RESEARCH ARTICLE

Metachromatic Leukodystrophy in the Navajo Indian Population: A Splice Site Mutation in Intron 4 of the Arylsulfatase A Gene

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Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder of myelin metabolism, resulting from the inability to properly degrade 3-sulfogalactosylceramide (sulfatide). This metabolic block is often due to defective functioning of the lysosomal enzyme arylsulfatase A (ARSA). Unmetabolized sulfatide accumulates in the white matter of the CNS and in the peripheral nerves, leading to progressive demyelination and death. Late infantile, juvenile and adult clinical variants of MLD have been described. A Navajo Indian child was diagnosed with late infantile MLD (LIMLD), and his ARSA gene was amplified in three overlapping regions by the PCR and sequenced. A single mutation was found: a G → A transition in the first nucleotide of intron 4 (IVS4nt1), which abolishes the 5' splice site consensus sequence. Negligible amounts of ARSA mRNA were observed in Northern blots. However, PCR amplification and sequencing of the ARSA cDNA showed that all of the mRNA species from the patient have exon 4 deleted. A new reading frame is thus established which results in a premature stop codon within exon 5. A minority of transcripts had additional splicing errors. Both parents carry this mutation, and the father also carries the pseudodeficiency (PD) allele. Three additional unrelated Navajo LIMLD patients were found to be homozygous for the same MLD-causing mutation by allele-specific oligonucleotide (ASO) hybridization. This method could be used for carrier and patient identification in this population. © 1994 Wiley-Liss, Inc.

KEY WORDS: Metachromatic leukodystrophy, Arylsulfatase A, Pseudodeficiency, Splice site mutation, Alternative splicing, Navajo Indians

INTRODUCTION

Metachromatic leukodystrophy (MLD) is characterized by the storage of 3-sulfogalactosylceramide (sulfatide) in the CNS white matter and in the peripheral nerves, which leads to demyelination, progressive neurological degeneration, and death (for review, see Kolodny, 1989). Sulfatide is a sphingolipid component of normal myelin, and is present in other tissues throughout the body. In patients with MLD sulfatide is excreted in their urine. MLD is the most common lysosomal storage disorder with neurological complications and mental retardation. It is a pan-ethnic disease which has an incidence of 1/40,000 births, and is inherited in an autosomal recessive fashion. Most patients with MLD have a defective activity of the lysosomal enzyme arylsulfatase A (ARSA, EC 3.1.6.1) (Austin et al., 1963), whose gene has been localized to chromosome 22. The ARSA nucleotide sequence and its genomic organization are known (Stein et al., 1989; Kreysing et al., 1990). Some patients with MLD are deficient in sphingolipid activator protein-1, which is required for the in vivo interaction of ARSA with its sulfatide substrate (Shapiro et al., 1979; Hahn et al., 1982).

Three major clinical subtypes of the disease are recognized: late infantile, juvenile, and adult MLD. The most prevalent form is late infantile MLD (LIMLD), with onset between the first and second years of life. Its major clinical manifestations are developmental delay, ataxia, weakness, optic atrophy, loss of speech, and progressive spastic quadriparesis. Death usually occurs by the end

of the first decade. The juvenile variant has its onset between 4 and 12 years of age, presenting with intellectual deterioration, ataxia, and a more protracted progression than that of LIMLD. Symptoms of adult MLD begin at any time after puberty, and include dementia, psychosis, ataxia, weakness, incontinence, and progressive spastic quadriparesis, a course that leads to death within 5 years to several decades. Recent mutational analyses show that the clinical variation results from the underlying genetic heterogeneity of the ARSA alleles that cause this disease (Polten et al., 1991).

The diagnosis of MLD is usually based on the finding of low ARSA activity, elevated CSF protein, decreased nerve conduction velocities, and increased urinary sulfatide (Kolodny, 1989). However there are individuals who are clinically normal but have low in vitro ARSA activity (Dubois et al., 1977; Butterworth et al., 1978). This could reflect the presence of the ARSA pseudodeficiency (PD) allele (Gieselmann et al., 1989). Patients with MLD owe their low activity to having two mutant alleles at the ARSA locus, whereas so called pseudodeficient individuals can either be homozygous for the PD allele or heterozygous for both the PD allele and an MLD-causing allele. The carrier rate for the PD allele is approximately 1/7 (Herz and Bach, 1984). In families in which both PD and MLD-causing alleles occur, measurement of ARSA activity alone cannot be used for carrier and patient identification.

This laboratory has diagnosed eight LIMLD patients among the Navajo Indian population. Initially, one of these patients was found to be homozygous for a novel point mutation in the ARSA gene, while his father, who has low ARSA activity, also carries the PD allele. DNA analyses of other Navajo MLD patients and available family members confirmed that all patients had the same mutation. A DNA-based test can now be used to accurately identify patients and carriers in this population.

MATERIALS AND METHODS
Subjects

A blood sample from a 20-month-old Navajo Indian child (subject II-6 in Family H0119, Fig. 1), who presented with loss of developmental milestones and neurological signs typical of LIMLD, was sent to this laboratory for testing. He is the brother of a patient diagnosed as having MLD by this laboratory in 1980. The parents had four additional unmonitored pregnancies which resulted in the birth of unaffected children. Four additional Navajo Indian families with members having
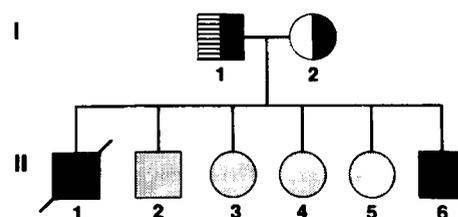
FIGURE 1. Pedigree of Family H0119. Black filled symbols represent an MLD-causing allele. The lined symbol indicates the presence of the PD allele, whereas healthy members of unknown genotypes are indicated by gray filled symbols.

LIMLD diagnosed by this laboratory have also been studied (Table 1).

Diagnostic Tests

Leukocytes were isolated from heparinized blood using dextran sedimentation (Skoog and Beck, 1956). Cultured skin fibroblasts from skin biopsies were grown in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 1% gentamicin (all supplied by Gibco, Gaithersburg, MD). The ARSA activity was measured according to previously described methods (Baum et al., 1959; Wenger and Williams, 1991). The sulfatide loading test was performed on cultured skin fibroblasts from patient II-6 and his father (Family H0119, Fig. 1) according to methods developed in this laboratory (Kudoh and Wenger, 1982).

Purification of Genomic DNA

Genomic DNA was prepared from leukocytes or from cultured skin fibroblasts from patients, their families, and control individuals (Louie et al., 1991; Ausubel et al., 1992). In addition, blood samples were spotted onto filter paper (Whatman 1 Chr, Maidstone, England), air dried, and used for obtaining genomic DNA (Berlin and Kazazian, 1992). The tools used to handle the blotted samples were depurinated to prevent cross contamination (Jinks et al., 1989).

Amplification and Cloning of ARSA Genomic DNA Fragments

The entire ARSA gene was amplified in three overlapping fragments, as shown in Figure 2, by the polymerase chain reaction (PCR) using the primer oligonucleotides summarized in Table 2 (Saiki et al., 1988). The primer sequences were obtained either from the literature (Fluharty et al., 1991) or were designed by this laboratory according to the genomic ARSA sequence available through GenBank (accession no X521151) (Stein et al., 1989; Kreysing et al., 1990). These oligo-

TABLE 1. ARSA Values, Clinical Status, and Genotype From Navajo Indian Families With LIMLD Affected Members

Subjects	ARSA activity[a]	Clinical status	Genotype[b]
Family H0119			
II-1	3.0	Affected	NA
II-6	2.8	Affected	IVS4nt1/IVS4nt1
I-1 (father)	8.7	Healthy	PD/IVS4nt1
I-2 (mother)	35.4	Healthy	NL/IVS4nt1
Family C2118			
Patient	6.1	Affected	IVS4nt1/IVS4nt1
Father	28.6	Healthy	NL/IVS4nt1
Mother	31.9	Healthy	NL/IVS4nt1
Family J0913			
Patient	4.4	Affected	IVS4nt1/IVS4nt1
Mother	31.5	Healthy	NL/IVS4nt1
Family F0120			
Patient	4.7	Affected	IVS4nt1/IVS4nt1
Father	24.0	Healthy	NL/IVS4nt1
Mother	6.0	Healthy	PD/IVS4nt1
Family Y0126			
Patient	6.8	Affected	NA
Mother	30.5	Healthy	NL/IVS4nt1
Half-Sister	27.3	Healthy	NL/IVS4nt1
Controls[c]	71.1 ± 11.4		
Obligate carriers[d]	33.5 ± 8.8		
Patients[c]	6.4 ± 3.0		

[a]Activity in leukocyte sonicates, as nmol nitrocatechol sulfate hydrolyzed per hour per mg protein.
[b]NA, sample not available for DNA analysis; IVS4nt1 = G → A mutation at nucleotide 1 of ARSA IVS4; PD, pseudodeficiency allele; NL, normal allele.
[c]Wenger and Williams (1991).
[d]Mean of 51 obligate carriers not using those with values under 20% of normal mean (Louie et al., 1991).

FIGURE 2. ARSA gene organization and strategy for PCR amplification. The ARSA gene structure is presented. The exons are numbered and indicated by boxes, the start and stop codons are indicated by vertical lines in exon 1 and 8, respectively. The solid lines denote the position of the PCR-amplified fragments, whereas the primers used in the amplification reactions are summarized in Table 2. The site of the G → A mutation (first base of intron 4) is indicated by an asterisk (*).

nucleotides were synthesized on a Du Pont Coder 300 DNA synthesizer, and purified by passage through Sephadex G-25 columns according to the manufacturer's instructions (Pharmacia, Piscataway, NJ). The amplification was performed on a Perkin-Elmer Cetus Thermal Cycler using the GeneAmp PCR reagent kit purchased from Perkin-Elmer-Cetus (Norwalk, CT). After an initial denaturation step at 95°C for 5 min, 2.5 U of AmpliTaq DNA polymerase was added to each reaction. Thirty amplification cycles followed under specific conditions for each fragment: Fragment D1, 94°C for 1 min, 54°C for 1 min, 72°C for 2.5 min; Fragment D2, 94°C for 1 min, 57°C for 1 min, 72°C for 2 min; Fragment D4 94°C for 1 min, 55°C for 1 min, 72°C for 2 min; Fragment C, 94°C for 1 min, 54°C for 1 min, 72°C for 2 min. The products of these reactions were separated on a 0.8–1.1% agarose gels (SeaKem GTG, FMC Bio-Products, Rockland, ME) and were visualized under UV light after staining with ethidium bromide (Sambrook et al., 1989). The PCR products were either directly cloned into TA vector (Invitrogen, San Diego, CA), or gel purified (Ultra-free-MC 0.45-μm filter unit, Milipore, Bedford, MA), di-

gested with the appropriate restriction endonucleases (Boehringer Mannheim, Indianapolis, IN), and cloned into pBluescript using T4 ligase (Stratagene, La Jolla, CA).

Sequencing of PCR Amplified Products

A Sequenase kit (United States Biochemical, Cleveland, OH) was used to sequence the cloned PCR products using [α-^{35}S]dATP (Du Pont, Boston, MA), according to the dideoxy termination method (Sanger et al., 1977). Universal and reverse primers provided by the kit were used for sequencing, as well as internal primers derived from the available ARSA sequence. The products were electrophoresed on a denaturing 6% polyacrylamide gel (Sequagel-6, National Diagnostics, Atlanta, GA) at 60–70 W for 2–5 hr (Sambrook et al., 1989). After drying the gel, the sequence was visualized by placing it in contact with X-Omat AR film (Kodak, Rochester, NY) at room temperature for over 18 hr.

RNA Isolation, cDNA Synthesis, and PCR Amplification

Total RNA was obtained from cultured skin fibroblasts from patient H0119 II-6 and control individuals using the acid guanidium thiocyanate-phenol chloroform method (Chomczynski and Sacchi, 1987). Poly(A)$^+$ RNA was prepared from total RNA on a single oligo(dT)-cellulose column from an mRNA purification kit (Pharmacia) following the procedure recommended by the manufacturer. cDNA was prepared from total RNA using a reverse transcription kit, according to the provided instructions (Promega, Madison, WI). The cDNA was dissolved in 20 μl of sterile distilled water, and 2–5 μl of that volume was used

TABLE 2. PCR Products and Amplification Primers Used in This Study

Product (size)	Primers	Primer sequence (5' to 3')
D1 (1,133)	ASA1ER1c[a]	TCGAATTCTGCTGGAGCCAAGTAGCCCT
	ASA730BH1nc	GAGGATCCTGAACGCTCTGCAAAGCTC
D2 (1,084)	ASA620ER1c	TCGAATTCCCATGACCTCATGGCCGAC
	ASA17BH1nc[a]	GAGGATCCCAGTGCAGGAGGCACTGAGG
D4 (402)	ASA620ER1c	TCGAATTCCCATGACCTCATGGCCGAC
	ASA22BH1nc	GAGGATCCGGCAAGCAGCTGAACTGCAA
C (1,158)	ASA19ER1c[a]	CGGAATTCTTGATGGCGAACTGAGTGAC
	ASA20H3nc[a]	GCGAAGCTTCCTCATTCGTACCACAGG
W[b] (1,668)[c]	ASA0ER1c	CGGAAAGAGCCTGCTGGAGC
	ASA8BH1nc	GCGAAGCTTCTCCAGACACCTGAGCCT

[a]Primer sequences obtained from the literature (Fluharty et al., 1991). The restriction sites added to the ARSA primer sequences are shown: ER1, EcoRI; BH1, BamHI; H3, HindIII. Orientation of the primers: c, coding sequence; nc, noncoding sequence.
[b]This fragment is obtained when cDNA is used as the template for amplification.
[c]Size of the product when control cDNA is used as a template.

for PCR amplification. The entire coding region was amplified in one piece (Fragment W) using the primers presented in Table 2. The PCR protocol for this fragment has a denaturing step for 5 min at 95°C. After the addition of 2.5 U of AmpliTaq polymerase, amplification was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min. The equipment and protocols for visualization, cloning, and sequencing of the PCR products have been described in previous sections.

Northern Blot Analysis

Total and poly(A)$^+$ RNA samples (10 μg and approximately 3.3 μg per lane, respectively) were denatured and separated on a 1.2% denaturing agarose gel containing formaldehyde (Ausubel et al., 1992). The RNA from the gel was blotted onto Hybond-N-membrane (Amersham, Arlington Heights, IL) in 20 × SSC (Sambrook et al., 1989), and fixed to the membrane by baking at 80°C for 2 hr. The prehybridization, hybridization, and washing conditions were obtained from the literature (Stein et al., 1989). The ARSA 1.6-kb cDNA probe (Stein et al., 1989) and the human β-actin 1.9-kb probe (Clonetech, Palo Alto, CA) were radioactively labeled by the random primer method (Feinberg and Vogelstein, 1983, 1984) with [α-^{32}P]dCTP (Du Pont) using the materials and methods provided by a commercially available kit (Boehringer Mannheim).

Screening for the MLD-Causing Mutation

The ARSA D4 fragment (Fig. 2, Table 2) was amplified from genomic DNA by PCR using the conditions described above. The amplified fragments (approximately 0.5 μg of DNA) were denatured under alkaline conditions and applied to Nylon membrane (Boehringer Mannheim) using the Minifold II slot blot apparatus (Schleicher

and Schuell, Keene, NH). The DNA was UV-crosslinked to the membrane with the Stratalinker 1800 autocrosslink program (Stratagene). The reagents and procedures for prehybridization, hybridization, labeling, washing, and detection in this assay followed the protocol recommended by Boehringer Mannheim for the nonradioactive Genius System. Two allele-specific oligonucleotides, 5'-AGACAATGGGTATGCCAGC-3' and 5'-AGACAATGGATATGCCAGC-3', were used as probes for the detection of normal and mutant sequences, respectively. These oligonucleotides were synthesized and purified in the same fashion as the PCR primers, and were 3'-end labeled with digoxigenin-ddUTP (DIG-ddUTP) using terminal transferase according to the Genius System protocol. The blots were hybridized to either probe (1 pm/ml) at 50°C for 16–18 hr. The filters were incubated twice at room temperature in wash solution (2 ×), and twice at 68–70°C in wash solution (0.5 ×). The detection steps consisted of several incubations at room temperature, as follows: (1) buffer 1 for 1 min, (2) buffer 2 for 30 min (blocking step), (3) 1/5,000 dilution of anti-DIG-Fab fragments (conjugated with alkaline phosphatase) in buffer 2 for 30 min, (4 and 5) buffer 1 for 15 min, and (6) buffer 3 for 2 min. The hybridization results were visualized by placing the filters in contact with Lumi-Phos 530 and exposing them for 15–60 min at room temperature to X-Omat AR film (Kodak).

RESULTS

ARSA Activity Values and Clinical Status in Navajo Indian Families of Patients Affected With LIMLD

In Family H0119 (Fig. 1), patient II-6 has clinical features typical of LIMLD and low ARSA ac-
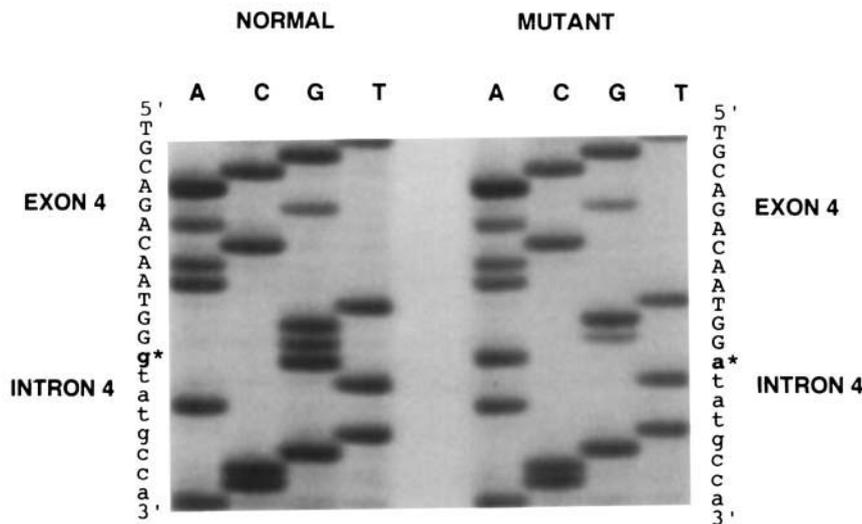
FIGURE 3. Partial ARSA sequence from PCR-amplified genomic DNA from subject H0119 I-2 (obligate carrier). The normal and mutant sequences at the exon 4/intron 4 boundary are shown. The exonic sequence is indicated by upper case letters, and the intronic sequence by lower case letters.

tivity (Table 1). The mother (I-2) has a typical carrier value, while the healthy father (I-1) has low ARSA activity indicating that he is probably a carrier of a PD allele in addition to the MLD-causing allele. The finding of such a parent complicated the use of ARSA activity measurement alone for patient and carrier identification in the above family. The sulfatide loading test was carried out in cultured fibroblasts from the patient and his father. This test confirmed that the patient cannot metabolize sulfatide, while the father metabolizes it normally (data not shown). The ARSA values from four additional Navajo Indian families studied are also presented in Table 1. The mother of Family F0120 is healthy but also has low ARSA values, indicating that she is probably also a carrier of the PD allele in addition to the MLD-causing allele.

Sequence Analysis of the ARSA Gene in the H0119 Family

The entire 3 kb ARSA gene from the patient (II-6) was amplified from genomic DNA isolated from the leukocyte sonicates used for the enzyme assays. Upon sequencing, a G → A transition was identified at the first base of intron 4, which abolishes the highly conserved 5′ splice junction consensus sequence (Mount, 1982). The mutation, here listed as IVS4nt1, was detected in the homozygous state in the patient and in the heterozygous state in both parents. The normal and mutant sequences from the mother (I-2) are shown in Figure 3. Sequence analysis (not shown) also demonstrated that the father is a carrier of the typical PD

allele already described in the Caucasian population, which consists of two G → A transitions, one of which abolishes an N-glycosylation site and another which changes the consensus polyadenylation signal (Gieselmann et al., 1989).

RNA Isolation and Northern Blot Analysis of the ARSA Total and Poly(A)$^+$ RNA

Steady-state ARSA mRNA was analyzed by Northern blot using fibroblast total RNA and poly(A)$^+$ RNA from patient II-6 (Family H0119) and healthy controls. In total RNA from control individuals, three major ARSA mRNA species of 4.8, 3.7, and 2.1 kb were observed as described by Gieselmann et al. (1989, 1991). RNA from the patient showed only one major species at 4.8 kb (data not shown). In a blot of poly(A)$^+$ RNA all three species were severely decreased in the patient sample, while the control only lost the signal of the two larger transcripts (Fig. 4). When normalized to β-actin, the deficiency of the 2.1 kb species in the patient was obvious.

The IVS4nt1 Mutation Causes Exon 4 Skipping

Even though the Northern blot of poly(A)$^+$ RNA from the patient (II-6) did not detect any ARSA transcript, the entire cDNA coding region was amplified in one piece (Fragment W) from the patient's total RNA (Table 2). In an agarose gel (not shown), the PCR products from the patient appeared as a diffuse population of bands between 1.4 and 1.7 kb. This area of the gel was excised in one piece and the PCR products obtained from it
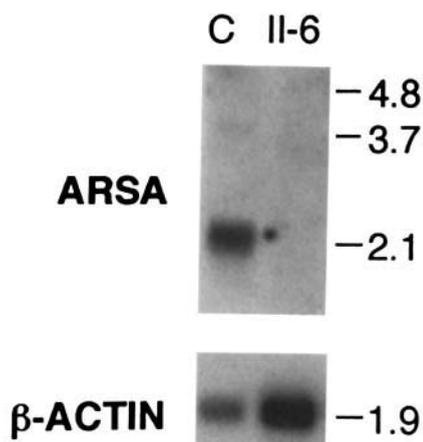
FIGURE 4. Northern blot analysis of poly(A)$^+$ RNA. The RNA from control (C) and patient (II-6, Family H0119) were detected using an ARSA cDNA probe and a control β-actin cDNA probe. The sizes of the transcripts are indicated in kb.

were directly cloned and sequenced revealing that the exon 4 sequence was absent from all the clones from the patient, and that exon 3 was spliced directly to exon 5, which in turn produced a shift in the reading frame of the ARSA cDNA leading to the creation of a stop codon within exon 5 (Fig. 5). Other less abundant ARSA cDNA forms were characterized, and are thought to have arisen from aberrant splicing. One such species lacked both exons 4 and 5, splicing exon 3 directly to exon 6 which also creates a reading frame shift and a premature stop codon within exon 6. Another species retained the entire intron 1 and intron 2 sequences while splicing out exon 4. Yet another species retained intron 2 and spliced out exon 4. Figure 6 shows the splicing pattern of normal ARSA RNA and the most abundant IVS4nt1 ARSA mRNA species.

Rapid Detection of the Mutant Allele in Navajo Indian Patients and Their Families

This mutation does not create or abolish a restriction site and therefore we developed a nonradioactive ASO hybridization assay to detect the G → A mutation in this population. A sample of the results obtained by ASO hybridization is presented in Figure 7. Table 1 summarizes the enzyme-based as well as the DNA-based test results obtained during these investigations. Both parents (Family H0119) were found to be carriers of the IVS4nt1 mutation (Fig. 7), and three additional unrelated Navajo Indian children with LIMLD were found to be homozygous for this mutation. The mother of one of these patients and the family members of a
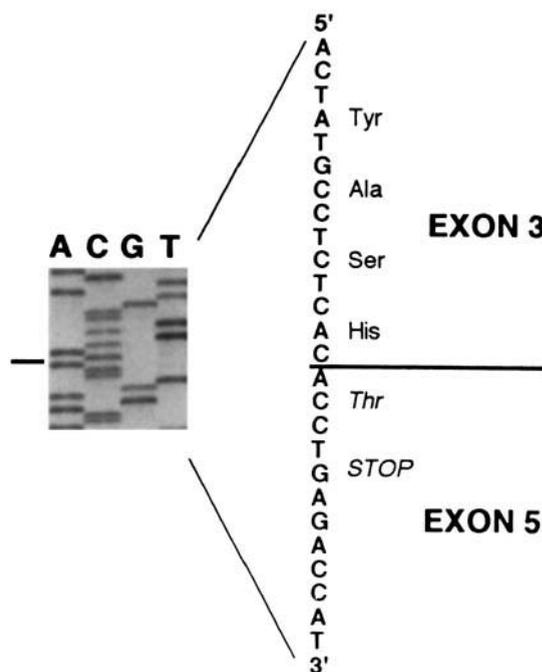
FIGURE 5. Partial ARSA sequence from PCR-amplified cDNA from patient H0119 II-6. Exon 4 is absent from the patient's cDNA. The lines represent the exon 3–exon 5 boundary. The new codons created by the aberrant splicing are indicated in italics.
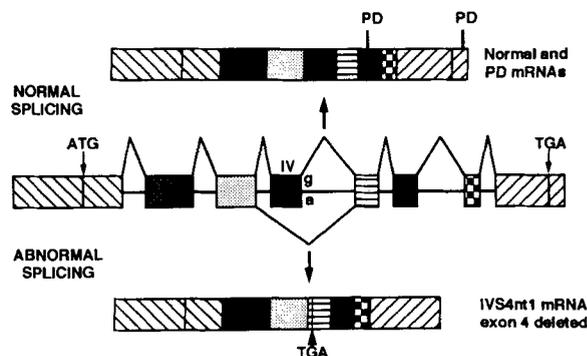
FIGURE 6. Diagram of the intron/exon organization of the normal and mutant ARSA gene and potential mRNA products. The lines connecting the exon boxes show the splicing patterns. The first nucleotide of intron 4 is shown for both normal (g) and mutant (a) alleles. Also shown is the location of the mutations present in the PD allele. The most abundant abnormal message has an exact exon 4 deletion, which establishes a new reading frame that results in a stop codon within exon 5 (arrowhead).

deceased patient were also determined to be carriers of this mutation. The IVS4nt1 change was not found in other non-Navajo LIMLD patients or in control individuals screened in this laboratory.
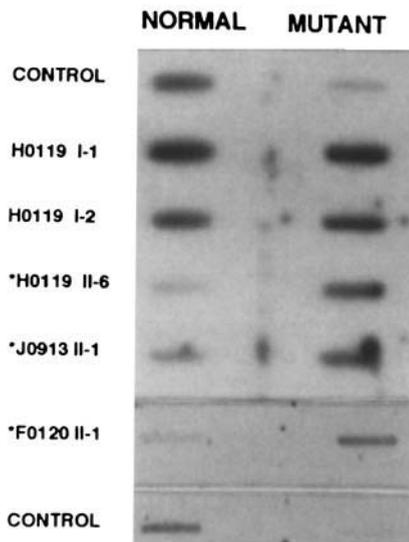
FIGURE 7. ASO hybridization of the PCR-amplified ARSA D4 fragments of three unrelated Navajo Indian LIMLD patients (denoted by the asterisks), two obligate carriers, and controls. The DIG-labeled normal and mutant probes were 19-base oligonucleotides which differed only by the G → A transition at the exon 4/intron 4 boundary.

DISCUSSION

Eight Navajo Indian patients with LIMLD have been diagnosed in this laboratory over the past 15 years. These patients fit the classic clinical picture of the late infantile form of the disease. In order to determine which mutation was responsible for MLD in this population, one family with two affected children was chosen for study. A G → A mutation at the first nucleotide of intron 4 (IVS4nt1) of the ARSA gene was identified in the homozygous state in the living affected child in this family. This mutation has not been found in any other patient reported in the literature. A DNA-based test for the IVS4nt1 mutation was developed, which involves ASO hybridization of a PCR-amplified ARSA gene fragment (D4). The source of genomic DNA for this test need only be a drop of blood dried onto a piece of filter paper (Jinks et al., 1989; Berlin and Kazazian, 1992) or left over leukocyte sonicates (Louie et al., 1991). The IVS4nt1 mutation was found in the homozygous state in three additional unrelated Navajo Indian children, using genomic DNA from various sources. Family members of a fifth already deceased LIMLD patient from the same ethnic background were found to be carriers of this mutation.

In addition, ARSA pseudodeficiency was found in two families in this population, which compli-

cates the enzyme-based testing for carrier and patient identification. It is interesting that the typical PD allele is found in nearly all ethnic groups studied to this day. This points to the early appearance of this polymorphism in human evolution, and it underlines the need for care in patient and carrier identification in this ethnic group.

The IVS4nt1 mutation abolishes the 5' donor splice site consensus sequence and causes exon 4 skipping, which in turn produces a reading frame shift and creates a stop codon within exon 5. There are two other MLD causing mutations at 5' donor splice sites of the ARSA gene, one in intron 2 (allele I) (Polten et al., 1991), and the other in intron 7 (E7S2195) (Bohne et al., 1991; Fluharty et al., 1991). Patients homozygous for allele I show a clinical picture of LIMLD and their ARSA RNA levels are severely reduced in a fashion similar to the results presented here. On the other hand, the E7S2195 mutation does not seem to depress the synthesis or the stability of the major (2.1 kb) ARSA transcript (Gieselmann et al., 1989 and 1991; Fluharty et al., 1991). The importance of having a G at the 5'-end of an intron for proper splicing has been established (Mount, 1982). It is also known that this nucleotide directly interacts with the U1 small nuclear ribonucleoprotein particle, but the role of this first base of the intron in the splicing process is not completely understood (for review refer to Moore et al., 1993). Recently there has been a report that this first intronic base may participate in an essential non-Watson–Crick interaction with the last nucleotide of the intron during splicing (Parker and Siciliano, 1993).

The decreased levels of ARSA mRNA in the patient could be explained by the inability of the mutant pre-mRNA to be properly recognized by the splicing machinery. It is known that, in mammalian cells, if an RNA contains a mutation at either the 5'-donor or 3'-acceptor splice site, most of the pre-mRNA will be retained in the nucleus and either degraded or spliced using alternative or cryptic splice sites (Chang and Sharp, 1989). The aberrantly spliced cDNAs from the patient probably represent a very small percentage of ARSA transcripts that survive degradation, and whose protein products are truncated and therefore are most likely enzymatically inactive.

At present, there is no specific therapy that will completely stop the progression and fatal outcome of MLD. Bone marrow transplantation has been successful in a few cases of MLD who were early in

the course of the disease when they received this treatment (Krivit et al., 1990). Our findings will lead to an accurate test for the identification of carriers and patients in this population, and to improved genetic counseling. In addition, use of this test may result in opportunities for early therapeutic intervention if patients are diagnosed before neurologic damage becomes evident.

ACKNOWLEDGMENTS

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