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Atypical presentation of Leigh syndrome associated with a Leber hereditary optic neuropathy primary mitochondrial DNA mutation

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ABSTRACT

Leber hereditary optic neuropathy (LHON) is caused by point mutations in mitochondrial DNA (mtDNA), and is characterized by bilateral, painless sub-acute visual loss that develops during the second decade of life. Here we report the case of a five year old girl who presented with clinical and neuroradiological findings reminiscent of Leigh syndrome but carried a mtDNA mutation m.11778G>A (p.R340H) in the *MTND4* gene usually observed in patients with LHON. This case is unusual for age of onset, gender, associated neurological findings and evolution, further expanding the clinical spectrum associated with primary LHON mtDNA mutations.

1. Introduction

Mutations in mitochondrial DNA (mtDNA) are associated with a spectrum of clinical syndromes including among others, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) and Leber hereditary optic neuropathy (LHON) [1]. The m.11778G>A transition changes a conserved arginine to a histidine residue within the most evolutionarily conserved region in the *MTND4* gene encoding a component of complex I of the mitochondrial respiratory chain [2]. This mutation is typically associated with the LHON phenotype, and is by far the most common LHON mutation, accounting for approximately 70% of cases observed among Caucasian populations [3]. It generally causes the most severe visual failure with only about 4% of patients experiencing visual recovery. The penetrance for developing optic atrophy differs depending on the gender of the individual carrying this mutation; the risk is 43% for males and 11% for females [4]. However, the risk of visual failure in both genders increases when this mutation arises in haplogroup J, and decreases when the mutation arises in haplogroup H [5]. The m.3460G>A is the second most common mtDNA mutation causing LHON, and also causes severe visual failure, although more than 20% of patients experience visual recovery. The m.14459G>A is the most pathogenic mtDNA mutation causing LHON, and is associated with visual loss, dystonia, a generalized movement disorder, pseudobulbar syndrome, and myopathic features among other clinical manifestations [6].

Leigh syndrome is usually a devastating neurodegenerative disease (also called subacute necrotizing encephalopathy) that presents mostly in infancy. From a neuroradiological perspective, it consists of almost identical lesions in the central nervous system, e.g., focal, bilaterally symmetrical lesions, particularly in the basal ganglia, thalamus, and brainstem [7,8]. The clinical presentation of Leigh syndrome is highly variable with considerable heterogeneity and is characterized by a wide variety of clinical features. Most frequently, the central nervous system is affected, with psychomotor retardation, epilepsy, nystagmus, ophthalmoparesis, optic atrophy, ataxia, dystonia, or respiratory failure associated with brainstem dysfunction. Some patients also present with involvement of the peripheral nervous system, including polyneuropathy, myopathy, or non-neurological abnormalities such as diabetes, short stature, hypertrichosis, cardiomyopathy, anemia, renal failure, vomiting, or diarrhea (atypical Leigh syndrome) [9]. The disease is usually fatal [10]. Leigh syndrome can be caused by mutations affecting either the mitochondrial or the nuclear genome leading to dysfunction of subunits of the respiratory chain (particularly complexes I, II, IV, or V), coenzyme Q_{10} deficiency, pyruvate dehydrogenase complex or pyruvate carboxylase deficiencies [9,11].

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Although various neurological features have been reported to be associated with the m.11778G>A and other primary LHON mutations, atypical Leigh syndrome is not a common feature.

Here we report the case of a child with a clinical phenotype suggestive of atypical Leigh syndrome associated with a primary mtDNA mutation typically observed in cases of LHON.

2. Materials and methods

All of the investigations in this study were undertaken as part of clinical diagnosis and medical management. No research investigations were performed, although informed consent approved by the Baylor College of Medicine Internal Review Board for the publication of this report was obtained from the family.

2.1. Mitochondrial DNA common mutation analysis

The proband's peripheral blood sample was screened for common mtDNA point mutations (m.3243A>G, m.3271T>C, m.8344A>G, m.8356T>C, m.8993T>G, m.8993T>C, m.8363G>A, m.11778G>A, m.3460G>A, m.13513G>A, m.13514A>G, m.14459G>A, and m.14484T>C) by multiplex PCR/allele-specific oligonucleotide (ASO) dot-blot analysis as previously described [12,13]. ARMS (allele refractory mutation system) qPCR targeted to the m.11778G>A mutation was performed to quantify the mutation heteroplasmy [14]. mtDNA deletions and rearrangements were analyzed by restriction enzyme digestion followed by Southern blot analysis [13].

2.2. Sequence analysis

Sequencing of the *PDHA1*, *POLG1*, *PC*, *SURF1*, *NDUFS2*, *NDUFS8*, *NDUFS7*, *NDUFV1*, *NDUFS3*, *NDUFS6*, *NUDFS4*, *NDUFAF2*, *NDUFA1*, *NDUFAF4*, *NDUFA7*, and *NDUFS5* genes was performed on the proband's peripheral blood sample. All coding exons plus 50 basepairs of the flanking introns were PCR amplified using sequence specific primers covalently linked at the 5′ end to M13 universal primers. The entire mitochondrial genome was amplified from the proband's skeletal muscle sample using 24 pairs of sequence specific overlapping primers [11,15,16].

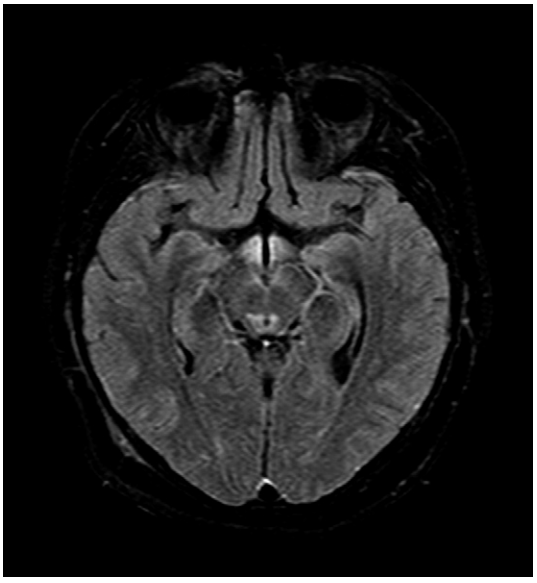

Fig. 1. MRI of the brain Axial T2 weighted FLAIR (FLuid Attenuated Inversion Recovery) image demonstrating an increased signal returned from the periaqueductal gray matter as well as the hypothalamus and deep gray matter surrounding the anterior aspect of the third ventricle, without evidence for mass effect.

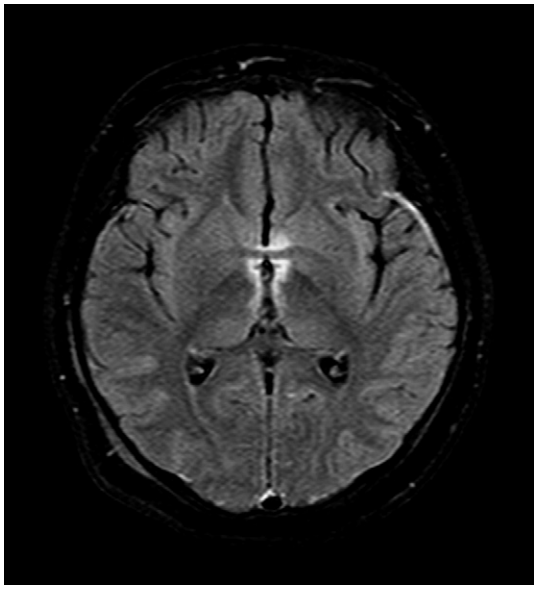

Fig. 2. MRI of the brain Axial T2 weighted FLAIR image confirming hyperintensity returned in a very symmetric fashion from the hypothalamic region as well as paralleling the third ventricle and straddling the anterior commissure. There was no enhancement following the administration of gadolinium (not shown).

Sequencing reactions were performed on purified PCR products using the BigDye Terminator Cycle Sequencing kit, and analyzed on an ABI3730XL automated DN sequencer. Sequences were analyzed using Mutation Surveyor version 3.20. GenBank sequences NM_000284.1, NM_002693.1, NM_000920.3, NM_003172.2, NM_004550, NM_002496.3, NM_024407.4, NM_007103.2, NM_004551.1, NM_004553.3, NM_002495.2, NM_174889.3, NM_004541, NM_014165.1, NM_005001.2, NM_004552.1 and NC_001807.4 were used as reference sequences for *PDHA1 POLG1*, *PC*, *SURF1 NDUFS2*, *NDUFS8*, *NDUFS7*, *NDUFV1*, *NDUFS3*, *NDUFS6*, *NUDFS4*, *NDUFAF2*, *NDUFA1*, *NDUFAF4*, *NDUFA7*, *NDUFS5* and the whole mitochondrial genome respectively.

2.3. Electron transport chain enzyme analysis

Spectrophotometric analysis of the respiratory chain complexes was performed on the proband's skeletal muscle sample. The electron transport chain enzymes were assayed at 30 °C using a temperature-controlled spectrophotometer (Tecan M200 Microplate Reader, Durham, NC). The activities of complex I (NADH:ferricyanide reductase), complex II (succinate dehydrogenase), rotenone sensitive complex I + III (NADH:cytochrome *c* reductase), complex II + III (succinate:cytochrome *c* reductase), and complex IV (cytochrome *c* oxidase) were measured using appropriate electron acceptors/donors [17]. Each assay was performed in duplicate [18,19].

2.4. Mitochondrial DNA copy number analysis

Mitochondrial DNA copy number in the proband's muscle sample was determined using real-time quantitative PCR as previously reported [20]. All samples were assayed in triplicate. Fluorescent signal intensities of PCR products were recorded and analyzed on a 7900HT Fast RT_PCR system (Applied Biosystems, Foster City CA). The mtDNA content (referred to as the mtDNA copy number relative to single copy nuclear genes) was calculated based on the difference in threshold cycle numbers between the nuclear and mitochondrial genes of the patient, followed by comparison to the mtDNA content of age and tissue matched pooled control [21].