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SHORT COMMUNICATION

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Metachromatic leukodystrophy: a 12-bp deletion in exon 2 of the arylsulfatase A gene in a late infantile variant

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Abstract Sequencing of the arylsulfatase A gene in a late infantile metachromatic leukodystrophy patient showed the presence of a 12-bp deletion in exon 2. This deletion was found in a compound heterozygous state with the previously described 287 C→T transition.

The nucleotide sequence data have been deposited with the EMBL data bank and are available under the accession number: X82845

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Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessive neurological disease characterized by demyelination and storage of sulfatides in the central and peripheral nervous systems. It is nearly always caused by a defi-

Table 1 Activity of ARSA in leukocytes and cultured fibroblasts and determination of sulfatide excretion in urine (n.d. not determined)

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes</th>
<th>Cultured fibroblasts</th>
<th>Sulfatide excretion in nmol/24h Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>4</td>
<td>11</td>
<td>334</td>
</tr>
<tr>
<td>Father</td>
<td>32</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Mother</td>
<td>32</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Reference range</td>
<td>35–110</td>
<td>300–1100</td>
<td>1–60</td>
</tr>
</tbody>
</table>

Fig. 1 A SSCP analysis of the amplimers of exon 2. Lane 1 Patient, lane 2 father, lane 3 mother, lane 4 control.
B Agarose gel electrophoresis of the amplimers of the normal exon 2 lanes 2, 3 and of the exon that has the deletion (lanes 4–6), digested with SauI (lanes 2, 5) and BstEII (lanes 3, 6). Lane 7 φX 174/HaeIII length marker. Restriction sites: cDNA: SauI, 388–394; BstEII, 312–318
Fig. 2  A Sequence of the region of the coding strand of exon 2 in which the deletion is found. B Chou-Fasman and Garnier-Osguthorpe-Robson prediction of the polypeptide of ARSA. 1 Normal, 2 deletion. Arrow Region of deletion.

no 5' GTG GGG CCT GAG GGG GCC TTC CTG CCC CCC CAT 3'
val gly pro glu gly ala phe leu pro pro his
mu 5' GTG GGG CCT G
val gly pro VAL
tgl 128 129 130 131 132 133 134 135 136 137 138
ciency of the lysosomal enzyme arylsulfatase A (ARSA); a few cases have been described in which the decreased hydrolysis and subsequent storage of sulfatides is the result of the absence of the sphingolipid activator protein saposin B (Luijten 1991).

In the ARSA gene, 37 different mutations have now been described, of which three are deletions (Gieselmann et al. 1994). In this paper, we present an MLD patient with five different mutations in the ARSA gene.

**Patient**

The patient, a boy, presented with motor disturbances at the age of 12 months. He never started walking. He gradually developed a spastic tetraplegia and mental retardation followed by regression that ended in a vegetative state at the age of four. Magnetic resonance imaging of the brain showed extensive demyelination.

On electrophysiological examination of peripheral nerves, a decreased conduction velocity was found, indicating a demyelinating neuropathy. The diagnosis of MLD was made by determination of the activity of ARSA in leukocytes and cultured fibroblasts, and by determination of the sulfatide excretion in urine (Table 1).

**Materials and methods**

The ARSA gene was amplified in two fragments (exons 1–4 and exons 5–8), by the polymerase chain reaction (PCR), from genomic DNA of leukocytes. Individual exons with their intron boundaries were subsequently amplified, with these two PCR fragments as a template. Primers used for PCR amplification and sequence analysis of exon 2 were: forward primer, 5’CTGCTGCTGTCAGGGGACTCTG3’ (nucleotide position: 332–351); reverse primer, 5’TGGGGCTGCGGGACAGTCGA3’ (nucleotide position: 684–665). Nucleotide position refers to gene nucleotide numbering, with the A of ATG as no. 1.

Amplimers were subjected to single-stranded conformation polymorphism (SSCP) analysis using the Pharmacia Phast System (conditions for exon 2: 12.5% polyacrylamide gel, 20°C, 175 Vh). Cycle sequencing of the coding and the noncoding strands of all eight exons, and of exon 2 after ligation into the pCR-Script™ SK(+) cloning vector, was carried out by the Taq Dye Deoxy Terminator method using an ABI DNA sequencer. The amplimers obtained by PCR amplification of the cloned exon 2 were digested with the restriction enzyme Smal and with the restriction enzyme Hpal as control.

**Results and discussion**

SSCP analysis of the amplimer of exon 2 of the patient showed bands not present in the control. One band with a shift in mobility was present in the SSCP of the patient and the father, whereas two different bands were present in the SSCP of the patient and the mother. The SSCP of exon 2 of the patient therefore indicates the presence of two different mutations on separate alleles (Fig. 1A).

Direct sequencing of the eight exons suggested a deletion in one allele of exon 2, both in the patient and the mother. The patient and the father had a heterozygous nucleotide (287C→T) in exon 2 in common (data not shown).

The identity of both mutations was secured by cloning exon 2 of the patient. As the deletion results in the loss of the restriction site of the Smal enzyme, it was possible to identify which exon 2 had the deletion, by means of restriction enzyme digestion (Fig. 1B).

Sequencing of the separate alleles of exon 2 demonstrated two independently inherited mutations: (1) In the maternal allele, a deletion of 12 bp (cDNA 392-403) (Fig. 2A), resulting in one amino acid substitution (E131V), and a deletion of four amino acids (G132, A133, F134, and L135) were present. (2) In the paternal allele, a 287C→T transition leading to an amino acid substitution (S96F, data not shown) was found. In the maternal allele that had the deletion, an additional point mutation occurred in exon 7 (a 1172C→G transversion), resulting in an amino acid substitution (T391S), a polymorphism described as part of the I allele (Polien et al. 1991). A 1049A→G transition and a 1620A→G transition, the two mutations of ARSA pseudodeficiency, were found in the paternal allele (data not shown). The finding of the 287C→T transition on the background of the pseudodeficiency mutations has been described previously (Gieselmann et al. 1991). Direct sequencing of the remaining exons demonstrated no further mutations.

Secondary structure prediction according to both Chou-Fasman 1974 and Garnier-Osguthorpe-Robson 1978 demonstrated one turn in the normal ARSA molecule (Fig. 2B1) which has not been found in the ARSA polypeptide with the deletion (Fig. 2B2).

It can be assumed that the deletion is a disease-causing mutation because it is located in the exon 2 and exon 3 region of the enzyme. This region shows a high degree of conservation among sulfatases (Gieselmann et al. 1994). The 287C→T missense mutation has previously been shown to lead to a complete loss of enzyme activity in an expression study (Gieselmann et al. 1991). The deletion was not found in twenty other families with MLD investigated by means of SSCP analysis of exon 2.

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**References**


