

Pearson's Marrow-Pancreas Syndrome

A Multisystem Mitochondrial Disorder in Infancy

Agnès Rötig, Valérie Cormier, Stéphane Blanche, Jean-Paul Bonnefont, Françoise Ledeist, Norma Romero,*

Jacques Schmitz, Pierre Rustin, Alain Fischer, Jean-Marie Saudubray, and Arnold Munnich

Unité de Recherches sur les Handicaps Génétiques de l'Enfant, Institut National de la Santé et de la Recherche Médicale (INSERM) U-12 et Département de Pédiatrie, Hôpital des Enfants-Malades, 75743 Paris, France; and *Unité de Recherches de Biologie et Pathologie Neuromusculaires, INSERM U-153, 75005 Paris, France

Abstract

Pearson's marrow-pancreas syndrome (McKusick No. 26056) is a fatal disorder of hitherto unknown etiology involving the hematopoietic system, exocrine pancreas, liver, and kidneys. The observation of high lactate/pyruvate molar ratios in plasma and abnormal oxidative phosphorylation in lymphocytes led us to postulate that Pearson's syndrome belongs to the group of mitochondrial cytopathies. Since rearrangements of the mitochondrial genome between direct DNA repeats were consistently found in all tissues tested, our results show that this disease is in fact a multisystem mitochondrial disorder, as suggested by the clinical course of the patients. Based on these observations, we would suggest giving consideration to the hypothesis of a defect of oxidative phosphorylation in elucidating the origin of other syndromes, especially those associated with an abnormal oxidoreduction status in plasma. (*J. Clin. Invest.* 1990. 86:1601-1608.) Key words: Pearson's syndrome • lactate/pyruvate molar ratios • mitochondrial cytopathies • rearrangement of the mitochondrial DNA

Introduction

Pearson's disease (McKusick No. 26056) is a new syndrome of refractory sideroblastic anemia in childhood with vacuolization of marrow precursors and exocrine pancreatic dysfunction. Severe, transfusion-dependent, macrocytic anemia begins in early infancy and is associated with a variable degree of neutropenia and thrombopenia (1, 2). A simple metabolic screening, based on the determination of oxidoreduction status in plasma, led us to consider the possibility of a multisystem disorder of oxidative metabolism in Pearson's syndrome and to ascribe this fatal disease of hitherto unknown origin to a widespread mitochondrial respiratory enzyme defect. In addition, we describe here the rearrangements of the mitochondrial (mt)¹ genome between directly repeated DNA

sequences as a consistent feature of this disease. Based on these observations, we hypothesize that disorders of mitochondrial energy metabolism could possibly account for several other syndromes of hitherto unexplained origin in man.

Methods

Case reports

Patient 1. A 2,990-g boy was born after an uneventful pregnancy to healthy, unrelated parents. There were no siblings and the family history was negative. Pallor, vomiting, diarrhea, and growth failure were initially noted at 8 wk of age and worsened rapidly. At 4 mo of age a severe sideroblastic anemia with reticulocytopenia and persistent diarrhea necessitated repeated transfusions and institution of a gluten-free diet. Neutropenia and thrombopenia were noted. Bone marrow aspirate was normally cellular, with striking vacuolization of both erythroid and myeloid precursors. At 7 mo of age liver involvement was first noted (elevated transaminases, hyperbilirubinemia). By 1 yr of age he had persistent diarrhea (three to five semiliquid stools/day) and hepatomegaly became gradually prominent with decreased coagulation factors. Steatorrhea was initially moderate (4 g/d, normal below 3), but duodenal aspiration after stimulation later showed a severe exocrine pancreatic insufficiency. Permanent metabolic acidosis, hyperlactatemia, and hypercalciuria were first observed at that age and rapidly worsened (Table I). He died at 14 mo of age after an acute episode of metabolic acidosis with liver failure. At autopsy, the liver was noted to be enlarged with nodular cirrhosis. The pancreas was poorly lobulated and atrophic.

Patient 2. A 2,550-g girl was born after a term pregnancy to unrelated, healthy parents. An older sibling was healthy. Anorexia and vomiting were noted at 1 mo of age and led to recommendation of exclusive breast feeding until the age of 6 mo. At 6.5 mo of age an episode of lethargy and dehydration occurred. Shortly thereafter, a severe pancytopenia of unknown origin was diagnosed. A marked vacuolization of erythroid and myeloid precursors was found in her bone marrow aspirate. At 10 mo of age another episode of lethargy, dehydration, and fever occurred. This led to the discovery of a permanent metabolic acidosis and hyperlactatemia (Table I). At 18 mo a severe exocrine pancreatic insufficiency was observed. She developed major liver enlargement with progressive hepatic failure and died at 30 mo of age.

Patient 3. A 3,300-g girl was born after normal pregnancy and delivery. An older sibling was healthy. A severe anemia with hydrops fetalis necessitated blood exchange in the first few hours of life for putative blood group incompatibility. A transfusion-dependent anemia with neutropenia and thrombopenia at 1 mo of age could not be explained, but a vacuolization of both myeloid and erythroid precursors was noted in bone marrow aspirate. Mild permanent metabolic acidosis was also observed (plasma bicarbonates 15-20 mM) (Table I). Anorexia with diarrhea, hepatomegaly, and failure to thrive appeared at 8 mo of age and worsened rapidly. At 17 mo she progressively developed total anorexia, recurrent infections, and tubulopathy. Parenteral nutrition precipitated fatal liver failure at 25 mo of age.

Patient 4. A 3,460-g girl was born after a term pregnancy to healthy, unrelated parents. One older brother was healthy. She developed nor-

Address reprint requests to Dr. Arnold Munnich, Unité De Recherches Sur Les Handicaps, Genetiques De L'Enfant, INSERM U-12, 149 Rue De Sevres, 75743 Paris Cedex 15, France.

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1. Abbreviations used in this paper: mt, mitochondrial; nt, nucleotide; PCR, polymerase chain reaction.

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Table I. Laboratory Investigations in Five Patients with Pearson's Syndrome

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Control
Blood						
Anemia (Hb g/dl)	5.9–9	6.8–8.2	7.4	6	7.8	12–14
Reticulocytopenia (elts/mm ³)	3,700	23,000	>5,000	5,000	5,000	50,000
Granulocytopenia (elts/mm ³)	1,500–3,000 at 1 yr	4,400 at 18 mo	400 at 3 mo	3,500	800	7,000
Thrombopenia (platelets/mm ³)	20,000 at 1 yr	42,000 at 18 mo	18,000 at 3 mo	50,000–100,000	105,000	>150,000
Bone marrow						
Cellularity	Normal	Normal	Normal	Increased	Normal	
Ringed sideroblasts (%)	18	26	—	45	10	0
Vacuolization	Yes	Yes	Yes	Yes	Yes	0
Exocrine pancreatic function (μmol/ml per min)						
Lipase	0	0	ND	ND	ND	1–3
Chymotrypsin	0	0	ND	ND	ND	0.8–1
Redox status in plasma						
Lactatemia (mM)	11.4	6.7	11.9	7–9.8	3.5	0.6–2.4
Pyruvicemia (mM)	0.216	0.200	0.215	ND	0.16	0.045–0.190
L/P ratio	>30	>30	>30	25	22	<20
3 OH butyrate (mM)	0.195	3.54	0.72	ND	0.19	0.02–0.09
Acetoacetate (mM)	0.041	0.44	0.17		0.05	0.016–0.04
3 OH butyrate/ acetoacetate	4.7	8	4.2	ND	3–5	<2

L, lactate; P, pyruvate.

mally until the age of 7 mo, when an acute episode of watery diarrhea and dehydration occurred. At that time intestinal biopsy showed partial villous atrophy. By 1 yr of age a severe pancytopenia required repeated transfusions, while recurrent episodes of diarrhea and dehydration necessitated parenteral nutrition. She progressively developed liver failure with tubulopathy, punctuate keratitis, and metabolic acidosis (Table I). She died at 30 mo of age after an acute episode of dehydration with metabolic acidosis and collapse.

Patient 5. A 3,150-g boy was born to unrelated, healthy parents after a normal pregnancy. He developed normally until 9 mo of age, when pallor and failure to thrive were noted and led to the discovery of a pure aregenerative anemia, requiring iterative transfusions. The diagnosis of Blackfan–Diamond syndrome was first considered and a corticotherapy (2 mg/kg per d) was therefore initiated. 6 mo later recurrent episodes of fever, drowsiness, and diarrhea occurred (three to eight stools/day). At 17 mo of age diarrhea became permanent; steatorrhea (9–10 g/d), neutropenia, and thrombopenia were noted. The liver gradually enlarged with hepatocellular dysfunction and cytolysis (four- to eightfold increase of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, and gamma glutamyl transpeptidase). Nuclear magnetic resonance examination performed for investigating pancreas structure gave poor results due to a major hemochromatosis of liver, spleen, and kidneys. Peculiar erythematous lesions of the forearms were suggestive of a photosensitization. A severe bacterial infection (*Hemophilus influenzae*) occurred and he died at 2 yr of age.

Metabolic investigations in patients with mitochondrial cytopathies

Oxidative phosphorylation includes the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP. During the oxidation process, reducing equivalents are transferred to oxygen via the energy-transducing complexes of the mitochondrial respiratory chain (NADH oxidase activity via complexes I, III, and IV for the NADH-producing substrates, succinate oxidase activity via complexes

II, III, and IV for succinate). Consequently, a disorder of oxidative phosphorylation should result in an increase of reducing equivalents in both mitochondria and cytoplasm. Therefore, an increase of both ketone body and lactate/pyruvate molar ratios might be found in the plasma of affected individuals. For this reason, in patients likely to suffer from mitochondrial cytopathies our current screening for inborn errors of oxidative phosphorylation includes the enzymatic determination of blood lactate/pyruvate and ketone body molar ratios, after deproteinization by perchloric acid, in both fasted and fed individuals.

Biochemical and histopathological investigations

Muscle mitochondria from a 300-mg deltoid biopsy under local anesthesia were prepared as described by Bookelman et al. (3). Oxymetric and spectrometric studies on muscle mitochondria were carried out as described (4, 5). The processing of biopsy specimens for light microscopic histochemistry was performed according to Dubowitz and Brooke (6).

For measurement of oxygen consumption by intact cells, lymphocytes were first isolated from 10 ml heparinized venous blood on Ficoll cushion (7) and counted. Then oxygen consumption by 20×10^6 intact lymphocytes was measured with a Clark-type oxygen electrode (Hansatech, Norfolk, UK) in a water-jacketed reaction chamber containing 0.3 M mannitol, 5 mM MgCl₂, 10 mM KCl, 10 mM Na₂HPO₄, pH 7.4, 250 μg BSA in a final volume of 0.5 ml. The rotenone-sensitive NADH oxidase activities, the antimycin-sensitive succinate oxidase activities, and the KCN-sensitive cytochrome *c* oxidase activities were measured after disruption of 20×10^6 lymphocytes (–20°C, overnight) in the presence of specific substrates (0.32 mM NADH, 10 mM succinate, or 50 mM reduced cytochrome *c*) and inhibitors as described (4, 5). EBV-transformed cell lines were submitted to B cell line cloning as described (8, 9).

Molecular analysis of the mt DNA

For Southern blotting, total DNA for different tissues (5 μg) was digested, separated by agarose (0.7%) gel electrophoresis, and transferred

onto nylon filters (Gene Screen Plus; New England Nuclear, Boston, MA). The filters were hybridized with [³²P]dCTP-labeled single-strand mt DNA probes (2 × 10⁶ cpm/ml) cloned in our laboratory and identified by comparison with available sequences (10).

For characterization of the nucleotide (nt) sequence at the boundaries of mt DNA deletions, 1 µg total DNA from lymphoblastoid cell lines was submitted to polymerase chain reaction (PCR) amplification (30 cycles) with two oligonucleotide primers (patient 1: primer A = nt 9,507–nt 9,527, primer B = nt 15,021–nt 15,001; patient 2: primer A = nt 11,113–nt 11,133, primer B = nt 14,257–nt 14,236; patients 3, 4, and 5: primer A = nt 7,598–nt 7,618, primer B = nt 14,257–nt 14,236). DNA was amplified by mixing 100 pmol of each primer with 2.5 U Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) as described (11). Amplified fragments were recovered on low-melting point gel agarose, then cloned in the phage M13 mp18 at the Sma I site of the polylinker, and sequenced by the Sanger technique.

Results

Metabolic investigations, immunological studies, and enzyme activities. All five patients presented with permanent metabolic acidosis and increased ketone body or lactate/pyruvate molar ratios in plasma (Table I). Polarographic studies showed that oxygen consumption by intact lymphocytes was defective and that oxidation of NADH in fragmented lymphocytes was greatly reduced (Table II). Oxidation of succinate and cytochrome *c* was altered to a varying extent. The absolute number of T cells (CD3) and B cells (surface immunoglobins) was otherwise normal. The levels of serum immunoglobulin and mitogen- and antigen-induced lymphocyte proliferation, measured as described (8), were normal as well. On the other hand, oxygen consumption and enzyme activities on muscle mitochondria were normal in vivo (Table II), and all the previous tests were normal in parents and siblings (not shown).

Histopathological observations. Several muscle fibers were found to contain small vacuoles in patients 2 and 3 (at 12 and 18 mo of age, respectively), when stained with hematoxylin eosine. These vacuoles were filled with lipid material that stained positive with Sudan red and Sudan black, especially in type I fibers. In contrast, no lipidosis but a mild selective atrophy of type II fibers was found in the muscle of patient 1 (Fig. 1). No ragged red fibers were seen with the modified Gomori trichrome staining, and no decrease of the oxidative enzyme activities was noted in any patient. In circulating lymphocytes from patient 3, electron microscopy showed normal mitochondria (Fig. 1). Finally, intestinal biopsy displayed normal (patient 3) or partially atrophic villi (patients 1 and 5, not shown).

Molecular analysis of the mt DNA. Southern blot analysis of lymphoblastoid cell line DNA digested with restriction enzyme Bam HI (cleavage at position 14,258) and hybridization using either a Cox 2 or a cytochrome *b* probe showed that patients 2–5 had two populations of mt DNA, one normal (16.5 kb) and one partly deleted (Fig. 2). Initial data in patient 1 were also consistent with a simple heteroplasmic deletion, but digestion with enzyme Bam HI (which normally cleaves the mt DNA at a site located in the deletion) and hybridization with a cytochrome *b* probe detected a mixed population of normal and high molecular weight mt DNA chromosomes (24 kb; Fig. 3, lane 3), while hybridization with a ND4 probe only revealed a normal Bam HI restriction fragment (Fig. 3, lane 4). Finally, evidence for symmetry of the rearrangement was given by the detection of a normal and one single abnormal Eco RI fragment with either a Cox 2 or a cytochrome *b* probe (Fig. 3, lane 5). These experiments confirmed the assumption of a complex rearrangement in patient 1, involving both an insertion and a deletion of the mt chromosomes, and suggested

Table II. Investigation of Oxidative Phosphorylation in Four Patients with Pearson's Syndrome

	Patient 1	Patient 2	Patient 3	Patient 5	Control
O ₂ consumption by intact lymphocytes (nmol O ₂ min ⁻¹ 4.10 ⁻⁷ cells)	1.7	3.5	2.9	5.9	14.06±2.15
O ₂ consumption in fragmented lymphocytes (nmol O ₂ min ⁻¹ 4.10 ⁻⁷ cells)					
NADH ox		1.2	0.1	1.07	5.2±1.75
Succ ox	ND	1.2	1.8	0.89	6.6±2.4
Cyt c ox		4.2	2.7	1.07	11.11±5.02
O ₂ consumption on muscle mitochondria (nmol O ₂ min ⁻¹ mg prot ⁻¹)					
Mal-Pyr	27.3*	66.7	70.2		67±20.6
Succ	23.9*	270	188.6	ND	119±42.8
Asc-TMPD	171.2*	435	423.8		182±67.4
Enzyme activities on muscle mitochondria					
Cyt c ox		4,538			1,352±351
Succ Cyt c red	ND	1,154	ND	ND	185±12
NADH ox		727			503±65

NADH ox, NADH oxidase; Succ ox, succinate oxidase; Cyt c ox, cytochrome *c* oxidase; Succ Cyt c red, succinate cytochrome *c* reductase; mal-pyr, malate-pyruvate; Asc-TMPD, ascorbate-*N,N,N',N'*-tetramethyl-*p*-phenylenediamine. Control values are mean±1 SD for at least 15 individual determinations. * Measured on post-mortem tissue.

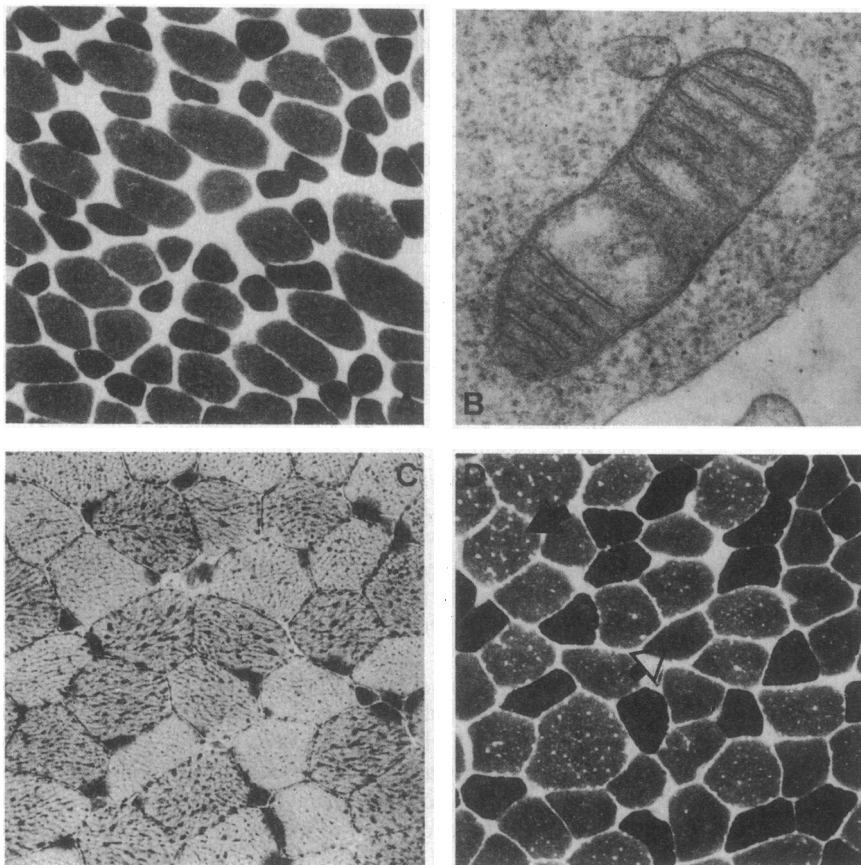


Figure 1. Histopathological observations in muscle and lymphocytes of patients with Pearson's syndrome. *A*, *C*, and *D*, Light micrographs of deltoid muscle fibers from patients 1–3. Note slight atrophy of type II fibers in patient 1 (*A*; ATPase staining) and the mild lipodosis in patients 2 and 3 (*C* and *D*; Sudan red and ATPase staining, respectively). The open arrow in *D* shows a normal fiber. The solid arrow indicates a fiber with mild lipodosis. *B*, Electron micrograph of lymphocyte mitochondria from patient 3. Note very regular distribution of cristae and inner and outer membranes.

the model of a direct duplication of deleted mt genomes (Fig. 3).

In contrast, no rearrangements of the mt genome were found in lymphocytes or cultured fibroblasts from six unrelated patients with Shwachman syndrome (McKusick No. 26040).

PCR amplification and sequence analysis, carried out to characterize the endpoints of the rearrangements in Pearson's syndrome, showed that the deletions in patients 3–5 spanned 4,977 bp, from nt 8,482 to nt 13,460 (Fig. 4 *C*), while patient 2 had a shorter deletion, spanning only 2,748 bp, from nt 11,232 to nt 13,980 (Fig. 4 *B*). The deletion in patient 1 was intermediate in size, spanning 4,192 bp, from nt 10,676 to nt 14,868 (Fig. 4 *A*). Finally, sequence analysis showed that directly repeated sequences of 9–13 bp were present in the wild-type mt genome at the boundaries of the deletions in all five patients (Fig. 4, *A–C*). None of the patient's mothers or siblings were found to bear rearranged molecules, even when their lymphocyte DNA was submitted to PCR amplification using specific oligomers (not shown).

A mixed population of normal and deleted mt genomes was present in all the tissues tested, but different proportions of deleted mt DNA molecules were noted. Indeed, the amount of deleted mt DNA species ranged from 80–90% in the more severely affected tissues (bone marrow, polymorphonuclears, T and non-T lymphocytes, and gut; see Fig. 5, lanes 1–4 and 7) to only 50% in muscle (Fig. 5, lane 6). In addition, the amount of deleted mt DNA molecules was related to a certain extent to the level of the NADH oxidase activity in cultured fibroblasts (ninth subculture) and lymphoblastoid cell lines (normal

NADH oxidase activity and 20–50% of deleted mt genomes were found in both cell cultures; Fig. 5, lanes 5 and 8).

To approach the partitioning of the rearrangement in cultured lymphocytes, transformed cell lines were submitted to limit dilutions and subcultures. 10 subcultures were tested, and all 10 were found to contain a double population of mt DNA molecules (Fig. 5, lane 9).

Discussion

All patients reported here presented with an unexplained syndrome of sideroblastic anemia with neutropenia, thrombopenia, and vacuolization of marrow precursors. Only at the point when exocrine pancreatic dysfunction became apparent could the diagnosis of Pearson's syndrome be made (1). In this study we have been able to describe involvement not only of bone marrow and pancreas but also of liver (hepatic failure), kidney (proximal tubulopathy), gut (watery diarrhea), skin (patchy erythematous lesions, photosensitivity), and possibly other tissues as well. For this reason, Pearson's syndrome should now be regarded as a multisystem disorder in infancy.

The cause of the disease has been unknown, but permanent metabolic acidosis and hyperlactatemia in our patients pointed to a possible disorder of the mitochondrial energy supply and led us to investigate their oxidoreduction status. Elevated lactate/pyruvate and ketone body molar ratios in plasma were consistent with a defect of oxidative phosphorylation and prompted us to investigate the tissue that first expressed the disease, namely the hematopoietic system. This approach resulted in the identification of Pearson's syndrome

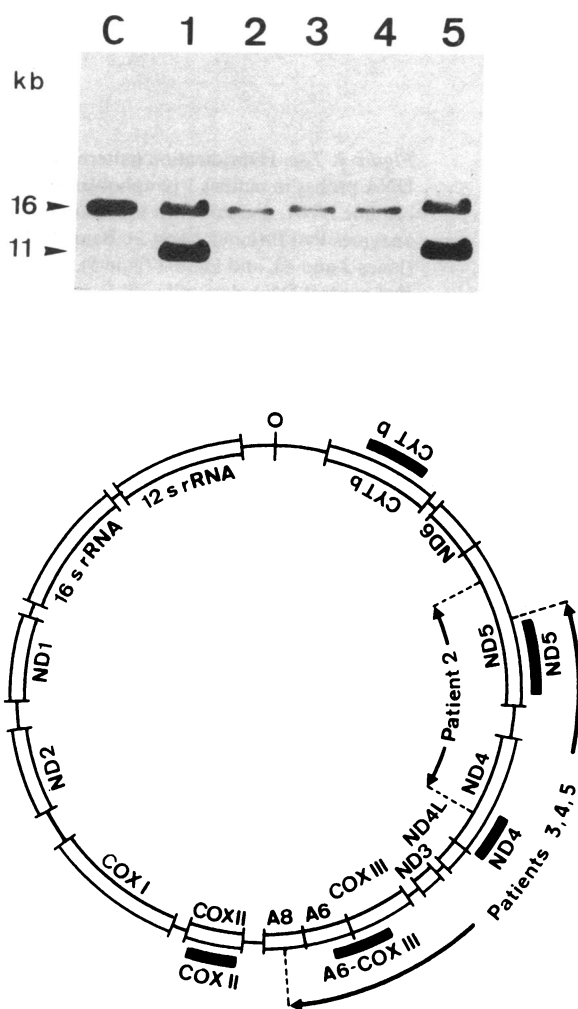


Figure 2. Hybridization patterns of mt DNA probes in patients 2-5 and control lymphoblastoid cell line DNA. (Top) Hybridization to an 11-kb abnormal fragment occurred in patient 3 with a Cox 2 probe (lane 1) and a cytochrome *b* probe (*cyt b*, lane 5). Only one fragment was detected using the ATPase 6-Cox 3 probe (lane 2) and the ND4 or ND5 probes (lanes 3 and 4, respectively). An identical pattern of hybridization was observed in patients 4 and 5. (Bottom) Map of human mt DNA showing the localization of the probes used for hybridization (thick lines) and the extension of the deletions found in patient 2 (shorter deletion) and patients 3-5 (larger deletion).

as the first mitochondrial respiratory enzyme defect of non-neuromuscular expression.

Since the mt genome encodes several polypeptides of the respiratory chain, we first studied its organization in Pearson's syndrome. We were able to describe major rearrangements of mt DNA in all patients, namely, deletions (patients 2-5) or deletion-duplication (patient 1). All the tissues and clones tested contained two populations of mt DNA molecules, one normal and one deleted (heteroplasmy). Moreover, we have recently been able to confirm the presence of a mt DNA deletion in the lymphoblastoid cell line from a patient originally reported by Howard Pearson (see reference 1, courtesy of Charles Mize, Dallas, TX).

Rearrangements of the mt genome are therefore consistent features of Pearson's syndrome, but a number of questions

remain unanswered: What is the mechanism of the progressive organ involvement in this disease and why does the severity of clinical symptoms differ among affected tissues? What are the molecular mechanisms of the rearrangements and how does one explain the sporadic nature of this disease?

The major clinical feature of Pearson's syndrome is the increasing number of tissues affected in the course of the disease. This progressive organ involvement is intriguing and could very well be related to the rearrangements of the mt DNA that we have observed in all tissues and clones tested. One could, for example, consider the hypothesis of a selective advantage of deleted molecules during replication of mt DNA. Along the same lines, the duplication of the origins of replication in patient 1 might progressively favor the preponderance of rearranged molecules. Heretofore it has been considered possible that Pearson's syndrome is related to Shwachman syndrome, another syndrome of pancreatic insufficiency and bone marrow dysfunction (McKusick No. 26040) (12). However, no progressive organ involvement is observed in Shwachman disease and none of the patients with this disease had rearrangements of their mt genome in our study. These data suggest that Pearson's syndrome and Shwachman syndrome are indeed distinct entities, as suggested in Pearson's original report (1).

Why the amount of rearranged molecules differs among affected tissues is also questionable. Multiple copies of mt genomes are normally present in mitochondria, and all the tissues tested contained both normal and rearranged mt DNA in our patients. However, the ratio of mutant to normal molecules differed greatly among tissues. This intriguing feature should be related to either selection or random partitioning of mitochondria during cell divisions and development (mitotic segregation). Whatever the mechanism, it is interesting to note that rearranged mt DNA species were predominant in the most severely affected organs, namely, bone marrow and gut. Indeed, a relatively high level of normal mt DNA molecules was found in skeletal muscle, which was clinically, biochemically, and morphologically normal in our patients (except for mild lipodosis). It seems, therefore, that the phenotypic expression of Pearson's syndrome requires a minimal threshold number of rearranged molecules. Finally, why the defect of oxidative phosphorylation in lymphocytes did not apparently alter mitochondrial structures or immune functions is also unclear and should be related to the short life span of lymphocytes, compared with hepatic or pancreatic cells.

Why all our cases of Pearson's syndrome were sporadic is also surprising, especially as mitochondria are known to be maternally inherited in man (13). The absence of either maternal inheritance or familial forms of the disease is suggestive of de novo mutations. In addition, the observation of widespread multitissular mt DNA deletions in our patients, without rearrangement in peripheral blood cells from their parents and siblings, also favors the view that de novo mutations have arisen during either oogenesis or early development of fertilized eggs. Along the same lines, the direct repeats found at the boundaries of all deleted sequences might have promoted intramolecular recombinations of the mt genome (Fig. 4 D), as commonly occurs in yeast (14). How these rearrangements occur is still open to debate and might possibly involve either breakage-reunion or replication-slippage (15), especially as two direct repeats (*A* and *B*) were one nucleotide away from the limits of the deleted region. Whatever the mechanism, the

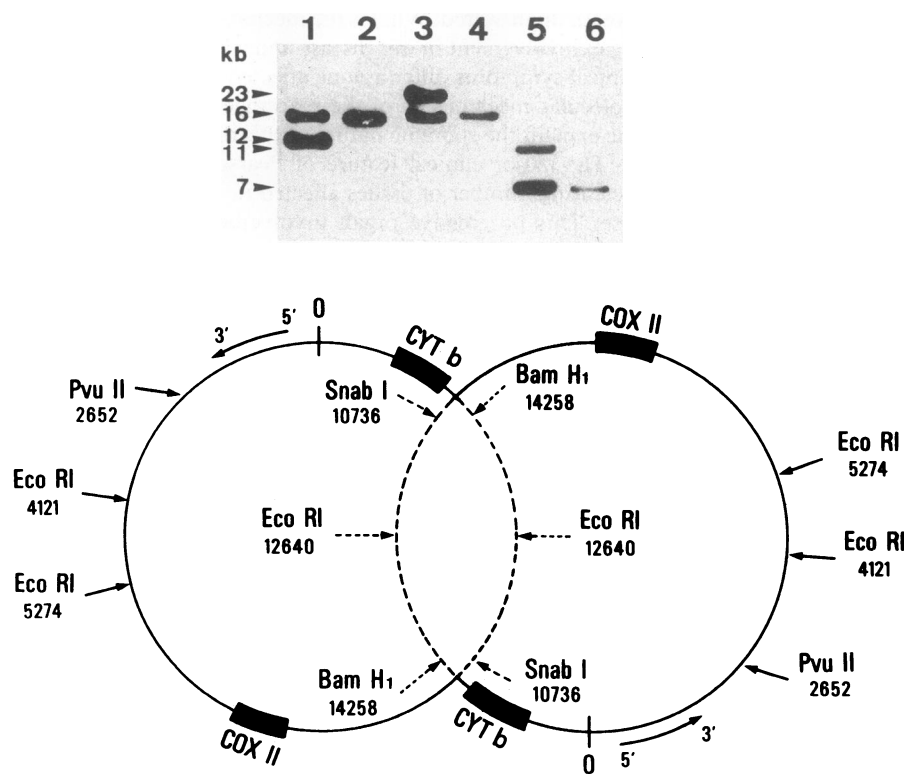


Figure 3. Top, Hybridization pattern of mt DNA probes in patient 1 lymphoblastoid cell line DNA, digested with the restriction enzymes Pvu II (lanes 1 and 2), Bam HI (lanes 3 and 4), and Eco RI (lane 5), and in a control DNA digested with Eco RI (lane 6). A normal (16.5-kb) and an 11-kb abnormal Pvu II fragment were detected using a cytochrome *b* probe (lane 1). Only the normal Pvu II fragment was detected using ND4 (lane 2), ND5, or ND6 probes (not shown). A cytochrome *b* probe revealed two Bam HI species of 16.5 and 24 kb (lane 3). Only the normal Bam HI fragment was revealed using ND4 (lane 4), ND5, or ND6 probes (not shown). Hybridization using a Cox II probe revealed two Eco RI fragments (lane 5) instead of one single 7-kb Eco RI fragment in normal control (lane 6). Bottom, A model of direct duplication of deleted mt DNA molecules which accounts for the results obtained in patient 1 (hatched lines, extent of the mt DNA deletion).

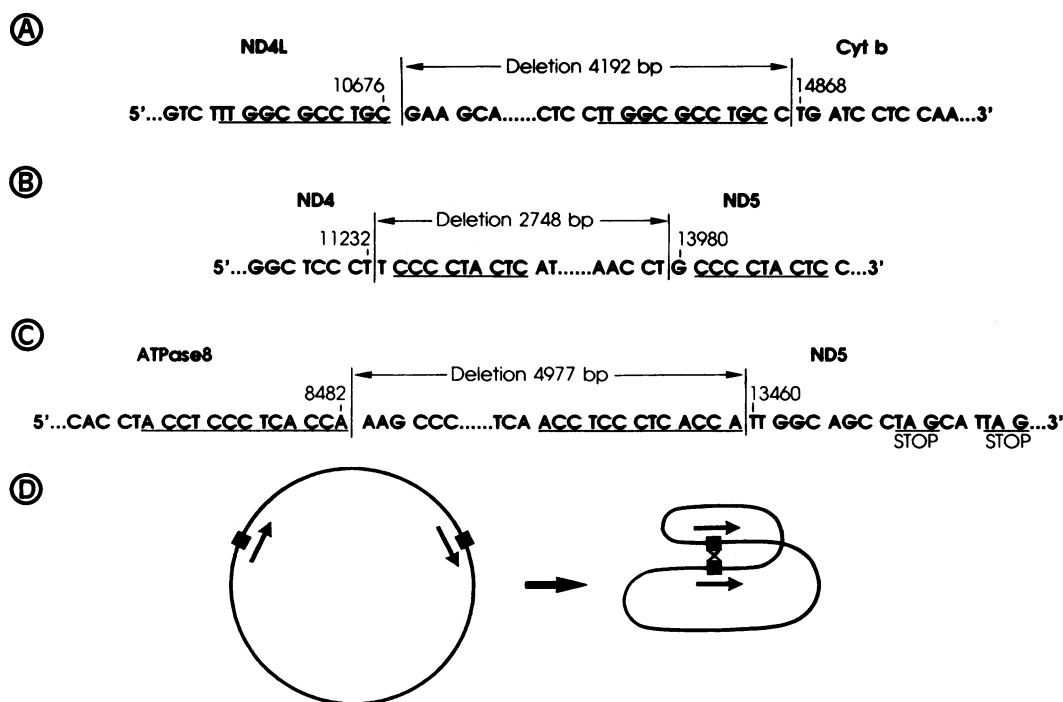


Figure 4. Characterization of the nucleotide sequence at the boundaries of the mt DNA deletions in five cases of Pearson's syndrome. A, An 11-bp direct repeat in the wild-type mt DNA (underlined) was found to flank a 4,192-bp deletion in patient 1. B, A 9-bp direct repeat in the wild-type mt DNA sequence (underlined) was found to flank a 2,748-bp deletion in patient 2. C, A 13-bp direct repeat in the wild-type mt DNA sequence (underlined) was found to flank a 4,977-bp deletion in patients 3-5. D, Intramolecular recombination between the directly repeated sequences is possibly involved in the deletions of the mt DNA genome. These sequence data are available from EMBL/GenBank/DBJ under accession number M125L8.

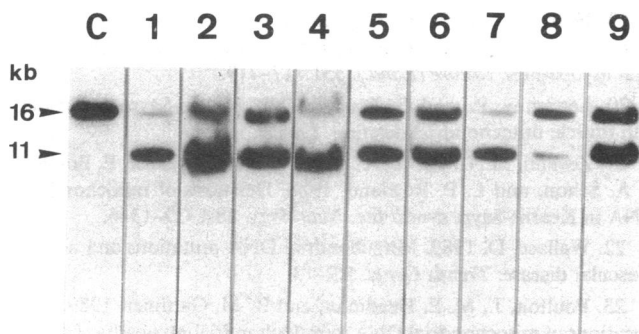


Figure 5. Relative proportions of normal and rearranged mt chromosomes in different tissues from a patient with Pearson's disease (patient 3) and in a control subject. C, Control. Lanes 1–9, tissues derived from patient 1 in vivo. Lane 1, bone marrow; lane 2, polymorphonuclears; lane 3, T lymphocytes; lane 4, non-T lymphocytes; lane 5, lymphoblastoid cell lines; lane 6, muscle; lane 7, small intestine; lane 8, cultured skin fibroblasts; lane 9, cloned lymphoblasts.

strict identity of the rearrangements in patients 3–5 raises the intriguing possibility that the deletions might not occur randomly. There may be instead several sites in this genome that are more prone to rearrangements than other regions of the mt DNA. In keeping with this, it is remarkable to consider that the same genotype is associated with completely different phenotypes in man. Indeed, the same mt DNA deletions (Fig. 4 C) have now been found in three strikingly different disorders; namely, Kearns-Sayre syndrome, mitochondrial myopathies, and Pearson's disease (16–18). Random partitioning of mitochondria during development can possibly account for such a broad spectrum of clinical presentations, ranging from isolated myopathies and Kearns-Sayre syndromes (19–22) to Pearson's syndrome and other progressive multitissular defects (23). Yet the question of why pancytopenia and exocrine pancreatic dysfunction are the first targeted defects in Pearson's syndrome remains intriguing. This observation points toward the possibility that nuclear genes or environmental factors play important roles in the expression of these diseases (24, 25).

The identification of Pearson's syndrome as a multisystem disorder of mitochondrial energy metabolism has several important implications for the clinical management of the patients. First, the increasing number of organs affected in the course of the disease makes bone marrow transplantation illusive and even dangerous in pancytopenic children. Second, a carbohydrate-rich diet apparently precipitated hepatic failure, which was the major cause of fatal outcome in our series. One can easily imagine that a high-glucose diet is a "metabolic challenge" (sometimes performed as a diagnostic test) in patients with a defect of oxidative phosphorylation, especially as glucose oxidation is largely aerobic in the liver. Based on our present experience, we strongly suggest avoiding a hypercaloric diet and parenteral nutrition in these patients and recommend a low-carbohydrate diet in addition to the symptomatic treatment of exocrine pancreatic dysfunction, pancytopenia, and metabolic acidosis.

Finally, as far as prenatal diagnosis of the disease is concerned, this study supports the view that Pearson's syndrome results from de novo mutations. However, the hypothesis of a specific mode of inheritance (particularly a maternal transmission with germinal mosaicism for the mt DNA deletions)

cannot be ruled out. Moreover, because of the random partitioning of mitochondria during embryogenesis, the results of prenatal diagnosis on chorion villi or amniotic cell fluid would be unreliable. Consequently, one must be particularly cautious in delivering genetic counseling for this disease.

In conclusion, this study shows that a careful investigation of oxidoreduction ratios in plasma led us to identify Pearson's syndrome as a mitochondrial disorder of nonneuromuscular expression. Based on these observations, we would suggest giving consideration to the hypothesis of a defect of oxidative phosphorylation in elucidating the origin of other diseases, especially those associating seemingly unrelated symptoms.

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