants and children.5,9–11 Myocardial β-oxidation requires active transport of long-chain fatty acids and carnitine across the cardiomycocyte sarcolemma, activation by esterification to CoA, and the mitochondrial inner membrane fatty acyl-CoA/carnitine shuttle to deliver fatty acids to the matrix. The mitochondrial fatty acid β-oxidation spiral involves 4 enzymatic steps, the first of which is catalyzed by 4 different acyl-CoA dehydrogenases with overlapping substrate specificities. These are very-long-chain acyl-CoA dehydrogenase (VLCAD) with substrate specificity for fatty acids of 14 to 20 carbons, long-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase (MCAD), and short-chain acyl-CoA dehydrogenase. These 4 acyl-CoA dehydrogenases share extensive homology. Each cycle of β-oxidation shortens the fatty acid, producing acetyl-CoA and reducing equivalents that participate in the citric acid cycle, electron transport chain, and oxidative phosphorylation to generate ATP.
Sudden death in infancy, cardiomyopathy, acute metabolic crises (hepatic encephalopathy with hypoketotic hypoglycemia), and skeletal myopathy are recognized features of human fatty acid oxidation disorders.1–11 These recessively inherited disorders often present in infants who are critically ill after a period of relatively poor intake during an intercurrent illness. Other environmental factors precipitating illness include exercise, stress, and exposure to cold.

We describe here clinical features and molecular genetic analysis of the largest series of infants and children with mutations in VLCAD. We demonstrate that VLCAD deficiency is heterogeneous with multiple different mutations, that CM is the most common presentation, and that initial mortality is high. However, long-term survival, with reversal of CM, occurs.

**Methods**

**Patient Selection and Samples**

Patients were studied on the basis of clinical features and history. Thirty-seven infants and children with ≥1 of the listed features suggestive of a fatty acid oxidation defect were investigated.4–9,10–11 These included (1) unexplained congestive heart failure and dilated or hypertrophic CM on echocardiography in infancy; (2) nonketotic hypoglycemia, abnormal liver function tests, and dicarboxylic aciduria during an acute illness; (3) skeletal muscle weakness or exercise intolerance with myoglobinuria; or (4) sudden, unexpected death in early childhood with postmortem hepatic and/or myocardial steatosis and abnormal biochemical findings (see below). In 19 patients, no VLCAD mutations were found, and no definitive diagnosis has been made.

Fibroblast cell lines derived from skin biopsies from 16 patients and blood samples from 9 were sent directly for molecular genetic analysis (to A.W.S.). In 2 families, we could analyze only samples from the parents of the index patient who had died. Postmortem frozen liver from 12 patients was analyzed for fatty acid, glucose, and carnitine concentrations; and bile acyl-carnitine profiles (by P.R.) were determined by electrospray–tandem mass spectrometry.12–14 Fibroblast lines were analyzed for VLCAD enzyme activity by the ETF reduction assay with palmitoyl-CoA as the substrate.15

**Cell Lines and DNA and RNA Isolation**

Fibroblast cell lines were maintained in Dulbecco’s modified Eagle’s medium (GIBCO) with 10% FBS, 20 mmol/L glutamine, antibiotics, and 2% fetal calf serum (GIBCO) with 10% FBS, 20 mmol/L glutamine, antibiotics, and 2% fetal calf serum (GIBCO). Whole blood, fibroblasts, or tissue samples were analyzed for VLCAD expression, not present in our amplified products. Ethidium bromide staining before and after transfer demonstrated similar amounts of RNA loaded and complete transfer.16

**Polymerase Chain Reaction Amplification and Single-Stranded Conformational Variance Analysis**

We previously isolated the human VLCAD cDNA and gene and reported the complete nucleotide sequences.9 All 20 VLCAD exons in patients’ genomic DNA were amplified in the presence of [a-32P]-dCTP by the polymerase chain reaction (PCR) under standard conditions9 with the use of 27-bp-long intronic primer pairs (sequences available on request). Exons 3 and 4, 12 and 13, 14 and 15, 16 and 17, and 19 and 20 were amplified together. The PCR products analyzed by single-stranded conformational variance (SSCV)17 ranged from 212 to 420 bp in length.

**Sequencing Analysis and RNA Blots**

Exonic DNA exhibiting altered band mobilities compared with amplified control DNA on SSCV analysis was sequenced, either directly or after fragment subcloning. A full-length VLCAD cDNA insert (2.2 kb) labeled with α-[32P]-dCTP was used as a probe for RNA blot analysis performed with total RNA extracted from patient fibroblasts or tissue samples.16 Ethidium bromide staining before and after transfer demonstrated similar amounts of RNA loaded and complete transfer.16

**Results**

**Genomic PCR, SSCV Screening, and Delineation of VLCAD Mutations**

We studied 37 patients with clinical and/or biochemical features suggestive of a long-chain fatty acid oxidation defect. SSCV analysis of amplified PCR products revealed aberrantly migrating bands in 26 patients. DNA sequencing revealed that 2 differences at nonconsensus intronic sites, including C or T at position +6 after exon 16 and C or T at position –8 before exon 8, accounted for 10 SSCV patterns. These were frequently present in normal DNA and likely represent inconsequential polymorphisms.

Twenty-nine sequence differences, present in 18 different individuals, were in the VLCAD exonic coding regions and altered amino acids or occurred in consensus donor or acceptor splice sites (Tables 1 and 2). These likely represent disease-causing mutations. VLCAD mutations in both alleles were defined in 11 patients, including 2 homozygous individuals. In 12 families (67%), mutations were confirmed in parental DNA. Only a single mutation was delineated in 7 patients, even though direct sequence analyses of all exons was done. These undefined mutations may occur in regulatory domains or intronic regions crucial for VLCAD gene expression, not present in our amplified products.

**Table 1. Mutational Spectrum in Human VLCAD Deficiency**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>ΔG1-1, exon 8‡</td>
<td>C1837T, R573W</td>
</tr>
<tr>
<td>2</td>
<td>T848C, V243A</td>
<td>G1468C, A450P</td>
</tr>
<tr>
<td>3</td>
<td>Δ386-88, Δ689</td>
<td>41-bp insertion, exon 7</td>
</tr>
<tr>
<td>4†</td>
<td>G+1A, exon 11‡</td>
<td>Homozygous‡</td>
</tr>
<tr>
<td>5</td>
<td>C779T, T220M</td>
<td>Δ689-3, ΔΔK258</td>
</tr>
<tr>
<td>6*</td>
<td>Δ386-88, Δ689</td>
<td>Δ6162‡</td>
</tr>
<tr>
<td>7</td>
<td>G637C, A173P</td>
<td>Homozygous</td>
</tr>
<tr>
<td>8</td>
<td>G1322A, G401D</td>
<td>C1837T, R573W</td>
</tr>
<tr>
<td>9</td>
<td>Δ687-88</td>
<td>?</td>
</tr>
<tr>
<td>10*</td>
<td>Δ689-3, ΔΔK258</td>
<td>?</td>
</tr>
<tr>
<td>11*</td>
<td>A-2C, exon 8‡</td>
<td>Δ689-1-3, ΔΔK258</td>
</tr>
<tr>
<td>12</td>
<td>G-1A, exon 8</td>
<td>Δ1932</td>
</tr>
<tr>
<td>13</td>
<td>A739G, K207E</td>
<td>Δ689-1-3, ΔΔK258</td>
</tr>
<tr>
<td>14</td>
<td>T1372C, F418L</td>
<td>?</td>
</tr>
<tr>
<td>15</td>
<td>G1844A, R575Q</td>
<td>?</td>
</tr>
<tr>
<td>16</td>
<td>G1600A, E454K</td>
<td>?</td>
</tr>
<tr>
<td>17</td>
<td>G1600A, E454K</td>
<td>?</td>
</tr>
<tr>
<td>18</td>
<td>G1280A, W347Ter</td>
<td>?</td>
</tr>
</tbody>
</table>

Mutations are designated by nucleotide number from the start codon and amino acid number from the mature N-terminus. Patients 1 through 18 identify the same individuals in all figures and tables.

‡Mutations not defined by Hashimoto and coworkers.23

†Mutations previously reported by us.9
Heterogeneity and Types of VLCAD Mutations
Fifteen mutations, representing 52% of mutant alleles, were found only once (Table 2). Two patients carried the R573W mutation in exon 20; 2 patients had the 3-bp in-frame DE89 deletion in exon 6; 4 patients shared the DK258 3-bp deletion; and 2 patients had the E454K mutation in exon 16. None of these shared heterozygous mutations were in patients who were related, as assessed by country of origin and family history. Both homozygous patients (patients 4 and 7, Table 1) were products of consanguineous marriages. Overall, we detected 21 different mutations among the 29 abnormal alleles, demonstrating the genetic heterogeneity of VLCAD deficiency.

These 21 mutations were of various types (Table 2). Three splice-acceptor consensus site mutations were noted: deletion of a single G at the −1 position before exon 6, an A-to-C mutation at the −2 position preceding exon 8, and a G-to-A mutation at −1 before exon 8. The single consensus donor splice-site mutation (patient 4), G1280A, was reported previously.9 Three molecular defects were small deletions within exons that resulted in a shift in the reading frame. These were D887–888 in exon 10, DG1621 in exon 17, and D891–893, DEK258 in exon 6. One patient had a 41-bp insertion, a duplication of coding sequence, in exon 7. Only 1 premature termination mutation, W387ter, was detected. These 9 mutations (43%) would likely cause instability of the mutant VLCAD mRNA because premature termination codons would be generated either directly or secondary to precursor mRNA missplicing.

The remaining 12 mutations were in-frame deletions or missense mutations. To prove that these were not common polymorphisms, >100 normal alleles were examined by SSCP for all 12 sites. No SSCP differences among these normal individuals were observed. Two mutations, DE89 and DK258, would delete single amino acids but maintain the reading frame. There were 10 missense mutations. Four (K207E, G401D, R575Q, and E454K) would result in changes in amino acid charge. Two (A173P and A450P) substitute proline for alanine, an alteration that might interrupt helical structures. Two (R573W and F418L) introduce or remove a bulky aromatic amino acid, mutations often associated with generation of unstable conformations. Two (V243A and T220M) represent relatively conservative changes.

Among the 11 patients with 2 defined mutations (Table 1), 5 had two missense mutations or in-frame deletions that might allow production of stable VLCAD mRNA. The remaining 6 and another individual with a single known splice-site mutation would likely have unstable VLCAD mRNA from ≥1 allele.

Northern Blot Analysis and VLCAD Enzyme Activity
To examine the mechanisms by which mutations caused VLCAD deficiency, we analyzed VLCAD mRNA levels in 10 available cell lines or tissues by RNA blot (Figure 1). In 5 samples, moderate to severe reduction in mRNA levels occurred. Three of these individuals (patients 4, 6, and 9) carried an exonic deletion or a splice-site mutation on ≥1 allele.
### Table 3: Clinical Features and Outcome in VLCAD Deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Presentation</th>
<th>Outcome (Years After Diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 mo</td>
<td>F</td>
<td>DCM, hepatic</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>22 mo</td>
<td>M</td>
<td>Hepatic</td>
<td>Asymptomatic (9)</td>
</tr>
<tr>
<td>3</td>
<td>8 mo</td>
<td>F</td>
<td>DCM</td>
<td>Asymptomatic*</td>
</tr>
<tr>
<td>4</td>
<td>1 mo</td>
<td>M</td>
<td>HCM, hepatic</td>
<td>HCM, myopathy (16)</td>
</tr>
<tr>
<td>5</td>
<td>2 mo</td>
<td>M</td>
<td>DCM</td>
<td>HCM, myopathy (9)</td>
</tr>
<tr>
<td>6</td>
<td>6 wk</td>
<td>M</td>
<td>DCM, hepatic</td>
<td>Asymptomatic (11)</td>
</tr>
<tr>
<td>7</td>
<td>1 d</td>
<td>M</td>
<td>Sudden death</td>
<td>Died</td>
</tr>
<tr>
<td>8</td>
<td>1 y</td>
<td>M</td>
<td>DCM</td>
<td>Asymptomatic (18)</td>
</tr>
<tr>
<td>9</td>
<td>1 y</td>
<td>F</td>
<td>DCM</td>
<td>Asymptomatic (4)</td>
</tr>
<tr>
<td>10</td>
<td>2 mo</td>
<td>M</td>
<td>Hepatic</td>
<td>Died</td>
</tr>
<tr>
<td>11</td>
<td>6 mo</td>
<td>M</td>
<td>DCM</td>
<td>Myopathy*</td>
</tr>
<tr>
<td>12</td>
<td>3 mo</td>
<td>M</td>
<td>DCM</td>
<td>Died</td>
</tr>
<tr>
<td>13</td>
<td>2 d</td>
<td>F</td>
<td>Sudden death</td>
<td>Died</td>
</tr>
<tr>
<td>14</td>
<td>4 mo</td>
<td>F</td>
<td>DCM</td>
<td>Asymptomatic*</td>
</tr>
<tr>
<td>15</td>
<td>2 wk</td>
<td>M</td>
<td>DCM</td>
<td>Died</td>
</tr>
<tr>
<td>16</td>
<td>3 d</td>
<td>F</td>
<td>DCM</td>
<td>Died</td>
</tr>
<tr>
<td>17</td>
<td>13 mo</td>
<td>F</td>
<td>Sudden death</td>
<td>Died</td>
</tr>
<tr>
<td>18</td>
<td>12 mo</td>
<td>M</td>
<td>Hepatic</td>
<td>Asymptomatic (7)</td>
</tr>
</tbody>
</table>

DCM indicates dilated CM; HCM, hypertrophic CM.
*Only acute history is available; patient was lost to our long-term follow-up.

VLCAD-deficient patients.19

### Demographic and Clinical Features of Patients

Antenatal, neonatal, and family histories were obtained from the referring physicians for all patients (Table 3). Among the 18 patients with documented VLCAD gene mutations, 11 were male and 7 were female; their ages at presentation ranged from the first day of life to 22 months. The racial distribution included 2 African-American and 16 European-American patients. CM, diagnosed with standard echocardiographic criteria for abnormal left ventricular systolic perfor-

mance and structure, was the presenting feature in 12 patients (67%). Initially, dilated CM was demonstrated in 11 individuals, and hypertrophic CM was seen in 1. The youngest patient with CM was 3 days old, and all 12 patients with cardiac manifestations presented in the first year of life. Four patients with CM died despite medical treatment to augment cardiac contractility. Of the 8 surviving patients in this group, 6, including 1 treated with extracorporeal membrane oxygenation as ventricular support, had normal systolic function at follow-up. One patient later developed hypertrophic CM. Thus, 67% of those with CM are improved and active, with dramatic reversal of cardiac dysfunction.

Six patients presented in a metabolic crisis with hypoke
totic hypoglycemia and deranged liver function tests, with 5 becoming ill in the first year. Three had simultaneous CM. Two died. After recovery from the initial event, 3 patients developed skeletal myopathy with episodic exercise intoler-
ance, hypotonia, and muscle weakness, including 1 with myoglobinuria (Table 3). All 3 patients in this group had initially presented with CM. Three patient samples were analyzed after sudden, unexplained death in infancy or early childhood. Two died in the first days of life; the other died at 15 months of age. Postmortem histopathological study revealed macrovesicular and microvesicular hepatic steatosis in 2. In 1, VLCAD deficiency was suspected only after a bile acylcarnitine profile was obtained.14 These results support our view that sudden infant death has a metabolic and genetic cause in some cases.9,12-14,18

Thus, CM was the most common clinical manifestation among patients with VLCAD deficiency. Although both dilated and hypertrophic CM occurred, the former was more commonly seen initially. Liver disease was common but not universal at the time of diagnosis. Most patients with VLCAD deficiency presented early, in the first year of life (16 of 18). CM was severe with substantial mortality (33%). The overall mortality among these VLCAD deficiency patients was 8 of 18 (44%).

### Genotype-Phenotype Correlation in VLCAD Deficiency

Of the mutations described here, 80% (17 of 21) were associated with CM. Liver disease was associated with 10 mutations. Skeletal myopathy developed late in 3 patients, all of whom initially had other system involvement. Thus, we could not identify a correlation between any gene mutation and any particular organ involvement or death. However, our enzymatic and RNA blot data (Figure 1) document that most affected patients had very severe VLCAD deficiency, consistent with early presentation and high mortality. Milder phenotypes with missense mutations may occur in other VLCAD-deficient patients.19

### Outcome and Long-Term Management

Among the 10 patients surviving the initial episode, long-
term follow up of 4 to 18 years is available for 7. In all, the initial severe CM has improved, and all have nearly normal cardiac function, as documented by measured left ventricular chamber dimensions and ejection fraction on echocardiogra-
phy. All are being treated by avoidance of fasting and a
low-fat diet with frequent meals and vigilance during intercurrent illness. Early institution of intravenous glucose treatment to abort metabolic and myopathic crises may have reduced the frequency and severity of these life-threatening episodes. All patients are usually well, although episodic skeletal myopathy continues to limit exercise tolerance in 3 patients.

Discussion

In this study, we defined the molecular genetic basis in VLCAD deficiency, a mitochondrial long-chain fatty acid oxidation enzyme, in 18 patients, the largest series reported. The major findings of this study are that 21 different mutations cause VLCAD deficiency, documenting genetic heterogeneity, and that cardiac presentation is almost universal, with CM, arrhythmia, and sudden, unexpected death in infancy. Although mortality with VLCAD deficiency is high, recovery with favorable long-term outlook can occur.

Deficiency of β-oxidation enzymes and transporters should be suspected in infants and young children with an acute metabolic crisis, particularly hypoketotic hypoglycemia; CM; or sudden, unexplained death with organ steatosis at autopsy. Plasma and urine metabolite analyses are crucial in suggesting a possible fatty acid oxidation disorder. However, abnormal metabolites may be detected in body fluids only at the height of an acute crisis, and biochemical screening may be uninformative in asymptomatic patients. Enzymatic analysis in fibroblasts or tissue is helpful, but because of overlapping substrate specificities of multiple enzymes catalyzing each step, definitive recognition of a specific enzyme defect may be problematic. In fact, many patients with reduced activity with palmitoyl-CoA substrate in crude fibroblast extracts were initially incorrectly believed to have long-chain acyl-CoA dehydrogenase deficiency because VLCAD had not yet been identified and because the specificity of human LCAD for intermediate fatty acid substrates of 10 to 14 carbons had not been clarified. Therefore, verification of a tentative diagnosis of VLCAD deficiency that is based on clinical presentation, enzyme assay, and/or acyl-glycine or acyl-carnitine profiles requires molecular genetic analysis. Study of genomic DNA is preferable because many mutations result in undetectable levels of mutant mRNA expression. This conundrum is demonstrated by the fact that several mutations defined here with genomic DNA were not previously detected by reverse-transcriptase PCR analysis of mRNA.

One major conclusion from our results is that VLCAD deficiency is highly heterogeneous at the molecular level. Since our original delineation of 3 mutations in 2 patients with VLCAD deficiency, others defined 16 VLCAD mutant alleles in 13 patients. These reports used PCR amplification from fibroblast mRNA, not genomic DNA, so that only mutations expressed at the mRNA level would be detected. Four reported mutations (G401D, V243A, T22M, and ΔE89) are identical to those described here but occurred in different individuals. Eleven additional mutations—A241D, R326C, ΔE341, R410H, T158N, G290D, G294E, L562I, K341Q, V277A, and a 4-bp insertion in exon 18—were defined. Altogether, including our results, 32 different mutations, totaling 49 alleles, in 31 VLCAD-deficient patients have been determined, including 22 different missense or single amino acid deletion mutations, 4 splice-site abnormalities, and 6 frame-shift mutations. In 13 individuals, only 1 mutation has been found. These results clearly demonstrate molecular heterogeneity in human VLCAD deficiency.

In contrast, 2 other β-oxidation enzymatic defects, MCAD and long chain 3-hydroxacyl-CoA dehydrogenase (LCHAD) (the α-subunit of trifunctional protein) deficiency, result from a common mutation, presumably derived by a founder effect. In MCAD deficiency, the K304E mutation comprises 80% to 90% of mutant alleles. In LCHAD deficiency, the E474Q mutation represents ~60% of abnormal alleles.

The extensive amino acid homology shared by the 4 known acyl-CoA dehydrogenases, including MCAD and VLCAD, suggests that structural features are likely conserved within the enzymatic domains. The molecular structure of MCAD has been defined by crystallographic methods at 0.2-nm resolution (Figure 2). Amino acid residues 56 through 440 of VLCAD share 30% similarity with MCAD. Alignment of these residues into the computer-generated structural model of an MCAD monomer allows delineation of the locations of the missense and single amino acid deletions that we have described and may assist in our understanding of the mechanisms by which these mutations affect the VLCAD protein (Figure 2). The MCAD monomer consists of an extended domain of α-helices (A through L in yellow) and another domain of β-sheets (1 through 7 in purple), connected by random coils (blue), and separated by the active site pocket and flavin cofactor binding region. All VLCAD missense and in-frame deletion mutations that we have characterized are predicted to lie within the β-sheets or random coils (Figure 2). None are within the fatty acyl-CoA binding pocket (active site) or flavin binding regions where mutations would dra-
tically interfere with enzyme activity. We suggest that improper folding and interaction of the α-helical or β-sheet domain likely result from the VLCAD mutations that we defined, causing the mature protein to be unstable or decay prematurely. Transfection and pulse-chase studies with the R573W, ΔE89, K341Q, and ΔK258 mutations prove that steady-state levels of these mutant proteins are reduced secondary to more rapid intramitochondrial degradation. These results are consistent with the severe deficiency of VLCAD enzyme activity measured (Figure 1) in many of our patients. Thus, for the missense and single amino acid deletion mutations, VLCAD instability is the likely mechanism producing deficiency.

The most striking finding of this study is that CM and sudden, unexplained death in early childhood, perhaps secondary to cardiac arrhythmia, are the most common presentations of VLCAD deficiency (Table 3). Careful histological study, including routine fat stains, a systematic biochemical screen of postmortem liver and body fluids, and a high index of suspicion, are essential to prove that sudden death is secondary to a fatty acid oxidation defect. It is also intriguing that both dilated and hypertrophic CM were seen, with the former being more common at initial presentation but with development of hypertrophic CM later. CM with VLCAD deficiency presents in infancy and has a high mortality (33%). However, with early diagnosis and aggressive therapy, improved cardiac function and activity were noted. Overall, among all VLCAD-deficient patients (this study and References 23 through 25), 88% had CM; sudden, unexplained death with cardiac steatosis; or cardiac arrhythmia, either in the index patient or in siblings who died. Thus, cardiac involvement is virtually universal in VLCAD deficiency.

The clinical presentation and outcome results (Table 3) emphasize 2 additional points. In several patients, sudden death or severe CM occurred in the first few days of life. This early onset demonstrates the severe degree of VLCAD enzyme deficiency and the crucial role of fatty acid oxidation in the fetal-neonatal transition because the heart and other highly oxidative tissues switch from using maternally derived glucose, the sole fetal energy substrate, to long-chain fatty acids as the major energy source. Often, symptoms in patients with fatty acid oxidation defects are triggered by environmental events, such as intercurrent illness with diminished oral intake, exercise, or fasting, which increase dependence on fatty acid metabolism for energy. The neonate often does not receive adequate caloric intake in the first few days of life, especially with breast feeding, and this may trigger the catastrophic events leading to death and CM in some VLCAD-deficient newborns.

CM is also seen in deficiencies of the plasma membrane carnitine transporter, carnitine palmityl transferase-II, carnitine/acetyl-carnitine translocase, LCHAD, and trifunctional protein. The mechanism of myocardial injury or arrhythmogenesis in these long-chain fatty acid metabolic deficiencies may be accumulation of toxic long-chain acyl-carnitines.

Our study demonstrates that human VLCAD deficiency is heterogeneous at the molecular genetic level and that cardiac involvement is universal. Both dilated CM and hypertrophic CM may occur. Early recognition of this and other genetic deficiencies in the mitochondrial fatty acid oxidation pathway can allow effective treatment and a good long-term outcome. These results emphasize that genetic causes of CM and sudden death in infants and newborns secondary to defects in cardiac energy production are likely more common than previously recognized.

Acknowledgments

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References


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