SHORT COMMUNICATION

Molecular bases of metachromatic leukodystrophy in Polish patients

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Our preliminary studies on 43 unrelated Polish patients suffering from different types of metachromatic leukodystrophy (MLD) showed that four mutations in the *ARSA* gene accounted for 55% of mutated alleles (c.459+1G>A, p.P426L, p.I179S and c.1204+1G>A). Subsequently, we reported six additional mutations jointly accounting for 10% of mutated alleles. Further sequence analysis of the *ARSA* gene performed on DNA samples of Polish MLD patients with unidentified alleles revealed eight rare mutations on 10 alleles: p.R390W, p.E382K, p.R390Q, p.R288C, p.H397Y, p.F247S, p.D335V and g.561_562insC, responsible together for 12% of the examined alleles. We have not identified any frequent mutation in the *ARSA* gene, which would be typical or unique for Polish patients. In this report, we describe the results of this and summarize the results of this and our previous studies.

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INTRODUCTION

Metachromatic leukodystrophy (MLD; OMIM 250100) is a metabolic disease affecting white matter of central and peripheral nervous systems. It is caused by a deficiency of a lysosomal hydrolase arylsulfatase A (ARSA; EC 3.1.6.8), which leads to intralysosomal accumulation of galactosylceramide sulfatide and other sulfated sphingolipids. This process results in a progressive demyelination and a clinical picture characteristic of leukodystrophy. A variant of MLD caused by the deficiency of saposin B is also known (OMIM 249900).¹

So far, over 150 mutations in the *ARSA* gene have been identified (<http://www.hgmd.cf.ac.uk/ac/index.php>). Three of them are most frequently found in Caucasian MLD patients: c.459+1G>A, p.P426L and p.I179S.¹ Other mutations in the *ARSA* gene are private or were described rarely. There is also a common polymorphism named ARSA-pseudodeficiency allele.

Our preliminary studies on 43 unrelated Polish patients suffering from different types of MLD revealed that four mutations (c.459+1G>A, p.P426L, p.I179S and c.1204+1G>A) together accounted for 55% of mutated alleles.² After sequence analysis of eight exons of the *ARSA* gene, six additional mutations were found³ which were together responsible for 10% of MLD alleles. Thus, 35% of MLD alleles remained unidentified.

In this study, we report the results of sequence analysis of the *ARSA* gene performed on DNA samples of Polish MLD patients with unidentified alleles. The aim of this study was to elucidate the

molecular bases of MLD in these individuals and to summarize mutation profile of our cohort of patients.

MATERIALS AND METHODS

Patients were diagnosed by biochemical assay of ARSA activity in isolated peripheral blood leukocytes with 4-nitrocatechol sulfate as a substrate.⁴ The sequence analysis of *ARSA* gene was performed with primers described previously.⁵ PCR products encompassing exons and exon/intron boundaries were sequenced in both directions with the DYEnamic ET Cycle Sequencing Kit (Amersham Biosciences, Little Chalfont, UK) and analyzed on ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). In patients with ARSA-pseudodeficiency allele the diagnosis of MLD was confirmed by a sulfatide excretion test.

An informed consent was obtained from the patients or their parents, and the study was approved by the local ethical committee.

RESULTS

Eight MLD causing mutations of the *ARSA* gene were found among 10 alleles in this study. Mutation p.R390W occurred in a heterozygous state in two patients: one with late infantile and the other with early juvenile type of the disease. In a single patient with early juvenile-type MLD, a homozygous mutation p.R390Q was detected. Thus, the prevalence of these two mutations in the examined group of 43 unrelated Polish MLD patients is approximately 2% (the cohort was described in detail in Ługowska *et al.*²). All other identified mutations were found in heterozygous state and always in single patients: the

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Table 1 Mutations of ARSA gene detected in Polish MLD patients

Late infantile (n=14)		Early juvenile (n=11)		Late juvenile (n=8)		Adult (n=10)	
No. of patient	Genotype	No. of patient	Genotype	No. of patient	Genotype	No. of patient	Genotype
1	c.459+1G>A/c.459+1G>A	1	c.459+1G>A/p.P426L	1	c.459+1G>A/p.I179S	1	p.P426L/p.P426L
2	c.459+1G>A/c.459+1G>A	2	c.459+1G>A/p.P426L	2	c.1204+1G>A/ p.I179S	2	p.P426L/p.P426L
3	c.459+1G>A/c.459+1G>A	3	c.459+1G>A/X	3	p.I179S/X _{PD}	3	p.P426L/p.P426L
4	c.459+1G>A/c.459+1G>A	4	p.P426L/X	4	p.I179S/X	4	p.P426L/p.P426L
5	c.459+1G>A/c.459+1G>A	5	p.P426L/ g.2590_2591dupC	5	p.I179S/X	5	p.P426L/p.P426L
6	c.459+1G>A/X	6	X/g.445_446dupG _{PD}	6	p.I179S/p.F247S	6	c.459+1G>A/p.I179S
7	c.1204+1G>A/c.1204+1G>A	7	X/X _{PD}	7	p.P426L/p.H397Y	7	p.I179S/p.D335V
8	c.1204+1G>A/p.G293C	8	X/X	8	p.I179S/p.C493F	8	p.I179S/X
9	p.E382K/g.445_446dupG _{PD} ^a	9	X/X			9	p.I179S/ g.561_562insC
10	p.G122C/p.G122C	10	p.R390Q/p.R390Q			10	X/X
11	p.R114X/p.R114X	11	p.R390W/p.R288C				
12	X/X						
13	c.459+1G>A/p.R390W						
14	X/X						

PD—ARSA-pseudodeficiency allele.

X—unidentified MLD causing alleles (lack of the material for further analysis).

^amutation p.N350S in the ARSA-PD allele.

p.E382K mutation in a late infantile-type patient, the p.R288C mutation in an early juvenile-type patient; the p.H397Y and p.F247S mutations in late juvenile-type patients; the g.561_562insC insertion and the p.D335V substitution in adults. Thus, in the Polish group of MLD patients the frequency of each of those mutations is approximately 1%. The rare MLD causing mutations identified in this study accounted together for 12% of MLD alleles in 43 Polish patients.

The DNA samples from 13 patients among whom there were 20 alleles without identified mutations were no more available for the analysis (leaving 23% of the examined MLD alleles unidentified).

All ARSA mutations detected in Polish patients with different forms of MLD are summarized in Table 1.

DISCUSSION

As a result of our previous studies, 35% of mutated alleles in Polish MLD patients remained unidentified. In this study, eight rare but already described MLD mutations were found on 10 chromosomes thus representing 12% of all examined alleles. These results are summarized in Figure 1. In our study, we did not identify mutations on 23% of the examined MLD alleles. It should be emphasized that these DNA samples were all screened for the four most frequent mutations (c.459+1G>A, p.P426L, p.I179S and c.1204+1G>A) but because of lack of DNA in none of them, the full sequence analysis was completed. It was not possible to obtain new samples because of lack of consent or death of the patients. In the future, we plan to finalize the molecular analysis by using samples from parents of probands.

The p.R390W mutation was originally described by Barth *et al.*⁶ in a patient with severe phenotype. We have found this variant in a late infantile patient in a compound heterozygous state with another severe mutation—c.459+1G>A and in an early juvenile-type MLD patient in a compound heterozygous state with the p.R288C mutation. The latter amino acid substitution was originally described by Gieselmann *et al.*⁷ in a Caucasian MLD patient. As no data are available

Figure 1 Contribution of the ARSA gene mutations to the molecular basis of MLD in Polish patients with different types of the disease. The following recently described mutations were found: g.445_446dupG (frequency of 2% in the whole group of MLD patients), p.G122C (2%), p.R114X (2%), p.G293C (1%), p.C493F (1%) and g.2590_2591dupC (1%). Among rare ARSA gene mutations there were: p.R390Q (frequency of 2% in the whole group of MLD patients), p.R390W (2%), p.E382K (1%), p.R288C (1%), g.561_562insC (1%), p.D335V (1%), p.H397Y (1%) and p.F247S (1%).

on the influence of p.R288C on the phenotype, it can be assumed that this is a type R mutation as we found it in a patient with early juvenile-type MLD together with a severe mutation p.R390W. The p.E382K mutation was originally described in homozygous state in a juvenile patient.⁸ We have found this variant in a subject with late infantile MLD together with a recently described severe MLD mutation g.445_446dupG on the other allele.³ The p.R390Q mutation identified by Coulter-Mackie⁹ in a juvenile MLD patient in compound heterozygous state with p.P426L (type R) was detected in this study in homozygous state in an early juvenile MLD patient. The p.H397Y is another mild mutation detected earlier by Coulter-Mackie⁹ and found

also in this study—it was present in a late juvenile MLD patient together with p.P426L. Unfortunately, no information is available on the ethnic origin of MLD patients examined by Dr Coulter-Mackie (personal information). In three of our patients with late juvenile or adult type of the disease, severe mutations p.F247S,¹⁰ g.561_562insC¹¹ and p.D335V¹² were detected in compound heterozygous state with p.I179S. The latter is a frequent mutation, which always associates with a mild type MLD with characteristic predominance of psychotic changes in the clinical picture.

In conclusion, this study and our previous work showed that in Polish MLD patients, similarly to other Central European cohorts, the p.P426L, c.459+1G>A and p.I179S mutations were most prevalent. The frequency of c.1204+1G>A may be higher in Polish MLD patients when compared with other cohorts: 5%² vs 2%.¹³ All the remaining ARSA mutations were rare—they were found in one or two of the investigated patients, so their prevalence in the examined group ranged from 1 to 2%. All of them have been already described. We have not identified any frequent mutation in the ARSA gene, which would be typical or unique for Polish patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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