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Short Communication

Leigh syndrome associated with mitochondrial complex I deficiency due to novel mutations In *NDUFV1* and *NDUFS2*☆,☆☆

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ABSTRACT

Leigh syndrome (LS) is a progressive neurodegenerative disease caused by either mitochondrial or nuclear DNA mutations resulting in dysfunctional mitochondrial energy metabolism. Mutations in genes encoding for subunits of the respiratory chain or assembly factors of respiratory chain complexes are often documented in LS cases. Nicotinamide adenine dinucleotide (NADH):ubiquinone oxidoreductase (complex I) enzyme deficiencies account for a significant proportion of mitochondrial disorders, including LS. In an attempt to expand the repertoire of known mutations accounting for LS, we describe the clinical, radiological, biochemical and molecular data of six patients with LS found to have novel mutations in two complex I subunits (*NDUFV1* and *NDUFS2*). Two siblings were homozygous for the previously undescribed R386C mutation in *NDUFV1*, one patient was a compound heterozygote for the R386C mutation in *NDUFV1* and a frameshift mutation in the same gene, one patient was a compound heterozygote for the R88G and R199P mutations in *NDUFV1*, and two siblings were compound heterozygotes for an undescribed E104A mutation in *NDUFS2*. After the novel mutations were identified, we employed prediction models using protein conservation analysis (SIFT, PolyPhen and UCSC genome browser) to determine pathogenicity. The R386C, R88G, R199P, and E104A mutations were found to be likely pathogenic, and thus presumably account for the LS phenotype. This case series broadens our understanding of the etiology of LS by identifying new molecular defects that can result in complex I deficiency and may assist in targeted diagnostics and/or prenatal diagnosis of LS in the future.

1. Introduction

Leigh syndrome (LS) is a progressive neurodegenerative disorder, also known as subacute necrotizing encephalomyelopathy. The syndrome was first described by Leigh (1951) based on the pathological findings of widespread focal, bilaterally symmetrical subacute necrotic lesions extending from the thalamus to the spinal cord in a 7-month-old who presented with rapidly progressive neurological deterioration.

The incidence of LS is 1:77,000 live births (Rahman et al., 1996) and the prevalence is estimated at 1:34,000 (Fernandez-Moreira et al., 2007). The incidence of LS is higher in males (male-to-female ratio = 3:2), which is not solely explained by the X-linked inheritance of pyruvate dehydrogenase complex (PDHC) E1 alpha and complex I subunit *NDUFA1*-associated defects (Fernandez-Moreira et al., 2007; Rahman et al., 1996). The onset of clinical features is typically between 3 and 12 months of age (Rahman et al., 1996; Thorburn and Rahman, 2003); however, a later onset (including onset in adulthood) has been described in 25% of patients (Goldenberg et al., 2003; Huntsman et al., 2005). Initial features may be non-specific, including failure to thrive and/or persistent vomiting. Decompensation during intercurrent illness

Abbreviations: A, Alanine; C, Cysteine; CSF, Cerebrospinal fluid; E, Glutamic acid; F, Phenylalanine; G, Glycine; L, Leucine; LS, Leigh syndrome; MRI, Magnetic resonance imaging; MRS, Magnetic resonance spectrometry; mtDNA, Mitochondrial DNA; NAA, N-acetylaspartic acid; NADH, Nicotinamide adenine dinucleotide; nDNA, Nuclear DNA; NDUFA, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex; NDUFS, NADH-ubiquinone oxidoreductase Fe–S protein; NDUFV, NADH dehydrogenase (ubiquinone) flavoprotein; NDUFS2, NADH dehydrogenase (ubiquinone) Fe–S protein 2; nsSNP, Non-synonymous single nucleotide polymorphism; P, Proline; PDHC, Pyruvate dehydrogenase complex; POLG, Polymerase gamma; R, Arginine; SIFT, Sorting intolerant from tolerant.

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is common, with associated psychomotor regression and the presentation of new neurological features, and often incomplete recovery (Thorburn and Rahman, 2003). The most common neurological features are developmental delay (86%), seizures (79%), and altered level of consciousness (57%) (Huntsman et al., 2005). Other associated neurological features include abnormalities in tone, muscle weakness, movement disorders, ataxia, tremor, peripheral neuropathy, central respiratory disturbance, bulbar symptoms (dysarthria, dysphagia), and abnormalities of thermoregulation (Lee et al., 2009; Rahman et al., 1996; Thorburn and Rahman, 2003). Extraneurologic features include diabetes, short stature, hypertrichosis, anemia, cardiomyopathy (hypertrophic or dilated), hepatomegaly, renal tubulopathy or diffuse glomerulocystic kidney damage, and optic atrophy, retinitis pigmentosa, and ophthalmoplegia to varying degrees (Agapitos et al., 1997; Lee et al., 2009; Leshinsky-Silver et al., 2003; Tay et al., 2005; Yamakawa et al., 2001). The course of illness is one of episodic deterioration interspersed with plateaus, during which development may be staple or even progress. Ultimately, death occurs within early childhood (50% by 3 years of age) due to respiratory insufficiency or cardiac failure (Lee et al., 2009; Rahman et al., 1996; Thorburn and Rahman, 2003).

The diagnosis of LS requires a combination of clinical features, serologic and CSF investigations, radiological and pathological features. The criteria for LS have been outlined (Rahman et al., 1996), which require progressive neurologic disease with motor and intellectual developmental delay, signs and symptoms of brainstem and/or basal ganglia disease, raised lactate concentration in blood and/or CSF and one or more of characteristic features on neuroimaging. Neuropathological changes include multiple focal symmetric lesions in the basal ganglia, thalamus, brainstem, dentate nuclei and optic nerves with a spongiform appearance characterized by demyelination, gliosis and vascular proliferation and relative sparing of neurons. Typical MRI findings include bilateral symmetrical T2-weighted imaging hyperintensities in the brainstem and/or basal ganglia, particularly in the dorsal aspects of pons and medulla and putamen (Arii and Tanabe, 2000; Lee et al., 2009). MRS may reveal regional elevations in brain lactate levels.

Deficits of respiratory chain complex subunits (complex I, II, IV, and V) and their cofactors (e.g. co-enzyme Q10), mtDNA encoded tRNA or the PDHC are known causes of LS. Greater than 100 mutations have been identified thus far associated with a LS phenotype, both in mitochondrial DNA (30%) and nuclear DNA (Finsterer, 2008). Nicotinamide adenine dinucleotide (NADH):ubiquinone oxidoreductase (complex I) is the largest enzymatic complex (45 subunits) of the mitochondrial respiratory chain and deficiencies of complex I account for the majority of mitochondrial disorders, including LS (Smeitink and van den Heuvel, 1999). In recent years, mutations have been described in more than ten nuclear-encoded subunits of complex I, which account for the majority of complex I deficient cases in infancy and childhood with known molecular characterization.

We describe the clinical, radiological, biochemical and molecular data of six patients with LS found to have novel mutations in three complex I subunits (*NDUFV1* and *NDUFS2*). Two siblings were homozygous for the previously undescribed R386C mutation in *NDUFV1*, one patient was a compound heterozygote for a R386C mutation in *NDUFV1* and a frameshift mutation (753delCCCC), one patient was a compound heterozygote for R88G and R199P mutations in *NDUFV1* and two siblings were compound heterozygotes for the F84L and an undescribed E104A mutation in *NDUFS2*. After the novel mutations were identified, we employed prediction models using protein conservation analysis (SIFT, PolyPhen and UCSC genome browser) to determine pathogenicity of the novel mutations.

2. Methods

A retrospective chart analysis was conducted on six patients from four families with a diagnosis of LS known to have novel mutations in complex I subunits who presented to McMaster University Medical Center from 1999 to 2009.

All sequencing was completed using PCR amplification and sequencing of exons and 50 bp into the intronic region with dye-labeled primers for the target genes of for the NDUF mutations described below. Sequencing was completed using an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Protein conservation analysis was performed using the sorting intolerant from tolerant (SIFT) software (Kumar et al., 2009; Ng and Henikoff, 2001, 2002). The SIFT algorithm predicts whether an amino acid substitution in a protein of interest will have a tolerated or deleterious effect on protein function. This is accomplished by aligning similar protein sequences to the sequence in question and calculating normalized probabilities for all possible substitutions from the alignment to determine the evolutionary conservation status of the amino acid of interest. Normalized probabilities less than 0.05 are predicted to be deleterious, while probabilities greater than 0.05 are predicted to be tolerated. The sensitivity and specificity of SIFT in predicting the pathogenicity of single nucleotide polymorphisms associated with disease is 69% and 13%, respectively (Flanagan et al., 2010; Ng and Henikoff, 2002).

Polymorphism phenotyping (PolyPhen) (Ramensky et al., 2002; Sunyaev et al., 2000, 2001) was used for further prediction of pathogenicity. PolyPhen is a tool for predicting whether an amino acid substitution in a protein of interest will be benign or damaging. It focuses on non-synonymous single nucleotide polymorphism (nsSNP) effects by applying empirical rules to the sequence, phylogenetic, and structural information characterizing the amino acid substitution. A position-specific independent counts (PSIC) profile score is calculated that allows for the categorization of amino acid substitutions as benign, possibly damaging or probably damaging. The sensitivity and specificity of PolyPhen in predicting the pathogenicity of single nucleotide polymorphisms associated with disease is 68–82% and 16%, respectively (Flanagan et al., 2010; Ramensky et al., 2002).

Amino acid conservation was further sought from the UCSC genome browser (http://genome.ucsc.edu/) (Fujita et al., 2011; Kent et al., 2002). UCSC genome browser gives a pictorial representation of the sequence of the protein of interest in humans and 46 other vertebrate species and leaves the reader to assess whether or not a specific amino acid is conserved but does not itself calculate tolerability.

3. Clinical vignettes

3.1. Case 1

Case 1 is a male who was born at term to non-consanguineous parents of Southeast-Asian descent. Family history was non-contributory. He had an unremarkable medical and developmental history before he presented at 2.8 years with abnormal posturing of his left upper and lower extremity. Over the following 12 months, he developed developmental regression in a step-wise fashion associated with intercurrent illness, left eye esotropia, significant dysphagia requiring gastrostomy tube placement, ataxia, left hemi-body dystonic posturing, generalized spasticity, diffusely brisk reflexes, and extensor plantar responses. As a result of a high suspicion of a mitochondrial disorder, he was started on co-enzyme Q10, creatine monohydrate, riboflavin, thiamine, vitamin E, and vitamin C.

Investigations revealed a mildly elevated serum lactate concentration (3.1 mmol/L, NR<2.2 mmol/L) and a cranial CT scan showed bilateral symmetrical signal hypoattenuation involving the periventricular white matter and basal ganglia. MRI revealed bilateral symmetric hyperintense signal on T2-weighted imaging in the basal ganglia, thalamus, brainstem, corpus callosum and periventricular white matter, associated with cystic necrosis and a high lactate peak in affected areas on MR spectroscopy. A repeat MRI at 4 years showed significant improvement in the T2 hyperintense signal abnormalities and resolution of the lactate peak on