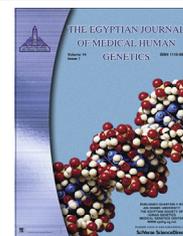




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## CASE REPORT

# Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes in a Japanese child: Clinical, radiological and molecular genetic analysis

Laila Selim <sup>a</sup>, Dina Mehaney <sup>b,\*</sup>

<sup>a</sup> *Pediatrics Neurology Department, Faculty of Medicine, Cairo University, Egypt*

<sup>b</sup> *Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt*

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**Abstract** Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes is a mitochondrial multisystem disorder. This disease has mainly been associated to the mitochondrial DNA mutation A3243G located in the tRNA Leucine gene. In this article, we report the clinical, radiological and molecular results of a 10 years old Child with the classical Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes phenotype. A 10 years old male Japanese child presented with recurrent episodes of headache, nausea and vomiting of 5 years duration and hyperlactic acidemia. These episodes were associated with motor weakness on the right side, with difficulties in language and memory and visual disturbance. Neurological examination revealed generalized muscle weakness with mild right sided hemiparesis. The Magnetic Resonance Imaging revealed infarct like lesions in the left occipital regions and the left medial temporal. The mitochondrial DNA mutations A3243G, T3271C and G13513A were tested using Polymerase Chain Reaction- Restriction Fragment Length Polymorphism analysis and direct sequencing. The heteroplasmic A3243G mutation was detected in the blood of the patient and his mother. L-Arginine is reported to be beneficial for the patients and a preventive treatment was given in the form of arginine 500 mg twice per day.

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## 1. Introduction

Mitochondria are key to many cellular processes. Oxidative phosphorylation (OXPHOS) is one of the most important mechanisms resulting in the production of cellular energy in the form of adenosine triphosphate (ATP) [1,2].

Disorders of mitochondrial origin are a heterogeneous group of diseases commonly manifesting in high-energy demanding tissues such as muscles, brain, heart and nerves, hence the name “mitochondrial encephalomyopathies” [3].

\* Corresponding author. Tel.: +201222889195; fax: +20233453797.  
E-mail address: drdinamehaney@kasralainy.edu.eg (D. Mehaney).  
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The clinical presentations of mitochondrial diseases (MCDs) are highly variable and the symptoms are often vague and non-specific [4,5]. The clinical recognition of MCDs is often challenging [6]. These diseases should be considered in patients with apparently unexplained combinations of symptoms and signs, especially if neurological features are present [1].

The mitochondrial genome encodes 13 essential polypeptides of the OXPHOS system and the necessary RNAs machinery. The remaining structural proteins and those involved in import, assembly and mitochondrial DNA (mtDNA) replication are encoded by the nuclear DNA (nDNA) and are targeted to the mitochondria [7].

More than 100 point mutations of mtDNA that are known to cause mitochondrial dysfunction were identified [8]. Mutations in mtDNA are more common than in nDNA. mtDNA mutates 10–17 times faster than nDNA due to the absence of chromatin and histones. Also the continuous generation of reactive oxygen species (ROS) and the lack of an efficient repairing mechanism further increase the mutation rate [9]. Recent developments in the molecular diagnostics allowed for the exploration of many of pathogenic mutations, thus providing more clues about the molecular basis of these disorders.

Mitochondrial genetics is characterized by maternal inheritance, mitotic segregation, threshold effect and heteroplasmy [10]. Since mitochondria are inherited only from the mother, mtDNA defects result in pedigrees exhibiting a pattern of maternal inheritance [7]. Because there are hundreds or even thousands of mitochondria in each cell, mutation in mtDNA may result in two populations of mtDNA (wild and mutant), a condition known as heteroplasmy [7]. The phenotypic expression of a mtDNA mutation is regulated by the threshold effect, the mutant phenotype is expressed in heteroplasmic cells only when the relative proportion of mutant mtDNAs reaches a certain value [11]. A respiratory chain (RC) defect may become manifest in some tissues, but not in others, if the number of mutant mtDNA exceeds a certain critical threshold [12]. The threshold level for the expression of mtDNA mutations is usually high (85–95%), but varies with different mutations. The threshold varies among tissues, depending on the oxidative energy requirements [7].

Because both mtDNA replication and mitochondrial division are random processes unrelated to cell division, a dividing cell donates variable numbers of mitochondria and mtDNAs to its progeny [11]. This process, known as mitotic segregation, can be important clinically if a patient harbors heteroplasmic populations in tissues, resulting in changing mutation loads in consecutive generations and increasing the phenotypic variation of MCDs [7].

Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome is one of the most frequently occurring MCDs [3]. Patients with MELAS syndrome are usually normal at birth and develop normally during the first years of life [1]. The patients often have episodes of vomiting with severe headache [3] leading to somnolence and coma [1]. Some of these episodes may lead to severe generalized seizures with stroke-like episodes of hemiparesis [3]. Ataxia [13], dementia [14], muscle weakness, sensorineural deafness, exercise intolerance [1] and diabetes mellitus are frequently seen [15]. Cardiomyopathy may also develop in the late stages of the disease [1].

Although nearly 80% of the coding part of the mtDNA is allocated to protein-coding genes and around 10% to tRNA genes, most of the pathogenic point mutations so far described affect tRNA genes [16]. Pathogenic mutations have been identified in most of the 22 tRNA genes, however, some tRNA genes are more frequently affected than others. Among these are the tRNA Leucine (Leu), tRNA –Lysine (Lys) and tRNA-Isoleucine (Ile). The most common tRNA mutation is tRNA Leu A3243G, which is typically associated with MELAS syndrome [17]. Other tRNA Leu mutations (G3244A, T3258C, C3256T, T3271C, T3291C), mutations in tRNA Val [18] and tRNA His [19] were also found to be associated with the MELAS syndrome [8].

Molecular causes of MELAS other than tRNA mutations have been described [20]. Mutations in the nicotinamide adenine dinucleotide dehydrogenase (NADH) 5 gene of mitochondrial DNA are important. The G13513A mutation emerged as a hotspot. It is therefore important to consider this mutation in patients with Leigh Syndrome (LS), or overlapping features of the MELAS and leigh syndromes [20].

In this article, we report the clinical, radiological and molecular results of a 10 year old child with the classical MELAS phenotype.

## 2. Case report

A 10 year old male child, born to a Japanese father and Chinese mother, presented to the Inherited Metabolic Disease Unit at the Cairo University Children's hospital with recurrent episodes of headache, nausea and vomiting of 5 years duration. These episodes were associated with motor weakness on the right side with difficulties in language and memory and visual disturbance, mostly right sided homonymous hemianopia. Neurological examination revealed generalized muscle weakness, with mild right sided hemiparesis. These clinical manifestations were reliable to the Magnetic Resonance Imaging (MRI) showing infarction of left posterior parietal, left occipital and left medial temporal regions, without visible vascular abnormality at the Magnetic Resonance Angiography (MRA) (Fig. 1A, B). Laboratory Investigations revealed hyperlactic acidemia and a discrete increase in hepatic transaminases. The patient was clinically suspected to have MELAS syndrome. The mother reported that her brother suffered from the same clinical picture and died by the age of 19 years but the cause of death is not clear (Fig. 2).

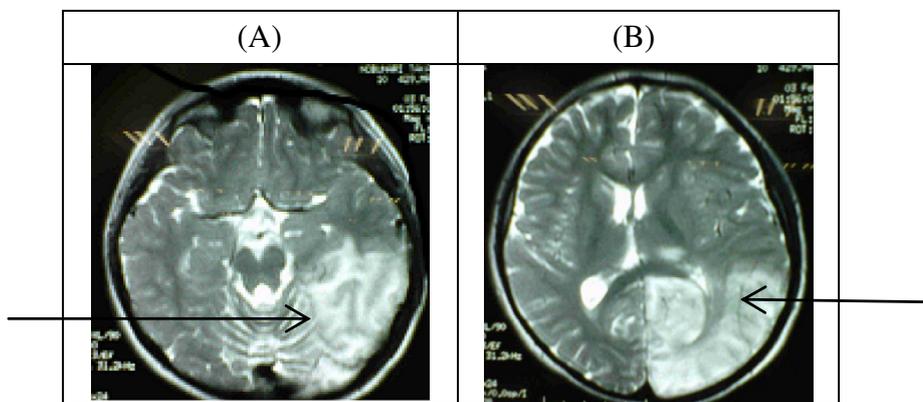
## 3. Methods

### 3.1. Ethical issue

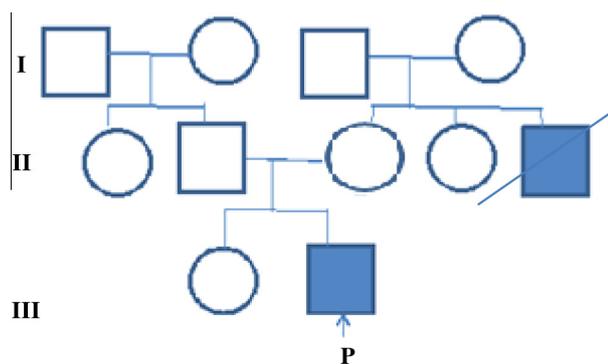
Written informed consent was obtained from the parents for all the procedures performed.

### 3.2. DNA extraction from the whole blood samples

Total genomic nDNA and mtDNA were extracted from peripheral blood leukocytes of the patient and his mother and healthy control age and sex matched child. The control was attending the hospital for other different reasons. He reported no symptomatic metabolic, genetic, or ocular disorders regarding family history, past medical problems, and



**Figure 1** MRI brain showing infarction of the left medial temporal (A) and left occipital regions (B) (arrows).



**Figure 2** Family pedigree of the studied patient. P: Proband.

current health. Extraction was done using the commercially available DNA isolation kits (QIAamp DNA Blood Mini Kit) from Qiagen (TM), Germany, and following the manufacturer’s instructions.

*3.3. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis*

The most common point mutations related to MELAS A3243G, T3271C, G13513A were tested as previously described [21,22,2]. The PCR products were digested using HaeIII, DraI and BpuAI, respectively. Digested products were separated at 120 V for 20–30 min by 12% polyacrylamide gel electrophoresis using the BioRad Mini-Protean tetra gel system (Bio-Rad, Hercules, CA, USA) and stained by 0.5 mg/ml with ethidium bromide. A 50 bp DNA ladder (MBI Fermentas) was used as a size marker. The gel was visualized using UV transilluminator (BIORAD, USA). The wild and mutant patterns are shown in Table 1.

Mutation	PCR product size	Wild type (bp)	Mutant type (bp)
A3243G	230	166 + 64	93 + 73 + 64
T3271C	145	123 + 22	145
G13513A	210	189 + 21	210

Bp: base pair.

*3.4. Direct sequencing*

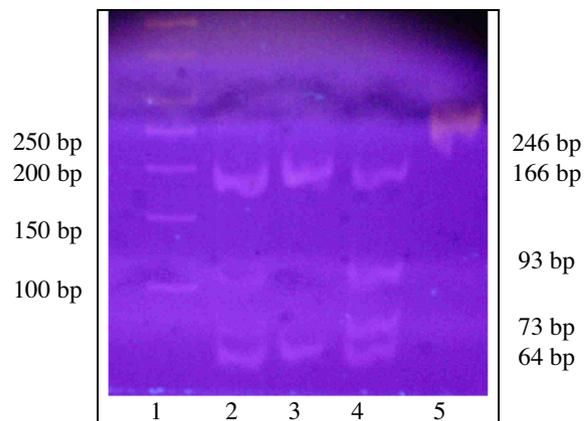
To confirm the presence of the mutation A3243G, direct sequencing was performed using the Applied Biosystems (ABI) automated Sequencer (ABI 310, Applied Biosystems, Foster City, CA) and the dideoxy chain terminators kit (BigDye Terminator Cycle Sequencing Reaction Kit; ABI). The sequence was compared to the revised Cambridge reference sequence [24].

**4. Results**

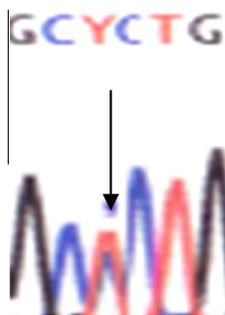
PCR/RFLP analysis for three MELAS associated mtDNA mutations revealed positive heteroplasmic pattern for the A3243G mutation in the patient and his mother (Fig. 3). The mutation was confirmed by direct sequencing (Fig. 4). The T3271C and the G13513A mutations were negative.

**5. Discussion**

MELAS syndrome is one of the most common clinical entities caused by mtDNA mutations [27,28]. A diagnosis of MELAS is based on clinical features, including recurrent stroke-like



**Figure 3** RFLP analysis showing positive 3243 heteroplasmic mutation in the patient and his mother along with negative control. Lane 1: 50 base pair (bp) ladder, lane 2: mother, lane 3: negative control, lane 4: the patient and lane 5: uncut PCR product.



**Figure 4** A3243G mutation confirmed by direct sequencing (arrow).

episodes preceded by vomiting and headache [29], MRI abnormalities of infarct-like lesions that are not confined to the major vascular territories [30] and biochemical evidence for mitochondrial defects, such as lactic acidosis or Ragged Red Fibers (RRF) in the muscle biopsy [31,32]. The proband of the present study expressed the classical picture of MELAS syndrome.

MELAS usually affects patients by the age of 5–15 years [33]. In concordance with the present study, the proband started to manifest by the age of 5 years.

The high plasma lactate in the proband was in agreement with the fact that high lactate is a marker of MCDs [3,7,34]. In MELAS, lactic acidosis correlates with the severity of neurological impairment [35].

Cerebral imaging plays a central role in further diagnostics relating to suspected or certain MCDs in children [36]. Cortical changes are especially prominent in MELAS and Alpers syndrome [37]. Infarct-like lesions not confined to vascular territories are the imaging hallmark of MELAS syndrome [35]. Concomitant with these findings, the MRI of the proband of the present study showed infarct like lesions of the left posterior parietal, left occipital and left medial temporal regions, without visible vascular abnormality at the Magnetic Resonance Angiography.

At least 30 kinds of gene mutations had been reported in MELAS patients ([www.mitomap.org](http://www.mitomap.org)). The A3243G mutation is found in 80% of the patients [15,27,38,39]. The A3243G MELAS mutation is also found in some patients with maternally inherited diabetes/deafness syndrome (MIDD) [40] and many other neuromuscular disorders [41]. In a cohort of 1340 unrelated Chinese patients suspected of having mitochondrial disease, the detection rate of A3243G was 9.4%, and it was the most frequent mutation (79.3%) in the samples with mtDNA mutations. This high detection rate suggests the importance of this mutation especially in Chinese patients with MELAS [42]. In the present study, the proband and his mother were positive for the 3243 mutation confirming the theory of maternal inheritance of MCDs. The mother and his sister were phenotypically normal and this could be explained by the peculiar genetics of MCDs (threshold effect).

A similar study had been conducted by Qi et al. [43] to investigate the spectrum of common mitochondrial mutations in Northern China during the years of 2000–2005. Five hundred and 52 patients were clinically diagnosed as MELAS. The A3243G mutation was detected in 10% of the cases.

Ten Brazilian patients suffering from MELAS were studied by Lorenzoni et al. [39]. Similar to the present study, MRI

revealed a stroke-like pattern in all patients and molecular analysis of tRNA Leu gene by PCR/RLFP showed the A3243G mutation in 40% of the patients. They recommended that PCR/RLFP for the A3243G mutation must be the first simple molecular test when MELAS is suspected.

The T3271C mutation is the second most common mutation [39], presenting in about 10% of MELAS patients [44,45], predominantly in Japanese patients [15,46]. This mutation was reported in only 11 MELAS patients outside Japan (North America, Europe and Brazil) [15,38,47,48]. This mutation was not detected in 522 Chinese patients with encephalomyopathy, nor in 114 clinically diagnosed MELAS patients [49]. This mutation was negative in the proband of the present study (Chinese mother), this confirms the finding that T3271C may be uncommon in Chinese patients with MELAS and mitochondrial encephalomyopathy [49].

G13513A mutation was also reported in MELAS patients [20,23]. It was described in 21 patients; seven with MELAS [23,50–52] and 14 with LS [53–57]. Despite being negative in the proband of the present study, this mutation had been tested in our study in agreement with several authors who recommended that this mutation should be routinely tested in patients with MELAS even in the absence of a biochemical complex I deficiency [20,53,56,58].

Contrary to the results of the present study, Shanske et al. [58] suggested that G13513A mutation is a common cause of MELAS even in the absence of marked complex I deficiency, clear history of maternal inheritance or pathological evidence in muscle. However, many studies reported that 13513 is a rare cause of MELAS syndrome or mitochondrial encephalomyopathy in Chinese patients [46,49,59].

Many treatment options of RC disorders had been recently reviewed [60]. Several lines of treatment were proposed for the treatment of MELAS patients [59]. L-Arginine was reported to be one of the promising therapeutic options for MELAS patients [60]. Accordingly a preventive treatment was given to the proband described in the present study in the form of arginine 500 mg twice per day.

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