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Pearson Syndrome and Mitochondrial Encephalomyopathy in a Patient with a Deletion of mtDNA

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Summary

A patient is described who has features of Pearson syndrome and who presented in the neonatal period with a hypoplastic anemia. He later developed hepatic, renal, and exocrine pancreatic dysfunction. At the age of 5 years he developed visual impairment, tremor, ataxia, proximal muscle weakness, external ophthalmoplegia, and a pigmentary retinopathy (Kearns-Sayre syndrome). Muscle biopsy confirmed the diagnosis of mitochondrial myopathy. Analysis of mtDNA from leukocytes and muscle showed mtDNA heteroplasmy in both tissues, with one population of mtDNA deleted by 4.9 kb. The deleted region was bridged by a 13-nucleotide sequence occurring as a direct repeat in normal mtDNA. Both Pearson syndrome and Kearns-Sayre syndrome have been noted to be associated with deletions of mtDNA; they have not previously been described in the same patient. These observations indicate that the two disorders have the same molecular basis; the different phenotypes are probably determined by the initial proportion of deleted mtDNAs and modified by selection against them in different tissues.

Introduction

Pearson syndrome comprises refractory sideroblastic anemia requiring transfusion, thrombocytopenia, neutropenia, pancreatic insufficiency, and hepatic dysfunction. Onset is in infancy, and many patients die before the age of 3 years (Pearson et al. 1979; Stoddard et al. 1981). Some of the features of Pearson syndrome are observed individually in cases of mitochondrial myopathy, a clinically and biochemically diverse group of disorders which may first give rise to symptoms in infancy, childhood, or adult life and are characterized histologically by the presence of ragged red fibers in biopsied muscle stained by the Gomori trichrome method. Clinical presentations include infantile lactic acidosis with failure to thrive, progressive external ophthalmoplegia (PEO), proximal myopathy with weakness enhanced by exercise, and multisystem neurological syndromes mainly or exclusively affecting the central nervous system (CNS) and causing seizures, ataxia, dementia, movement disorders, and strokelike episodes. Short stature, retinopathy, deafness, peripheral neuropathy, cardiac conduction defects, and endocrine, hematological, and renal dysfunction also occur (Di Mauro et al. 1985; Petty et al. 1986). The last two include sideroblastic anemia and renal tubular acidosis (Rawles and Weller 1974; van Biervliet et al. 1977).

Among mitochondrial myopathies, one particularly striking syndrome is that of Kearns and Sayre (KSS), comprising PEO and retinopathy developing before the age of 20 years and later accompanied by ataxia and/or a cardiac conduction defect. The cerebrospinal fluid (CSF) protein concentration is often increased (Berenberg et al. 1977). Until recently it was thought that Pearson syndrome was an autosomal recessive disorder (McKusick 1988). It has since become apparent that cases of Pearson syndrome and approximately 30% of those with mitochondrial myopathies share an underlying genetic defect, large deletions of one population of mtDNA (Holt et al. 1988, 1989; Moraes et al. 1989; Rotig et al. 1989). mtDNA is exclusively maternally inherited and encodes 13 of the 67 or so subunits of

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the mitochondrial respiratory chain and oxidative phosphorylation system, as well as 22 tRNAs and two rRNAs. The present paper describes the clinical and molecular genetic features of a child who presented with Pearson syndrome as an infant and survived to develop the features of mitochondrial myopathy, specifically those of KSS, in childhood.

Patients and Methods

Case Report

This 8-year-old boy was born at 38 wk gestation by cesarean section for breech presentation. Birth weight was 3.4 kg. On day 8 he was noted to be pale and lethargic. Investigation showed hypoplastic anemia, and bone marrow examination showed abundant sideroblasts and vacuolation of early and late myeloid cells and of early lymphoid cells. He required blood transfusions every 6 wk until the age of 2 years. In his first year he also developed diarrhea, failed to thrive, and was found to have pancreatic exocrine insufficiency. At 2½ years a transient period of hepatic dysfunction occurred and liver biopsy showed mild fibrosis with no deposition of copper. Patchy vitiligo and chronic candida infection had been noted since the age of 3 years. Fanconi renal tubular acidosis was recognized at age 4 years. Glomerular function at this point, however, was normal, and renal biopsy showed only mild interstitial fibrosis. Further bone marrow aspiration showed no evidence of cystine crystal deposition. By the age of 6 years, progressive renal and visual impairment was recognized, and he developed a tremor. He then became increasingly unsteady, with deterioration in writing and slow speech. His reading skills had not progressed since the age of 5 years. There was no relevant family history.

On examination, height and weight were both below the third percentile for age; head circumference was on the 50th percentile. He was hirsute and had patchy vitiligo on the arms and back. A resting tremor with head titubation was noted. He was cooperative and had a scanning dysarthria. Visual acuity was reduced at 6/24 (right) and 6/36 (left). There was bilateral ptosis, external ophthalmoplegia, and a pigmentary retinopathy. He had mild bilateral facial weakness, and limb power was reduced, especially proximally. There was no sensory loss. He showed marked limb ataxia and a wide-based ataxic gait. CT scan showed cerebral atrophy. Electroencephalography showed an excess of irregular slow activity, with a poverty of appropriate rhythmic activity. The electroretinogram was absent, but the flash visual evoked response was normal. Nerve conduction velocities and electromyography were normal. Hemoglobin was low at 7.3 g/dl, with mild macrocytosis. Platelet count was slightly low at 125 $\times$ 10^9/dl (normal 150–400 $\times$ 10^9/dl). Leukocytes were normal. Urea was elevated at 19.2 mmol/liter (normal 2.3–6.7), creatinine elevated at 276 μmol/liter (normal 30–70), calcium low at 2.06 mmol/liter (normal 2.2–2.5), phosphate elevated at 1.83 mmol/liter (normal 0.83–1.49), alkaline phosphatase low at 84 u/liter (normal 250–800), and transaminases showed a slight elevation of AST, at 60 u/liter (normal 15–45). Plasma amino acids showed branched-chain amino acids at the lower range of normal, and urine metabolic screen showed a pattern consistent with Fanconi syndrome, with excretion of glucose (50 mmol/liter) and mild aminoaciduria, especially alanine and glutamine. A glucose load (2 g/kg) produced a diabetic blood glucose response, with a persistently elevated plasma lactate. CSF lactate was significantly elevated at 5.9 mmol/liter (normal 0.5–3.4 mmol/liter), and CSF protein was elevated at 1.72 g/liter (normal <0.4 g/liter). Needle muscle biopsy showed the classical features of mitochondrial myopathy, with ragged red fibers, increased glycogen and fat, and some cytochrome oxidase negative fibers.

Methods

DNA was extracted from blood and muscle according to a method described elsewhere (Holt et al. 1989) and digested with a number of restriction endonucleases (from Bethesda Research Laboratories or Northumbria Biologicals Limited) prior to Southern blotting, hybridization to human mtDNA labeled with ^{32}P by the random primer method, and autoradiography (Holt et al. 1989). The proportions of normal and abnormal mtDNA were quantified using an LKB Ultroscan densitometer.

Following restriction mapping, the deleted region of mtDNA was amplified by means of the polymerase chain reaction (Sakai et al. 1988), using thermostable *Taq* polymerase (Perkin Elmer—Cetus), primers TGCTCTGAAATCTGTGGAGC (bp 8171–8190) and CGATGATGTGGTCTTTGGAG (bp 13525–13506), and a Hybaid intelligent heating block. DNA was amplified in 36 three-step cycles: denaturation (91.6°C for 80 s), annealing (56°C for 100 s), and extension (71.5°C for 120 s). A final extension step of 20 min was allowed to fill in ragged ends. The amplified DNA was sequenced by the direct sequencing method (Schon et al. 1989). The primer 8171–8190 was used in conjunction with

a Sequenase kit (USB) and alpha ^{35}S-dATP (Amersham) by the dideoxy method (Sanger et al. 1977). Labeled oliogonucleotides were resolved on an 8% polyacrylamide gel by electrophoresis at 2,500 V for 3 h.

Results

Two populations of mtDNA were observed in muscle and blood from this patient, one of which was deleted by approximately 5 kb (fig. 1). The proportion of deleted mtDNA was 63% in blood and 95% in muscle. Restriction mapping showed that the deletion was in the same region as that most frequently observed in patients with mitochondrial myopathy (Holt et al. 1989; Mita et al. 1990). Analysis of the deletion junction showed sequences matching the published mtDNA sequence up to bp 8482 and down from bp 13460. These nucleotides were bridged by a 13-nucleotide (nt) direct repeat occurring at bp 8470–8482 and bp 13447–13459 of the published sequence. As this 13-nt sequence may be derived from either side of the flanking region or contributed in part by both sides, the exact breakpoint of the deletion could not be determined.

No deletion of mtDNA was detectable in the patient's mother's blood either by Southern blot analysis (fig. 1) or after mtDNA amplification across the deletion junction by means of the polymerase chain reaction (30 cycles).

Discussion

The case described exhibited the phenotype first of Pearson syndrome and later that of KSS. Both syndromes have been noted to be associated with deletions of mtDNA but have not been previously described in the same patient, although the clinical features of older children with Pearson syndrome have not been reported. The deletion delineated in this patient is identical both to that most commonly reported in mitochondrial myopathies, including KSS (Holt et al. 1989; Mita et al. 1990), and to that reported in another case of Pearson syndrome (Rotig et al. 1989). The finding of directly repeated sequences flanking mtDNA deletions in a high proportion of cases (Mita et al. 1990), including that reported here, suggests that they arise because of either replication slippage or intramolecular recombination. The timing of the deletion is unclear, but it probably arises in the oocyte or zygote. Maternal transmission of identical mtDNA deletions has not been reported, and no abnormal mtDNA could be detected in the mother of our patient, even after amplification.

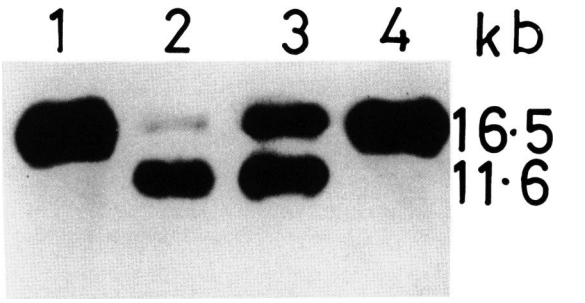

Figure 1 Restriction-fragment patterns of mtDNA after digestion with *Bam*HI, Southern blotting, and hybridization to ^{32}P-labeled human cell mtDNA from control muscle (lane 1), patient's muscle (lane 2), patient's blood (lane 3), and his mother's blood (lane 4). The normal population of mtDNA in each sample is represented by the 16.5-kb fragment, and in the patient's blood and muscle there is an additional fragment of 11.6 kb which represents the deleted population of mtDNA.

The functional effects of deleted mtDNAs must depend on their distribution within mitochondria, muscle fibers, and different tissues; this is presumably determined by their initial amount and, possibly, by random partitioning during early cell division. The majority of patients with mitochondrial myopathies have high proportions of deleted mtDNAs in muscle, but these are rarely detectable in leukocytes by Southern blotting techniques (Holt et al. 1989; Moraes et al. 1989). Deleted mtDNAs were present in high amounts in both tissues in both this and other cases (Rotig et al. 1989) of Pearson syndrome, corresponding to their marked hematological dysfunction. The differences between these two phenotypes can probably be explained on the basis of patients with Pearson's syndrome having a higher proportion of deleted mtDNAs in tissues overall than those who present with mitochondrial myopathy in later life.

Bone marrow, pancreatic, hepatic, and renal cells undergo frequent division throughout life, allowing selection against cells containing genetically defective mitochondria. Selection could be complete in mitochondrial myopathies, leading to normal function of rapidly dividing tissues. In contrast, initial persistence of a higher proportion of defective mitochondria might give rise to the features of Pearson syndrome. It is notable that the patient reported here did not require transfusions after the age of 2 years, and this has been observed in other cases (Pearson et al. 1979); this may be because the number of hemopoietic cells containing a high proportion of deleted mtDNAs decreased with time as a result of selection.

Persistent survival of deleted mtDNA molecules in both muscle and CNS is expected, given that cell division is complete after early fetal life, and this may be enhanced by a replicative advantage conferred by shorter length. Our patient had a lower proportion of deleted mtDNAs in blood (63%) than in muscle (95%). The proportion of deleted muscle mtDNAs in cases of mitochondrial myopathy is 20%–90% (Holt et al. 1989). These observations are consistent with the hypothesis that the phenotypes associated with deleted mtDNAs are determined by their initial amount and distribution but are modified by selection processes involving both mitochondria within cells and cells within different tissues.

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