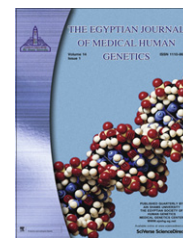




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## CASE REPORT

# Hypothyroidism could be the only manifestation of mitochondrial T8993C mutation in Leigh syndrome

Hoda Tomoum <sup>a</sup>, Solaf M. Elsayed <sup>b,\*</sup>, Elizabeth Berry-Kravis <sup>c</sup>

<sup>a</sup> Department of Pediatrics, Ain Shams University, Cairo, Egypt

<sup>b</sup> Genetics Unit, Department of Pediatrics, Ain Shams University, Cairo, Egypt

<sup>c</sup> Departments of Pediatrics, Neurological Sciences, and Biochemistry, Rush University Medical Center, Chicago, USA

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### KEYWORDS

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**Abstract** Mitochondrial DNA-associated Leigh syndrome is a part of a continuum of progressive neurodegenerative disorders caused by abnormalities of mitochondrial energy generation. Mitochondrial T8993C and T8993G mutations account for 10–20% of these cases. T8993C is generally associated with milder phenotype than T8993G mutation. Here we report an Egyptian family with T8993C mutation with unusual early onset of severe phenotype in three sisters (consisting of regression of previously acquired motor and mental milestones after an attack of viral infection) and hypothyroidism as the only presenting symptom in their brother. The mother (like her son) carried the T8993C mutation and was asymptomatic. This unusual lack of manifestation could be attributed to different percentages of mutated mitochondrial DNA in the brain or muscle or perhaps to some unknown protective factor. The hypothyroidism could be a simple association, but to the best of our knowledge, no previous reports have described hypothyroidism in carriers of this mutation.

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### Case report

We report an 8 year old Egyptian girl, 4th child of non consanguineous parents, born by elective cesarean section with unremarkable antenatal and postnatal periods. Her milestones

\* Corresponding author. Address: Medical Genetics Center, 27A Baghdad Street, Korba, Cairo, Egypt. Tel.: +20 2 4151999; fax: +20 2 4150977.

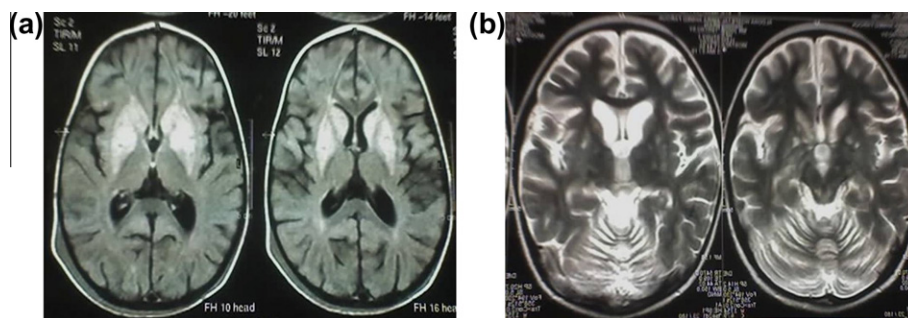
E-mail address: [elsayed683@yahoo.com](mailto:elsayed683@yahoo.com) (S.M. Elsayed).

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were achieved very satisfactorily until the age of 4 years when she developed severe upper respiratory tract infection and after 2 weeks developed hematemesis and melena, and became floppy, with marked regression in her previously acquired developmental milestones. She became aphasic together with gradual deterioration of the level of consciousness. She was admitted to the intensive care unit for 45 days without the need for mechanical ventilation and was discharged non-ambulatory. Her condition has been stable since then and she has even shown some improvement. On examination, her weight and length were below the 5th percentile. She was alert, and could fix and follow visual stimuli. She responded to auditory stimuli and interacted appropriately with her surroundings. She was aphasic though she understood and performed simple orders.



**Figure 1** T2WI FLAIR (a) and T2WI (b) magnetic resonance imaging showing focal zones of abnormal signal (high on T2-weighted images) within the basal ganglia.

Cranial nerve examination and formal ophthalmic examination showed no abnormality. Her muscle bulk was clearly reduced with atrophy and a generalized increase in muscle tone and weakness. Though, she was moving freely in bed, she could barely move her arms and thighs against gravity. Her hand manipulation and fine hand movements were better than her proximal movement. Deep tendon reflexes were brisk with left patellar clonus, bilaterally positive Babinski and intact abdominal reflexes. The girl was non-ambulatory with no evidence of limb ataxia. There was no limb deformity or abnormal involuntary movements. She could appreciate pain in all sensory dermatomes.

Chest heart and abdominal examination was clinically free.

An initial work up was done including serum electrolytes, liver and kidney function tests, and ammonia level which all revealed normal results. Serum lactate was elevated (50 mg/dl and 40 mg/dl in two separate occasions). Screening for amino-acidopathies and fatty acid oxidation defects was normal. Also, urine organic acid testing showed no abnormalities.

Needle EMG and conduction studies were not suggestive of any definite pathology. MRI brain showed mild diffuse volume loss with prominent cortical sulci and extraaxial CSF spaces and cerebellar folia. Bilateral focal zones of abnormal signal within the basal ganglia were present. These zones appeared with high signal on T2-weighted images, low signal on T1 and showed diffusion restriction producing intense bright signals on diffusion-weighted images (Fig. 1).

The child had a very significant family history. Her eldest brother was diagnosed soon after birth in the National neonatal screening program with congenital hypothyroidism and received L-thyroxine since then and was otherwise comparable to peers. The second sib was a female who was also normal until the age of 8 months when she developed upper respiratory tract infection, vomiting and diarrhea which did not respond to treatment. After 30 days, she developed fever and seizures, and progressed to loss of consciousness. She was admitted to the PICU where she developed thrombocytopenia and hepatocellular failure and died 13 days after admission. The third sib was also a female, who was normal until the age of 1.5 years when she also developed upper respiratory tract infection and irritability then started to have vomiting and diarrhea for 20 days, then progressed to fever and loss of consciousness. She died within 2 months of progression of her illness. During the period of her admission, investigations including complete blood count, electrolytes, blood sugar, liver and kidney function tests, and CSF analysis (for protein, sugar and chloride, and culture), were within normal. Serum ammonia, plasma

and urine amino acid profile were within normal. However, serum lactate showed mild elevation (26 mg/dl). MRI brain showed multiple signal alteration (hyperintense in T1 and T2-W) areas in the basal ganglia on both sides and central portions of midbrain, suggestive of Leigh syndrome.

Thus taking all data into consideration, the patient and her older two sisters seemed to fit the stringent diagnostic criteria for Leigh syndrome that were defined by Rahman et al. [1]. And so molecular testing for mitochondrial DNA-associated Leigh syndrome was done.

#### Molecular testing methods

Whole cellular DNA was extracted from blood or tissue and subjected to amplification by PCR using primers which flank position 8993 in the mitochondrial DNA. The PCR product was digested with restriction endonuclease Msp I which cuts the product if the nucleotide at position 8993 is mutated from T to G or T to C. After digestion, the DNA products were subjected to electrophoresis through agarose and visualized with ethidium bromide. When analyzed by this method, DNA from normal individuals gives only one band, of 551 base pairs. DNA from individuals carrying an 8993 NARP mutation gives three bands, the normal 551 base pair band and two abnormal bands, 345 and 206 base pairs in length, which result from cleavage of the mutated portion of the products by Msp I. In this case an abnormal banding pattern was seen, and so the T to G mutation was differentiated from the T to C mutation by digestion of the PCR product with Ava I, which cuts at position 8993 only if the T to G mutation is present.

Molecular diagnosis revealed mitochondrial T8993C mutation within the gene coding for ATPase 6 in the girl, her brother, and her mother but not her father thus confirming mitochondrial DNA-associated Leigh syndrome in this family. As the patient's two sisters were already deceased at the time of diagnosis and there was no DNA material preserved, molecular testing was not done to them. The patient and her brother show roughly 90% mutated mitochondrial DNA while the mother had only 50% mutated DNA.

#### Discussion

Complex V (ATP synthase) of the mitochondrion comprises 10–16 subunits encoded by nuclear DNA and 2 subunits (ATPase 6 and ATPase 8) encoded by mtDNA. Subunit 6 of

mitochondrial ATP synthase (complex V) is encoded by nucleotides 8527–9207 of the mitochondrial genome. Tatuch et al. [2] and Shoffner et al. [3] demonstrated that the nucleotide 8993 mutation can cause Leigh syndrome. Defective catalytic properties of the enzyme complex may result either from an impairment of proton transport or from impaired coupling of proton translocation with ATP synthesis [4–7]. White et al. [8] described 13 pedigrees with mtDNA mutations at nucleotide 8993: 10 pedigrees with 8993T-G (OMIM 516060.0001) and 3 with 8993T-C (OMIM 516060.0002).

Two main phenotypes were identified: the typical Neuropathy Ataxia Retinitis Pigmentosa (NARP syndrome) [9–11] and maternally inherited Leigh syndrome [12,13], most of the cases reported were Juvenile onset type of Leigh syndrome.

Our patient had T8993C mutation which together with T8993G account for 10–20% of mitochondrial DNA-associated Leigh syndrome. Though the T8993C mutation is generally considered to be clinically milder than the T8993G mutation, the scenario of events in the deceased sisters with earlier onset of the disease (before their second birthday) contradicts this statement. The increased severity of the disease in the proband in this case may relate to the very high percentage of mutated mtDNA (90%).

Although the patient's brother has the same percentage of mutated mitochondrial DNA (90%), he had hypothyroidism which could be the only manifestation of the T8993C mutation. This unusual lack of manifestation could be attributed to different percentages of mutated mitochondrial DNA in the brain or muscle or perhaps to some unknown protective factor. The hypothyroidism could be a simple association, but to the best of our knowledge, no previous reports have described hypothyroidism in carriers of this mutation.

Fujii et al. [14] reviewed 10 Leigh syndrome patients with the 8993T-C mutation and compared them with 18 reported cases with Leigh syndrome caused by the 8993T-G mutation (516060.0001). Leigh syndrome with the 8993T-C mutation was characterized by a significantly higher frequency of ataxia. None of the reviewed 8993T-C Leigh syndrome patients had retinitis pigmentosa, which is one of the characteristic findings in Leigh syndrome caused by the 8993T-G mutation. The milder symptoms of 8993T-C Leigh syndrome may be explained by the milder complex V dysfunction.

Debray et al. reviewed 20 Leigh syndrome patients with the 8993T-C mutation. Only half (10/20) of the patients fulfilled the criteria of Rahman et al. [1] for typical Leigh syndrome. Eighty-five percent (17/20) survived a median follow-up time of 16 years and 41% (7/20) did not have mental retardation. The authors concluded that a favorable outcome can be observed in a significant percentage of Leigh syndrome patients with the 8993T-C mtDNA mutation [15]. A similar observation was previously noted by Santorelli and coworkers [16].

In conclusion, we report a family with 8993T-C mitochondrial DNA mutation associated with severe (early onset) Leigh syndrome in one member and hypothyroidism as the only presenting symptom in another member of the family.

#### Declaration of conflicting interests

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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