Am. J. Hum. Genet. 50:364–370, 1992

Maternally Inherited Duplication of the Mitochondrial Genome in a Syndrome of Proximal Tubulopathy, Diabetes Mellitus, and Cerebellar Ataxia

Agnès Rötig,* Jean-Louis Bessis,‡ Norma Romero,† Valérie Cormier,* Jean-Marie Saudubray,* Philippe Narcy,* Gérard Lenoir,* Pierre Rustin,* and Arnold Munnich*

*Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U.12, and Département de Pédiatrie, Hôpital des Enfants Malades, and †Centre de Recherches de Biologie et Pathologie Neuromusculaires, INSERM U.153, Paris; and ‡Hôpital Pasteur, Cherbourg

Summary

Two sisters in the first year of life presented with a proximal tubulopathy of unknown etiology. They subsequently developed a pluritissular disorder including diabetes mellitus, skin abnormalities, mitochondrial myopathy with ragged-red fibers, and cerebellar ataxia. Their mother had ptosis, ophthalmoplegia, and muscle weakness. Analysis of the mitochondrial respiratory chain showed a complex III deficiency in both skeletal muscle and lymphocytes of the second girl. Southern blot analysis provided evidence for a heteroplasmic partial duplication of the mtDNA (26 kb), involving one full-length and one partly deleted mitochondrial genome and with one single abnormal junction between the genes for ATPase 6 and cytochrome b. Using PCR amplification of lymphocyte DNA, we were able to detect minute amounts of duplicated molecules in the mother, which provided evidence for maternal inheritance of the partial duplication. While maternal transmission of point mutations have been reported in Leber disease, retinitis pigmentosa, and MERRF disease, this observation is, to our knowledge, the first example of a maternally inherited duplication of the mitochondrial genome in man.

Introduction

The mitochondrial respiratory chain catalyzes oxidative phosphorylation, i.e., the oxidation of fuel molecules by oxygen and the concomitant energy transduction into adenosine triphosphate (ATP). The impairment of this metabolic pathway results in a group of clinically heterogeneous disorders usually involving skeletal muscle (Sengers et al. 1984) but sometimes dominated by nonneuromuscular manifestations. In the past few years, sporadic and autosomal rearrangements of the mtDNA, a maternally inherited 16.5-kbp circular genome, have been reported in several conditions including mitochondrial myopathies (Holt et al. 1988), Kearns-Sayre syndrome (Lestienne and Ponsot 1988), progressive external ophthalmoplegia (Zeviani et al. 1988), and Pearson syndrome (Rötig et al. 1990). On the other hand, maternal inheritance of point mutations and deletions has been reported in Leber disease (Wallace et al. 1988), retinitis pigmentosa, muscle weakness and sensory neuropathy (Holt et al. 1990), mitochondrial myopathy with ragged-red fibers (MERRF) (Shoffner et al. 1990), MELAS syndrome (Goto et al. 1990), and mitochondrial myopathy (Poulton et al. 1991). To date, however, no maternal transmission of an mtDNA duplication has been described. We report here partial duplication of the mitochondrial genome in two siblings with proximal tubulopathy as the onset symptom and in their mildly affected mother. This is, to our knowledge, the first evidence for maternal transmission of an mtDNA duplication in man.

Received April 22, 1991; revision received October 14, 1991.

Address for correspondence and reprints: Arnold Munnich, INSERM U.12, Hôpital des Enfants Malades, 149 rue de Sèvres. 75743 Paris, Cedex 15, France.

© 1992 by The American Society of Human Genetics. All rights reserved.

0002-9297/92/5002-0012$02.00

Patients and Methods

Patient 1

A 2,550-g girl (III1) was born after a term pregnancy to unrelated parents. She was small (height 46 cm;

head circumference 33 cm) for gestational age, for no obvious reason. Her father was healthy, but her mother (II2) gradually developed bilateral extraocular muscle palsy with lid ptosis and micropunctated tapetoretinal degeneration in early adulthood. The mother also experienced myopia, hypoacousia, and muscle weakness after her first pregnancy. The girl's grandmother (I1) and maternal aunt (II1) were healthy. The proband did relatively well during the first 9 mo of life, but she failed to thrive thereafter (weight −3.5 SD; height −4 SD; head circumference −2 SD at 30 mo of age). These features were first related to a severe proximal tubulopathy with major polyuria (1.5 liters/d) and urinary loss of potassium (185 mmol/liter), sodium (85 mmol/liter), calcium (24 mmol/liter), and chlorine (160 mmol/liter). No renal failure, glycosuria, or proteinuria was noted. At that age, a mottled pigmentation was present in the photoexposed areas (forearms, legs, and cheeks; fig. 1), and iterative episodes of cold-triggered erythrocyanosis involving toe and finger extremities occurred. Total anorexia and severe constipation required continuous enteral nutrition, and she gained weight regularly for the next 2 years. At 5 years of age, however, she suddenly developed a fatal episode of diarrhea, vomiting, and dehydration. No blood or tissue sample was conserved.

Patient 2

The second child (III2) was born after a 38-wk pregnancy. She too was small (birth weight 2,500 g; height 46 cm) for gestational age, but she did well during the first 12 mo of life, and she could sit, stand, and walk within normal time limits. However, she then displayed severe anorexia, failure to thrive, skin abnormalities, and proximal tubulopathy closely similar to those observed in her older sister. At 3 years of age, she also experienced a severe episode of diarrhea and dehydration. She survived this episode but subsequently developed progressive multiorgan involvement with extraocular muscle palsy, lid ptosis, micropunctated pigmentary deposits, and extinguished electroretinogram. Shortly thereafter, major osteoporosis and rickets were detected (fig. 1), and liver enlargement with persistent hyperlactatemia and normal lactate/pyruvate (L/P) and ketone-body molar ratio were noted (lactate 3–3.6 mmol/liter [normal <2]; L/P 15–16 [normal <20]; ketone-body molar ratio 1 [normal <2]). During her fifth year, insulin-de-

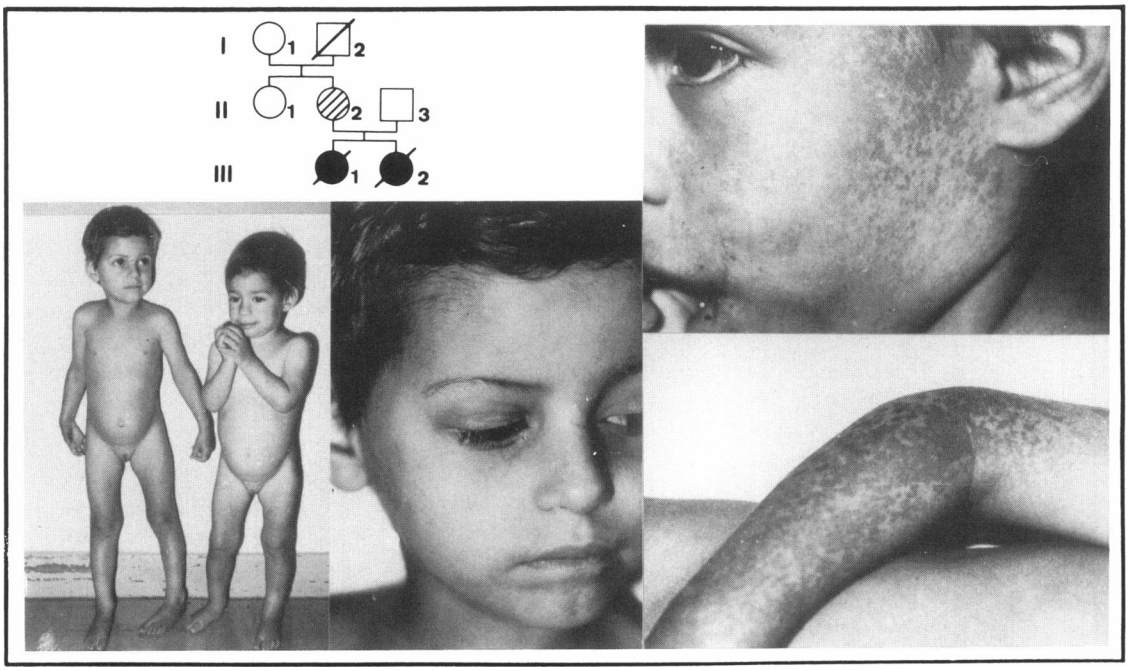

Figure 1 Pedigree and clinical aspect of patients III1 and III2. Note their satisfactory general appearance, mild rickets, and mottled pigmentation in the photoexposed areas (forearms, cheeks, and legs). Also note the absence of muscle atrophy and ptosis.

pendent diabetes mellitus was diagnosed, and her neurological condition markedly worsened. She gradually developed major cerebellar ataxia and hypotonia with bilateral deafness, total blindness, myoclonic jerks, psychomotor regression, and cortical atrophy. No spasticity or heart involvement was noted. Finally, she developed major obesity and lost the ability to stand and walk. She died at 8 years of age after an acute episode of dehydration and collapse. No tissue sample was taken after death.

Methods

Spectrometric studies on frozen muscle (30 mg) taken during life were performed according to a method described elsewhere (Chrétien et al. 1990), and results were presented as ratios rather than as absolute enzyme activities, as balanced respiratory-enzyme activities are required for functional oxidative phosphorylation in vivo (Rustin et al. 1991). Oxygen consumption by intact lymphocytes was measured according to a method described elsewhere (Rötig et al. 1989). The processing of skeletal biopsy specimens (deltoid) for light microscopic histochemistry was performed according to the method of Romero et al. (1989).

For Southern blotting, total DNA (5 μg) derived from different tissues was digested, separated by agarose (0.7%) gel electrophoresis, and transferred onto nylon filters (Hybond N^+; Amersham). The filters were hybridized with [^{32}P]dCTP-labeled single-strand mtDNA probes (2×10^6 cpm/ml) cloned in our laboratory and identified by comparison with available sequences (Anderson et al. 1981). For characterization of the nucleotide sequence at the boundaries of the partial duplication, 1 μg total DNA was submitted to PCR amplification (30 cycles) with two oligonucleotide primers (primer A, nt8335–nt8353; primer B, nt15480–nt15460). DNA was amplified by mixing 100 pmol of each primer with 2.5 U *Taq* polymerase (Perkin Elmer—Cetus) according to a method described elsewhere (Erlich et al. 1988). Amplified DNA was recovered on agarose gel and then was directly sequenced by the Sanger technique.

Results

Evidence for complex III deficiency in III2 was provided by markedly decreased succinate cytochrome *c* reductase activity (26 nmol cytochrome *c*/min/mg protein [control mean ± 1 SD = 51 ± 17]) and elevated cytochrome *c* oxidase (Cox)/succinate cytochrome *c* reductase and Cox/NADH cytochrome *c* reductase ratios in muscle homogenate (IV/II + III = 7.6 [control mean ± 1 SD = 3.3 ± 0.8]; IV/I + III = 5.6 [control mean ± 1 SD = 3.7 ± 0.7]). On the other hand, Cox, succinate PMS-DCPIP reductase activities, and their ratios were normal (Cox 198 nmol cytochrome *c*/min/mg protein [control mean ± 1 SD = 165 ± 76]; succinate PMS-DCPIP reductase 33 nmol DCPIP/min/mg protein [control mean ± 1 SD = 24 ± 10]; Cox/succinate PMS-DCPIP reductase 6.88 [control mean ± 1 SD = 6.8 ± 1.22]); in keeping with this, oxygen consumption by intact lymphocytes was defective in III2 (5 nmol O_2/min/4.10^7 cells [control mean ± 1 SD = 14 ± 2]).

Histopathological studies of the muscle by using the modified Gomori trichrome method showed both increased subsarcolemmal and intermyofibrillar red material (ragged-red fibers; fig. 2*a*) and an intense reaction for succinic dehydrogenase in many fibers (fig. 2*b*). With myofibrillar ATPase stain, a predominance of type I muscle fibers was found (data not shown). With the Sudan red stain, lipid droplets were detected in both ragged-red fibers and in normal fibers. Histochemical studies revealed the decrease of Cox activity in several muscle fibers (fig. 2*c*). However, immunocytochemical techniques using polyclonal anti-Cox protein antibodies gave a positive signal in all muscle fibers, including Cox-negative fibers. Ragged-red fibers showed an increased intensity of immunofluorescence to Cox in the subsarcolemmal regions (data not shown).

Southern blot analysis of lymphocyte DNA digested with restriction enzyme *Pvu*II (cleavage at nt2652) and hybridization using either a Cox subunit II (Cox II) or a cytochrome *b* (Cyt *b*) probe showed that III2 had two populations of mtDNA, one normal (16.5 kb) and one rearranged (10 kb; fig. 3, lane 2). Subsequent hybridization with either an NADH dehydrogenase 4 (ND4) or a ND5 probe only revealed a normal *Pvu*II restriction fragment (fig. 3, lane 3) These initial data are consistent with a simple heteroplasmic deletion encompassing the ND4 and ND5 genes in III2. It is surprising that digestion with enzyme *Bam*HI (cleavage at nt14258) and hybridization with either a Cyt *b* or Cox II probe detected a mixed population of normal and high-molecular-weight (26 kb; fig. 3, lane 6) mtDNA molecules. Similar results were obtained with enzyme *Xho*I, which cleaves the mtDNA at nt14955 (not shown). These data provided evidence for a complex rearrangement involving both an insertion and a deletion of the mitochondrial genome.

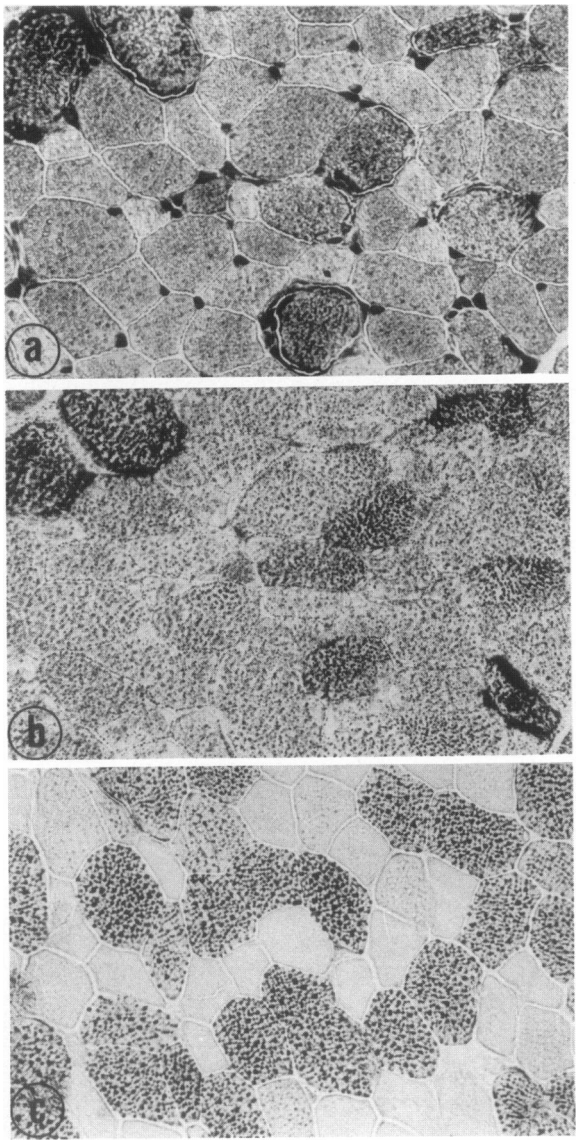

Figure 2 Histopathological observations in serial sections of muscle biopsy from patient III2. *a*, Modified Gomori trichrome stain. Note the "ragged-red fibers" with subsarcolemmal and intermyofibrillar accumulations of abnormal mitochondria. *b*, Succinate dehydrogenase stain. Note the increased activity in the "ragged-red fibers." *c*, Histochemical Cox stain. Note the decreased activity of Cox in some muscle fibers.

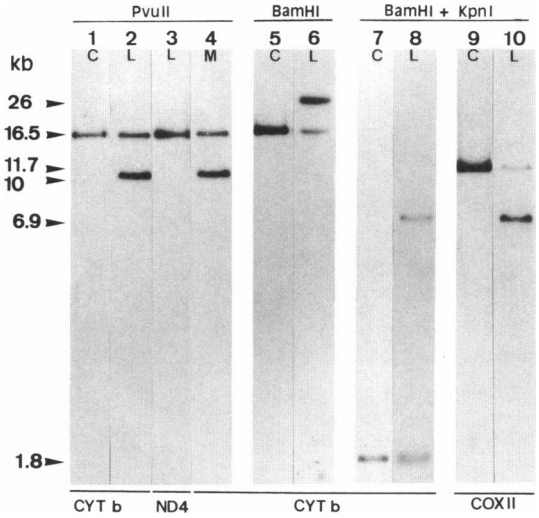

Figure 3 Southern blot analysis of mtDNA from patient III2 leukocytes (L) or muscle (M) and of control leukocyte DNA (C). Lanes 1, 2, and 4–8, Hybridization using Cyt *b* probe. Lane 3, Hybridization using ND4 probe. Lanes 9 and 10, Hybridization using Cox II probe. The same pattern was obtained with a Cox II probe for lanes 1, 2, and 4–6.

Further experiments suggested the model of a direct duplication involving one full-length and one partly deleted mtDNA molecule with a unique abnormal junction between the two mtDNA species. Indeed, double digestion with enzymes *Bam*HI + *Kpn*I (cleavage at nt2577, nt16052, and nt16133; fig. 4) and hybridization using either a Cyt *b* or a Cox II probe revealed the same abnormal fragment (6.9 kb; fig. 3, lanes 8, 10). Considering that the genes for Cox II and Cyt *b* are normally located on both sides of the unique *Bam*HI site, the detection of one single abnormal *Bam*HI-*Kpn*I fragment by using either probe gave strong support to the view that the deletion involved the *Bam*HI site (nt14258) and the *Xho*I site (nt14955) and that the abnormal junction was located between the gene for Cox II and that for Cyt *b* (fig. 4). In fact, PCR amplification using nucleotide primers A and B, normally separated by 7.145 bp, gave rise to an abnormal 624-bp fragment (fig. 4). Sequence analysis carried out to characterize the endpoints of the partial duplication showed that the abnormal junction was located between ATPase 6 and Cyt *b* and resulted in an in-frame ATPase 6-Cyt *b* chimeric gene (fig. 4). Sequence analysis also showed that two directly repeated sequences of 4 bp (AGGA) were present in the wild-type mitochondrial genome at the boundaries of the deletion (fig. 4). Moreover, since the 3′ repeat was located two nucleotides away from the endpoint of the deletion, these results provided evidence for retention of the distal repeat in the partial duplication. Similar patterns of PCR amplification or restriction analysis were obtained in patient III2, regardless of the tissue tested (e.g., see fibroblasts, muscle, and lymphocytes; fig. 4).

Finally, Southern blot analysis did not detect any

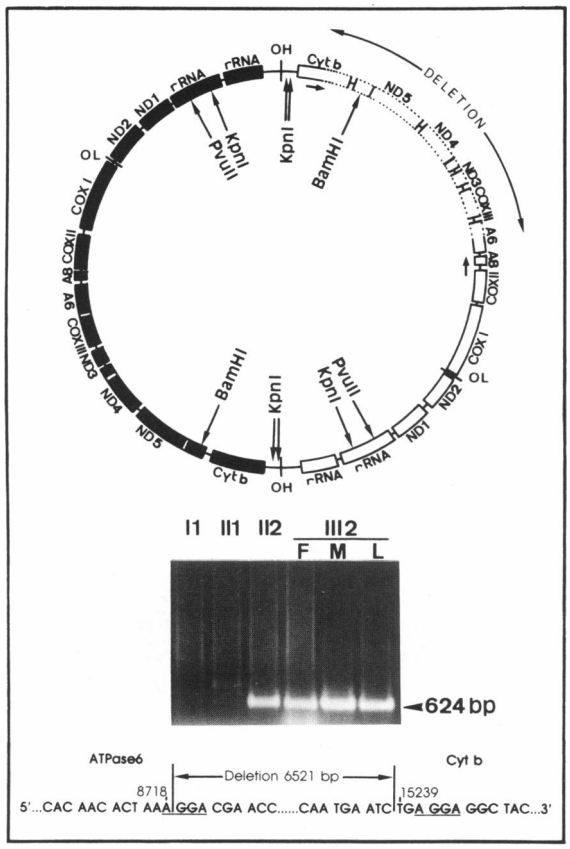

Figure 4 *Top,* Schematic representation of duplicated mitochondrial genome in II2 and III2. The map shows that the mtDNA partial duplication involved one normal (blackened boxes) and one deleted mitochondrial genome (unblackened boxes). The extent of the deletion, the position of the restriction sites mentioned in the study, and the positions of the oligonucleotide primers used for amplification of the abnormal junction are also given (primer A, nt8335–nt8353; primer B, nt15480–nt15460). A6 and A8 = subunits 6 and 8, respectively, of ATPase; OH and OL = heavy- and light-strand replication origins, respectively. *Middle,* PCR amplification of abnormal junction in lymphocyte DNA of mother (II2) and in muscle (M), fibroblasts (F), and lymphocytes of patient III2 (L). Note the absence of amplification in I1 and II1. *Bottom,* Characterization of nucleotide sequence at boundaries of mtDNA rearrangment in patient III2.

rearranged mtDNA molecules in leukocyte DNA from the affected mother (II2), healthy maternal aunt (II1), or healthy maternal grandmother of the proband (not shown). It is most interesting, however, that we were able to detect the abnormal 624-bp junction in the patient's mother (II2) by using PCR amplification of her leukocyte DNA (fig. 4). This test was negative in the maternal grandmother (I1) and the maternal aunt (II1) of the patient (fig. 4).

Discussion

The present study reports on a partial duplication of the mitochondrial genome in a kindred with proximal tubulopathy and photosensitization in early infancy. The cause of the disease in proband III1 was not known, and only at the point when a progressive multiorgan involvement occurred in the second child (III2) could the hypothesis of a genetic defect of oxidative phosphorylation be considered. Subsequently, there was successive involvement of kidney (proximal tubulopathy), gut (watery diarrhea), skin (mottled pigmentation in photoexposed areas), skeletal muscle (mitochondrial myopathy with ragged-red fibers), endocrine pancreas (diabetes mellitus), retina (tapetoretinal degeneration), ear (bilateral deafness), cerebellum (cerebellar ataxia), and cortex (myoclonic jerks, psychomotor regression, and cortical atrophy) in this syndrome. The progressive organ involvement and the observation of a mild hyperlactatemia in III2 pointed to a possible disorder of the mitochondrial energy supply and led us to finally identify a complex III deficiency in the patient. On the basis of this observation, we would suggest giving consideration to the hypothesis of a defect of oxidative phosphorylation in elucidating the origin of other unexplained diseases, especially those with seemingly unrelated concomitant symptoms.

We and others have already described elevated oxidoreduction ratios in plasma as useful clues to the diagnosis of this condition (Robinson 1989). In the present study, however, lactate levels were moderately elevated, and the L/P ratios remained in the normal range (<20). This feature could be related either to proximal tubulopathy, which lowers blood lactate, or to diabetes mellitus, which hampers entry of pyruvate into the Krebs cycle. A similar observation has been made in a patient with Cox deficiency presenting as de Toni-Fanconi-Debré syndrome with Leigh disease (Ogier et al. 1988). Whatever the mechanism, it is important to be aware that proximal tubulopathy and/or insulinopenia can be pitfalls in the investigation of mitochondrial energy metabolism in vivo.

In this syndrome, as in other mitochondrial disorders, an increasing number of tissues were involved in the course of the disease. The mechanism of this progressive organ involvement remains largely unknown. In the present case, however, it could be related to the partial duplication of the mitochondrial genome that we observed in all tissues. Indeed, our patient showed heteroplasmy in mtDNA, with normal

and duplicated mtDNA molecules being present in blood leukocytes, muscle, and cultured skin fibroblasts. Since each tandem duplication included both origins of replication and the entire D-loop, one could consider the hypothesis of a selective advantage of duplicated molecules that progressively favors the preponderance of rearranged mitochondrial genomes.

Yet, why a partial duplication of the mitochondrial genome should result in a severe disease is surprising, especially as the partial duplication included one normal copy of every gene. One can hypothesize that the interrupted Cyt *b* gene produced a chimeric protein containing a Cyt *b* moiety joined to an ATPase 6 moiety, since the deletion did not suppress the reading frame of the rearranged mitochondrial genome. Such a protein could compete with the normal counterpart and alter the function of the corresponding complexes, particularly complex III which was markedly deficient in III2. Alternatively, one can suppose that the excess of particular gene products altered either assembly or kinetic properties of several complexes, as suggested by negative Cox staining in several fibers. However, it is worth noting that Cox staining was positive in about half of all muscle fibers, a feature which might be related to the largely normal Cox activity in the bulk of III2 skeletal muscle. It is therefore difficult to decide whether the disease resulted from complex III deficiency only or whether Cox deficiency in some tissues also played a role in the clinical expression of the illness.

In our opinion, however, the main feature of the present study is the observation of a maternal transmission of the duplication over two generations. Theoretically, this mother-to-child transmission could be accounted for either by a cytoplasmic transmission or by a dominant transmission of the trait. It is worth remembering that all autosomal dominant pedigrees reported thus far were associated with multiple mtDNA deletions (Zeviani et al. 1989; Cormier et al. 1991). In the present study, only one type of rearranged mtDNA molecule was detected in the different tissues over two generations, thus supporting the view that a maternal inheritance was involved. While maternal transmission of deletions (Poulton et al. 1991) and point mutations have been reported in Leber disease (Wallace et al. 1988), retinitis pigmentosa, muscle weakness (Holt et al. 1990) and MERRF (Shoffner et al. 1990), this observation is, to our knowledge, the first example of maternal inheritance of a major mtDNA partial duplication in man. The mtDNA duplication was not easy to detect in the mildly affected mother, and only PCR amplification allowed detection of minute amounts of abnormal mitochondrial genome in her circulating lymphocytes. A mosaicism, including germ-line mosaicism, is therefore likely to be involved, and the variable proportion of abnormal mtDNA in the different tissues may explain the mild phenotype in the mother.

Yet, the question of how the duplication has arisen remains unanswered. It might involve circular dimerization during replication followed by deletion via either recombination between homologous sequences (replication-slippage) or nonhomologous breakage and reunion, especially as two 4-bp direct repeats were found in the wild-type mitochondrial genome, at the boundaries of the deleted region. In keeping with this, it is interesting to note that the 3′ repeat was located two nucleotides away from the endpoint of the partial duplication, a result which supports our previous observation that distal repeats are usually retained in mtDNA deletions (Rötig et al. 1991). Duplications of the mitochondrial genome have already been described in yeast, fungi (Dujon and Belcour 1989), and lizards (Moritz and Brown 1987). Although normal cells may contain catenated mtDNA molecules, only in the case of malignant cells have total duplications of mtDNA been reported in man (Clayton and Vinograd 1967; Hudson and Vinograd 1967; Clayton 1982). The only cases of partial duplication of the human mitochondrial genome hitherto reported have been described in two unrelated patients with diabetes mellitus, small stature, ataxia, and mitochondrial myopathy (Poulton et al. 1989*a*, 1989*b*). In that interesting study, however, no proximal tubulopathy or photosensitization was observed, and no evidence for maternal inheritance of the partial duplication was noted.

While most rearrangements of the mitochondrial genome are usually regarded as sporadic events, the possibility of germ-line mosaicism for mtDNA, proposed by Poulton et al. (1989*a*) and reported by the present study raises the difficult issue of genetic counseling in mitochondrial disorders. In our study, however, PCR detection of the abnormal junction enabled rapid diagnosis of relatives at risk.

Acknowledgments

We gratefully acknowledge Professors P. Royer, J. Frézal, Y. de Prost, and J. L. Dufier for referring the patients to us and for their constant support. We are thankful to J. Poulton for helpful discussion, to M. Poussière and A. Strickland for

their help in preparing the manuscript, and to Association Francaise contre les Myopathies for its constant support.

References

Anderson S, Bankier AT, Barrell MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, et al (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Chrétien D, Bourgeron T, Rötig A, Munnich A, Rustin P (1990) The measurement of the rotenone sensitive NADH cytochrome c reductase activity in mitochondria isolated from minute amount of human skeletal muscle. Biochem Biophys Res Commun 173:26–33

Clayton DA (1982) Replication of animal mitochondrial DNA. Cell 28:693–705

Clayton DA, Vinograd J (1967) Circular dimer and catenate forms of mitochondrial DNA in human leukaemic leukocytes. Nature 216:652–657

Cormier V, Rötig A, Tardieu M, Colonna M, Saudubray JM, Munnich A (1991) Autosomal dominant deletions of the mitochondrial genome in a case of progressive encephalomyopathy. Am J Hum Genet 48:643–648

Dujon B, Belcour L (1989) Mitochondrial DNA instabilities and rearrangements in yeasts and fungi. In: Berg DE, Howe MM (eds) Mobile DNA. American Society for Microbiology, Washington, DC, pp 861–878

Erlich HA, Gelfand DH, Saiki RK (1988) Specific DNA amplification. Nature 331:461–462

Goto Y, Nonaka I, Horai S (1990) A mutation in the tRNA-leu(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348:651–653

Holt IJ, Harding AE, Morgan-Hughes JA (1988) Deletion of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature 331:717–719

——— (1990) A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 46: 428–433

Hudson B, Vinograd J (1967) Catenated circular DNA molecules in HeLa cell mitochondria. Nature 216:647–652

Lestienne P, Ponsot G (1988) Kearns-Sayre syndrome with muscle mitochondrial DNA deletion. Lancet 1:885

Moritz C, Brown WM (1987) Tandem duplications in animal mitochondrial DNAs: variation in incidence and gene content among lizards. Proc Natl Acad Sci USA 84:7183–7187

Ogier H, Lombes A, Scholte HR, Poll-The BT, Fardeau M, Aicardi J, Vignes B, et al (1988) de Toni-Fanconi-Debré syndrome with Leigh syndrome revealing severe muscle cytochrome c oxidase deficiency. J Pediatr 112:734–739

Poulton J, Deadman ME, Gardiner RM (1989*a*) Duplications of mitochondrial DNA in mitochondrial myopathy. Lancet 1:236–239

——— (1989b) Tandem direct duplications of mitochondrial DNA in mitochondrial myopathy: analysis of nucleotide sequence and tissue distribution. Nucleic Acids Res 24:10223–10229

Poulton J, Deadman ME, Ramacharan S, Gardiner RM (1991) Germ-line deletions of mtDNA in mitochondrial myopathy. Am J Hum Genet 48:649–653

Robinson BH (1989) Lactic acidemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited diseases, 6th ed. McGraw-Hill, New York, pp 869–888

Romero N, Lestienne P, Marsac C, Paturneau-Jouas M, Nelson I, François D, Eymard B, et al (1989) Immunocytological and histochemical correlation in Kearns-Sayre syndrome with mtDNA deletion and partial cytochrome c oxidase deficiency in skeletal muscle. J Neurol Sci 93: 297–309

Rötig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, Schmitz J, et al (1990) Pearson's marrow-pancreas syndrome: a multisystem mitochondrial disorder in infancy. J Clin Invest 86:1601–1608

Rötig A, Cormier V, Koll F, Mize E, Saudubray JM, Veerman A, Pearson HA, et al (1991) Site-specific deletions of the mitochondrial genome in the Pearson marrow-pancreas syndrome. Genomics 10:502–504

Rustin P, Chrétien D, Bourgeron T, Rötig A, Munnich A (1991) Assessment of the mitochondrial respiratory chain. Lancet 2:60

Sengers RCA, Stadhouders AM, Trijbels JMF (1984) Mitochondrial myopathies: clinical, morphological and biochemical aspects. Eur J Pediatr 141:192–207

Shoffner JM, Lott MT, Lezza AMS, Seibel P, Ballinger SW, Wallace DC (1990) Myoclonic epilepsy and ragged-red-fiber disease (MERRF) is associated with a mitochondrial DNA tRNAlys mutation. Cell 61:931–937

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AMS, Elsas LJ, et al (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427–1430

Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP (1988) Deletions of mitochondrial DNA in Kearns-Sayre syndrome. Neurology 38: 1339–1346

Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, DiDonato S (1989) An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. Nature 339:309–311