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1 **Title:** The First Japanese Case of Leukodystrophy with Ovarian Failure Arising from Novel
2 Compound Heterozygous AARS2 Mutations.

3
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24
25 **Running title:** The First Japanese Case of AARS2 Mutation

26
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1 **Abstract**

2 Even now, only a portion of leukodystrophy patients are correctly diagnosed, though
3 various causative genes have been identified. In the present report, we describe a case of
4 adult-onset leukodystrophy in a woman with ovarian failure. By whole exome sequencing, a
5 compound heterozygous mutation consisting of NM_020745.3 (AARS2_v001):c.1145C>A and
6 NM_020745.3 (AARS2_v001):c.2255+1G>A was identified. Neither of the mutations has been
7 previously reported, and this is the first report of *AARS2* mutation in Asia. We anticipate that
8 further studies of the molecular basis of leukodystrophy will provide insight into its
9 pathogenesis and hopefully lead to sophisticated diagnostic and treatment strategies.

10 **Key words:** *AARS2*/compound heterozygous mutation/leukodystrophy/ovarian failure

11

1 **Introduction**

2 Leukodystrophy is a syndrome with progressive white matter degeneration. Its pathogenic
3 background and clinical presentation are variable. Genetic rather than environmental factors are
4 associated with its variability.¹ More than a hundred subtypes of leukodystrophy have been
5 reported, and many causative genes have been identified.² However, the heterogeneity and
6 complexity of leukodystrophy make a definitive diagnosis difficult. Indeed, only half of
7 leukodystrophy patients receive a specific diagnosis.³

8 Here, we report a Japanese woman presenting with adult-onset leukodystrophy and ovarian
9 failure in her thirties. By whole exome sequencing (WES), we detected novel compound
10 heterozygous mutations in alanyl-transfer RNA (tRNA) synthetase 2 (*AARS2*) (OMIM
11 *612035).

12

13 **Case report**

14 A 31-year-old Japanese woman first visited our hospital in 2010. She was born healthy
15 with a normal delivery to non-consanguineous parents. She developed normally and graduated
16 from college. She worked until she was 30 years old, when she suddenly quit her job and had
17 reduced interactions with her surroundings. She developed cognitive decline and began
18 abnormal behaviors (e.g., buying the same thing on consecutive days). She had difficulty using

1 her left limbs, and she tended to lean to the left while sitting. She became totally incontinent. No
2 similar symptoms occurred in her parents or her older sister.

3 A general examination at age 33 revealed secondary amenorrhea, although she had normal
4 menstrual cycles until then with normal secondary sexual characteristics. The unelevated
5 gonadotropin level indicated the impairment of reactive gonadotropin release by hypothalamus
6 and pituitary, suggesting the secondary ovarian failure. She also had generalized atopic
7 dermatitis.

8 Neurological examination revealed significant decline in frontal lobe function. The patient
9 had a Mini-Mental State Examination score of 19/30 and a Frontal Assessment Battery score of
10 4/18. Left hand ideomotor apraxia, hyperreflexia, and rigidity of neck and limbs were observed
11 more on the left side. At the most recent examination at age 38, the patient was aphasic and
12 almost bedridden.

13 Brain magnetic resonance imaging (MRI) showed diffuse cerebral white matter
14 abnormalities without gadolinium enhancement. Patchy, diffusion-restricted areas were present
15 in the abnormal white matter. Thinning of corpus callosum was also observed (Figure 1).

16 Cardiac ultrasound and electrocardiography showed no abnormalities.

17 Laboratory workups including plasma/cerebrospinal fluid lactate, serum arylsulfatase A,
18 galactocerebrosidase, very long chain fatty acids, and cholestanol were within normal ranges.

1 Gene analyses of colony stimulating factor 1 receptor (*CSF1R*) and eukaryotic translation
2 initiation factor 2B (*EIF2B*) were normal. We performed WES on genomic DNA.

3

4 **Genetic analysis**

5 Genetic analysis by WES was approved by the Tokyo Women's Medical University Ethical
6 Committee, and written informed consent was obtained from the patient's parents. DNA
7 samples from the patient and her parents were analyzed by WES, as previously described.⁴
8 Detailed methods of WES and prioritization of variants are described in the supplementary
9 information.

10 We focused on *de novo* and recessive mutations. We found novel compound heterozygous
11 mutations in AARS2, NM_020745.3 (AARS2_v001):c.1145C>A and NM_020745.3
12 (AARS2_v001):c.2255+1G>A. These were the missense mutation (p.T382K) and the splice-site
13 mutation, respectively. These two mutations were not registered in the dbSNP137, NHLBI-ESP
14 6500, HGVD, or our in-house 575 Japanese control exome databases. Sanger sequencing
15 confirmed that the mutations were transmitted maternally and paternally, respectively (Figure 2),
16 and the *in silico* analysis for the pathogenicities is described in the supplementary information.

17 Next, we performed messenger RNA (mRNA) analysis around the segments harboring
18 each mutation (Figure 3a). We could not detect any aberrantly-spliced mRNA caused by the

1 splice-site mutation (Figure 3b). Direct sequence analysis of cDNA revealed that the patient was
2 heterozygous at the locus for c.1145, and the sequence around c.2255 was normal without any
3 aberrant insertion or truncation (Figure 3c).

4

5 **Discussion**

6 The causative gene in a Japanese leukodystrophy patient with ovarian failure was identified
7 as a compound heterozygous *AARS2* mutation.

8 We initially considered the patient's diagnosis as hereditary diffuse leukoencephalopathy
9 with axonal spheroids, which is most prevalent in women in their 30s and 40s and frequently
10 includes corpus callosum thinning.⁵⁻⁷ We also suspected vanishing white matter disease, which
11 includes ovarian failure as one of its features.⁸⁻¹¹ However, no mutation in causative genes for
12 these diseases were identified.

13 Therefore, we performed a comprehensive genetic workup and, by use of WES, compound
14 heterozygous mutations, c.1145C>A (p.T382K) and c.2255+1G>A, were detected. These
15 mutations were inherited as an autosomal recessive trait. Both mutations were novel and
16 predicted as pathogenic *in silico*. In the analysis of the patient's mRNA, we could not detect any
17 misspliced mature mRNA caused by the splice-site mutation, c.2255+1G>A. However, it is
18 predicted that the mutation introduced a premature stop codon when the splicing at the

1 exon16-intron16 boundary failed (Figure 3a). Then this transcript would be a target for the
2 nonsense-mediated mRNA decay and should be degraded.

3 *AARS2* encodes mitochondrial alanyl-tRNA synthetase, an enzyme that charges a specific
4 tRNA with its cognate amino acid, alanine. *AARS2* mutation was recently shown to cause
5 mitochondrial dysfunction and reported in 6 patients with adult-onset leukodystrophy¹². In that
6 report, two specific clinical phenotypes were described: characteristic MRI features and ovarian
7 failure. Common MRI features are striking white matter tract involvement and presence of spots
8 of restricted diffusion in the cerebral white matter. Corpus callosum thinning and the strip of
9 signal abnormality in the splenium were also seen. Ovarian failure was present in all of the
10 previously reported 5 female cases; 1 case had secondary amenorrhea like the present patient,
11 and 4 cases had primary amenorrhea.

12 *AARS2* mutations have another subtype, severe infantile cardiomyopathy with early fatal
13 outcome.¹³⁻¹⁵ In contrast to this infantile subtype, the later-onset subtype, which includes the
14 present case, has no signs of cardiomyopathy. Therefore, the question arises why *AARS2*
15 mutations cause two very different subtypes with dissimilar tissue involvement. In 2015, Euro et
16 al. gave the elegant explanation for the phenomenon.¹⁶ *AARS2* is unique among the human
17 mitochondrial aminoacyl-tRNA synthetases because it contains an editing domain for
18 deacylating mischarged tRNAs in addition to the aminoacylation domain. All patients with

1 infantile-onset cardiomyopathy had the c.1774C>T (p.R592W) mutation located in the editing
2 domain in at least one allele, which severely compromises aminoacylation. On the other hand,
3 none of the missense mutations in adult-onset leukodystrophy patients were in the editing
4 domain, while at least one missense mutation was in the aminoacylation domain, leading to
5 relatively partial reduction in aminoacylation activity. The location of the compound
6 heterozygous mutations in the present case is consistent with the theory.

7 In conclusion, we describe a Japanese woman with novel compound heterozygous
8 mutations in *AARS2*, the first report of leukodystrophy caused by *AARS2* mutations in Asia.
9 This is also the subsequent report after the first description in 2014 for 6 adult-onset
10 leukodystrophy patients by *AARS2* mutations. All reported cases including the present case are
11 apparently sporadic without family history and caused by compound heterozygous mutations,
12 making the correct diagnosis very difficult. Therefore, if you will notice the characteristic MRI
13 findings and, especially in female, the existence of ovarian failure, you should examine the
14 *AARS2* gene.

15 Further identification of disease-causing mutations with detailed clinical descriptions
16 including MRI and menstrual history will provide diagnostic clues, and will contribute to
17 unraveling the disease mechanism, and hopefully lead to the development of treatment
18 strategies.

1

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12

13 **Conflict of Interest**

14 The authors declare no conflict of interest.

15

16 Supplementary information accompanies the paper on Journal of Human Genetics website.

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1 **Titles and legends to figures**

2 **Figure 1. Brain MRI of the patient at age 33**

3 The fluid attenuated inversion recovery (FLAIR) images show periventricular white matter
4 abnormalities predominant at the parietal and frontal area, which revealed low signal lesions in
5 the T1-weighted image (arrows in images a, b and c). The diffusion-weighted image shows
6 patchy areas of restricted diffusion in the abnormal white matter, which are confirmed by low
7 signal of the corresponding areas on the apparent diffusion coefficient map (arrows in image d,
8 e and f). The sagittal FLAIR image shows thinned corpus callosum, especially in the splenium
9 (arrow in image g) and affected white matter structures in a tract-like manner (arrow in image h).
10 The MR angiography showed no stenosis of the major vessels (image i).

11

12 **Figure 2. Sanger sequencing of the patient and her parents**

13 The mutations were confirmed by Sanger sequencing. c.1145C>A (p.T382K) was transmitted
14 maternally, and c.2255+1G>A was transmitted paternally.

15

16 **Figure 3. mRNA analysis**

17 (a) Genomic structure of *AARS2* was illustrated. Arrowheads indicate primer pairs. Triplets
18 under bases indicate reading frames and, assuming that the mutation c.2255+1G>A causes

- 1 splicing error, a premature stop codon will appear just afterwards (blue). (b) Image of PCR
- 2 products of cDNA including each mutation (PCR1 and PCR2). (c) Direct sequence analysis of
- 3 patient's cDNA.

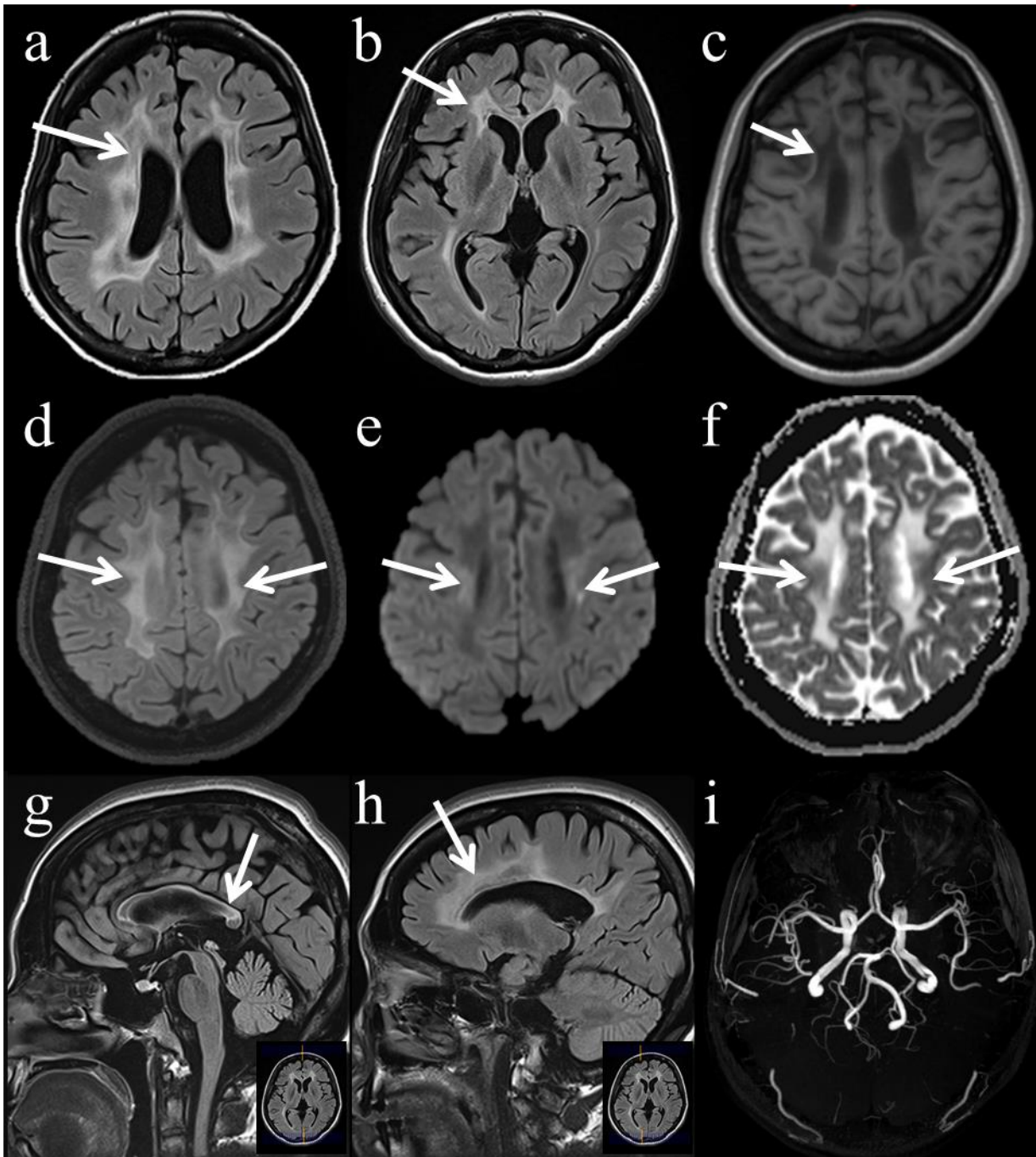
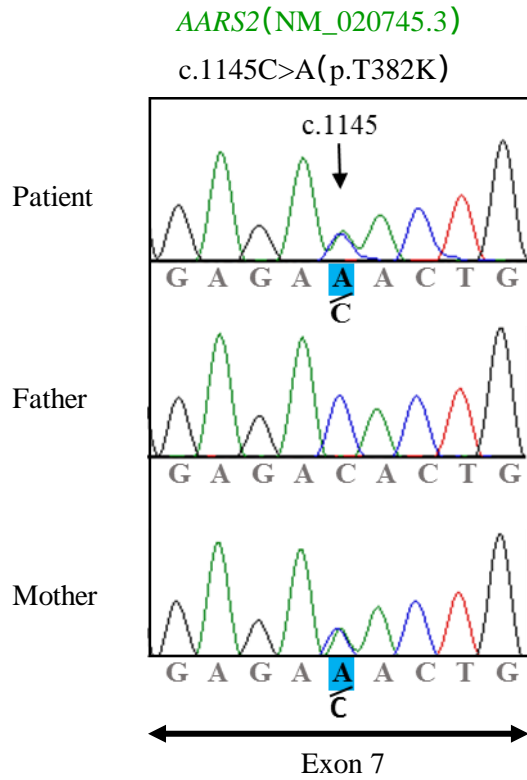
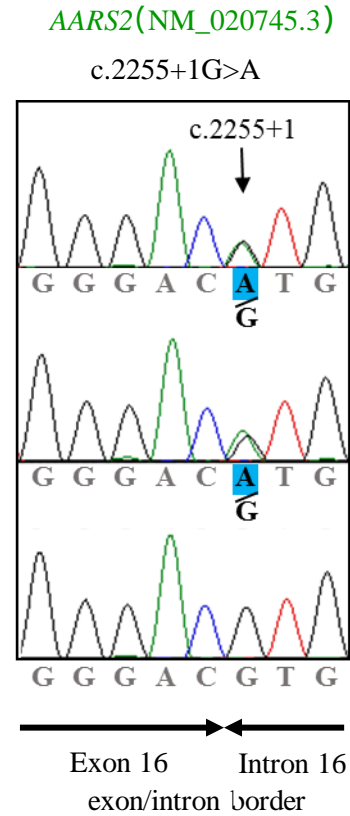


Figure 1



**Maternal
inheritance**



**Paternal
inheritance**

Figure 2

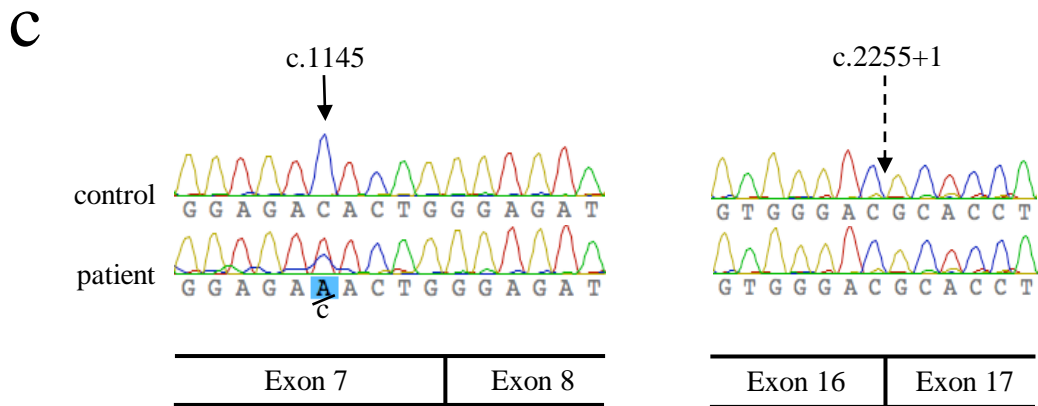
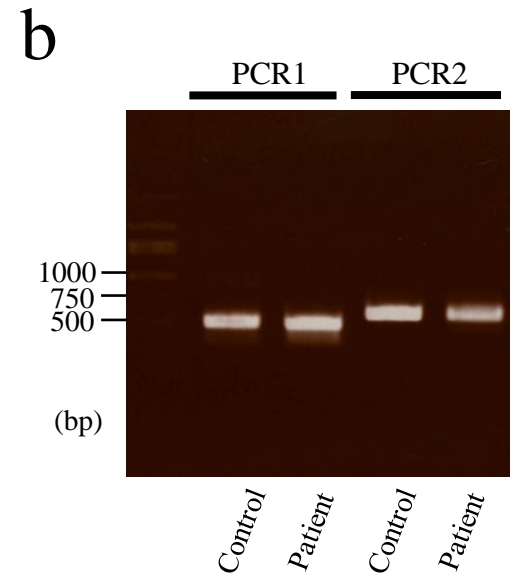
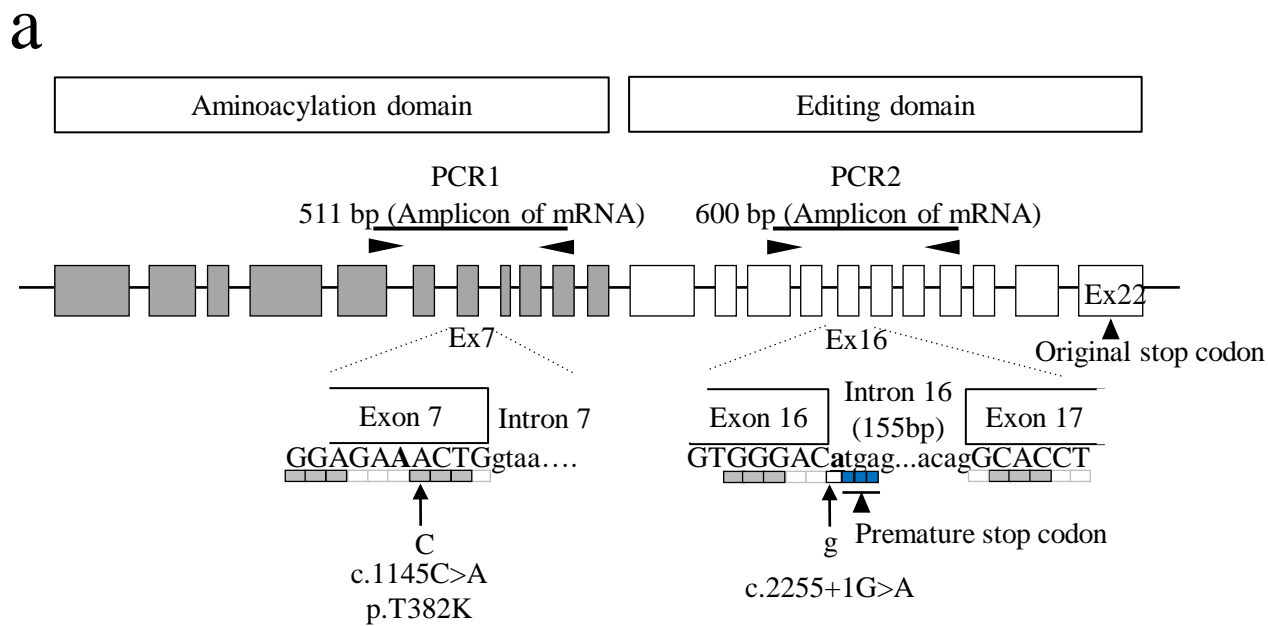


Figure 3

Supplementary information

Whole exome sequencing and *in silico* analysis for the pathogenicities

DNA samples from the patient and her parents were analyzed by whole exome sequencing (WES) as previously described. Genomic DNA was captured by using the SureSelect Human All Exon v5 (50Mb) Kit (Agilent Technologies, Santa Clara, CA). Captured DNA was sequenced on a HiSeq2500 (Illumina, San Diego, CA) with 101 bp paired-end reads and 7 bp index reads. The mean read depth against RefSeq coding sequence (CDS) was 38.18–158.75 reads with 74.6–95.5% of CDS being covered by 20 or more reads. Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software (v1.8) (Illumina). The quality controlled reads were mapped to the human reference genome (UCSC hg19, NCBI build 37.1) by using Novoalign (v3.00.02). After the removal of PCR duplication by using Picard (v1.55), single nucleotide variants (SNVs) and short insertions and deletions (Indels) were identified by using Genome Analysis Toolkit (GATK) (v1.6-5) and annotated by using ANNOVAR (2013 jun).

Out of all variants within exons and regions ± 30 bp from the exon-intron boundaries, those registered common single nucleotide polymorphisms (SNP) with minor allele frequency of $>1\%$ in dbSNP137, and common SNP containing six or more individuals in our in-house database (exome data from 575 Japanese individuals), and synonymous variants were all removed. The variants were confirmed by Sanger sequencing with an ABI PRISM 3500xl or ABI3130xl autosequencer (Life Technologies, Carlsbad, CA).

We focused on *de novo* and recessive mutations, then we found novel compound heterozygous mutations in *AARS2*. The missense mutation (p.T382K) was predicted as deleterious (0.01) by SIFT (<http://sift.jcvi.org/>), possibly damaging (0.712) by Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>). The splice-site mutation (c.2255+1G>A) was predicted as abolishing a donor site by BDGP (http://www.fruitfly.org/seq_tools/splice.html) and NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2/>), and as altering the donor site and most probably affecting splicing (86.07 to 59.23) by Human Splicing Finder (<http://www.umd.be/HSF3/>).

mRNA analysis

Total RNA was extracted from the white blood cells of the patient and a healthy control by using QIAamp RNA Blood Mini and RNase-Free DNase set (QIAGEN). cDNA was synthesized with random primers by use of First-Strand cDNA Synthesis Kit (GE Healthcare). cDNA of *AARS2* was amplified by polymerase chain reaction (PCR) using the primer pairs, 5'-GCCGCCTTTCTGAACTTCTT-3', and 5'-GGCAGCTTCCAGGTCAGTAG-3'. Then, segments harboring c.1145C>A or c.2255+1G>A were amplified by nested-PCR using the primer pairs, 5'-CTGCAAGGCAAACACTCCAC-3' and 5'-CCAGGTCTCCACACAGTGAC-3', 5'-AGGGCTCCCATCTCAATCCT-3' and 5'-GCTGCAGCATCTTCACTGTG-3', respectively (Figure 3a).