Korean J Lab Med 2010;30:516-20
DOI 10.3343/kjlm.2010.30.5.516

Identification of a Novel Splicing Mutation in the *ARSA* Gene in a Patient with Late-infantile Form of Metachromatic Leukodystrophy

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Metachromatic leukodystrophy (MLD; MIM 250100), a severe neurodegenerative disorder inherited as an autosomal recessive trait, is caused by mutations in the arylsulfatase A *(ARSA)* gene. Although several germ line *ARSA* mutations have been identified in patients with MLD of various ethnic backgrounds elsewhere in the world, no genetically confirmed cases of MLD have been reported in Korea. Recently, we identified a mutation in the *ARSA* gene of a Korean male with MLD. A male infant with late-infantile form of MLD had been admitted to our hospital for further examination. His neuromuscular symptoms, which included inability to walk at the age of 12 months, gradually worsened, even after allograft bone marrow transplantation; he died at the age of 9 yr. His elder brother had also been diagnosed with MLD. To confirm the presence of a genetic abnormality, all the coding exons of the *ARSA* gene and the flanking introns were amplified by PCR. A molecular analysis of the *ARSA* gene revealed both a novel heterozygous splicing mutation (c.1101+1G>T) in intron 6 and a heterozygous missense mutation in exon 2 (c.296G>A; Gly99Asp). The patient's elder brother who had MLD is believed to have had the same mutation, which may be correlated with a rapidly deteriorating clinical course. This study identified a novel mutation in the *ARSA* gene, related to a late-infantile form of MLD with a lethal clinical course and suggested that molecular diagnosis of patients may be useful in early diagnosis and for deciding intervention measures for their family members. *(Korean J Lab Med 2010;30:516-20)*

Key Words : *Metachromatic leukodystrophy, ARSA, Novel mutation, Korean*

INTRODUCTION

Metachromatic leukodystrophy (MLD; MIM 250100) is a severe neurodegenerative disorder caused by decreased activity of arylsulfatase A (ARSA; EC 3.1.6.8), a lysosomal enzyme involved in the catabolism of cerebroside sulfatide [1, 2]. MLD is inherited as an autosomal recessive trait [1]. The accumulation of sulfatide leads to demyelination in the central and peripheral nervous systems, resulting in a variety of neurological symptoms [1].

The worldwide prevalence of MLD is very low (1:40,000 live births), and its prevalence among Koreans is unknown [3]. MLD is usually subdivided into 3 clinical subtypes, on the basis of the age at onset: a late-infantile form (50-

Received : April 19, 2010 Manuscript No : KJLM10-070
Revision received : July 13, 2010
Accepted : August 17, 2010
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ISSN 1598-6535

60% of cases), in which the first symptoms develop at around 2 yr of age; a juvenile form (20-30% of cases), in which patients present with the symptoms between 3 and 16 yr of age; and an adult-onset form (15-20% of cases), with manifestations appearing after 16 yr of age [4].

The late-infantile form is the most common type of MLD. Onset is characterized by ataxia and loss of acquired motor skills. Weakness and hypotonia are present in most patients, and disease progression is accompanied by mental deterioration until patients are no longer able to establish any contact with their surroundings. This form of MLD has a short course, and death generally occurs within 7 yr after onset [5].

Some Korean cases of MLD have been reported, including 2 cases of the late-infantile form (one in a 2-yr-old girl, and another in a 4-yr-old girl), which showed typically clinical, neurologic, pathologic, and laboratory findings [6-8].

The human *ARSA* gene maps to the long arm of chromosome 22, in the region of q13, and contains 8 exons that encode a 1521-nucleotide-long mRNA [3, 9]. More than 110 different mutations causing MLD have been identified in the *ARSA* gene in patients with various ethnic backgrounds worldwide [4]. However, thus far, no case of genetically confirmed MLD has been reported in Korea. Here, we describe a Korean case of late-infantile MLD, with a novel *ARSA* splicing mutation as a causative factor.

CASE REPORT

A Korean male infant was one of the 2 children born to non-consanguineous parents. Pregnancy and labor progressed without complications. At the age of 3 months, the patient was tested for ARSA because of a family history of MLD. The patient's fibroblast ARSA level was 0.082 nmol/min/mg protein (reference value: 0.5-1.5 nmol/min/mg protein), which was consistent with infantile-onset MLD. He showed normal development until 12 months but was never able to walk independently. At 17 months, he developed spastic lower limbs. At 20 months, he was no longer able to sit, and by 2 yr, he was unable to turn his face down or grasp items. At 2 yr of age, he received allograft bone marrow transplantation (BMT). Although his ARSA level slightly increased at 1 month after the transplantation, it never reached normal values. At the age of 3 yr, he developed seizures.

After being in a bed-ridden state due to progressive quadriparesis with spastic and flaccid characteristics, the patient died at the age of 9 yr.

Brain magnetic resonance imaging showed bilateral, diffuse hyperintensities in the periventricular white matter and cerebral atrophy, consistent with a demyelinating disease (Fig. 1).

For 3 generations, no definite MLD history was established in the patient's family, excluding his elder brother. The patient's elder brother was tested for ARSA at the age of 23 months because of generalized weakness and spasticity of the lower limbs. His enzyme level was 0.049 nmol/min/mg protein (reference value: 0.5-1.5 nmol/min/mg protein), and he was diagnosed with late-infantile MLD. He eventually reached a vegetative state and died at the age of 5 yr.

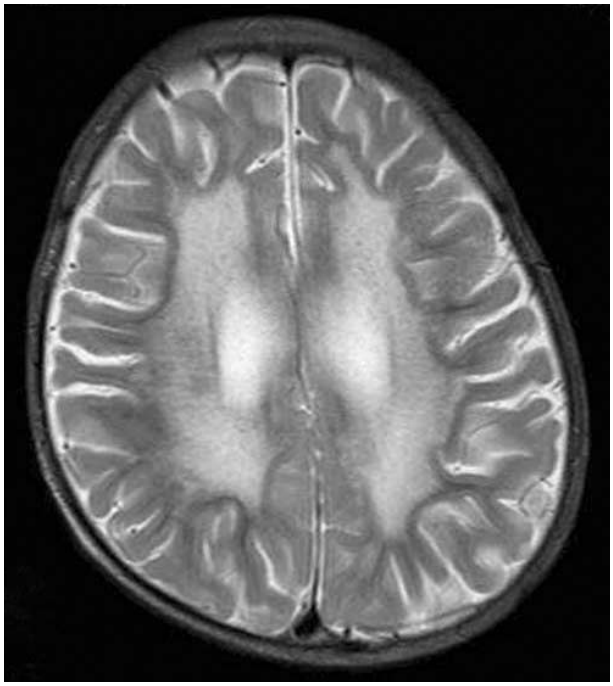

Fig. 1. Brain magnetic resonance image. Axial T2-weighted image shows bilateral, diffuse confluent hyperintensities in the periventricular white matter and cerebral atrophy.

After obtaining informed consent from the parents, we obtained blood samples from the patient and his parents. The genomic DNA was isolated from peripheral blood leukocytes using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA), according to manufacturer's instruction. All the coding exons and the flanking intronic regions of the *ARSA* gene were amplified using primer sets designed by the authors (sequences available upon request). PCR was performed using a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA). Direct sequencing was performed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the Big-Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). Potential mutations were defined by exclusion from the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk) and the previously reported mutations on PubMed (http://www.ncbi.nlm.nih.gov/PubMed/). All novel mutations were confirmed by sequencing 100 control chromosomes.

Direct sequencing of the *ARSA* gene revealed a novel heterozygous mutation affecting the consensus splicing donor site in intron 6 (c.1101+1G>T) and a heterozygous mutation altering the coding sequence of exon 2 (c.296G>A; Gly99Asp) (Fig. 2). Screening of the *ARSA* gene in 50 normal control subjects revealed no mutant alleles in 100 screened chromosomes. Further genetic analysis of the patient's parents showed that the *ARSA* genes of his father and mother had c.296G>A and c.1101+1G>T, respectively. The result of *ARSA* gene analysis of his parents suggested an autosomal recessive inheritance pattern.

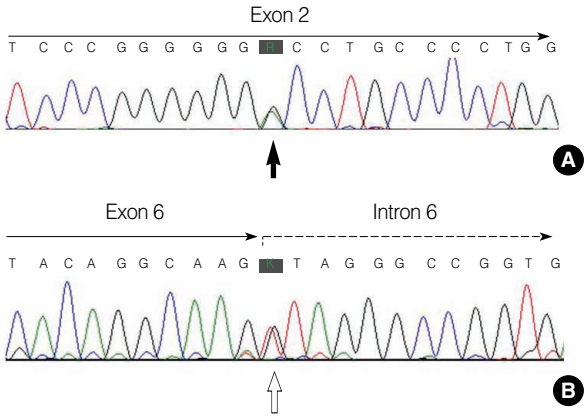

Fig. 2. Mutation analysis of the arylsulfatase A (*ARSA*) gene in a Korean patient with metachromatic leukodystrophy. Direct sequencing of the *ARSA* gene shows overlapped peaks (solid arrow) at the nucleotide position 296 due to a heterozygous G-to-A transition (c.296G>A; Gly99Asp) (A) and overlapped peaks (open arrow) at the consensus splicing donor site in the intron 6 due to a heterozygous G-to-T transversion (c.1101+1G>T) (B).

The genomic sequence spanning an interval of 50 nucleotides up- and downstream of the mutation at the splicing site in intron 6 was evaluated by information analysis of the splice donor and acceptor sites [10]. The individual information content of each natural and variant primary and secondary splice site was analyzed by measuring their Ri values expressed in bits of information using the Scan program (from the Laboratory of Computational and Experimental Biology at the National Institutes of Health). The effect of nucleotide substitution on splicing information was predicted on the basis of the Ri values.

The c.1101+1G>T variation weakened the natural splicing donor site of intron 6 [initial information content score (Ri), 8.6 bit; final Ri, 0.8 bit] and strengthened a site 5 nucleotides downstream, a preexisting cryptic donor site of exon 6 (4.7 bit).

DISCUSSION

MLD is a lysosomal storage disease caused by a deficiency in ARSA. It is characterized by the accumulation of sulfatide-the ARSA substrate-in the white matter of the central nervous system and in the peripheral nerves [11]. A pathological hallmark of the disease is demyelination, causing various ultimately lethal neurological symptoms [4].

Patients homozygous for alleles that do not allow synthesis of functional ARSA always develop the most severe late-infantile form of the disease, whereas those homozygous for alleles allowing the expression of residual enzyme activity are associated with the later-onset juvenile or adult forms [4, 12, 13]. In this study, we report a compound heterozygous mutation in the *ARSA* gene in a patient with late-infantile onset MLD, with lethal clini-

cal characteristics. An amino acid substitution of Asp for 99Gly resulted from a G-to-A transition in exon 2. Because the substitution was from a neutral amino acid to an acidic one, the structure of the ARSA protein was thought to be possibly altered. In fact, a transient expression study by Kondo et al. [11] confirmed that such a mutation abolishes ARSA activity.

In addition, a mutation affecting the donor consensus splice site of intron 6, which was identified in this study, is a novel alteration in this gene. The most common *ARSA* mutations in patients with MLD differ depending upon ethnicity. Four mutations, including c.459+1G>A, p.Pro426Leu, c.1204+1G>A, and p.Ile179Ser, are common in European populations, whereas 3 other mutations, p.Gly99Asp, p.Gly245Arg, and p.Thr409Ile, are common in Japanese populations [14]. These mutations are thought to have spread by the founder effect.

Splice-site mutations involving introns 1-7, but not intron 6, have already been identified. The c.1101+1G>T mutation identified in this study results in an altered splice-recognition sequence between exon 6 and the subsequent intron 6, which may be responsible for the diminished function of ARSA. Thus, the combination of the 2 mutations found in the present case led to the most severe late-onset form of MLD.

Because ethnic differences exist in the type and frequency of mutations in the *ARSA* gene, novel genetic changes may be identified in the Korean population. In the present case, while disease progression was slowed to some extent after BMT, the patient died at the age of 9 yr. MLD cannot be treated effectively by BMT if neuropsychologic and/or neurologic signs are advanced, or if MLD is in its late-infantile form [15, 16]. In this study, BMT was performed after the onset of symptoms. Best outcomes are noted in MLD patients who undergo BMT before the symptoms occur, slowing disease progression, although BMT does not seem to alleviate peripheral nervous system manifestations [14]. Therefore, identification of the disease-causing mutation can enable establishing a genotypic diagnosis of MLD, providing important information to both families and physicians.

This study identified a novel splicing mutation of the *ARSA* gene that was found to be related to late-infantile onset MLD with a lethal clinical course. Molecular diagnosis may be beneficial in the early diagnosis of MLD patients and for deciding intervention measures for their family members.

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