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BRIEF REPORT

Mitochondrial DNA deletion in a girl with Fanconi's syndrome

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Abstract We report a sporadic large-scale mitochondrial deletion in a paediatric patient with Fanconi's syndrome. Renal biopsy disclosed chronic interstitial nephritis. Ultrastructural examination of the renal tissue showed many giant atypical mitochondria. Histochemical stains revealed markedly reduced cytochrome *c* oxidase (COX). Genetic analysis disclosed a novel mitochondrial deletion of 7.3 kb in both peripheral blood and renal tissue. Mitochondrial diseases have heterogeneous clinical phenotypes; mutation analysis has proved to be an effective tool in confirming the diagnosis.

Keywords mtDNA deletion · Fanconi's syndrome · Cytochrome *c* oxidase deficiency · Mitochondriopathy

Introduction

Large-scale mitochondrial DNA (mtDNA) deletions are usually associated with mitochondrial myopathies (MMs), progressive external ophthalmoplegia (PEO) and Kearns–Sayre syndrome (KSS) [1]. However, in infants and young children, the clinical phenotypes of mtDNA deletions are heterogeneous and unrelated to PEO/KSS [2]. It has been reported that renal diseases of unknown origin in children may be related to mitochondrial disorders (MDs) [3–10]. A study has shown that renal involvement is frequent and is present in about half of the children with MD; four of the five patients with Fanconi's syndrome (FS) harboured single mtDNA deletions [10]. There are also phenotypic differences of identical deletion in adults and children, due to differential tissue distribution. A common 4977 bp deletion is found in PEO/KSS and in infants with Pearson's syndrome, causing bone marrow and pancreatic dysfunction [11]. In PEO/KSS mtDNA deletions are predominantly in the brain and muscle, whereas, in Pearson's syndrome, the deletions are found mainly in the peripheral blood [12]. In young subjects with mtDNA deletions the mutant mtDNA is found in various tissues, including blood, and the deletions are usually novel and sporadic instead of the common 5 kb deletion [2].

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Case report

This 6-year-old girl was the only child born to non-consanguineous parents. She showed normal development and had an unremarkable history. At the age of 2 years she developed upper respiratory tract infection, poor feeding, diarrhoea and vomiting. Laboratory investigations showed metabolic acidosis, hypoglycaemia and deranged liver function test results. She recovered after intravenous fluid supplement. However, her symptoms recurred 1 month later, with poor feeding, vomiting and polyuria. Regression in development was also observed, and she could speak only a few words. She was seen by a herbalist and was treated with traditional Chinese medicine for 2 to 3 months. She then developed generalised weakness and became bed bound. She also failed to thrive, with a body

weight less than the 3rd percentile. She was admitted to hospital in Shengzhen (China) for further treatment. Results of computer tomography (CT) and magnetic resonance imaging (MRI) of the brain were normal. She was noted to have metabolic acidosis, glycosuria and proteinuria and was suspected to have FS. Supportive treatment was given. However, she further deteriorated, becoming confused, and developed generalised tonic clonic convulsion. She was brought to Hong Kong for medical advice. Laboratory investigations showed normal anion gap, metabolic acidosis, normal liver function and low plasma phosphate concentration. Her serum urea and creatinine were 4.4 mmol/l and 83 μmol/l, respectively. Estimated glomerular filtration rate (GFR) was 60 ml/min per 1.73 m^2. Blood lactate level was increased on one occasion to 4.1 mmol/l (reference range 0.7–2.1 mmol/l), with an elevated lactate/pyruvate ratio of 19.1 (reference range 11–18). Concentration of plasma free carnitine was low at 3.6 μmol/l (reference range 19.3–53.9 μmol/l). Blood ammonia, lead, mercury and copper levels were normal. Laboratory investigations (Table 1) for tubule function revealed generalised dysfunction of the proximal tubule. Sodium bicarbonate loading test demonstrated a fractional excretion of bicarbonate of 22% after adequate bicarbonate load compatible with proximal renal tubular acidosis. Tubular reabsorption of phosphate was only 11%, suggesting phosphate wastage. Urine analysis also showed glycosuria, proteinuria, generalised amino-aciduria, hypercalciuria, hyperlactaturia and ketonuria. She was diagnosed to have Fanconi's syndrome of unknown aetiology. Ultrasound (USG) of the abdomen demonstrated bilateral renal parenchymal disease. MRI of the brain showed extensive leukoencephalopathy. Results of genetic tests for mitochondrial DNA point mutations for MELAS A3243G, MERRF A8344G and NARP T8993G/C were all negative. Her condition improved after correction of the acidosis and electrolyte imbalance and carnitine supplementation and nutritional support. She gradually improved, with neurological recovery; she could speak phrases and walk with support. Her body weight reached the 50th percentile. The follow-up MRI scan showed overall reduction in the extent of periventricular leukoencephalopathy.

She was referred to our hospital while she was 4 years old for the investigations of progressive renal insufficiency and FS. Her serum urea and creatinine levels were 8.9 mmol/l and 103 μmol/l, respectively. The estimated GFR was 45 ml/min per 1.73 m^2. Blood lead, mercury and cadmium levels were normal. Renal biopsy was performed and showed chronic interstitial nephritis. Twenty-six glomeruli were sampled; one glomerulus was globally sclerosed. The remaining glomeruli were unremarkable. There were moderate-to-marked tubular atrophy, moderate interstitial lymphocytes infiltration and fibrosis. The blood vessels were unremarkable. Direct immunofluorescence studies showed no significant immunoglobulin and complement deposition. Histochemical staining showed markedly reduced cytochrome *c* oxidase (COX) activity (Fig. 1a,b). Tissue for ultrastructural examination showed four glomeruli, which were unremarkable. The tubules showed mild tubulitis and many giant atypical mitochondria. Some mitochondria showed amorphous materials, while some displayed circular or stacks of parallel cristae (Fig. 1c). Occasional large electron-dense granules were present. In the patient's renal and peripheral blood DNA, long-distance polymerase chain reactions (PCRs) using the primer pairs AB and CD showed a single large-scale deletion, and no deletion was observed in the primer pair of EF (Figure not shown). PCR from primers GH showed a deletion of 7.3 kb in both the renal and peripheral blood samples of the patient (Fig. 2a). Direct sequencing of the PCR products (forward and reverse) revealed an identical gross deletion of 7.315 kb (nt 7325–14639) (Fig. 2b). No deletion was observed in the mother's blood sample (Figure not shown). Our findings support the notion that our patient's Fanconi's syndrome was related to mitochondrial respiratory chain deficiency caused by this mtDNA deletion.

She was treated accordingly with mega-vitamin supplement. She is now 6 years old with cognitive function appropriate for her age. However, her fine motor and self-care ability is mildly retarded at the age of 4–5 years old, and

Table 1 Urine investigations for tubule function of the patient (*FE HCO_3* fractional excretion of bicarbonate, *FE Na* fractional excretion of sodium, *RTA* renal tubular acidosis)

Investigations	Results	Reference ranges
Glomerular filtration rate	60→45 ml/min per 1.73 m^2	Normal 100±25 at >2 years old
FE HCO_3 (Bicarbonate loading test)	22%	Normal <5%>15% indicates proximal RTA
FE Na	5.6%	Normal <1%
Tubular reabsorption of phosphate	11%	Normal >85%
Calcium/creatinine (mmol/mmol)	7.6	Hypercalciuria when >0.7
Protein/creatinine (mg/mg)	3.9	Nephrotic range when >2
Glycosuria	+	
Generalised amino-aciduria	+	

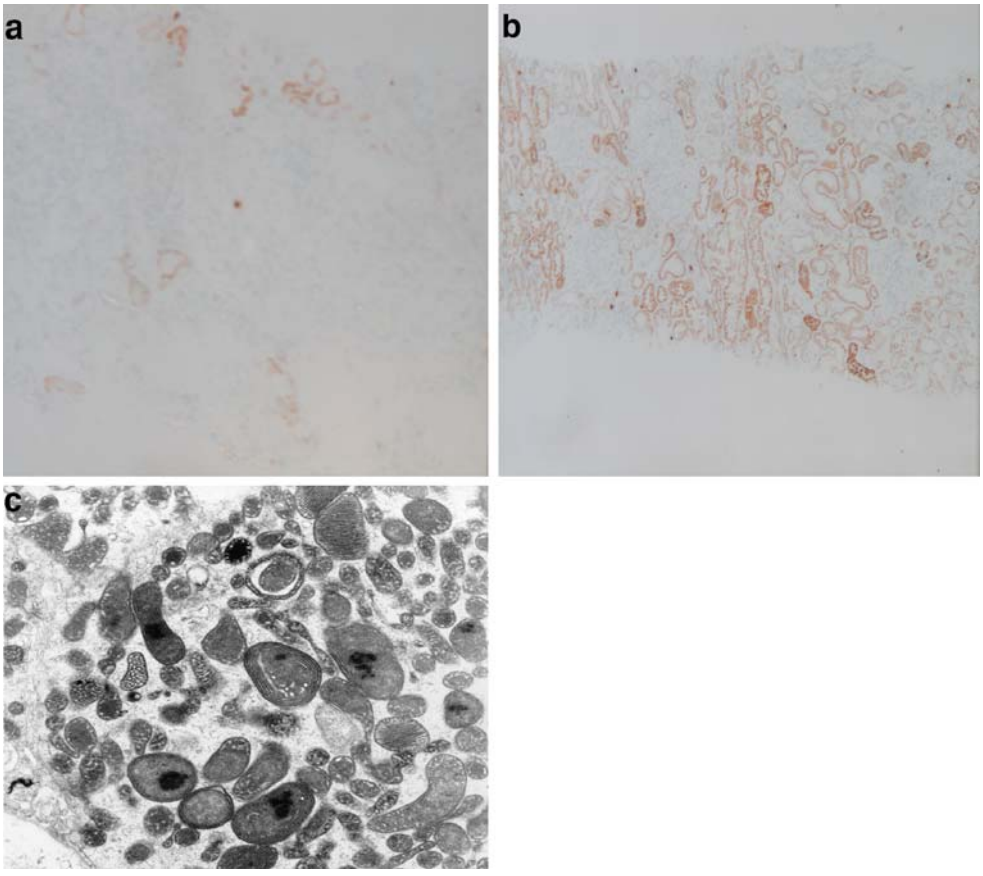

Fig. 1 COX stain of renal tubule cells showing that COX reactivity was markedly reduced in the patient (**a**) compared with the control (**b**). **c** Electron micrograph showing abnormal mitochondria with circular and stacks of parallel cristae. There were irregular electron-dense amorphous materials in the centre

her gross motor function is that of only a 3-year-old child. Her latest serum creatinine concentration was 168 μmol/l, and she is on supportive treatment for her chronic renal failure.

Mutational analysis

Total DNA was extracted from peripheral blood and renal biopsy with a QIAamp DNA blood mini-kit and a QIAamp DNA mini-kit (Qiagen, Germany). Amplification of mitochondrial DNA was carried out with long-distance PCR [13–15] with three primer pairs, AB-(F/R): nt 2695–2720 and nt 16459–16436; CD-(F/R): nt 571–598 and 16220–16192; EF-(F/R): nt 7018–6989 and nt 15320–15348. To locate the site of deletion, primer pair GH-(F/R): nt 15348–15320 and nt 6991–7020 was designed to amplify in a direction opposite to that of primer EF. Direct sequencing (forward and reverse strands) of the PCR products was performed. Results were compared with the reference sequence (MITOMAP revised Cambridge reference sequence).

Discussion

MDs in children are clinically heterogeneous and multisystem disorders. The central nervous system, skeletal muscles, cardiac conduction system and renal tissues require high-energy supply from the mitochondria; thus, encephalopathy, myopathy and cardiac dysfunction are commonly seen in MDs. Although renal involvement is rare in adults, it appears to be more common in children [10]. It may present as FS, chronic renal failure, Bartter syndrome, nephrotic syndrome secondary to focal segmental glomerulosclerosis (FSGS) and tubulo-interstitial nephritis. The commonest presentation is FS. Our patient presented initially with encephalomyopathy, regression and extensive encephalodystrophy of the brain. She also developed mild lactic acidosis, glycosuria, proteinuria, generalised amino-aciduria, hyperlactaturia and ketonuria, suggesting that her FS might have had a mitochondrial respiratory chain defect as an underlying cause. The initial investigations for common mtDNA mutations were negative for MELAS A3243G, MERRF A8344G and NARP T8993G/C. Genetic test for mtDNA deletion showed a large-scale 7.3 kb deletion, which was not detected in the asymptomatic mother's peripheral blood; it was consistent with a sporadic mutation, as commonly seen in mtDNA deletions [16]. The deleted site of nt 7325–14639 encompasses cytochrome *c* oxidase (I, II, and III), several tRNAs, ATPase 8, ATPase 6, and NADH dehydrogenase (3, 4L, 4, 5 and 6). Therefore, children with renal involvement of unknown origin should be investigated for common

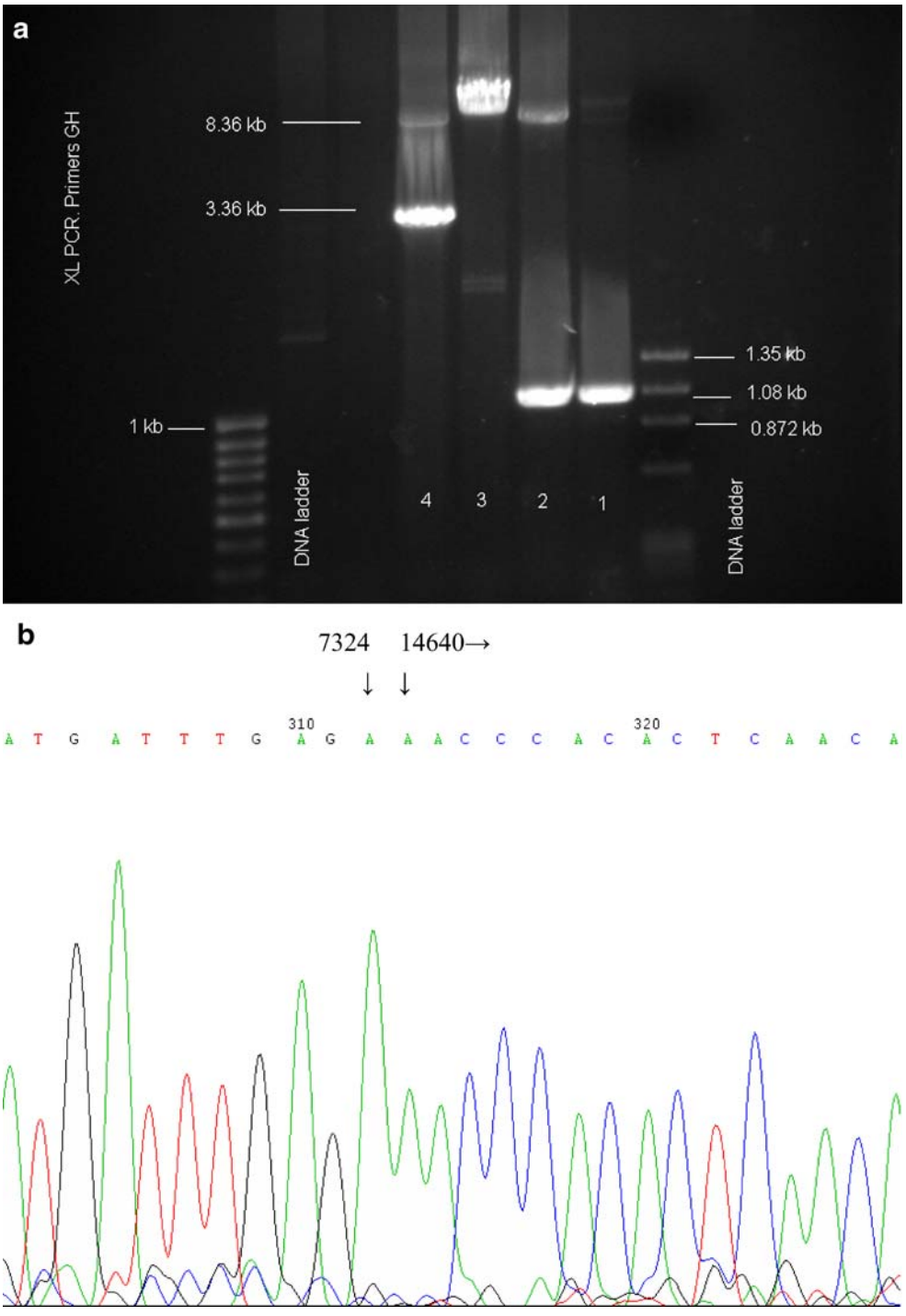

Fig. 2 **a** Analysis of primer pairs GH showing a 7.3 kb deletion in both the renal and blood DNA of the patient. *Lane 1* renal tissue, *lane 2* blood, *lane 3* normal control, *lane 4* 4,977 bp mtDNA deletion QC. **b** Sequence analysis (forward) of PCR product from primer GH showing the deletion site of mtDNA nt 7325–14639

mitochondrial mutations, large-scale deletions and other rare mtDNA mutations when needed.

MDs have been reported in children with FS [5–7, 10], and they are usually sporadic, heteroplasmic, and unique, suggesting that they are caused by de novo rearrangements during oogenesis or early development. As in our case, MDs usually encompass several coding genes and tRNA genes, making it difficult for one to correlate the clinical presentation and the nature or extent of the deletions. Study showed no correlation between a specific clinical presentation and a specific respiratory chain defect [10]. Heteroplasmy of deleted mtDNA and the variations of mutant loads in different tissues or individuals further explain the clinical diversity of the patients affected. However, there are some non-specific clinical similarities in addition to FS in these patients. As in our patient, some of them presented with encephalopathy, mild lactic acidosis, hyperlactaturia, hypoglycaemia and vomiting [5–7, 10]. However, our

patient had no clinical features of Pearson's syndrome, KSS, sideroblastic anaemia or pigmentary retinopathy. Our patient exhibited prominent deleted mtDNA in renal tissue while retaining a small portion of intact mtDNA in blood (Fig. 2a); this may explain the dominant renal involvement. Study on renal involvement on 42 children with MDs showed a high frequency (50%) of renal disorders [10]; and the commonest presentation was FS. Six patients had single mtDNA deletions, four of them had FS. Moreover, four of the five patients with FS harboured single deletions. This association of single mtDNA deletions and FS has also been documented elsewhere [4–7] and was also observed in our patient.

In conclusion, we report a large-scale deletion in mtDNA in a 6-year-old girl, associated with FS. She showed partial resolution of symptoms under conservative treatment. However, it is possible that she may go into end-stage renal failure secondary to ongoing interstitial nephritis. As renal disease appears to be more prominent in multi-system disorders associated with mtDNA deletions in children, long-term monitoring of her renal progression as well as symptoms involving other organs is essential.

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