BRIEF COMMUNICATION

Identification of a Novel Mutation in Patients with Medium-Chain Acyl-CoA Dehydrogenase Deficiency

A novel mutation was identified in two unrelated patients with medium-chain acyl-CoA dehydrogenase deficiency. First, a 19-year-old Caucasian female presented with a devastating illness, resulting in sudden death in adulthood which is unusual. The second patient, now a 3.5-year-old male, presented at 17 months of age with a hypoglycemic seizure and dehydration. Sequence analysis revealed a novel mutation G617T in exon 8 resulting in an arginine to leucine substitution at codon 206 (R206L). Both patients were compound heterozygous for this G617T and the common mutation A985G. © 2000 Academic Press

Key Words: MCAD deficiency; mitochondrial β-oxidation; mutation.

Mitochondrial β-oxidation plays a major role in energy production, especially during fasting periods. Inherited mitochondrial fatty acid oxidation disorders consist of 17 autosomal recessive defects, of which medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (MIM 201450) is the most common disorder with a carrier frequency of approximately 1:80 in most Caucasian populations (1,2). MCAD deficiency is characterized by episodes of illness in early childhood associated with fasting resembling Reye’s syndrome: coma, hypoglycemia, hyperammonemia, and fatty liver. A common mutation A985G has been identified among patients with MCAD deficiency, which account for 88.9% of the mutant alleles (3–7). There is significant phenotypic heterogeneity in MCAD deficiency, even within the same family. The affected children may have only one episode of illness or multiple recurrences. In some cases, the first episode can be devastating, resulting in sudden death. On the other hand, they may appear to be asymptomatic (8). Most children present with acute illness between 3 and 15 months of age. There are few reports of first symptoms after age 4 years. In the present study, we report two patients with MCAD deficiency, including an unusual adult patient, and their molecular defects.

SUBJECTS AND METHODS

Patients

Patient 1. The proband, a 19-year-old previously healthy Caucasian female, is the daughter of healthy nonconsanguineous parents. She presented to a hospital for alteration in consciousness. One day prior to presentation she developed nausea, vomiting, and drowsiness after camping for a day with her friends and drinking alcoholic beverages. She had stable vital signs after IV hydration. On neurologic examination, she was oriented only to place and person and did not follow any commands. Cranial nerves were intact. She responded to painful stimulation. Deep tendon reflexes were normal. Normal laboratory data included glucose, blood alcohol level, and kidney and liver function tests. Her WBC count and serum uric acid levels were mildly elevated. Electrolytes revealed mild metabolic acidosis and mild ketonuria. Brain computerized axial tomography scan was normal. A few hours after admission the patient was found pulseless and unresponsive. Resuscitation efforts were unsuccessful. On autopsy, liver pathology revealed macroversicellular steatosis. Plasma acylcarnitine profile showed highly elevated octanoylcarnitine, indicating MCAD deficiency. The family later volunteered a history of Reye-like attack at 18 months of age.

Patient 2. The proband, now a 3.5-year-old male, is the only child of a healthy Caucasian mother and an African-American father with apparent Noonan’s syndrome. The patient presented for the first time,
at 17 months of age, with vomiting and a hypoglycemic seizure. He also had microcephaly and a facial profile similar to his father’s, suggesting dominant transmission of Noonan’s syndrome. Urine organic acids showed increased quantities of both hexanoylglycine and suberylglycine and the presence of phenobarbital. Plasma acylcarnitine profile showed elevated C6, C8, and C10:1 consistent with MCAD deficiency.

Preparation of Genomic DNA

Genomic DNA was isolated from postmortem formalin-fixed liver from patient 1 by standard methods using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer’s procedure. Genomic DNA was also isolated from peripheral blood obtained from patient 2, the parents of patients, and normal controls. DNA was prepared by a standard method using the QIAamp blood kit (Qiagen, Inc. Valencia, CA).

Sequence Analysis of MCAD

All 12 MCAD exons were amplified from genomic DNA by the use of specific intronic primers, derived from the 5’ and 3’ intronic sequences and AmpliTaq (PE Applied Biosystems). The DNA fragments were amplified using 50 ng of genomic DNA in a 50-μl reaction volume consisting of 1× PCR buffer (Perkin-Elmer), 2.5 units of Taq polymerase, 0.25 mM dNTPs, and 20 pmol of each relevant primer. PCR cycling conditions were as follows: after heating the reactions for 1 min at 94°C, 30 cycles were performed at 94°C for 15 s, 56°C for 15 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. The corresponding PCR products were purified by agarose gel electrophoresis and QIAEX II gel extraction kit (Qiagen). Direct sequencing of PCR products was performed with dRhodamine terminator cycle sequencing kit (PE Applied Biosystems) on an ABI Prism 377 DNA sequencer (PE Applied Biosystems).

Restriction-Endonuclease Analysis of Mutations

Genomic DNA was isolated and PCR was carried out in the manner described above. Because the MCAD G617T mutation did not create or eliminate a restriction site for a suitable endonuclease, PCR amplification was performed with mismatched primers.

Sense primer: 5’ TGTATCTCTTAGGTATTTTTTATTGACAC 3’;
Antisense primer: 5’ AGGCTTTATTAGCAGGAGCTTTA 3’.

The sense primer was a modified primer in which G-613 was artificially substituted with A. The amplified fragments were then digested with an endonuclease AflIII, subjected to electrophoresis on 12% polyacrylamide gel, stained with 0.5 mg/ml ethidium bromide, and visualized with UV light.

RESULTS

Mutation Analysis by Sequencing

Patient 1 was a late-onset MCAD deficiency with a common mutation A985G that was previously detected by multiple primer extension assay, which was developed in our laboratory (data not shown). To screen for the second mutation within the MCAD gene, all 12 exons and their flanking intronic sequences were amplified from genomic DNA. The PCR products were purified and sequenced.

Sequence analysis revealed a G to T substitution at nucleotide position 617 (Fig. 1A), resulting in an arginine to leucine substitution at codon 206 (R206L).
Patient 2 was a 17-month-old male. The exons of the MCAD gene, including their flanking intronic sequences, were amplified and sequenced as described above. The G617T mutation as well as the A985G mutation (data not shown) was also identified indicating that patient 2, like patient 1, was a compound heterozygote. The presence of the G617T mutation was identified on one allele from the patient’s father. He did not carry the A985G mutation. DNA from the patient’s mother was not available.

Mutation Test by PCR/Restriction-Endonuclease Analysis

To confirm the G617T mutation by PCR/restriction-AflIII analysis, a 68-bp fragment was amplified by PCR with mismatched primers. The G617T mutant allele results in the loss of an AflIII restriction site. The amplified fragments were digested with AflIII and then subjected to electrophoresis on 12% polyacrylamide gel. The PCR/restriction enzyme digestion results were consistent with the sequencing results (data not shown). The G617T mutation was not identified in any control individuals, suggesting that it does reflect a pathological variation.

DISCUSSION

MCAD is one of the most frequently inherited metabolic disorders among Caucasians. In most cases, acute illness presents between 3 and 15 months of age. Once the diagnosis is established, the treatment and appropriate management are effective. In contrast, without diagnosis, most children with MCAD deficiency are at significant risk of sudden death with either the initial or a later episode. MCAD deficiency is found almost exclusively among Caucasians, particularly those of Northwestern European origin (9–12). There are isolated cases of Southern European and North African origin. Interestingly, the father of patient 2, who is a G617T carrier, is African-American. In addition this is the first reported mutation located in exon 8, which is the region believed to be important for FAD–polypeptide interaction and for the electron transfer from FAD bound to MCAD to electron transferring flavoprotein (ETF) (13).

MCAD is a homotetrameric enzyme located in the mitochondrial matrix. The MCAD monomer is composed of three domains of approximately equal size. The N-terminal and the C-terminal domains are α-helices packed together and the middle domain consists of two β-sheets (13). The two packed β-sheets are encoded by exons 6–8. The mutation of G617T which changes the polar residue arginine-206 to the hydrophobic residue leucine may affect the folding of the β-sheet domain. This region where arginine-206 is located is also important for the FAD–polypeptide interaction. The flavin ring is locked in the crevice between the β-domain and the C-terminal domain of one subunit and the C-terminal domain of a neighboring subunit. Fourier mapping also showed tubular electron densities near the FAD ring and between the β-sheet domain and the two α-helix domains (13,14). Therefore, this region is believed to be important for the electron transfer from FAD bound to MCAD to ETF.

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REFERENCES

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