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A Tunisian patient with Pearson syndrome harboring the 4.977 kb common deletion associated to two novel large-scale mitochondrial deletions

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ABSTRACT

Pearson syndrome (PS) is a multisystem disease including refractory anemia, vacuolization of marrow precursors and pancreatic fibrosis. The disease starts during infancy and affects various tissues and organs, and most affected children die before the age of 3 years. Pearson syndrome is caused by de novo large-scale deletions or, more rarely, duplications in the mitochondrial genome. In the present report, we described a Pearson syndrome patient harboring multiple mitochondrial deletions which is, in our knowledge, the first case described and studied in Tunisia. In fact, we reported the common 4.977 kb deletion and two novel heteroplasmic deletions (5.030 and 5.234 kb) of the mtDNA. These deletions affect several protein-coding and tRNAs genes and could strongly lead to defects in mitochondrial polypeptides synthesis, and impair oxidative phosphorylation and energy metabolism in the respiratory chain in the studied patient.

1. Introduction

Large-scale mitochondrial DNA (mtDNA) deletions have been associated with myopathies including chronic progressive ophthalmoplegia (CPEO) and Kearns–Sayre syndrome (KSS) [1–3] as well as with non-muscular, multi-organ diseases such as Pearson's syndrome [4,5] and in some cases of maternally inherited adult-onset diabetes and deafness [6,7].

mtDNA deletions differ in both size and location, but are confined to a region delineated by the H-strand and L-strand origins of replication which is the major arc of the mtDNA. The deletions include several tRNA and protein-coding genes [8,9] and often are flanked by direct repeats [10]. In most cases, mtDNA deletions are spontaneous events that occur either in the oocyte or during early stages of embryonic development. The variable clinical presentation presumably depends on the distribution and the level of heteroplasmy.

Pearson syndrome (PS), first described in 1979, is a multisystem mitochondrial cytopathy resulting from severe deficiency in mitochondrial energy supply in different organs. This syndrome includes refractory anemia, vacuolization of marrow precursors and pancreatic fibrosis [11]. The disease starts during infancy and affects various tissues and organs. Although a few neonatal cases have been described, the first manifestations, including hematologic features and/or growth retardation secondary to exocrine pancreatic dysfunction, generally occur in early infancy. These hematologic signs include macrocytic sideroblastic anemia, associated or not with neutropenia or thrombocytopenia. These manifestations are usually associated with hyperlactacidaemia and an increased lactate/pyruvate ratio. Other signs, such as tubulopathy and aminoaciduria, hepatomegaly, cytolysis and cholestasis, endocrine gland disturbances, neuromuscular manifestations, cardiac involvement or splenic atrophy can be described, simultaneously or during the course of the disease [12].

Pearson syndrome is often fatal in infancy and death often occurs before the age of 3 years, due to septicemia, metabolic acidosis or hepatocellular insufficiency. Moreover, survivors may later develop the features of Kearns–Sayre syndrome (KSS) [5].

Several studies revealed the molecular and genetic defect of Pearson syndrome which is the presence of de novo large-scale deletions or, more rarely, duplications in the mitochondrial genome [13,4,14] leading to an impaired ATP production [15]. The most common reported deletion is the 4977 bp deletion. This deletion has been identified in more than 80% of the affected children, but several other mtDNA deletions were also found (www.mitomap.org).

In the present study, we reported a 2-years-old girl presenting clinical features of Pearson syndrome. The mitochondrial genome

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studies revealed the presence of multiple deletions. In fact, we detected the common 4.977 kb deletion in association of two novel mitochondrial heteroplasmic deletions: 5.030 kb (8131–13160) and 5.234 kb (8051–13284).

2. Materials and methods

2.1. Patient

The proband was the third child of healthy non consanguineous parents. She was born after an uncomplicated pregnancy and delivery on term in 2009. Family history was negative. Her birth weight was 3100 g and her head circumference was 35 cm. She had two sisters who are healthy. Perinatal and neonatal periods were without any serious problem. Growth and development of the patient were normal until the age of 40 days.

At the age of 2 months, she suffered from anemia and she received red blood cell transfusions. In fact, her complete blood count (CBC) was: white blood cell (WBC): 5900/mm^3, hemoglobin (Hb): 5.6 g/dl, Platelet count: 171000/mm^3, Reticulocyte count: 33400/mm^3, VGM: 99 μm^3, PNN: 1180/mm^3.

Clinical examination revealed a significant mucocutaneous pallor and the absence of hepatomegaly and splenomegaly. These symptoms were associated with high folate >45 (normal values: 3.4–38.4) and a ferritenemia: 303 ng/ml (normal values: 10–280). The infectious screening was negative for parvovirus B19.

The first bone marrow aspirate revealed dyserythropoesis and dysgranulopoesis at the age of 2 months and she developed a pancytopenia found in many complete blood count.

At the age of 7 month, a marked vacuolization of erythroid and myeloid precursors was found in the second marrow aspirate. At the age of 13 months, biochemical analysis revealed a metabolic acidosis with high lactate concentration in blood of 7.23 mmol/l (normal values: 0.6–2), hyperglycemia: 4.3 g/l and glucosuria. These symptoms indicated a mitochondrial disease, thus, a diagnosis of Pearson syndrome was established.

The patient was hospitalized for a bony marrow graft and she died at the age of 21 months.

2.2. Controls

In addition, 200 Tunisian healthy individuals from the same ethnocultural group were tested as controls. These controls should have no personal or family history of PS or any other disorder. All individuals (patient and controls) provided informed consent.

3. Methods

3.1. DNA extraction

After getting informed consent from the family members, total genomic DNA was extracted from peripheral blood using phenol–chloroform standard procedures [16].

3.2. Screening of the m.3243A > G mutation in the tRNA$^{Leu(UUR)}$ gene

PCR amplification of the mitochondrial tRNA$^{Leu(UUR)}$ (MT-TL1) was performed using the following primers: 5′-TCTAGAGTCCATATCAACAA-3′ (nt 2953–2972) and 5′-TTTGGTGAAGAGTTTTATGG-3′ (nt 3480–3461) in a total volume of 50 μL as described previously [17]. After PCR amplification, the 527 pb generated fragment containing the entire tRNA$^{Leu(UUR)}$ gene (3230–3304) and part of its flanking MT-ND1 (NADH dehydrogenase, downstream) and MT-RNR2 (16S rRNA, upstream) genes was analysed by PCR-RFLP with *ApaI* (Promega) to detect the m.3243A > G mutation.

3.3. Mutational screening of the mitochondrial tRNA^{Glu} gene

The mitochondrial tRNA^{Glu} (MT-TE) was PCR amplified using the following primers: 5′-ACCACACCGCTAACAATCA-3′ and 5′-TTGATGAAAAGGCGGTTGA-3′. The PCR amplification was performed using the previous conditions (previous paragraph).

3.4. Long-range PCR amplification

Long-range PCR was performed using Long PCR Enzyme Mix (# K0182) (Fermentas) using different combinations of primers (Table 1). The PCR amplification was performed using the Long PCR Enzyme Mix. The conditions for the PCR reaction were: initial denaturation at 93 °C for 3 min, followed by 10 cycles: 30 s at 93 °C, 30 s at 58.5 °C and 12 min at 68 °C and then 25 cycles at 93 °C for 30 s, 58.5 °C for 30 s, 68 °C for 12 min and 10 s, and a final extension at 68 °C for 11 min. Products were separated on 0.8% agarose gel and visualized with ethidium bromide.

3.5. PCR amplification and sequencing of the mitochondrial DNA

PCR amplification of the entire mtDNA was carried out by amplifing 24 overlapping segments as described elsewhere [18]. Regions containing putative novel variations were amplified and sequenced again on both strands to exclude that they were PCR artefacts.

Direct sequencing of PCR products was performed with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM/Biosystems) and the products were resolved on ABI PRISM. The blast homology searches were performed using the program available at the National Center for Biotechnology Information Web site in comparison with the updated consensus Cambridge sequence (GenBank Accession No. NC_012920).

4. Results

We described a Tunisian girl with typical features of Pearson syndrome including anemia, lactic acidosis and diabetes. Thus, we performed a mutational screening of the two most mitochondrial genes associated to diabetes, tRNA$^{Leu(UUR)}$ and the tRNA^{Glu}, but the results revealed the absence of reported mutations especially the m.3243A > G and the m.14709T > C mutations. However, the sequencing of the whole mitochondrial genome in this patient with Pearson syndrome showed the presence of many known mitochondrial variations m.8104T > C (MT-CO2), m.8227T > C (MT-CO2), m.12609 T > C (MT-ND5), m.12705C > T (MT-ND5),

Table 1
Primers used for the long-range PCR amplifications.

Size	Primer	Sequences	Nucleotidic positions
10162 bp	M9F	5′-GAGGCCTAACCCCTGTCTTT-3′	5835–5854
	M22R	5′-AGCTTTGGGTGCTAATGGTG-3′	15997–15978
8089 bp	M12F	5′-ACGAGTACACCGACTACGGC-3′	7908–7927
	M22R	5′-AGCTTTGGGTGCTAATGGTG-3′	15997–15978
6379 bp	M12F	5′-ACGAGTACACCGACTACGGC-3′	7908–7927
	M20R	5′-AGAGGGGTCAGGGTTGATT-3′	14287–14269
5618 bp	M12F	5′-ACGAGTACACCGACTACGGC-3′	7908–7927
	M19R	5′-TCGATGATGTGGTCTTTGGA-3′	13526–13507