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Mitochondrial Encephalomyopathies: Clinical and Molecular Analysis

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The classification of mitochondrial encephalomyopathies relied upon clinical, biochemical, and histological features until the discovery of mitochondrial DNA defects in 1988. Since then, an outburst of molecular genetic information has aided our understanding of the pathogenesis and the classification of these heterogeneous disorders. Novel concepts of maternal inheritance, mitochondrial DNA (mtDNA) heteroplasmy, tissue distribution, and threshold have explained many of the clinical characteristics. The discovery of point mutations, large-scale mtDNA deletions, duplications, and autosomally inherited disorders with multiple mtDNA deletions have revealed new genetic phenomena. Despite our rapidly expanding understanding of the molecular genetic defects, many questions remain to be explored to fill the gap in our knowledge of the relationship between genotype and clinical phenotype.

KEY WORDS: Brain; KSS; Leigh; LHON; maternal inheritance; MELAS; MERRF; mitochondrial DNA; muscle; NARP; oxidative phosphorylation; PEO; respiratory chain.

INTRODUCTION

Mitochondrial encephalomyopathies are a diverse group of disorders characterized by defects in mitochondrial function. Inherited defects causing mitochondrial dysfunction can be due to mutations either in nuclear DNA (nDNA) or in mitochondrial DNA (mtDNA). To make matters even more complicated, some mitochondrial diseases due to mtDNA mutations are not inherited at all, while others are caused by environmental factors.

The human mitochondrial genome is a 16.6-kb circle of double-stranded DNA (Anderson *et al.*, 1981), and contains only 37 genes (see Fig. 1). Two genes encode ribosomal RNAs (rRNAs), 22 encode transfer RNAs (tRNAs), and 13 encode polypeptide-coding messenger RNAs (mRNAs). Both the rRNAs and tRNAs are required for the translation of the mRNAs, all of which specify components of the respiratory chain/oxidative phosphorylation system. These components include seven subunits of Complex I, or NADH dehydrogenase-ubiquinone oxidoreductase (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6); one subunit of Complex III, or ubiquinone-cytochrome *c* oxidoreductase (cytochrome *b*), three subunits of Complex IV, or cytochrome *c* oxidase (CO I, CO II, and CO III); and two subunits of Complex V, or ATP synthetase (ATPase 6 and ATPase 8). The respiratory complexes also contains subunits encoded by nDNA; these are synthesized on cytoplasmic ribosomes and are subsequently imported into the organelle, where they are assembled together with the mtDNA-encoded subunits into the respective holoenzymes. Complex II, or succinate dehydrogenase-ubiquinone oxidoreductase, contains only nDNA-encoded subunits.

Mitochondria, and mtDNAs, are unique in that they are inherited only from the mother (Giles *et al.*, 1980). Thus, most (but not all) pathogenic errors in mtDNA are maternally inherited: women may transmit the defect to all of their children, but only their daughters can transmit the defect to their progeny. Since a cell contains multiple mitochondria

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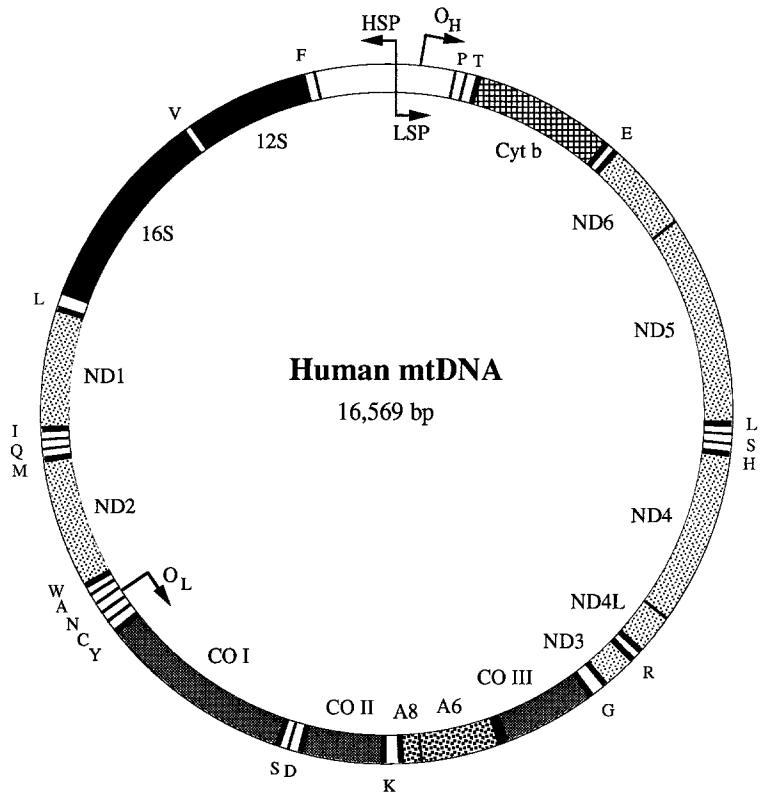

Fig. 1. The human mitochondrial genome (Anderson *et al.*, 1981). The structural genes for the 12S and 16S ribosomal RNAs (rRNA), the subunits of NADH-coenzyme Q oxidoreductase (ND), cytochrome *c* oxidase (CO), cytochrome b (Cyt b), and ATP synthetase (A), and 22 tRNAs (one-letter amino acid nomenclature) are shown. The origins of light-strand (O_L) and heavy-strand (O_H) replication, and of the promoters for initiation of transcription from the light-strand (LSP) and heavy-strand (HSP) are shown by arrows.

(hundreds or thousands) and since each mitochondrion contains multiple mtDNAs [about 5, on average (Satoh and Kuroiwa, 1991)], both normal and mutated mtDNAs may coexist within the patient's tissues, a condition known as heteroplasmy. If a patient contains a heteroplasmic population of normal and mutated mtDNAs, the phenotypic expression of a pathogenic mutation may vary among tissues or may change within a tissue during the course of time, depending on the relative (and sometimes the absolute) amount of mutated mtDNAs present. Virtually every human organ system has expressed clinical symptoms and signs in mitochondrial disorders; however, muscle and brain have extremely high energy requirements. Relatively low levels of mutated mtDNAs can affect the respiratory capacity of these tissues, and high levels can be extremely devastating. It is therefore no surprise that mitochondrial disorders are predominantly encephalomyopathies. The common clinical manifestations of the mitochondrial encephalomyopathies include ocular myopathies (ophthalmoplegia and ptosis), skeletal muscle weakness, seizures, dementia, and stroke-like episodes.

The two main biochemical features in most mitochondrial diseases are respiratory chain deficiency and lactic acidosis. Morphologically, patients with mitochondrial diseases often display ragged-red fibers (RRF) in the muscle biopsy (see the article by Shoubridge, this issue). Not all mitochondrial diseases display RRF, but the presence of RRF is an important indicator of the presence of a mitochondrial disorder.

DISEASES ASSOCIATED WITH MATERNALLY INHERITED POINT MUTATIONS IN mtDNA

Most maternally inherited mitochondrial disorders are associated with mtDNA point mutations.

Table 1. Phenotypes Associated with mtDNA Point Mutations

Nucleotide	Mutation[a]	Gene location	Phenotype	Reference[b]
1555	A → G	12S rRNA	AID	Prezant *et al.*, 1993
3243	A → G	tRNA-Leu(UUR)	MELAS/PEO/diabetes/hearing loss	Goto *et al.*, 1990
3250	T → C	tRNA-Leu(UUR)	Myopathy	Goto *et al.*, 1992b
3251	A → G	tRNA-Leu(UUR)	MELAS	Sweeney *et al.*, 1993
3252	A → G	tRNA-Leu(UUR)	MELAS	Morten *et al.*, 1993
3256	C → T	tRNA-Leu(UUR)	Multisystem/PEO	Moraes *et al.*, 1993b
3260	A → G	tRNA-Leu(UUR)	Cardiomyopathy/myopathy	Zeviani *et al.*, 1991
3271	T → C	tRNA-Leu(UUR)	MELAS	Goto *et al.*, 1991
3302	A → G	tRNA-Leu(UUR)	Myopathy	Bindoff *et al.*, 1993
3303	C → T	tRNA-Leu(UUR)	Cardiomyopathy	Silvestri *et al.*, 1993b
3394	T → C	ND1	LHON	Brown *et al.*, 1992b
3460	G → A	ND1	LHON	Huoponen *et al.*, 1991
4160	T → C	ND1	LHON	Howell *et al.*, 1991
4216	T → C	ND1	LHON	Mackey and Howell, 1992
4269	A → G	tRNA-Ile	Multisystem/cardiomyopathy	Taniike *et al.*, 1992
4917	A → G	ND2	LHON	Johns and Berman, 1991
5244	G → A	ND2	LHON	Brown *et al.*, 1992c
5703	G → A	tRNA-Asn	Myopathy (PEO)	Moraes *et al.*, 1993b
7444	G → A	COX I	LHON	Brown *et al.*, 1992b
8344	G → A	tRNA-Lys	MERRF	Shoffner *et al.*, 1990
8356	T → C	tRNA-Lys	MERRF	Silvestri *et al.*, 1992
8993	T → G	ATPase 6	NARP/MILS	Holt *et al.*, 1990
8993	T → C	ATPase 6	NARP/MILS	deVries *et al.*, 1993
11084	A → G	ND4	MELAS	Lertrit *et al.*, 1992
11778	G → A	ND4	LHON	Wallace *et al.*, 1988
14484	T → C	ND6	LHON	Johns *et al.*, 1992
13708	G → A	ND5	LHON	Johns and Berman, 1991
15257	G → A	Cyt b	LHON	Johns and Neufeld, 1991
15812	G → A	Cyt b	LHON	Johns and Neufeld, 1991
15923	A → G	tRNA-Thr	Infantile respiratory deficiency	Yoon *et al.*, 1991
15990	C → T	tRNA-Pro	Myopathy	Moraes *et al.*, 1993a

[a] L-strand sequence.
[b] First published article.

To date, more than 30 point mutations have been described (Table I), associated with the clinical phenotypes below.

Leber's Hereditary Optic Neuropathy (LHON)

LHON is a maternally inherited form of blindness due to an optic neuropathy predominantly affecting men, with onset in the second or third decade of life. The vision loss begins in the central visual field, usually in one eye, and subsequently affects the other eye weeks to months later. Recovery of vision has been reported in some patients and seems to depend on the particular pathogenic mtDNA mutation present. Asymptomatic individuals and acutely affected patients frequently have peripapillary telangiectasia (Nikoskelainen *et al.*, 1987). While LHON is predominantly an ocular disease, patients can have a detectable deficiency of Complex I in muscle (Larsson *et al.*, 1991).

LHON was the first mitochondrial disease to be defined at the molecular level: Wallace *et al.* (1988) found a G → A transition at mtDNA position 11778, in codon 340 of the ND4 gene of Complex I, in LHON pedigrees displaying maternal inheritance. The mutation was nonconservative, and converted the codon from Arg → His. Since the discovery of the nt-11778 mutation, a number of other mutations have now been found in other Complex I genes, as well as in Complex III and Complex IV genes (reviewed in Wallace, 1992; see Table I). The degree of heteroplasmy of these mutations can shift rapidly among generations (Bolhuis *et al.*, 1990; Howell *et al.*, 1992), a finding which has been confirmed with other pathogenic mtDNA point mutations. The increased penetrance of LHON in

males may be due to a locus on the X-chromosome that interacts with the mutated LHON mtDNA genotype (Vilkki *et al.*, 1991; Bu and Rotter, 1991); this finding, however, is controversial (Sweeney *et al.*, 1993).

The analysis of the LHON mutations has provided us with new insight into the phenotypic expression of mtDNA mutations: it appears that specific mutations may act together, perhaps synergistically, to produce the LHON phenotype (Howell *et al.*, 1991; Mackey and Howell, 1992; Brown *et al.*, 1992b). This "interactive mutation" model may help explain some of the variability associated with the expression of mitochondrial diseases.

Myoclonus Epilepsy with Ragged-Red Fibers (MERRF)

MERRF is characterized by myoclonus, generalized seizures, ataxia, and myopathy with ragged-red fibers (Fukuhara *et al.*, 1980). Onset is usually in childhood, but adult onset has been described. Clinical features which are less consistently seen include dementia, short stature, hearing loss, neuropathy, and optic atrophy (Silvestri *et al.*, 1993a). The family history is typically consistent with maternal inheritance; however, some of the individuals who harbor the mtDNA mutations associated with MERRF may be asymptomatic or oligosymptomatic (Silvestri *et al.*, 1993a). Pathological studies have revealed neuronal loss in the dentate nucleus, inferior olivary nucleus, diffuse cerebellar and brain stem white matter gliosis, and spinal cord posterior column degeneration (Fukuhara *et al.*, 1980).

MERRF has been associated with an A → G transition at nt-8344 in the $tRNA^{Lys}$ gene (Shoffner *et al.*, 1990; Yoneda *et al.*, 1990). A second mtDNA mutation has also been found in MERRF patients, and interestingly, it too is in the $tRNA^{Lys}$ gene, at nt-8356 (Silvestri *et al.*, 1992; Zeviani *et al.*, 1993). A C → T transition at nt-3256 in the $tRNA^{Leu(UUR)}$ gene has been associated with a MERRF overlap syndrome characterized by MERRF plus optic neuropathy, ophthalmoparesis with ptosis, retinopathy, and diabetes (Moraes *et al.*, 1993b). All of these mutations are heteroplasmic.

Other clinical phenotypes have been associated with the nt-8344 mutation, including Leigh's syndrome, myoclonus or myopathy with truncal lipomas, and limb-girdle myopathy (Silvestri *et al.*, 1993a; Holme *et al.*, 1993).

Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-Like Episodes (MELAS)

MELAS has been clinically defined by the following criteria: (1) stroke at a young age (i.e., before age 40); (2) encephalopathy characterized by seizures, dementia, or both; and (3) evidence of mitochondrial dysfunction with lactic acidosis, ragged-red fibers, or both (Hirano *et al.*, 1992). The strokes commonly cause hemianopia and cortical blindness because the posterior cerebral hemispheres are particularly vulnerable. Other frequent clinical features include: migraine-like headaches, recurrent vomiting, exercise intolerance, limb weakness, and short stature. Like MERRF pedigrees, MELAS families often have oligosymptomatic and asymptomatic maternal relatives (Ciafaloni *et al.*, 1992).

Stroke is the clinically distinctive feature of MELAS. The etiology of the stokes is not known. Computerized tomography and magnetic resonance imaging studies have shown brain lesions that do not conform to the distribution of major cerebral arteries (Matthews *et al.*, 1991). Excessive accumulations of mitochondria have been seen in the walls of small arteries and capillaries in muscle and pia mater (Ohama *et al.*, 1987; Hasegawa *et al.*, 1991). These small vessel abnormalities might be contributing to the stroke pathogenesis in MELAS.

MELAS is associated with at least four different point mutations. The first mutation is an A → G transition at nt-3243 in the $tRNA^{Leu(UUR)}$ gene (Goto *et al.*, 1990; Kobayashi *et al.*, 1990), and is found in about 80% of cases. The second mutation is a T → C transition at nt-3271, also in the $tRNA^{Leu(UUR)}$ gene (Goto *et al.*, 1991), and is found in about 10% of cases. Yet a third (A → G) transition in the $tRNA^{Leu(UUR)}$ gene, at nt-3252, has been reported (Morten *et al.*, 1993). A point mutation in a polypeptide-coding gene, at nt-11084 in ND4, was reported to be associated with MELAS (Lertrit *et al.*, 1992), but there is evidence that this is a nonpathogenic neutral mutation (Ozawa *et al.*, 1991; Sakuta *et al.*, 1993). All the mutations in MELAS are heteroplasmic, with a high proportion (> 80%) of mutated mtDNAs in muscle (Goto *et al.*, 1992a; Ciafaloni *et al.*, 1992).

Maternally Inherited Progressive External Ophthalmoplegia (PEO)

Progressive external ophthalmoplegia (PEO) is characterized by childhood or adolescent onset of extraocular muscle weakness with ptosis and is often accompanied by limb weakness. Rarely, PEO can be seen in patients with otherwise typical MELAS (Hirano *et al.*, 1992) and MERRF (Silvestri *et al.*, 1993a). PEO is also seen in the multisystem disorder called Kearns–Sayre syndrome [KSS (see below)] which is typically sporadic; however, patients with an intermediate disorder between pure PEO and KSS have been described as PEO-plus.

PEO has been associated with two different defects of mtDNA. About one-half of all patients have large-scale deletions of mtDNA (see below), which can occur either sporadically (Holt *et al.*, 1989; Moraes *et al.*, 1989) or are inherited in an autosomal dominant manner (Zeviani *et al.*, 1989, 1990; Servidei *et al.*, 1991). Of the remaining half, about one-third had a positive family history, and interestingly, many of these patients harbor the nt-3243 mutation, which is more commonly associated with MELAS (Goto *et al.*, 1990; Johns and Hurko, 1991; Moraes *et al.*, 1993c). It appears that there are significant differences in the localized concentration of the nt-3243 mutation at the single muscle fiber level in PEO as compared to MELAS patients, implying that the pattern of the spatial distribution of mutant mtDNAs at the cellular level leads to clinically distinct phenotypes (V. Petruzzella and E. A. Schon, unpublished).

Another point mutation associated with PEO is a C → T transition at nt-5703 in the $tRNA^{Asn}$ gene (Moraes *et al.*, 1993b). Two other mutations, at nt-12308 in the $tRNA^{Leu(CUN)}$ gene and at nt-15904 in the $tRNA^{Thr}$ gene, had been claimed to be etiologic of PEO (Lauber *et al.*, 1991), but are probably neutral polymorphisms (van den Ouweland *et al.*, 1992a).

NARP and MILS

Neuropathy, ataxia, and retinitis pigmentosa (NARP) are characterized by developmental delay, dementia, retinal pigmentary degeneration, seizures, ataxia, proximal neurogenic muscle weakness, and sensory neuropathy (Holt *et al.*, 1990). Lactic acidosis was noted in one of three patients in the original pedigree (Holt *et al.*, 1990). The clinical features of this maternally inherited disorder are variable. Ragged-red fibers are not seen in NARP; however, electron microscopy revealed occasional mitochondria with abnormal cristae and small subsarcolemmal aggregates of mitochondria (Holt *et al.*, 1990).

NARP is associated with two different mutations, both of which are at the same nucleotide (nt-8993) in the ATPase 6 gene (i.e., subunit 6 of Complex V). The more common mutation is a T → G transversion which converts codon 156 from Leu → Arg (Holt *et al.*, 1990). The less frequent mutation is a T → C transition which converts codon 156 from Leu → Pro (deVries *et al.*, 1993; Santorelli *et al.*, 1994). Because the mutation is in the ATPase 6 gene, it has been postulated that the fundamental defect in NARP lies in the proton channel, or F_0 segment, of complex V (Tatuch *et al.*, 1992). Support for the pathogenicity of the T → G mutation came from analysis of a directed mutation of the analogous position in the *E. coli* homologue of human ATPase 6 (i.e., ATPase *a*) gene; this mutation abolished detectable ATP synthesis via oxidative phosphorylation (Hartzog and Cain, 1993).

When the proportion of the T → G mutation at nt-8993 is very high (> 90%), the clinical phenotype is not NARP but a fatal encephalopathy—maternally inherited Leigh's syndrome (MILS) (Tatuch *et al.*, 1992; Shoffner *et al.*, 1992; Ciafaloni *et al.*, 1993; Santorelli *et al.*, 1993). Leigh's syndrome (LS) is a multisystem degenerative disorder with onset usually in the first year of life characterized by developmental delay, psychomotor regression, hypotonia, seizures, myoclonus, ataxia, brainstem dysfunction, optic atrophy, and peripheral neuropathy. The diagnosis can be confirmed by the characteristic pathological findings of symmetric necrotic foci in the basal ganglia, thalamus, brainstem, and dentate nuclei. Three mitochondrial enzyme defects, which are presumed to be autosomally inherited, have been associated with Leigh's syndrome: pyruvate dehydrogenase complex (PDHC), cytochrome *c* oxidase (COX), and NADH-CoQ reductase. Leigh's syndrome patients with the nt-8993 mutation had an earlier onset and a more rapid deterioration than did the individuals with COX and PDHC deficiencies (Santorelli *et al.*, 1993). In addition, retinal pigmentary degeneration, present in about 40% of the MILS patients, can be another distinguishing clinical feature (Santorelli *et al.*, 1993).

Aminoglycoside-Induced Deafness (AID)

The first known mutation in a mitochondrial ribosomal RNA gene, an A → G transition at

nt-1555 of the 12S rRNA gene, causes maternally inherited aminoglycoside-induced deafness and familial nonsyndromic deafness (Prezant *et al.*, 1993; Hutchin *et al.*, 1993). The mutation causes hypersensitivity to aminoglycoside drugs such as streptomycin, kanamycin, and gentamicin. By analogy to the binding of aminoglycosides to small rRNAs in lower organisms, the nt-1555 mutation probably lies at or near a step-loop structure in 12S rRNA associated with the binding of these drugs (Prezant *et al.*, 1993; Hutchin *et al.*, 1993).

Other Disorders

Limb-girdle myopathies have been associated with a T → C transition at nt-3250 in the $tRNA^{Leu(UUR)}$ gene (Goto *et al.*, 1992b), A → G transitions at nt-3251 (Sweeney *et al.*, 1993; see Moraes *et al.*, 1993b) and nt-3302 in the $tRNA^{Leu(UUR)}$ gene (Bindoff *et al.*, 1993), and a G → A transition at nt-15990 in the anticodon stem of $tRNA^{Pro}$ (Moraes *et al.*, 1993a); myopathy plus cardiomyopathy with an A → G transition at nt-3260 in the $tRNA^{Leu(UUR)}$ gene (Zeviani *et al.*, 1991); cardiopathy plus a multisystem disorder with an A → G transition at nt-4269 in the $tRNA^{Ile}$ gene (Taniike *et al.*, 1992); and fatal infantile respiratory chain defects with two A → G transitions in the $tRNA^{Thr}$ gene, at nt-15923 and, less definitively (Brown *et al.*, 1992a), at nt-15924 (Yoon *et al.*, 1991).

Diabetes and deafness (DAD) have been noted in maternally related individuals of pedigrees with the nt-3243 mutation that is usually found in patients with MELAS (Reardon *et al.*, 1992; van den Ouweland *et al.*, 1992b; Dunbar *et al.*, 1993; Gerbitz *et al.*, 1993; Kadowaki *et al.*, 1993; Onishi *et al.*, 1993; Remes *et al.*, 1993).

DISEASES ASSOCIATED WITH SPONTANEOUS ERRORS IN mtDNA

One class of mtDNA mutations can arise spontaneously, with no apparent genetic component: large-scale partial deletions and duplications of mtDNA. These sporadic mtDNA rearrangements are almost never found in the parents or siblings of affected patients.

The combination of retinitis pigmentosa, external ophthalmoplegia, and complete heart block was first recognized as a clinical syndrome in 1958 (Kearns and Sayre, 1958). Twenty-five years later, Rowland and colleagues defined the Kearns–Sayre syndrome (KSS) by the invariant triad of ophthalmoplegia, pigmentary retinopathy, and onset before age 20, with at least one of the following additional features: cardiac conduction block, cerebellar syndrome, or cerebrospinal fluid protein greater than 100 mg/dl (Rowland *et al.*, 1983). Other associated clinical features of KSS include hearing loss, proximal limb weakness, endocrinopathies (particularly hypoparathyroidism and diabetes mellitus), and renal tubular dysfunction.

In 1988, giant deletions of mtDNA (Δ-mtDNAs) were observed in KSS and ocular myopathy (OM), two mitochondrial disorders associated with PEO (Zeviani *et al.*, 1988; Holt *et al.*, 1989; Moraes *et al.*, 1989). Later, the same Δ-mtDNAs were found in a rare hematopoetic disorder called Pearson's marrow/pancreas syndrome (Rötig *et al.*, 1991). Pearson's syndrome typically begins in infancy or early childhood with sideroblastic anemia and exocrine pancreatic dysfunction (Pearson *et al.*, 1979). The anemia can be fatal despite blood transfusion therapy. A few patients who presented with Pearson's syndrome later developed KSS (McShane *et al.*, 1991). In these disorders, the Δ-mtDNAs can be observed easily by Southern blot hybridization analysis as a large population (up to 80% of total mtDNA) of a single species of mtDNA migrating in electrophoretic gels more rapidly than full-length mtDNAs.

Duplications of mtDNA have also been identified in KSS (Poulton *et al.*, 1989a, b), but not as frequently as deletions. The relationship between duplications and deletions is not clear, but it may well be that duplications are recombination intermediates which can be resolved into deletions (Poulton *et al.*, 1993).

One hallmark of sporadic mtDNA deletions and duplications is that there is only one specific type of mtDNA rearrangement in each patient. Moreover, the particular type of deletion can be quite different among patients; on the other hand, about one-third of all KSS/PEO patients harbor the same deletion, called the "common deletion" (Schon *et al.*, 1989; Mita *et al.*, 1990), which removes 4,977 bp of mtDNA between the ATPase 8 and ND5 genes (see Fig. 1). These findings imply that the population of rearranged mtDNA molecules in any one patient with a sporadic rearrangement is a clonal expansion of a single spontaneous deletion or duplication event occurring early in oögenesis or embryogenesis.

Since they were first discovered in 1988 (Holt *et*

al., 1988), about one hundred species of Δ-mtDNAs and about a dozen species of duplicated mtDNAs have been described.

DISEASES ASSOCIATED WITH MATERNALLY INHERITED REARRANGEMENTS OF mtDNA

While most DNA rearrangements arise spontaneously, there is no theoretical reason why they could not be transmitted subsequently as a maternally inherited trait. In fact, a mother with sporadic PEO and deleted mtDNA transmitted the mtDNA rearrangement to her child, who was diagnosed as having Pearson's syndrome (Bernes *et al.*, 1993).

In addition, maternally inherited mtDNA duplications have been observed in a syndrome of brain, kidney, and endocrine dysfunction (Rötig *et al.*, 1992). This latter case is remarkable for at least one feature that seems quite common in mitochondrial disorders: diabetes. Diabetes, often accompanied by deafness, has been observed in a number of cases of maternally transmitted mtDNA duplications (Ballinger *et al.*, 1992; Dunbar *et al.*, 1993) and in pedigrees with the nt-3243 mutation, as previously noted.

DISEASES ASSOCIATED WITH MENDELIAN-INHERITED ERRORS IN mtDNA

To date, there are only two types of errors in mtDNA that are inherited in a mendelian fashion. The first type is an autosomal dominant-inherited myopathy characterized by PEO, in which affected family members harbor large quantities of multiple species of deletions of mtDNA in their muscle, and which are apparently generated during the lifespan of the patient (Zeviani *et al.*, 1989, 1990). Aside from the pattern of inheritance, the clinical presentation is fundamentally identical to maternally inherited PEO (i.e., point mutations) or sporadic PEO (i.e., clonal rearrangements).

The second type of disorder is actually a quantitative error in mtDNA copy number. In these mtDNA depletion syndromes affected tissues are characterized by respiratory chain defects, massive mitochondrial proliferation, and a severe depletion in the amount of mtDNA present. On the other hand, there is no observable biochemical or genetic defect in unaffected tissues. The presence of mtDNA depletion has been documented by quantitative Southern blot hybridization analysis, by *in situ* hybridization of affected muscle sections with mtDNA probes, and by immunohistochemistry of affected muscle sections using anti-DNA antibodies. In the latter assay, nuclei stain positively in both control and patient muscle sections, but the reticulated staining of the cytoplasm (due to immunoreactivity to mtDNA within the mitochondria) is observable only in control sections, and is absent or deficient in fibers from the patients.

In the early-onset form of the disease, symptoms begin at birth, with death ensuing within a few months (Moraes *et al.*, 1991). The degree of depletion is severe—up to 98% reduction in mtDNA as compared to controls—but is confined to only one or two tissue types (e.g., muscle alone, liver alone, muscle and kidney). If the depletion is in muscle, it is uniform, that is, all muscle fibers examined are devoid of observable COX enzyme activity and have little or no immunoreactive mtDNA.

In the later-onset form, symptoms begin around one year of age, and death ensues some years later; all late-onset described to date have been myopathies (Tritschler *et al.*, 1992). The depletion in the late-onset cases is less severe (between 70 and 90% reduction). Moreover, affected muscle in these cases show a segmental pattern of depletion, that is, some fibers appear normal while others are COX- and mtDNA-deficient.

DISEASES ASSOCIATED WITH ENVIRONMENTALLY INDUCED ERRORS IN mtDNA

Besides mendelian-inherited mtDNA depletion, there also exists an environmental cause of mtDNA depletion: zidovudine myopathy (Dalakas *et al.*, 1990). Some AIDS patients treated long-term with zidovudine (azidothymidine or AZT) develop a myopathy with RRF. The muscle mtDNA in these patients is severely reduced—up to 78% depletion as compared to controls (Arnaudo *et al.*, 1991)—and appears to be due specifically to the AZT treatment (i.e., it was not a secondary effect of the HIV infection). The depletion is apparently reversible.

The reason for mtDNA depletion in zidovudine myopathy is likely due to AZT's structure. AZT is a nucleoside analog that inhibits the replication of the mitochondrial genome by interfering with the action of the mitochondrial DNA polymerase (DNA polymerase γ). AZT is incorporated into the mtDNA, leading to premature termination of the elongating mtDNA daughter strands (Simpson *et al.*, 1989).

CONCLUDING REMARKS

The identification of mtDNA mutations in mitochondrial disorders has opened a new field of investigation in a diverse group of clinical diseases. Despite the recognition of these mtDNA mutations, the pathogenic mechanisms leading to the clinical phenotypes have not been characterized well. Further exploration of the cellular sequelae resulting from mtDNA mutations is likely to provide new insight into mitochondrial functions and perhaps provide the basis for more rational therapies for the patients.

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