Novel 5.712 kb mitochondrial DNA deletion in a patient with Pearson syndrome: A case report

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Abstract. Pearson marrow-pancreas syndrome (PS) is a progressive multi-organ disorder caused by deletions and duplications of mitochondrial DNA (mtDNA). PS is often fatal in infancy, and the majority of patients with PS succumb to the disease before reaching three-years-of-age, due to septicemia, metabolic acidosis or hepatocellular insufficiency. The present report describes the case of a four-month-old infant with severe normocytic normochromic anemia, vacuolization of hematopoietic precursors and metabolic acidosis. After extensive clinical investigation, the patient was diagnosed with PS, which was confirmed by molecular analysis of mtDNA. The molecular analysis detected a novel large-scale (5.712 kb) deletion spanning nucleotides 8,011 to 13,722 of mtDNA, which lacked direct repeats at the deletion boundaries. The present report is, to the best of our knowledge, the first case reported in South Korea.

Introduction

Pearson marrow-pancreas syndrome (PS; MIM #557000), was initially described in 1979, and is a progressive multi-organ disorder caused by deletions and duplications of mitochondrial DNA (mtDNA) (1). The presenting symptoms include hematological features, and/or growth retardation, secondary to pancreatic exocrine dysfunction (1). The onset of PS generally occurs in early infancy. The hematological features include macrocytic sideroblastic anemia and vacuolization of bone marrow (BM) precursors, which are sometimes associated with neutropenia or thrombocytopenia (1). Other symptoms include

tubulopathy and aminoaciduria, hepatomegaly, cytolysis and cholestasis, endocrine gland disturbances, neuromuscular manifestations, cardiac involvement or splenic atrophy, which may develop simultaneously or during the course of the disease (2). PS is often fatal in infancy, and the majority of the reported patients with PS succumb to the disease before reaching three-years-of-age, due to septicemia, metabolic acidosis or hepatocellular insufficiency (2). Previous studies have demonstrated that surviving infants exhibit hematological improvement; however, they were eventually shown to develop the typical features of Kearns-Sayre syndrome, which is characterized by a triad of symptoms: Progressive external ophthalmoplegia, pigmentary degeneration of the retina and cardiomyopathy (3-5). Numerous studies have identified genetic defects associated with PS, which include the presence of the common 4.977 kb deletion, *de novo* large-scale deletions or rare duplications in the mitochondrial genome (6-8), which lead to impaired production of ATP (9).

The present report describes the case of a four-month-old infant who presented with the clinical features of PS. Molecular analysis identified a novel 5.712 kb deletion between nucleotides 8,011 and 13,722 in mtDNA.

Case report

Patient and clinical findings. The female patient was the first-born child of healthy non-consanguineous Korean parents. She was born following an uncomplicated pregnancy and was delivered at term (40+4 weeks). Her birth weight was 2,400 g and the first months of her life were uneventful. Family members typically did not show signs and symptoms associated with PS. At the age of four months, the patient visited Seoul St. Mary's Hospital (Seoul, South Korea) presenting with severe pallor. Mucocutaneous pallor was observed upon physical examination. Physical and psychomotor development was shown to be within the normal range and no clinical signs of neuromuscular disease were present. Blood examination detected severe normocytic normochromic anemia. Complete blood count data were as follows: White blood cell count, 4,390/mm^3; hemoglobin, 5.0 g/dl; platelets, 180,000/mm^3; mean corpuscular volume, 95.7 fl; mean corpuscular hemoglobin concentration, 33.1%; and reticulocytes 0.79%. The

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Table I. List and sequence of primers used for the long-distance polymerase chain reaction of mitochondrial DNA.

| Pair	Name	Primer sequence (5'→3')	Position	Region	Size (bp)
1	M9F	GAGGCCTAACCCCTGTCTTT	5835-5854	tRNA^{Tyr}	10162
	M22R	AGCTTTGGGTGCTAATGGTG	15997-15978	tRNA^{Pro}	
2	M12F	ACGAGTACACCGACTACGGC	7908-7927	COII	8089
	M22R	AGCTTTGGGTGCTAATGGTG	15997-15978	tRNA^{Pro}	
3	M12F	ACGAGTACACCGACTACGGC	7908-7927	COII	6379
	M20R	ACGAGTACACCGACTACGGC	14287-14269	ND6	
4	M12F	ACGAGTACACCGACTACGGC	7908-7927	COII	5618
	M19R	TCGATGATGTGGTCTTTGGA	13526-13507	ND5	
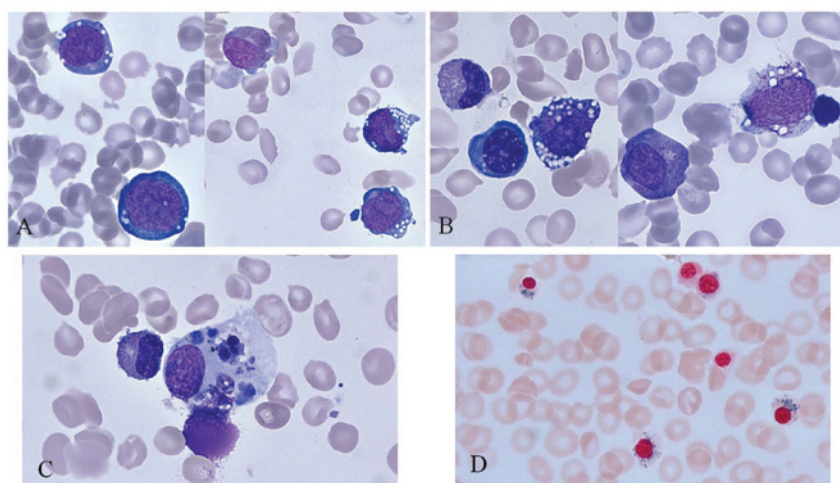
Figure 1. Bone marrow smears obtained from the patient with Pearson syndrome at 4.5-months-of-age. (A) Erythroid and (B) myeloid precursors presented with numerous vacuoles, which appear as variably-sized, round cytoplasmic 'holes' (Wright-Giemsa stain; magnification, x1,000). (C) Increased erythrophagocytosis was also observed (Wright-Giemsa stain; magnification, x1,000) (D) Prussian blue staining demonstrated that certain erythroid precursors formed ring sideroblasts, with five or more siderosomes encircling at least one-third of the nucleus (magnification, x1,000).

results of other routine laboratory tests, including blood glucose, pH, blood gas analysis, electrolytes, blood urea nitrogen, creatinine, aspartate transaminase, alanine transaminase, creatine kinase, lactate dehydrogenase, prothrombin time, activated partial thromboplastin time and urinalysis were all within the normal ranges. No viral pathogens were detected, in particular, infections with parvovirus B19, Epstein-Barr virus or cytomegalovirus were excluded. A BM aspirate was performed at 4.5 months. Erythroid and myeloid precursors exhibited numerous cytoplasmic vacuoles (Fig. 1A and B). Histiocytes exhibited erythrophagocytosis (Fig. 1C) and ringed sideroblasts were observed following Prussian blue staining (Fig. 1D). Karyotyping and a chromosome instability assay revealed normal results. Echocardiography demonstrated normal ventricular systolic and diastolic function, without any definite structural anomalies. Abdominal ultrasonography demonstrated mild pelviectasia in both kidneys. Brain magnetic resonance imaging detected no abnormal signal in the brain parenchyma.

At the age of 23 months, biochemical analyses detected metabolic acidosis with a high pyruvic acid concentration of 1.07 mg/dl (reference range 0.3-0.9). A blood organic acid profile revealed increased 3-hydroxybutyric acid (512.3 μmol/l; reference range, 22-213 μmol/l) and palmitic acid (267.9 μmol/l; reference range, 27-222 μmol/l) levels. A urinary organic acid profile revealed an elevated excretion of 3-hydroxybutyric acid (224.7 mmol/mol creatinine; normal <62.6 mmol/mol), 3-methylglutaconic acid (61.3 mmol/mol creatinine; normal <6.0 mmol/mol), 3-hydroxyglutaric acid (11.6 mmol/mol creatinine; normal: Not detected), 4-hydroxyphenylacetic acid (105.7 mmol/mol creatinine; normal <69.9 mmol/mol), and 4-hydroxyphenyllactic acid (73.3 mmol/mol creatinine; normal <19.6 mmol/mol). These clinical features indicated the presence of a mitochondrial disease, such as PS.

mtDNA deletion analysis. A molecular analysis was performed in order to confirm the diagnosis of PS. Written informed consent was obtained and mtDNA was extracted from the peripheral blood of the patient using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany). Amplification of mtDNA was performed using long-distance polymerase chain reaction (PCR) (Expand Long Template PCR system; Roche Diagnostics, Basel, Switzerland) with various combinations of primers, as previously described (10) (Table I). Direct sequencing of the PCR products was performed using the
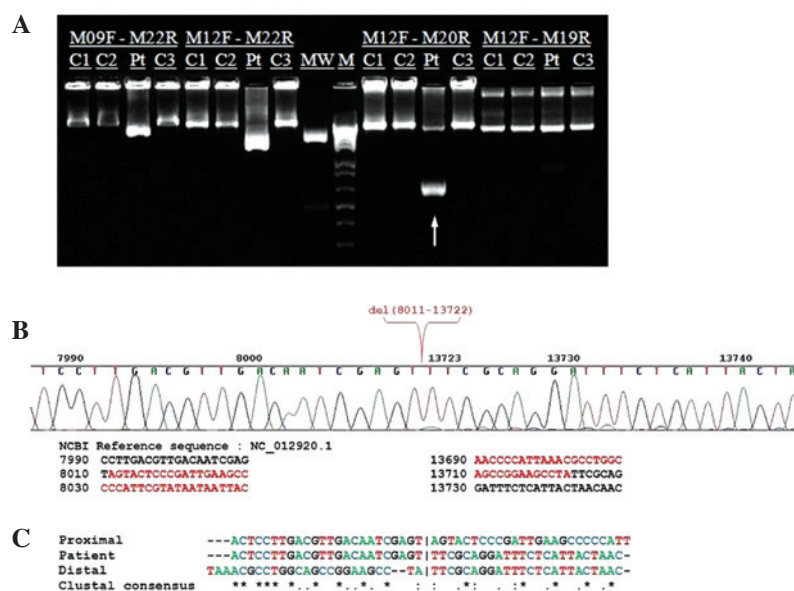
Figure 2. Molecular genetic analysis of the mitochondrial (mt)DNA in a patient with Pearson syndrome (PS). (A) Long-distance polymerase chain reaction amplification with M12F and M20R primers detected the presence of a large-scale mitochondrial deletion (arrow) in the Korean patient with PS. C1, normal control 1; C2, normal control 2; Pt, patient; C3, normal control 3; MW, GeneRuler™ 1 kb DNA Ladder, 250-10,000 bp; M, molecular DNA ladder 100 bp. (B) Electropherogram from Sanger DNA sequencing, representing the breakpoints of the 5.712 kb deletion spanning nucleotides 8,011-13,722 of mtDNA. (C) Sequence homology analysis of the 49 nucleotides surrounding the deletion junction revealed no perfect (class I) or imperfect (class II) repeats at the deletion boundaries. No base pair repeats were detected (class III). Vertical bars (|) indicate the breakpoints.
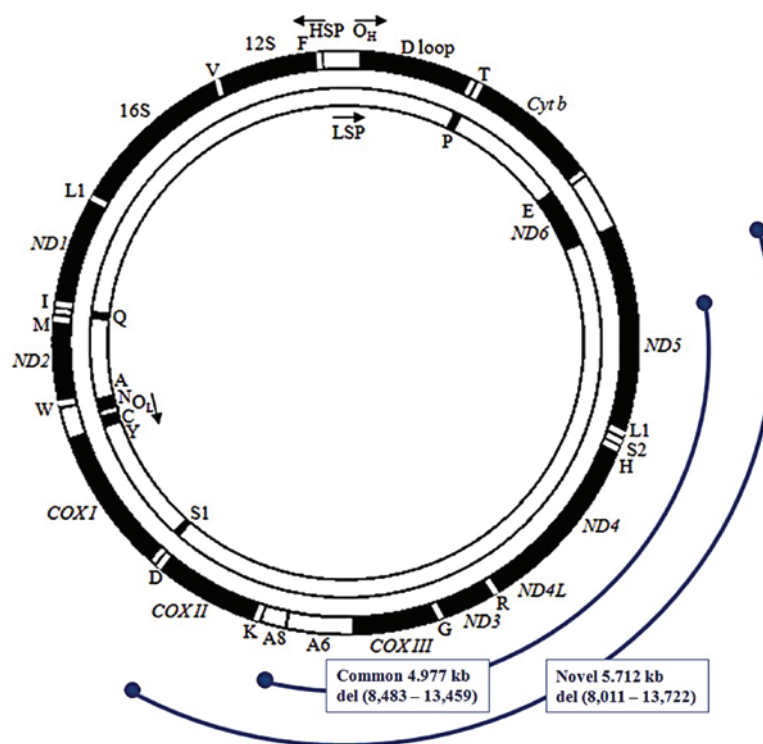

Figure 3. Comparison of the defective region of mtDNA caused by common and novel large-scale deletions. The novel large-scale deletion in a Korean patient with Pearson syndrome spanned nucleotide position 8,011-13,722 of mtDNA, and removed part of the *COXII* gene and part of the *ND5* gene. The novel deletion covered a larger defective region, particularly the first part of *COXII* (8,011-8,269), all of *tRNA^Lys*, the first part of *ATPase 8* (8,369-8,482), and the last part of *ND5* (13,460-13,722), as compared with the defective area caused by the common deletion. O_H, the origin of the heavy strand replication; D loop, the displacement loop or non-coding control region; OL, the origin of the light strand replication.

BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems Life Technologies, Foster City, CA, USA) and the products were resolved on an ABI PRISM 3130 Genetic analyzer (Applied Biosystems). Following long-distance PCR

amplification using M12F and M20R primers a large-scale mtDNA deletion was detected in the patient, as compared with the normal control (Fig. 2A). Sanger sequencing identified the breakpoints of the 5.712 kb deletion, and the deletion was shown

to span between the nucleotide positions 8,011 and 13,722 of mtDNA (Fig. 2B). No perfect or imperfect repeats were detected at the boundaries of the mtDNA deletion (Fig. 2C). This heteroplasmic deletion removes part of the *COXII* gene, the entire *tRNA^Lys*, *ATPase 8*, *ATPase 6*, *COXIII*, *tRNA^Gly*, *ND3*, *tRNA^Arg*, *ND4L*, *ND4*, *tRNA^His*, *tRNA^Ser(AGY)*, *tRNA^Leu(CUN)* genes and part of the *ND5* gene (Fig. 3). The sequence homology in the 49 nt region (24 nt on each side of the breakpoint nucleotide) of the proximal and distal sequences was analyzed using Clustal Omega software (11), with default parameters (http://www.ebi.ac.uk/Tools/msa/clustalo/), and was compared with the updated consensus Cambridge sequence (GenBank Accession no. NC_012920.1).

At the time of initial organic acid analysis and mtDNA evaluation, the patient had been admitted to hospital with a fever, poor oral intake and decreased activity, which persisted despite administration of fluids and broad spectrum antibiotics. Blood culture was negative and urine culture was positive for *Pseudomonas aeruginosa* infection, which was treated with ceftazidime. The patient's fever did not remit and her overall condition failed to show improvement, therefore she was transferred to another institution for further care.

Discussion

The present report describes the case of a female patient with PS harboring a large-scale mitochondrial deletion, which is to the best of our knowledge, the first case reported in South Korea. Molecular investigation of mtDNA extracted from a peripheral blood sample detected a novel large-scale heteroplasmic mitochondrial deletion without direct repeats. This deletion was shown to affect numerous tRNA and protein-coding genes, which may lead to defects in mitochondrial polypeptide synthesis, and impaired oxidative phosphorylation and energy metabolism in the respiratory chain.

Usually, mtDNA in humans is ~16.6 kb in length, and encodes numerous enzymes of the respiratory chain and oxidative phosphorylation system, ribosomal RNAs, and various tRNAs (12). Impairment of the mitochondrial respiratory chain may lead to lactic acidemia, high plasma lactate/pyruvate molar ratios, and even fatal metabolic acidosis (13). Previous studies have reported various mitochondrial deletions in patients with PS (4-10). A common 4.9 kb deletion spanning the *ATPase 8* gene to the *ND5* gene, between 13 bp direct repeats has previously been frequently observed (14). This deletion has been identified in >80% of affected children; however, numerous other mtDNA deletions have also been detected (www.mitomap.org). Mitochondrial deletions differ in size and location; however, they are confined to a region delineated by the heavy- and light-strand origins of replication, which is the major arc of mtDNA (14). The known deletions include numerous tRNA and protein-coding genes (15), and are often flanked by direct repeats (16). In the majority of cases, mtDNA deletions are spontaneous events that occur either in the oocyte or during early stages of embryonic development (17).

The underlying mechanisms regarding the regulation of tissue distribution of deleted mtDNA molecules, or why the rate of disease progression and the degree of disease severity is variable, even for the same deletions, remains to be elucidated. No strict correlations have been observed between heteroplasmy in blood cells and the severity of hematopoietic features. Furthermore, no obvious genotype-phenotype correlation has been identified regarding the appearance of hematological manifestations in PS and the mtDNA deletion (18). The different phenotypic expression of the same mtDNA defects may be associated with nuclear modifier genes, polymorphisms and environmental factors (19).

No specific treatment is currently available for patients with PS, therefore awareness of possible complications, and early intervention may minimize PS-associated mortality and morbidity. Red blood cell transfusions are often required to manage macrocytic anemia, and patients may be dependent on these transfusions (20). Pancreatic enzyme replacement is required for patients with malabsorption due to exocrine pancreatic dysfunction (20). Intermittent metabolic crises are managed with hydration, correction of electrolyte abnormalities, and correction of acidosis (20). Bicarbonate supplementation and dichloroacetic acid have also been used to treat persistent metabolic acidosis. Previous studies have reported the case of two patients with PS, who were treated with allogeneic hematopietic stem cell transplantation (HSCT) (21,22). The procedure was shown to correct the hematological and metabolic abnormalities in the two patients; therefore, allogeneic HSCT may be a viable treatment option for patients with PS whose prognosis is uniformly fatal if left untreated.

In conclusion, to the best of our knowledge, the present study was the first to report a novel mtDNA deletion causing Korean PS. It is necessary to consider mitochondrial disorder in infants who present with persistent hematological abnormalities, such as hypoplastic anemia. Due to the often fatal course of PS, molecular analysis of possible mtDNA deletions may be beneficial for the diagnosis and prognosis of PS, and may also aid in the genetic counseling of relatives.

Acknowledgements

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