A NOVEL MUTATION OF THE ABCD1 GENE IN SERBIAN X-ADRENOLEUKODYSTROPHY

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ABSTRACT

X-linked adrenoleukodystrophy (XALD), the most common inherited peroxisomal disorder, is characterized by central nervous system demyelination, primary adrenal failure and the systemic accumulation of saturated very long chain fatty acids (VLCFAs). We describe a novel mutation of the ABCD1 gene in a Serbian patient with this disorder. The affected boy developed Addison's disease and neurological symptoms at 6 years of age and had a bone marrow transplant 2 years later. His plasma level of saturated VLCFAs, ratios of C24:0/C22:0 and C26:0/C22:0, were all significantly elevated. Direct sequencing of the ABCD1 gene detected the point mutation 1519 (G>A) in exon 6, which changes a glycine at position 507 into serine (G507S). This is the first report of genetically confirmed X-adrenoleukodystrophy in Serbia.

Key words: ABCD1 gene, Point mutation, X-linked adrenoleukodystrophy (XALD), Peroxisome.

INTRODUCTION

Adrenoleukodystrophy (ALD) comprises two genetically determined disorders with a different mode of inheritance, *i.e.*, X-linked (XALD) and neonatal autosomal recessive ALD (1). Both are

characterized by varying degrees of central nervous system demyelination and adrenal dysfunction as well as systemic accumulation of very long chain fatty acids (VLCFAs, fatty acids with more than 22 carbon atoms). X-linked ALD (MIM 300100) is the most common inherited peroxisomal disorder and is due to mutations or defects in the ABCD1 gene that codes for ALDP (ALD protein) (XALD) which is located in the peroxisomal membrane and functions as a crucial transporter of VLCFAs that are metabolized exclusively in peroxisomes by β -oxidation. Lacking ALDP, VLCFAs fail to enter the peroxisomes and to be activated to their coenzyme A (CoA) derivatives by VLCFA acyl-CoA synthetase (VLCS) for further catabolism (2). The mechanism of how ALDP and VLCS interact remains to be elucidated. Diagnosis relies on clinical presentation, biochemical findings of primary adrenal failure, elevated plasma level of VLCFAs, characteristic leukodystrophic changes in brain magnetic resonance imaging (MRI) and is complemented by genetic analysis. The ABCD1 gene spans about 22 kb of DNA on chromosome Xq28 and contains 10 exons. To date, more than 944 ABCD1 gene mutations have been registered in a database (<http://www.x-ald.nl>). We have identified a novel mutation in a Serbian family with XALD.

CASE REPORT

The patient is a 6-year-old boy, the first child of unrelated parents. He was born at 40 weeks gestation by spontaneous delivery. There were no perinatal complications. Physical examination revealed

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increased pigmentation of the lips, tongue and buccal mucosa. Early neurological symptoms were behavioral disturbances, deterioration of vision and impaired auditory discrimination. Our patient had the following biochemical abnormalities: low morning plasma cortisol level 1.0 µg/dL (reference range 5-20 µg/dL) and an elevated ACTH level of 62.4 ng/L (reference range 9-52 ng/L), respectively. A 1 µg-short synacthen test showed a peak cortisol response of 6.1 µg/dL (reference range 5-20 µg/dL), confirming the primary adrenal cortex failure. Fasting plasma VLCFA levels of C22:0, C24:0 and C26:0 were 18.0 (control 22.5 ± 8.0), 39.8 (control 21.6 ± 8.0) and 1.5 µg/mL (control 0.35 ± 0.08), respectively. Ratios of C24/C22 and C26/C22 were significantly elevated at 2.21 (normal 0.74-1.627) and 0.083 (normal 0.014-0.063). Cerebral MRI typically showed extensive demyelination in the occipital periventricular white matter.

MATERIALS AND METHODS

Genomic DNA was extracted from the peripheral blood leukocytes using a standard phenol-chloroform extraction method (3). The splice junctions and all coding regions of the ABCD1 gene of the proband were amplified by polymerase chain reaction (PCR) using primers ALDe6-F (5'-CGC TCT CTG GCG TCA GCG-3') and ALDe6-R (5'-TGG TGT TGG TCC TCC CTG-3'). The conditions for

PCR were as follows: initial denaturation for 15 seconds at 96°C, followed by 25 cycles of denaturation for 15 seconds at 96°C, annealing for 15 seconds at 55°C and a final extension for 4 min. at 60°C. The reaction mixture at final volume (100 µL) contained 1X PCR buffer II (PE Applied BioSystems, Foster City, CA, USA), 1.5 mM MgCl₂, 0.5 M betaine (Sigma, St. Louis, MO, USA), 200 µM dNTP, 1 µM of each primer, two units Taq polymerase and 100 ng of DNA template. The PCR products were sequenced directly using the Dye™ Terminator cycle sequencing kit (PE Applied BioSystems). Restriction enzyme digestions were performed using 40 µL of the initial PCR products of exon 6 using primers ALDe-F and ALDe6-R, and were incubated at 37°C overnight with five units of *Hha*I restriction enzyme. The restriction fragments were separated by 3% agarose gel electrophoresis under 100 V for 4 hours.

RESULTS AND DISCUSSION

Peroxisomal disorders are an extremely heterogeneous group of genetic disorders without strict correlation between clinical picture and biochemical abnormalities. The natural history of the disease course is known to be unpredictable. The analysis of VLCFAs in plasma is necessary as the first screening test for diagnosis of XALD.

Sequence analysis (Figure 1) of the ABCD1 mutant allele in the proband revealed a novel point

Figure 1. Partial sequence determination of the 1519 (G>A) point mutation of the ABCD1 gene for the hemizygous proband.

mutation 1519 (G>A) in exon 6, causing the change of glycine at position 507 into serine (G507S). So far, no established correlation between genotype and phenotype has been reported (4).

The range of phenotypic expression in XALD and the prognosis for an affected male are unpredictably variable and cannot be predicted through levels of VLCFA in plasma or cultured skin fibroblasts, the residual VLCFA β -oxidation activity present in XALD skin fibroblasts, the family history or the nature of the mutation identified in the ABCD1 gene of the patient (5). The same mutation can be associated with each of the known clinical phenotypes.

The affected boy had developed neurological symptoms and Addison's disease at 6 years old and a bone marrow transplant at age of 8 without any effect. This mutation in exon 6 caused the changes in the intracellular domain of ALDP and may lead to instability or loss of function of ALDP resulting in accumulation of VLCFAs. Further investigation of ALDP stability in this patient is needed to clarify the impact of this ABCD1 mutation. The childhood cerebral form of XALD is a severe metabolic disease without a definite effective therapy. Mutations in the ABCD1 gene have been identified in the majority of XALD patients, and almost 60% of mutations are non recurrent. In conclusion, analyses of mutations in patients with XALD is necessary, especially for carrier diagnosis and genetic counseling, since more than 900 different mutations in the gene have been reported.

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