Immunochemical Characterization of Variant Long-Chain Acyl-CoA Dehydrogenase in Cultured Fibroblasts from Nine Patients with Long-Chain Acyl-CoA Dehydrogenase Deficiency

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ABSTRACT. Long-chain acyl-CoA dehydrogenase (LCAD) deficiency is a disorder of mitochondrial fatty acid oxidation that is characterized by hypoglycemia, muscle weakness, and hepato- and cardiomegaly. To characterize variant LCAD, we first carried out preliminary experiments using pure enzyme preparations. Despite the significant sequence similarity of LCAD to medium-chain acyl-CoA dehydrogenase, the antibody raised against rat LCAD was monospecific for human and rat LCAD and did not cross-react with either human or rat medium-chain acyl-CoA dehydrogenase. Immunoblot analysis of variant LCAD in cultured fibroblasts from nine patients with LCAD deficiency revealed a single LCAD band in all nine LCAD-deficient cell lines. Each variant LCAD was comparable in molecular size and quantity to normal LCAD, suggesting that the LCAD mutation in each of these cell lines is likely to be a point mutation that produces a stable variant LCAD. The uniform nature of variant LCAD suggests that only a single, or at most a few, prevalent point mutations may be found in the majority of LCAD-deficient patients. If this is the case, it should be possible to devise a molecular diagnostic method for LCAD deficiency. (Pediatr Res 30: 211–215, 1991)

Abbreviations

LCAD, long-chain acyl-CoA dehydrogenase
MCAD, medium-chain acyl-CoA dehydrogenase
SCAD, short-chain acyl-CoA dehydrogenase

LCAD deficiency is an autosomal recessive disorder of fatty acid metabolism. It was first described in three infants in 1985 by Hale et al. (1). Subsequently, 11 additional patients have been identified (2). The main clinical features seen in these patients were severe hypoglycemia, skeletal muscle weakness, and hepatomegaly; cardiomegaly was a significant feature in eight patients. Six of them died in early infancy; however, the clinical manifestations in some other cases were much milder (1, 2), suggesting

heterogeneity. Heterogeneity of LCAD deficiency was also suggested by a biochemical assay (3). As in the case of MCAD deficiency (4), some patients with LCAD deficiency abruptly died with few prodromal symptoms, mimicking sudden infant death syndrome (5). No urinary metabolites are specific for this disease, making it difficult to establish the diagnosis. At present, the diagnosis of LCAD deficiency is made by the assay of LCAD activity in cells such as fibroblasts and leukocytes or in solid tissues; however, methods for the assay of LCAD and other acyl-CoA dehydrogenase activities are cumbersome and time-consuming.

As the first step in the molecular characterization of mutations of human LCAD that cause LCAD deficiency, we studied the variant LCAD protein in cultured fibroblasts from nine patients with LCAD deficiency using immunoblot analysis.

MATERIALS AND METHODS

Materials. Purification of MCAD and LCAD from rat liver mitochondria and the preparation of the respective antibodies were previously reported by Ikeda et al. (6). These antibodies also recognized the respective human enzymes as well (7). Purification of human MCAD was reported by Finocchiaro et al. (8). Tissue culture materials were obtained from GIBCO (Grand Island, NY). Inactivated *Staphylococcus aureus* cells were from Bethesda Research Laboratories (Gaithersburg, MD).

Source of fibroblast cultures. Cell cultures were obtained from nine unrelated LCAD-deficient patients who have been reported previously (1, 2). All patients were unrelated. The identification of the LCAD-deficient cells is as follows: YH1414, J-1; YH1418, A-1; and YH1435, R-1, as reported in reference 1. The identification numbers of the six other LCAD-deficient cell lines are identical to those in the original report (2), except that each number is preceded by the two letters CP. The deficiency of LCAD activity in these cells was confirmed using the electron transfer flavoprotein-dependent fluorometric assay. Clinically, YH1414, YH1418, YH1435, CP2, CP3, and CP8 belong to the first type, which is characterized by early onset, high mortality, and cardiac involvement. CP9 and CP11 belong to the second type, which is acute and of early onset but presents no cardiac dysfunction or muscle weakness. C14 belongs to the third type, which is characterized by a late onset (2). Normal cell cultures were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ).

Cell culture and protein chemistry. Cell culture and extraction of cell proteins were carried out as previously described (9). The protein concentration was determined by the method of Branford (10) using bovine gamma globulin as a standard.

Immunoabsorption of pure enzyme preparations and cell extracts with antibody. The indicated amount of pure enzyme preparations was dissolved in 100 µL of the extraction buffer, to which 0.1% BSA was added as a carrier. The enzyme solution was mixed with 2-µL of the antibody against the enzyme to be removed and left at 4°C overnight. In the case of cell extracts, insoluble materials were first removed by centrifugation, and the supernatant was similarly treated with antibody. Fifty µL of 10% *S. aureus* suspension were then added, and allowed to react for 15 min at room temperature. The immune complex bound to *S. aureus* cells was removed by centrifugation. The supernatant was used for further analysis by SDS-PAGE.

SDS-PAGE and immunoblot analysis. SDS-PAGE was carried out on 10% polyacrylamide-gel slabs with a 5% stacking gel, using the discontinuous buffer system described by Laemmli (11). When pure enzyme was used as standard, 1 mg/mL of BSA was included in the standard solution to avoid the loss of antigen due to adsorption. After electrophoresis, gel was electroblotted onto a nitrocellulose (Schleicher and Schuell, Keene, NH) or Immobilon-P sheet (Millipore, Bedford, MA), according to the method described by Towbin *et al.* (12). The blot was treated as described previously (13), except that incubation with the specific antibody was done for 1 h. The antigen/antibody complex was detected using the alkaline phosphatase-conjugated goat anti-rabbit IgG system (Bio-Rad Laboratories, Richmond, CA).

RESULTS

Characterization of interactions of rat and human LCAD and MCAD with homologous and heterologous antibodies raised against rat enzymes. We previously observed that, unlike other human acyl-CoA dehydrogenases, human LCAD was not immunoprecipitated as native protein by the antibody raised against its rat counterpart (7). Therefore, [^{35}S]methionine-labeling/immunoprecipitation of human LCAD could not be performed. However, we subsequently found that immunoblotted human LCAD was strongly recognized by anti-rat LCAD antibody, possibly due to unfolding of the protein by SDS.

To characterize the immunoreactions of LCAD and MCAD, both human and rat, we first carried out three sets of preliminary experiments with the pertinent antibodies for the following reasons. First, although rat LCAD did not cross-react with anti-MCAD antibody nor did MCAD cross-react with anti-LCAD antibody as native proteins (6), their cross-reactivity in the denatured state was unknown. Furthermore, the electrophoretic mobility of authentic human LCAD was unknown, inasmuch as human LCAD has never been purified. The electrophoretic mobilities of rat LCAD and MCAD were indistinguishable, but other acyl-CoA dehydrogenases can be readily distinguished from each other by slight but distinct differences in their electrophoretic mobility (6).

In the first set of experiments, we carried out immunoabsorption experiments using the purified rat enzymes to test whether it is necessary to remove a small amount of contaminating enzyme that may be present in the purified LCAD or MCAD preparations and may interfere with the immunoreactions. These experiments served also to characterize the specificity of antibodies toward denatured acyl-CoA dehydrogenases. We first carried out preabsorption of pure rat MCAD by incubating it with anti-LCAD antibody or preimmune serum, followed by mixing with *S. aureus* cell suspension; in this system, the immune complex binds to Protein A in *S. aureus* cells and precipitates. The supernatant was electrophoresed and electroblotted, and the proteins detected using anti-MCAD antibody as probe. When the pure rat MCAD preparation[2] was directly applied to the gel

[2] Despite their high purity, most of the rat and human MCAD preparations exhibited double bands when analyzed on SDS-PAGE (6, 8). The double bands can be immunoprecipitated by anti-rat MCAD antibody with equal deficiency, indicating their identity. The determination of the amino terminal sequence indicated that the double band is due to a loss of several amino acid residues from the amino terminus in some of the MCAD molecules (6).

without preabsorption, MCAD above the amount of 10 ng was readily detectable (Fig. 1*A, lanes 1* and *2*). When 133 and 13.3 ng of pure rat MCAD were subjected to preabsorption with preimmune serum followed by electrophoresis and electroblot, a recognizable loss of MCAD occurred, but both bands were readily detectable (Fig. 1*A, lanes 3* and *5*). When the same amounts of the MCAD preparation were preabsorbed with anti-LCAD antibody, the amount of MCAD detected was comparable
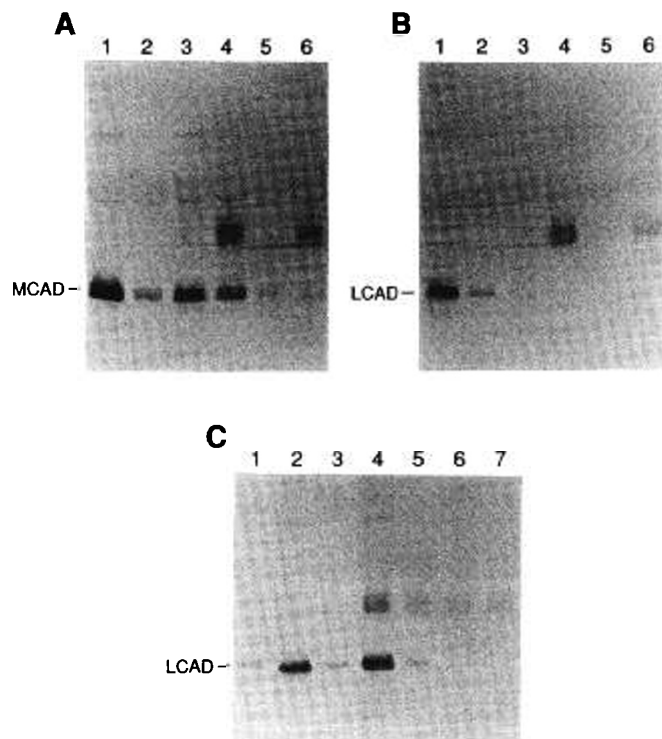

Fig. 1. Characterization of antibody by preabsorption and immunoblot analysis. Pure enzymes (400 or 40 ng) in 100 µL of buffer were preabsorbed with 2 µL of preimmune, anti-MCAD, or anti-LCAD antibody, followed by mixing with 50 µL of 10% *S. aureus* suspension and centrifugation. One third of each supernatant was subjected to immunoblot analysis as described in Materials and Methods. *A,* Immunodetection of purified rat MCAD using anti-MCAD antibody as a probe with and without preabsorption with anti-LCAD antibody. *Lanes 1* and *2,* 100 and 10 ng, respectively, of purified rat MCAD were directly applied as standard; *lanes 3* and *4,* 133 ng of rat MCAD after preabsorption with preimmune rabbit serum and anti-LCAD antibody, respectively; *lanes 5* and *6,* 13.3 ng of rat MCAD after preabsorption with preimmune rabbit serum and anti-LCAD antibody, respectively. The intense bands above the MCAD bands (*lanes 3–6*) are the heavy chain of IgG derived from the preimmune serum or the antibodies used. The broad, faint bands seen in each lane are a result of BSA being used as a carrier. *B,* Immunodetection of purified rat MCAD using anti-LCAD antibody as a probe after preabsorption with anti-LCAD antibody. This experiment was designed to test whether the rat MCAD preparation is cross-reactive to anti-LCAD antibody. *Lanes 1* and *2,* 100 and 10 ng, respectively, of purified rat LCAD were directly applied as an electrophoretic standard; *lanes 3* and *4,* 133 ng of purified rat MCAD after preabsorption with preimmune rabbit serum and anti-LCAD antibody, respectively; *lanes 5* and *6,* 13.3 ng of pure rat MCAD after preabsorption with preimmune rabbit serum and anti-LCAD antibody, respectively. *C,* Immunodetection of purified rat LCAD, with and without preabsorption with anti-LCAD antibody, using anti-LCAD antibody as a probe. *Lane 1,* 15 ng of rat LCAD was directly applied as a standard; *lanes 2* and *3,* 133 and 13.3 ng, respectively, of rat LCAD after preabsorption with preimmune rabbit serum; *lanes 4* and *5,* 133 and 13.3 ng, respectively, of pure rat LCAD after preabsorption with anti-MCAD antibody; *lanes 6* and *7,* 133 and 13.3 ng, respectively, of pure rat LCAD after preabsorption with anti-LCAD antibody.

to that detected after absorption with preimmune serum (Fig. 1A, lanes 4 and 6), indicating that anti-LCAD antibody does not precipitate rat MCAD.

In the second experiment of this set, the same aliquots of the same MCAD preparation were analyzed by electrophoresis and electroblotted in the same manner as in the first experiment, but immunodetection of proteins was done using anti-LCAD antibody as a probe (Fig. 1B). In lanes 1 and 2, 100 and 10 ng, respectively, of the purified rat LCAD preparation was directly applied to SDS-PAGE as a standard without preabsorption. When the rat MCAD preparation was preabsorbed with either anti-LCAD antibody or preimmune serum and probed with anti-LCAD antibody, no band was detectable (Fig. 1B, lanes 3-6), indicating that rat MCAD, even at 133 ng, did not cross-react with anti-LCAD antibody. In the third experiment, the rat LCAD preparation was immunoblotted after preabsorption with preimmune serum (lanes 2 and 3), anti-MCAD (lanes 4 and5) or anti-LCAD antibody (lanes 6 and 7), and probed with anti-LCAD antibody (Fig. 1C). In lane 1, 15 ng of pure rat LCAD was directly applied to the gel as a standard. As can be seen in lanes 6 and 7, 133 and 13.3 ng of rat LCAD was completely removed by preabsorption with 2 μL anti-LCAD antibody, indicating the strong potency of this antibody. In contrast, no detectable amount of rat LCAD was removed by preabsorption with preimmune serum (lanes 3 and 4) or anti-MCAD antibody (lanes 4 and 5). These results demonstrate that the antibodies used in this study were indeed monospecific and potent.

In the second set of experiments, immunotitration of rat LCAD (Fig. 2A) and MCAD (Fig. 2B) was carried out using a serially diluted crude rat liver mitochondrial extract as antigen. Preabsorption with 2 μL of anti-LCAD antibody removed most of the LCAD from the mitochondrial extract containing 50 and 25 μg protein, and all of the LCAD from the mitochondrial extract containing 12.5 μg protein (Fig. 2A, row a). However, preabsorption with anti-MCAD antibody did not reduce LCAD to any recognizable degree (Fig. 2A, row b). Preabsorption with 2 μL of anti-MCAD antibody similarly removed most of the MCAD proteins from mitochondrial extracts containing 25 μg protein and all of the MCAD from mitochondrial extracts containing 6.25 μg protein (Fig. 2B, row a), but preabsorption with anti-LCAD antibody did not reduce the amount of MCAD (Fig. 2B, row b). These results again demonstrated the high specificity and strong potency of the antibodies used in the present study.

In the third set of experiments, we tested the cross-reactivity of human MCAD with anti-LCAD antibody. The purified human MCAD preparation was probed with anti-MCAD (Fig. 3A) or anti-LCAD antibody (Fig. 3B) with and without preabsorption with anti-MCAD antibody. The data shown in Figure 3A demonstrate that considerably more than 40 ng of MCAD could be precipitated with 2 μL of anti-MCAD antibody. No band could be detected with anti-LCAD antibody either with or without preabsorption with anti-MCAD antibody (Fig. 3B). Similar experiments on human LCAD could not be carried out because it has not been purified.

Analysis of skin fibroblasts from patients with LCAD deficiency. First, immunoreactive LCAD and MCAD proteins in cultured skin fibroblasts from the three LCAD-deficient patients originally described by Hale *et al.* (1) were analyzed with and without preabsorption with anti-MCAD antibody (Fig. 4, A and B). For comparison, a normal (GM05756) and an MCAD-deficient cell line (YH1475) were included in this experiment. When immunoblots were probed with anti-LCAD antibody, all three LCAD-deficient cells showed a clear single variant LCAD band that was indistinguishable in size from normal LCAD. The mobility of the LCAD band in the cell extract appeared slightly slower than that of pure rat LCAD on SDS-PAGE. The intensity of variant LCAD bands in the three LCAD-deficient cell lines was also similar to that in normal cells. When the same samples were analyzed for MCAD protein without preabsorption with anti-MCAD antibody, a normal MCAD band was detected in
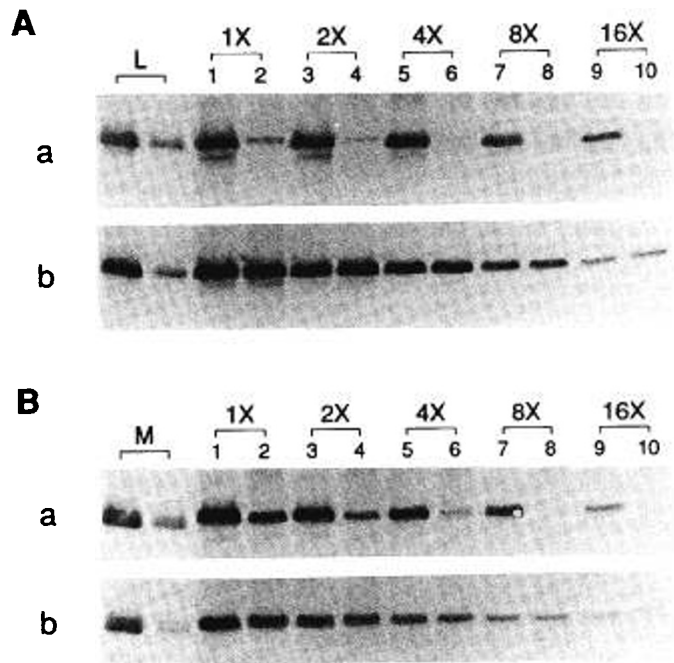

Fig. 2. Immunotitration of rat mitochondrial extract: characterization of antibodies. Serial 2-fold dilutions of a rat mitochondrial extract containing 50 μg of protein were preabsorbed with an appropriate antibody, and one third of each supernatant was subjected to immunoblot analysis. In each panel, 100 and 10 ng of an appropriate purified rat enzyme (L, LCAD; M, MCAD) were applied at the left end. The degree of dilution is indicated at the top. A, Detection of LCAD using anti-LCAD antibody as a probe. a, After preabsorption with preimmune rabbit serum (odd-numbered lanes) or anti-LCAD antibody (even-numbered lanes). b, After preabsorption with preimmune rabbit serum (odd-numbered lanes) or anti-MCAD antibody (even-numbered lanes). L, 100 ng and 10 ng of rat LCAD. B, Detection of MCAD using anti-MCAD antibody as a probe. a, After preabsorption with preimmune rabbit serum (odd-numbered lanes) or anti-MCAD antibody (even-numbered lanes). b, After preabsorption with preimmune rabbit serum (odd-numbered lanes) or anti-LCAD antibody (even-numbered lanes). M, 100 and 10 ng of rat MCAD.
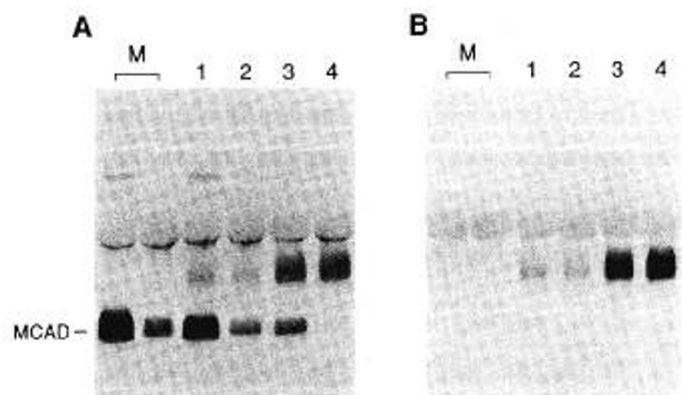

Fig. 3. Immunoreactions of human MCAD with anti-rat MCAD (A) and anti-rat LCAD antibodies (B). A, Pure human MCAD (133 and 13.3 ng) was detected using anti-MCAD antibody as a probe after preabsorption with preimmune rabbit serum (lanes 1 and 2) or anti-MCAD (lanes 3 and 4) antibody. M, 133 and 13.3 ng of human MCAD were directly applied as standard. B, Pure human MCAD (133 and 13.3 ng) was probed with anti-LCAD antibody probe after preabsorption with preimmune rabbit serum (lanes 1 and 2) or anti-MCAD (lanes 3 and 4) antibody. M, 133 and 13.3 ng of human MCAD were directly applied as standard.
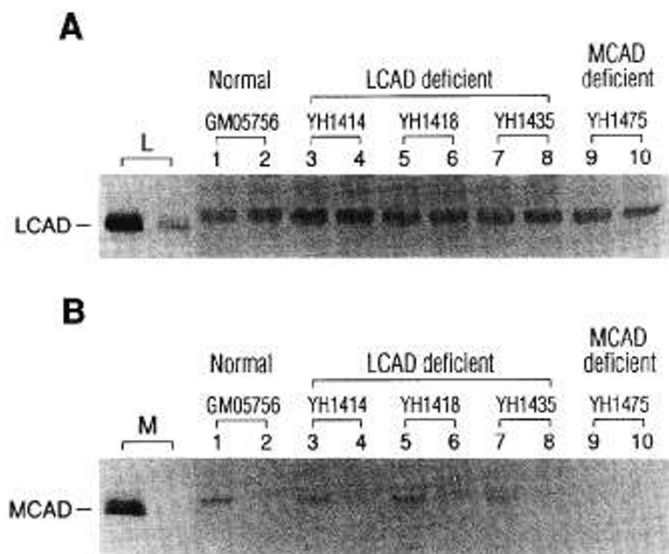
A

B

Fig. 4. Immunoblot analysis of variant LCAD in skin fibroblasts from three patients with LCAD deficiency. A confluent culture of normal or mutant fibroblasts in two 150-cm² flasks was harvested and treated with a detergent. The cell extract (300 μg protein in 100 μL) was preabsorbed with preimmune rabbit serum (*odd-numbered lanes*) or anti-MCAD antibody (*even-numbered lanes*). One third of each supernatant was subjected to the immunoblot analysis. *A*, LCAD detected using anti-LCAD antibody as a probe; *B*, MCAD detected using anti-MCAD antibody as a probe. At the *left side of each panel*, 100 and 10 ng of a purified rat enzyme are spotted. *L*, LCAD; *M*, MCAD.
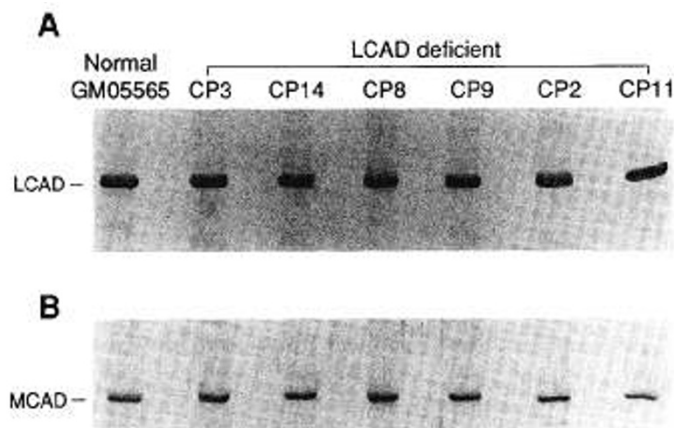
A

B

Fig. 5. Immunoblot analysis of variant LCAD in six additional LCAD-deficient cell lines. The skin fibroblast extract (100 μg protein) was subjected to the immunoblot analysis without preabsorption with antibodies. *A*, Variant LCAD detected using anti-LCAD antibody as a probe; *B*, MCAD detected using anti-MCAD antibody as a probe.

the normal and the three LCAD-deficient cell lines, but the MCAD band was detectable in none of them after preabsorption with anti-MCAD antibody. In the MCAD-deficient cells, no immunoreactive MCAD band was detectable even without preabsorption, indicating that YH1475 is negative for cross-reactive materials. These data also provided evidence that LCAD and MCAD proteins were well distinguished in the present analytical system, despite the close sequence similarity (14–16; Indo Y, Glassberg R, Tanaka K, unpublished data).

Variant LCAD in six additional LCAD-deficient cell lines was further analyzed without preabsorption using anti-MCAD antibody as a probe (Fig. 5). Again, a single variant LCAD band was detected in all six cell lines. The molecular size was indistinguishable from and the amount was comparable to that of LCAD in normal cells.

DISCUSSION

There has been a controversy concerning whether LCAD and MCAD are immunologically cross-reactive. Previously, Furuta *et al.* (17) observed that their purified rat LCAD and MCAD proteins each cross-reacted with antibody raised against the other. We have subsequently demonstrated using immunotitration and Ouchterlony double diffusion methods (6) that none of our purified preparations of rat acyl-CoA dehydrogenases, including LCAD and MCAD, cross-reacted with antibodies raised against the other acyl-CoA dehydrogenases (6). The data presented here conclusively confirm that rat LCAD and MCAD are indeed immunologically distinct, not only in their native forms but also in their denatured forms. Thus, the cross-reactivity of rat LCAD and MCAD preparations with the respective antibodies observed by Furuta *et al.* (17) was due to cross-contamination of their preparations. Although human LCAD and MCAD are homologous, sharing 30.4% identical residues in their sequences (Indo Y, Yang-Feng T, Glassberg R, Tanaka K, unpublished data), the present data suggest that they do not share common epitopes. This is probably because the stretches of consecutive identical amino acid residues shared by human LCAD and MCAD are short as in a pair of any two acyl-CoA dehydrogenases from the same species (17), usually extending only two to three residues, with the longest stretch being seven residues. In contrast, the stretches of consecutive identical amino acid residues shared by the same acyl-CoA dehydrogenase, including LCAD, from human and rat are generally much longer, often extending 15 to 17 residues. In the case of human and rat LCAD, the longest stretch was 42 residues (Indo Y, Yang-Feng T, Glassberg R, Tanaka K, unpublished data), rendering cross-reactivity to anti-rat LCAD antibody against human LCAD.

The characteristics of the variant LCAD protein studied here not only provide important insights into the nature of the biochemical basis of this disorder, but also contribute useful information for the molecular study of LCAD mutations. It was notable that the variant LCAD protein of normal size was detected in an amount comparable to normal in all nine unrelated LCAD-deficient cell lines regardless of their clinical phenotypes, suggesting that the LCAD mutation in these patients is likely to be a point mutation that produces a stable variant LCAD. This is in contrast to the findings with other acyl-CoA dehydrogenase deficiencies. In the study of 15 patients with isovaleric acidemia, at least five distinct types of variant isovaleryl-CoA dehydrogenase alleles were recognized with regard to the presence and absence of variant isovaleryl-CoA dehydrogenase proteins and their molecular size (18). In three known patients with SCAD deficiency, all variant SCAD were of normal size as tested with [³⁵S]methionine-labeling, suggesting that they are point mutations (19). However, the study of stability (9) and the sequence study (20) indicated that these SCAD mutations were extremely heterogenous. The pattern of variant MCAD is to some degree similar to that of variant LCAD. In the study of 13 MCAD-deficient cell lines, we previously showed using [³⁵S] methionine-labeling and immunoprecipitation that all of them synthesized a normal-size variant MCAD (21), but when one of these cell lines (YH1475) was tested by immunoblot analysis in the present study, no variant MCAD band was detectable, as shown in Figure 4. In the sequence study of nine independent MCAD-deficient cell lines including YH1475, a common point mutation, $^{985}A \rightarrow G$ transition, was detected in 17 variant alleles (22). In a subsequent study of 55 independent MCAD-deficient patients, we found that the prevalence of $^{985}A \rightarrow G$ was 89.1% of variant MCAD alleles (Yokota I, Coats PM, Hale DE, Rinaldo P, Tanaka K, unpublished results). Absence of cross-reactive variant MCAD in $^{985}A \rightarrow G$ homozygous cells have been confirmed in a much larger number of cell lines (Coates PM, Indo Y, Young D, Hale DE, Tanaka K, unpublished results).

The uniform nature of variant LCAD in terms of their size and stability suggests that only a single or a few prevalent point

mutations may be found in the majority of LCAD-deficient patients. This is despite the apparent clinical and biochemical heterogeneity among patients. If this is the case, it should be possible to devise a fast molecular diagnostic method for LCAD deficiency that may make prompt diagnosis possible in most of the cases.

REFERENCES

1. Hale DE, Batshaw ML, Coates PM, Frerman FE, Goodman SI, Singh I, Stanley CA 1985 Long-chain acyl coenzyme A dehydrogenase deficiency: an inherited cause of nonketotic hypoglycemia. Pediatr Res 19:666–671
2. Hale DE, Stanley CA, Coates PM 1990 The long-chain acyl-CoA dehydrogenase deficiency. In: Tanaka K, Coates PM (eds) Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspects. Alan R Liss, Inc, New York, pp 303–324
3. Amendt BA, Moon M, Teel L, Rhead WL 1988 Long-chain acyl-coenzyme A dehydrogenase deficiency: biochemical studies in fibroblasts from three patients. Pediatr Res 23:603–605
4. Roe CR, Coates PM 1989 Acyl-CoA dehydrogenase deficiencies. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic Basis of Inherited Disease. McGraw-Hill, New York, pp 889–914
5. Allison F, Bennett MJ, Variend S, Engel PC 1988 Acylcoenzyme A dehydrogenase deficiency in heart tissue from infants who died unexpectedly with fatty change in the liver. Br Med J 296:11–12
6. Ikeda Y, Okamura-Ikeda K, Tanaka K 1985 Purification and characterization of short chain, medium chain, and long chain acyl-CoA dehydrogenases from rat liver mitochondria: isolation of the holo- and apoenzymes and conversion of the apoenzyme to the holoenzyme. J Biol Chem 260:1311–1325
7. Ikeda Y, Tanaka K 1987 Immunoprecipitation and electrophoretic analysis of four human acyl-CoA dehydrogenases and electron transfer flavoprotein using antibodies raised against the corresponding rat enzymes. Biochem Med Metab Biol 37:329–334
8. Finocchiaro G, Ito M, Tanaka K 1987 Purification and properties of short chain acyl-CoA, medium chain acyl-CoA, and isovaleryl-CoA dehydrogenases from human liver. J Biol Chem 262:7982–7989
9. Naito E, Indo Y, Tanaka K 1989 Short chain acyl-coenzyme A dehydrogenase (SCAD) deficiency. Immunochemical demonstration of molecular heterogeneity due to variant SCAD with differing stability. J Clin Invest 84:1671–1674
10. Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
11. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
12. Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from acrylamide gels to nitrocellulose sheet: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354
13. Indo Y, Kitano A, Endo F, Akaboshi I, Matsuda I 1987 Altered kinetic properties of the branched-chain α-keto acid dehydrogenase complex due to mutation of the β-subunit of the branched-chain α-keto acid decarboxylase (E₁) component in lymphoblastoid cells derived from patients with maple syrup urine disease. J Clin Invest 80:63–70
14. Kelly DP, Kim JJ, Billadello JJ, Hainline BE, Chu TW, Strauss AW 1987 Nucleotide sequence of medium-chain acyl-CoA dehydrogenase mRNA and its expression in enzyme-deficient human issue. Proc Natl Acid Sci USA 84:4068–4072
15. Matsubara Y, Kraus JP, Ozasa H, Glassberg R, Finocchiaro G, Ikeda Y, Mole J, Rosenberg LE, Tanaka K 1987 Molecular cloning and nucleotide sequence of cDNA encoding the entire precursor of rat liver medium chain acyl coenzyme A dehydrogenase. J Biol Chem 262:10104–10108
16. Matsubara Y, Indo Y, Naito E, Ozasa H, Glassberg R, Vockley J, Ikeda Y, Kraus J, Tanaka K 1989 Molecular cloning and nucleotide sequence of cDNAs encoding the precursors of rat long chain acyl-coenzyme A, short chain acyl-coenzyme A, and isovaleryl-coenzyme A dehydrogenases. J Biol Chem 264:16321–16331
17. Furuta S, Miyazawa S, Hashimoto T 1981 Purification and properties of rat liver acyl-CoA dehydrogenases and electron transfer flavoprotein. J Biochem 90:1739–1750
18. Ikeda Y, Keese SM, Tanaka K 1985 Molecular heterogeneity of variant isovaleryl-CoA dehydrogenase from cultured isovaleric acidemia fibroblasts. Proc Natl Acad Sci USA 82:7081–7085
19. Naito E, Ozasa H, Ikeda Y, Tanaka K 1989 Molecular cloning and nucleotide sequence of complementary DNAs encoding human short chain acyl coenzyme A dehydrogenase and the study of the molecular basis of human short chain acyl-coenzyme A dehydrogenase deficiency. J Clin Invest 83:1605–1613
20. Naito E, Indo Y, Tanaka K 1990 Identification of two variant short chain acyl-coenzyme A dehydrogenase alleles, each containing a different point mutation in a patient with short chain acyl-coenzyme A dehydrogenase deficiency. J Clin Invest 85:1575–1582
21. Ikeda Y, Hale DE, Keese SM, Coates PM, Tanaka K 1986 Biosynthesis of variant medium chain acyl-CoA dehydrogenase in cultured fibroblasts from patients with medium chain acyl-CoA dehydrogenase deficiency. Pediatr Res 20:843–847
22. Yokota I, Indo Y, Coates PM, Tanaka K 1990 Molecular basis of medium chain acyl-coenzyme A dehydrogenase deficiency. An A to G transition at position 985 that causes a lysine-304 to glutamate substitution in the mature protein is the single prevalent mutation. J Clin Invest 86:1000–1003