Diagnostic Whole Exome Sequencing Identifies Alterations in the Novel Gene, *WARS2*, in a Patient with Severe Infantile-Onset Leukoencephalopathy

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BACKGROUND

- Whole exome sequencing (WES) has been shown to be an effective tool in diagnosing genetic disorders, with approximately 25-30% of patients undergoing clinical WES receiving a molecular diagnosis with mutations in characterized genes (Farwell, 2014; Yang, 2014).
- By analyzing the ~15,000 genes not currently known to be associated with a genetic disorder, we are able to increase the diagnostic yield of WES and identify new candidate genes for rare and unknown genetic disorders.
- The mitochondrial aminoacyl-tRNA synthetases (mt aaRSs) are involved in activation and transfer of amino acids to the appropriate tRNAs during translation of mitochondrial genes and protein synthesis (Diodato, 2014).
- Mutations in 18 of the 19 mt aaRS genes have been reported to cause autosomal recessive, early onset disorders with diverse clinical presentations, including neurological and muscular phenotypes.

CLINICAL HISTORY

- 24-year-old male of mixed European ancestry
- Profound intellectual disability, postnatal microcephaly, intractable epilepsy
  - Presented at 6 months with motor delay and seizures
  - No language, but is socially interactive
  - Unable to sit without support
- Neurologic exam: spastic quadriplegia with central hypotonia, tremor, dysmetria, muscle wasting, and contractures of arms and legs
  - Hyperreflexia of upper extremities and knees, however ankles are areflexive and loose, suggestive of peripheral neuropathy
- Ophthalmology exam: intermittent exotropia and amblyopia
- Brain MRIs: diffuse cerebral atrophy and extensive bilateral diffuse periventricular T2 hyperintensities
- EEGs: multifocal spikes, diffuse slowing, intermittent rhythmic delta activity
- Family history: negative for similarly affected individuals or consanguinity
- Previous genetic testing: uninformative
  - Karyotype, SNP chromosomal microarray, Fragile X testing, Angelman FISH, mitochondrial DNA deletions
  - Skin fibroblast mitochondrial enzyme activities

METHODS

- Diagnostic WES was performed on genomic deoxyribonucleic acid (gDNA) isolated from whole blood from the proband and both parents. Targeted Sanger sequencing was performed on gDNA isolated from whole blood from the brother. Informed consent was obtained from all family members involved in the testing process.
- Exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described (Farwell, 2014).
- See Poster # 651 for criteria for analysis and interpretation of novel gene findings.

RESULTS

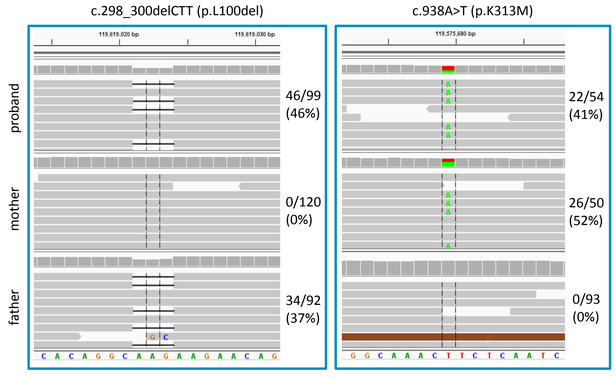
Gene Symbol	Characterized/Novel Gene*	Protein Change	Nucleotide Change	Genotype	Alteration Type	Alteration Classification	Gene Overlap
WARS2	Novel	p.K313M	c.938A>T	Heterozygous, maternal	Missense	Uncertain	Likely Positive
		p.L100del	c.298_300delCTT	Heterozygous, paternal	In-frame Deletion	Uncertain	

TABLE 1: Variant Filtering and Analysis

	Inheritance Model Filtering	Medical Review				Candidates
		Alteration Review	Clinical Association Review			
Inheritance	Total	Total	Characterized	Clinically novel	Total	Total
Autosomal Dominant	8 (8)	3 (3)	0 (0)	2 (2)	2 (2)	0 (0)
Autosomal Recessive	5 (9)	2 (3)	0 (0)	1 (2)	1 (2)	1 (2)
X-Linked Recessive	2 (2)	1 (1)	0 (0)	1 (1)	1 (1)	0 (0)
X-Linked Dominant	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Y-Linked	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Autosomal Dominant (reduced penetrance)	17 (17)	11 (11)	2 (2)	0 (0)	2 (2)	0 (0)
X-Linked (reduced penetrance)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
All Models	**31 (36)**	**16 (18)**	**2 (2)**	**4 (5)**	**6 (7)**	**1 (2)**

- Diagnostic WES did not identify any candidate alterations among characterized genes.
- Novel gene analysis identified two compound heterozygous alterations in the *WARS2* gene.

FIGURE 1: *WARS2* Alterations



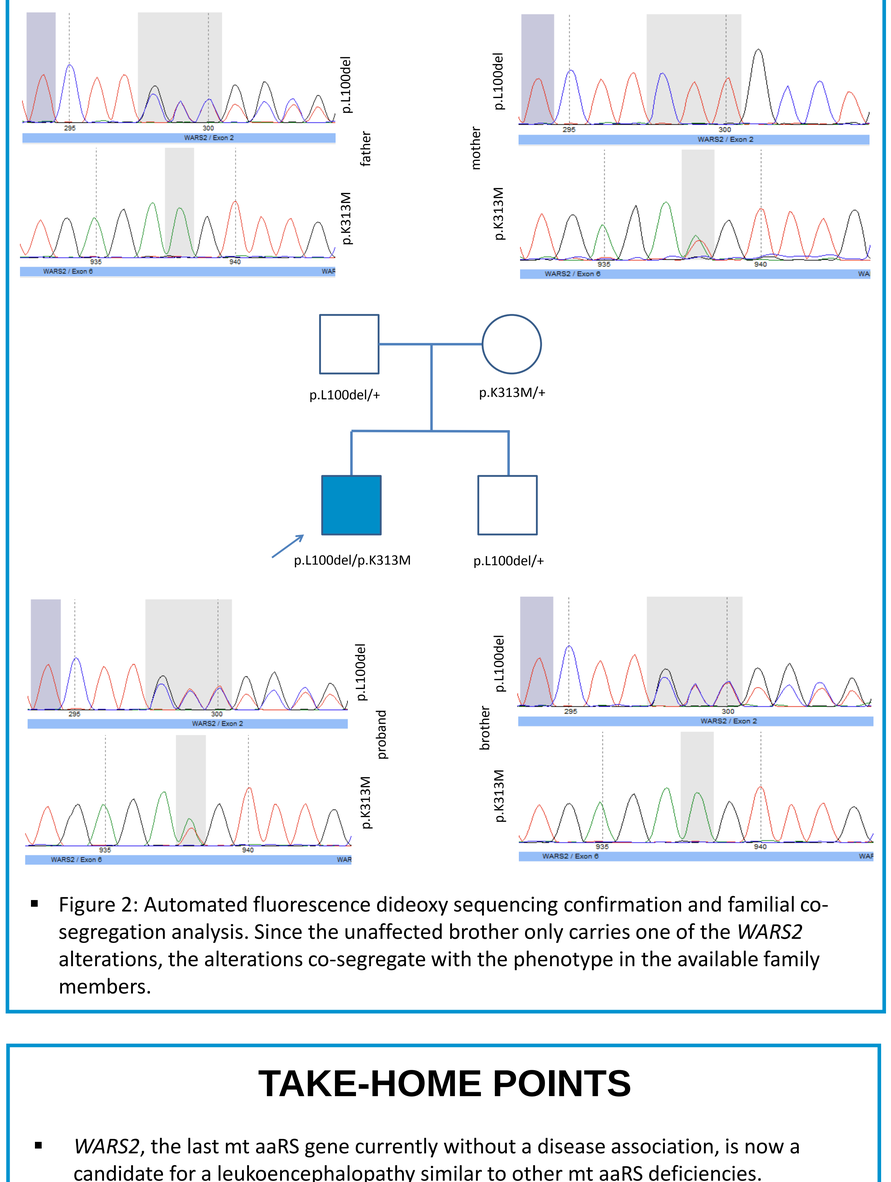
- Figure 1: Next-generation sequencing reads with ratios and percentages of each alteration. Since mother and father are each heterozygous for one *WARS2* alteration, the proband is compound heterozygous for the two *WARS2* alterations.

TABLE 2: mt aaRSs, Associated Phenotypes, and Overlap with Our Patient

Gene	Protein	Phenotype	Year first reported	ID/DD	microcephaly	seizures	spasticity	tremor	muscle atrophy	contractures	brain atrophy	abnormal EEG	neuropathy
Neurodegenerative													
DARS2	mt aspartyl-tRNA synthetase	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation	2007	x			x	x	x	x	x		x
RARS2	mt arginyl-tRNA synthetase	Pontocerebellar hypoplasia, type 6	2007	x	x	x	x				x		
MARS2	mt methionyl-tRNA synthetase	Spastic ataxia 3, autosomal recessive	2012	x			x				x		
FARS2	mt phenylalanine-tRNA synthetase	Combined oxidative phosphorylation deficiency (global developmental delay, refractory seizures, and lactic acidosis)	2012	x	x	x					x	x	
EARS2	mt glutamyl-tRNA synthetase	Combined oxidative phosphorylation deficiency (leukoencephalopathy with thalamus and brainstem involvement and high lactate)	2012	x		x	x						
CARS2	mt cysteinyl-tRNA synthetase	Neurodegenerative disorder	2014	x		x	x						
NARS2	mt asparaginyl-tRNA synthetase	Alpers syndrome	2015	x	x	x	x		x		x	x	
PARS2	mt prolyl-tRNA synthetase	Alpers syndrome	2015	x	x	x					x	x	
Neurodegenerative with Myopathy													
YARS2	mt tyrosyl-tRNA synthetase	Myopathy, lactic acidosis, and sideroblastic anemia 2	2010						x				
AARS2	mt alanyl-tRNA synthetase	Combined oxidative phosphorylation deficiency (lethal infantile hypertrophic cardiomyopathy)/Leukoencephalopathy, progressive, with ovarian failure	2011/ 2014	x			x	x			x	x	
TARS2	mt threonyl-tRNA synthetase	Combined oxidative phosphorylation deficiency (mitochondrial encephalomyopathy)	2014	x									
VARS2	mt valyl-tRNA synthetase	Combined oxidative phosphorylation deficiency (mitochondrial encephalomyopathy)	2014	x	x	x							
Neuropathy													
GARS	glycyl-tRNA synthetase	Charcot-Marie-Tooth disease, type 2D/Neuropathy, distal hereditary motor, type VA	2003						x				x
KARS	lysyl-tRNA synthetase	Charcot-Marie-Tooth disease, recessive intermediate, B/Deafness, autosomal recessive 89	2013						x				x
IARS2	mt isoleucyl-tRNA synthetase	Cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss, and skeletal dysplasia	2014							x			x
Perrault syndrome													
HARS2	mt histidyl-tRNA synthetase	Perrault syndrome 2	2011										
LARS2	mt leucyl-tRNA synthetase	Perrault syndrome 4	2013										
Other													
SARS2	mt seryl-tRNA synthetase	Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis	2011	x									
This Patient													
WARS2	**mt tryptophanyl-tRNA synthetase**	**none yet reported**	**this patient**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**

- The patient's overlapping features within the gene family include seizures, developmental delay, tremor, exotropia and nystagmus, central hypotonia with spastic quadriparesis, contractures of arms and legs, hyperreflexia, areflexia of ankles (suggesting peripheral neuropathy), cerebral atrophy with ventriculomegaly, periventricular leukomalacia, and myelination abnormalities.

FIGURE 2: Familial Co-segregation Analysis



- Figure 2: Automated fluorescence dideoxy sequencing confirmation and familial co-segregation analysis. Since the unaffected brother only carries one of the *WARS2* alterations, the alterations co-segregate with the phenotype in the available family members.

REFERENCES

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TAKE-HOME POINTS

- *WARS2*, the last mt aaRS gene currently without a disease association, is now a candidate for a leukoencephalopathy similar to other mt aaRS deficiencies.
- Diagnostic WES is a tool for novel gene discovery.
- Novel gene analysis can be implemented in the clinical laboratory to provide diagnoses for patients with previously undiagnosed genetic disorders.