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Brief Communication

Novel mutations in arylsulfatase A gene in three Ukrainian families with metachromatic leukodystrophy

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Abstract

Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by the deficiency of arylsulfatase A (ARSA) or saposin B. The majority of mutations identified in patients with MLD are unique within individual families. Here, we report on the novel missense mutations (F247S, D381E, and A469G) and the known mutations "A" allele and P136S in the ARSA gene in three unrelated Ukrainian families with MLD. The mutations F247S and P136S were found in compound heterozygous with the "A" allele in two patients with juvenile onset MLD. The clinical features of the typical patient with genotype D381E/A469G (early onset with very rapid manifestation of disease) suggest the reason to distinguish an early infantile MLD variant.

Keywords: Metachromatic leukodystrophy; Arylsulfatase A; Mutations; Genotype–phenotype correlation

Introduction

Metachromatic leukodystrophy (MLD, MIM# 250100) is an autosomal recessively inherited lysosomal storage disorder resulting from a deficiency of activity of arylsulfatase A (ARSA; EC 3.1.6.1) [1]. On the other hand, saposin B may be also responsible for a rare type of MLD [2]. The estimated frequency of this disorder is 1:100,000 [2]. Three major MLD variants have been characterized to date: late infantile, juvenile, and adult. There is considerable clinical heterogeneity within these variants, with respect to the age of onset and the severity of symptoms.

The ARSA gene (*ARSA*, GenBank No. 30016942) maps to chromosome 22q13, covers 3.2 kb genomic DNA, and includes eight exons [3]. So far, 95 MLD-relevant mutations have been identified. There are two common *ARSA* mutations which are frequently involved in the severe deficiencies of arylsulfatase A leading to MLD. A splicing mutation 459 + 1G>A, the "I" allele (MIM#250100.0003), is associated with severe late infantile onset MLD, and a missense mutation P426L in exon 8, the "A" allele (MIM#250100.0004), is more frequently found in juvenile and adult onset cases [1].

Although the mutations of *ARSA* have been extensively analyzed, reports from the former USSR are relatively rare. Here, we report on the identification of novel mutations of *ARSA* (F247S, A469G, and D381E) in Ukrainian patients with MLD.

Materials and methods

Case histories

Three unrelated MLD Ukrainian families were included in this study.

The patient of *family 1* (Proband 1) was a girl with juvenile MLD variant with onset at the age of 5 years.

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The patient of *family 2* (Proband 2) was a girl with juvenile MLD variant with onset at the age of 7 years.

The patient of *family 3* (Proband 3) was a girl with atypical late infantile onset MLD variant. The obvious symptoms of MLD were diagnosed at the age of 6 months. During the first 6 months of the disease the patient lost skills such as sitting and taking meals. She lost interest in toys and was unable to recognize her parents. Mental regression was obvious. Very quickly this patient became quadriplegic. She suffered from severe tonic seizures. The optic atrophy was diagnosed at the last stages of the disease.

Brain MRI scans of all patients demonstrated diffuse high intensity signals in the cerebral white matter, especially in the periventricular zones.

Biochemical studies

Patients were diagnosed biochemically by standard method of ARSA activity determination in leukocytes [4] and by qualitative TLC studies of sulfatide excretion in urine [5]. All patients demonstrated low arylsulfatase A activity (3–25 nmol/h/mg protein, control range is 118.5 ± 25.8 nmol/h/mg protein) and elevated sulfatide excretion in urine (Fig. 1A) (see Table 1).

Mutation analysis

Genomic DNA was extracted from frozen leukocyte pellets of patients using standard methods described by Sambrook et al. [6]. PCR amplifications were performed

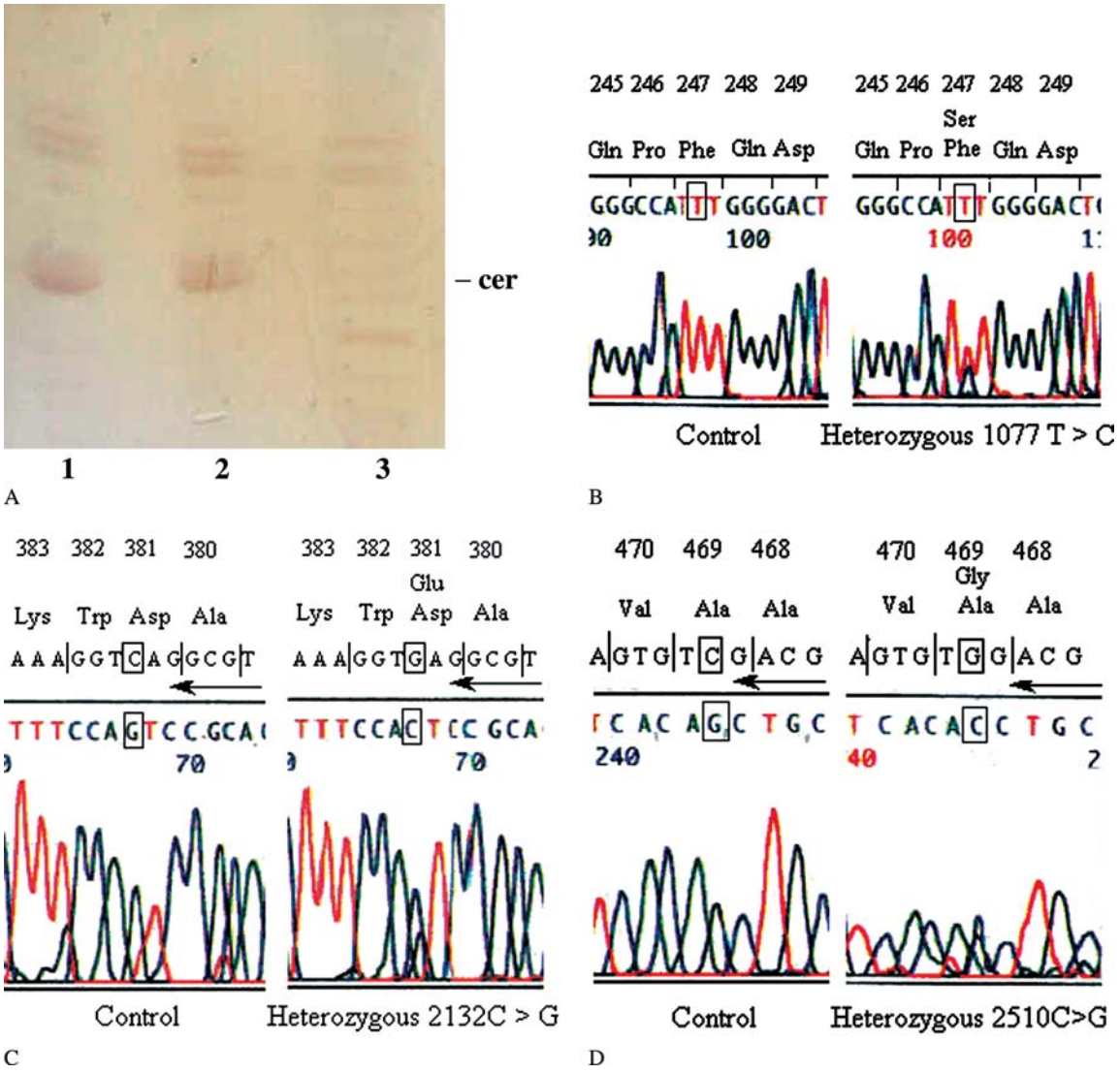

Fig. 1. (A) TLC analysis of urinary sulfatide excretion in atypical case of MLD (Proband 3): 1, Proband 3; 2, positive control (classical MLD, genotype I/I); 3, negative control (healthy individual). (B) Direct nucleotide sequencing of exon 4 of *ARSA* (Proband 2): left panel is the reference sequence; at right panel the transition T → C which generated the F247S alteration is shown. (C) Direct nucleotide sequencing of exon 7 of *ARSA* (Proband 3): left panel is the reference sequence (reverse); at right panel the transversion G → C which generated the D381E alteration is shown. (D) Direct nucleotide sequencing of the exon 8 of *ARSA* (Proband 3): left panel is the reference sequence (reverse); at right panel the transversion G → C which generated the A469G alteration is shown.

Table 1
The genotype–phenotype correlations in Ukrainian MLD patients

Probands	The MLD variants	Arylsulfatase A activity (nmol/h/mg protein)	Urinary sulfatide excretion	Mutations in the ARSA gene
Proband 1	Juvenile	25	Elevated	P426L/P136S
Proband 2	Juvenile	3	Elevated	P426L/F247S
Proband 3	Early infantile	15	Elevated	D381E/A469G

using standard procedures described by Innis et al. [7]. "I" and "A" alleles were identified by polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) method with the use of restriction enzymes *Mva*I and *Pst*I (Fermentas, Lithuania), respectively, as described by Coulter-Mackie et al. [8]. The ARSA pseudodeficiency allele was also determined as described by Gieselmann [9]. The PCR conditions and primers used for the amplification of various regions of the *ARSA* for the mutation analysis were as described by Marcao et al. [10]. The coding sequence for *ARSA* was directly sequenced with Big-Dye Terminator Cycle Sequencing Kit and run on an ABI 310 automatic sequencer (PE Applied Biosystems).

Population studies in the screening for the novel mutations

Blood samples from 50 Ukranian healthy individuals were randomly collected after obtaining their informed consent.

Results and discussion

All members of each family were screened for the pseudodeficiency mutations in the *ARSA* gene. In *families 1*, *2*, and *3* the pseudodeficiency allele was not found.

So far, 95 mutations in *ARSA* have been identified. Two of them, "I" and "A" alleles, are the most common in the European population [11]. Therefore, we initially screened these major mutations in unrelated Ukrainian MLD patients. Through this screening, Proband 1 and Proband 2 were found to be heterozygous for the "A" allele. In Proband 3 the major mutations were not found.

To identify mutations in another allele in Probands 1 and 2, and the MLD-causing mutations in Proband 3, we have sequenced all eight exons of ARSA gene.

The sequencing of exon 2 of *ARSA* in Proband 1 revealed a C to T transition at nucleotide (nt.) 555 in a heterozygous state, which leads to a P136S alteration (nt. 1 is the first coding nucleotide of *ARSA*). The mutation P136S was first described in a Spanish patient with juvenile onset MLD [12]. In our study, this mutation was associated with the "A" allele in a patient with the same MLD variant (Proband 1).

The sequencing of exon 4 of *ARSA* in another patient with juvenile onset MLD (Proband 2) revealed a T to C transition at nt. 1077, which leads to a novel alteration F247S and creates a restriction site for *Bcc*I (New England Biolabs, MA, USA) restriction site (Fig. 1B). Thus, correlation of the "A" allele with late onset MLD variants has been confirmed.

The sequencing of exon 7 of *ARSA* in Proband 3 revealed a C to G transversion at nt. 2132, which leads to a novel alteration D381E and loses *Tth*111I (Promega, WI, USA) restriction site (Fig. 1C). The sequencing of exon 8 of *ARSA* in this patient revealed a C to G transversion at nt. 2510 at heterozygous state, which leads to a novel alteration A469G and loses *Pvu*II (Promega) restriction site (Fig. 1D). It is interesting that Proband 3 presented with a late infantile MLD variant with very early onset (at 6 month) and very rapid manifestation of the disease. The classic late infantile variant of MLD begins between the age of 12 and 30 months [2]. Therefore, this clinical case is most likely attributable to the early infantile MLD variant.

The novel mutations were proved by restriction enzyme digestion (RFLP). The screening for mutations F247S, D381E, and A469G in 50 healthy donors (100 alleles) suggested that these mutations were not polymorphic. These results and the fact that the mutations we described were the only found in the investigated individuals, allow to confirm that the novel mutations are MLD-causing.

Although the number of cases is limited, results of our study are consistent with the observation that the majority of MLD mutations are unique within families. Furthermore, it is worthy of note that the Proband 3 (compound heterozygous mutations; D381E/A469G) showed a very early onset of the clinical symptoms from the infantile period. The recent elucidation of the three-dimensional structure of arylsulfatase A [12] allows to suggest the possible effect of mutations leading to amino acid substitutions at structural level. None of the mutations described by us in Proband 3 is situated in the active center but nearness of both mutations to the hydrogen bridge and mutation A469G to the S–S bridge may influence the tertiary and quaternary structure of the enzyme molecule. Future investigations of the transient transfection of novel mutations allow to analyze in detail of its structural effects in arylsulfatase A. These results presented here may provide useful information for the study of other MLD patients, as well as new insights about the effect of mutations on the structure and function of ARSA gene.

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