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Autosomal Dominant Deletions of the Mitochondrial Genome in a Case of Progressive Encephalomyopathy

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Summary

Multiple deletions of the mitochondrial genome were found in a family in which the proband had ataxia and ketoacidotic comas. A progressive multiorgan involvement appeared in the course of the disease, and histopathological investigation demonstrated mitochondrial myopathy features with ragged red fibers. A defect of oxidative phosphorylation was found in both skeletal muscle and lymphocytes. It is surprising that various mtDNA deletions were detected both in the proband and in his healthy mother and maternal aunt but not in the rest of the maternal progeny. All the deletions were located between Cox II and cytochrome b genes, and short (4-5 bp) repeated sequences were consistently present at the boundaries of the rearrangements in different tissues. Therefore, the deletions appear not to be transmitted per se but to be inherited in a Mendelian manner, being possibly dominant. Both the Mendelian inheritance of the trait and the variety of the deletions in carriers suggest that a nuclearly encoded factor(s) might be involved in the triggering of the deletions. However, the presence of the rearrangements in healthy individuals raises the question of whether mtDNA deletions actually cause the clinical expression of the disease.

Introduction

In the past 2 years, several rearrangements of the mitochondrial genome have been reported in neuromuscular and hematological disorders, namely, mitochondrial myopathies (Holt et al. 1988), Kearns-Sayre syndrome (McKusick 16510; Zeviani et al. 1988), and Pearson's marrow-pancreas syndrome (McKusick 26056; Rötig et al. 1988, 1989). While most rearrangements were sporadic, familial forms have occasionally been reported. The mitochondrial inheritance of an mtDNA deletion has been hypothesized in one family (Ozawa et al. 1988). On the other hand, an autosomal dominant inheritance has been demonstrated in a large pedigree, providing genetic evidence for the possible involvement of nuclearly encoded factors in these rearrangements (Zeviani et al. 1989). In the present study we describe multiple autosomal dominant deletions of the mtDNA genome in a family in which the proband presented with recurrent episodes of ataxia and ketoacidotic comas. However, since not all carriers were clinically affected in this family, the present study raises the question of whether deletions are actually causal in the clinical expression of the disease.

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Case Report

M (III-1) is a 9-year-old boy born to healthy unrelated parents. At 5 years of age he first presented with ketoacidotic comas with repetitive attacks of ataxia and drowsiness. Metabolic acidosis, hypoglycemia, and hyperlactatemia were noted (plasma bicarbonates 5.7 mM, pH 7.19, P_{CO_2} 15 Torr). He progressively lost the ability to walk and stand. Then, he developed a bilateral cerebellar ataxia and a pyramidal syndrome with muscle weakness. Ptosis with ocular paralysis and deafness are now present. He has marked growth

failure (length and weight below − 2 SD) and mental retardation but no cardiac involvement or retinitis pigmentosa. Histopathological examination showed typical mitochondrial myopathy features with ragged red fibers (not shown), and the computed-tomography scan revealed low-density areas in basal ganglia, centrum ovale, and peduncle. High blood lactate and elevated lactate/pyruvate (i.e., 24; normal <16) and elevated ketone body molar ratios in plasma (3OH butyrate/acetoacetate = 2; control <2) were suggestive of a defect of oxidative phosphorylation and prompted us to investigate enzyme activities in lymphocytes and muscle. A marked hypoparathyroidism (P 2.21 mM, Ca 1.91 mM, PTH 3 pg/ml) was present, but no other endocrine disorder was noted. He is doing relatively well on coenzyme Q treatment. His 19-year-old brother (III-2) and his mother (II-5), maternal aunts (II-3 and II-4), and maternal uncle (II-2) are healthy.

Methods

The mitochondrial respiratory chain catalyzes the oxidation of reduced molecules by oxygen and the energy transduction into ATP. During this process, reducing equivalents (NADH, succinate) are transferred to oxygen via four multienzymatic complexes, i.e., NADH-CoQ-reductase (complex I), succinate-CoQ-reductase (complex II), $CoQH_2$-cytochrome *c*–reductase (complex III), and cytochrome oxidase (complex IV).

For determination of the enzyme activities, muscle mitochondria from a 300-mg biopsy were prepared according to a method described by Bookelman et al (1978). Oxymetric and spectrophotometric studies were carried out according to the method of Sottocasa et al. (1967). Oxygen consumption by intact lymphocytes was measured according to a method described elsewhere (Rötig et al. 1988). In brief, lymphocytes were isolated from 10 ml heparinized venous blood on a Ficoll cushion, and oxygen consumption by 20 × 10^6 cells was measured with a Clark-type oxygen electrode (Hansatech, Norfolk, England) in a water-jacketed reaction chamber containing 0.3 M mannitol, 5 mM $MgCl_2$, 10 mM KCl, 10 mM Na_2HPO_4, pH 7.4 , and 250 μg serum albumin in a final volume of 0.5 ml.

Total DNA from skeletal muscle (deltoid), leukocytes, and fibroblasts was extracted in 7 M urea, 10 mM EDTA, 10 mM Tris-HCl (pH 7.4), 0.3 M NaCl, proteinase K (20 μg/ml), and 0.1% SDS for one night at 37°C. DNA was subsequently purified by phenol-chloroform extraction and ethanol precipitation. Total DNA (5 μg) was digested with *Pvu*II, was electrophoresed on agarose gel (0.7%), and was transferred onto nylon filters (Hybond N^+) for 2 h. The deletions were mapped by using either human or mouse mtDNA probes (human Cox II, nucleotides [nt] 7561–8308; human ATPase–Cox III, nt 8581–9296; human ND5, nt 12860–13801; human cyt b, nt 14726–15760; and a whole mouse mtDNA probe reported by Lutfalla et al. [1985, fig 2]). The filters were hybridized with ^{32}P dCTP–labeled probes (2 × 10^6 cpm/ml) and were autoradiographed. The boundaries of the mtDNA deletions were submitted to PCR amplification using *Taq* polymerase (Perkin Elmer–Cetus). Different pairs of oligonucleotides were used for each patient: II-4—5′ nt 9507–9527 and 3′ nt 16190–16170; II-5—5′ nt 7598–7618 and 3′ nt 16062–16042; and III-1—5′ nt 7598–7618 and 3′ nt 16062–16042, and 5′ nt 7923–7943 and 3′ nt 16062–16042. Amplified DNA was then purified on low-melting-temperature agarose gel, was cloned in phage M13mp18 at the *Sma*I site, and was sequenced by the Sanger technique.

Results

Investigation of the respiratory chain in muscle tissue revealed a reduced NADH-CoQ-reductase (complex I) activity (28.7 nmol O_2/min/mg protein; normal mean ± SD 67 ± 20.6), as estimated by measuring malate oxidation. The cytochrome composition of the respiratory chain was not modified. Oxygen consumption by intact lymphocytes from the proband (III-1) was also markedly decreased (6.58 nmol O_2/min/4 × 10^7 cells; normal mean ± SD 14.06 ± 2.15).

Southern blot analysis of mtDNA digested with the restriction enzyme *Pvu*II (cleavage at position 2652) and hybridization using either a cytochrome oxidase (Cox) II probe or a whole mouse mtDNA probe (Lutfalla et al. 1985) showed two bands in the muscle of the proband (III-1), one normal (16.5 kb) and one of approximately 10 kb (fig. 1), as well as two abnormal fragments, one of 8 kb and one of 10 kb, in his leukocytes (fig. 1). No rearrangement of mtDNA was found in his cultured fibroblasts (fig. 1). The proband's healthy mother (II-5) presented an abnormal fragment of approximately 8 kb in circulating leukocytes (fig. 1). Moreover, multiple deleted species ranging in size from 8 kb to 11 kb were found in the circulating leukocytes of his healthy aunt (II-4) (fig. 1). The other rela-

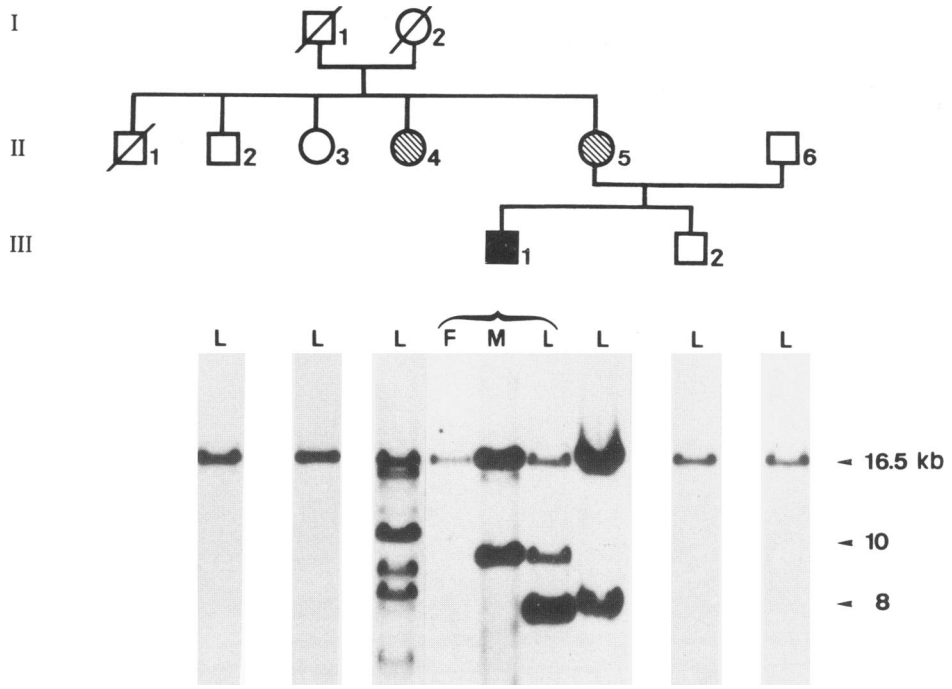

Figure 1 *Top,* Family pedigree. The blackened symbol indicates a clinically affected individual; the hatched symbols indicate healthy individuals who have mitochondrial rearrangements. A diagonal slash through a symbol indicates that the individual is deceased. Roman numerals indicate generations. *Bottom,* Hybridization pattern of *Pvu*II-digested total DNA from leukocytes (L), fibroblasts (F), and muscle (M) of healthy and affected individuals of family, by Cox II probe. Only one 16.5-kb normal fragment was detected in II-2, II-3, II-6, and III-2. A normal and at least one abnormal fragment were present in II-4, II-5, and III-1. Similar results were obtained with a whole mouse mtDNA probe (not shown).

tives (II-2, II-3, II-6, and III-2) had no major mtDNA rearrangements in their leukocytes, and no other tissue specimens could be studied in this family.

The deletions were then mapped by using different mtDNA probes (Cox II, Cox III–ATPase, ND5, and cytochrome b; fig. 2). The deletions extended from the Cox II gene to the cytochrome b gene, both in the proband (III-1) (fig. 2, Ⓐ and Ⓑ in leukocytes and Ⓑ in muscle) and in his mother II-5 (fig. 2Ⓒ). In the maternal aunt (II-4), all the deletions were included between the Cox III gene and the far end of the cytochrome b gene. Her two largest deletions are presented in figures 2Ⓓ and 2Ⓔ.

PCR amplification and sequence analysis carried out to characterize the boundaries of the rearrangements showed that the 8-kb abnormal fragment found in leukocytes from the proband (III-1) was accounted for by at least two deletions, one spanning 7,916 bp and one spanning 7,830 bp. These two rearrangements shared the same distal endpoints (figs. 3Ⓐ and 3Ⓑ). In order to investigate the proband's 10-kb abnormal fragment, we used another pair of oligonucleotide primers (5′ nt 7923–7943 and 3′ nt 16503–16483), and we were able to demonstrate that the same pattern of amplification was present in both muscle and leukocytes (not shown), suggesting that the 10-kb abnormal fragment was identical in these tissues. The deletion found in the proband's mother (II-5) was different and spanned 7,413 bp (fig. 3Ⓒ). For the maternal aunt, only the two largest deletions were sequenced; one spanned 6,220 bp (fig. 3Ⓓ), and one spanned 5,835 bp (fig. 3Ⓔ). Finally, in all cases, short repeated sequences (4–5 bp) were present in the wild-type mitochondrial genome at the boundaries of the deletions. These direct repeats were either perfect or imperfect and were found to be either direct or inverted (fig. 3). In three rearrangements the deletions caused a frameshift, resulting in the termination of the translation of the specific mRNA (figs. 3Ⓐ and 3Ⓒ).

Finally, in an attempt to detect minute amounts of deleted mtDNA molecules in apparently healthy relatives, PCR amplification was performed in II-2, II-3,

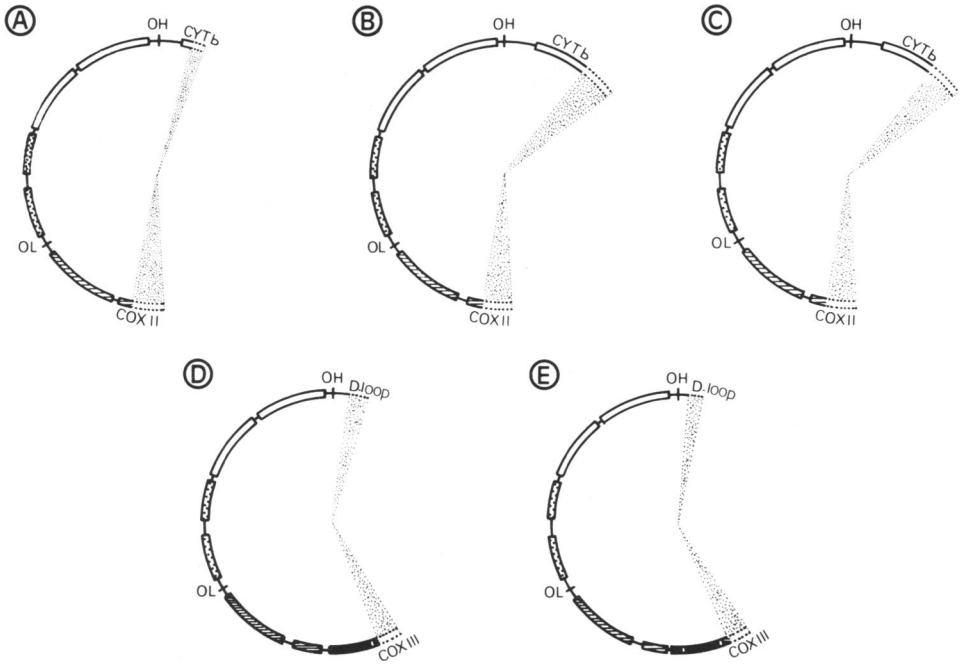

Figure 2 Localization of mtDNA deletions in III-1 leukocytes (Ⓐ and Ⓑ) and muscle + leukocytes (Ⓑ) and in II-5 (Ⓒ) and II-4 (Ⓓ and Ⓔ). The larger open wedges of the pie denote the localization and size of the deletions, and the small lightly stippled wedges of the pie denote the upper and lower limits. OH = origin of heavy-strand replication; OL = origin of light-strand replication. Cox II = subunit II of cytochrome c oxidase; Cox III = subunit III of cytochrome c oxidase; Cyt b = cytochrome b.

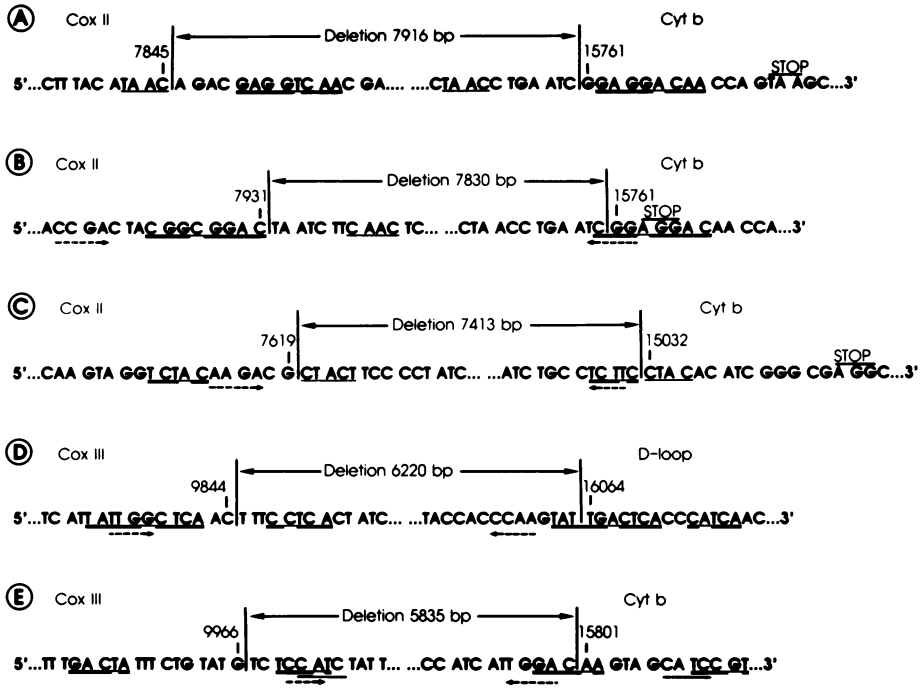

Figure 3 Characterization of nucleotide sequences at boundaries of five different mtDNA deletions. Ⓐ and Ⓑ, sequenced deletions from patient III-1. Ⓒ, Sequenced deletion from individual II-5. Ⓓ and Ⓔ, Sequenced deletions from individual II-4. Regions of homology, at each side of the rearrangements, are underlined (——). Inverted repeats are hatched underlined (----→ and ←----).

II-6, and III-2 by using the oligonucleotide pairs from III-1. No amplification product was detected (not shown).

Discussion

In the disease reported here, the proband has had iterative attacks of ataxia and ketoacidotic comas since the age of 5 years. Both the progressive involvement of seemingly unrelated organs and the observation of a markedly abnormal oxydoreduction status in plasma led us to consider the hypothesis of a mitochondrial cytopathy and to finally identify a defect of oxidative phosphorylation in both skeletal muscle and circulating lymphocytes of the proband. Since the 16.5-kb mtDNA encodes 13 polypeptides of the respiratory chain in man, we first looked at the organization of the mitochondrial genome in our patient; and we were able to demonstrate multiple mtDNA deletions in both tissues.

However, the major feature of the present study is the variety of the mtDNA deletions in the two tissues. It is, to our knowledge, the first report on multiple mtDNA deletions in several tissues derived from one single individual. This feature could hardly be accounted for by independent molecular events during embryogenesis and strongly supports the involvement of an unknown factor favoring the rearrangements of the mitochondrial genome.

Since multiple heteroplasmic deletions were found not only in the proband but in his mother (II-5) and maternal aunt (II-4) as well, the present study raises the question of the inheritance of the trait. Two distinct mechanisms could theoretically account for familial deletions of the mitochondrial genome: (1) maternal inheritance of the rearrangement or (2) transmission of a nuclearly encoded factor triggering deletions of the mitochondrial chromosome.

Maternal inheritance seems unlikely, as the deletions differed among carrier individuals and no rearrangements were found in the rest of the maternal progeny (II-2 and II-3). One cannot rule out, however, a maternal transmission of the trait in case of germinal mosaicism. Nevertheless, the mtDNA deletions in this pedigree appear instead to be inherited in a Mendelian manner, being possibly dominant. Indeed, the diversity of the rearrangements in various tissues and in different individuals suggests that a nuclear mutant gene triggers multiple deletions in this pedigree. Recently, Zeviani et al. (1989) have reported the autosomal dominant transmission of mtDNA deletions in a family with progressive external ophthalmoplegia. In that study, however, maternal inheritance could easily be ruled out by the recurrent transmission of the trait to patrilinear descendants. Taken together, our two independent observations provide genetic evidence that a nuclearly encoded factor(s) may be involved in the occurrence of the mtDNA deletions.

All the deletions identified in this pedigree were found between COX II and cytochrome b genes. Sequence analysis of these deletions showed short repeats (4–5 bp) at the endpoints of the rearrangements. Directly repeated sequences are frequently associated with mtDNA deletions in human diseases (Mita et al. 1990). In both the present study and the case reported by Zeviani et al. (1989), the directly repeated sequences are located imprecisely relative to the edges of the deletions. In addition, they are shorter than the 9–18-bp direct repeats flanking deletions in sporadic rearrangements such as Pearson syndrome (Rötig et al. 1990), Kearns-Sayre syndrome (Zeviani et al. 1988), and mitochondrial myopathy (Holt et al. 1989). These repeats could possibly be involved in homologous recombination of the mitochondrial genome via a nuclearly encoded factor(s) promoting mtDNA breakage-reunion of the homologous sequences. Alternatively, this putative factor could be involved in the jumping of the DNA replication machinery from one repeated sequence to the other.

The question of why the two carriers in the present study displayed absolutely no clinical symptoms is intriguing. The comparison of the modified sequences in the proband (III-1) and in his unaffected relatives (II-4 and II-5) does not account for these discrepancies, since the deletions resulted in a frameshift in all but one rearrangement. This clinical heterogeneity among carriers not only raises the hypothesis of variable expressivity of the trait in different tissues but also addresses the question of whether the deletions of the mtDNA actually cause the clinical expression of the disease.

Finally, the autosomal dominant transmission of the trait should prompt us to initiate linkage studies in informative families as a first step toward the ultimate goal of identifying the still unknown proteins triggering the mtDNA deletions in these patients. However, genetic heterogeneity among dominant forms might possibly exist, and one must be particularly cautious when pooling pedigrees for linkage study, especially when clinical profiles are different.

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References

Bookelman H, Trijbels JMF, Sengers RCA, Janssen AMJ (1978) Measurement of cytochromes in human skeletal muscle mitochondria, isolated from fresh and frozen stored muscle specimens. Biochem Med 19:336–373

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

——— (1989) Deletions of muscle mitochondrial DNA in mitochondrial myopathies: sequence analysis and possible mechanisms. Nucleic Acids Res 17:4465–4469

Lutfalla G, Blanc H, Bertolotti R (1985) Shuttling of integrated vectors for mammalian cells to E-coli is mediated by head-to-tail multimeric inserts. Somatic Cell Mol Genet 11:223–238

Mita S, Rizzuto R, Moraes CT, Shanske S, Arnaudo E, Fabrizi GM, Koga Y, et al (1990) Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA. Nucleic Acids Res 18:561–567

Ozawa T, Yoneda M, Tanaka M, Ohno K, Sato W, Suzuki H, Nikishimi M, et al (1988) Maternal inheritance of deleted mitochondrial DNA in a family with mitochondrial myopathy. Biochem Biophys Res Commun 154: 1240–1247

Rötig A, Colonna M, Blanche S, Fischer A, Le Deist F, Frézal J, Saudubray JM, et al (1988) Deletion of blood mitochondrial DNA in pancytopenia. Lancet 2:567–568

Rötig A, Colonna M, Bonnefont JP, Blanche S, Fischer A, Saudubray JM, Munnich A (1989) Mitochondrial DNA deletions in Pearson's marrow-pancreas syndrome. Lancet 1:902–903

Rötig A, Cormier V, Blanche S, Bonnefont JP, Le Deist 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

Sottocasa GL, Kuylenstierna AB, Ernster L, Bergstrand A (1967) An electron transport system associated with the outer membrane of liver mitochondria. J Cell Biol 32: 415–438

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