http://pmjournal.ir Original Article

Summer 2020, Volume 5, Issue 18 (14-16)

ARSA gene mutation analysis in 5 patients with metachromatic leukodystrophy

Sahar Hassannejad[1*], Seyed Reza Hayatgheibi[2], Massoud Houshmand[2], Roya Amirinezhad[3]

[1] Researcher and lecture assistant at the Medical Laboratory Science Department, Knowledge University, Kurdistan Region, Erbil, Iraq.

[2] Department of Medical genetic, national Institute for genetic Engineering and Biotechnology, Tehran, Iran.

[3] Personalized Medicine Research Center of AmitisGen, Tehran, Iran.

*Corresponding author: Sahar Hassannejad, Researcher and lecture assistant at the Medical Laboratory Science Department, Knowledge University, Kurdistan Region, Erbil, Iraq. Sahar.najad@Knowledge.edu.krd

DOI: 10.22034/PMJ.2020.46381

Submitted: 2020-06-14
Accepted: 2020-08-30

Keywords:
Metachromatic leukodystrophy
ARSA gene
Mutation
Genetic counseling

Abstract

Metachromatic leukodystrophy is a kind of lysosomal storage disorder caused by a deficiency in the ARSA enzyme, which is involved in the metabolism of membrane sulfatides into galactosylceramide. ARSA gene mutation analysis is likely to be in increasing demand for the accurate detection of heterozygous carriers and for prenatal diagnosis in families with a metachromatic leukodystrophy proband. This study examined the sequence of the ARSA gene in 7 patients with metachromatic leukodystrophy by exon Sanger sequencing. Eleven mutations were found in the ARSA gene. It was further found that five mutations were probably damaging to the protein activity with a high score and specificity. The results pave the way to a more effective method for Carrier diagnosis, genetic diagnosis, and the counseling of Iranian patients with MLD disorder.

INTRODUCTION

Metachromatic leukodystrophy (MLD) is a rare hereditary disease characterized by the accumulation of fats called sulfatides. The frequency of MLD is approximately 1 in 40,000 worldwide (1). It causes the destruction of the protective fatty layer (myelin sheath) surrounding the nerves in both the central and the peripheral nervous systems. There are three forms of metachromatic leukodystrophy which involve different age ranges: late infantile form, juvenile form, and adult form (2). Signs and symptoms can vary. The infantile form is the most common and progresses more rapidly than the other forms. Most individuals with metachromatic leukodystrophy have mutations in the ARSA gene, which provides instructions for making the enzyme arylsulfatase A (3). This enzyme is located in cellular structures called lysosomes, which are the cell's recycling centers. Within lysosomes, arylsulfatase A helps break down sulfatides (4). A few individuals with metachromatic leukodystrophy have mutations in the PSAP gene. Mutations in the ARSA or PSAP genes result in a decreased ability to break down sulfatides, resulting in the accumulation of these substances in cells (5). Excess sulfatides are toxic to the nervous system. The accumulation gradually destroys myelin-producing cells, leading to the impairment of nervous system function that occurs in metachromatic leukodystrophy (6). This condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. Clinical examination and MRI are often the first steps in a MLD diagnosis. MRI can be indicative of MLD but is not adequate as a confirming test (7). An ARSA-A enzyme level blood test with a confirming urinary sulfatide test is the best biochemical test for MLD. Confirming urinary sulfatide is important to distinguishing between MLD and pseudo-MLD blood results. There is no cure for MLD. Treatment for the condition focuses on the management of symptoms and improvement of the quality of life. In some people, a bone marrow or cord blood transplant may be effective in slowing the progression of the disease (8). This current study examined the sequence of the ARSA gene in 7 patients with metachromatic leukodystrophy.

METHODS AND MATERIALS

Seven MLD patients from seven unrelated families were enrolled in this study. All patients were clinically diagnosed with MLD based on the manifestation, classical MRI feature, and ARSA enzyme deficiency in leukocytes. Genomic DNA was extracted from peripheral blood leukocytes of patients based on the standard protocols of the GenExTM Blood, Sx (GeneAll, Korea.). Each exon and the exon-intron boundaries of the ARSA gene were amplified by polymerase chain reaction using oligonucleotide primers described in Table 1. The reaction program was 94 °C for 5 minutes, then 34 cycles at 94 °C for 1

minute, 58 °C for 15 seconds, and 1 minute at 72 °C. The polymerase chain reaction products were purified and sequenced using either sense or antisense primer by the BigDye Terminator cycle sequencing kit in the ABI PRISM 3130 genetic analyzer.

Table 1. Primer sequencing of five primer pairs used to amplify ARSA gene exons

Exon	Primer sequence	Product size
1	5'-aagaccgcagccaacagcctc-3' 5'-cagagtccctgagacagacagaaatg-3'	879
1+2	5'-ttgcccgtccgcccaacatcgtg-3' 5'-ccctggtcacagccaccgtcgcaag-3'	737
2+3+4	5'-gatttctaggcatcccgtactc-3' 5'-ccctcacccactatgttcttg-3'	706
5+6+7	5'-gccaagaacatagtgggtgagg-3' 5'-ggtagaagaagagagactgccgag-3'	860
7+8	5'-gcaagaagcggtgcacgtcc-3' 5'-ccacgacaccagggttcaaatcc-3'	916

Table 2. Mutations in the ARSA gene in Iranian MLD patients

Patients ID	Nucleotide change	Amino acid change	Status
1	c.218C>T	p.Pro73Leu	Hetro
	c.911A.G	p.K304R	Hetro
2	c.302G>T	pGly101Val	Hetro
3	c.925G>A	p.Glu309Lys	Hetro
4	C.610C>G	p.Arg204Gly	Hetro
5	c.739G.A	p.G247R	Hetro
	c.1160G>T	p.387Gly>Val	Hetro
6	c.917C>T	P.Thr306Met	Hetro
	c.827C>T	P.Thr276Met	Hetro
	c.383T>C	p.Leu128Pro	Hetro
7	c.257G>A	p.Arg86Gln	Hetro

RESULTS

Eight exons of the ARSA gene were examined in 7 patients with MLD symptoms. Eleven mutations were found in the ARSA gene (Table 2). After predicting the effect of the amino acid changes in ARSA using the web server PolyPhen-2, five mutations were found to be probably damaging to the protein activity with a high score and specificity. All 11 mutations were inherited as heterozygotes.

DISCUSSION

MLD is a kind of lysosomal storage disorder caused by a deficiency in the ARSA enzyme, which is involved in the metabolism of membrane sulfatides into galactosylceramide (9). Progressive demyelination and dysfunction of the peripheral and central nervous systems are the symptoms of this disease, as the undergraded sulfatides require time to accumulate in oligodendrocytes and Schwann cells. ARSA, the disease-causing gene of MLD, is located on chromosome 22q13, has a total length of 3.2 kb, contains eight exons, and is transcribed into three mRNA species (10). To date, the Human Gene Mutation Database has reported a total of 217 ARSA mutations, comprising 161 missense mutations, 14 splicing-site mutations, 20 small deletions, 12 small insertions, 4 small indels, 2 gross deletions, 2 complex rearrangements, 1 gross insertion, and 1 regulatory mutation. ARSA gene mutation analysis is likely to be in increasing demand for the accurate detection of heterozygous carriers and for prenatal diagnosis in families with a metachromatic leukodystrophy proband (11). Therefore, ARSA gene mutation analysis was performed on seven Iranian patients with metachromatic leukodystrophy. Eleven mutations were found in the ARSA gene (Table 2). After predicting the effect of the amino acid change in ARSA, it was found that five mutations were probably damaging to the protein activity with a high score and specificity, and all 11 mutations were inherited as heterozygotes. Among these mutations, all 5 known mutations, c.917C>T, c.827C>T, c.257G>A, c.925G>A, and c.302G>T, could destroy the function of the ARSA protein and were considered disease-causing mutations. The results of this research have broadened the genotypic spectrum of Iranian patients

with MLD, paving the way to a more effective method for Carrier diagnosis , genetic diagnosis, and counseling of Iranian patients with MLD disorder.

REFERENCE

1. An Egmond ME, Pouwels PJ, Boelens JJ, et al. Improvement of white matter changes on neuroimaging modalities after stem cell transplant in metachromatic leukodystrophy. JAMA Neurol. 2013;70(6):779–782.

2. Wang RY, Bodamer OA, Watson MS, Wilcox WR; ACMG Work Group on Diagnostic Confirmation of Lysosomal Storage Diseases. Lysosomal storage diseases: diagnostic confirmation and management of presymptomatic individuals. Genet Med. 2011;13(5):457–484.

3. Cheon JE, Kim IO, Hwang YS, et al. Leukodystrophy in children: a pictorial review of MR imaging features. Radiographics. 2002;.

4. Eichler F, Grodd W, Grant E, et al. Metachromatic leukodystrophy: a scoring system for brain MR imaging observations. AJNR Am J Neuroradiol. 2009;30(10):1893–1897.

5. Molzer B, Sundt-Heller R, Kainz-Korschinsky M, Zobel M. Elevated sulfatide excretion in heterozygotes of metachromatic leukodystrophy: dependence on reduction of arylsulfatase A activity. Am J Med Genet.

6. Gort L, Coll MJ, Chabás A. Identification of 12 novel mutations and two new polymorphisms in the arylsulfatase A gene: haplotype and genotype-phenotype correlation studies in Spanish metachromatic leukodystrophy patients. Hum Mutat. 1999;14(3):240–248.

7. Kreysing J, von Figura K, Gieselmann V. Structure of the arylsulfatase A gene. Eur J Biochem. 1990;191(3):627–631.

8. Polten A, Fluharty AL, Fluharty CB, Kappler J, von Figura K, Gieselmann V. Molecular basis of different forms of metachromatic leukodystrophy. N Engl J Med. 1991;324(1):18–22

9. Berger J, Löschl B, Bernheimer H, et al. Occurrence, distribution, and phenotype of arylsulfatase A mutations in patients with metachromatic leukodystrophy. Am J Med Genet. 1997;69(3):335–340.

10. Schestag F, Yaghootfam A, Habetha M, et al. The functional consequences Of mis-sense mutations affecting an intra-molecular salt bridge In arylsulphatase A. Biochem J.2002;367(Pt 2):499–504.

11. Gieselmann V, Franken S, Klein D, et al. Metachromatic leukodystrophy: consequences of sulphatide accumulation. Acta Paediatr Suppl. 2003;92(443):74–79; discussion 45.