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Illness-induced exacerbation of Leigh syndrome in a patient with the *MTATP6* mutation, m. 9185 T>C

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Leigh syndrome; brainstem; magnetic resonance imaging; complex V

Introduction

Leigh syndrome is a common and distinctive mitochondrial disease syndrome. However, before modern neuroimaging, only pathological analysis could verify disease (Leigh, 1951). The advent of nuclear magnetic resonance imaging (MRI) has virtually supplanted pathological verification of Leigh syndrome (Saneto et al., 2008; Rhaman et al., 1996). Furthermore, strict criteria have narrowed phenotype expression, but multiple genetic etiologies and other unknown processes likely influence the range of phenotypic variability (Finsterer, 2008; Rhaman et al. 1996). Whether environmental factors alter phenotypic variability is unknown. Typical onset is usually within the first 2 years of life with heterogeneous involvement of the basal ganglia, brainstem, or white matter on MRI (Barkovich et al., 1993; Rossi et al., 2003; Ostergaard et al., 2007). The variability of MRI findings may be related to the genetic mutation location.

The common pathway of disease is defective function in one or more of the electron transport chain complexes caused by mutations in mitochondrial DNA (mtDNA) or nuclear genes (Finsterer, 2008). Although multiple mtDNA mutations have been reported, by far the most common mutation resides in the *MTATP6* gene, m. 8993 T>G (Rahman et al., 1996). The *MTATP6* gene is one of two mtDNA genes that comprise the F0 domain of complex V that is embedded in the mitochondrial inner membrane and conducts protons from the intermembrane space into the matrix.

We report a patient with nearly 100% homoplasmy, m. 9185 T>C mutation with Leigh syndrome. His initial disease presentation and subsequent exacerbations corresponded to changes seen on MRI scan and febrile viral-like illness.

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Case Report

Our patient is the second child of unrelated parents of European decent (Figure 1). His older brother is healthy. Of the maternal relatives, the only exception of chronic illness is the grandmother's brother (II-3) who was mentally retarded and died at age 60 years. Our patient was a product of a normal pregnancy and delivery. Psychomotor development was normal until the age of 3 years, when he developed a febrile illness with viral illness-like symptoms. After two months of lingering symptoms of fatigue and episodic cough, within a two week period he developed unexplained changes of abnormal breathing pattern, hyperphagia, bilateral ptosis, ataxia, wide-based gait with lumbar lordosis, and coughing with meals. He was referred to our hospital. Testing demonstrated lactate level of 3.5 mmol/L (normal value, < 2.1 mmol/L). Quantitative plasma amino acids demonstrated an elevation of alanine levels and urine organic acids showed elevated lactate levels. Serum coenzyme Q10 and plasma acyl carnitine levels were normal. Electroencephogram (EEG) showed generalized slowing without epileptiform discharges. MRI scan of the brain demonstrated T2 and Fluid Attenuated Inversion Recovery (FLAIR) hyperintensity within the periaqueductal region and bilateral basal ganglia (Figure 2). Three days after admission, he suffered a probable pulmonary hemorrhage with acute hypotension and required mechanical ventilation. Due to poor oxygenation, extracorporeal membrane oxygenation (ECMO) and cardiovascular inotropic support were started. ECMO was weaned over the next 7 days. Oscillating ventilation was required for the next 3 days, with a subsequent day on normal mechanical ventilation. He was discharged from the hospital after muscle biopsy. Lactate levels had normalized. Muscle biopsy was performed with demonstrated normal electron transport chain enzymology and electron microscopy findings (data not shown). Common mitochondrial DNA (mtDNA) mutations were negative.

Repeat MRI scan of the brain two years later demonstrated resolution of the periaqueductal T2/FLAIR changes, but putamen and caudate head changes seen on the previous MRI scan remained (Figure 2). He remained healthy for the next 5 years making developmental gains. Unfortunately, his muscle endurance difficulties remained. He described difficulty keeping up with and quitting activities before his peers. He required extra help at school and was in special education in math and reading.

During the summer of his 8th year, he went to Europe with his family and developed a febrile viral-like illness, with resulting increased muscle fatigue and decreased endurance. Parents noticed increased clumsiness when walking distances. His speech became more dysarthric with a scanning quality. The summer's elevated environmental temperatures exacerbated muscle fatigue. Within a month, due to worsening symptoms, he was hospitalized. Upon admission, venous lactate and cerebral spinal fluid (CSF) lactate levels were normal. His MRI demonstrated progressive signal hyperintensities within the putamen and caudate, along with new T2/FLAIR changes in the midbrain and right cerebellar hemisphere (Figure 2). Proton magnetic resonance imaging (MRS) demonstrated lactate peaks confirming oxidative phosphorylation derangement (Figure 4). He rapidly improved with normal saline containing 5% dextrose and L-carnitor (50 mg/kg) containing fluids and was discharged within 4 days. Before discharge, blood lymphocytes were sent for complete mtDNA sequencing and an apparent homoplasmic mutation, m.9185 T>C (p.L220P) in *MTATP6*, was found. Mother was tested and found to have a low level of heteroplasm (30%) of the m. 9185 T>C mutation.

After recovery to near baseline, two years later he developed increased gait ataxia, decreased muscle endurance, and cognitive slowing in the context of another febrile viral illness. MRI scan demonstrated an increase in signal in the basal ganglia, midbrain, right cerebellum, and new changes in the left cerebellar hemisphere (Figure 3). The MRS scan demonstrated the

presence of lactate peaks (Figure 4) demonstrating defects in oxidative phosphorylation. Clinically, although there has been some recovery, there have been progressive muscular and cognitive deficits. His gait is more ataxic and wide-based and his teachers report more time is required to process information. Handwriting is more difficult to decipher showing increased problems with fine motor skills and likely reduced muscle endurance. He requires more rest periods throughout the day due to fatigue.

Analysis of mtDNA

Peripheral blood leukocytes were used for total DNA extraction and entire mtDNA genome was PCR amplified using overlapping primers. Each fragment was sequenced in the forward and reverse directions using automated fluorescent dideoxy sequencing (Liang and Wong, 1998; Baylor College of Medicine, Medical Genetics Laboratory, Houston, TX). Nucleotide numbering is in accordance to the revised Cambridge sequence (Anderson et al., 1999). Heteroplasmy was determined by conventional methods (Bai and Wong, 2005; Baylor College of Medicine, Medical Genetics Laboratory, Houston, TX).

Biochemical and morphological analyses

Muscle was obtained from the vastus lateralis and portions were prepared for histochemical and electron microscopy analysis. Another portion was quickly frozen at -70°C and later used for enzyme analysis. Histochemical analysis and electron microscopy was performed using standard procedures. Spectrophotometric enzyme analysis was performed by standard protocols (Marin-Garcia et al., 1999; The Molecular Cardiology and Neuromuscular Institute, Highland Park, NJ).

Results

Biochemical and morphological analyses: Enzyme activities of complex I, II, I/III, III and IV were all within normal control values (data not shown). Complex V could not be measured as the muscle sample was frozen before assay. Mitochondria were seen in normal numbers without histochemical or structural abnormality. Ragged red fibers were not identified.

Analysis of mtDNA: Initial mtDNA testing for common mutations at 3243, 3271, 8344, 8993 did not reveal abnormality. Sequence analysis of the entire mtDNA genome revealed an apparent homoplasmic T>C substitution at nucleotide position 9185, m. 9185 T>C, in *MTATP6*. This mutation replaces a highly conserved leucine to proline at codon 220 (p.L220P). The amino acid leucine is conserved from *Drosophila* to human. The mutation was also present in the blood sample of the patient's asymptomatic mother at a level of approximately 30%.

The boy's mtDNA was found to have known polymorphism naturally occurring in the *MTATP6* of m. 8860 A>G. The *MTATP6* polymorphisms of m.9055 G>A, m.9010 T>C, m. 9101 G>A, m. 9163 G>A, m.9176 T>C, and m. 9181 A>G were not present.

Discussion

Our patient has an apparent homoplasmic missense mutation at m.9185 T>C found in the mtDNA *MTATP6* gene. His mother had approximately 30% heteroplasmy. This mutation and similar phenotypic description to Leigh syndrome has been previously reported as a single case report (Moslemi et al., 2005) and in multiple family members in another study (Castagna et al., 2007). Consistent with the previous studies, early development is normal until the initial exacerbation of disease. However, unlike the previous studies of Moslemi et al. (2005) and Castagna et al. (2007), our patient presented at an earlier age, 3 years versus 7

years. His disease exacerbations were temporally related to febrile viral-like illness, with exacerbations occurring years apart. The clinical exacerbations were also related to progressive changes on MRI (Figures 2 and 3) and brain lactate noted on MRS (Figure 4). An MRI scan performed during a quiescent disease period demonstrated some, but not full, resolution. The latter would strongly suggest that MRI changes were not progressive without environmental stressor of a febrile viral-like illness. Febrile illness was also associated in some patients, but not all patients, in the previous reports (Moslemi et al., 2005; Castagna et al., 2007). It is not clear why our patient's clinical symptoms and MRI findings seem sensitive to febrile illness. More patients and studies are needed to understand this finding.

The exact mechanism of how the m. 9185 T>C (p.L220P) mutation alters the F1F0 ATPase (complex V) in the induction of disease is not known. Castagna et al. (2007) proposed that the proline substitution alters the alpha-helix structure and interferes with the proton pump. However, ATP synthesis in lymphoblast mitochondria isolated from the proband in their study did not show abnormality. Although completely without support, the correlation between febrile illness and disease exacerbation seen in our patient might suggest conformational change that alters stability of the protein.

Our patient presented later than the average age of Leigh syndrome patients, 3 years instead of 2 years, but earlier than the previously reported m. 9185 T>C patients. The most likely explanation for our patient's earlier onset, compared to the other patients, may be the 100% homoplasmy of his mutation. The larger family study demonstrated that those family members with higher heteroplasmy, >85%, produced more severe and earlier disease (Castagna et al., 2007). The degree of heteroplasmy is also related to disease severity and onset by other mutations in the *MTATP6*. The most common mutation, m. 8993 T>G, is expressed as Leigh syndrome at high heteroplasmy (>90%) and at lower heteroplasmy with neurogenic muscle weakness, ataxia, and retinitis pigmentosa (Carelli et al., 2002; Tatuch et al., 1992; Holt et al., 1990).

Most mtDNA disorders are heteroplasmic with higher heteroplasmy inducing more involved disease (Carelli et al., 2002; Tatuch et al., 1992; Holt et al., 1990). However, there are mtDNA disorders that are 100% homoplasmy for pathological mutations yet have somewhat favorable outcomes (McFarland et al., 2007; Chapiro et al., 2002). Some 100% homoplasmic mtDNA pathological mutations have severe outcomes with death at very young ages (Taylor et al., 2003; McFarland et al., 2002). The reason for the dichotomy is not entirely clear. Some mutations are tissue specific which may impart favorable outcome, such as in Leber Hereditary Optic Neuropathy (Wallace et al., 1988). Other homoplasmic mutations are hypothesized to induce maturational impairment leaving residual activity, but the exact mechanism remains unclear (McFarland et al., 2007). We were unable to test for residual complex V activity of our patient, so the exact mechanism of his more favorable outcome remains to be elucidated.

The clinical course of this mutation is variable, but seems to be progressive. The normal early psychomotor development has been reported in all patients thus far. In many, but not all patients with m. 9185 T>C, a febrile illness was the disease trigger. Degree of heteroplasmy may dictate, to some extent, disease progression. At higher heteroplasmy, onset of disease may be fulminant as demonstrated by our patient and the proband case described by Castagna et al. (2007). Our patient was aggressively treated with ECMO and cardiovascular inotropic medications with good recovery. As demonstrated with the current long term survival of our patient, aggressive medical intervention is warranted with this mutation.

Progressive cerebellum involvement was demonstrated by clinical changes and MRI scans in our patient. Cerebellum changes and clinical symptoms were also described by Castagna et al. (2007). Changes in cerebellum were originally described by Leigh (1951) but in patients with mutations at m. 9185 T>C, cerebellar symptoms seem more pronounced. The reason is not clear, but even with lower heteroplasmy, the cerebellum is a major brain region affected. Our patient also demonstrated that as his disease progressed, more involvement of the basal ganglia was noted on MRI. This suggests that the cerebellum as well as basal ganglia, putamen and caudate, become more involved as disease progresses. The relationship between changes on MRI and other genetic etiologies of Leigh syndrome has been described but limited to a few gene mutations (Saneto et al., 2008; Ostergaard et al., 2007; Bohm et al., 2006; Rossi et al., 2003). The variety of MRI changes suggests that various brain regions may have different bioenergetic sensitivity. One wonders if sensitivity is mutation specific in general and in particular with intragenetic *MTATP6* mutations. This remains to be tested.

Our patient demonstrates that fulminate onset and disease exacerbation are related to febrile illness, affecting the basal ganglia and cerebellum, in the context of m. 9185 T>C. The mechanism of how febrile illness alters mitochondrial function in the context of this particular mutation is not clear. The outcome for patients seems to be favorable, although aggressive medical intervention may be required.

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Figure 1.

Diagram shows the family pedigree. The maternal relatives are all healthy with the exception of the maternal grandmother's brother (II-3) who was mentally retarded and died at age 60 years. The maternal great grandmother (I) died at age 98 years. Mitochondrial DNA sequencing was only done on the index case (our patient) and his mother. The only inherited disorder in the family is ductal breast cancer; the mother (III-2), maternal grandmother (II-2) and maternal grandmother's sister (II-4) have been diagnosed. All are currently in remission and are long term survivors (>5 years). There is no history of musculoskeletal, neuropathy, or cognitive problems (with the exception of the maternal grandmother's brother).

Figure 2.

MRI scans are shown from the initial exacerbation of his disease and during a quiescent period. At 3-years of age he had his initial exacerbation (2002). The MRI represents an axial T2 weighted image taken after hospitalization (A). This image demonstrates punctuated hyperintensity within the putamina (small arrows) and caudate heads (large arrows, A) and periaqueduct gray (small arrows, B). A two year follow-up MRI is shown during a quiescent period. These scans represent T2 weighted images. There was stable appearance of the putamina (small arrows) and caudate head (large arrows) regions resembling the 2002 scan (C) with resolution of the periaqueduct changes (small arrows, D) compared to the 2002 scan.

Figure 3.

MRI scans are shown from the subsequent two disease exacerbations. In 2007 he suffered another exacerbation of his disease. The MRI scan shows an increased T2 signal involving more of the putamina (small arrows) and caudate heads (large arrows, A) with new involvement of the right cerebellar hemisphere (large arrows, B). Two years later, 2009 he had another exacerbation of his disease with new T2 weighted changes in the putamina (small arrows) and caudate heads (large arrows, C) and now left cerebellar hemisphere (large arrows, D).

Figure 4. MRS scans are shown representing our patient's disease exacerbations in 2007 and 2009. The MRS scan in Figure 4A represents the 2007 exacerbation. A large lactate peak (1.33

ppm) can be seen demonstrating abnormal oxidative phosphorylation within the left basal ganglia. The spectra was acquired at 1.5 T (Siemens Avanto scanner, TE 30, TR 1.5 sec, 16×16 matrix, interpolated to 32×32 , 16 cm) showing this dramatic elevation of lactate. There is also a decrease in the N-acetyl-L-aspartate (NAA) peak (2.02 ppm) suggesting neuronal or axonal loss. The MRS scan represented in Figure 4B is of the scan taken during the 2009 exacerbation. In this scan, we summed the region depicted by the square outline, which represents the acquisition from the complete summed region of the 16×16 matrix (Siemens 3T, TE 30, TR 1700, 16×16 matrix). The CSF analysis did not detect significant lactate. We had to use this large summed voxel to detect lactate peak (1.33 ppm), due to low concentrations. The presence of lactate in the brain parenchyma demonstrates oxidative phosphorylation defects, although not as significant as the 2007 study.