

Original Article

A novel ARSA gene mutation c.302delG in a Chinese patient with metachromatic leukodystrophy

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Abstract: Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder mainly caused by the arylsulfatase A (ARSA) gene mutations, which results in ARSA activity deficient to accumulate sulfatide in the oligodendrocytes and in the Schwann cells. On the basis of the age of onset, MLD is characterized by three clinical subtypes: late infantile, juvenile, and adult. In this manuscript we report a novel ARSA gene mutation c.302delG in a Chinese late infantile form MLD patient. The frameshift mutation c.302delG changes translated amino acid sequence and creates a premature stop codon in exon 2 at residue 107 (G101Afs*7) according to Mutation Taster Database analysis. Moreover, the mutation c.302delG also damages the protein structure in comparison to that of wild type ARSA protein through SWISS-MODEL. Combined with the patient's typical late infantile presentation, we speculate it may be the cause of MLD.

Keywords: Metachromatic leukodystrophy, arylsulfatase A, mutation, Chinese

Introduction

Metachromatic leukodystrophy (MLD; OMIM 250100) is a rare lysosomal storage disease caused by the deficiency of arylsulfatase A (ARSA) or sphingolipid activator protein B (SAP-B) activity [1]. It will result in accumulation of sulfatide in the oligodendrocytes and Schwann cell and cause progressive demyelination. The incidence of MLD regarded as "orphan disease" is 1/40000~1/120000 live births [1]. According to the age of onset, MLD is subdivided into three forms: late infantile, juvenile, and adult forms [2]. The late infantile form is the most common subtype of MLD, accounting for 50%~60% of cases [2, 3], in which the clinical symptoms are severest [4]. Patients with the late infantile MLD present between one and three years of age [1, 2]. Difficulty walking and hypotonia are the most common clinical symptoms.

The low ARSA enzymatic activity and clinical symptoms are the main diagnostic basis of MLD. However, low ARSA activity could occur in six conditions, including: 1) MLD, 2) ARSA pseu-

dodeficiency, 3) saposin B deficiency, 4) multiple sulfatase deficiency, 5) compound heterozygosity for a null and pseudodeficiency alleles of the ARSA gene (without white matter disease), 6) the 22q13 deletion syndrome [5]. The ARSA pseudodeficiency (PD) allele is frequently found to decrease ARSA enzymatic activity. And the majority of pseudodeficiency alleles identified are Asn350Ser and c.2723A>G [6]. So direct DNA sequencing is helpful for us to make a definitive diagnosis of MLD. Up to date, more than 150 ARSA gene mutations causing MLD have been reported [2]. The ethnicity is a major factor of the most common ARSA mutations in patients with MLD. Three ARSA gene mutations, including -c.459+1G>A, p.P426L, and p.I179S, are common in Poland populations, whereas three other mutations, p.Gly245Arg, p.Gly99-Asp, and p.Thr409Ile, are responsible for MLD patients in Japanese [7, 8]. However, few cases confirmed MLD by mutation analysis of the ARSA gene have been reported in China.

In this study, we report a novel ARSA gene mutation c.302delG in a Chinese late infantile form metachromatic leukodystrophy patient by using

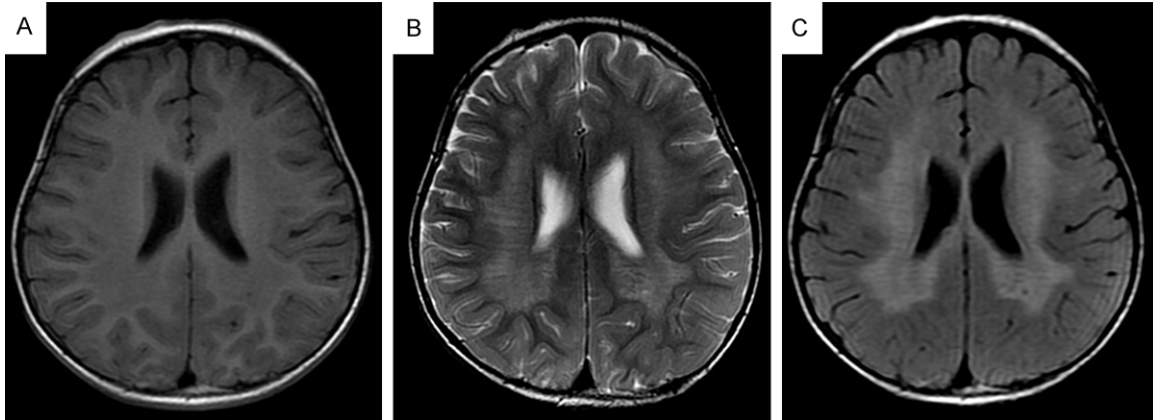


Figure 1. The brain MRI performed when the patient was at the age of 32 months. Tigroid pattern of white matter involvement in the bilateral periventricular showed low signal intensity in FSE/T1-weighted image (A), but confluent hyperintense lesions in FSE/T2-weighted image (B) and T2-FLAIR image (C).

PCR and direct DNA sequencing to extend the knowledge about molecular basis of MLD.

Materials and methods

Patient

The patient is a boy, born as the second child to young, nonconsanguineous patients. After birth, his growth was normal, but his psychomotor development was slightly delayed. He gained head control, sitting, and standing at the age of 6, 9, 14 months, respectively. He started to speak some simple words at the age of 13 months old. But he couldn't walk until the age of two years.

He was admitted to referring hospital and was misdiagnosed as cerebral palsy. However, the treatment efficacy was poor and clinical symptoms progressively deteriorated. He was hospitalized in our hospital at 2 years and 8 months of age. He presented with language impairment, lower limbs stiffness, deterioration of mental function and foot thumbs spontaneity dorsiflexion. And he presented the bilateral Babinski sign. Moreover, he developed generalized seizures during hospitalization and seizures frequency decreased after treatment for levetiracetam. The neuron-specific enolase was significantly higher. Genetic metabolism examination showed blood carnitine deficiency. The cerebrospinal fluid pressure was 150 mmH₂O, in which protein content was high and cell count was normal. The activity of arylsulfatase A in blood leukocytes was 0.5 nmol/mg/h

(normal range 3.7~28.2 nmol/mg/h). The electroencephalogram showed more high amplitude 4~4.5θ activities and spikes in the forehead. The MR imaging detected abnormal signal in the bilateral periventricular and in the corpus callosum (**Figure 1**). The tigroid pattern of white matter involvement was highly suggestive of metachromatic leukodystrophy.

No other family members had clinical symptoms as the same as the patient's. The mother and brother were healthy, but the mother's arylsulfatase A activity showed slightly low. The father was recently diagnosed with acute lymphoma and undergoing chemotherapy. He failed to detect arylsulfatase A activity and ARSA gene.

Determination of ASA activity

The activity of arylsulfatase A was determined in leukocyte according to the method of Bognar et al [9] and Chang et al [10]. Determination of the protein content in leukocyte homogenates was detected by using the Bradford assay with bovine serum albumin as the standard.

Mutation screening

Genomic DNA was prepared from venous blood of the patient's family members but not the father by using the extraction kit (QIAGEN) according to the manufacturer's protocol. Using 2XPCR MasterMix polymerase (TIANGEN), the eight ARSA gene exons were amplified by PCR. PCR products were sequenced on an ABI PRISM

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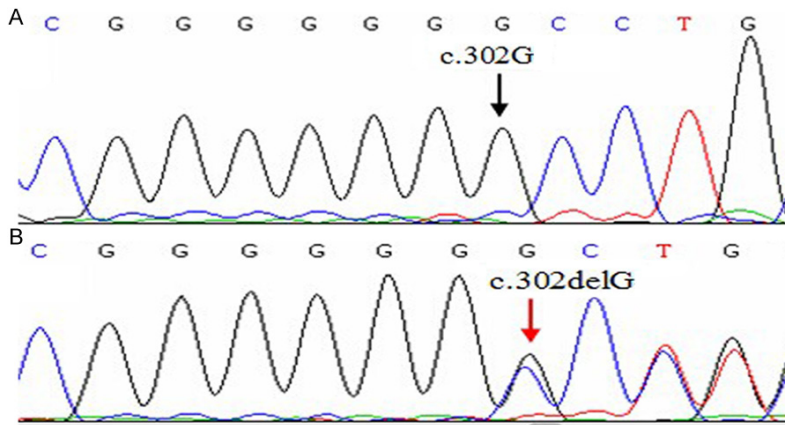


Figure 2. The novel frameshift mutation c.302delG found in the patient. A. Normal sequence around c.302delG in exon 2. B. c.302delG frameshift mutation sequenced using sense primer. Black arrow, normal sequence; red arrow, abnormal sequence.

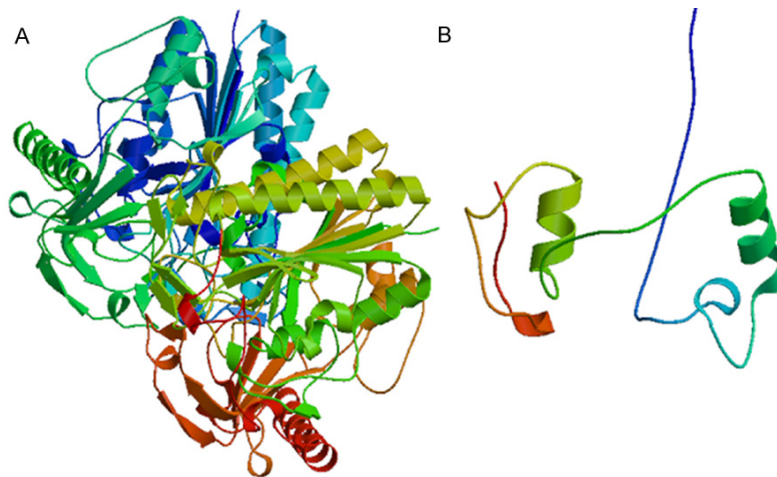


Figure 3. The protein tertiary structure of normal ARSA protein (A) and mutant ARSA protein (B) according the SWISS-MODEL analysis.

3500xl Genetic Analyzer (American Life Technology Company).

Result

We investigated ARSA gene mutations of this family member except the father by direct screening. Sequence analysis demonstrated two mutations in exon 2 of ARSA gene in the patient, including a known heterozygous missense mutation c.293C>T which was also found in his mother and a novel frameshift mutations c.302delG (Figure 2). None of them was also found in the patient's brother and 200 healthy children, indicating them being not polymorphisms. Further, we found that the frameshift

mutation c.302delG resulted in a frame shift beginning at the amino acid position 101 and creating a premature stop codon at residue 107 (G101Afs*7), which is predicted to impact the protein function according to Mutation Taster Database analysis (<http://www.mutationtaster.org/>) and SWISS-MODEL (<http://www.swissmodel.expasy.org/>) (Figure 3).

Discussion

Metachromatic leukodystrophy (MLD) is an autosomal recessive genetic disease caused by lack of ASA activity, resulting in sphingolipid cerebroside-3-sulfate (sulfatide) hardly being hydrolyzed to deposit in oligodendrocytes and Schwann cells [11]. The characteristic pathological changes of MLD are that brownish or red square metachromatic substances stained by toluidine blue or cresyl violet are found in oligodendrocytes and Schwann cells. According to age of onset, MLD is characterized by three clinical types: late infantile, juvenile and adult form MLD. The late infantile MLD is the most common form and its clinical symptoms are more severe. The late infantile MLD patients may manifest difficult walking, knee hyperextension, mental retardation, irritability, hypotonia as the first clinical signs. As it progresses, patients develop muscle atrophy, spastic paralysis, generalized tonic-clonic seizures, nystagmus, optic atrophy, and aphasia [1]. The late infantile MLD patients usually die within the first decade as no causal therapy is yet available [12-14]. In this study, the patient presented psychomotor retardation after birth as the first clinical symptom, and then progressively deteriorated to difficulty with speech, dyskinesia, spastic paralysis and generalized

tonic-clonic seizures. And brain MRI showed typical white matter demyelination change and leukocyte ASA activity was significantly deficient. These typical clinical symptoms were consistent with the diagnosis of late infantile MLD.

In this study, we have identified a heterozygous missense mutation c.293C>T of ARSA gene in the patient and the mother by PCR direct sequencing. The missense mutation c.293C>T had been found by Gieselmann et al in 1991 [15], which leads to replacement of a Ser residue by a Phe residue at position 98. The mutation was identified to signify the arylsulfatase A pseudodeficiency allele, meaning that it is associated with low arylsulfatase A activity but does not cause MLD. So the phenotype of the mother is normal and leukocyte ASA activity is slightly low, which is consistent with previous findings. Further, we also found a novel frameshift mutation c.302delG in exon 2 in the patient. Up to date, the frameshift mutation c.302delG has not been reported. According to Mutation Taster database analysis and SWISS-MODEL, the frameshift mutation c.302delG changes translated amino acid sequence, which accounts for the first 100 amino acids normal and the following amino acid sequence aberrant. Additionally, the mutation creates a premature stop codon in exon 2 at residue 107 (G101Afs*7). Moreover, the structure of the mutant protein has been damaged in a largely extent. So c.302delG frameshift mutation has a possibility of radically altering the structure of the ARSA protein that may cause arylsulfatase A deficiency. Combined with the patient's typical late infantile form MLD presentation, we confirm that the frameshift mutation c.302delG may be responsible for the late infantile form MLD. As this mutation was not found in the proband's mother or brother, it may derived from his father. But it cannot be confirmed in this study due to a lack of DNA sample of his father. It's interesting to note that there are two known mutations have been reported on the same nucleotides, resulting in a different amino acid substitution c.302G>T (p.Gly101Val) [16] and c.302G>A (p.Gly101Asp) [17]. The first substitution resulted in changes in the polarity or charge in the residue G101V, indicating that it may be a disease-causing mutation, which is needed to identify by expression study [16].

Kondo et al confirmed that the second mutation c.302G>A was to be the cause of MLD according to the transient expression studies, in which COS cells transfected with the mutant cDNA carrying gly99-to-asp did not show increase of ARSA activity [17].

This study identified a novel frameshift mutation of the ARSA gene that was in relation to late infantile type MLD. On the basis of analysis of Mutation Taster database and SWISS-MODEL, we hypothesize it probably cause the late infantile type MLD. Our current knowledge of the molecular mechanisms of MLD is still not complete; however, this study may make us a better understanding and serve as reference for family counseling.

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Disclosure of conflict of interest

None.

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References

- [1] Lugowska A, Mierzewska H, Bekiesińska-Figatowska M, Szczepanik E, Goszczańska-Ciuchta A and Bednarska-Makaruk M. A homozygote for the c.459+1G>A mutation in the ARSA gene presents with cerebellar ataxia as the only first clinical sign of metachromatic leukodystrophy. *J Neurol Sci* 2014; 338: 214-217.

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- [2] Luzi P, Rafi MA, Rao HZ and Wenger DA. Sixteen novel mutations in the arylsulfatase A gene causing metachromatic leukodystrophy. *Gene* 2013; 530: 323-328.
- [3] Kang DH, Lee DH, Hong YH, Lee ST, Jeon BR, Lee YK, Ki CS and Lee YW. Identification of a novel splicing mutation in the ARSA gene in a patient with late-infantile form of metachromatic leukodystrophy. *Korean J Lab Med* 2010; 30: 516-520.
- [4] Kehrer C, Groeschel S, Kustermann-Kuhn B, Bürger F, Köhler W, Kohlschütter A, Bley A, Steinfeld R, Gieselmann V and Krägeloh-Mann I. Language and cognition in children with metachromatic leukodystrophy: onset and natural course in a nationwide cohort. *Orphanet J Rare Dis* 2014; 9: 18.
- [5] Artigalás O, Paskulin G, Riegel M, Burin M, Saraiva-Pereira ML, Maluf S, Kiss A and Schwartz IV. A patient presenting a 22q13 deletion associated with an apparently balanced translocation t(16;22): An illustrative case in the investigation of patients with low ARSA activity. *Genet Mol Biol* 2012; 35: 424-427.
- [6] Dorboz I, Eymard-Pierre E, Kefi R, Abdelhak S, Miladi N and Boespflug-Tanguy O. Identification of a new Arylsulfatase A (ARSA) gene mutation in Tunisian patients with metachromatic leukodystrophy (MLD). *J Neurol Sci* 2009; 287: 278-280.
- [7] Fluharty AL. Arylsulfatase A Deficiency. 1993.
- [8] Lugowska A, Berger J, Tyłki-Szymańska A, Löschi B, Molzer B, Zobel M and Czartoryska B. Molecular and phenotypic characteristics of metachromatic leukodystrophy patients from Poland. *Clin Genet* 2005; 68: 48-54.
- [9] Bognar SK, Foretic B, Furac I, Vukelic Z and Grubescic Z. Kinetics and activity of arylsulfatase A in leukocytes derived from patients with cerebral palsy. *Acta Pharm* 2006; 56: 95-104.
- [10] Chang PL and Davidson RG. Complementation of arylsulfatase A in somatic hybrids of metachromatic leukodystrophy and multiple sulfatase deficiency disorder fibroblasts. *Proc Natl Acad Sci U S A* 1980; 77: 6166-6170.
- [11] Barboura I, Hadded S, Chebel S, Ben Mansour R, Chahed H, Gueddiche MN, Frih-Ayed M, Ferchichi S and Miled A. Brain MRI and biological diagnosis in five Tunisians MLD patients. *Diagn Pathol* 2012; 7: 11.
- [12] Groeschel S, í Dali C, Clas P, Böhringer J, Duno M, Krarup C, Kehrer C, Wilke M and Krägeloh-Mann I. Cerebral gray and white matter changes and clinical course in metachromatic leukodystrophy. *Neurology* 2012; 79: 1662-1670.
- [13] Kehrer C, Blumenstock G, Gieselmann V and Krageloh-Mann I. The natural course of gross motor deterioration in metachromatic leukodystrophy. *Dev Med Child Neurol* 2011; 53: 850-855.
- [14] Gieselmann V and Krageloh-Mann I. Metachromatic leukodystrophy—an update. *Neuropediatrics* 2010; 41: 1-6.
- [15] Gieselmann V, Fluharty AL, Tonnesen T and Von Figura K. Mutations in the arylsulfatase A pseudodeficiency allele causing metachromatic leukodystrophy. *Am J Hum Genet* 1991; 49: 407-413.
- [16] Gort L, Coll MJ and Chabas 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: 240-248.
- [17] Kondo R, Wakamatsu N, Yoshino H, Fukuhara N, Miyatake T and Tsuji S. Identification of a mutation in the arylsulfatase A gene of a patient with adult-type metachromatic leukodystrophy. *Am J Hum Genet* 1991; 48: 971-978.