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Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency: Diagnosis by Acylcarnitine Analysis in Blood

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Summary

Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency is a disorder of fatty acid catabolism, with autosomal recessive inheritance. The disease is characterized by episodic illness associated with potentially fatal hypoglycemia and has a relatively high frequency. A rapid and reliable method for the diagnosis of MCAD deficiency is highly desirable. Analysis of specific acylcarnitines was performed by isotope-dilution tandem mass spectrometry on plasma or whole blood samples from 62 patients with MCAD deficiency. Acylcarnitines were also analyzed in 42 unaffected relatives of patients with MCAD deficiency and in other groups of patients having elevated plasma C8 acylcarnitine, consisting of 32 receiving valproic acid, 9 receiving medium-chain triglyceride supplement, 4 having multiple acyl-coenzyme A dehydrogenase deficiency, and 8 others with various etiologies. Criteria for the unequivocal diagnosis of MCAD deficiency by acylcarnitine analysis are an elevated C8-acylcarnitine concentration (>0.3 μM), a ratio of C8/C10 acylcarnitines of >5, and lack of elevated species of chain length >C10. These criteria were not influenced by clinical state, carnitine treatment, or underlying genetic mutation, and no false-positive or false-negative results were obtained. The same criteria were also successfully applied to profiles from neonatal blood spots retrieved from the original Guthrie cards of eight patients. Diagnosis of MCAD deficiency can therefore be made reliably through the analysis of acylcarnitines in blood, including presymptomatic neonatal recognition. Tandem mass spectrometry is a convenient method for fast and accurate determination of all relevant acylcarnitine species.

Introduction

Disorders of fatty acid oxidation are recognized as a contributing cause of sudden unexplained death in infancy (Roe and Coates 1989). Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency is the most common disorder of fatty acid oxidation and is one of the most frequent of all inborn errors of metabolism, with an estimated incidence of 1:20,000 among Caucasians (Blakemore et al. 1991; Matsubara et al. 1991). The classical clinical presentation consists of a Reye-like syndrome with stupor and hepatomegaly associated with hypoglycemia, hypoketonemia, hypocarnitinemia, elevated transaminases, and mild hyperammonemia (Roe and Coates 1989). However, it is increasingly apparent that clinical presentations can range from asymptomatic to isolated hypoglycemia to sudden unexplained death (Roe et al. 1986). Most cases present between infancy and the second year of life, often during metabolic stress resulting from prolonged fasting or infection. Cases presenting during the neonatal period (Catzeflis et al. 1990) and up to 13 years of age are also known. Up to 25% of patients with MCAD deficiency die during their first episode of illness (Roe and Coates 1989). With careful treatment consisting of avoidance of fasting, dietary restriction of fat, and L-carnitine therapy, patients do well, and morbidity and

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mortality can be prevented (Roe and Coates 1989). A method for early detection, applicable to neonatal screening, is therefore of major importance ("Medium chain acyl CoA dehydrogenase deficiency" 1991).

Several diagnostic tests for MCAD deficiency are available, but only a few are applicable to small blood volumes. A single point mutation of adenine to guanine at position 985 (designated "A985G mutation") in the MCAD gene complementary DNA sequence accounts for almost 90% of all MCAD mutations. Affected patients are either homozygous (81%), heterozygous (18%), or negative (1%) for this common mutation (Matsubara et al. 1990; Yokota et al. 1991; Workshop on Molecular Aspects of MCAD Deficiency 1992). The PCR-based assay for this mutation can be performed on small blood volumes, including filter paper blood spots (Matsubara et al. 1991). Carriers and compound heterozygotes must, however, be distinguished by a different test, preferably one applicable to filter paper blood spots. The estimated carrier frequency is as high as 1:70 in the general population (Matsubara et al. 1990; Blakemore et al. 1991).

Plasma levels of *cis*-4-decenoic acid, a diagnostic metabolite, are elevated in MCAD-deficient patients, as determined by gas chromatography/mass spectrometry (Duran et al. 1988; Heales et al. 1991). This method has also been applied to filter paper blood samples (Morton and Kelley 1990; Heales and Leonard 1992). For large-scale neonatal screening, chromatographic methods suffer from time constraints that limit the sample throughput.

The accumulation of medium chain-length acylcarnitines, especially octanoylcarnitine, is potentially diagnostic for MCAD deficiency (Roe et al. 1985, 1986). Recent improvements in nonchromatographic mass spectrometry methods developed in this laboratory have enabled the detection and quantitative analysis of specific acylcarnitines from small volumes of plasma and blood, including filter paper blood spots (Millington et al. 1989, 1990, 1991). The objective of this study was to define a set of criteria to enable the unequivocal diagnosis of MCAD deficiency from the analysis of acylcarnitines, with absolute discrimination from other patients with elevated levels of octanoylcarnitine, including patients with multiple acyl-coenzyme A dehydrogenase deficiency (MADD) and those receiving valproic acid or medium-chain triglyceride (MCT) supplement. The influence of clinical state, carnitine treatment, and underlying mutation was evaluated.

Subjects, Material, and Methods

Patients

Patients for this study were selected from a total data base of >7,000 patients for whom samples for diagnostic tests were sent to Duke Medical Center since 1984. A total of 126 patients with MCAD deficiency were identified from these records. Of these, 54 were excluded because no plasma or blood sample was available. A further six were excluded because the only sample available was a blood spot collected on a nonstandard type of filter paper from which the amount of blood extracted could not be quantified. Four were excluded because the signal-to-noise criteria for the diagnostic signals were not met and because there was insufficient sample for a repeat assay. For the remaining 62 patients, the first sample of blood or plasma received by the laboratory was used whenever possible. These patients included 28 patients asymptomatic at the time the blood sample was obtained, 30 with acute illness, and 4 with insufficient information. There were 24 patients known to have received carnitine prior to obtaining the blood sample and 18 known to have received no carnitine. In every case, the diagnosis of MCAD deficiency was confirmed by at least one additional test. DNA analysis confirmed the diagnosis in 30 patients who were homozygous for the common A985G mutation, and 6 patients were heterozygous for this mutation. In three of these compound heterozygotes, the mutation in the second allele was identified (Ding et al. 1992). One patient was negative for the A985G mutation. In all nonhomozygous patients, diagnostic urine organic acids, including acylglycines (Roe et al. 1985; Rinaldo et al. 1989), were present. In several cases, additional positive evidence was available from one or more of the following tests: phenylpropionic acid loading (Seakins and Rumsby 1988), carnitine loading (Roe et al. 1985, 1990), plasma *cis*-4-decenoate assays (Heales et al. 1991), and in vitro enzyme assays (Stanley et al. 1983; Coates et al. 1985; Moon and Rhead 1987). Original neonatal screening (Guthrie) cards were available from eight of the aforementioned patients with MCAD deficiency.

Relatives, including siblings and parents, of affected patients identified as A985G homozygotes were employed as the controls ($n = 42$). This group included 21 unaffected heterozygotes and 21 relatives lacking the A985G mutation. Patients receiving 2-propylpentanoic (valproic) acid ($n = 32$) and four patients with MADD diagnosed by analysis of urinary organic acids were in-

cluded in the study, in addition to nine patients receiving a formula containing at least 40% MCT. Other miscellaneous cases with elevated plasma C8 acylcarnitine were identified from the patient records. These included two patients with cystinosis and one each with transient hypertrophic cardiomyopathy on 3′-azido-3′-deoxythymidine therapy; argininosuccinate lyase deficiency; myopathy, mental retardation, seizures, and dysmorphic features; intermittent ataxia; encephalopathy, myopathy, and headaches; and mitochondrial respiratory-chain disorder with ataxia and retinopathy.

Sample Preparation and Instrumentation

Analysis of acylcarnitines in plasma was performed by liquid secondary-ion mass spectrometry combined with tandem mass spectrometry, as described in detail elsewhere (Millington et al. 1989, 1991). The mass spectrometer employed was of the triple quadrupole type, a VG QUATTRO (Fisons Instruments, Boston) equipped with a cesium ion gun. Methanol extracts of either blood spots (2 punches of each $^3/_{16}$ inches, equivalent to 12 μL vol) collected on filter paper (type 903; Schleicher and Schuell, Keene, NH) or plasma samples (100 μL) were used for analysis. The equivalent of 5 μM acetyl-[$^2H_3$-methyl]-carnitine and 1 μM octanoyl-[$^2H_3$-methyl]-carnitine were added to each sample as internal standards, for the quantification of individual acylcarnitines. After conversion to methyl esters, the addition of 1% octylsodium sulfate to the liquid matrix used for liquid secondary ionization improved the detection limits for acylcarnitines (Millington et al. 1991).

Calculations

Each acylcarnitine exhibits a signal, represented on the mass scale, corresponding to the mass-to-charge ratio (m/z) of the intact molecule. The minimum criterion for an acceptable signal from the internal standards, acetyl-[$^2H_3$-methyl]-carnitine and octanoyl-[$^2H_3$-methyl]-carnitine, was a ratio to the baseline "noise" of at least 5:1. All other signals were recorded only if the signal-to-noise ratio exceeded 2:1. The response ratio for octanoylcarnitine to its internal standard (m/z 302/305) was calibrated by standard addition of octanoylcarnitine to aliquots of normal plasma containing 1 μM internal standard. The response was linear from 0.2–20 μM (r^2 = .99). A similar calibration curve was obtained for acetylcarnitine. By using the reasonable assumption, in the absence of available standards, that equimolar amounts of acylcarnitines of acyl-group chain-length C8, C10, and C10:1 produce equal signal intensities in the mass spectrum, the concentrations of C10 and C10:1 acylcarnitines were estimated. In a few MCAD patient samples, no internal standards were added, and quantitative data were therefore not available.

Also calculated were the ratios of concentrations of octanoylcarnitine to acetylcarnitine (C8/C2) and of octanoylcarnitine to decanoylcarnitine (C8/C10). In the latter case, the absolute ratio of the signals at m/z 302 and at m/z 330 was calculated, obviating the internal standard requirement. To make this calculation, a minimum signal-to-noise ratio of 5:1 was required for the m/z 302 signal.

Statistical Methods

Results for the various groups were expressed as median and range, because the data did not follow a normal distribution. Comparison of the concentration of individual acylcarnitines, and ratios thereof, between MCAD patients when sick and when well, with or without carnitine supplement, or according to underlying mutation, was done by the Mann-Whitney U test. Calculations were done using The True Epistat™ (Epistat Σ Services, Richardson, TX) computer software package.

Results

Plasma acylcarnitine profiles from an unaffected control and from a patient affected with MCAD deficiency (asymptomatic and untreated) are shown in figure 1*A* and *B*, respectively. These typical patterns represent the intact molecular species of all the acylcarnitines present in the sample, in the order of their increasing molecular weights. A representative acylcarnitine profile from the original neonatal screening (Guthrie) card of an asymptomatic patient with MCAD deficiency is shown in figure 1*C*. This patient died suddenly and unexpectedly on the third day of life. The profile in figure 1*D* is from one of the patients on valproate therapy, who has the highest signal level for C8 acylcarnitine. These profiles are characterized by the absence of signals derived from plasma constituents other than acylcarnitines, a feature of the extraordinarily high specificity of this analytical method for acylcarnitines. The signal for acetylcarnitine occurs at m/z 218, with that of the acetyl-[$^2H_3$-methyl]-carnitine internal standard at m/z 221. Other signals correspond to propionyl- (232), hexanoyl- (274), octanoyl- (302), decanoyl- (330), octenoyl- (300), and decenoyl- (328) car-

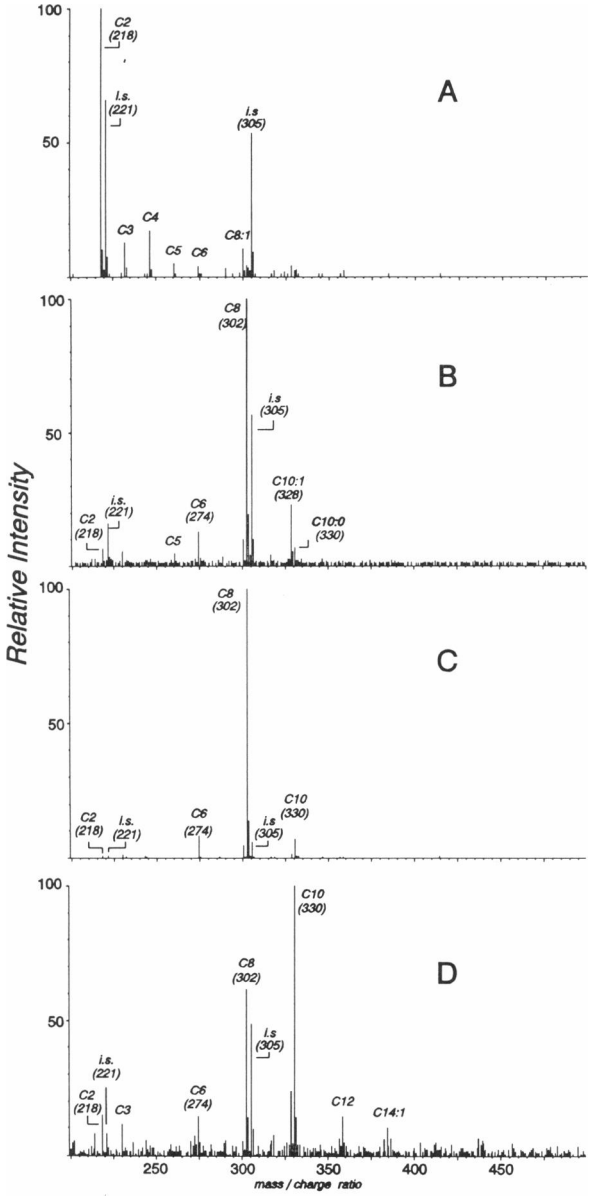

Figure 1 Analyses of acylcarnitines in blood, using tandem mass spectrometry. The signals detected represent the intact molecular species of acylcarnitine methyl esters and form a homologous series from C2 (acetylcarnitine, m/z 218) through C6 (m/z 274) and C8 (m/z 302), and so on. Isotopically labeled internal standards (*i.s.*; m/z 221 and m/z 305) were added for quantitation of individual species. *A*, Acylcarnitine profile from a typical normal patient, showing mostly acetylcarnitine (C2). *B*, Profile from patient with MCAD deficiency, showing accumulation of C6, C8, and C10:1 species with high C8/C10 ratio and no elevated species of chain-length higher than C10. *C*, Profile from original Guthrie card obtained at 2 d of age from an asymptomatic patient with MCAD deficiency who died suddenly at the age of 3 d. *D*, Profile from one of the patients receiving valproate therapy, showing elevated C10 and higher chain-length species, in addition to C8. This sample had the most-elevated C8 signal in this group of patients.

nitines. The signal at m/z 305 is derived from the octanoyl-[$^2H_3$-methyl]-carnitine internal standard.

In the control group, the plasma concentrations of C8, C10, and C10:1 species were all <0.3 μM, with most <0.1 μM (table 1). In all MCAD patients, the concentration of C8 acylcarnitine was elevated (0.5–16 μM) relative to the controls. As summarized in table 2, there was no significant difference in the C8 acylcarnitine concentration between MCAD patients when asymptomatic or when sick, with or without carnitine supplement, or according to the underlying genetic mutation (Mann-Whitney U test; $P > .05$). The concentration of C8 acylcarnitine was also elevated in 10/32 patients on valproate therapy, in 4/4 patients with MADD during acute episodes, and in some patients on MCT supplement (table 1). When asymptomatic, patients with MADD sometimes exhibited a normal acylcarnitine profile. When elevated (>0.3 μM) C8 acylcarnitine was used as the sole criterion, there was overlap between 60% of the MCAD samples and others having elevated C8 species. The highest levels of C8 acylcarnitine in non-MCAD patients were seen in MADD patients (≤2.6 μM); in most other cases they were <1 μM.

In most of the acylcarnitine profiles from MCAD patients the signals corresponding to C10:1 and C6 acylcarnitines were also elevated, and the signal from acetylcarnitine was reduced relative to controls. The C8/C2 ratio was calculated for all patients. This criterion represents a large improvement in specificity for MCAD diagnosis, compared with measurement of only octanoylcarnitine concentration, but four patients in the MCAD-deficiency category overlapped with other patient categories (table 1). This ratio was also influenced by the clinical state of the patient (table 2).

The most striking feature of the acylcarnitine data is the fact that, in MCAD deficiency, the concentration of C10 was always much less than that of C8, whereas in all other cases, the signals for C8 and C10 were in the same order of magnitude. This is apparent from the comparison of profiles of MCAD-deficient and valproate-treated patients in figure 1*B* and *D* and is expressed as the C8/C10 ratio in table 1. The ratio was >5:1 in all 62 patients with MCAD deficiency but was ≤3:1 in any of the other patients. Thus, the C8/C10 ratio of plasma acylcarnitine signals unequivocally discriminates MCAD-deficiency patients from all other patients with elevated C8 signals (fig. 2). This ratio was not influenced by clinical state (symptomatic vs. asymptomatic), by the use of carnitine supplement, or by the

Table 1

Concentrations of C8, C10:1, C10, and C2 Acylcarnitines in Blood or Plasma

Acylcarnitine Species	Concentration (Median) and Range[a]					
	MCAD[b]	VALP	MADD	MCT	OTHER	CONTROLS
C8	2.04 (53) .5–15.6[c]	.19 (32) <.1–1.22	.79 (4) .4–2.6	.23 (9) <.1–.57	.42 (8) .16–.9	NA (42) <.3
C10:1	.46 (48) <.1–3.50	.17 (22) <.1–.65	.46 (4) .14–.89	.19 (8) <.1–.60	.36 (8) .2–.6	NA (42) <.3
C10:0	.18 (48) <.1–1.33	.22 (22) <.1–2.02	.98 (4) .46–1.50	.21 (8) .14–.41	.47 (8) .21–.84	NA (42) <.3
C8/C10	12.4 (62) 5.6–87.0	1.1 (22) 1.6–2.8	1.04 (4) .4–1.7	1.26 (8) .65–2.0	.92 (7) .8–1.1	NA
C2	3.53 (50) .3–17.4	8.4 (32) 1.8–65.0	21.4 (4) 4.6–57.5	28.7 (9) 7.9–50.6	10.6 (8) 3.1–20.6	7.5 (42) 1.2–15.4
C8/C2	.67 (44) .10–9.3	NA (32) <.17	NA (4) <.18	NA (9) <.06	NA (8) <.11	NA (42) <.1

[a] Concentrations in μM. MCAD = MCAD deficiency; VALP = valproate treated; MADD = MADD; MCT = MCT supplemented; OTHER = other patients with elevated C8 acylcarnitine with miscellaneous diagnoses; and CONTROLS = DNA-proved unaffected relatives of the patients with MCAD deficiency. Results in μM units are expressed as median and range because the data did not follow a normal distribution. NA = Not applicable.

[b] All patients with MCAD deficiency had elevated C8-acylcarnitine, but there was overlap with certain patients in some other groups.

[c] Results from one patient were .32 μM. The sample was stored for 4 years and was probably degraded.

[d] The ratio of C8/C10 acylcarnitines provided a complete differentiation of MCAD patients from all other patients.

underlying DNA mutation (Mann-Whitney U test; $P > .05$) (table 2).

Acylcarnitine profiles were obtained from six original Guthrie cards retrieved from patients already diagnosed, plus two further cases where deaths had occurred within 4 d and the acylcarnitine profile on the neonatal screening card provided the primary diagnosis (e.g., see fig. 1C). These patients were subsequently confirmed as being homozygous for the MCAD A985G mutation, by DNA analysis. Some of the Guthrie cards had been in storage up to 2 years prior to testing. In all eight cases, the acceptable criteria of C8 > 0.3 μM and C8:C10 > 5:1 were met (table 3). In the analysis of over 150 neonatal screening cards from normal neonates, including some from unaffected heterozygotes for the MCAD A985G mutation, neither of these criteria for MCAD deficiency was met.

Discussion

The acyl-coA dehydrogenases are a group of mitochondrial enzymes with overlapping substrate specificities. The saturated substrates for short-, medium- and long-chain dehydrogenases are acyl-coAs with chain lengths of C4–C6, C6–C12, and C10 and higher, respectively (Dommes and Kunau 1984; Ikeda et al. 1985). In vitro studies have documented the accumulation of C6–C10 acyl-coAs in cells deficient in MCAD activity (Kler et al. 1991). The two mitochondrial acylcarnitine transferases, carnitine acetyltransferase and carnitine palmitoyltransferase, have broad chain-length specificities for acyl-coA that together cover the range C2–C18 (Bieber 1988). Accumulating acyl-coAs can thus exit the mitochondria as the corresponding acylcarnitines and then appear in the plasma and urine.

In MCAD deficiency, elevated concentrations of C6, C8, and C10:1 acylcarnitines have been observed in the urine (Roe et al. 1985) and plasma of affected patients. The long-chain acyl-coenzyme A (LCAD) enzyme has much lower activity toward 4-*cis*-decenoyl-coA than does MCAD (Dommes and Kunau 1984); therefore, accumulation of C10:1 from an unsaturated precursor, such as linoleic acid, would be expected in MCAD deficiency.

The acylcarnitine profile with added internal standards provides quantitative information on acylcarnitine species in plasma. Furthermore, the ratio of C8 to C10 acylcarnitines, which does not require absolute

Table 2

Plasma Concentrations of C2, C8, C10, and C10:1 Acylcarnitines

Patient Category	Concentration (Median) and Range[a]					
	C2	C8	C10:1	C10:0	C8/C10	C8/C2
Asymptomatic	3.94 (26) 1.88–17.4	2.8 (25) .53–15.6	.50 (23) <.1–3.5	.26 (24) <.1–1.29	12.2 (28) 7.2–30	.55 (24) .11–4.6
Symptomatic	3.16 (20) .88–6.4[b]	1.54 (24) .32–15.6	.46 (22) <.1–2.17	.16 (21) <.1–1.33	12.91 (30) 5.57–87	1.12 (16) .25–3.11[c]
Untreated	3.23 (16) .33–17.4	1.48 (15) .53–15.6	.43 (13) .12–2.40	.26 (12) <.1–1.29	13.4 (18) 5.6–42.7	1.1 (13) .11–9.3
On L-carnitine	3.99 (21) 1.17–9.5	2.83 (19) .66–15.6	.58 (19) .18–3.5	.26 (18) .04–1.33	12.2 (24) 7.2–87	.73 (18) .14–42
A985G Homozygotes	4.36 (28) 1.5–17.4	2.8 (27) .53–15.6	.50 (25) <.1–3.5	.31 (24) <.1–1.29	12.8 (30) 7.2–42.7	.65 (26) .11–4.60
A985G Compound Heterozygotes	3.08 (7) 1.25–7.4	3.50 (7) .76–3.9	.32 (7) .12–1.9	.23 (7) <.1–0.47	11.5 (7) 7.6–17.5	1.15 (7) .25–2.76

[a] Plasma concentrations in μM expressed as median and range, for patients with MCAD deficiency. The ratios of C8/C10 and of C8/C2 acylcarnitines are also tabulated.

[b] Comparisons were made between asymptomatic and symptomatic patients at the time of blood sampling, as well as between untreated patients and those receiving carnitine at the time of blood sampling. Further comparison was made between patients homozygous for the A985G mutation in the MCAD gene and affected (compound) heterozygotes. The only significant correlations using the Mann-Whitney U test were a lower acetylcarnitine concentration in symptomatic vs. asymptomatic patients ($P = .03$).

[c] A similar difference was found for the C8/C2 ratio ($P = .05$).

quantitation of individual acylcarnitines, unequivocally distinguishes all MCAD-deficient patients from other groups with elevated C8 acylcarnitine. This observation is readily explained by the fact that, when MCAD activity is reduced, the oxidation of C10 acyl-coA is dependent on the LCAD activity. In all other conditions except MCAD deficiency, the activities of several dehydrogenases, including that of LCAD, are affected. Therefore, C10 and higher chain-length species accumulate with C8. Acylcarnitines with chain lengths >C10 would not be expected to accumulate in patients with MCAD deficiency.

The diagnostic criteria for MCAD deficiency, which use the plasma or blood acylcarnitine profile, are therefore summarized as follows: the C8 acylcarnitine concentration must exceed 0.3 μM, the ratio of C8/C10 must exceed 5:1, and no significant accumulation of species of chain length longer than C10 must be observed. These criteria were satisfied by all 62 patients with MCAD deficiency in this study and were independent of the underlying genetic mutation, clinical condition, or treatment regimen, indicating the absence of a false-negative test in this larger cohort of patients with proved MCAD deficiency. These criteria were not met by any of the other patients for whom acylcarnitine profiles of blood were available (>1,000) in the large and diverse population base of this laboratory and in over 7,000 neonates and sudden infant death syndrome patients analyzed, illustrating the extremely low incidence of false-positive diagnoses. The incidence of false-negative or false-positive diagnoses must therefore be considered very low and has, so far, not been encountered by us while using these criteria. The acylcarnitine profile in blood reliably diagnoses MCAD deficiency, without fasting or loading tests and even in asymptomatic newborns.

Recent improvements in methodology have enabled quantitative analysis of acylcarnitines in small volumes of plasma and blood, including filter paper spots (Millington et al. 1990, 1991). The convenience of filter paper blood sampling is complemented by the speed of sample preparation and analysis, which takes <2 h from receipt of sample. In addition to the specific diagnosis of MCAD deficiency, this test also recognizes related disorders presenting with similar symptoms, including LCAD and long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency (Millington et al. 1992). The only conceivable situation in which the test

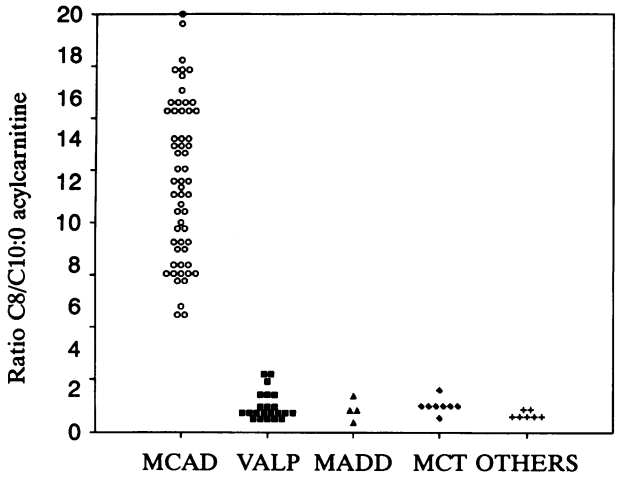

Figure 2 Distribution of C8/C10 acylcarnitine ratios among patients with MCAD deficiency (MCAD), valproic acid–treated (VALP), MADD deficiency (MADD), MCT supplemented (MCT), and various other diagnoses (OTHERS). This ratio was higher in MCAD patients, and there was no overlap with other groups. Four MCAD patients with C8/C10 ratios >20 are not shown.

would not be diagnostic is one in which the minimum criterion for signal intensity is not met. This could happen if the acylcarnitine signals are very low because of profound carnitine deficiency, for which there has been no clear precedent in our experience, or if a sample is not handled appropriately before analysis, and the acylcarnitines are degraded. Long-term storage or repeated thawing and refreezing of plasma or blood samples might cause such degradation. The safest and most convenient method of collection is the fresh filter paper blood spot. Although interpretable acylcarnitine profiles have been obtained from filter paper blood spots stored up to 2 years, the long-term stability of individual acylcarnitines has not been systematically evaluated. Only the standard paper used in neonatal screening laboratories in the United States is recommended (see Sample Preparation and Instrumentation subsection), for which excellent recovery has been documented (Millington et al. 1990). The few uninterpretable profiles from known MCAD patients were from blood spots on a different type of filter paper, for which no basis for the quantification or stability of acylcarnitines has yet been established.

The high diagnostic value and the convenience of this test make it a valuable addition in the workup of the patient with a suspected fatty acid–oxidation disorder. Its application can avoid the need, in many patients, to use the currently frequently applied fasting test, with its concurrent risk of death, in the workup for possible disorders of fatty acid oxidation (Hale and Bennett 1992).

The successful application of the aforementioned criteria for MCAD deficiency to the original screening cards of asymptomatic neonates indicates the potential of this method for neonatal screening. Neonatal screening for MCAD deficiency by using the DNA method is now under serious consideration in several laboratories. If this method is adopted, then heterozygotes for the common mutation could all be screened by the acylcarnitine profile, to differentiate unaffected carriers from compound heterozygotes. Alternatively, acylcarnitine analysis could be the primary screening method, which has the advantage that other disorders of fatty acid and amino acid catabolism, including phenylketonuria, are detected (Millington et al. 1992; Chace et al. 1993). The methodology for automation of acylcarnitine and amino acid analysis from filter paper spots, to facilitate the analysis of >400 samples per day, has also been developed (Millington et al. 1989;

Table 3

Concentrations of C2, C8, C10, and C10:1 Acylcarnitines in Blood from Original Neonatal Phenylketonuria Screening Cards

	Concentration (Median) and Range					
	C2	C8	C10:1	C10:0	C8/C10:0	C8/C2
MCAD-deficient	3.4 (8) 1.25–17.9	3.5 (7) .43–15.6	.29 (7) .13–.43	.4 (6) <.1–1.29	12.4 (8) 6.5–17.5	1.5 (7) .14–4.6
Control		<0.3 (500)	<0.1 (500)	<0.1 (500)	NA (500)	NA (500)

NOTE.—The criteria set above for the diagnosis of MCAD deficiency (C8 acylcarnitine > 0.3 μM, C8/C10 > 5, and no significant species of higher chain length than C10) were fulfilled in all patients.

Chace et al. 1993). Other methods that depend on chromatographic separation (Heales et al. 1991; Schmidt-Sommerfeld et al. 1992) would seriously restrict the sample throughput because of the additional time requirement.

We conclude that the analysis of acylcarnitines in blood is a safe and reliable method for diagnosis of MCAD deficiency. The applicability of this new method to neonatal samples offers the opportunity to facilitate the diagnosis of this disorder, plus perhaps other disorders of fatty acid oxidation, before the onset of life-threatening symptoms.

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