Detection of Platelet Mitochondrial DNA Deletions in Kearns-Sayre Syndrome

Yoshie Ora, Masashi Tanaka, Wataru Sato, Kinji Ohno, Tomoko Yamamoto, Mitsuo Maehara, Tamiko Negoro, Kazuyoshi Watanabe, Shinobu Awaya, and Takayuki Ozawa

To establish a noninvasive genetic diagnosing method for Kearns-Sayre syndrome, the authors used the polymerase chain reaction (PCR) technique for detecting mitochondrial DNA (mtDNA) deletions in the platelets and directly sequenced the crossover regions of the deleted mtDNA using the fluorescence-based automated sequencing system. The mtDNA deletions were identified in the platelets of three of four patients. The sizes and locations of deletions were determined by the nesting primer PCR method, in which the primary PCR products derived from deleted mtDNAs undergo reamplification using a series of nesting primers. With the fluorescence-based sequencing of templates amplified by the asymmetric PCR method, deleted mtDNA was sequenced directly without cloning. In patient 1, guanine (G) was found at the boundaries of a deleted segment spanning 8400 base pairs (bp) between the CO1 and ND6 genes. In patient 2, a 9-bp directly repeated sequence of 5'-ACCTCCCTC-3' (where A = adenine, C = cytosine, and T = thymine) was found at the boundaries of a deleted segment spanning 7221 bp between the CO1 and ND5 genes. In patient 3, an 8-bp sequence of 5'-ICGCTGTC-3' was found at the boundaries of a deleted segment spanning 4664 bp between the ATPase6 and ND5 genes. Deletions were not detected in the mtDNA of patient 4 or in that of the mothers of the patients. Previously, the genetic diagnosis of this syndrome required muscle biopsy specimens and the use of Southern blot analysis. However, this method requires neither muscle biopsy nor isotopes and is more rapid than the Southern blot method. These results indicate that this method is useful for noninvasive genetic diagnosis of Kearns-Sayre syndrome. Invest Ophthalmol Vis Sci 32:2667-2675, 1991

Mitochondria are unique organelles because they have their own DNA genome. The human mitochondrial genome is a closed circular DNA of 16,569 base pairs (bp) that encodes for 13 subunits of the oxidative phosphorylation complexes (Complexes I, III, IV, and V) involved in adenosine triphosphate generation, two rRNAs, and 22 tRNAs of the mitochondrial protein synthesizing system. Inheritance of mitochondrial DNA (mtDNA) is characterized by its maternal pattern. Various neuromuscular diseases recently were associated with mutations in mtDNA.

Mitochondrial myopathies share a common feature of major structural mitochondrial abnormality in skeletal muscle biopsy specimens, an abnormality characterized by accumulations of morphologically abnormal mitochondria. The Kearns-Sayre syndrome (KSS) or Kearns-Shy syndrome is one of the distinct syndromes among mitochondrial myopathies, and it is characterized by a triad involving onset before age 20 yr, progressive external ophthalmoplegia, and pigmentary degeneration of the retina, with one of the following: heart block, cerebellar syndrome, or high cerebrospinal fluid protein (over 100 mg/dl). This syndrome often is associated with perceptive hearing loss, skeletal muscle weakness, and mental debility. It sometimes is associated with disorders in the endocrine systems, such as hypogonadism and diabetes. Although mtDNA deletions were detected in the biopsy specimens of muscles from...
many patients with mitochondrial myopathy including KSS,6–15 those in the blood cells were reported in only a few cases.16,17 A sequence of deleted blood cell mtDNA was reported in only one case with Pearson’s marrow/pancreas syndrome.23 To diagnose this disease noninvasively, we attempted to detect mtDNA deletions in the blood cells of patients with KSS by using the polymerase chain reaction (PCR) method and a fluorescence-based automated sequencing system. We also examined the blood cells of the patients’ mothers to determine whether or not there is maternal transmission of this genetic disorder.

Patients and Methods

Patients

Patient 1: This patient was a 17-yr-old woman who was short in stature from age 3 yr. Slight ptosis and limitation of eye movements in all directions started at age 10 yr. She had chorioretinal atrophy, constriction of visual field in both eyes, and perceptual deafness. Electromyography showed a myogenic pattern. Needle biopsy of the skeletal muscle at age 10 yr showed ragged-red fibers. Diabetes mellitus was found and treated with insulin from age 14 yr. There was no family history of this disorder.

Patient 2: This patient was a 40-yr-old woman. She had chronic progressive external ophthalmoplegia and ptosis from age 3 yr with chorioretinal atrophy and perceptual deafness. Needle biopsy of the skeletal muscle revealed ragged-red fibers. Her daughter also had slight deafness, but she had no ophthalmoplegia or retinopathy. Other members of her pedigree were normal.

Patient 3: This was a 30-yr-old woman. Bilateral ptosis and limitations of external ocular movement were noticed at age 19 yr. Muscle weakness and night blindness occurred at age 20 yr. Funduscopy revealed chorioretinal atrophy. Electromyography at age 21 yr showed a myogenic pattern. A needle biopsy of skeletal muscle revealed ragged-red fibers. Cardiac conduction block was found at age 24 yr, and a pacemaker was implanted at age 25 yr. She had tremor and ataxia from age 26 yr. There were no family history of ptosis or external ophthalmoplegia.

Patient 4: This patient was a 19-yr-old man. He was short in stature from age 8 yr. Reduction of visual acuity and chorioretinal atrophy were found at age 11 yr. At age 13 yr, he had ptosis, limitation of eye movement, muscle weakness, slurred speech, and cerebellar ataxia. A needle biopsy of the muscle revealed ragged-red fibers. At age 15 yr, electrocardiography showed complete right bundle branch block. At age 16 yr, perceptual deafness was found. He had diabetes mellitus and was treated with insulin from age 18 yr. There was no known family history of this disorder.

We also tested one control subject who was a normal 31-yr-old woman. Blood samples were obtained only after the procedure was explained fully, and informed consent was given.

Isolation of Platelets

Blood samples were collected from patients, their mothers, and the control in 10–20-ml volumes in plastic tubes containing heparin. After addition of ethylenediaminetetraacetic acid (EDTA) to a concentration of 4.6 mM, the samples were centrifuged at 1400 × g for 3 min at room temperature. The supernatant was transferred to another tube and was centrifuged for 15 min at 2250 × g. The resultant pellet was resuspended in 10 ml of prewarmed washing buffer containing 4.3 mM K2HPO4, 4.3 mM Na2HPO4, 113 mM NaCl, 4.4 mM NaH2PO4, and 5.5 mM glucose, pH 6.5, and centrifuged at 120 × g for 7 min. The pellet (leukocyte fraction) was separated. The supernatant was transferred to another tube and centrifuged at 2000 × g for 15 min. The pellet (platelet fraction) was resuspended in 1 ml of buffer containing 4.3 mM K2HPO4, 4.3 mM Na2HPO4, 113 mM NaCl, 4.4 mM NaH2PO4, and 5.5 mM glucose, pH 6.5.

Extraction of DNA

The platelet suspension (100 μl) was digested in 400 μl of a buffer (10 mM Tris HCl, pH 7.5, 5 mM MgCl2, 0.32 M sucrose, and 1% Triton X-100 (BCL)) containing 100 μg/ml of proteinase K and 0.5% sodium dodecyl sulfate for 10 min at 37°C. The DNA was extracted twice with phenol–chloroform–isoamyl alcohol (25:25:1) and once with chloroform–isoamyl alcohol (25:1).

Preparation of Primers for PCR

Primers for PCR were synthesized using either a model NS-1 DNA synthesizer from Shimadzu (Osaka, Japan) or model 380B DNA synthesizer from Applied Biosystems (Foster City, CA) and purified on oligonucleotide purification cartridges from Applied Biosystems. In asymmetric PCR amplification for producing template for the fluorescence-based automated DNA sequencing, we used limiting primers with the M13–21 universal sequence (Table 1). They also were synthesized and purified as described.

Conditions for PCR

The extracted DNA suspension (5 μl) was amplified in 50 μl of reaction mixture containing 200 μM of each deoxynucleotide triphosphate, 1 μM of each
primer (Table 1), 1.25 units of Taq DNA polymerase (AmpliTaq; Cetus, Emeryville, CA), and PCR buffer containing 50 mM KCl, 10 mM Tris HCl, pH 8.3, 1.5 mM MgCl₂, and 0.01% gelatin. The mixture reacted for a total of 30 cycles using a thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT). The cycle times were as follows: denaturation, 15 sec at 94°C; annealing, 15 sec at 55°C; and primer extension, 40 sec at 72°C.

**Nesting Primer PCR Method**

The primary PCR product (5 μl) was reamplified in a final volume of 50 μl that included the reagents described previously. In this method, the regions of deletion were determined by testing whether the primary PCR product had a complementary site for each primer. The combination of primers used for each patient is shown in Table 2. The PCR was done for a total of 30 cycles as described.

**Asymmetric PCR Amplification for Template Formation of Fluorescence-Based Sequencing**

Asymmetric amplification was done on 1 μl of the primary PCR product in a final volume of 100 μl that included the reagents described, 0.01 μM of limiting primers with the M13–21 universal sequence (Table 1), and 1 μM of another primer with three units of Taq DNA polymerase. The primer pairs used for each patient were the closest primers to the starting and end points of the deletions expected after the nesting primer method. In patients 1, 2, and 3, the primer pairs FL596 and H1479, FL596 and H1363, and FL909 and H1393 were used, respectively. The PCR was done for a total of 30 cycles as described. The PCR product containing single-stranded DNA was precipitated with 10 μl of sodium acetate (pH 7.4) and 200 μl of ethanol. After incubation at −20°C for 1 hr, the mixture was centrifuged at 11,000 x g for 10 min. The pellet was rinsed with 100 μl of 70% ethanol, dried in a vacuum chamber for 15 min, and dissolved in 10 μl of distilled water.

**Fluorescence-Based Sequencing**

Dye primers and a Taq sequencing kit were obtained (Applied Biosystems). For the sequencing reactions with the JOE dye primer (for A) and the FAM primer. The combination of primers used for each patient is shown in Table 2. The PCR was done for a total of 30 cycles as described.
dye primer (for C), the following reagents were mixed to a total volume of 15 μl: 1.5 μl of PCR-generated single-stranded DNA template, 2.0 μl of each dye primer (dissolved to a concentration of 0.4 pmol/μl), 2.0 μl of the 5 × Taq sequencing buffer (50 mM Tris HCl, pH 8.5, 50 mM MgCl₂, and 250 mM NaCl), 2.0 μl of each mixture of deoxynucleoside–dideoxynucleoside triphosphate and 5.0 μl of distilled water, and 0.5 μl of Taq DNA polymerase (5 units/μl; Promega, Madison, WI). For the sequencing reactions with TAMRA dye primer (for G) and ROX dye primer (for T), the amounts of reagents were doubled to a total volume of 30 μl. Ten cycles of denaturation at 90°C for 15 sec with annealing and extension at 70°C for 60 sec were conducted in the thermal cycler. The contents of the four tubes were pooled into a tube containing a mixture of 9.0 μl of sodium acetate (pH 5.2) and 180 μl of ethanol. After incubation at −20°C for 10 min, the mixture was centrifuged at 11,000 × g for 10 min. The pellet was rinsed with 100 μl of 70% ethanol and dried in a vacuum chamber for 10 min. Just before electrophoresis, the pellet was dissolved in 5 μl of denatured formamide and 1 μl of 50 mM EDTA (pH 8.0). The DNA samples were denatured by heating at 90°C for 2 min, then immediately cooled on ice, and loaded onto a 6% acrylamide gel that had undergone electrophoresis. Fluorescence-based DNA automated sequence analysis was done using a 373A DNA Sequencer (Applied Biosystems) with the manufacturer’s version 1.0.1 software.

Results

Primary PCR Amplification of Deleted mtDNA

Most of the mtDNA deletions reported in mitochondrial myopathies, including KSS, retain the ori-

![Image](url)

**Fig. 1.** Detection of mtDNA deletions by PCR in platelets. Primer pairs used are indicated below each lane. Lane 1, patient 1 (P1); lane 2, mother of patient 1 (M1); lane 3, normal individual (C); lane 4, patient 2 (P2); lane 5, normal individual (C); lane 6, patient 3 (P3); lane 7, mother of patient 3 (M3); lane 8, normal individual (C). Fragments are amplified with mtDNA of Patients 1, 2, and 3 (lanes 1, 4, and 6) while no fragments were amplified from the mothers and normal individual (lanes 2, 3, 5, 7, and 8).

Table 3. Size of the fragment amplified with various combinations of the primers in patients 1–3

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Primer distance (kb)</th>
<th>Fragment size (kb)</th>
<th>Deletion size (kb)</th>
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</thead>
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<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary amplification</td>
<td>L568 + H1534</td>
<td>9.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Reamplification</td>
<td>L596 + H1338</td>
<td>9.3</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>L625 + H1534</td>
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<td>3.3</td>
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<tr>
<td></td>
<td>L596 + H1506</td>
<td>9.1</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>L596 + H1479</td>
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<td>0.5</td>
</tr>
<tr>
<td></td>
<td>L596 + H1450</td>
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<tr>
<td>Patient 2</td>
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<tr>
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<td></td>
<td>L881 + H1420</td>
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<td></td>
<td>L909 + H1420</td>
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</tr>
<tr>
<td>Patient 3</td>
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<td>Primary amplification</td>
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<td>6.3</td>
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<tr>
<td></td>
<td>L790 + H1363</td>
<td>5.7</td>
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</tr>
</tbody>
</table>

Figures are in kb.
result was consistent with a 7.2-kb deletion (Table 3). No fragments were amplified from mtDNA extracted from the platelets of the control using the same pair of primers (Fig. 1, lane 5). In patient 3, deleted mtDNA was amplified using the pair of primers L790 and H1420, the distance between which was 6.3 kb. A single band of 1.6 kb (Fig. 1, lane 6) was consistent with a 4.7-kb deletion (Table 3). No fragments were amplified from the mtDNA extracted from the platelets of the mother of patient 3 or the control (Fig. 1, lanes 7–8). In patient 4 and his mother, we tried to detect mtDNA deletion using the primer pairs listed in Table 2, but we could not amplify any abnormal fragments.

Localizatiöm of mtDNA Deletion by Nesting Primer PCR Method

To confirm that the primary PCR products were derived from deleted mtDNAs and localize the deleted regions precisely, we analyzed the primary PCR products by the nesting primer method. In patient 1, the 1.3-kb fragment obtained using primers L568 and H1534 underwent the nesting primer PCR method. When primer L596 was used in place of L568 for reamplification, a 1.0-kb fragment was amplified (Fig. 2, lane B). This fragment was 0.3 kb shorter than the primary PCR product and was consistent with an 8.3-kb deletion (Table 3). This result indicates that the deleted mtDNA retained the complementary site for L596. When primer L625 was used for reamplification along with H1534, a diffuse band, probably single-stranded DNA, was amplified below the 1.3-kb primary PCR product (Fig. 2, lane C), suggesting that the deleted mtDNA lost the complementary site for L625. Thus, the starting point of the mtDNA deletion in patient 1 was located between positions 5961 and 6251.

To localize the end point of the mtDNA deletion in patient 1, primers H1506, H1479, and H1450 were used in place of H1534 for reamplification of the 1.3-kb primary PCR product along with primer L596. These results are summarized in Table 3. Using primers H1506 and H1479 with L596, 0.8-kb and 0.5-kb fragments were amplified, respectively (Fig. 2, lanes D–E). Because the distances between primers H1506 and L596 and between H1479 and L596 were 9.1 kb and 8.8 kb, respectively, all results were consistent with an 8.3-kb deletion. However, when primer H1450 was used, a diffuse band of single-stranded DNA was amplified below the 1.3-kb primary PCR product (Fig. 2, lane F), suggesting that the deleted mtDNA lost the complementary site for primer H1450. Thus, the end point of the deletion was localized between positions 14,301 and 14,791. These results indicate that the size of the deletion in patient 1 was 8.3 kb.

In patient 2, the 1.0-kb fragment obtained using primers L596 and H1420 underwent the nesting primer PCR method. To localize the start point of the mtDNA, L625 was used for reamplification along with H1420. A diffuse band of single-stranded DNA was amplified below the 1.0-kb primary PCR product (Fig. 3, lane B), suggesting that the deleted mtDNA lost the complementary site for L625. Thus, the starting point of the mtDNA deletion in patient 2 was located between positions 5961 and 6251.

To localize the end point of the mtDNA deletion in patient 2, primers H1393, H1363, and H1338 were used in place of H1420 for reamplification of the 1.0-kb primary PCR product along with primer L596. These results are summarized in Table 3. Using primers H1393 and H1363 with L596, 0.8-kb and 0.5-kb fragments were amplified, respectively (Fig. 3, lanes C–D). Because the distances between primers L596 and H1393 and between L596 and H1363 were 8.0 kb and 7.7 kb, respectively, these results were consistent with a 7.2-kb deletion. However, when primer
Fig. 3. Localization of mtDNA deletion in patient 2 by the nesting primer method. Primary amplification product was reamplified as indicated in the figure above the photograph of the gel beside the names of the lanes: lane A, primary amplification using primers L596 and H1420; lanes B–E, reamplification using primers indicated in the figure. Fragments are amplified in lanes C and D, though no distinct fragment was amplified in lanes B and E.

H1338 was used, a diffuse band of single-stranded DNA was amplified below the 1.0-kb primary PCR product (Fig. 3, lane E), suggesting that the deleted mtDNA lost the complementary site for primer H1338. Thus, the end point of the deletion was localized between positions 13,381 and 13,631.

In patient 3, the 1.6-kb fragment obtained using primers L790 and H1420 was analyzed by the nesting primer PCR method. To localize the starting point of the mtDNA deletion in patient 3, primers L820, L853, L881, L909, and L929 were used in place of L790 for reamplification of the 1.6-kb primary PCR product along with primer H1420. These results are summarized in Table 3. Using primers L820, L851, L881, and L909 with H1420, 1.3-kb, 1.0-kb, 0.7-kb, and 0.4-kb fragments were amplified, respectively (Fig. 4, lanes B–E). Because the distances between primers L820 and H1420, between L853 and H1420, between L881 and H1420, and between L909 and H1420 were 6.0 kb, 5.7 kb, 5.4 kb, and 5.1 kb, respectively, these results are consistent with a 4.7-kb deletion (Table 3). However, when primer L929 was used with H1420, only the 1.6-kb primary PCR product was detected (Fig. 4, lane F), suggesting that the deleted mtDNA lost the complementary site for primer L929. Thus, the starting point of the deletion was localized between positions 9091 and 9291.

To localize the end point of the mtDNA deletion in patient 3, H1393 and H1363 were used in place of H1420 for reamplification of the 1.6-kb primary PCR product along with primer L790. These results are summarized in Table 3. Using primer H1393 with the primer L790, a 1.3-kb fragment was amplified (Fig. 4, lane G). Because the distances between primers L790 and H1393 was 6.0 kb, this result was consistent with a 4.7-kb deletion. However, when primer H1363 was used, only the 1.6-kb primary PCR product was visible (Fig. 4, lane H), suggesting that the deleted mtDNA lost the complementary site for primer H1363. These results indicate that the size of the deletion in patient 3 was 4.7 kb and that the deletion started in the ATPase6 or CO3 gene and ended in the ND5 gene.

Fluorescence-Based Automated Sequencing

In patient 1, a single-stranded DNA template for sequencing was amplified using primer FL596 and
H1479. Sequencing revealed that the crossover sequence was found to involve a one-base repeat of guanine (G, Fig. 5A) located in the boundaries of the deletion between the CO1 and ND6 gene. The deletion spanned 8400 bp from position 6023 to position 14,423. When the wild-type sequences of the CO1 and ND6 genes were compared with each other, no significant homologies were found in the region surrounding the deletion.

In patient 2, a single-stranded DNA template for sequencing was amplified using primer FL596 and H1363. Sequencing revealed that the crossover sequence was found to involve a 9-bp directly repeated sequence of 5'-ACCTCCCTC-3' (where A = adenine, C = cytosine, and T = thymine, Fig. 5B) located in the boundaries of the deletion between the CO1 and ND5 gene. The deletion spanned 7221 bp from position 6226 to position 13,447. When the wild-type sequences of the CO1 and ND5 genes were compared with each other, no significant homologies were found in the region surrounding the directly repeated sequence.

In patient 3, a single-stranded DNA template for sequencing was amplified using primer FL909 and H1393. Sequencing revealed that the crossover sequence was found to be a 8-bp directly repeated sequence of 5'-TCGCTGTC-3' (Fig. 5C) located in the boundaries of the deletion between the ATPase6 and ND5 genes. The deletion spanned 4,664 bp from position 9,144 to position 13,808. When the wild-type sequences of the ATPase6 and ND5 genes were compared with each other, no significant homologies were found in the region surrounding the directly repeated sequence.

**Discussion**

In this study, we identified suitable PCR primers and reaction conditions for the detection of mtDNA deletions in platelets. We also confirmed that the abnormal bands were derived from deleted mtDNA by using the fluorescence-based automated sequencing system without cloning. This method greatly simplifies genetic diagnosis of KSS.

**Fig. 5a-c.** Comparison of the nucleotide sequences of the wild-type genes with those of the hybrid genes in the deleted mtDNAs from patients 1–3. The sequence of the wild-type genes is shown above the sequence of the hybrid gene that resulted from deletion of mtDNA in each patient. Bold and underlined letters indicate the directly repeated sequences. Numerals indicate the nucleotide numbers.\(^1\)
The mtDNA deletion in KSS was reported to be widely distributed among various organs. Although a high proportion of deleted mtDNA to normal mtDNA was observed in the skeletal muscle, heart, and brain, only a small proportion was detected in cultured muscle cells or fibroblasts. In blood cells, the mtDNA deletion frequently was not detected by Southern blot analysis. These observations could be explained by assuming that cells with deleted mtDNA tend to be eliminated from tissues composed of mitotically active cells. By PCR amplification, however, we can detect mtDNA deletion in platelets.

We used platelet DNA as the PCR template because platelets are rich in mtDNA with little contamination of nuclear DNA, and therefore, possible interference by the nuclear DNA would be eliminated. When leukocyte DNA from these patients was used as the template for amplification of deleted mtDNAs, however, the same fragments as in the platelets were amplified, and no additional bands were observed. Therefore, the unseparated fraction containing both leukocytes and platelets can be used as the template if the amount of blood samples is limited.

Because the specimens obtained by previous needle biopsy were used for morphologic studies, we attempted to detect mtDNA deletions by the gene amplification method using PCR without information on the site of the deletions. For this purpose, we adjusted the PCR conditions, especially the denaturing time and the extension time, so that long (5-8 kb) mtDNA fragments could be amplified. The mtDNA deletions in KSS patients are not localized to any single region of the mitochondrial genome.6-15 Most of the deletions reported reside in the regions between the replication origin for the heavy strand and that for the light strand. When screening the blood samples, therefore, we chose the primer pairs for the primary amplification that had complementary sites that were on these spared sites.

Our analysis showed that replication origins for both the heavy and light strands were conserved in the deleted mtDNA as expected. The three patients had different deletions, and these deletions occurred between the directly repeated sequences present in wild-type mtDNA (Figs. 5, 6). They were not identical with the directly repeated sequences present in wild-type mtDNA (Figs. 5, 6). The extent of the deleted region of each patient's mutant DNA is indicated by the shadowed boxes. Corresponding nucleotide numbers appear below each region. 12S and 16S. 12S and 16S rRNAs; ND, NADH dehydrogenase subunits; CO, cytochrome c oxidase subunits; Cyt b, cytochrome b.

**Fig. 6.** Schematic presentation of the deleted regions of the patients' mtDNA as determined by the fluorescence-based sequencing. The extent of the deleted region of each patient's mutant DNA is indicated by the shadowed boxes. Corresponding nucleotide numbers appear below each region. 12S and 16S. 12S and 16S rRNAs; ND, NADH dehydrogenase subunits; CO, cytochrome c oxidase subunits; Cyt b, cytochrome b.

The starting and end points of the deletions predicted by the nesting primer method were consistent with the results of the sequence except for the end point of the patient 1. In this patient, although the end point of the deletion was predicted by the nesting primer method to be between positions 14,501 and 14,791 (Table 3, Fig. 2), the sequence indicated that the deletion ended at position 14,423. Primer H1450 spans position 14,501-14,520. The distance between the end point of deletion and the primer H1450 was 78 bp. In patients 2 and 3, the distances between the end points of deletions and heavy chain primers were 184 bp and 123 bp. When we anticipate the end points of the deletions by the nesting primer method, there may be experimental errors. No inconsistencies were found in the starting points of the deletions. The distances between the starting point of the deletions and the end points of the light chain primers in patients 1, 2, and 3 were 42, 245, and 34 bp respectively.

Deletions of mtDNA were not detected in patient 4 despite the fact that he had severe clinical symptoms. One possibility is that the amount of deleted mtDNA in the platelets was below the sensitivity of this PCR method although a deleted mtDNA may be present in other tissues, eg, skeletal muscle. Alternatively, a different genetic lesion might be responsible for the ocular myopathy of this patient, and our selection of primer pairs may be inadequate for finding a deletion in this patient.

Inheritance of mtDNA is characterized by its maternal pattern. Some mitochondrial genetic disorders were reported to be inherited maternally.6,5,15 Although we examined the mothers of three of four patients, we could not detect the mtDNA deletions found in the patients.

Our method was valuable, especially when a muscle biopsy sample is not available. These patients had a long history of this disease, and the muscle specimens obtained by needle biopsy were used for histologic studies. Screening for deleted mtDNA using blood samples is important in the diagnosis of patients who have not consented to rebiopsy of skeletal muscle. The PCR analysis has advantages over the Southern blot method; it is faster, more sensitive, and does not require radioisotopes. In Southern blot analysis, the region of uncertainty at each end of the dele-
tion cannot be narrowed by less than the length of the probe used. In addition, our method is so sensitive that total DNA extracted from only 10 ml of blood is sufficient for the determination and localization of the deleted region of mtDNA. Because the primers close to the deleted region can be determined by the nesting primer PCR method, the precise localization and directly repeated sequences of the deletions can be identified easily by direct sequencing of the PCR product using these primers.

Others reported successful treatment of a patient with Complex III deficiency with a combination of menadione and ascorbic acid. In another study, significant improvement was found in skeletal muscle work after administration of coenzyme Q10 in a patient with Complex I deficiency associated with mtDNA deletion. We also observed a marked decrease in lactic acid elevation after exercise loading in patients with cytochrome c oxidase deficiency. Although the principle of treatment of mitochondrial myopathies must be established, our method of screening for deleted mtDNA in blood samples will be useful in choosing appropriate medical treatment based on precise genetic diagnosis.

Key words: mitochondrial DNA, polymerase chain reaction, fluorescence-based automated sequencing system, platelet, Kearns-Sayre syndrome

Acknowledgments

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References