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Case report

Pearson's marrow/pancreas syndrome: a histological and genetic study

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Abstract. A patient with features of Pearson's syndrome who presented with transfusion-dependent severe macrocytic anaemia, neutropoenia, thrombocytopoenia, and insulin-dependent diabetes mellitus in the neonatal period is described. His bone marrow was characterized by marked vacuolization of myeloid precursors and ringed sideroblasts. Autopsy examination revealed fibrosis and steatosis of the liver, reduction in the size and number of the islets, fibrosis and acinar atrophy of the pancreas, vacuolation of renal tubules, glomerulosclerosis, and "ragged red" fibres of skeletal muscles. Analysis of mitochondrial DNA (mtDNA) from the autopsied liver and skeletal muscle showed mtDNA heteroplasmy in both tissues, with one population of mtDNA deleted by 7374 bp. The deleted region was bridged by a single nucleotide, C, in normal mtDNA.

Key words: Pearson's syndrome – Mitochondrial DNA – Diabetes mellitus – Ragged-red fibre

Introduction

Pearson's marrow-pancreas syndrome is a fatal disorder of unknown aetiology involving the haemopoietic system, exocrine pancreas, liver, and kidneys (Pearson et al. 1979). The observation of high lactate/pyruvate molar ratio in plasma and abnormal oxidative phosphorylation in lymphocytes led Rötig et al. (1990) to postulate that Pearson's syndrome belongs to the group of mytochondrial cytopathies. We report a case of Pearson's syndrome associated with diabetes mellitus (DM), in which deletion of mitochondrial DNA (mtDNA) without flanking direct repeat and "ragged-red" fibres (RRF) in the skeletal muscle were detected.

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Case report

A male infant (2.21 kg) was born after an uneventful pregnancy to healthy, unrelated parents. An older sibling was healthy. Pallor was noted during the neonatal period. Anaemia and icterus were noted at 1 month of age (RBC $100 \times 10^4/mm^3$, Hb 3.9 g/dl, haematocrit 11.4%). Both direct and indirect Coombs' tests were negative. Neutropoenia and thrombocytopoenia were also noted. The bone marrow aspirate showed normal cellularity, with striking vacuolation of myeloid precursors (Fig. 1) and ringed sideroblasts (13.2% of erythrocytes). Serum levels of iron, ferritin, vitamin B_{12}, and folic acid were high.

At 7 weeks of age, laboratory data revealed γ-glutamyltransferase of 614 U/l, aspartate transaminase of 161 U/l, and alanine transaminase of 34 U/l. At 4 months of age, liver biopsy showed bridging fibrosis of Glisson's sheath and increased deposition of iron in the hepatocytes. By 8 months of age, there was marked growth failure despite normal thyroid function. By 16 months of age, weight loss, hypophosphataemia, hypokalaemia, panaminoaciduria, and proteinuria suggestive of the Fanconi syndrome were noted.

At 19 months of age, ketonuria and glucosuria were found and DM was diagnosed by glucose tolerance test and a glucagon tolerance test. His condition continued to deteriorate and he died

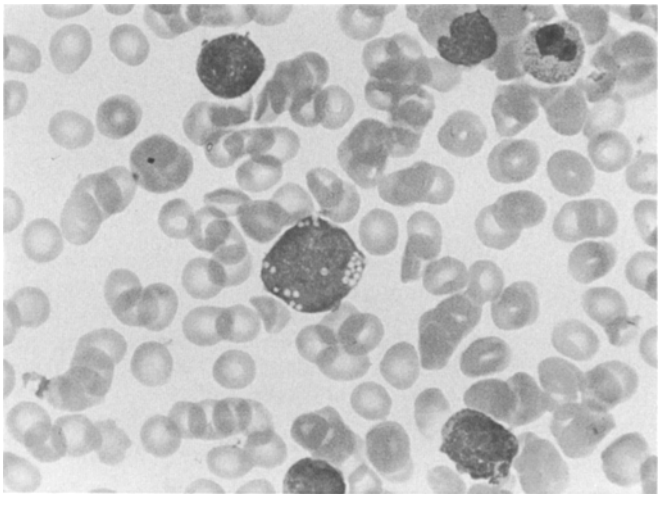

Fig. 1. Vacuolated myeloid precursor. May-Giemsa ×870

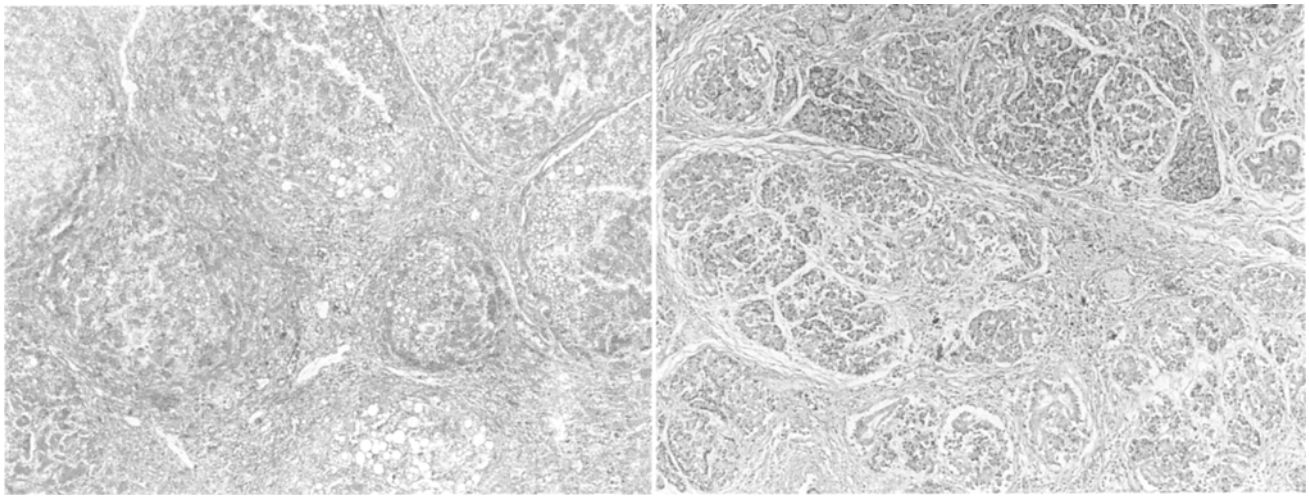

Fig. 2. Fibrosis in the liver and pancreas. Hepatic fibrosis and steatosis. Fibrous septa connect the portal areas and almost all hepatocytes contain large lipid vacuoles *(left)*. Atrophy of pancreatic acini with fibrosis *(right)*. HE, × 40

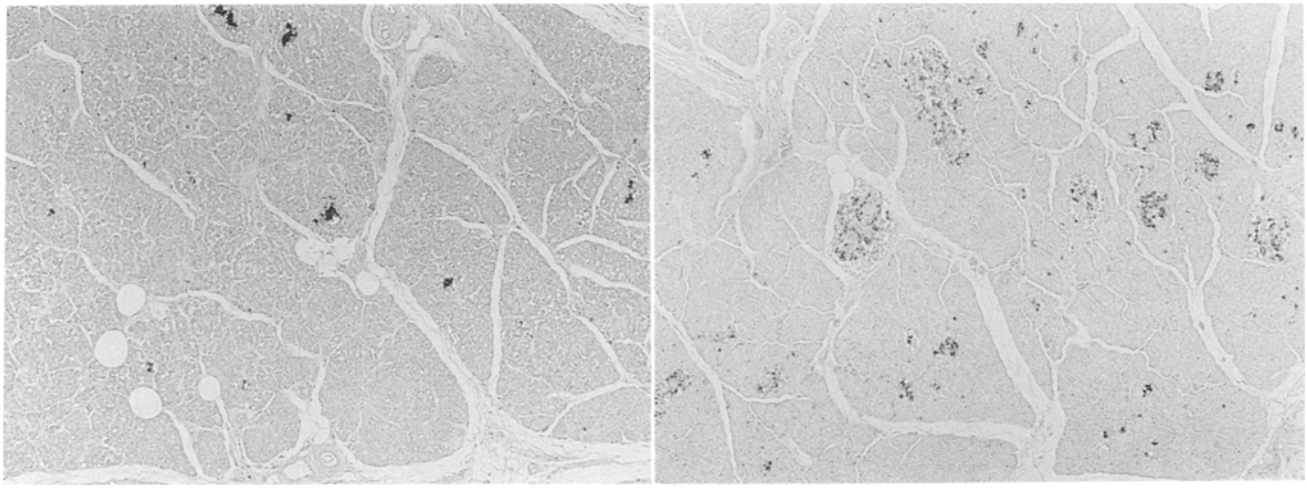

Fig. 3. Immunohistochemical demonstration of insulin in the islets of this patient *(left)* and of another patient who died of unrelated disease *(right)*. Note the reduction in size and number of islets in addition to the decrease of insulin-producing cells. Immunoperoxidase, nuclear counterstain with methyl green, × 50

of renal failure at 26 months of age. Post-mortem examination, excluding the brain, was performed immediately after death.

Materials and methods

Tissues were fixed in 10% formalin and embedded in paraffin. Sections 4 μm thick were prepared for staining with haematoxylin-eosin (HE) and for immunohistochemical examination.

Immunohistochemical staining was performed by the indirect method. Briefly, preparations were treated with 3% hydrogen peroxide in absolute methanol for 30 min. After treatment with normal rabbit serum, the preparations were incubated with guinea pig anti-insulin antibody (DAKO Japan, Kyoto) for 30 min, and then incubated sequentially with peroxidase-labelled rabbit anti-guinea pig immunoglobulin antibody (DAKO Japan) for 30 min. The peroxidase reaction was developed with 0.06% 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M TRIS buffer (pH 7.7) containing 0.03% hydrogen peroxide. Methyl green was used for counterstaining.

Liver and skeletal muscle from the patient were stored frozen at −80° C until they were used for the analyses of mitochondrial

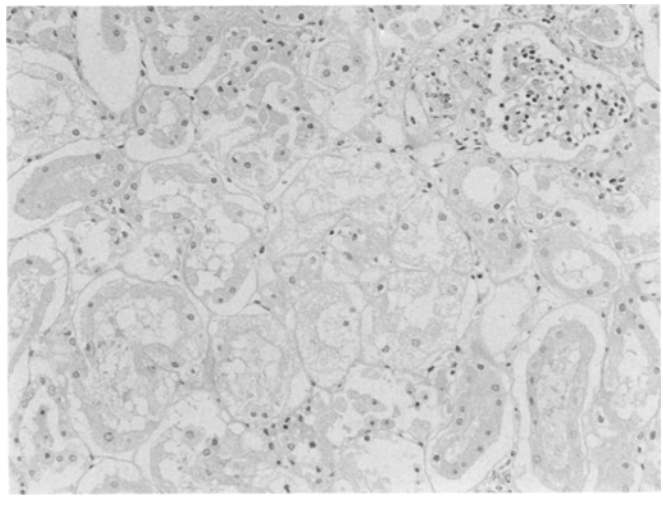

Fig. 4. Renal cortex with considerable vacuolation of tubules. HE, × 80

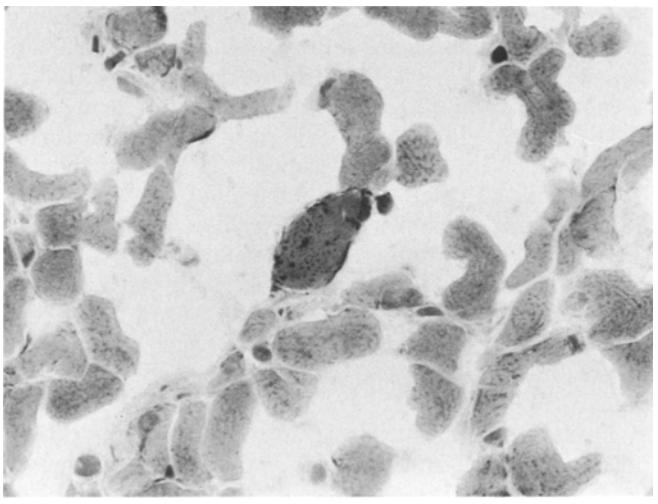

Fig. 5. Skeletal muscle fibres of this patient. The darkly stained fibre is a basophilic "ragged-red" fibre. Gomori modified trichrome, × 550

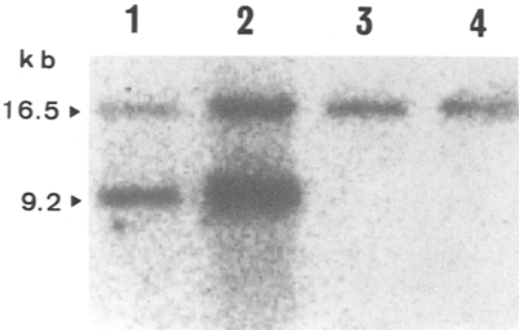

Fig. 6. Hybridization patterns of mitochondrial (mt)DNA probe to normal and deleted populations of liver and muscle mtDNA digested with *PvuII*, which cleaves mtDNA and normally produces a single linear fragment of 16.5 kb. The liver *(lane 1)* and skeletal muscle *(lane 2)* of the patient had wild-type genomes of 16.5 kb with deleted genomes of 9.2 kb. Control muscles (*lane 3* and *4*) had only wild-type of mtDNA

genomes. Total DNA was isolated from the tissue by the use of proteinase K. After digestion of the sample with the endonuclease *Pvu II*, the sample was subjected to Southern blot analysis. The hybridization probe, dAAAGACGTTAGGTCAAGGTGTAG from nucleotide number 1317–1339, was synthesized with an automatic DNA synthesizer (Model 380B, Applied Biosystems, Foster City, Calif., USA) and then labelled at the 5′- end with [γ^{32}P]ATP and T4-polynucleotide kinase. For quantitative analyses of the deleted genomes, the radioactivity of each band in the gel was estimated with a Fujix Bioimaging analyser (BAS 2000, Fuji, Tokyo, Japan).

The nucleotide sequences at the boundaries of the deletion were analysed. The deleted mtDNA fragments were amplified by polymerase chain reaction (PCR) with 100 ng of total DNA as templates with two primers that held the deleted region. The amplified DNA fragments were subjected to asymmetric PCR to produce single-stranded DNA fragments as sequencing templates. The nucleotide sequences of both strands were determined by using fluorescent dye dideoxynucleotide terminators (Applied Biosystems). The sequence was read by a DNA sequencer (Applied Biosystems).

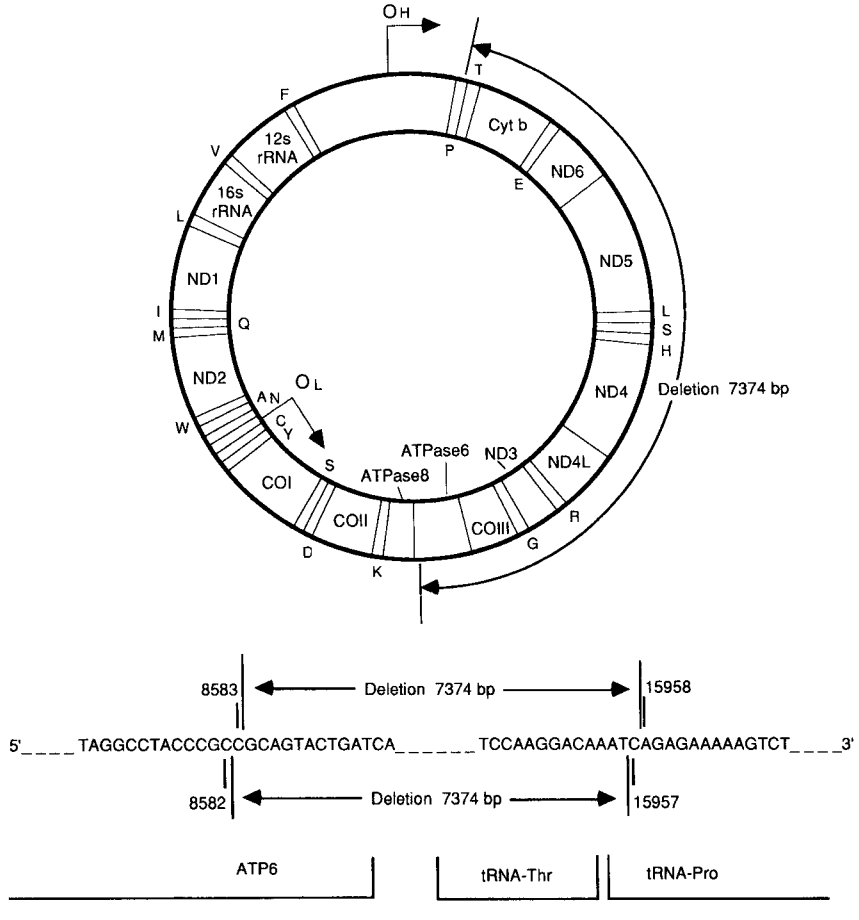

Fig. 7. Upper: map of human mtDNA showing the extent of the deletion found in this patient. Lower: characterization of the nucleotide sequences at the boundaries of the mtDNA deletions in this patient. No direct repeat, but a common single nucleotide, C, was detected at the boundaries of the deleted segment between ATPase 6 and tRNA-pro genes

Results

The liver weighed 290 g and showed many fibrous septa surrounding pseudolobules. There was moderate centrilobular deposition of large fat droplets (Fig. 2, left). The pancreas weighed 20 g with reduction in the size and number of islets and immunohistochemical staining for insulin revealed a decrease in insulin-producing cells (Fig. 3). In part of the pancreatic body, fibrosis and atrophic acini were demonstrated (Fig. 2, right). The spleen was 35 g in weight and congested. The left and right kidneys weighed 45 g and 50 g, respectively. Global sclerosis of about 15 to 20% of glomeruli, dilatation and vacuolation of tubules (Fig. 4) and slight interstitial lymphoid cell infiltration were seen. No significant changes were observed in other organs, including the thyroid and parathyroid glands. Skeletal muscle alterations were the same as those in other types of mitochondrial myopathy except for an increase in the amount of interstitial adipose tissue; in modified Gomori trichrome-stained sections of skeletal muscle, subsarcolemmal collections of mitochondria formed irregular red deposits which have been referred to as RRF (Fig. 5) and strong activity for succinate dehydrogenase was demonstrated in these muscle fibres.

Southern blot analysis after digestion of mtDNA with *PvuII* revealed two populations of mtDNA, one being partly deleted (Fig. 6). PCR amplification and sequence analysis showed that the deletion spanned 7374 bp from nucleotide 8582 to nucleotide 15958 (Fig. 7).

Discussion

Pearson's syndrome is a fatal disorder characterized by refractory sideroblastic anaemia in childhood with vacuolation of marrow precursors and exocrine pancreatic dysfunction. The condition was first described by Pearson et al. (1979), and there have been a number of reports of this disorder (Demeocq et al. 1983; Favareto et al. 1989; Sansone et al. 1989; Stoddard et al. 1981). More recently, the pathogenesis of the syndrome was postulated to be a mutation of mtDNA, and major rearrangements of mtDNA in patients with Pearson's syndrome, characterized by deletion (Jakobs et al. 1991; McShane et al. 1991; Rötig et al. 1990) or deletion-duplication (Rötig et al. 1990; Superti-Furga et al. 1991) flanked by direct repeats, have been described.

Several mechanisms have been proposed for the origin of the deletion of mtDNA; homologous recombination, topoisomerase cleavage, and slip-replication (Wallace 1992). In Pearson's syndrome, the deletion of mtDNA results from homologous recombination because of the deletion flanking direct repeats. However, no flanking direct repeat was detected in mtDNA of our patient. This finding suggests that homologous recombination is not the only deletion mechanism of mtDNA in this disorder.

Hayakawa et al. (1991) reported that mitochondrial myopathy expressed in many patients with acquired immunodeficiency syndrome treated with long-term azidothymidine results from the conversion of deoxyguanosine to 8-hydroxy-deoxyguanosine, and suggested that conformational changes in the DNA helix secondary to the adducted purine would promote deletion of mtDNA, which is a common finding in degenerative neuromuscular diseases. However, Mita et al. (1990) have reported that transfer RNA (tRNA) sequences may participate in the process of deletion not flanked by any obviously unique repeat element, because they can form secondary structures which may bring the breakpoint regions into close proximity (Turker et al. 1987). In our case, the finding that the 3′-end of the deletion was in the tRNA-proline gene, and the tRNA-threonine gene was at 4 bp upstream of the 3′-breakpoint, supports Mita's hypothesis.

Pearson's syndrome is characterized by severe sideroblastic anaemia and exocrine pancreatic dysfunction (Pearson et al. 1979). Although it has often been reported that Kearns-Sayre syndrome (KSS), a mitochondrial myopathy characterized by ophthalmoplegia, retinal pigmentary degeneration, and a conduction block (Wallace 1992), is associated with DM (Egger et al. 1981; Tanabe et al. 1988) and RRF (Egger et al. 1981; Olson et al. 1972; Tanabe et al. 1988) in skeletal muscle, cases of Pearson's syndrome with DM and RRF are rare (Favareto et al. 1989; Superti-Furga et al. 1991). However, our case was associated with insulin-dependent DM, and RRF was detected in skeletal muscle. The findings that KSS is often associated with DM and RRF, and that patients who survive the Pearson's syndrome of infancy later develop KSS (Larsson et al. 1990; McShane et al. 1991) suggest that our patient may have developed KSS in early infancy.

It has been suggested that the phenotype of a mtDNA deletion disorder can change with time and is governed by the fractional concentration of mtDNA with deletion in different tissues (Larsson et al. 1990). Some phenotypes of the mtDNA deletion disorders may be explained by different tissue distributions of the mtDNA deletion. Advances in genetic biology including our results may lead to revision of the traditional pathological and aetiological classification of mtDNA deletion disorders, including KSS and Pearson's syndrome.

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