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Mitochondrial DNA Mutations in Diseases of Energy Metabolism

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A variety of degenerative diseases involving deficiencies in mitochondrial bioenergetics have been associated with mitochondrial DNA (mtDNA) mutations. Maternally inherited mtDNA nucleotide substitutions range from neutral polymorphisms to lethal mutations. Neutral polymorphisms are ancient, having accumulated along mtDNA lineages, and thus correlate with ethnic and geographic origin. Mildly deleterious base substitutions have also occurred along mtDNA lineages and have been associated with familial deafness and some cases of Alzheimer's Disease and Parkinson's Disease. Moderately deleterious nucleotide substitutions are more recent and cause maternally-inherited diseases such as Leber's Hereditary Optic Neuropathy (LHON) and Myoclonic Epilepsy and Ragged-Red Fiber Disease (MERRF). Severe nucleotide substitutions are generally new mutations that cause pediatric diseases such as Leigh's Syndrome and dystonia. MtDNA rearrangements also cause a variety of phenotypes. The milder rearrangements generally involve duplications and can cause maternally-inherited adult-onset diabetes and deafness. More severe rearrangements frequently involving detetions have been associated with adult-onset Chronic Progressive External Ophthalmoplegia (CPEO) and Kearns–Sayre Syndrome (KSS) or the lethal childhood disorder, Pearson's Marrow/Pancreas Syndrome. Defects in nuclear–cytoplasmic interaction have also been observed, and include an autosomal dominant mutation causing multiple muscle mtDNA deletions and a genetically complex disease resulting in the tissue depletion of mtDNAs. MtDNA nucleotide substitution and rearrangement mutations also accumulate with age in quiescent tissues. These somatic mutations appear to degrade cellular bioenergetic capacity, exacerbate inherited mitochondrial defects and contribute to tissue senescence. Thus, bioenergetic defects resulting from mtDNA mutations may be a common cause of human degenerative disease.

KEY WORDS: Mitochondrial DNA; oxidative phosphorylation (OXPHOS); nucleotide substitutions; DNA rearrangements; neurodegenerative diseases; adult-onset diabetes; Leber's Hereditary Optic Neuropathy (LHON).

INTRODUCTION

The demonstration in 1988 that mitochondrial DNA (mtDNA) base substitution (Wallace *et al.*, 1988) and deletion (Holt *et al.*, 1988b) mutations can cause human disease provided a new dimension to the study of bioenergetics and offered a new perspective on the study of degenerative diseases and aging. In the subsequent five years, a broad spectrum of mtDNA base substitutions have been identified ranging from ethnically associated neutral polymorphisms to lethal childhood mutations, and a wide variety of mtDNA rearrangements have been identified in diseases ranging from adult-onset diabetes mellitus to lethal childhood pancytopenia (Wallace *et al.*, 1994). Moreover, somatic mtDNA mutations have been found to accumulate in quiescent tissues with age, in parallel with a decline

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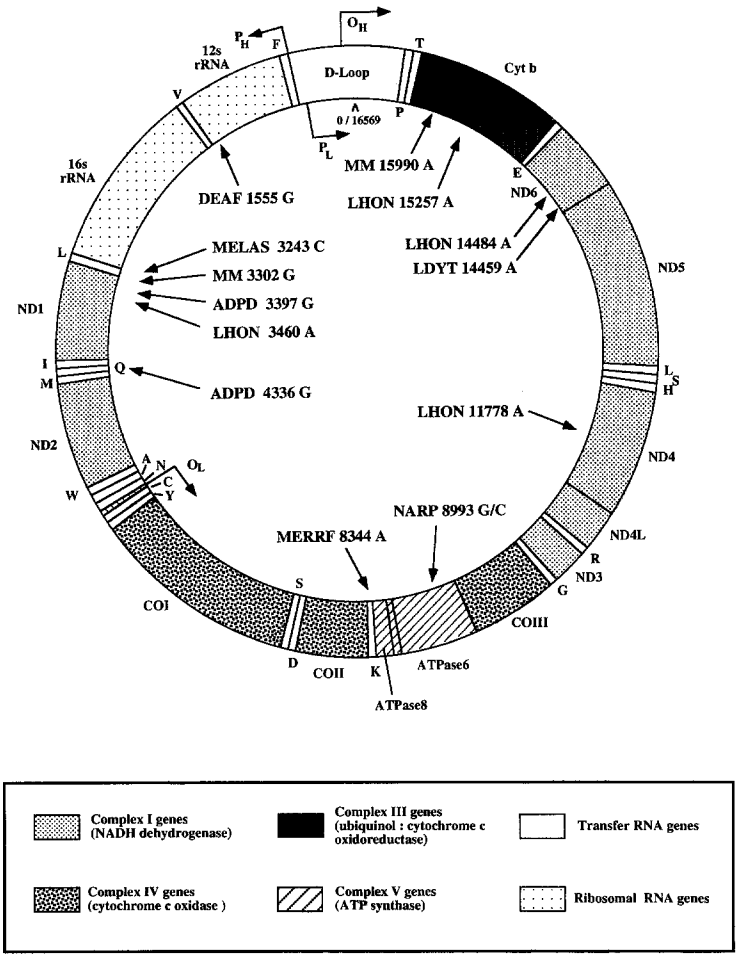

Fig. 1. The human mtDNA map showing the location of the genes and the pathological mutations described in the text (Wallace *et al.*, 1994b).

in the mitochondrial energy generating pathway, oxidative phosphorylation (OXPHOS). This somatic tissue energetic decline may augment the effects of inherited mtDNA mutations causing the onset and progression of degenerative diseases and contribute to the tissue decline in aging.

MITOCHONDRIAL GENETICS

The mtDNA is a closed circular molecule of 16,569 nucleotide pairs (nps) which encodes a 12S and 16S rRNA, 22 tRNAs, and 13 polypeptides (Fig. 1). These polypeptides are all components of the mitochondrial energy-generating pathway OXPHOS. OXPHOS encompasses five multiple polypeptide enzyme complexes: Complex I (NADH: ubiquinone oxidoreductase), Complex II (succinate: ubiquinone oxidoreductase), Complex III (ubiquinol: cytochrome *c* oxidoreductase), Complex IV (cytochrome *c* oxidase), and Complex V (ATP synthase). This pathway utilizes the energy released by the oxidation of fatty acids and sugars by the electron transport chain (Complexes I to IV) to synthesize ATP through Complex V. Mitochondrial ATP is then exchanged for cytosolic ADP by the adenine nucleotide translocator (ANT) (Wallace, 1992b).

Complex I is composed of about 40 polypeptides, 7 (ND 1, 2, 3, 4, 4L, 5, 6) from the mtDNA; Complex II is assembled from four nuclear polypeptides; Complex III involves about 10 polypeptides, 1 (cytb) from the mtDNA; Complex IV is composed of 13 subunits, 3 (COI, II, III) from the mtDNA; while Complex V contains 13 polypeptides, 2 (ATPase 6 and 8) from the mtDNA. The mtDNA genes are transcribed from two adjacent promoters located in the control region, one for the guanine-rich heavy (H)-strand and the other for the cytosine-rich light

(L)-strand. These promoters generate continuous, polycistronic transcripts which are processed into mature rRNAs, tRNAs, and mRNAs. The control region also contains the H-strand origin (O_H) and the associated displacement (D)-loop. The L-strand origin (O_L) is located 2/3 of the way around the genome. Hence, replication is bidirectional but asynchronous (Wallace, 1992b).

The cytoplasmic location and high copy number of the mtDNA gives it a unique genetics. First, the mtDNA is transmitted through the oocyte cytoplasm. Hence, it is maternally inherited (Giles *et al.*, 1980). Second, the occurrence of a new mtDNA mutation creates a mixed intracellular population of mutant and normal molecules called heteroplasmy. As heteroplasmic cells divide during mitosis or meiosis the mutant and normal mtDNAs are randomly distributed to the daughter cells and the mitochondrial genotype drifts. This replicative segregation ultimately results in cells with pure mutant or normal mtDNAs, a state called homoplasmy. Third, increasingly severe mtDNA defects reduce cellular energy outputs until they decline below the minimum energy level (energetic threshold) for normal tissue function. Energetic thresholds differ between tissues, with the brain, heart, muscle, kidney, and endocrine organs being most reliant on mitochondrial energy. Fourth, the mtDNA has a very high mutation rate, which affects both germline and somatic tissue mtDNAs. Germline line mutations result in maternally transmitted diseases or predispose individuals to late-onset degenerative diseases. Somatic mutations accumulate in stable tissues and exacerbate the OXPHOS defects inherited through the germline (Wallace, 1992a,b).

MITOCHONDRIAL DNA SEQUENCE VARIATION

Base substitution mutations occur randomly throughout the mtDNA, and can either alter a noncoding nucleotide and be selectively neutral or change an essential function and be deleterious. Once a new mtDNA mutation arises in a maternal lineage, it becomes progressively enriched through meiotic segregation. If the mutation is neutral, it will eventually segregate to homoplasmy and become established in that maternal lineage. The chance effects of genetic drift will then dictate whether the new mtDNA haplotype will increase to polymorphic frequencies in the general population. By contrast, if the mutation is severely deleterious, it will greatly reduce the fitness of carriers as the cellular proportion approaches homoplasmy. Consequently, maternal lineages which acquire severely deleterious mutations will eventually die out and the mutant mtDNA will be lost. Occasionally, a mutation will arise that is of intermediate severity. Such mildly deleterious mutations do not substantially reduce fitness when homoplasmic and become fixed in the population as a low-frequency variant that predisposes carriers to late-onset degenerative disease (Wallace *et al.*, 1994a).

Neutral mtDNA Variants

Over the course of human radiation, the high mtDNA mutation rate has resulted in the accumulation of a wide range of neutral, population-specific, sequence polymorphisms. Since the mtDNA is maternally inherited, mtDNAs from different maternal lineages do not mix at fertilization and thus cannot recombine. Hence, these neutral mutations have accumulated sequentially along radiating maternal lineages, creating groups of related haplotypes (haplogroups).

This population-specific, neutral, mtDNA variation can be conveniently detected by surveying the mtDNA restriction site variants or by determining the nucleotide sequence of the hypervariable control region. Such studies have revealed that much of the neutral mtDNA variation in populations involves base substitutions in third codon positions or in noncoding regions. Analysis of the mtDNA variation in several thousand individuals from populations throughout the world has shown that the mtDNA sequence variation correlates highly with the ethnic and geographic origin of the sample (Cann *et al.*, 1987; Johnson *et al.*, 1983, Merriwether *et al.*, 1991), with approximately 72% of all extant mtDNAs harboring continent-specific sequence variants.

A number of the population-specific variants are particularly informative. A polymorphic DdeI restriction site at np 10394 subdivides the populations of all continents and thus must predate the radiation of the races (Torroni *et al.*, 1994a,c; Ballinger *et al.*, 1992c; Torroni *et al.*, 1992). A HpaI restriction site at np 3592 (morph 3) delineates 75% of the African mtDNAs (Denaro *et al.*, 1981; Johnson *et al.*, 1983; Chen, Torroni, and Wallace, unpublished), and over 60% of European mtDNAs are contained within four distinct mtDNA lineages: the first lacking an AluI site at np 7025; the second defined by a DdeI site loss at np

1715, an AluI site gain at np 10028, a HaeII site loss at np 4529, an AvaII site gain at np 8249, and a BamHI/MboI site gain at np 16389; the third identified by site losses for BstNI at np 13704 and RsaI at np 16065; and the fourth delineated by a concomitant HaeII np 9052/HhaI np 9053 site loss (Torroni *et al.*, 1994b). Important Asian mtDNA polymorphisms include an associated DdeI site at np 10394 and AluI site at np 10397 which subdivide all Asian mtDNAs (Denaro *et al.*, 1981; Torroni *et al.*, 1994c), a HaeIII site gain at np 663 which defines haplogroup A, a 9 np intergenic deletion between MTTK and MTCO2 that delineates haplogroup B (Cann and Wilson, 1983; Wallace *et al.*, 1994b), a HincII np 13259 site loss which defines haplogroup C (Wallace *et al.*, 1985), and an AluI site loss at np 5176 that defines haplogroup D (Torroni *et al.*, 1994c; Wallace *et al.*, 1994b). Haplogroup B is found in a large proportion of Pacific Islanders (Ballinger *et al.*, 1992a; Hertzberg *et al.*, 1989; Stoneking *et al.*, 1990), and haplogroups A, B, C, and D account for virtually all Native American mtDNAs (Ballinger *et al.*, 1992a; Schurr *et al.*, 1990; Torroni *et al.*, 1993a,b, 1994d).

Knowledge of these ethnic-specific variants and their associated haplotypes is of great importance in searching for possible pathological mutations in the mtDNA disease. Special care must be exercised to avoid accidentally attributing a pathological role to a population-specific neutral variant, and determination of the mtDNA haplotypes of different patients with a specific pathological mutation provides information on the number of times a particular mutation has arisen in the population.

Mildly Deleterious Mutations

While most population variants are neutral, some are mildly deleterious and predispose individuals to late life, progressive, neurodegenerative diseases. Since these mutations do not significantly reduce the reproductive fitness of individuals, they are generally the result of mutations that occurred as a single founder mutation multiple generations in the past. Consequently, these mutations are homoplasmic and associated with a particular mtDNA haplogroup.

Mildly deleterious mutations have been reported in rRNA, tRNA, and protein-coding genes. A 12S rRNA mutation at np 1555 (MTRNR1*DEAF1555G) has been shown to predispose some families to maternally transmitted neurosensory hearing loss. In other individuals it imparts an increased propensity for aminoglycoside-induced deafness (Prezant *et al.*, 1993).

A mildly deleterious $tRNA^{Glu}$ gene mutation at np 4336 (MTTQ*ADPD4336G) has been associated with an increased risk for developing Alzheimer's Disease (AD) and Parkinson's Disease (PD). This mutation is found in 5.2% of Caucasian patients, but only 0.3% of Caucasian controls, and it defines a mtDNA lineage at increased risk for late onset dementias and movement disorders. Other mildly deleterious mutations have also been found with this mutation. One missense mutation in ND1 at np 3397 (MTND1*LHON3397G) changes a highly conserved methionine to a valine and has occurred two independent times in AD families, one with the MTTQ*ADPD4336G mutation and one without. Another 12S rRNA mutation involves a five-base-pair insertion at np 956–965 which also arose in the MTTQ*LHON4336G lineage (Shoffner *et al.*, 1993a).

Moderately Deleterious Mutations

The somewhat more severe moderately deleterious mtDNA mutations generally cause disease in young adults. Since these diseases are relatively deleterious, families with these mutations eventually die out. Hence, depending on severity, families can represent new, heteroplasmic mutations, or older homoplasmic mutations associated with specific mtDNA lineages.

The majority of the mtDNA mutations responsible for Leber's hereditary optic neuropathy (LHON) fall into this category. LHON involves the mid-life onset of acute or subacute central vision loss leading to optic nerve death and central scotoma. Thirteen mtDNA missense mutations have been associated with this disease, scattered throughout the subunits of the electron transport chain enzymes. These mutations can be subdivided into two classes. Four of the mutations are considered primary because they have a high propensity for causing blindness and are sufficient in themselves to cause disease. Nine other mutations are considered secondary since they are unable to cause blindness on their own, but are found at increased frequencies in LHON patients suggesting that they may contribute to the pathology (Wallace *et al.*, 1994b). Of the four primary LHON mutations, the most common and severe mutation occurs in the ND4 gene at np 11778 (MTND4*LHON11778A). This mutation, changes a highly conserved arginine to a histidine (Wallace *et al.*, 1988), is often heteroplasmic (Lott *et al.*, 1990), occurs on different mtDNA

backgrounds (Singh *et al.*, 1989), and is sufficient in itself to cause LHON. In Europe, the second most common primary LHON mutation occurs in ND1 at np 3460 (MTND1*LHON3460A) (Huoponen *et al.*, 1991; Howell *et al.*, 1991). This mutation converts a moderately conserved alanine to a threonine, is sufficient to cause LHON, and is occasionally heteroplasmic (Huoponen *et al.*, 1993). The third primary cause of LHON is a mutation in the ND6 gene at np 14484 (MTND6*LHON14484C) (Johns *et al.*, 1992a; Mackey and Howell, 1992). This mutation changes a weakly conserved methionine at codon 64 to a valine. This mutation is most commonly found on a limited number of mtDNA haplotypes in association with specific secondary LHON mutations (MTND5* LHON13708A, MTCYB*LHON15257A, MTND1* LHON3394C) (Brown *et al.*, 1992a,b). The fourth LHON mutation occurs in cytochrome *b* at np 15257 (MTCYB*LHON15257A) (Brown *et al.*, 1992a; Johns and Neufeld, 1991). This mutation converts a highly conserved aspartate to an asparagine, is invariably homoplasmic, and has consistently been found on the same mtDNA lineage together with MTND5* LHON13708A and MTND6*LHON14484A (Brown *et al.*, 1992a).

Two tRNA mutations are representative of the more severe mutations of this class of mtDNA disease. The first mutation occurs at np 8344 in the $tRNA^{Lys}$ gene and causes myoclonic epilepsy and ragged red fiber disease (MERRF) (MTTK* MERRF8344A) (Wallace *et al.*, 1988; Shoffner *et al.*, 1990). Heteroplasmic patients with different percentages of mutant mtDNAs can have a wide range of symptoms from normal, through hearing loss and mild myopathy, to uncontrolled jerking and multiple system failure. The severity of the symptoms is a function of the percentage of mutant mtDNAs the individual inherits and his/her age, with older individuals developing progressively worsening disease (Wallace *et al.*, 1994a). The second mutation alters np 3243 in the $tRNA^{Leu(UUR)}$ and causes mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (Goto *et al.*, 1990). This heteroplasmic mutation can present as adult onset diabetes (Van den Ouweland *et al.*, 1992), cardiomyopathy (Corral-Debrinski *et al.*, 1991), MELAS, or ocular myopathy (Goto *et al.*, 1990).

Severely Deleterious Mutations

The severe mtDNA mutations generally result in lethal childhood disease as they approach homoplasmic. Because of their reduced fitness, maternal lineages harboring these mutations rapidly die out. Consequently, each family represents a new, heteroplasmic, mutation on a different mtDNA haplotype.

A mutation in ND6 at np 14459 (MTND6* LDYT14459A) is at the mild end of this group of mutations. This missense mutation converts a moderately conserved alanine to a valine, eight codons from the np 14484 mutations and in the most highly conserved region of this protein. As the mutation approaches homoplasmy, it causes pediatric dystonia associated with bilateral basal ganglia degeneration. Individuals with a lower percentage of mutant mtDNAs present with LHON (Jun *et al.*, 1994).

A pair of more severe missense mutations occurs in the ATPase 6 gene at np 8993 (MTATP6* NARP8993 G or C). These mutations convert the highly evolutionarily conserved leucine at position 156 to either an arginine or a proline (Holt *et al.*, 1990; De Vries *et al.*, 1993). Both mutations produce a range of clinical manifestations from mild retinitis pigmentosa; through mental retardation, macular degeneration, and olivopontocerebellar atrophy; to lethal Leigh's disease (Tatuch *et al.*, 1992; Shoffner *et al.*, 1992; Ortiz *et al.*, 1993).

Several severe tRNA mutations have also been reported. The $tRNA^{Leu(UUR)}$ gene mutation at np 3302 (MTTL1*MM3302G) alters a nucleotide at the end of the amino acid acceptor stem and inhibits the processing of the tRNA in muscle (Bindhoff *et al.*, 1993; Shoffner *et al.*, 1993b). A mutation in $tRNA^{Pro}$ at np 15990 (MTTP*MM15990A) converts the proline anticodon (UGG) to serine (UGA) (Moraes *et al.*, 1993).

MITOCHONDRIAL DNA REARRANGEMENTS

A wide spectrum of mtDNA rearrangements has also been identified. MtDNA rearrangements can involve deletions, duplications, or a combination of the two with a common breakpoint (Poulton *et al.*, 1993). To date, all rearrangements have been heteroplasmic, and deletions which come to predominate retain both O_H and O_L, permitting their replication, and thus map in two arcs delineated by the origins (Wallace, 1992a).

Three phenotypes have been shown to be caused by mtDNA rearrangements, diabetes and deafness (Ballinger *et al.*, 1992b), ocular myopathy (Moraes *et al.*, 1989), and Pearson's syndrome (Rötig *et al.*, 1988,

1989). Diabetes and deafness patients are frequently members of maternally inherited pedigrees, while ocular myopathy and Pearson's syndrome patients are generally isolated cases resulting from new mutations.

Maternally transmitted, adult-onset, diabetes mellitus and deafness is the mildest clinical phenotype that has been associated with mtDNA rearrangements (Ballinger *et al.*, 1992). Molecular analysis of the mtDNAs in two pedigrees has shown that one involves the maternal transmission of normal plus a partially duplicated mtDNA (Dunbar *et al.*, 1993) while the other involves a trimolecular heteroplasmy of normal, deleted, and duplicated molecules (Ballinger *et al.*, 1994). In the trimolecular heteroplasmy, the deleted and duplicated molecules have the same breakpoint junction and hence must be interrelated, with the proportion of the three molecules differing between tissues and individuals (Ballinger *et al.*, 1994).

Detailed physiological analysis of several members of the trimolecular heteroplasmic pedigree has shown that all have significant skeletal muscle OXPHOS defects, but without the mitochondrial myopathy commonly seen in other mtDNA rearrangement syndromes. The systemic OXPHOS defect is inherited together with an inability to respond to hyperglycemia with increased insulin production, even though insulin production during euglycemia can be normal (Ballinger *et al.*, 1994). This defect in the "glucose sensor" is also seen in maturity-onset diabetes of the young (MODY) which results from mutations in the pancreatic islet cell glucokinase gene. Islet cell glucokinase has a higher K_m than other cellular hexokinases and hence is only active during hyperglycemia (Stoffell *et al.*, 1992; Gidh-Jarn *et al.*, 1993; German, 1993). Since most islet glucokinase is attached to the mitochondrial outer membrane by porin and porin interacts with the ANT of the inner membrane (Malaisse-Lagae and Malaisse, 1988; Adams *et al.*, 1991), it appears that glucose sensing involves the linkage between glucokinase and OXPHOS.

The ocular myopathies are much more severe and minimally present with ophthalmoplegia (paralysis of the eye muscles), ptosis (droopy eye lids), and mitochondrial myopathy which involves the degeneration of muscle fibers, accumulation of abnormal mitochondria, and staining of the aggregated mitochondrial red by Gomori-modified trichrome (ragged red fibers, RRF). Milder forms of this class of disease are designated chronic progressive external ophthalmoplegia (CPEO), while severe forms involving multisymptom failure are called the Kearn-Sayre syndrome (KSS) (Wallace, 1992b). Ocular myopathy patients show a progressive accumulation of rearranged mtDNAs in skeletal muscle over time (Larsson *et al.*, 1990), with the mutant mtDNAs accumulating in discrete regions along the muscle fibers (Mita *et al.*, 1989; Shoubridge *et al.*, 1990) as if selectively amplified in these areas.

The molecular basis for the selective amplification of the deleted mtDNAs in muscle cells is unknown. However, since all of the replication proteins and most of the mitochondrial structural proteins are encoded by the nucleus, it most likely involves modulation of nuclear gene expression in response to a mitochondrially generated signal. One such signal could be the cytosolic concentration of NADH. Cells need to increase their mitochondrial number under either highly oxidizing or highly reducing conditions. Promoter analysis of several nuclear encoded OXPHOS genes has recently revealed a pair of positive, overlapping, cis-control elements, the OXBOX and REBOX, which could have this function. The OXBOX enhances OXPHOS gene expression in muscle differentiation, while the REBOX appears to modulate nuclear OXPHOS gene expression in response to environmental changes. In gel shift experiments, the REBOX factor was found to bind under both highly oxidizing (no NADH) and highly reducing (15 mM NADH) conditions, but not to bind at the more physiological intermediate concentrations (Chung *et al.*, 1993). The increased NADH which accumulates in the vicinity of the mutant mitochondria could activate the REBOX binding protein, thus stimulating the transcription of nuclear OXPHOS and mitochondrial biosynthetic genes. This compensatory response would cause the preferential expansion of the mutant mtDNAs, further exacerbating the respiratory defect.

The Pearson marrow/pancreas syndrome is the most severe of the mtDNA rearrangement syndromes. It is associated with pancytopenia in children resulting from the accumulation of rearranged mtDNAs in the bone marrow precursor cells (Wallace, 1992).

MITOCHONDRIAL DNA MUTATIONS IN SOMATIC CELLS

MtDNA mutations also arise in somatic cells, but since they occur in quiescent tissues they cannot

be diluted out by replicative segregation. Consequently, a heterogeneous array of somatic mutations accumulates throughout the life of the individual, in parallel with the decline in tissue OXPHOS (Trounce *et al.*, 1989; Yen *et al.*, 1989; Cooper *et al.*, 1992) and may provide an aging clock which accentuates inherited OXPHOS defects. MtDNA rearrangements (Cortopassi and Arnheim, 1990; Corral-Debrinski *et al.*, 1991; Cortopassi *et al.*, 1992; Zhang *et al.*, 1992) and base substitutions (Munscher *et al.*, 1993) have been detected in somatic tissues, with the highest levels of mtDNA damage accumulating in the basal ganglia and cortical regions of the brain (Corral-Debrinski *et al.*, 1992a, Soong *et al.*, 1992). MtDNA mutations also accumulate in skeletal muscle (Simonetti *et al.*, 1992), heart (Corral-Debrinski *et al.*, 1991, 1992b; Hayakawa *et al.*, 1992), and other tissues (Cortopassi *et al.*, 1992).

MtDNA damage appears to accumulate progressively with age (Corral-Debrinski *et al.*, 1992b). This suggests that the mutant mtDNAs may proliferate, possibly due to the same mechanism that causes the regional accumulation of deleted mtDNAs in the ocular myopathies.

NUCLEAR–CYTOPLASMIC INTERACTIONS

While the high mutation rate and vital genes of the mtDNA mean that it plays an important role in mitochondrial disease, most of the genes for mitochondrial functions are encoded by the nucleus. These include most of the polypeptides for OXPHOS, as well as the genes for mitochondrial metabolism and biogenesis. Hence, we can expect that nuclear mutations will play an important role in bioenergetic disease.

Several diseases have already been identified in which nuclear mutations impact on mtDNA biogenesis. In one set of families a nuclear, autosomal dominant mutation results in the accumulation of multiple mtDNA deletions in muscle. These mtDNA deletions, in turn, reduce muscle energy metabolism, causing mitochondrial myopathy (Zeviani *et al.*, 1989, 1990). Presumably, this disease results from a mutation in a nuclear gene involved in mtDNA metabolism. In a second set of pedigrees, the affected individuals show a substantial depletion in the amount of mtDNA in specific tissues, with associated tissue-specific pathology. In one pedigree, two affected individuals were related through a male (Moraes *et al.*, 1991; Otsuka *et al.*, 1990; Tritscher *et al.*, 1992). Hence, this disease is most likely due to a variable-penetrance, autosomal dominant mutation in a gene which regulates mtDNA copy number.

Nuclear mutations which affect mitochondrial biogenesis are only one group of mutations that can be expected to affect mitochondrial bioenergetics. Virtually any nuclear mutation that alters a pathway in intermediate metabolism which interfaces with the mitochondria could also interact with OXPHOS gene polymorphisms and lead to disease. The fact that both glucokinase gene and mtDNA mutations can result in adult-onset diabetes mellitus gives an indication of the complex interactions that will be found as more diseases of energetics become understood at the biochemical and molecular level.

A BIOENERGETIC HYPOTHESIS FOR DEGENERATIVE DISEASES AND AGING

These observations suggest a hypothesis that might explain a number of the perplexing features of degenerative diseases and aging.

It is proposed that an individual's inherited OXPHOS genotype interacts synergistically with lifelong acquired somatic mtDNA mutations to define the energetic capacity of his tissues and organs. The inherited genotype defines the initial energetic capacity of the individual, while the accumulated somatic mutations erode this capacity throughout life until the energy output of the cells falls below the minimum energetic threshold necessary for normal tissue and organ function (Bandy and Davidson, 1990; Harman, 1972; Linnane *et al.*, 1989; Miguel *et al.*, 1980; Wallace, 1992a,b).

Individuals who inherit robust OXPHOS genotypes maintain adequate energetic capacities until late in life, manifesting symptoms only in old age. Individuals who inherit mildly deleterious mutations develop late-onset diseases like AD and PD; individuals who inherit moderately deleterious mutations manifest adult-onset diseases like Type II diabetes, deafness, or blindness; while individuals who inherit severely deleterious mutations manifest debilitating pediatric-onset myopathies, dystonias, and Leigh's syndrome. Thus, aging and the common degenerative diseases are envisioned to result from the same process, energetic decline. The nature of the symptoms and the age of onset are defined by the inherited array of energy gene alleles, but the onset of symptoms and the subsequent progression of the disease are determined by the accumulation of somatic mutations (Wallace, 1992a,b).

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