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DATA REPORT

A novel *DARS2* mutation in a Japanese patient with leukoencephalopathy with brainstem and spinal cord involvement but no lactate elevation

Keiko Shimojima[1], Takafumi Higashiguchi[2], Kanako Kishimoto[2], Satoko Miyatake[3], Noriko Miyake[3], Jun-ichi Takanashi[4], Naomichi Matsumoto[3] and Toshiyuki Yamamoto[1]

The mitochondrial aspartyl-tRNA synthetase 2 gene (*DARS2*) is responsible for leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL). A Japanese patient with LBSL showed compound heterozygous *DARS2* mutations c.358_359delinsTC (p.Gly120Ser) and c.228-15C>G (splicing error). This provides further evidence that most patients with LBSL show compound heterozygous mutations in *DARS2* in association with a common splicing mutation in the splicing acceptor site of intron 2.

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Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL, MIM#611105) is an autosomal recessive disorder of the brain white matter and clinically characterized by slowly progressive pyramidal, cerebellar, and dorsal column dysfunction involving the legs more than the arms.[1] Distinct radiological findings include heterogeneous cerebral white matter abnormalities accompanied by a selective involvement of the brainstem and spinal cord tracts. Elevated lactate levels in magnetic resonance spectroscopy of the abnormal white matter is also characteristic. It has been reported that the clinical spectrum may vary from early-onset severe disease, which is sometimes even fatal within the first few years, to an adult-onset type.[1] Variable clinical manifestations, including delayed intellectual and/or motor development, cognitive impairment, epilepsy, and peripheral neuropathy, may also occur. Definite diagnosis of LBSL is established by the demonstration of mutations in the mitochondrial aspartyl-tRNA synthetase 2 gene (*DARS2*), which encodes mitochondrial aspartyl-tRNA synthetase, the enzyme that attaches the amino acid aspartate to the correct mitochondrial transfer RNA.[2] Aspartyl-tRNA is necessary in the translation of mitochondrial messenger RNA into protein.[3] The majority of the LBSL cases is due to compound heterozygous *DARS2* mutations.

Here we present a male with LBSL in whom a novel *DARS2* mutation was identified. At present, the patient is 27 years of age. He is the second child of healthy and non-consanguineous Japanese parents after an uneventful pregnancy and delivery. He has two healthy siblings. He started to walk at 1 year and 8 months. It was noted that he easily fell to the ground when he was 3.5 years. At that time, brain computed tomography was performed, and leukoencephalopathy was suggested. From the age of 4 years, he started to require support for walking. A walking aid and a wheelchair were needed from the age of 6 years and 10 years, respectively. Subsequently, nystagmus was noted at 13 years. His motor ability gradually deteriorated and he, at present, can use only his upper limbs. Intellectual ability has also declined. Routine laboratory examination, including metabolic screening and cerebrospinal fluid testing, showed no abnormalities involving lactic and pyruvate acids. Enzymatic analyses for arylsulfatase A and galactocerebrosidase were also normal. Brain magnetic resonance imaging examined at 27 years revealed white matter abnormalities in association with cystic findings (Figures 1a–d). The spinal cord was also involved. These findings were typical for LBSL. Although an elevation of lactate level was not clear by magnetic resonance spectroscopy at the same time (data not shown),[4,5] such a pattern is not unusual. There are some LBSL patients who show normal lactate levels, which might occur due to advanced stage or a late onset of symptoms.[6–8]

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Tokyo Women's Medical University. After obtaining written informed consent, blood samples were obtained from the patient, his mother, and his younger sister. The father's sample was not obtained, because the couple was divorced. Although LBSL was suspected based on the abovementioned findings, whole-exome sequencing was performed. Briefly, DNA samples were captured using the SureSelect Human All Exon Kit v5 (Agilent Technologies, Santa Clara, CA) and sequenced on an Illumina HiSeq 2500 system (Illumina, San Diego, CA) with 101 bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed as previously described.[9] The average read depth of protein-coding regions ranged from 87.29 to 118.99× (mean coverage against coding regions: 118.99× (proband), mother: 87.29× (mother), 87.76× (sister)), and at least 91.2% of target bases were sequenced with 10 or more reads for each sample (96.5% (proband), 91.2% (mother), and 92.5% (sister)). Common single-nucleotide polymorphisms with minor allele frequencies ⩾1% in dbSNP 135 and variants observed in more than five subjects of our in-house exome database ($n=575$ controls) were

[1]Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan; [2]Department of Pediatrics, Japan Community Health Care Organization Osaka Hospital, Osaka, Japan; [3]Department of Genetics, Yokohama City University, Yokohama, Japan and [4]Department of Pediatrics, Tokyo Women's Medical University Yachiyo Medical Center, Yachiyo, Japan.
Correspondence: T Yamamoto (yamamoto.toshiyuki@twmu.ac.jp)
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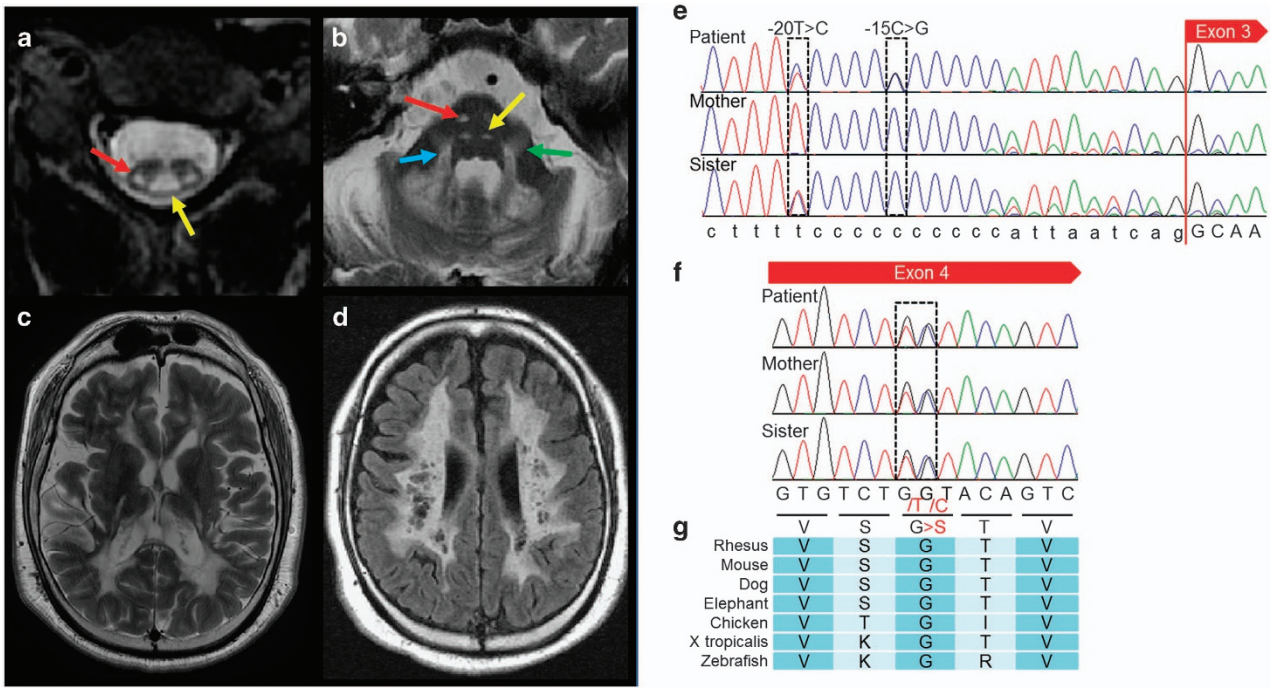

Figure 1. Radiological findings and results of molecular analysis. (**a**–**d**) Axial magnetic resonance images examined at 27 years. T2-weighted axial images (**a**–**c**) and a fluid-attenuated inversion recovery image (**d**). (**a**) In the spinal cord, the posterior columns (yellow) and the lateral corticospinal tracts (red) are involved. (**b**) At the level of the pons, high signal intensity is shown in bilateral lesions in the pyramidal tracts (red), medial lemniscus (yellow), superior cerebellar peduncles (blue), and intraparenchymal trajectory of the trigeminal nerve (green). (**c**) At the level of the basal ganglia, the posterior limb of the internal capsule and periventricular white matter are affected. (**d**) High signal intensity is shown in the periventricular white matter associated with multiple small cysts. (**e**, **f**) Electropherograms of Sanger sequencing for the patient, his mother, and the sister. Although a c.228-20T > C polymorphism is shown in both the patient and his sister (**e**), c.228-15C > G is only present in the patient. c.358_359delinsTC (p.Gly120Ser) is commonly shown in all samples (**f**), and the affected amino acid is conserved among species (**g**).

filtered out. We evaluated the remaining variants using SIFT (http://sift.jcvi.org/) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) prediction software. Particular attention was given to mutations found in genes known to cause neurodegenerative diseases when mutated. The samples from the mother and the sister were used for filtering. Finally, two heterozygous mutations in *DARS2*, NM_018122.4: c.228-15C > G and c.358_359delinsTC (p.Gly120Ser) remained, and mutations in the other candidate genes for leukoencephalopathy were ruled out. p.Gly120Ser was detected in the mother and healthy sister (Figures 1e and f). Although p.Gly120Ser has never been reported previously (Supplementary Table S1), the affected codon is conserved among species (Figure 1g) and prediction scores including SIFT and Polyphen-2 suggested that this mutation is 'damaging.' Therefore, we concluded that this mutation is pathogenic. On the other hand, the other mutation, c.228-15C > G, has been reported previously as a pathogenic splicing mutation that causes predisposition to p.R76SfsX5. As this splicing mutation was not detected in the mother and healthy sister, this was suspected to be inherited from his father or to be a *de novo* mutation. From these findings, we concluded that LBSL features in this patient are derived from compound heterozygous mutations in *DARS2*.

In 2007, Scheper *et al.*[2] reported that mutations in the *DARS2* gene are responsible for this disease. Since then, more than 50 types of *DARS2* mutations have been identified.[3,4,6,8,10–19] It is striking that most patients have been reported to have compound heterozygous mutations in *DARS2* and that one of the mutations is often located on the splicing acceptor site in intron 2 (the region neighboring exon 3).[2,3] Because the patient showed the same pattern, this report suggests additional evidence supporting this finding.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.1726 and http://dx.doi.org/10.6084/m9.figshare.hgv.1729.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv)