Perinatal Manifestations of DARS2-Associated Leukoencephalopathy With Brainstem and Spinal Cord Involvement and Lactate Elevation (LBSL)

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Abstract
Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is a progressive disorder associated with deficiency of mitochondrial aspartyl-tRNA synthetase, a homodimer encoded by the gene DARS2. There is a wide range in age of onset of symptoms, typically from childhood to adulthood, with very few cases of infantile onset disease reported. We report a child at age 10 years with perinatal onset of symptoms evidenced by congenital microcephaly with progression to severe but non-lethal epileptic encephalopathy and spastic quadriplegia. A comprehensive epilepsy focused gene panel performed as a trio with parents detected a novel homozygous DARS2 variant. This variant is located at the dimer interface in a critical catalytic domain and is expected to result in markedly reduced enzyme activity which likely explains the severe and early onset symptoms in this case.

Keywords
DARS2, LBSL, leukoencephalopathy, epileptic encephalopathy, spasticity, neuroimaging, mitochondrial disease

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DARS2 encodes the mitochondrial aspartyl-tRNA synthetase (mtAspRS) which acts to incorporate aspartic acid into mitochondrial DNA-encoded proteins. MtAspRS functions as a homodimer, and pathogenic variants in DARS2 have been demonstrated to disrupt enzyme activity in several ways including catalytic activity, protein expression or dimerization.1 Autosomal recessive DARS2-related disorder, also known as leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), exhibits a wide phenotypic spectrum and age of onset, which does not always correlate with the specific variant or variants and enzyme activity.1 Classic features include ataxia, spasticity especially of the lower limbs, epilepsy, leukoencephalopathy, and dorsal column dysfunction. The purpose of this report is to present a child with congenital microcephaly and progressive epileptic encephalopathy associated with a novel homozygous DARS2 variant.

Case
The parents provided written consent for publication of this report.

A 10-year-old female of Mexican ancestry presented for evaluation of severe microcephaly with occipitofrontal circumference (OFC) of 45 cm (Z = -5.15 Nellhaus curve), intractable epilepsy, cortical blindness, severe spastic quadriplegia and hypotonia. She was the youngest of 3 siblings. Family history was negative for other similarly affected individuals. Her parents denied consanguinity. Pregnancy was uncomplicated and she was born at term with normal birth weight (3.77 kg) and

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birth length (50 cm). Birth OFC was 31 cm (Z = -2.43 World Health Organization curve) consistent with congenital microcephaly. Evaluation for congenital infection in early infancy was negative. Seizure onset was at age 4 months consisting of tonic seizures treated with levetiracetam. By age 3 years there were frequent myoclonic jerks and choreiform movements of the hands and tongue and topiramate was added. The first generalized tonic-clonic seizure was at age 9 years. She was never diagnosed with infantile spasms. She did not meet any developmental milestones except for nonreciprocal smile. Her course was further complicated by chronic lung disease secondary to dysphagia/aspiration leading to gastrostomy tube placement at age 2.5 years. On examination, weight was 21.2 kg (Z = -2.6) and length 124 cm (Z = -3.71, estimated due to severe lower limb contractures). She was nonambulatory and nonverbal with no apparent cognition. She had severe microcephaly with small forehead, generalized hirsutism with hair on back, forehead, and proximal phalanges of hands and feet. Palpebral fissures were normal length, with a small epicanthal fold on the left. Hands and feet were small with normal palm and digital creases, and shortened toes 4 and 5 on left foot. She exhibited nystagmus, dysconjugate gaze, axial hypertonia, severe spastic quadriplegia, hyperreflexia, significant scoliosis, and upper and lower limb contractures.

The most recent brain magnetic resonance imaging (MRI) with and without contrast demonstrated severe supratentorial white matter volume loss, ex vacuo enlargement of cerebral ventricles and extra axial spaces with relative sparing of the basal ganglia and cerebellum. There are patchy areas of increased T2 signal seen in the periventricular white matter with some associated diffusion restriction including the posterior limb of the internal capsule. Representative MRI images at age 9 days (Figure 1A) and 9 years (Figure 1B) are included. Spine MRI was not performed. Magnetic resonance spectroscopy (MRS) was not performed. The most recent EEG at age 10 years showed many electroclinical tonic seizures, poor background organization, diffuse background slowing, poorly sustained posterior dominant rhythm, rudimentary sleep architecture and focal epileptiform discharges predominantly in the right and left frontal and left parietal areas consistent with severe global neuronal dysfunction/encephalopathy, cortical hyperexcitability and ongoing seizures.

Chromosomal microarray detected no pathogenic copy number variants, with absence of heterozygosity calculated at 93%, confirming that the parents are not closely related (fifth degree relatives or beyond). A comprehensive epilepsy gene panel detected a novel homozygous variant of uncertain significance (VUS) in DARS2 [NM_018122.4] exon 9 denoted c.785C>T p.Ala262Val (A262V). This testing was performed as a trio with both parents to allow for segregation analysis to confirm each parent was a heterozygous carrier of A262V. DARS2 protein is a homodimer and the Ala262 amino acid is located at the protein dimer interface (magenta, Figure 2A-B). The Ala262 falls within a critical stretch of amino acids that are evolutionarily conserved (Figure 2C-E). Comparison of A262V to all other ClinVar variants for DARS2 shows a high variant impact score (combination of conservation, PolyPhen2, Provean, SIFT, and Align-GVGD scores) comparable to other pathogenic variants (Figure 2F-G), including the pathogenic R263Q seen in a patient with LBSL (red, Figure 2A-E). Details of the bioinformatics methods have been previously published.3

Discussion

Most patients with LBSL have childhood to adolescent onset of symptoms associated with compound heterozygous variants. It was originally hypothesized that since mtAspRS functions as a homodimer, homozygosity could result in significant reduction in function that may lead to early lethality.4 However, several individuals and families with homozygous DARS2 variants have been reported with variable symptoms and severity.5-7 Finsterer et al. documented the phenotypic variability of both homozygous and compound heterozygous DARS2 variants.8 There are now several reports of patients with symptom onset in infancy,5,6,8 but neonatal-onset symptoms are exceptional. Steenweg et al. used MRI criteria for LBSL to review previously undiagnosed cases of leukoencephalopathy and presented 6 new cases with compound heterozygous DARS2 variants.9 This included one similar infant to our case with severe hypotonia, onset of seizures at 5 months and no developmental progress. That infant died at age 20 months. MRI criteria for the diagnosis of LBSL include signal abnormalities of the cerebral white matter with relative sparing of subcortical white matter, dorsal column and corticospinal tracts of the spinal cord, and pyramids at the level of medulla oblongata or decussatio of the medial lemniscus or both as major criteria. Minor criteria include signal abnormalities in the splenium of corpus callosum and posterior limb of the internal capsule, superior and inferior cerebellar peduncles, intraparenchymal trigeminal nerve, mesencephalic trigeminal tracts, anterior spinocerebellar tracts of the medulla oblongata and cerebellar white matter. If no spinal MRI is
available, signal abnormality at the pyramids and decussation of medial lemniscus are both required for MRI diagnosis.\textsuperscript{9} Progressive atrophy of cerebral white matter over time was documented in the eldest sibling reported by Yamashita et al.\textsuperscript{6} Brain MRI imaging in this case fulfills some major and minor criteria for LBSL. Our patient demonstrates the major criterion of signal abnormalities in the cerebral white matter; however, due to severe congenital white matter atrophy, sparing of U-fibers cannot be adequately assessed. Our patient also demonstrates the minor criterion of involvement of the posterior limb of the internal capsule. However, the available imaging does not meet other major or minor infratentorial white matter criteria. Due to such severe white matter atrophy, white matter changes in the corpus callosum are also difficult to assess. The brain imaging in this case demonstrates profound cerebral dysgenesis, white matter atrophy and persistent diffusion weighted imaging changes rather than more typical MRI findings of LBSL. These observations may expand the spectrum of LBSL imaging abnormalities given this

Figure 2. A) Structural model of DARS2 homodimer, with one monomer in gray and the other shown as a surface plot in cyan. Amino acids in red are known pathogenic variants and the patient variant in magenta. B) Zoom in view of A262 (magenta) and R263 at the dimer interface. C) Deep evolutionary analysis of DARS2 in 259 species on a 21-codon window. D) Conservation scores of amino acids around site 262. E) Alignment data for position 262. F) Impact scores based on PolyPhen2, Provean, SIFT, Align-GVGD, conservation score, and 21 codon linear motif scores for ClinVar Pathogenic/likely pathogenic (red), patient (magenta), and uncertain significance (VUS, black). G) Impact score of A262V (magenta) relative to ClinVar Pathogenic/likely pathogenic (red), and VUS (black).
severe clinical phenotype. If LBSL is considered as a potential diagnosis, results from comprehensive imaging including brain and spine MRI and MRS is useful for planning appropriate genetic testing, and for correlation with such results, especially in patients without classic clinical features.

Based on the clinical findings, imaging, protein modeling, evolutionary analysis, and comparison of the variant A262V to other reported variants, we suggest that our patient is consistent with perinatal onset LBSL. Further support for the pathogenicity of the A262V variant is comparison to the variant R263Q for which functional data is available. A262V and R263Q are located at the dimerization interface and within the catalytic domain of mtAspRS (Figure 2). Van Berge et al. studied the effect of R263Q and other reported missense variants of mtAspRS and documented a 135-fold reduced aminoacylation activity in human cell culture of R263Q compared to wildtype, and documented decreased dimerization of R263 with wildtype mtAspRS and with one other mutant. Further functional studies of the A262V variant would be useful to allow reclassification of this variant as pathogenic. Finally, homozygosity of this A262V in a family without close consanguinity suggests that it is a regional founder variant.

**Author Contributions**

All authors contributed to the to the concept or design of this work, acquisition, analysis or interpretation of data, drafted the article and/or revised it critically for important intellectual content. All authors approve the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Ethical Approval**

This report is waived from Institutional Review Board approval.

**Declaration of Conflicting Interests**

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