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Severe Infantile Leigh Syndrome Associated With a Rare Mitochondrial *ND6* Mutation, m.14487T>C

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We describe a case of severe infantile-onset complex I deficiency in association with an apparent de novo near-homoplasmic mutation (m.14487T>C) in the mitochondrial *ND6* gene, which was previously associated with Leigh syndrome and other neurological disorders. The mutation was near-homoplasmic in muscle by NextGen sequencing (99.4% mutant), homoplasmic in muscle by Sanger sequencing, and it was associated with a severe complex I deficiency in both muscle and fibroblasts. This supports previous data regarding Leigh syndrome being on the severe end of a phenotypic spectrum including progressive myoclonic epilepsy, childhood-onset dystonia, bilateral striatal necrosis, and optic atrophy, depending on the proportion of mutant heteroplasmy. While the mother in all previously reported cases was heteroplasmic, the mother and brother of this case were homoplasmic for the wild-type, m.14487T. Importantly, the current data demonstrate the potential for cases of mutations that were previously reported to be homoplasmic by Sanger sequencing to be less homoplasmic by NextGen sequencing. This case underscores the importance of considering mitochondrial DNA mutations in families with a negative family history, even in offspring of those who have tested negative for a specific mtDNA mutation.

Key words: Leigh disease; deep sequencing; homoplasmy; mitochondrial disease

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INTRODUCTION

Leigh syndrome is a severe, childhood-onset, mitochondrial disorder leading to psychomotor regression and characteristic MRI features. Complex I deficiency is a common subgroup of Leigh syndrome with mutations identified in both the nuclear-encoded (i.e., *NDUFS1*, *S3*, *S4*, *S7*, *S8*), and the mitochondrial DNA (mtDNA)-encoded (i.e., m.11777C>A, m.14459G>A) complex I genes. The specific mutation does not usually predict the severity of the disorder; however, some mtDNA mutations, such as the 8993T>C/G in a complex V gene, show a fairly predictable genotype–phenotype correlation [Tatuch et al., 1992; Fujii et al., 1998]. In the case of m.8993T>G, low heteroplasmy (< 60%) often is asymptomatic, intermediate levels 70–95% are associated with neurogenic ataxia and retinitis pigmentosa (NARP) or movement disorders (bilateral striatal necrosis, BSN), and high mutational load (>95%) is associated with Leigh syndrome [Tatuch et al., 1992].

The m.14487T>C mutation in the mtDNA *ND6* gene encodes a structural complex I subunit, and has been reported in the neurological disorders of optic atrophy, BSN, childhood-onset dystonia, progressive myoclonic epilepsy, and Leigh syndrome [Solano et al., 2003; Ugalde et al., 2003; Malfatti et al., 2007; Wang et al., 2009; Dermaut et al., 2010]. Among cases labeled with Leigh syndrome, the degree of mutant heteroplasmy in muscle was 95–100% in three cases [Malfatti et al., 2007; Wang et al., 2009; Dermaut et al., 2010], but only 65% in one case [Ugalde et al., 2003]. Lesser degrees of mutant heteroplasmy were also reported in cases labeled as BSN (93% [Solano et al., 2003]), and optic atrophy [Malfatti et al., 2007]. Also consistent with the fact

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that higher levels of heteroplasmy are generally associated with a more severe phenotype [Tatuch et al., 1992], in one family, homoplasmy (100% mutant) was associated with Leigh syndrome, 97–99% with progressive myoclonic epilepsy, and 8–35% with "migraine with aura, Leber hereditary optic neuropathy (LHON), sensorineural hearing loss and diabetes mellitus type 2," all in muscle [Dermaut et al., 2010]. In two of the cases evaluated, transmitochondrial cybrids showed a severe complex I deficiency [Solano et al., 2003; Ugalde et al., 2003].

In each case previously reported with m.14487T>C, the mother was heteroplasmic [Malfatti et al., 2007]. Also, in each case, recognition of an mtDNA mutation was facilitated by apparent maternal inheritance and/or obvious heteroplasmy in the proband's blood. We herein report a case of fatal Leigh syndrome in an infant with a negative family history, in which the patient was near-homoplasmic for m.14487T>C in muscle, and his mother and two siblings are homoplasmic for the wild-type m.14487C. This case supports the association of high-level heteroplasmy of m.14487T>C with Leigh syndrome, and underscores the importance of NextGen sequencing in differentiating near-homoplasmic from truly homoplasmic mutations in mitochondrial disease. Importantly, we also demonstrate the potential for mtDNA mutations to arise sporadically within a family.

PATIENTS AND METHODS

A 6.5-month-old male infant (proband) presented to the emergency room with progressive respiratory issues including feeding-induced cyanosis. He was found to be somnolent with low oxygen saturation, and was intubated and ventilated. The child was born at term without complications, and was delayed in gross motor development; by 6 months he was not rolling or making any attempts to push up when placed on his stomach. Hearing and vision appeared intact. There were no seizures. In the weeks prior to his emergency admission, he was noted to be taking deep breaths, feeding for short periods of time, pulling back, and gasping for air with progressive peri-oral cyanosis during feeding. He had dysconjugate eye movements. Lactate was 3.0 mmol/L in serum (normal <2.2 mmol/L). CT scan showed decreased attenuation in basal ganglia and thalamus suspicious for BSN and/or Leigh syndrome. MRI showed hyperintensity on T2-weighted imaging in the putamen and globus pallidus, thalami and brain stem consistent with Leigh syndrome. He was extubated a week later and sent home on gastrostomy tube feedings. Follow up neurological examination demonstrated nystagmus, poor head control, severe hypotonia with brisk knee and ankle reflexes, and an extensor plantar response. A muscle biopsy during the initial admission was normal at the light and electron microscopic level, but showed a mitochondrial complex I + III enzyme deficiency, confirmed on fibroblasts (see below).

When the proband was 8 months of age, his mother became pregnant and wished to have chorionic villous sampling and prenatal counseling. These tests confirmed a normal complex I activity (144 mU/U CS, NR = 80–260) [Robinson, 2001], and she subsequently gave birth to a healthy baby boy. The proband died at 13 months of age with increasing apneic episodes and respiratory insufficiency. Five months after this event, the parents became extremely anxious about the possibility that the brother may still develop complex I-associated Leigh syndrome. Consequently, a needle muscle biopsy and fibroblast culture were obtained showing a normal lactate to pyruvate ratio in fibroblasts and normal complex I + III activity with the muscle biopsy being normal at the light and electron microscopic level and showing normal complex I + III activity (0.75 mol/min/g, NR = 0.5–1.9).

The brother went on to have normal development and is currently healthy at age 10, as is his sister, currently age 14. The mother is healthy with no neurologic issues. Muscle DNA from the proband and his brother was extracted according to conventional methods. The mitochondrial DNA from both was selectively amplified using proprietary primers and sequenced by Courtagen Laboratories using Next Generation sequencing technology and Illumina's Miseq instrumentation. The samples were sequenced to an average depth of 10,000×, enabling detection of heteroplasmic variants at levels <1%. In addition, the sequence for approximately 1,100 nuclear-encoded genes, comprising essentially all known mitochondrial-located proteins and including all MitoCarta genes (http://www.broadinstitute.org/pubs/MitoCarta/human.mitocarta.html) was obtained for the proband. Any clinically relevant mutations were verified by Sanger sequencing. Muscle biopsy respiratory chain enzyme activity for complex I + III, II + III, IV and citrate synthase were measured as previously described [Safdar et al., 2010], and fibroblast culture was established with enzyme activity for complex I, I + III, II + III, IV, citrate synthase determined as previously described [Robinson, 1994].

RESULTS

Biochemical analysis of the muscle from the proband demonstrated an isolated complex I deficiency with a compensatory increase in citrate synthase (complex I + III = 0.32 [NR = 0.5–1.9]; complex II + III = 2.3 [0.6–2.3]; cytochrome *c* oxidase = 4.7 [0.6–2.5]; citrate synthase = 15.2 [0.8–5.5]) and fibroblasts (complex I = 8.2 [18.9–44.2]; complex II + III = 63.9 [41.8–74.4]; complex IV = 183 [147–252]; citrate synthase = 213 [144–257]). In contrast, the unaffected brother showed normal complex I + III activity (0.75 [0.5–1.9]) in muscle and normal complex I + III in fibroblasts (44.0 [36.5–42.6]) as well as normal complex I activity in the chorionic villus sample in utero (data above).

No heteroplasmic sequence variants were found in muscle samples from the proband, his brother or their mother by NextGen sequencing. Each sample showed identical homoplasmic sequence variants: m.5293G>A; Ser275Asn and m.13135G>A; Ala276Thr, and were from haplogroup H1 (m.3010G>A). The sequences were compared, and the only difference was that the proband showed near homoplasmy for m.14487T>C (99.4% mutant; Met63Val [appearing homoplasmic by Sanger sequencing]; yet, neither the white blood cell-derived DNA from his mother nor the muscle-derived DNA from his brother showed the m.14487T>C transition mutation using both NextGen and Sanger sequencing (Table I).

DISCUSSION

The current case confirms pathogenicity of the m.14487T>C sequence variant previously described as a cause of Leigh syn-

TABLE I. Next Gen Sequencing of the m.14487T>C Mutation

Sample	# Reads = T (wt)	# Reads C	# Reads G	# Reads A	Heteroplasmy (%)
Patient	33	5951	0	0	99.4
Mother	4,929	7	6	12	<0.1
Brother	5,453	1	2	4	<0.1

Data are the number of reads for the specific nucleotide (A, C, G, T) with T as the wild-type (wt) and C as the mutant. The % heteroplasmy is the ratio of mutant/wt × 100. For the mother and the brother, the very low proportion of C reads were no different than the G and A reads, and likely represent nonspecific sequence "noise;" that is the true % heteroplasmy is less than 0.1% and likely represents true homoplasmy for the wild-type.

drome and other neurological phenotypes [Solano et al., 2003; Ugalde et al., 2003; Malfatti et al., 2007; Wang et al., 2009; Dermaut et al., 2010]. This conclusion is strengthened because essentially every mitochondrial gene encoded by both mtDNA (in muscle) and nuclear DNA was sequenced with high coverage and no other mutations were identified. This case also provides support for higher degrees of heteroplasmy resulting in more severe clinical phenotypic severity, with infantile-onset Leigh syndrome generally associated with m.14487T>C heteroplasmy greater than 95%.

The unique aspect of the current case report is that the near-homoplasmic m.14487T>C mutation appears to have arisen from a mother who did not have the mutation somatically. In all previous cases of a m.14487T>C mutation, mothers of affected offspring showed heteroplasmy in blood of 26–65% [Solano et al., 2003; Ugalde et al., 2003; Malfatti et al., 2007; Dermaut et al., 2010]. The complete absence of the mutation in skeletal muscle from an unaffected sibling implies the possibility of either germ line mosaicism in the mother or a spontaneous de novo mutation arising during embyrogenesis in the proband.

Pre-natal diagnosis for mothers harboring heteroplasmic mtDNA mutations remains a challenge. Much of the positive experience comes from the m.8993 Leigh/NARP mutations where the mutational burden of the chorionic villus or amniocentesis can be used to predict the phenotypic severity of the offspring, and, in general, women with lower mutational burdens are at lower risk for the transmission of very high heteroplasmic mutational load associated with Leigh syndrome [White et al., 1999; Thorburn and Dahl, 2001]. In the case of m.14487T>C, the heteroplasmic load of the mothers in previous reports (26–65%) could not be used to predict mutational load/phenotypic expression in the offspring [Solano et al., 2003; Ugalde et al., 2003; Malfatti et al., 2007; Dermaut et al., 2010], and this case further highlights this observation in that a mother with essentially 0% heteroplasmy gave birth to a son with severe Leigh disease and 99.4% mutant heteroplasmy. In this case, the chorionic villus sample provided an accurate representation of the clinical phenotype (normal complex I in both CVS and childhood fibroblasts associated with absence of syndrome); however, the absence of a CVS sample from the proband in utero, and limited sample size leaves us unable to comment on the possibility of the universality of the relationship (i.e., false negative or false positive rates, in the former and later situation, respectively). Unfortunately, we did not have any of the CVS sample remaining to confirm the absence of the m.14487T>C in the brother in utero; however, complete absence of the mutation in his muscle biopsy using NextGen sequencing (<0.1% mutant) implied that he did not inherit the mutation.

In summary, our findings, in combination with previous reports [Dermaut et al., 2010], demonstrate that the m.14487T>C mutation is associated with Leigh syndrome and additional neurological phenotypes on a phenotypic spectrum of severity likely influenced by the mutational heteroplasmy. Leigh disease occurs under the condition of homoplasmy or near-homoplasmy, which, like complex I deficiency with LHON (m.11778G>A, m.3460G>A, m.14484T>C), reversible infantile COX deficiency (m.14674T>C/G), and m.1555A>G-related deafness, demonstrates the importance of assessing for pathological mutations among homoplasmic sequence variants. Finally, most cases of homoplasmy are reported using Sanger sequencing or chip-based technology [Dermaut et al., 2010], and this case highlights the value of NextGen sequencing in evaluating either very low or very high levels of mutant heteroplasmy undetectable by other methods.

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