Archives of Pediatrics and Neonatology
Volume 1, Issue 1, 2018, PP 4-8

Novel Mutation of DARS2 Gene Leading to a Rapidly Progressive Early onset Leukoencephalopathy

Geetanjali S Rathore, M.D[1], Jose E Morales, M.D[2], Paul D Larsen, M.D[1], William B. Rizzo, M.D[1], Lois J Starr, M.D[1]

[1]Department of paediatrics, University of Nebraska Medical Center, Nebraska medical centre, Omaha, Nebraska, 68198
[2]Department of Paediatrics, Saint Louis University School of Medicine, 1465 S. Grand Blvd., St. Louis, Mo 63104
***Corresponding Author:** Geetanjali S Rathore, M.D,[1]Department of paediatrics, University of Nebraska Medical Centre, Nebraska medical centre, Omaha, Nebraska, 68198*

ABSTRACT

Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is a rare autosomal recessive neurological disorder due to mutations in the DARS2 gene. The typical presentation is juvenile onset, with gradually progressive spasticity and ataxia. We describe a unique case of infantile-onset LBSL presenting with profound hypotonia and a rapidly progressive course. An extensive metabolic and initial genetic workup was unrevealing.A repeat brain MRI showed progressive confluent white matter T2 hyper-intensity within the brain and spinal cord. Re-analysis of the WES revealed compound heterozygous mutations in the DARS2 gene in trans. The first mutation (c.788G>A, p.Arg263Gln) has been previously reported as pathogenic. The second mutation (c.259G>A, p.Asp87Asn) is a novel mutation in the exon 3and reported likely pathogenic.

Our patient further expands the genotypic and phenotypic spectrum of LBSL. Additionally, this case emphasizes the significance of re-evaluation of WES if there are new clinical findings.

Keywords:*Leukodystrophy, DARS2, hypotonia, lactate elevation, LBSL*

INTRODUCTION

Leukoencephalopathy with brain stem involvement and elevated lactate (LBSL) is a rare autosomal recessive condition caused by mutations in the *DARS2* gene [1]. A vast majority of those affected are compound heterozygotes and havedifferent mutations on both copies of the DARS2 genes. These mutations lead to decreased activity of mitochondrial aspartyl tRNAsynthetase [2]. MRI signal abnormalities including low signal on T1-weighted images and high signal on T2-weighed images can be observed in the cerebral white matter, dorsal columns, lateral corticospinal tracts, and pyramids [3].

Individuals with this condition typically present with slowly progressive cerebellar ataxia, spasticity, sensory and position sense dysfunction. The lower extremities tend to have a greater degree of dysfunction than the upper extremities and those affected may become wheelchair dependent. Other associated symptoms include epilepsy, decreased cognitive skills, and dysarthria [4]. These symptoms present most commonly in late childhood and adolescence but have also been described in adults [5]. The progression of the disease is most often mild in adults but may be more aggressive in juvenile individuals [6].

Herein, we review the clinical course of a female patient with an atypical presentation who suffered the earliest age of death reported. She experienced rapid progression of her disease and was found to have a novel, but clearly pathogenicmutation in the *DARS2* gene. Her moderate to severe hypotoniainstead of spasticity, and normal blood lactate expands the phenotype for LSBL.

PRESENTATION OF PATIENT

We present a 6 month oldfemale who was evaluated by neurology for hypotonia, regression and developmental delay. She was born from an uncomplicated pregnancy and a full term vaginal delivery to non-consanguineous healthy parents. Development proceeded as normal during the first few months of life. She was noted to not be rolling over at her 6 month well child visit by her pediatrician

and was referred to neurology for further evaluation. She had a normal general exam. Head circumference was in the 80th percentile for age. On neurologic exam, she was alert and interactive with cooing and smiling. She had no cranial nerve deficits but exhibited severe axial and appendicular hypotonia with absent deep tendon reflexes. Additionally,she was unable to roll over and could not sit without support. Her plantar reflex was down-going and she had no ankle clonus. Her sensory exam was unremarkable and no ataxia or abnormal movements were noted.

An MRI of the brain with and without contrast was significant for abnormal periventricular parieto-occipital lobe white matter signal thought to be related to delayed myelination (Figure-1) and no structural anomalies. Due to her normal social development and non-specific MRI findings,a lower motor neuron disorder like spinal muscular atrophy (SMA), congenital myopathy, or a metabolic disorder was thought to be more likely than a primary CNS disorder. Creatine kinase and aldolase were normal. SMA testing was investigated and no molecular anomalies were found.

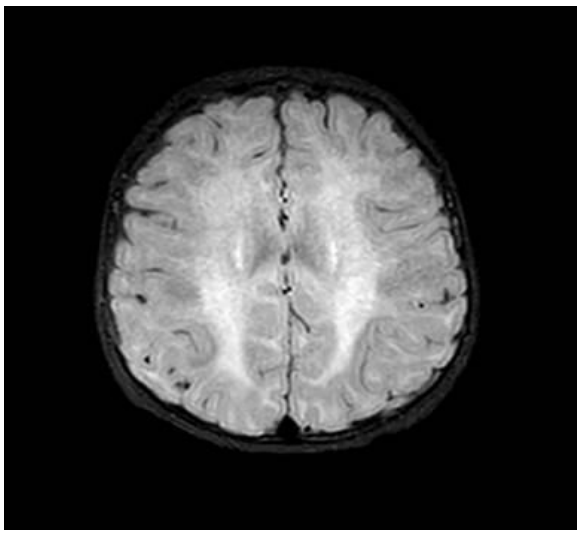

Figure1.*Initial axial T1 image showing delayed myelination*

In an eight week interval, our patient declined rapidly and was admitted due to failure to thrive and respiratory distress. She was having intermittent episodes of tonic stiffening with rolling her eyes back, and opisthotonus. On physical exam, she showed mild stridor in addition to continued hypotonia and absent deep tendon reflexes. An EEG revealed the presence of diffuse background slowing and disorganization without epileptiform discharges. Her episodes were more consistent with dystonia rather than seizures. On flexible laryngoscopy, she was noted to have pharyngo-laryngomalacia and eventually she was intubated for impending respiratory failure. Her respiratory condition and oral intake failed to improve and she underwent tracheostomy and gastric tube placement.

Genetic and metabolic studies were ongoing during her hospitalization and her subsequent discharge. High resolution karyotype, high density SNP array, plasma amino acids, TSH/free T4, ammonia and acylcarnitine profile were normal. She had a normal lipid panel, glycosylated transferrin, blood lactate, CK/Aldolase, and glycoseaminoglycans. Her urine revealed normal organic acids, free sialic acid, oligosaccharides, glycans, and had a normal N-glycan profile. Increased macular pigmentation seen on initial ophthalmologic examination was concerning for a cherry red spot. However, screening for lysosomal storage diseases revealed normal hexosaminidase A and B enzymes and no evidence for GM1 gangliosidosis, metachromatic leukodystrophy, Krabbe disease or beta-mannosidosis. Repeat ophthalmologic exam failed to reveal a cherry red spot. A lysosomal disease genetic panel (LysoSeek) returned negative.

Our patient's blood was sent for whole exome sequencing and was found to have four variants of unknown significance. The first was an*ATP7B* heterozygous mutation and the second was a *GBE1* heterozygous mutation. These have been linked to Wilson's disease and glycogen storage disease type 4 respectively. Her asymptotic mother proved to be a carrier for these mutations, thus it was deemed unlikely that these could be a source of pathogenicity in our patient. She also carried heterozygous mutations in the *COL6A1* and *COL6A2* genes which are associated with myopathy but not thought to be pathogenic in her case.

A repeat MRI of the brain and muscle biopsy was performed. The muscle biopsy revealed neurogenic atrophy and the second MRI showed progressive abnormal T2 hyper-intensity within the periventricular white matter with involvement of the corpus callosum, the internal capsule, and the dorsal aspect of the cervical spinal cord (Figure 2). These findings were suggestive of a progressive disease and the geneticist requested the exome be re-examined for a list of potentially contributing genes including *DARS2*.

This re-analysis revealed that the patient harbored compound heterozygous mutations in*DARS2* intrans. The first mutation (c.788G>A,

p.Arg263Gln) has been previously reported and involves a highly conserved amino acid residue in a known functional domain of exon 9 and was reported as a known pathogenic mutation. The second mutation (c.259G>A, p.Asp87Asn) is in exon 3 and has not been previously described in the literature. This mutation is also in a known functional domain and was reported as likely pathogenic due to its molecular properties and of note, *DARS2* has a very low rate of benign missense variation in both domains.

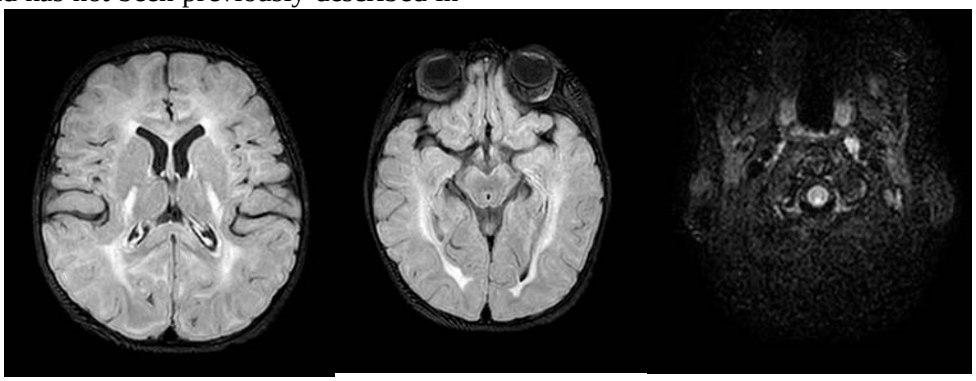

Figure2. *Abnormal T2 hyper-intensity within the periventricular white matter with involvement of the corpus callosum, the internal capsule, and the dorsal aspect of the cervical spinal cord.*

Our patient's genetic abnormalities and second MRI were consistent with LBSL and the diagnosis was finally established. Unfortunately, our patient's disease continued to progress rapidly and she died 6 monthsafter her initial presentation at 12 months of age.

DISCUSSION

The differential diagnosis for the infant with hypotonia is wide and includes muscular dystrophies, anterior horn cell disorders, neuromuscular junction disorders, congenital myopathies, cerebral palsy, structural abnormalitiesof the brain, and metabolic disorders. This variety of possibilities can make diagnostics an imposing task and can even be more difficult when, like our patient, the presentation is atypical.

LBSL was first described by van der Knaap and colleagues in 2003 [3] and has been reported to be the first disease to affect the genes encoding mitochondrial amino acyl-tRNAsynthetase.The exact mechanism that causes white matter involvement is unclear, but it is speculated that the high expression of amino acyl-tRNAsynthetase within white matter cells places this particular section of the brain and spinal cord at increased risk in the presence of *DARS2* mutations [7]. Thereported cases of LBSL have presented most commonly with slowly progressing cerebellar ataxia, impaired sensory function and spasticity that affects the lower extremities more than the upper extremities[4]. These deficits tend to present more often in childhood and adolescence rather than in infancy as our patient did.

Elevated lactate in the abnormal white matter on MRS (Magnetic resolution spectroscopy) tends to be present in almost all patients with LBSL, even when blood/CSF lactate levels are normal [1]. Our patient's serum lactate was persistently normal.Thus, LBSL would be one of those few conditions where MRS, in addition to the MRI, may help in making the diagnosis [1].

Individuals with LSBL become wheelchair bound in their teens and twenties and the severity of the disability correlates with the severity of white matter involvement [1,8].In contrast, our patient deteriorated over the course of a few months, requiring intubation, tracheostomy, and gastrostomy tube placement. This quick decline in function, in combination with the other atypical features of our patient's presentation, can perhaps be explained by the novel *DARS2* mutation in exon 3 or perhaps there is another contributing genetic component not identified.Previous studies have not been able to find a clear genotype-phenotype correlation, partially due to the autosomal recessive inheritance pattern of this condition and many combinations of mutations. All patients have noticeably lower mitochondrial aspartyl-tRNAsynthetase activity, when compared to controls. It can be postulated that our patient's phenotype could be a product of a more severe deficit in amino acyl-tRNAsynthetase, however in prior studies, no correlation could be established between the level of activity and severity of phenotype [6].

Our patient's initial MRI was also not consistent with the typical pattern of LBSL. The MRI changes in LBSL are very characteristic and can

be diagnostic. For the diagnosis of LBSL to be made on the basis of MRI, 3 major and 1 minor criteria must be met [7]. The first of the three major criteria is T2/FLAIR signal abnormalities in the cerebral white matter, which may be inhomogeneous and spotty or homogenous and confluent. This first criterion was met by our patient's first MRI as evidenced by the abnormal signal in the periventricular parieto-occipital lobe white matter. The second criterion involves signal abnormalities in the dorsal columns and lateral corticospinal tract of the spinal tract which may be seen in the cervical spinal cord. While the initial MRI did not show this particular change, the second MRI that was performed did show abnormal signal intensity within the visualized spinal cord. Lastly, the third major criterion consists of signal abnormalities in the pyramids of the medulla oblongata. This last major criterion was not met in our patient on either imaging. Several abnormalities seen on the subsequent MRI did meet the minor criteria. These changes included signal abnormalities in the splenium of the corpus callosum and posterior limb of the internal capsule.

Although there have been multiple cases of early onset LBSL, our patients seems to have suffered the most severe case yet reported with death by 12 monthsof age. The closest patient resembling was a femalewho exhibited symptoms of hypotonia, poor head control, and lack of development from birth. She died due to respiratory failure at 20 months of age [9]. There is another case report that was presented in 2015 that discusses a male who exhibited symptoms at 8 months of age and was eventually diagnosed with LBSL. However, his symptomsunexpectedly resolved and, except for some mild ataxic signs, his symptoms resolved [10]. At 15 months of age, another male in Brazil had some consistent symptoms and though genetic studies were not performed, his clinical, laboratory, and imaging findings were consistent with LBSL. His disease progressed and by 15 years of age he could not walk without support [11]. Yet another report discusses a malewith LBSL diagnosed by imaging and genetic studies who developed ataxia at 16 months of age. His disease progressed much more rapidly and he developed respiratory failure and died at 2 years of age [12]. In a cohort of 66 patients with LBSL that van Berge and her colleagues studied in 2014, only two patients had died. Both had experienced infantile onset and were dead by 2 years of age [6]. Although many of these cases are similar in severity and onset, none depict so quick a deterioration and asearly death as our patient.

A recent literature review by Finsterer et.al confirmed that the phenotypic spectrum is vaster than initially described. Some of the atypical features they found included- exercise-induced ataxia, organic psychosyndrome, absence of lactic acid elevation in the brain, optic atrophy, hypoacusis, ptosis, diplopia, myopathy (elevated creatin-kinase(CK), muscle weakness, muscle atrophy), polyneuropathy, anemia, and nephrolithiasis [13]

CONCLUSION

LBSL should be on the differential diagnosis for infants with rapidly progressive leukoencephalopathy, even with normal lactate. The atypical presentation of our patient, along with the rarity of LBSL, made our patient a diagnostic dilemmaand delayed the time to diagnosis. Furthermore, the severity, quick progression of her disease, and early death makes her case unique and adds to the phenotypic spectrum of LBSL. We also report a novel mutation that may be related to more aggressive course. The diagnostic process of this case alsoemphasizes the importance of updating clinical findings in guiding analysis of genetic studies and significance of re-evaluation of whole exome sequencing if there are new clinical findings, without which we would not have been able to confirm the diagnosis in our patient.

ACKNOWLEDGMENT

This case has been presented as a poster abstract at the annual AAN meeting on April 26th 2017, poster session IV, poster number 174 —‘ Novel mutation of DARS2 gene leads to a rare and aggressive presentation of leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL);Geetanjali Rathore, Lois Star, Paul Larsen and William Rizzo.’

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