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High Residual Arylsulfatase A (ARSA) Activity in a Patient with Late-Infantile Metachromatic Leukodystrophy

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Summary

We identified a patient suffering from late-infantile metachromatic leukodystrophy (MLD) who has a residual arylsulfatase A (ARSA) activity of about 10%. Fibroblasts of the patient show significant sulfatide degradation activity exceeding that of adult MLD patients. Analysis of the ARSA gene in this patient revealed heterozygosity for two new mutant alleles: in one allele, deletion of C 447 in exon 2 leads to a frameshift and to a premature stop codon at amino acid position 105; in the second allele, a G→A transition in exon 5 causes a Gly^{309}→Ser substitution. Transient expression of the mutant Ser^{309}-ARSA resulted in only 13% enzyme activity of that observed in cells expressing normal ARSA. The mutant ARSA is correctly targeted to the lysosomes but is unstable. These findings are in contrast to previous results showing that the late-infantile type of MLD is always associated with the complete absence of ARSA activity. The expression of the mutant ARSA protein may be influenced by particular features of oligodendrocytes, such that the level of mutant enzyme is lower in these cells than in others.

Introduction

Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by the deficiency of arylsulfatase A (ARSA). ARSA catalyzes the first step in the degradation pathway of galactosyl-3-sulfate ceramide (cerebroside sulfate or sulfatide), one of the major membrane lipids of the myelin sheaths. Deficiency of this enzyme causes the storage of the substrate in lysosomes and an increase of sulfatide in the myelin membranes of affected individuals. Although the storage can be found in many organs, it mainly affects the oligodendrocytes and Schwann cells of the nervous system. Patients develop a progressive demyelination and a wide variety of neurological symptoms. These include a delay in neurological development, weakness, ataxia, progressive spastic tetraparesis, optic atrophy, and dementia (for review, see Kolodny 1989). Patients usually die some years after the onset of the disease in a decerebrated state. Clinically the disease is heterogeneous. On the basis of the age at onset and the severity of the symptoms, three different forms can be distinguished: late infantile, juvenile, and adult.

A rare form of MLD is caused by the deficiency of a sphingolipid activator protein which physiologically solubilizes the hydrophobic substrates for the enzymatic action of ARSA. The clinical picture resembles that of a juvenile (Shapiro et al. 1979) or late-infantile (Wenger et al. 1989) form of MLD.

Until now, eight MLD-causing ARSA alleles have been characterized (for review, see Gieselmann et al. 1991*b*). These alleles can be divided into two groups, one of which is associated with the absence of enzyme activity and the other in which alleles encode for low residual enzyme activities. The distribution of these alleles among patients has revealed a genotype-phenotype correlation, such that patients with two alleles associated with no residual activity suffer from the severe, late-infantile type of the disease, whereas those with one or two alleles with low enzyme activity have the

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intermediate juvenile or mild adult type of the disease, respectively (Polten et al. 1991). Biochemical data have been presented that support this genotype-phenotype correlation (Kappler et al. 1991; Leinekugel et al. 1992).

Correlations between the residual activity of a lysosomal enzyme and the severity of the resulting storage disease have also been shown for β-hexosaminidase A (Leinekugel et al. 1992) deficiency, which causes another lysosomal storage disease, GM2 gangliosidosis. Additionally there is a very frequent (7.5%–15%) ARSA allele which is associated with a substantial ARSA deficiency (Herz and Bach 1984; Hohenschutz et al. 1989). Individuals homozygous for this allele have low residual ARSA activity but are clinically healthy. This condition has therefore been called "ARSA pseudodeficiency."

Obviously low amounts of ARSA activity in these individuals are sufficient to sustain a normal sulfatide metabolism. The mutations signifying this pseudodeficiency allele have been characterized, and the attenuated enzyme activity is explained by a reduced synthesis of the enzyme because of a mutation affecting a polyadenylation signal (Gieselmann et al. 1989). In order to test the general validity of the proposed genotype-phenotype correlation, we have measured ARSA activities in fibroblasts of patients with the late-infantile form of the disease, which are thought to have no residual enzyme activity. We have found one patient whose fibroblasts showed a substantial residual ARSA activity.

Material and Methods

Material

Enzymes used for in vitro mutagenesis were from New England Biolabs. α-S-dCTP was a generous gift from Dr. Eckstein, Max Planck Institute, Göttingen. *Taq* polymerase was from Bethesda Research Laboratories. [^{35}S]-Methionine (>800 Ci/mmol) and α[^{32}P]-dATP (>3,000 Ci/mmol) were from Amersham. Geneticin and *Staphylococcus aureus* bacteria that were used for immunoprecipitation were from Bethesda Research Laboratories. Oligonucleotides were synthesized according to the method described by Stein et al. (1989). The anti-ARSA antiserum was raised in goat and has been described by Waheed et al. (1982).

Description of the Patient's History

The patient presented with gait disturbances at the age of 18 mo. Subsequently he lost acquired capabilities such as walking and sitting. He developed a spastic paresis and was finally bedridden. He showed episodes of pain attacks occurring several times per hour. The electromyelography showed signs of denervation and decreased nerve conduction velocity. In a sural nerve biopsy, metachromatic granules were detected. Cerebroside sulfate excretion in urine was not determined.

Sequence Analysis of the ARSA Gene

The ARSA gene was amplified from genomic DNA and was sequenced completely, as has been described elsewhere (Fluharty et al. 1991; Gieselmann et al. 1991*a*).

Allele-specific Oligonucleotide Hybridization

The amplification of the ARSA gene in two overlapping fragments has been described by Polten et al. (1991). Amplified fragments were blotted onto Hybond-N filters and hybridized to [^{32}P]-labeled allele-specific oligonucleotides as described elsewhere (Gieselmann et al. 1989; Polten et al. 1991). The sequences of the oligonucleotides used to detect the mutations described were as follows: exon 2—normal allele, 5′CGGGGGGGCCTGCCCCTG3′ (62°C) and mutant allele, 5′CGGGGGGGCTGCCCCTGG3′ (62°C); and exon 5—normal allele, 5′ACCTACGAGGGCGGTGTC3′ (60°C) and mutant allele, 5′ACCTACGAGGGCAGTGTC3′ (60°C). The filters were washed for 10 min at the temperature indicated in parentheses, to give allele-specific signals.

In Vitro Mutagenesis and Transfection of Baby Hamster Kidney (BHK) Cells

The Gly^{309}→Ser mutation in exon 5 was introduced into the ARSA cDNA as described elsewhere (Nakamaye and Eckstein 1986). Cell culture conditions, transfection, and determination of ARSA activity have been described elsewhere (Stein et al. 1989). For the generation of stably transfected cells, a plasmid carrying a neomycin resistance gene was cotransfected, and cells were selected with 800 μg neomycin/ml (Southern and Berg 1982). Resistant colonies were screened for ARSA activity, to find overexpressing clones of transfected cells.

Immunoprecipitation of ARSA

The conditions for metabolic labeling of cultured cells and subsequent immunoprecipitation of ARSA from overexpressing BHK cells have been described by Gieselmann et al. (1992). When ARSA was immunoprecipitated from cultured fibroblasts, cells were harvested in 50 mM Tris-Cl pH 7.4, 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 5 mM iodoacetamide, 0.5%

Nonidet P 40. Cell lysates were freeze thawed and subsequently adjusted to 0.03% protamine sulfate to precipitate the DNA. This mixture was kept on ice for 10 min and centrifuged for 5 min at 14,000 *g* at 4°C in an Eppendorf centrifuge. After this we followed the protocol described elsewhere (Gieselmann et al. 1992). In experiments where immunoprecipitates from different flasks were compared, the incorporation of radioactivity was monitored by trichloroacetic acid precipitation, and the amount of cell lysate used was adjusted such that equal amounts of incorporated radioactivity were used for immunoprecipitations.

Indirect Immunofluorescence

Cells grown for 1–2 d on coverslips were used for indirect immunofluorescence staining with an affinity-purified antibody against human ARSA, raised in goat, and a rhodamine-labeled second antibody, as described elsewhere (Waheed et al. 1982, 1988).

Sulfatide Loading Assay

The sulfatide loading assay was performed with the sulfatide fluorescent analogue N-lissamine rhodaminyl-(12-aminododecanoyl)cerebroside 3-sulfate (LRh-sulfatide) as substrate, as described by Monti et al. (1992). Briefly, 10 nmol LRh-sulfatide complexed to albumin (Viani et al. 1989) were diluted with nutrient medium and added to 7-cm^2 dishes of confluent fibroblasts in a final volume of 2 ml. After a 7-h pulse, the medium containing the fluorescent sulfatide was replaced with fresh medium containing 15% FCS, and the cells were incubated for an additional 14 h. Cells were harvested by trypsinization, and the lipids were extracted from the cell pellet with 2 ml of chloroform:methanol (6:4 [v/v]). The identification and quantification of the metabolic products was carried out by thin-layer chromatography (silica gel 60 precoated plates) using chloroform:ethyl acetate:n-propanol:0.25% KCl:methanol (25:25:25:9:16 [v/v]) as solvent system. Fluorescent spots detected under UV light were scraped and extracted from the silica gel, and their fluorescence was measured. An excitation wavelength of 565 nm and an emission wavelength of 575 nm were used. Total fluorescence measured in all spots was taken as 100%. Cerebroside sulfate is first degraded to galactosylceramide and subsequently to ceramide. We were not able to detect galactosylceramide in the thin-layer chromatography, because it was completely converted to ceramide. The fluorescence intensity of the ceramide spot was therefore taken to calculate the degradation rate of cerebroside sulfate. It has been shown elsewhere that half of the intracellularly generated ceramide is released into the medium (Monti et al. 1992). This fraction of ceramide has not been taken into account in our experiments.

Results

ARSA Activity in Late-Infantile-MLD Patients

ARSA activity in cultured fibroblasts of patients suffering from late-infantile MLD was determined with the artificial substrate p-nitrocatecholsulfate. One patient (Wi.M.) showed significant residual ARSA activity of about 8% of normal (table 1). The clinical symptoms of this patient were typical for late-infantile MLD. The diagnosis was further based on metachromatic granules seen in a sural nerve biopsy. Fibroblasts of the patient and several individuals with known ARSA genotype were subjected to a sulfatide loading assay to clarify whether the fibroblasts of Wi.M. are able to degrade the natural sulfatide substrate. A sulfatide fluorescent analogue was added to the medium of the fibroblasts for 7 h. After this period the sulfatide-containing medium was replaced with fresh medium, and the cells were incubated for an additional 14 h. The percentage of sulfatide degradation in the cells was determined by thin-layer chromatography and subsequent quantification of fluorescent spots (see Material and Methods). The data are shown in table 1. As expected, the two patients (Mes. and Ass.) suffering from late-infantile MLD do not degrade any sulfatide. The patients with juvenile (Schn.) or adult (Lam.) MLD degrade small amounts of sulfatide. All cells from healthy individuals had a turnover rate of about 30%, confirming previous results showing that, when measured with the artificial substrate p-nitrocatecholsulfate, all cells with an ARSA activity above a critical level of about 10%–15% of the normal reach the normal sulfatide turnover (Leinekugel et al. 1992). The patient, Wi.M., with the late-infantile form of MLD shows three times more sulfatide degradation activity when compared with the patient with the adult type of MLD but only 40% of that of a normal individual.

Analysis of the ARSA Gene

DNA was prepared from cultured fibroblasts of the patient, and the ARSA genes were amplified in two overlapping fragments (Polten et al. 1991) which were subsequently subcloned into M13 mp18/19 and sequenced. Two mutations were found. A C deletion in exon 2 at nucleotide 447 causes a reading-frame shift and leads to a premature stop codon at amino acid position 105. The second mutation is a G→A transition in exon 5 at nucleotide 1574 which changes Gly^{309}

Table 1

ARSA Activities in Cultured Fibroblasts of Individuals with Different ARSA Genotypes

Cell Line	Genotype[a]	Phenotype	ΔmU ARSA/mg[b]
5188Q[c]	SD Ex2/SD Ex2	Late-infantile MLD	0
Wi.M.	$DelC^{447}/G^{309}\rightarrow S$	Late-infantile MLD	.7
Lau.	SD Ex2/PD	Healthy	.6
Nüs.	WT/WT	Healthy	8.3
			% Sulfatide Degradation[d]
Mes.	Del Ex8/SD Ex7	Late-infantile MLD	0
Ass.	SD Ex2/SD Ex2	Late-infantile MLD	0
Wi.M.	$DelC^{447}/G^{309}\rightarrow S$	Late-infantile MLD	12
Schn.	$SD\ Ex2/P^{426}\rightarrow L$	Juvenile MLD	2
Lam.	$P^{426}\rightarrow L/P^{426}\rightarrow L$	Adult MLD	4
Lau.	SD Ex2/Pd	Healthy	28
Schl.	PD/PD	Healthy	30
F6912	WT/WT	Healthy	30

[a] The ARSA alleles SD Ex2 (Polten et al. 1991; designated allele I in this reference), SD Ex7 (Fluharty et al. 1991), and Del Ex8 (Bohne et al. 1991) do not encode for any ARSA polypeptides or residual activities. The $Pro^{426}\rightarrow Leu$ (Polten et al. 1991; designated allele A in this reference) allele is associated with low residual enzyme activity. "PD" stands for the pseudodeficiency allele and "WT" for the normal ARSA allele.

[b] ARSA activities were measured in homogenates of cultured fibroblasts using the artificial substrate p-nitrocatecholsulfate.

[c] Cell line 5188Q is known to synthesize no ARSA polypeptides (Polten et al. 1991). The low amount of sulfatase activity found in these cells (not shown) is due to sulfatases other than ARSA. The value measured in these cells was taken as background value and subtracted from the activities of the other cell lines (mU). Activities are shown as mU/mg of cell protein.

[d] Cultured fibroblasts were exposed to a fluorescently labeled analogue of the natural substrate of ARSA cerebroside sulfate, and we measured how much of the substrate taken up by the cells was converted to its products galactosylceramide and ceramide.

to Ser. Figure 1 shows a schematic presentation of the alterations in the ARSA gene. The substituted Gly^{309} is conserved among five of the six sulfatases cloned so far. For a comparison of amino acid sequences, see Wilson et al. (1990).

Inheritance of the Two Mutations in the Family of the Patient

DNA was isolated from fibroblasts of the patient, his mother, his father, and his brother. The ARSA genes were amplified and analyzed for the presence of the two mutations by using an allele-specific oligonucleotide hybridization assay. The father is heterozygous for the C deletion and a normal ARSA allele, while the mother and the healthy brother are heterozygous for the G→A transition in exon 5 and a normal ARSA allele. The patient is heterozygous for the two mutant alleles, as expected (data not shown).

Frequency of the Alleles among MLD Patients

We have amplified the ARSA genes from 44 MLD patients with at least one unknown allele, and we searched for the presence of the C deletion in exon 2 and the $Gly^{309}\rightarrow Ser$ substitution by using allele-specific oligonucleotide hybridization. Two other patients carrying one allele with the C deletion were found. Both patients suffer from the juvenile form of MLD. The second allele from one patient is unknown, whereas the other patient carries the $Pro^{426}\rightarrow Leu$ substitution (Polten et al. 1991) on the second allele. None of the 44 patients carried the $Gly^{309}\rightarrow Ser$ allele.

Effects of the $Gly^{309}\rightarrow Ser$ Substitution on ARSA Polypeptides

The G→A transition found in exon 5 was introduced into the wild-type ARSA cDNA, and the mutated cDNA was cloned into the expression vector,

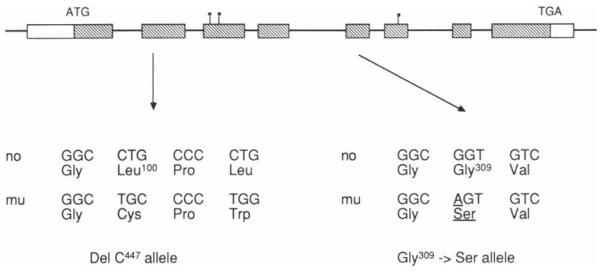

Figure 1 Schematic presentation of sequence alterations in the ARSA gene. *Top*, Schematic representation of structure of the gene. Boxes represent exons; lines represent introns; hatched boxes represent coding regions; and unblackened boxes represent 5′ or 3′ untranslated regions. "ATG" and "TGA" indicate initiation and termination codons, respectively. Small black squares indicate potential N-glycosylation sites. no = nucleotide and amino acid sequence of the normal ARSA allele in the regions which are of interest in this study. mu = sequence alterations occuring in the two mutant ARSA alleles of the patient. A C deletion at nucleotide 447 in exon 2 causes a frameshift at amino acid Leu^{100} in one allele, which causes a premature termination of translation at a position corresponding to amino acid 105 in the normal allele. A G→A transition at nucleotide 1574 changes amino acid Gly^{309} to Ser. Mutated nucleotide and substituted amino acid are underlined.

pBEH (Artelt et al. 1988). BHK cells were transiently transfected with this plasmid, a plasmid containing the wild-type ARSA cDNA, and with the pBEH vector without insert. Cells transfected with the mutated ARSA cDNA had enzyme activities higher than those of mock transfected cells but had only 13% of activity of cells expressing the wild-type ARSA cDNA (table 2). This indicates that the Gly^{309}→Ser substitution leads to a marked decrease, but not to a complete loss, of enzyme activity. In order to explain the low residual enzyme activity, we have examined the kinetic parameters, the intracellular localization, and the stability of the mutant ARSA polypeptides.

Table 2

ARSA Activities in Transiently Transfected BHK Cells

Transfected Plasmid	mU ARSA/mg	ΔmU ARSA/mg	% ARSA Activity
Vector	1.9		
WT-ARSA	8.2	6.3	100
Ser^{309}-ARSA	2.7	.8	13

NOTE.—The table shows the average values of five independent transfection experiments. ARSA activities were determined in cell homogenates and are expressed as mU/mg protein.

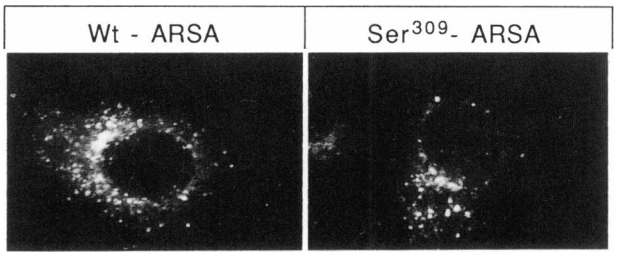

Figure 2 Immunofluorescent staining of ARSA in transfected BHK cells. BHK cells were stably transfected with the normal ARSA cDNA (*left*) or the mutant (Gly^{309}→Ser) ARSA (*right*). Cells were fixed and incubated with an ARSA-specific antibody. Both cells show the typical lysosomal granular perinuclear staining.

ARSA polypeptides were purified to homogeneity from BHK cells stably overexpressing the normal or mutant Ser^{309} ARSA, by using an immunoaffinity column. Kinetic parameters (K_m, V_{max}) and the specific activity of the mutant enzyme did not differ significantly from the normal ARSA (data not shown).

Intracellular localization was examined by immunofluorescent staining of ARSA-overexpressing BHK cells. The staining showed a perinuclear granular distribution of Ser^{309} ARSA typical for a lysosomal localization (fig. 2).

In order to determine whether the low enzyme activity is the consequence of a decreased protein stability, the ARSA half-life in fibroblasts of the patient was measured. ARSA from fibroblasts of the patient and a control individual was immunoprecipitated after [^{35}S]-methionine labeling and subsequent chase for up to 66 h. The results are shown in figure 3. In the patient's fibroblasts more than 50% of ARSA polypeptides disappeared after a chase of 18 h, whereas the ARSA polypeptides from control cells were stable over the entire chase period. Since cross-reacting material in the fibroblasts of the patient can only arise from the Gly^{309}→Ser allele, the data indicate that ARSA polypeptides expressed from this allele are unstable.

BHK cells stably overexpressing normal or mutant ARSA polypeptides were pulse labeled for 1 h and chased for 6 h in the absence or presence of ammonium chloride. Ammonium chloride interferes with the sorting of lysosomal enzymes and causes newly synthesized enzymes to be secreted. ARSA was immunoprecipitated from cells and media. In BHK cells expressing the mutant ARSA, the amount of ARSA in the medium corresponds to the amount of ARSA found intracellularly in the untreated cells (fig. 4). This suggests that the rapid breakdown of the mutant ARSA takes place in a

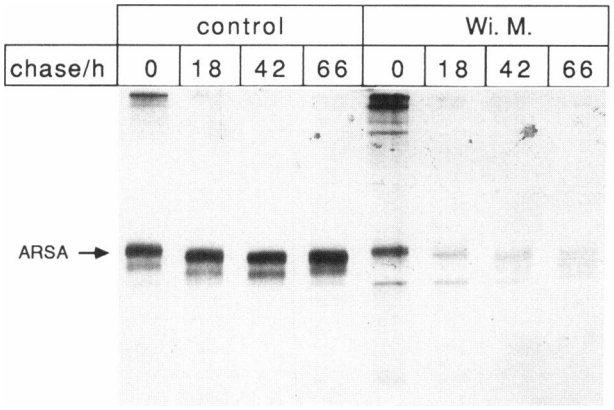

Figure 3 Pulse-chase experiment of normal and mutant cultured fibroblasts. Cells were labeled for 16 h with 50 μCi of [^{35}S]-methionine and chased for the times indicated. Cells were harvested, and ARSA polypeptides were immunoprecipitated and separated on an SDS-PAGE gel which was subjected to fluorography. *Left*, Experiment with normal control cells. *Right*, Experiment with mutant cells of the patient Wi.M. Arrow indicates ARSA polypeptides.

post-Golgi compartment after segregation of ARSA from the secretory route.

Discussion

In a number of MLD patients, evidence has been presented that the clinical phenotype is substantially influenced by the ARSA genotype of the patient. Homozygosity for alleles with mutations completely preventing the synthesis of ARSA mRNA or protein are usually associated with the severe late-infantile form of the disease, whereas presence of one allele with residual enzyme activity mitigates the course of the disease to the juvenile form. In the presence of two alleles with low residual enzyme activity, a gene dosage effect can explain the frequent association of this genotype with the mildest form of the disease, the adult type. Initially this model was proposed on the basis of genetic data (Polten et al. 1991), but in the meantime biochemical data have been presented supporting this genotype-phenotype correlation (Kappler et al. 1991; Leinekugel et al. 1992).

In the present study we have examined several cell lines from patients with a known genotype and different phenotypes, for their ability to degrade exogenously added cerebroside sulfate (see table 1). No sulfatide degradation was detected in two late-infantile patients with genotypes thought to be associated with no residual enzyme activity. Low levels of degradation could be detected in a juvenile patient who was a compound heterozygote having one allele with, and one allele without, residual enzyme activity. The adult patient who was homozygous for an allele with residual enzyme activity exhibited higher degradation rates than the patient with juvenile MLD. These data are in good agreement with the previously proposed genotype-phenotype relationship. Normal levels of sulfatide degradation were observed in individuals who were compound heterozygotes for an MLD and pseudodeficiency (PD) allele. When measured with the artificial substrate p-nitrocatecholsulfate, such individuals show enzyme activities in the range of 7%–15% (see table 1).

We have identified one late-infantile MLD patient who seems to present an exception to the rule that this form of the disease is associated with the lack of measurable residual enzyme activity. When the artificial substrate was used, this patient exhibited residual enzyme activity of about 10%, which is in the range of MLD/PD compound heterozygotes. In order to exclude the possibility that the mutation may specifically affect the enzyme's capability to degrade the natural substrate, we have examined the ability of the patient's fibroblasts to degrade exogenously added, fluorescently labeled sulfatide. These cells degraded three times more sulfatide than those of adult MLD patients and about half of the sulfatide of normal individuals. When the proposed residual ARSA activity–phenotype

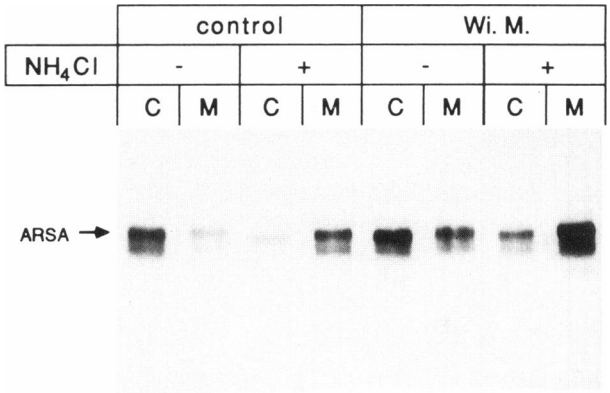

Figure 4 Mutant ARSA in cells and media of NH_4Cl-treated cells. BHK cells expressing the normal ARSA or the mutant (Gly^{309}→Ser) ARSA were labeled for 1 h with [^{35}S]-methionine and chased for 6 h in the presence or absence of 10 mM NH_4Cl. ARSA polypeptides were immunoprecipitated from cells (C) and media (M). Arrow indicates ARSA polypeptides. The distribution of ARSA polypeptides between the cells and the media is comparable in both the normal and mutant ARSA-expressing cells. The amount of ARSA found in the media of treated cells is about the same as that found in the cells of untreated controls.

relationship is extrapolated, one would have expected the patient to suffer from adult MLD with late onset or perhaps even be healthy. Three different reasons may explain why this patient does not fit into the residual ARSA activity-phenotype correlation: First, within a group of late-onset MLD patients having the identical ARSA genotype, the age at onset of the disease may vary from 8 to 23 years, which shows that besides the ARSA genotype other genetic or nongenetic factors significantly influence the clinical outcome of the disease (Polten et al. 1991). Because of those unknown factors, the patient described here may represent an extreme variation within the group of juvenile MLD patients with an identical genotype. Since we have not been able to identify any other patient carrying the $Gly^{309}\rightarrow Ser$ allele, we have no clinical data to prove or disprove this assumption. We think, however, that the residual ARSA activity–phenotype discrepancy cannot be explained in this way. Table 1 shows variations of only about 2% of catabolic activity detectable in the sulfatide loading assay in fibroblasts of patients with different clinical forms of the disease. In view of these small differences among patients with different genotypes and phenotypes, it is difficult to imagine how the great difference described here—0% catabolic activity expected versus 12% measured—can occur among patients with an identical genotype.

Alternatively, the $Gly^{309}\rightarrow Ser$ allele of the patient may represent another PD allele, and the symptoms of the patient may be unrelated to the ARSA genotype. This is highly unlikely, since the child showed typical clinical symptoms, a decreased nerve conduction velocity, and metachromatic deposits in a sural nerve biopsy.

A third explanation is that the mutation has such an effect on the protein that it particularly affects its function only in oligodendrocytes. We have shown that the specific activity, kinetic parameters, and intracellular sorting of the enzyme is normal. The attenuated activity can be explained by a decreased stability of the enzyme. Since, in ammonium chloride–treated cells, normal amounts of enzyme can be found in the media, the degradation has to occur in a post-Golgi compartment. Our data do not allow us to distinguish whether the degradation occurs in the lysosomes or already in a prelysosomal compartment. If one assumes that the instability of the mutant enzyme is the only cause for the enzyme deficiency, then one has to postulate mechanisms which, in an oligodendrocyte-specific manner, cause an even more rapid degradation of the ARSA. One can speculate that oligodendrocytes may express a distinct pattern of proteases which could cause a more rapid breakdown of the molecule. At this stage we do not have an experimental system that allows us to investigate whether the stability of mutant ARSA polypeptides in oligodendrocytes differs from that of other cell types.

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References

Artelt P, Morell C, Ansmeier M, Fitzek M, Hauser H (1988) Vectors for efficient expression in mammalian fibroblasts, myeloid, lymphoid cells via transfection. Gene 68:213–219

Bohne W, von Figura K, Gieselmann V (1991) A 11 bp deletion in the arylsulfatase A gene of a patient with late infantile MLD. Hum Genet 87:155–158

Fluharty AL, Fluharty CB, Bohne W, von Figura K, Gieselmann V (1991) Two new arylsulfatase A (ARSA) mutations in a juvenile metachromatic leukodystrophy (MLD) patient. Am J Hum Genet 49:1340–1350

Gieselmann V, Fluharty AL, Tønnesen T, Von Figura K (1991*a*) Mutations in the arylsulfatase A pseudodeficiency allele causing metachromatic leukodystrophy. Am J Hum Genet 49:407–413

Gieselmann V, Polten A, Kreysing J, Kappler J, Fluharty AL, Bohne W, von Figura K (1991*b*) Mutations in arylsulfatase A alleles causing metachromatic leukodystrophy. Brain Dysfunction 4:235–243

Gieselmann V, Polten A, Kreysing J, von Figura K (1989) Arylsulfatase A pseudodeficiency: loss of a polyadenylation signal and N-glycosylation site. Proc Natl Acad Sci USA 86:9436–9440

Gieselmann V, Schmidt B, von Figura K (1992) In vitro mutagenesis of potential N-glycosylation sites of arylsulfatase A. J Biol Chem 267:13262–13266

Herz B, Bach G (1984) Arylsulfatase A in pseudodeficiency. Hum Genet 66:147–150

Hohenschutz C, Eich P, Friedl W, Waheed A, Conzelmann E, Propping P (1989) Pseudodeficiency of arylsulfatase A: a common genetic polymorphism with possible disease implications. Hum Genet 82:45–48

Kappler J, Leinekugel P, Conzelmann E, Kleijer WJ, Kohlschütter A, Tonnessen T, Rochel M, et al (1991) Genotype phenotype relationship in various degrees of arylsulfatase A deficiency. Hum Genet 86:463–470

Kolodny EH (1989) Metachromatic leukodystrophy and multiple sulfatase deficiency: sulfatide lipidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. Vol 2. McGraw-Hill, New York, pp 1721–1750

Leinekugel P, Michel S, Conzelmann E, Sandhoff K (1992) Quantitative correlation between the residual activity of β-hexosaminidase A and arylsulfatase A and the severity of the resulting lysosomal storage disease. Hum Genet 88:513–523

Monti E, Preti A, Novati A, Aleo MF, Clemente ML, Marchesini S (1992) Uptake and metabolism of a fluorescent sulfatide analogue in cultured skin fibroblasts. Biochim Biophys Acta 1124:80–87

Nakamaye KL, Eckstein F (1986) Inhibition of restriction endonuclease NciI cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis. Nucleic Acids Res 14:9679–9698

Polten A, Fluharty AL, Fluharty CB, Kappler J, von Figura K, Gieselmann V (1991) Molecular basis of different forms of metachromatic leukodystrophy. N Engl J Med 324:18–22

Shapiro LJ, Aleck KA, Kaback MM, Itabashi H, Desnick RJ, Brand N, Stevens RL, et al (1979) Metachromatic leukodystrophy without arylsulfatase A defiency. Pediatr Res 13:1179

Southern PJ, Berg P (1982) Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV 40 early region promotor. J Mol Appl Genet 1:327–341

Stein C, Gieselmann V, Kreysing J, Schmidt B, Pohlmann R, Waheed A, Meyer HE, et al (1989) Cloning and expression of human arylsulfatase A. J Biol Chem 264:1252–1259

Viani P, Marchesini S, Cestaro B, Gatt S (1989) Correlation of the dispersion state of pyrene cerebroside sulfate and its uptake and degradation by cultured cells. Biochim Biophys Acta 1002:20–27

Waheed A, Gottschalk S, Hille A, Krentler C, Pohlmann R, Braulke T, Hauser H, et al (1988) Human lysosomal acid phosphatase is transported as a transmembrane protein to lysosomes in transfected baby hamster kidney cells. EMBO J 7:2351–2358

Waheed A, Hasilik A, von Figura K (1982) Synthesis and processing of arylsulfatase A in human skin fibroblasts. Hoppe Seyler Z Physiol Chem 363:425–430

Wenger DA, De Gala G, Williams C, Tayler HA, Stevenson RE, Pruitt JR, Miller J, et al (1989) Clinical pathological and biochemical studies on an infantile case of sulfatide/GM1 activator protein deficiency. Am J Med Genet 33: 255–265

Wilson PJ, Morris CP, Anson DS, Ochiodoro T, Bielicki J, Hopwood JJ (1990) Hunter syndrome: isolation of an iduronate-2-sulfatase cDNA clone and analysis of patient DNA. Proc Natl Acad Sci USA 87:8531–8535