Adult Onset Metachromatic Leukodystrophy Without Electroclinical Peripheral Nervous System Involvement

A New Mutation in the ARSA Gene

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**Background:** Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by the deficiency of arylsulfatase A (ARSA). Clinically, the disease is heterogeneous with respect to the age of onset, affection of peripheral and central nervous systems, and progression.

**Objectives:** To analyze mutations in the ARSA gene of a patient with adult-onset MLD with no signs of peripheral polyneuropathy and to emphasize the clinical, neuroradiologic, neuropathologic, and genetic features of the disease.

**Design:** Case study of a patient clinically presenting with rapidly progressive dementia and behavioral abnormalities. We report the findings of clinical evaluation and neurophysiologic and neuropathologic studies of peripheral nerves; we also performed DNA sequence analysis, transfections, metabolic labeling, and immunoprecipitation of mutant ARSA polypeptides.

**Setting:** Genetic research and clinical unit, university hospital.

**Results:** Genetic analysis revealed homozygosity for a novel mutation in exon 3 of ARSA (F219V). This substitution leads to a misfolded unstable enzyme with a specific activity less than 1% of normal. There were no clinical or neurophysiologic signs of peripheral nervous system dysfunction. Typical neuropathologic signs for MLD were absent from nerve biopsy specimens.

**Conclusions:** This novel mutation is associated with progressive psychocognitive impairment without clinical or electrophysiologic signs and only minor morphologic signs of peripheral nerve affection. The F219V substitution causes reduction in enzyme activity to an extent unexpected for an adult patient with MLD.


**METACHROMATIC LEUKODYSTROPHY (MLD)** (Online Mendelian Inheritance in Man 250100) is an autosomal recessive lysosomal storage disorder caused by the deficiency of arylsulfatase A (ARSA; E.C. 3.1.6.8). Arylsulfatase A catalyzes the first step in the intralysosomal degradation of 3-O-sulfogalactosylceramide (sulfatide). Sulfatide is found in high concentrations in myelin of the nervous system. Accumulation of sulfatide affects the oligodendrocytes and Schwann cells causing progressive demyelination. Patients with MLD who are homozygous for alleles that do not allow expression of any residual enzyme activity experience the most severe, late infantile form of the disease, whereas those who are homozygous for alleles allowing for the expression of residual enzyme activity in many cases have the mild adult forms of the disease. Patients heterozygous for these 2 kinds of alleles frequently have intermediate juvenile MLD. The diagnosis of MLD is based on the decreased activity of ARSA in leukocytes and/or fibroblasts and on the accumulation of sulfatides in urinary sediment. Histologically, sulfatide storage can be demonstrated in neuronal tissues (eg, in sural nerve biopsy specimens).

**REPORT OF A CASE**

A 37-year-old woman of Tamil origin presented with dysfunctional and bizarre behavior in 1996. She had been working as a housewife since 1989 and was described as well-organized and intelligent. Since 1997, she showed progressive apathy and loss of interest in daily living routines, including care of her 3 children. Memory functions deteriorated dramatically: she could recall neither the names...
nor birthdays of her relatives. She developed bladder incontinence.

Somatic neurologic examination revealed no abnormalities, especially no clinical signs of neuropathy. Muscle tone and gait were normal. Cognitive and mental functions were severely impaired, with fluctuating vigilance and loss of concentration. Immediate and delayed verbal recall was impaired. She showed frontal signs with grasping and perseveration (continuous drawing of Luria loops). Follow-up examinations in 1998 and 2000 revealed further cognitive decline but still no motor impairment.

LABORATORY FINDINGS

Arylsulfatase A activity in leucocytes was deficient (0.01 nmol/h per milligram of protein; normal range, 46.0-71.7 nmol/h per milligram of protein). Urine sulfatide excretion was severely elevated. Cerebrospinal fluid findings were normal. Testing included DNA sequence analysis, in vitro mutagenesis, ARSA activity determinations, metabolic labeling, and immunoprecipitations.3

MAGNETIC RESONANCE IMAGING FINDINGS

Brain magnetic resonance imaging with regular head coils (Magnetom 1.5-T scanner; Siemens AG, Erlangen, Germany), including axial native and gadolinium-enhanced T1-weighted imaging, axial and sagittal proton density/T2-weighted, fast spin-echo imaging, and coronal fluid-attenuated inversion recovery sequences, revealed marked subcortical brain atrophy and demyelination with pronounced periventricular T1-signal hypointensity and fluid-attenuated inversion recovery and PD/T2 hyperintensity of the white matter (Figure 1A). The anterior part of the corpus callosum was involved. Subcortical U fibers, basal ganglia, brainstem, and cerebellum showed normal signal intensities (Figure 1B).

ELECTROPHYSIOLOGIC EXAMINATIONS

Visual-evoked potential and tibial somatosensory-evoked potential findings were normal. Nerve conduction velocities of the right median and sural nerve were normal. Electroencephalography demonstrated focal slowing over the right midtemporal region.

NEUROPATHOLOGIC FINDINGS

Right sural nerve biopsy findings demonstrated reduction of nerve fiber density and signs of slight hypomyelination and remyelination without signs of metachromatic deposits (Figure 2A). Electron microscopy revealed complex lysosomal inclusions and cytoplasmic II granules in Schwann cells, with unspecific concomitant axonal and perineurial inclusions (Figure 2B). These neuropathologic findings, in contrast to normal neurophysiologic findings, indicate a slowly progressive demyelinating neuropathy.

DNA SEQUENCE ANALYSIS

Sequencing of the ARSA gene of the patient revealed homozygosity for a T→G transition at complementary DNA position 655, changing codon 219 in exon 3 from TTC coding for phenylalanine to GTC coding for valine. No other mutation was found (Figure 3).
To prove that the missense mutation affects ARSA activity, expression vectors coding for the wild-type, P425T, and F219V ARSA were transiently transfected into Syrian baby hamster kidney cells. The P425T substitution has recently been shown to be associated with residual enzyme activity. Almost no activity was expressed from the plasmid containing the F219V complementary DNA, whereas low levels of residual activity were found in cells expressing the P425T enzyme (Figure 4). Western blot analysis revealed the presence of ARSA polypeptides in all transfected cells, allowing the conclusion that F219V ARSA has severely reduced specific enzymatic activity (Figure 4, Table).

Pulse-chase experiments with stably transfected cells were performed to investigate the stability of the F219V ARSA. Whereas wild-type ARSA was stable during the chase period of 72 hours, most of the F219V ARSA was degraded after 6 hours. To analyze the structural integrity of the F219V ARSA, we immunoprecipitated wild-type, F219V, and P425T ARSA from transiently transfected, metabolically labeled baby hamster kidney cells with several monoclonal antibodies precipitating only the
native nondenatured enzyme. In contrast to the wild-type and P425T ARSA, the F219V ARSA could not be precipitated by any of the antibodies (Figure 5).

Degradation of mutant ARSA can occur in the endoplasmic reticulum (ER) and/or in the lysosomes. To investigate whether the F219V ARSA left the ER, we performed pulse-chase experiments in the presence of ammonium chloride, which targets newly synthesized lysosomal enzymes improperly to the secretory pathway. If on addition of ammonium chloride, mutant proteins were detected in the media of cultured cells, these enzymes must have passed the Golgi apparatus and thus left the ER. Cells were pulse labeled with [35S]-methionine and chased in the absence or presence of ammonium chloride. Arylsulfatase A was immunoprecipitated from cells and media (Figure 6). Without ammonium chloride, little ARSA was found in the medium of cells expressing wild-type or F219V ARSA. On addition of ammonium chloride, about 50% of the wild-type ARSA was found in the medium. In the presence of ammonium chloride, about 20% of the F219V ARSA was secreted, indicating that at least some but not all of the enzyme passed through the secretory pathway.

**COMMENT**

Adult MLD may present with behavioral abnormalities, emotional lability, psychiatric symptoms such as delusions or hallucinations, ataxia, extrapyramidal signs, progressive dementia, and signs of peripheral neuropathy. Magnetic resonance imaging reveals diffuse confluent white matter hyperintense signal alterations on T2-weighted images. Initially, the arcuate U fibers are spared.

Clinically, adult MLD shows considerable variability. Two groups can be distinguished: patients in whom psychiatric symptoms and impairment of intellectual functions dominate over sensorimotor symptoms and, in contrast, patients who present early with predominant motor impairment due to neuropathy. Patients with the former phenotype are frequently carriers of the I179S allele, suggesting a genotype-phenotype correlation. Although clinically the polyneuropathy is frequently not apparent, electrophysiologic and histologic signs of subclinical demyelinating polyneuropathy are usually found during the early course of late juvenile or adult MLD.

The patient described herein presented exclusively with severe cognitive and intellectual impairment 18 months after the onset of disease without clinical signs of peripheral nervous system involvement. Electrophysiologic findings of the nerves were normal, which is extremely rare in adult MLD. In addition, she was not ataxic, which is in accordance with the lack of alterations in the white matter of the cerebellum. The sural nerve biopsy revealed slight demyelination without metachromatic deposits. This relative preservation of function and the small extent of morphologic alterations may result from different kinetics of lysosomal storage of sulfatides in oligodendrocytes and Schwann cells, less-

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**Table. Arylsulfatase A (ARSA) Relative Specific Activity***

<table>
<thead>
<tr>
<th>ARSA Activity</th>
<th>WT</th>
<th>P425T</th>
<th>F219V</th>
<th>BHK Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARSA endogenous activity, µU</td>
<td>301.5</td>
<td>39</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td>Western blot, arbitrary units</td>
<td>2427</td>
<td>2654</td>
<td>3707</td>
<td>0</td>
</tr>
<tr>
<td>Relative specific activity, %</td>
<td>100</td>
<td>12</td>
<td>1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: BHK, Syrian baby hamster kidney; WT, wild type; NA, not applicable.

*Transiently transfected BHK cells were analyzed for ARSA activity and ARSA cross-reacting material by Western blot analysis. Relative specific activity was calculated as the quotient between ARSA activity and the Western blot signal.
pronounced segmental demyelination in the peripheral nerve, higher tolerance of peripheral nerve tissues for sulfate accumulation during adulthood, or other genetic or epigenetic factors.

This patient carries a new mutation causing the substitution of phenylalanine 219 by valine. Homozygosity is due to consanguinity of the parents (cousins). Surprisingly, the F219V substitution causes massive reduction of enzyme activity to an extent unexpected for an adult patient with MLD. A reduced specific activity and stability of the F219V ARSA accounts for this reduction.

The reasons for the discrepancy between the low enzyme activity and the mild phenotype of the patient remain unclear. Similar phenomena have been described in patients with early-onset MLD who display exceptionally high residual enzyme activities. In general, the genotypic-phenotypic correlation is variable, and the white matter changes on magnetic resonance imaging do not absolutely correlate with clinical symptoms. Since ARSA assays usually determine the activity with an artificial water-soluble substrate, it is likely that in some cases they do not reflect the in vivo situation in which an insoluble lipid substrate is degraded. On the other hand, it is known that genetic factors not linked to the ARSA locus can influence the phenotype of patients substantially.

Other parameters, however, also suggest that the F219V substitution affects the enzyme more severely than other adult mutations. Thus, for the most frequent adult P426L mutation, which accounts for up to 40% of all MLD mutations, it has been shown that the enzyme leaves the ER, is properly sorted in the Golgi apparatus, and is degraded in the lysosome owing to inability to octamerize. In contrast, the F219V enzyme is at least partially retained in the ER. This retention is in accordance with the severe structural alteration of the F219V ARSA as indicated by the lack of reactivity with structure-sensitive antibodies.

In summary, we describe an adult-onset case of MLD caused by a newly identified mutation in ARSA, F291V. This mutation leads to progressive psychogenic impairment without clinical or electrophysiological indications and only minor morphologic signs of peripheral nerve affection even though homozygous for an allele that expresses only low amounts of residual enzyme activity.

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