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MUTATION IN BRIEF

Two Intronic Mutations in the Adrenoleukodystrophy Gene

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INTRODUCTION

X-linked adrenoleukodystrophy (ALD) is an inherited disorder of peroxisomal β -oxidation of saturated very long chain fatty acids (VLCFA), and is characterized by progressive demyelination of the nervous system, and adrenocortical and gonadal insufficiency (Moser et al., 1989). The most frequent phenotypes are the rapidly progressive childhood cerebral ALD (CCALD) and the slowly progressive adrenomyeloneuropathy (AMN). Recently, the ALD gene encoding a peroxisomal membrane associated protein (ALDP) was cloned (Mosser et al., 1993, 1994). The ALDP belongs to the ATP-binding cassette (ABC) superfamily of transporter proteins, indicating that the primary defect in ALD interferes with a transport process essential for β -oxidation of VLCFA. So far, few chromosomal deletions (Mosser et al., 1993; Cartier et al., 1993) and two exonic point mutations (Cartier et al., 1993; Uchiyama et al., 1994) were reported, and a possible hot spot for mutations was identified in the fifth exon of the ALD gene (Kemp et al., 1994). We report two patients from two Dutch kindreds with mutations in introns of the ALD gene, leading to the use of novel or cryptic 3' splice acceptor sites and prematurely terminated proteins.

MATERIALS AND METHODS

In kindred ALD#1, a boy suffered from CCALD, and in kindred ALD#19 a patient had AMN. The following primers, derived from the human ALD cDNA sequence (EMBL database No. Z21876), were used for amplification of cDNA, chromosomal DNA, and for sequencing: ALD1880F: GGAAGGCATGCATCTGCTC;

ALD2061F: TACCCGGACTCAGTGGAGGA;
ALD2114R: CAGGTCCTGCTCCGAGTAGC;
ALD2204R: CGACAGGACGTCCTTCCAGT;
ALD2478R: GCTGCTTCTCCTCCGTCAG;
and ALD2669R: TGTGATCCGAGCTTGGGG.

Three ALD specific intronic primers, derived from the genomic ALD sequence (Sarde et al., 1994; Ligtenberg et al., 1995), were used to obtain ALD-specific PCR products for genomic sequencing. ALD544F*: CAGGGGCCCTGTCGTACAG (intron 7); ALD849F*: TGGAGGGTG-CACAGACTCTC (intron 8); and ALD1078R*: AGGCCACCTCCTCCCCTCAG (intron 9). Total chromosomal DNA and mRNA were isolated from fibroblasts, according to standard procedures. PCR and sequencing was performed as described (Kemp et al., 1994; Ligtenberg et al., 1995).

RESULTS AND DISCUSSION

In the CCALD patient from kindred ALD#1, a cDNA fragment generated with primers ALD1880F (exon 6) and ALD2204R (exon 8) was approximately 30–40 nucleotides shorter than control fragments (325 bp). By sequencing, we identified a deletion of the first 34 nucleotides of exon 7. Chromosomal DNA from this patient and his heterozygous mother was used to generate a PCR fragment containing the 336 bp intron 6 with primers ALD1880F (exon 6) and ALD2114R (exon 7). Sequencing of these fragments revealed that the invariant A at position -2 of the ctttc-

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ccacag/GC splice acceptor site of exon 7 was mutated into a G, creating cttcccacgg/GC, which cannot function as a splice acceptor site. In the patient's mother, both the normal and mutant allele were present.

Exon 7 of the ALD gene contains a sequence CTCCCTGCGTGACCAG/G, which serves as a cryptic splice site. Splicing at this site creates an mRNA of which 34 bp are deleted, which leads to a frameshift at amino acid Arg 545, immediately followed by a stop codon. Electrophoresis of the patient's PCR fragments, generated with primers ALD1880F and ALD2204R, showed mainly the 291-bp fragment, but also a small amount of a shorter fragment corresponding to an mRNA in which exon 7 had been skipped (179 bp). Skipping of exon 7 also leads to a frameshift at amino acid Arg 545 and a stop codon 6 amino acids downstream.

In the AMN patient from kindred ALD#19, a cDNA fragment generated with primers ALD2061F (exon 7) and ALD2669R (exon 10) contained an insertion of 8 bp at the start of exon 9. Sequencing of a chromosomal DNA fragment containing the 149-bp intron 8 revealed that the G at position -10 of the 3' splice acceptor site of exon 9 was substituted by an A. This mutation creates an upstream novel splice acceptor site (ctcgccccggacccccag/G into ctcggcccagACCCC-CAG/G). The insertion of the 8 bp leads to a frameshift at position Arg 622 and a premature stop codon 16 amino acids downstream. In this case only the cDNA fragment with the additional 8 bp was found.

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