Short Communication

Mitochondrial DNA Deletion in an 8-year-old Boy with Pearson Syndrome

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Pearson syndrome (PS) (McKusick 260560) is an often fatal disease of early childhood. We describe an 8-year-old boy with PS, who showed the typical haematological symptoms in early infancy. During his later course, increased CSF protein, ptosis and retinitis pigmentosa pointed to the Kearns–Sayre syndrome, another mtDNA deletion disorder. Analysis of leukocyte mtDNA revealed a 5.5-kb mtDNA deletion similar to that found in at least five other patients with PS (Rötig et al 1990).

CASE REPORT

G.P. was born in 1983 as the fifth child after normal pregnancy. The unrelated parents and four siblings are healthy. Birth weight was 3100 g, birth length 50 cm with normal neonatal adaptation. Anaemia (haemoglobin 138 g/L) was noted from birth. At age 1 month, haemoglobin (Hb) was 80 g/L. At 5 months, Hb was 66 g/L, reticulocytes 1.9%, haematocrit (Hct) 0.22, MCV 118 fl, granulocytes 0.79×10^9/L and thrombocytes 120×10^9/L. Substitution of iron was ineffective.

Clinical evaluation at 6 months gave no reason for the patient's pancytopenia. Blood values were as follows: Hb 56 g/L, erythrocytes 1.7×10^{12}/L, Hct 0.16, MCV 94 fl, reticulocytes 2.2%, Hb Fe 17%, serum-Fe 23.7 μmol/L, ferritin 123 μg/L, total leukocytes 4.6×10^9/L, granulocytes 0.37×10^9/L, thrombocytes 102×10^9/L, vitamin B_{12} 514 ng/L and folic acid > 24 μg/L. Coombs was negative, C-reactive protein < 3 mg/L. Bone marrow showed a marked vacuolization of myeloid precursor cells and erythroblasts. Transaminases were normal and serum antibodies for connatal infections (TORCH) and antinuclear antibodies were negative. Blood pH was 7.43, base excess −2 mmol/L, urine pH 6.4, with slight hyperaminoaciduria, especially increased alanine. Urinary orotic acid was normal and lactate in urine 0.13 mmol/mol creatinine. Sonography of the abdomen was normal. Nine transfusions of packed erythrocytes were necessary to stabilize haemoglobin during the next 16 months. During this period pretransfusional Hb-values were 49–79 g/L, granulocytes 0.18–1.01 $\times 10^9$/L and thrombocytes 59–110 $\times 10^9$/L; erythrocytes were macrocytic, MCV 93–100 fl.

Growth and psychomotor development were normal during the first 2 years. There were feeding difficulties and special food habits. Several infections such as obstructive bronchitis, rotavirus-enteritis, bronchopneumonia, angina follicularis and stomatitis aphthosa caused no special problems. From 22 months onwards, Hb and red cells normalized, with granulocytes and thrombocytes still in the lower normal range.

At the age of 3 years 6 months the patient showed failure to thrive (weight on 3rd centile, height P 10) and slight psychomotor retardation. EEG was unspecifically disturbed with no convulsive activity. Abdominal sonography showed increased echogenicity of the pancreas. Exocrine pancreatic function was normal to slightly decreased (slightly reduced chymotrypsin activity in stools but normal fat excretion). The sweat test was normal, also histology of the duodenal mucosa. There was slight metabolic acidosis (BE -6 mmol/L) and hyperalaninuria, but lactate was normal in urine.

In the following years, feeding habits and difficulties continued; failure to thrive progressed and growth retardation became evident.

At 7 years 2 months the patient was hospitalized during a metabolic crisis with apathy, muscle hypotonia and ataxia. Blood glucose was high (18.9 mmol/L) on admission, suggesting diabetes, but normalized spontaneously. There was lactic acidaemia (5.6 mmol/L), metabolic acidosis (pH 7.23), standard bicarbonate 3.3 mmol/L (BE -18 mmol/L), massive lactic aciduria (4490 mmol/mol creatinine). Clinically, ptosis, general muscular hypotonia, beginnings of salt and pepper fundus (retinitis pigmentosa) and peculiar brownish coloration of the skin were noted. The hearing threshold was slightly decreased. CSF protein (1.38 g/L) and also myelin basic protein were increased. Sonographically there was still hyperechogenicity of the pancreas. The EMG was normal, the brain appeared normal in CT and MRI, except for a slightly increased cysterna magna. Pearson syndrome was suggested for the first time and therefore mtDNA in leukocytes was analysed.

METHODS

mtDNA from leukocytes of the patient and controls were analysed by Southern blotting. The nucleotide sequence at the boundaries of the mtDNA deletion was characterized by PCR-amplification according to the methods described by Rötig et al (1990).

RESULTS

Southern blot analysis of leukocyte DNA digested with restriction enzyme PuvII (cleavage at nt 2652) and hybridization using a total mitochondrial probe showed that the patient had two populations of mtDNA: one normal (16.5 kb) and one partly deleted (11 kb).

In the patient's mtDNA probes (Figure 1, top part) hybridization to an 11-kb abnormal fragment occurred with cytochrome oxidase II probe (CoxII, lane 1) and cytochrome *b* probe (cytb, lane 5). Only one fragment was detected by ATPase 6-cytochrome oxidase III probe (A6-CoxIII, lane 2) and NADH dehydrogenase 4 and 5 probes (ND4 and ND5, lanes 3 and 4). The map of human mtDNA (Figure 1,
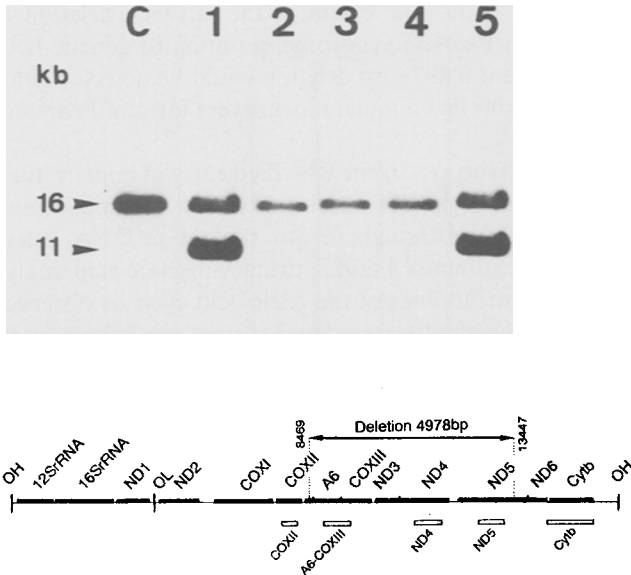
Figure 1 (*Upper*) Hybridization patterns of mtDNA probes in patient and control (C) leukocytes DNA. Lane 1, cytochrome oxidase II (COXII); lane 2, ATPase 6-cytochrome oxidase III (A6-COXIII); lanes 3 and 4, NADH dehydrogenase 4 and 5 (ND4 and ND5); lane 5, cytochrome *b* (cytb). For further explanation see text. (*Lower*) Map of human mtDNA showing the localization of the probes used for hybridization (open bars) and the extension of the 4978-bp deletion in the patient

bottom) shows the localization of the probes used for hybridization (open bars) and the extent of the deletion found in the patient.

The characterization of the nucleotide sequence at the boundaries of the mtDNA deletion revealed a 13-bp direct-repeat in the wild-type mtDNA sequence flanking a 4978-bp deletion.

DISCUSSION

Clinically our patient showed a mild form of Pearson syndrome (Pearson et al 1979) during early childhood with spontaneous remission of pancytopenia. At 7 years the neurological symptoms and increased CSF protein pointed to the Kearns–Sayre syndrome. Such a course of Pearson syndrome resembling the Kearns–Sayre syndrome in later childhood has recently been described by McShane et al (1991) in a 6-year-old boy. One patient with Kearns–Sayre syndrome had a similar anaemia in infancy as seen in Pearson syndrome (Larsson et al 1990). However, in Kearns–Sayre syndrome the mtDNA deletion is not expressed in leukocytes as in Pearson syndrome. Although Pearson syndrome is often lethal in early childhood, juvenile Pearson syndrome has been described (Blaw and Mize 1990). Neuromuscular involvement seems to be a late symptom in Pearson syndrome. However, Superti-Furga et al (1991) described neuromuscular signs in their patient as early as 1 year. In our patient muscular hypotonia and slight retardation of psychomotor development

were diagnosed at 3.5 years. The extent of the mtDNA deletion differs among the described patients with Pearson syndrome, pointing to genetic heterogeneity (Rötig et al 1991). In our patient a 4978-bp deletion could be detected similar to the finding in five other patients, who had a much more severe form of Pearson syndrome (Rötig et al 1990).

In a patient with Pearson syndrome who died early in infancy, the mtDNA deletion was expressed in several organs of which no clinical dysfunction was present during life (Cormier et al 1990). Although in our patient mtDNA deletion involved the NADH dehydrogenase subunits 4 and 5, urinary organic acid analysis did not reveal increased amounts of metabolites of the citric acid cycle as observed by Jacobs et al (1991). In patients previously diagnosed as 3-methyl-glutaconic aciduria and congenital neutropenia, Pearson syndrome could finally be demonstrated (G. Hoffmann, personal communication). From this and our findings it is clear that Pearson syndrome is a progressive generalized mitochondrial disorder with heterogeneity in clinical and genetic expression. Our observation raises again the question whether the two different clinical syndromes — Pearson and Kearns–Sayre — are related and might have a similar pathophysiological basis.

REFERENCES

Blaw ME, Mize CE (1990) Juvenile Pearson syndrome. *J Child Neurol* **5**: 186–190.

Cormier V, Rötig A, Quartino AR et al (1990) Widespread multitissue deletions of the mitochondrial genome in the Pearson marrow–pancreas syndrome. *J Pediatr* **117**: 599–602.

Jacobs C, Danse P, Veerman AJP (1991) Organic aciduria in Pearson syndrome. *Eur J Pediatr* **150**: 684.

Larsson NG, Holme E, Kristianson B et al (1990) Progressive increase in the mutated mitochondrial DNA fraction in Kearns–Sayre syndrome. *Pediatr Res* **28**: 131–136.

McShane MA, Hammans SR, Sweeney M et al (1991) Pearson syndrome and mitochondrial encephalomyopathy in a patient with a deletion of mtDNA. *Am J Hum Genet* **48**: 39–42.

Pearson HA, Lobel JS, Kocoskis SA et al (1979) A new syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic dysfunction. *J Pediatr* **95**: 976–984.

Rötig A, Cormier V, Blanche S et al (1990) Pearson's marrow–pancreas syndrome: A multisystem mitochondrial disorder in infancy. *J Clin Invest* **86**: 1601–1608.

Rötig A, Cormier V, Koll F et al (1991) Site specific deletions of the mitochondrial genome in the Pearson marrow–pancreas syndrome. *Genomics* **10**: 502–504.

Superti-Furga A, Schönle E, Tuchschmid P et al (1991) Pearson's bone marrow–pancreas syndrome with elevated HbF, lactic acidosis, renal Fanconi syndrome, and insulin-dependent diabetes mellitus caused by deletion-duplication of mitochondrial DNA. *Eur J Pediatr* **150**: 295.