Movement Disorders
CLINICAL PRACTICE

CASE SERIES WITH LITERATURE REVIEW

Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation: A Novel *DARS2* Mutation and Intra-Familial Heterogeneity

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ABSTRACT: Background: Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is characterized by slowly progressive spastic gait, cerebellar symptoms, and posterior cord dysfunction. *DARS2*, which encodes mitochondrial aspartyl tRNA synthase, is associated with the rare disease.
Cases: The proband had gait disturbance since age 56, while her younger brother had the gait problem since his 20s and needed cane-assistance at age 45. Both cases showed typical demyelinating features of LBSL on the magnetic resonance imaging (MRI) involving the periventricular white matter, brainstem, cerebellum and spinal cord. Sequencing of both cases showed compound heterozygous mutations: c.228-16C>A and c.508C>T in *DARS2*. The c.228-16C>A is a common mutation in splicing site of intron 2, which causes alternative splicing defect of exon 3, while the c.508C>T at the exon 6 is novel. Our patients are unique in the relative late onset and the apparent difference in disease progression.
Literature Review: Literatures from PubMed were reviewed. Five families showed intra-familial heterogeneity on age at onset or clinical severity.
Conclusion: We identified a family of LBSL with compound heterozygous mutations, and c.508C>T at the exon 6 is a novel one. Clinical heterogeneity was observed in the family and other literatures. Further research for underlying mechanism is required.

Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL, OMIM #611105) is a rare autosomal recessive disorder, typically characterized by child-onset, slowly progressive lower limb spasticity, cerebellar ataxia, and dysfunction of the posterior cord. It was firstly described in a case series of 8 patients by van der Knaap in the Netherlands in 2002.[1] Their brain MRIs showed abnormal T2 hyperintensities in periventricular and deep cerebral white matter, sparing the temporal lobes and U-fibers. Selective tract involvement includes the pyramidal tract, extending from internal capsule to the brainstem and the lateral corticospinal tract, as well as the sensory system from the posterior columns to medial lemniscus. The cerebellar peduncles are also affected. Elevated lactate in magnetic resonance spectroscopy (MRS) was found in the white matter lesions in most patients.

The causative mutations of LBSL were found at *DARS2* gene on chromosome 1 in 2007 through linkage analysis.[2] *DARS2* encodes mitochondrial aspartyl-tRNA synthetase, which conjugates aspartate to the cognate tRNA in the mitochondria. This enzyme is a kind of mitochondrial aminoacyl-tRNA synthetase (mt-aaRS), and plays an important role in mitochondrial translation machinery. Most *DARS2* mutations of LBSL are compound

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heterozygous, within which a splice mutation in intron 2 is almost invariably found. In this report we described two Taiwanese siblings with mutations, one in intron 2 and the other a novel mutation c.508C>T (p.R170W) in exon 6. Clinical and corresponding radiological heterogeneities were observed.

Case Series

Two of six siblings from a non-consanguineous marriage Taiwanese family were affected. Case 1 and 2 are the second and the fourth siblings. Both were born full term via uneventful spontaneous vaginal delivery. Neither of them is a vegetarian or a recreational drug user.

Case 1

The 57-year-old woman is the index patient. She had no motor developmental milestone delay according to her mother but had poor physical performance when she was a child. At age 55, she complained about unsteady gait, especially when going downstairs, with decreased sense of feedback when stepping on the ground. Occasional urinary incontinence was noticed at age 57. The patient denied any bulbar symptoms or upper limbs involvement.

Neurological examinations (Video 1) showed wide-based, ataxic gait, bilateral dysmetria on finger-nose-finger and knee-shin-heel tests, and mild hypometric saccade. Babinski sign was positive bilaterally, while spasticity was noted only at the right leg and the deep tendon reflex (DTR) decreased at her lower limbs. The muscle power was intact. Romberg's sign was positive and the sensitivity to vibration showed decrease at toes (2 out of 8) and indices (4 out of 8).

Case 2

The case 2 had poor physical performance in his childhood. He was unqualified for obligatory military service in our nation at age 18 and scissoring gait was described by his family. The patient however did not suffer from unsteadiness until age 45, especially in dark or narrow spaces. Falling frequency increased from once per month to once every 3 days at about age 50, and thus he started to use a walker. He also gradually had the problem of standing tall. He did not present to our hospital until age 52.

On examination (Video 2), the patient had pes cavus, severe genu recurvatum, and reduced muscle power in bilateral legs (4/5 in hip flexion, knee flexion and ankle dorsiflexion, and 2/5 in toe extension). Spasticity was noted at lower limbs and Babinski sign as well as Hoffmann sign was bilaterally positive. His DTR increases in upper limbs but decreased at lower limbs. He had prominent hypometric saccade, saccadic pursuit, dysdiadochokinesia, and bilateral dysmetria on finger-nose-finger and knee-shin-heel tests. His gait was wide-based with poor balance. The Romberg's sign was positive and there are also decreased sensitivity to vibration in both upper (6–7 out of 8) and lower limbs (5–6 out of 8).

Both cases have normal vitamin B12, folic acid, thyroid function, and serum lactic acid. Venereal disease research laboratory (VDRL) tests were negative. In case 1 we also examine the tumor markers, adrenal function, tandem mass spectrometry of serum metabolites, and the results were all normal. The cerebrospinal fluid of case 1 showed no pleocytosis, with total protein 54.0 mg/dL, and lactic acid level 2.00 mmol/L (reference range: 1.1–2.4 mmol/L).

Video 1. The video was recorded at age 57 of the case 1, showing cerebellar ataxia, and dysmetria on finger-nose-finger and knee-shin-heel tests.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13281

Video 2. The video was recorded at age 52 of the case 2, showing mixed waddling and cerebellar gait, severe genu recurvatum, dysmetria on finger-nose-finger test, and dysdiadochokinesia.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13281

The median somatosensory evoked potentials (SSEP) revealed bilaterally prolonged peak N9-N13 in both cases, and the tibial SSEP showed absence of evoked potentials in the case 1 and small amplitude with prolonged latencies in the case 2. These results suggested lesions in posterior cord in both cases.

MRI of brain and cervical cord in both cases showed signal abnormalities (T2 hyperintensity and T1 hypointensity) in the periventricular white matter, pyramidal tract (including the internal capsule, pyramids of medulla, and lateral corticospinal tract), dorsal column of spinal cord, medial lemniscus, middle cerebellar peduncle, inferior cerebellar peduncle, cerebellar white matter, anterior spinocerebellar tracts, spinal trigeminal nuclei, and intraparenchymal trigeminal tracts. In the case 1, there are cysts in the left middle cerebellar peduncle and

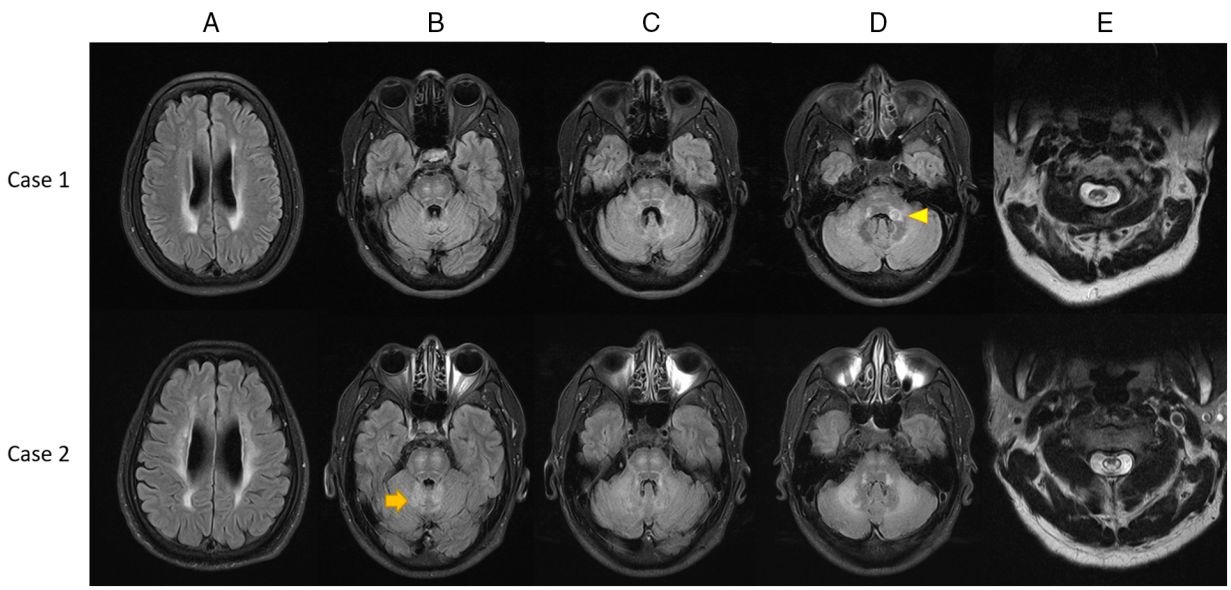

FIG. 1. MRI of brain and cervical cord of case 1 (age 57) and case 2 (age 52). There was more extensive cerebellar white matter involvement in the case 2 (**B**, arrow), and cysts in the middle cerebellar peduncle of the case 1 (**D**, arrowhead).

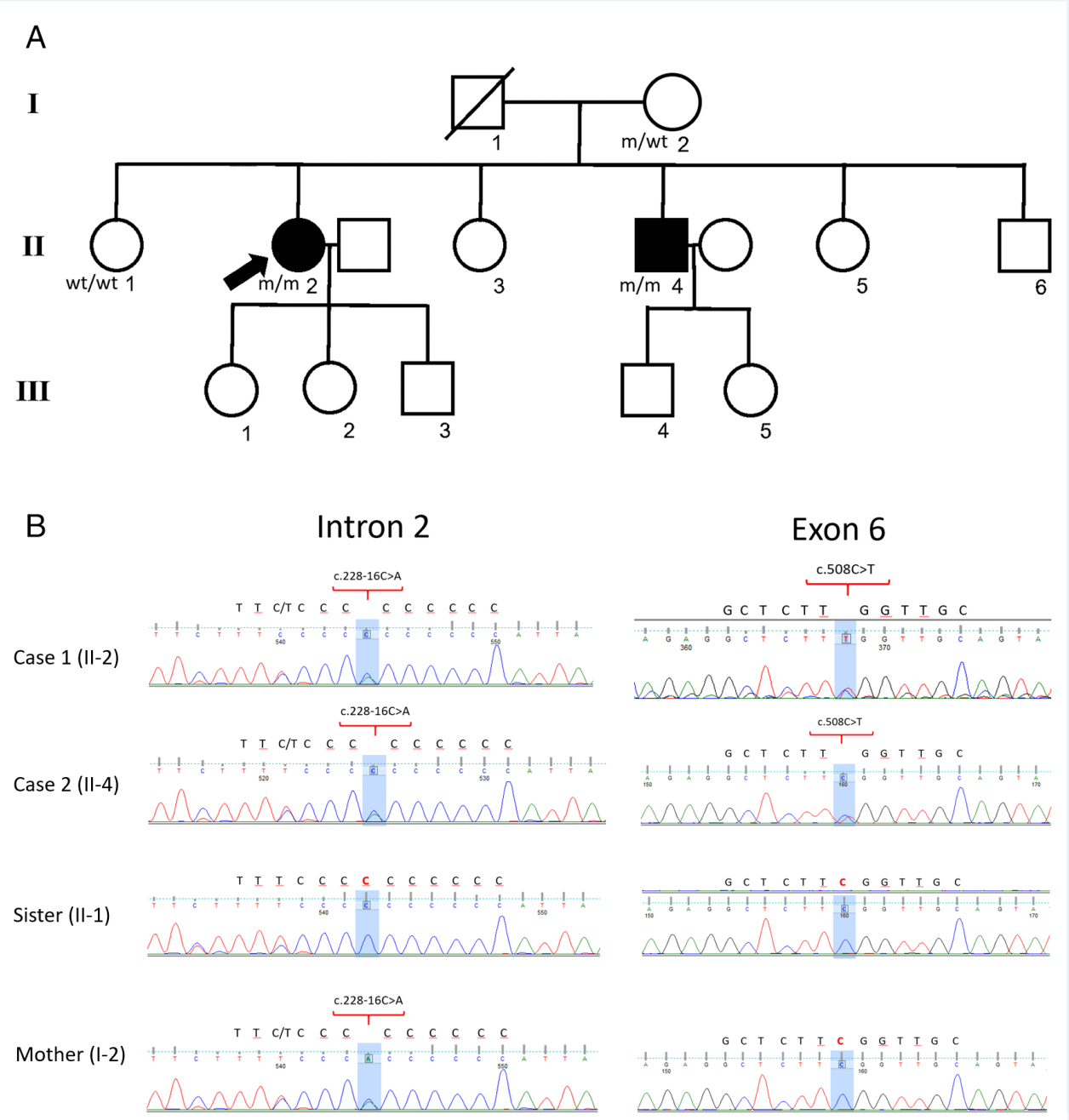

FIG. 2. (**A**) The family pedigree. (**B**) Segregation analysis with sanger sequencing showed both the case 1 (II-2, arrow) and case 2 (II-4) have the same compound heterozygous mutations. Their mother (I-2) carries only c.228-16C>A, and their sister (II-1) is normal.

cerebellar white matter. In the case 2, the involvement of cerebral and cerebellar white matter of the anterior lobe is more extensive, and the splenium is also affected (Fig. 1). The MRS using single-voxel spectroscopy (SVS) technique showed a small lactate peak by targeting the left middle cerebellar peduncle of the case 1, and no detectable lactate peak by targeting the left cerebellar white matter of the case 2 (Supplementary Figure S1).

With tentative diagnosis of demyelinating leukoencephalopathy and positive family history, whole exome sequencing was performed for the case 1. Two compound heterozygous variants (c.228-16C>A and c.508C>T) in *DARS2* were identified. The c.228-16C>A is a known mutation in splicing site of intron 2,[3,4] while the c.508C>T is a novel one which was predicted as deleterious by SIFT (Sorting Intolerant From Tolerant) program. To determine the pathogenicity of this novel

TABLE 1 Six families of LBSL showing intra-familial heterogeneity from literatures

Family No.	Sex	Age at Visit (yr)	Mutation 1	Mutation 2	Onset Age (yr)	Walking Aid (yr)	Wheelchair (yr)	Reference
1	F	57	c.228-16C>A	c.508C>T	55	–	–	Index report
	M	52	c.228-16C>A	c.508C>T	Teenage	50	–	
2	M	25	c.228-21_-20delTTinsC	c.492+2T>C	2	24	–	5
	M	22	c.228-21_-20delTTinsC	c.492+2T>C	15	–	–	
3	M	20	c.228-21_-20delTTinsC	c.787C>T	2	6	–	5
	F	24	c.228-21_-20delTTinsC	c.787C>T	1	14	22	
4	F	33	c.228-21_-20delTTinsC	c.788G>A	3	8	20	5
	F	29	c.228-21_-20delTTinsC	c.788G>A	5	28	–	
5	F	23	c.228-16C>G	c.745C>A	4	–	–	6
	F	28	c.228-16C>G	c.745C>A	Asymp.	–	–	
6*	F	15	c.1762C>G	c.563G>A	0.3	+	+	7
	F	18	c.1762C>G	c.563G>A	0.6	+	+	
	F	20	c.1762C>G	c.563G>A	Asymp	–	–	

*Consanguineous family; Asymp, asymptomatic; –, not affected; +, affected but not specified (both cases had motor delay but learned independent walking at age 6).

mutation, segregation analysis with Sanger sequencing was performed in the case 2, and their sister and mother (no available sample from the siblings' father who died of pancreatic cancer in his 50s without known gait problem). The latter two are normal under thorough neurological examination. Their mother carries c.228-16C>A only, and their sister has neither of these variants. In contrast, the case 2 has the same compound heterozygous variants as the case 1 (Fig. 2). The minor allele frequency of c.508C>T in *DARS2* is 0.00001622 worldwide according to gnomAD. In East Asia the frequency ranges from 0.00005564 to 0.0001718 (estimated by gnomAD and ExAC database respectively).[5] By applying the American College of Medical Genetics and Genomics (ACMG) guideline, the c.508C>T in *DARS2* is "likely pathogenic" (meeting criteria PM2, PM3, PP3, PP4).[6]

Literature Review

Although typically a childhood-onset, slowly progressive disease, the onset age and progression speed of LBSL are actually highly variable, ranging from infantile onset with great disability, to middle-age onset without loss of ambulation after decades.[7] Currently, there are more than 50 non-intron 2 mutations reported and according to a large case series from a European group, the genotype–phenotype correlation is not apparent.[7] Patients carrying c.228-21_-20delTTinsC in combination with c.455G>T showed various onset age ranging from 2 to 23 years. Similar situations could also be found in those in combination with c.492 +2T>C, with onset age 1–15 years. The duration of disease progression from onset to walking aids also varies from 7 to 22 years in these patients.[7]

To understand more about such variations, we reviewed all the case reports and case series of LBSL on the PubMed from April 2007 to March 2021. Sporadic cases or cases without clinical details were all excluded. Ages of onset/functional deficits are categorized into infancy (birth to 2 years), childhood (3–12 years), adolescence (13–20 years), early adulthood (21–40 years), and late adulthood (greater than 40 years). Siblings with ages of onset and functional deficits in different age categories were considered clinically heterogeneous and were therefore included.

Our report, along with five families from other studies, demonstrate intra-familial heterogeneity (Table 1),[7–9] suggesting some intrinsic mechanisms. Environmental factors such as well water, pesticide, smoking, alcohol, food and life style, at least in our cases, unlikely play crucial roles in clinical heterogeneity as both our cases were raised up together and lived nearby after marriage.

Discussion

DARS2 is responsible for conjugation of aspartate to the cognate tRNA. The intron 2 mutations that are invariably found in most LBSL cases cause skip of exon 3 through aberrant splicing,[10] probably leading to frameshifting or premature stop. Point mutations at non-intron sites, on the other hand, have been shown in association with decreased enzyme activities, decreased dimerization, decreased protein expression,[2,11] or alterations of other biophysical characteristics.[12] It is intuitive to consider that mitochondria aspartyl tRNA synthetase (mt-AspRS), the protein products of *DARS2*, plays an important role in intra-mitochondrial translation, and is therefore pivotal for

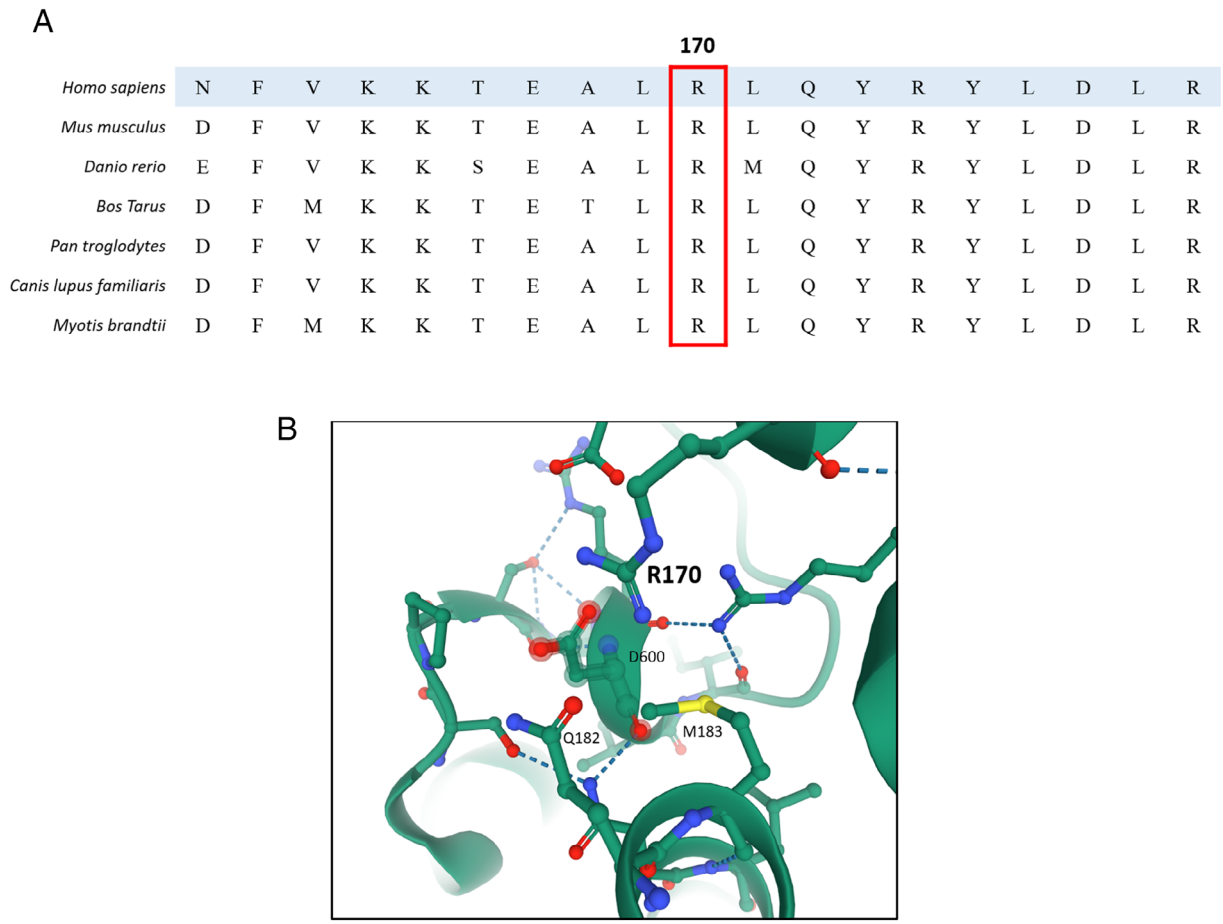

FIG. 3. The R170 residue is highly conserved across species (**A**) and has spatial proximity (<5 Å) with residues Q182, M183, and D600 (**B**), suggesting possible interactions with the dimerization helix and the catalytic domain.

mitochondria function. However, according to the work of Scheper and his colleagues, activities of mitochondria complex I-IV remained unaffected in fibroblasts and lymphoblasts derived from LBSL patients.[2] The contradictory results might be related to intrinsic differences among cell lineages. Van Berge et al. have shown that exon 3 preceded by mutant intron 2 will be spliced out more efficiently in neuronal than non-neuronal cell lines.[10] The leaky strategy might explain why the nervous system is more vulnerable than other systems, and probably also the tract-specific nature of LBSL. It is possible that the intra-familial heterogeneity is related to the splicing efficacy variations among individuals. Epigenetic regulation might contribute to the individual differences although the evidence remains limited. The RNA processing, supposedly associated with the aberrant splicing of *DARS2* mutants in intron 2, is regulated not only by the cis-sequences in pre-mRNA but also by many trans-acting factors, such as DNA methylation, histone modification, and non-coding RNAs.[13] Further studies about the underlying mechanisms may provide hints about treatment development in the future.[14]

Our newly identified mutation c.508C>T in *DARS2* causes R170W missense mutation. A similar variant c.509G>A (dbSNP: rs759658461) that causes R170Q is reported in the ClinVar database, but as no clinical detail is provided, the clinical significance is uncertain. Our report strengthens the importance of this residue. The mt-AspRS is mainly composed of five parts: mitochondria targeting sequence (aa 1–41), anticodon binding domain (aa 58–151), dimerization helix (aa 180–205), catalytic domain (aa 208–608), and C-terminal bacterial extension (aa 609–645).[15] The R170 residue resides in the hinge between the anticodon binding domain and dimerization helix, and is 80% conserved across species as the MiSynPat (http://misynpat.org/misynpat/) described.[16] Residues including Q182, M183, and D600 share spatial proximity (<5 Å) with the R170 according to the 3D modulation of Protein Data Bank (Fig. 3),[17] implying the possible interactions with dimerization helix and catalytic domain. These interactions to our knowledge have not been well-studied yet.

In conclusion, we identified a Taiwanese non-consanguineous family of LBSL showing prominent intra-familial heterogeneity.

Compound heterozygous mutations of one intron 2 mutation and one novel missense mutation in exon 6 were detected. The leakiness of aberrant splicing due to the intron 2 mutation might explain the clinical heterogeneity and provide clues for future gene therapies.

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Author Roles

(1) Research Project: A. Conception, B. Organization, C. Execution, D. Patient care, E. Evaluation of genetic findings; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript Preparation: A. Writing the first draft, B. Review and Critique.

J.L.L.: 1A, 1C, 1D, 3A
N.C.L.: 1E
P.S.C.: 1D
G.H.L.: 1D
R.M.W.: 1D, 3B

Disclosures

Ethical Compliance Statement: Declaration of consent for the publication of the medical data and the video from the two patients were obtained at the outpatient clinic of our university hospital. We confirm that the approval of an institutional review board is not required for this project. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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Supporting Information

Supporting information may be found in the online version of this article.

Figure S1. The MRS using the SVS technique showed a small lactate peak (arrow) at the left middle cerebellar peduncle of the case 1 (aged 57), and no lactate peak at the left cerebellar white matter of the case 2 (aged 52).