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Molecular and Genetic Analyses of Two Patients with Pearson's Marrow-Pancreas Syndrome

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ABSTRACT. Pearson's syndrome, a rare and fatal disorder characterized by refractory sideroblastic anemia and pancreatic insufficiency in infancy, is classified into mitochondrial cytopathies. To understand the molecular and genetic bases of this disorder, we have investigated the mitochondrial respiratory chain enzymes and the mitochondrial DNA (mtDNA) in two Japanese patients with Pearson's syndrome. Immunoblot analysis from various tissues showed the different grades of defects in the subunits of respiratory enzyme complexes. The analyses of mtDNA showed that the deletion in patient 1 spanned 4977 bp from the ATPase 8 gene to the NADH dehydrogenase 5 gene between 13-bp direct repeats, whereas the deletion in patient 2 spanned 3151 bp from the transfer RNA^{His} gene to the cytochrome *b* gene unrelated to any repeated sequences. The deleted mtDNA was heteroplasmic in all the analyzed tissues, but the proportions of deleted mtDNA were quite different. We observed a tendency for the tissue with low percentages of normal-sized mtDNA to show low contents of complex I subunits. Analysis of the entire sequence of both patient's mtDNA showed several nucleotide substitutions including alteration of the initiation codon of the NADH dehydrogenase 5 gene. Some of these nucleotide substitutions might contribute to the phenotypic expression of Pearson's syndrome synergistically with the deletion. (*Pediatr Res* 34: 105–110, 1993)

Abbreviations

mtDNA, mitochondrial DNA
CPEO, chronic progressive external ophthalmoplegia
KSS, Kearns-Sayre syndrome
MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes
MERRF, myoclonus epilepsy with ragged-red fibers
ND, NADH dehydrogenase
np, nucleotide position
PCR, polymerase chain reaction
rRNA, ribosomal RNA

Pearson's syndrome (McKusick 260560) is a distinctive clinical entity characterized by refractory sideroblastic anemia with vacuolization of marrow precursors, neutropenia, thrombocytopenia, exocrine pancreatic insufficiency, liver failure, and Fanconi's renal tubular acidosis (1, 2). Up to now, 20 cases have been reported (1–7). Rötig *et al.* (8) reported a large-scale deletion of mtDNA in a patient with Pearson's syndrome. Large-scale deletions of human mtDNA have also been reported in the muscle tissues from patients with CPEO including KSS (9–11). KSS is a multisystem disorder characterized by external ophthalmoplegia, pigmentary retinal degeneration, complete heart block, cerebellar dysfunction, and increase of cerebrospinal fluid protein concentration, but this syndrome generally has neither hematopoietic symptoms nor pancreatic insufficiency. These mitochondrial myopathies and Pearson's syndrome are usually associated with commonly reported deletions of mtDNA, but their clinical manifestations are quite different.

To clarify the molecular and genetic bases of Pearson's syndrome, we analyzed the respiratory enzyme complexes and mutations of mtDNA in various tissues from patients with Pearson's syndrome.

MATERIALS AND METHODS

Patient 1. This 23-mo-old boy was the second child of healthy and unrelated parents. His 3-y-old sister was healthy. Pregnancy and delivery were uneventful. He was born after 39 wk of gestation with a weight of 3920 g. On the 4th d of life, he was noted to be pale. Hb concentration was 106 g/L. One mo later, Hb concentration became lower than 60 g/L and this was accompanied by neutropenia. Throughout his first year, he frequently required blood transfusions. At the age of 23 mo, he was admitted to a hospital under the diagnosis of viral enterocolitis. Laboratory examinations at that time showed pancytopenia, elevation of ringed sideroblast (>15%) and myeloblast (11.8%) in the bone marrow, and lactic acidemia (blood lactate, 754 mg/L; normal value, <149 mg/L). Muscle biopsy showed a preponderance of cytochrome *c* oxidase-negative fibers. After admission, he became drowsy and later comatose. At the age of 28 mo, he died of septicemia with hypoglycemia and severe lactic acidemia.

Patient 2. This 3-mo-old boy was the second child of unrelated parents. His mother and 5-y-old brother were healthy. His father was a carrier of hepatitis B. Pregnancy and delivery were uneventful. He was born after 37 wk of gestation with a weight of 2860 g. He was noted to be pale at birth. On the 1st d of life, he had dyspnea. His blood examinations showed anemia (Hb, 82 g/L) and severe metabolic acidosis (pH, 7.02; base excess, −27.4). Although his symptoms improved and he was discharged on d 16, he was readmitted due to a relapse of severe anemia (Hb, 51 g/L) and lactic acidemia (blood lactate, 686 mg/L) at the age of 3 mo. From that point up to the present day, a period of 18 mo, he has had recurrent episodes of metabolic acidosis and severe anemia requiring transfusions.

Materials. The blood samples for the study were obtained from patient 2 and the family of patient 1. The other samples, including skeletal muscle, heart, liver, kidney, brain, and pancreas, were taken from autopsied tissues of patient 1 and an age-

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matched control after informed consent of the parents. The interval between death and autopsy was about 1 h in both cases.

Immunoblot analysis. Mitochondrial proteins from various tissues were separated by electrophoresis in 9.38 to 18.75% polyacrylamide linear gradient gels (12). The proteins on the gel were transferred to a Durapore filter (Millipore, Bedford, MA) (13) with an addition of 0.1% SDS. The binding of antibodies to the subunits of the mitochondrial enzyme complexes I, III, IV, and V on the filter was detected by the peroxidase-antiperoxidase method (14). Antibodies against mitochondrial enzymes were raised in rabbits by intradermal injections of 1 mg of protein for complexes I, III, IV, and V, which were isolated from beef heart mitochondria. Booster injections of the same mixture were administered 3, 5, and 7 wk later. Antisera were obtained 2 wk after the last injection.

The amounts of the immunochemically detectable subunits of complexes were determined by densitometry using a CS-9000 Dual Wavelength Frying Spot Scanner (Shimadzu, Kyoto, Japan). The amounts of the subunits of complexes in each tissue from patient 1 were compared with those in the same tissue from an age-matched control.

Southern blot analysis. To quantify the relative amounts of deleted mtDNA to total mtDNA, 5 μg of total DNA extracted from various tissues of patient 1 were digested with 50 units of restriction enzyme *Pvu*II (restriction site: np 2650) or *Bam*HI (restriction site: np 14258) (Takara, Kyoto, Japan) for 8 h, electrophoresed in a 0.8% agarose gel at 60 V for 5 h, and transferred to a nylon membrane (Hybond-N$^+$, Amersham, Buckinghamshire, UK) with an addition of 0.4 N NaOH. A 580-bp fragment corresponding to the D-loop region (from np 41 to np 620) of mtDNA was amplified by PCR as described below and was labeled with horseradish peroxidase using an Enhanced Chemiluminescence Hybridization Kit (Amersham). The membrane was hybridized with this probe at 42°C for 12 h. Hybridization and washing of the membrane were carried out according to the manufacturer's instructions. Luminol reaction was detected using an x-ray film. The x-ray film was scanned by densitometry (Shimadzu).

Oligonucleotide primers. Primers were synthesized using a model 391 DNA synthesizer (Applied Biosystems, Foster City, CA) and then purified with oligonucleotide purification cartridges from Applied Biosystems according to the manufacturer's instructions. L and H primers are 20-mer oligonucleotides possessing sequences specific for the light (L) strand and the heavy (H) strand of mtDNA.

PCR amplification. Thirty cycles of PCR amplification were performed with 10 ng of total DNA from skeletal muscles and leukocytes, 50 pmol of a pair of L primer and H primer, 100 mM of each deoxyribonucleoside triphosphate, 1.25 units of *Taq* DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT) in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin, in a total volume of 50 μL. This amplification was carried out using a Perkin-Elmer-Cetus thermal cycler with a cycle of denaturation at 94°C for 15 s, annealing at 50°C for 15 s, and extension at 72°C for 80 s. The amplified segments were electrophoresed in a 1.0% agarose gel in the presence of 0.5 μg/mL ethidium bromide at 100 V for 30 min.

Primer-shift PCR method. Deleted regions of mtDNA were analyzed by the primer-shift PCR method using several primer pairs (15). When a short fragment corresponding to deleted mtDNA was amplified, a second PCR amplification was performed with nesting primer pairs. If the second PCR amplification did not yield a shorter fragment, either of the primer pair was thought to be present in the deleted segment. If the second PCR amplification yielded a shorter fragment, a third PCR amplification was performed with other nesting primer pairs.

Fluorescence-based direct sequencing. The entire sequence of mtDNA from both patients was determined by the fluorescence-based direct sequencing method (16). Sequencing analysis was performed using a 373A DNA sequencer (Applied Biosystems). Sequencing reaction was carried out using asymmetrically amplified mtDNA fragments as templates with 10 cycles of denaturation at 90°C for 15 s and annealing and extension at 70°C for 1 min. A *Taq* sequencing kit was obtained from Applied Biosystems. All the nucleotides were compared with the published human mtDNA sequence (17). The entire sequence of mtDNA from 40 subjects including six healthy individuals and 34 patients with various mitochondrial diseases including MELAS, MERRF, KSS, hypertrophic cardiomyopathy, congenital complex I deficiency, Leber's hereditary optic neuropathy, or other myopathies were analyzed as controls.

RESULTS

Immunoblot analysis. The amounts of the subunits of the mitochondrial respiratory chain complexes I, III, IV, and V from various tissues of patient 1 were analyzed by immunoblotting. This analysis showed severe disproportionate deficiency of complex I subunits. Severe defects were predominantly observed in large molecular weight subunits. This disproportionate deficiency of complex I subunits was basically similar to that observed in tissues from patients with MELAS (14). The relative contents of complex I subunits will be compared with the proportion of deleted DNA present in the later section. The contents of the subunits of complexes III, IV, and V were slightly reduced (Figure 1 shows immunoblotting of complexes I and IV).

Southern blot analysis of mtDNA. Southern blot analysis after digestion with *Bam*HI revealed two fragments: a 16.6-kb fragment corresponding to normal-sized mtDNA and an 11.6-kb fragment corresponding to deleted mtDNA. The deleted mtDNA was present in all the analyzed tissues, but the proportions of deleted mtDNA were quite different (Fig. 2). Digestion with *Pvu*II also yielded two other fragments: one was 15.0 kb instead of 16.6 kb and the other was 10.0 kb instead of 11.6 kb. The ratio of the 10.0-kb band to the 15.0-kb band after digestion with *Pvu*II was equal to the ratio of the 11.6-kb band to the 16.6-kb band after digestion with *Bam*HI. A T-to-C transition at np 1005 in the 12S rRNA gene observed in patient 1 created a new *Pvu*II site (Fig. 3) and divided a 16.6-kb fragment into a 15.0-kb fragment and a 1.6-kb fragment. This T-to-C transition showed homoplasmy. The 1.6-kb fragment was not detected by the probe used here. The total amounts of mtDNA are not decreased in various tissues as judged by the density of bands in the Southern blot analysis.

Analysis of deleted regions. The size and site of deleted segments in the two patients were analyzed using the primer-shift PCR method. The deleted segment in patient 1 spanned 4977 bp from the ATPase 8 gene to the ND5 gene, whereas the deleted segment in patient 2 spanned 3151 bp from the transfer RNA^{His} gene to the cytochrome *b* gene (Fig. 3). Nucleotide sequences at the boundaries of the deleted regions were analyzed using the direct sequencing method. Thirteen-bp direct repeats were detected at the boundaries of the deleted region in patient 1, whereas neither direct nor indirect repeats were detected at the boundaries of the deleted region in patient 2.

Comparison between proportions of normal-sized mtDNA and contents of complex I subunits. Figure 4 shows a comparison between the proportions of the normal-sized mtDNA and the contents of complex I subunits among various tissues. The proportions of normal-sized mtDNA to total mtDNA (total mtDNA = normal-sized mtDNA + deleted mtDNA) were distributed in a wide range from 13% in the liver and pancreas to 57% in the heart. The contents of complex I subunits in the kidney showed the most obvious deficiency (10% of the control) in proportion to the severe reduction in the percentage of normal-sized mtDNA (22%), whereas the deficiencies of complex I subunits in the heart and the brain were mild (55 and 41% of the control, respectively) in proportion to the slight reduction in the percentage of normal-sized mtDNA (57 and 33%, respectively). In the skeletal muscle, the deficiency of complex I sub-

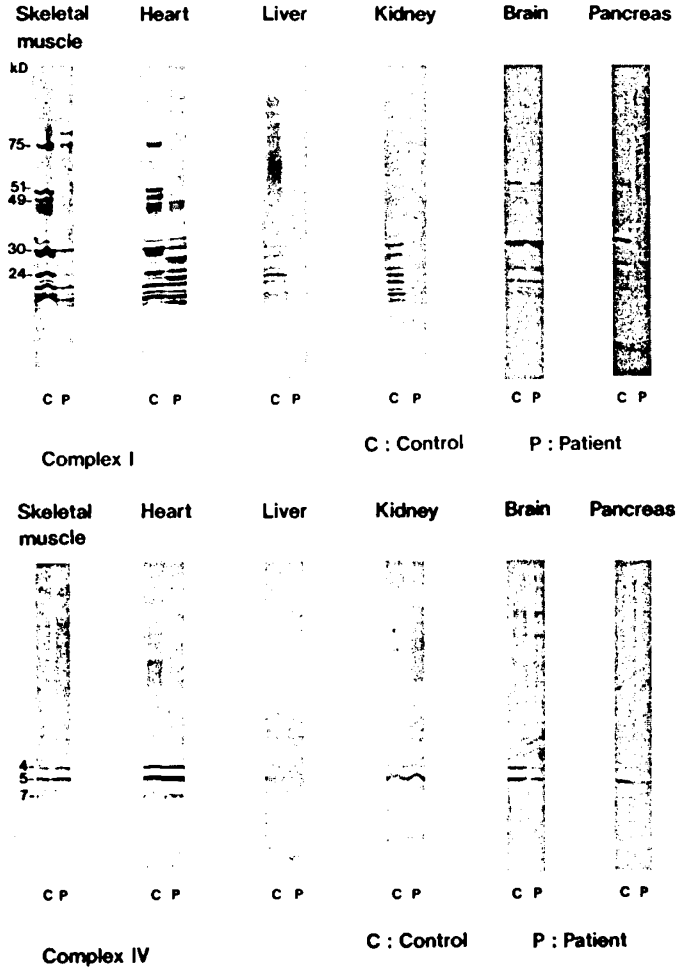

Fig. 1. Immunoblot analysis of complexes I and IV of mitochondrial respiratory chain in various tissues of patient 1. The amounts of subunits of complexes I and IV in each tissue from patient 1 were compared with those in the same tissue from an age-matched control. Immunoblot analysis showed severe disproportionate deficiency of complex I subunits. Severe defects were predominantly observed in large molecular weight subunits. The contents of complex IV subunits were slightly reduced. The amounts of loaded mitochondria protein were 40 μg for skeletal muscle, heart muscle, brain, and pancreas, and 80 μg for liver and kidney. The numbers *4*, *5*, and *7* on the left side of the immunoblot for complex IV denote the subunits 4, 5, and 7 of this complex.

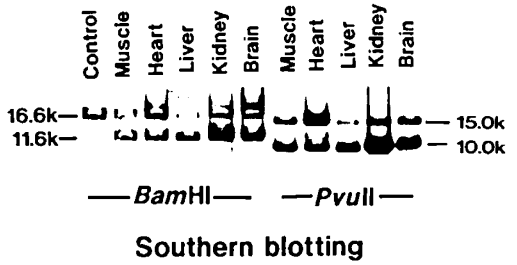

Fig. 2. Southern blot analysis of mtDNA in various tissues of patient 1. Total DNA from various tissues of patient 1 was digested with *Pvu*II or *Bam*HI and hybridized with a probe of 580-bp fragment corresponding to the D-loop region. Southern blot analysis after digestion with *Bam*HI revealed two fragments in all the analyzed tissues: one was 16.6 kb (normal-sized mtDNA) and the other was 11.6 kb (deleted mtDNA). The mtDNA after digestion with *Pvu*II also revealed two other fragments: one was 15.0 kb instead of 16.6 kb and the other was 10.0 kb instead of 11.6 kb.

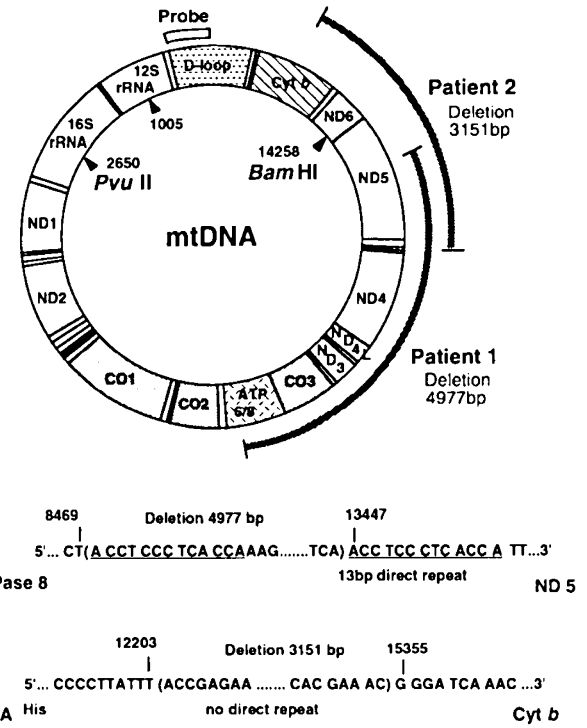

Fig. 3. The deleted regions and the sequences of the boundaries. The deletion in patient 1 spanned 4977 bp from the ATPase 8 (*ATP8*) gene to the ND5 gene between 13-bp direct repeats, whereas the deletion in patient 2 spanned 3151 bp from the transfer RNA^{His} gene to the cytochrome *b* (*Cyt b*) gene unrelated to any direct repeated sequences.

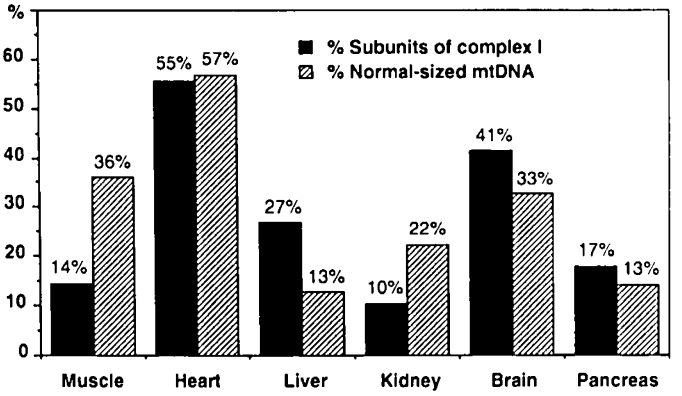

Fig. 4. Comparison between the proportions of normal-sized mtDNA and the contents of complex I subunits in various tissues. The tissues with a high proportion of normal-sized mtDNA contained high amounts of complex I subunits (heart, muscle, and brain), whereas the tissues with a low proportion of normal-sized mtDNA had a severe deficiency of complex I subunits (liver, kidney, and pancreas). In the skeletal muscle, the contents of complex I subunits (14% of the control) were distinctly less in comparison with the proportion of normal-sized mtDNA (36%). *% Subunits of complex I* represents the ratio of the amounts of complex I subunits in each tissue from patient 1 to those in the same tissue from an age-matched control.

units (14% of the control) was distinctly severe in comparison with the proportion of normal-sized mtDNA (36%).

Identification of point mutations in mtDNA. The entire sequence of mtDNA from the two patients was directly determined. All the nucleotide substitutions relative to the published human mtDNA sequence (17) were surveyed and compared with the sequences of 40 controls. Table 1 shows a summary of rare (found in less than 10% of the 40 controls) nucleotide substitutions in the entire mtDNA sequence that were commonly found

Table 1. *Rare* point mutations found in mtDNA of patients with Pearson's syndrome*

	Nucleotide position	Region	Nucleotide†			Frequency in controls‡	Amino acid				
			Std	P1	P2		Mut§	Human	Bovine	Mouse	Rat
Protein coding genes (replacement of amino acid)											
	13928	ND5	G	C	C	1	Thr	Ser	Thr	Ser	Ile
	12338	ND5	T	C	•	0	Thr	Met‖	Met	Ile	Met
	11150	ND4	G	A	•	0	Thr	Ala	Ile	Ile	Ile
	13708	ND5	G	A	•	0	Thr	Ala	Leu	Ala	Ala
	15714	Cyt *b*	C	T	•	0	Leu	Ser	Cys	Ile	Ile
	4732	ND2	A	•	G	1	Ser	Asn	Leu	Tyr	Tyr
	10609	ND4L	T	•	C	1	Thr	Met	Thr	Thr	Ser
	12406	ND5	G	•	A	1	Ile	Val	Phe	Ser	Thr
Protein coding genes (no replacement of amino acid)											
	3970	ND1	C	T	T	1		Leu			
	6392	CO1	T	C	C	2		Asn			
	10310	ND3	G	A	A	2		Leu			
	7828	CO2	A	G	•	0		Leu			
	10535	ND4L	T	C	•	0		Tyr			
	10586	ND4L	G	A	•	0		Ser			
	13722	ND5	A	G	•	0		Leu			
	3657	ND1	C	•	G	2		Leu			
	5049	ND2	C	•	T	0		Leu			
	5147	ND2	G	•	A	1		Thr			
	6962	CO1	G	•	A	2		Leu			
	10976	ND4	C	•	T	1		Leu			
	12633	ND5	C	•	T	1		Ser			
	12882	ND5	C	•	T	1		Phe			
rRNA genes											
	1005	12SrRNA	T	C	•	0					
	1824	16SrRNA	T	C	•	0					
D-loop region											
	16304	D-loop	T	C	C	1					
	16066	D-loop	A	G	•	0					
	16192	D-loop	C	T	•	1					
	16239	D-loop	C	T	•	0					
	16355	D-loop	C	T	•	1					
	16182	D-loop	A	•	C	2					
	16249	D-loop	T	•	C	1					
	16344	D-loop	C	•	T	1					
Noncoding regions											
	248	NCR	A	–	–	1					
	303.2	NCR	–	•	C	3					
	303.3	NCR	–	•	C	0					
	317	NCR	C	•	T	0					
	15954	NCR	A	•	G	0					

* Rare mutation indicates the mutation found in less than 10% of controls.

† Std represents the standard mtDNA sequence reported by Anderson *et al.* (17); P1 and P2 represent mtDNA sequences found in Patients 1 and 2. Minus sign represents deletion; dot indicates the same as that of the standard nucleotide.

‡ Control samples were obtained from 34 patients with MELAS, MERRF, KSS, hypertrophic cardiomyopathy, congenital complex I deficiency, Leber's hereditary optic neuropathy, or other myopathies and six healthy individuals. Number indicates the frequency of subjects with the same mutated mtDNA in 40 controls.

§ Mut represents resulting amino acid substitution.

‖ This methionine is the initiation codon of the ND5 gene.

in the two patients. Six common nucleotide substitutions (np 13928, 3970, 6392, 10310, 16304, and 248) were identified in these patients. Although a G-to-C transversion at np 13928 in the ND5 gene causes the replacement from Ser to Thr, Thr at this site can be observed in the bovine gene product. Five other nucleotide substitutions common to these patients were silent, causing no replacements of amino acid. Furthermore, 31 nucleotide substitutions were observed in either patient. A T-to-C transition was found at np 12338 in the ND5 gene in patient 1 but was not found in patient 2. It is noteworthy that this transition altered the initiation codon ATA for Met in the ND5 gene into ACA, which was normally a codon for Thr. This initiation codon for Met (or Ile) is conserved among four mammalian species. This T-to-C transition was not observed in the 40 controls or in six patients with CPEO. The ACA codon has never been observed in any initiation codons of vertebrate mitochondrial messenger RNA (18). Although six other transitions caused replacements of amino acids, these amino acids were not conserved among four mammalian species. No point mutations in the transfer RNA genes were found in either patient. Two other T-to-C transitions in the rRNA genes (np 1005 in the 12S rRNA gene and np 1824 in the 16S rRNA gene) were observed in patient 1, but not in the 40 controls or in patient 2. The transition at np 1005 created a new *Pvu*II site. Point mutations

that potentially induced the occurrence of deletions were not observed in either patient in the regions surrounding the deletions.

Analyses of mtDNA in family of patient 1. We analyzed blood samples from the parents and healthy sister of patient 1 to determine whether the deletion and point mutations identified in patient 1 were transmitted from his parents. Neither Southern blot analysis nor PCR amplification analysis could detect any deletions in the mtDNA from the peripheral blood cells of his parents and sister (data not shown). All the rare nucleotide substitutions identified in patient 1 were also observed in both his mother and sister in a homoplasmic fashion.

DISCUSSION

We have investigated the mitochondrial respiratory chain enzymes and the mtDNA in two patients with Pearson's syndrome. Although none of our patients had obvious pancreatic insufficiency, they were diagnosed as having Pearson's syndrome on the basis of hematopoietic symptoms and various laboratory findings. The amounts of the subunits of complexes were reduced in all the tissues analyzed in patient 1 (Fig. 1). Complex I showed the severest defect among the respiratory complexes. To clarify the pathogenesis of defects in the respiratory enzyme complexes in Pearson's syndrome, we analyzed the deleted mtDNA in various tissues of patient 1. The deleted mtDNA was heteroplasmic in all the analyzed tissues, but the proportions of deleted mtDNA were quite different (Fig. 2). Mitochondria have their own DNA coding for 13 subunits of enzyme complexes in the energy-transducing system. Complex I contains the largest number of mtDNA-encoded subunits among the mitochondrial respiratory complexes. The severe defect seems to reflect that complex I is most affected by mtDNA mutations or deletions.

Rötig *et al.* (8, 19) reported a large-scale deletion of mtDNA in a patient with Pearson's syndrome. In recent studies, several mutations of mtDNA associated with neuromuscular disorders have been reported. Deleted mtDNA was identified in patients with CPEO or KSS, whereas several point mutations of mtDNA were reported in patients with MELAS (16, 20, 21), MERRF (22, 23), or Leber's hereditary optic neuropathy (24, 25). In the present study, we analyzed both the deletions and the point mutations of mtDNA from two patients with Pearson's syndrome.

The onset of CPEO is usually before adolescence, whereas Pearson's syndrome is characterized by onset in infancy and early death before age 3 y. The clinical manifestations of CPEO and Pearson's syndrome are also quite different. The 4977-bp deletion observed in patient 1 (Fig. 3) is identical with the deleted region reported in six of 10 patients with Pearson's syndrome (6, 19) and in 13 of 30 patients with CPEO (26). Deleted regions in other cases of Pearson's syndrome are quite variable (sizes of deletions: 2.7–7.5 kb; sites of deletions: from the ATPase 8 gene to the cytochrome *b* gene). It has been reported that there is no correlation between the size or site of the deletion and clinical severity in KSS (27). In the present study, the 3-kb deletion found in patient 2 has never been reported in any other patient with Pearson's syndrome. Although both patients reported here had mtDNA deletions of different location and size, they showed similar clinical symptoms. In patients with CPEO, the deletion of mtDNA is reported to be associated with decreased activity of complex I (26). In the present molecular analysis, we have demonstrated the defects of complex I subunits in the patient with Pearson's syndrome. These observations suggest that CPEO and Pearson's syndrome may have common molecular and genetic mechanisms in pathogenesis. This idea is supported by two reported cases in which patients spontaneously recovered from Pearson's syndrome and later developed CPEO (5, 6).

In a case of KSS reported by Hurko *et al.* (28), the proportion of normal-sized mtDNA was lower in symptomatic postmitotic tissues, namely the brain, heart, and skeletal muscle, than in the liver and kidney. In the present case of Pearson's syndrome, patient 1, the proportion of normal-sized mtDNA was higher in the asymptomatic postmitotic tissues than in other tissues. We observed a tendency for the tissues with low percentages of normal-sized mtDNA to have low contents of complex I subunits (Fig. 4). However, the liver, which had the lowest proportion of normal-sized mtDNA, and the kidney, which showed the severest defects of complex I subunits, did not manifest severe symptoms. Although a low proportion of normal-sized mtDNA and severe defects of complex I subunits were observed in the pancreas, the exocrine function of the pancreas was normal in this patient. It is also noted that the defect of complex I subunits was distinctly severe in the skeletal muscle in comparison with the proportion of normal-sized mtDNA. These findings suggest that not only the proportion of deleted mtDNA and the degree of defect in the subunits of respiratory complexes but also the energy demands and reserve in each tissue are important in phenotypic expression of the mtDNA mutations.

It is suspected that the small population of deleted mtDNA from the peripheral blood of patients with CPEO is due to slower proliferation of cells with deleted mtDNA (29). Severe anemia in Pearson's syndrome is also explained by dysfunction of hematopoietic cells containing a high population of deleted mtDNA. Spontaneous remission of anemia in some cases of Pearson's syndrome is probably based on preferential selection of hematopoietic cells with a low population of deleted mtDNA. It has been reported that the proportion of deleted mtDNA in the muscle tissue increases with the progress of disease in patients with KSS (5). The gradual deterioration of muscles and the CNS in CPEO may be attributable partly to the gradual increase of deleted mtDNA and partly to the accumulation of deleterious effects of mtDNA deletions in postmitotic tissues. Compared with CPEO, the population of deleted mtDNA in patients with Pearson's syndrome may be much higher in the multiple organs, especially in the hematopoietic tissues, from an early stage of life. One explanation for the severe dysfunction of tissues in Pearson's syndrome is that once the population of deleted mtDNA in the hematopoietic organ and exocrine pancreas accumulates over each critical threshold, the functional impairment of these mitotically active tissues results in severer symptoms and earlier onset.

In the yeast *mit*$^-$ mutant, small and discrete mutations are associated with a significant rise in the rate of deletions (30). It is unknown whether some of the nucleotide substitutions found in the present patients increase the instability of mtDNA leading to deletions.

In a case of KSS without anemia, Fischel-Ghodsian *et al.* (31) recently reported that 75% of mtDNA from blood cells had a large-scale deletion. This case indicates that tissue distribution of deleted mtDNA may not always be a sufficient explanation for the different phenotypes between Pearson's syndrome and KSS.

We identified several nucleotide substitutions (Table 1) including a T-to-C transition at np 12338 associated with alteration of an initiation codon ATA for Met in the ND5 gene into a codon ACA for Thr. Most of the nucleotide substitutions that were commonly observed between two patients seem to be silent mutations. The G-to-C transition at 13928 causes a Ser-to-Thr replacement, but this Ser residue is not conserved among mammalian species. Nucleotide substitutions that were unique to each patient also caused amino acid replacements. The contribution of these nucleotide substitutions, in synergy with the deletion of mtDNA, will provide a possible explanation for distinct phenotypic expressions between KSS and Pearson's syndrome.

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