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Review

Mitochondrial DNA sequence variation in human evolution and disease

(mutations/human origins/Leber hereditary optic neuropathy/Alzheimer and Parkinson diseases/aging)

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ABSTRACT Germ-line and somatic mtDNA mutations are hypothesized to act together to shape our history and our health. Germ-line mtDNA mutations, both ancient and recent, have been associated with a variety of degenerative diseases. Mildly to moderately deleterious germ-line mutations, like neutral polymorphisms, have become established in the distant past through genetic drift but now may predispose certain individuals to late-onset degenerative diseases. As an example, a homoplasmic, Caucasian, $tRNA^{Gln}$ mutation at nucleotide pair (np) 4336 has been observed in 5% of Alzheimer disease and Parkinson disease patients and may contribute to the multifactorial etiology of these diseases. Moderately to severely deleterious germ-line mutations, on the other hand, appear repeatedly but are eliminated by selection. Hence, all extant mutations of this class are recent and associated with more devastating diseases of young adults and children. Representative of these mutations is a heteroplasmic mutation in *MTND6* at np 14459 whose clinical presentations range from adult-onset blindness to pediatric dystonia and basal ganglial degeneration. To the inherited mutations are added somatic mtDNA mutations which accumulate in random arrays within stable tissues. These mutations provide a molecular clock that measures our age and may cause a progressive decline in tissue energy output that could precipitate the onset of degenerative diseases in individuals harboring inherited deleterious mutations.

The human mtDNA sequence (Fig. 1) (1, 2) has a very high mutation rate, at least 10 times that of nuclear genes (1, 3, 4). Since mutations are random, any base in the mitochondrial genome, coding or noncoding, can change. Moreover, since every cell in the body harbors hundreds of mitochondria and thousands of mtDNAs, deleterious mtDNA mutations can occur in all human tissues, somatic and germline. However, the implications of the different types of mtDNA mutations and of their distribution between cells and tissues can be very different. Mutations which arise in the somatic tissues degrade cellular energy production but die with the individual. Mutations which arise in the female germ line are transmitted to the next generation, where they can be observed as new mtDNA polymorphisms or as devastating mtDNA disease. Thus, for somatic mtDNA mutations which are numerous, it is the quantitative differences between tissues that affects health, while for germ-line mtDNA mutations which are rare it is the qualitative nature of the mutation which is important for phenotype. It is the diversity of the germ-line mtDNA mutations and the diseases which they cause that is the focus of this review.

mtDNA Genetics

The human mtDNA is a closed circular molecule of 16,569 nucleotide pairs (np) which encodes a small (12S) and large (16S) rRNA, 22 tRNAs, and 13 polypeptides. All of the mtDNA-encoded polypeptides are subunits of the mitochondrial energy-generating pathway, oxidative phosphorylation. Oxidative phosphorylation encompasses five multisubunit enzyme complexes (I to V), arrayed within the mitochondrial inner membrane. Complex I (NADH:ubiquinone oxidoreductase or NADH dehydrogenase, EC 1.6.5.3) removes electrons from NADH while complex II (succinate:ubiquinone oxidoreductase, EC 1.3.5.1) collects electrons from succinate. Both enzymes then transport the electrons to coenzyme Q (CoQ). From CoQ, the electrons flow through complex III (ubiquinol:ferricytochrome-*c* oxidoreductase, EC 1.10.2.2) to cytochrome *c*, then to complex IV (ferrocytochrome-*c*:oxygen oxidoreductase or cytochrome-*c* oxidase, EC 1.9.3.1), and finally to oxygen to yield water. The energy that is released by this electron transport chain is used to pump protons out through the mitochondrial inner membrane, creating an electrochemical gradient. This gradient is utilized by complex V (H^+-translocating ATP synthase, EC 3.6.1.34) as a source of potential energy to condense ADP and P_i to make ATP. ATP and ADP are then exchanged across the mitochondrial inner membrane by the adenine nucleotide translocator (ANT) (1–3).

Complex I encompasses more that 40 polypeptides, seven (MTND1, -2, -3, -4, -4L, -5, and -6) from the mtDNA; complex II involves four nuclear polypeptides; complex III includes 11 polypeptides, one (MTCYB) from the mtDNA; complex IV encompasses 13 polypeptides, three (MTCO1, -2, and -3) from the mtDNA; and complex V involves at least 13 polypeptides, two (MTATP6 and -8) from the mtDNA. ANT is encoded by nuclear genes. The mtDNA rRNA and tRNA genes provide the structural RNAs for mitochondrial protein synthesis, which are required for the expression of the 13 mtDNA polypeptides (1, 3).

The mtDNA is replicated and transcribed by using an origin and a promoter for each of the two DNA strands, the G-rich heavy (H) strand and the C-rich light (L) strand. The H- and L-strand origins (O_H and O_L) are separated by 2/3 of the molecule, but the H- and L-strand promoters (P_H and P_L) are located adjacent to O_H in the approximately 1000-np noncoding control region, which also encompasses the triple-stranded D-loop (1, 2).

Most human cells contain hundreds of mitochondria and thousands of mtDNAs. Since the mtDNA is predominately transmitted through the oocyte cytoplasm, it is maternally inherited (5). As a consequence, maternal and paternal mtDNAs rarely mix in the same cytoplasm, and no recombination has been detected between different mtDNA lineages (6, 7). Thus, the only way that the mtDNA sequence can change is by the sequential accumulation of mutations along radiating maternal lineages.

The high mtDNA sequence evolution rate (E) is the product of both a high mutation rate (μ) and a high mutation

Abbreviations: np, nucleotide pair; LHON, Leber hereditary optic neuropathy; AD, Alzheimer disease; PD, Parkinson disease; MERRF, myoclonic epilepsy and ragged red fibers; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; LDYT, LHON and dystonia; NARP, neurogenic muscle weakness, ataxia, and retinitus pigmentosa and also Leigh syndrome; DEAF, deafness; MM, mitochondrial myopathy; MMC, MM and cardiomyopathy.

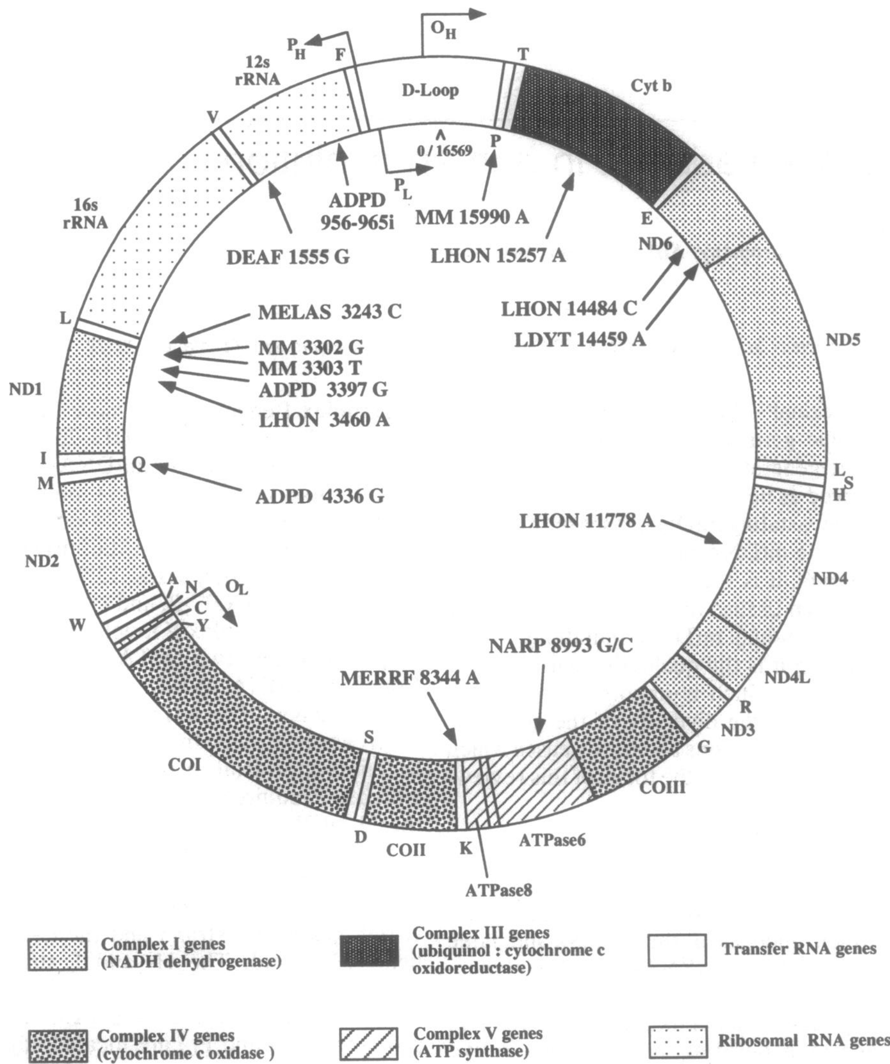

FIG. 1. Human mtDNA map showing locations of genes and mutations. Definitions of gene symbols and mutations are presented in the abbreviations footnote, the text, and refs. 1–3.

fixation rate (F), $E = \mu F$. The high mutation rate results in part from the mtDNA's lack of protective histones, inefficient DNA repair systems, and continuous exposure to the mutagenic effects of the oxygen radicals generated by oxidative phosphorylation (1, 3). The high mutation fixation rate (F) is due to the efficient intracellular sorting of mutant molecules in the female germ line and the rapid genetic drift of mtDNAs in the general population.

When a mtDNA mutation arises, it creates an intracellular mixture of mutant and normal molecules called heteroplasmy. Subsequently, as the mutant and normal mtDNAs are randomly distributed into daughter cells during mitotic or meiotic replication, the percentage of mutant and normal molecules drifts within the cell, toward either pure mutant or pure normal (homoplasmy), a process known as replicative segregation. Mitotic replicative segregation requires multiple cell divisions to approach homoplasmy, but meiotic replicative segregation can be quite rapid, with the proportion of mutant molecules changing from predominantly normal to essentially mutant in one or two generations (1, 3). This rapid germ-line segregation has been attributed to the rapid sorting of mitochondrial genomes within the female germ line during early embryogenesis. At fertilization, the oocyte contains approximately 200,000 mtDNAs, one per mitochondrion. Once fertilized, the nuclear DNA replicates and the oocyte cleaves, but the mtDNA does not replicate until after the blastocyst is formed. Since the blastocyst cells that are destined to become the female germ line sequester only a small number of the oocyte's mtDNAs, a large sampling error occurs (8). In most cases, only normal mtDNAs are sequestered in the germ-line progenitor cells. However, occasionally a mutant mtDNA is acquired, resulting in a large increase in the proportion of mutant mtDNAs in the oocytes of the next generation. Once a mutant mtDNA comes to predominate in the germ line, the next generation's mtDNA genotype can shift to virtually pure mutant (1, 3).

For a heteroplasmic mtDNA mutation, as the percentage of mutant molecules increases, the oxidative phosphorylation enzyme specific activities decrease until they fall below the cellular or tissue energetic threshold (1, 3). The cellular energetic threshold is that degree of inhibition of the respiratory complexes below which the overall efficiency of oxidative phosphorylation is impaired (9). For rRNA and tRNA mutations, the energetic thresholds can be quite precipitous (10). For example, the respiratory capacity of cells heteroplasmic for the *MTTK**-MERRF8344A and the *MTTL1**MELAS-3243C mutations[†] remain near normal until about 90% of the mtDNAs are mutant. Above 90%, oxidative phosphorylation drops off rapidly, reaching near zero above 95% mutant (11, 12). Once the proportion of mutant mtDNAs exceeds the cellular energetic threshold, the energetic capacities of the tissues and organs of the individual decline rapidly. Different tissues and organs rely on mitochondrial energy to different extents, in decreasing order of the central nervous system, heart, muscle, kidney, endocrine systems, and liver. Therefore, as the mitochondrial energy-generating capacity of the cell declines, the number and severity of the symptoms increases (1, 3). Organ energetic thresholds, like the cellular energy thresholds, are quite steep. In a family harboring the *MTTK**MERRF-8344A mutation, a 20- to 30-year-old with 95% mutant mtDNAs was severely affected, while a like-aged cousin with 85% mutant mtDNAs was clinically and biochemically normal (13, 14). This clinical threshold phenomenon, together with the high mtDNA segregation rate, can result in extreme variability in clinical symptoms of individuals within the same family. Moreover, for individuals whose mitochondrial genotypes are close to cellular and organ expression thresholds, small increases in the percentage of mutant mtDNAs can have large effects on clinical phenotypes. In these cases, small age-related accumulations of somatic mtDNA mutations may be sufficient to push the mtDNA genotype beyond the expression threshold, resulting in clinical failure (14, 15).

Somatic mtDNA Mutations

The capacity for oxidative phosphorylation has been shown to decline with age in a variety of postmitotic somatic tissues (16–18). This decline is paralleled by the age-related accumulation of somatic mtDNA mutations, both deletions (19–30) and point mutations (31). When quan-

[†]mtDNA disease mutation nomenclature. Example: *MTTK**MERRF8344A. MTTK is the altered mtDNA (MT) gene for $tRNA^{Lys}$ (TK), MERRF is the most characteristic clinical presentation, 8344 is the altered nucleotide, and A is the pathogenic base.

titated by using the common 5-kb mtDNA deletion, somatic mtDNA mutations are found to accumulate to their highest levels in the basal ganglia of the brain and next-highest levels in the various cortical regions. However, mtDNA deletions do not accumulate in the cerebellum (24, 25). mtDNA mutations also accumulate in skeletal muscle (30), in heart (22, 26), and in other tissues (23). These mutations are probably caused predominantly by oxygen radical damage, since 8-hydroxyguanosine levels are high in the mtDNAs of older individuals (32, 33), and mtDNA damage is greatly increased in ischemic heart disease, where cyclic ischemia and reperfusion stimulates oxygen radical production (22, 26). Since all forms of mtDNA damage that have been examined have been found to accumulate with age (22, 24, 26), and the 5-kb deletion reaches over 10% in the basal ganglia of an 85-year-old individual (26), it is likely that much of the mtDNA in the basal ganglia of the elderly is damaged (Table 1) (1, 3).

Assuming that the physiological effects of inherited and somatic mtDNA mutations are additive, the milder the inherited mitochondrial defect, the more somatic mtDNA damage that would be required before symptoms appear. For individuals born with normal or mildly deleterious mitochondrial genotypes, years of accumulated somatic mtDNA damage would be required before organ function would decline. However, for individuals inheriting moderately deleterious mutations, substantially less somatic mtDNA damage would be necessary for clinical symptoms to appear (Table 1) (1, 3).

Germ-Line mtDNA Mutations

While somatic mutations may determine the time and progression of mitochondrial disease, inherited mtDNA mutations define the nature and severity of the clinical manifestations. Germ-line genetic variation is the product of both ancient and modern mutations, which have been acted on by both genetic drift and organismal selection. Neutral mutations that arise in the general population are usually lost due to the chance effects of drift. Only rarely does genetic drift result in a mutation that reaches polymorphic frequencies. Hence most of the polymorphic mutations that exist in the general population today occurred long ago and have long since segregated to homoplasmy. By contrast, severely deleterious mutations are rapidly eliminated by selection in the form of genetic disease, and individuals harboring such mutations are quickly brought to the attention of the clinician. Therefore, mtDNA mutations that cause severe disease are generally new heteroplasmic mutations. Between these two extremes are the mild to moderately deleterious mutations which do not significantly decrease reproductive fitness. These mutations can become established in a population by drift, but individuals harboring these variants have slightly reduced energetic capacity, rendering them subject to premature degenerative disease.

Neutral mtDNA Variants

A high degree of mtDNA sequence variation exists between geographically separated populations. Initially detected by restriction fragment length polymorphisms (RFLPs) (34), much of this variation is selectively neutral, involving nucleotide substitutions in third-codon positions and in noncoding regions such as the control region. mtDNA sequence variation was first shown to correlate with the ethnic and geographic origin of the subjects by analyzing the *Hpa* I RFLPs from samples collected from Europe, Asia, and Africa (35). A four-fragment African *Hpa* I pattern (morph 3) was present in over 95% of !Kung Bushmen and Pygmies and 75% of Bantus, but was not present in nonadmixed European or Asian samples. A three-fragment Eurasian *Hpa* I variant (morph 2) differs from morph 3 by loss of one restriction site and was present in essentially 100% of Europeans and the majority of Asians. A two-fragment Asian *Hpa* I pattern (morph 1) resulted from the loss of a second *Hpa* I site and was present in various frequencies in Asian populations from a few percent in China to 30% in Vietnam (36, 37). The sequential nucleotide changes resulting in the *Hpa* I site losses, together with the maternal inheritance of the mtDNA, suggest that each of the new mutations became fixed along radiating female lineages as women migrated out of Africa and became dispersed among the various continents (6, 38, 39).

Analysis of restriction site changes for the highly polymorphic enzymes *Ava* II, *Bam*HI, *Hae* II, *Hpa* I, *Hha* I, and *Msp* I in 3065 mtDNAs from 62 populations throughout the world has revealed several basic principles about the evolution of human mtDNA diversity. First, mtDNA mutations accumulate sequentially along radiating maternal lineages. Second, mtDNA variation correlates highly with ethnic and geographic origin of the individual. Third, individual restriction site variants are either always associated or never associated, indicating that mtDNAs rarely if ever recombine. Fourth, there is only one mtDNA tree for humans, whose branches radiate out into the continents, implying that there was a single origin for *Homo sapiens*. Fifth, the mtDNA sequence diversity within individual populations is highest in African populations, next highest in Asian and European populations, and lowest in Native American populations. Assuming that mtDNA mutations and thus sequence diversity accumulate at a relatively constant rate, this implies that *Homo sapiens* originated in Africa, migrated through Asia and Europe, and recently arrived in the Americas (6).

These conclusions have been confirmed and extended by examining a greater proportion of the mtDNA sequence, using high-density restriction analysis and control region sequencing. For these analyses, the mtDNAs of subjects are amplified in nine overlapping segments by using the polymerase chain reaction (PCR). The PCR fragments are then digested with 14 restriction endonucleases, surveying approximately 20% of the mtDNA sequence. The hypervariable regions of the D-loop are also sequenced. The interrelationships between the mtDNA haplotypes (the aggregate of all nucleotide variants in a mtDNA) are then determined by generating sequential mu-

Table 1. mtDNA mutations in human evolution, aging, and disease

	Inherited mtDNA mutation			
Characteristics at time of analysis	Neutral	Mildly deleterious	Moderately deleterious	Severely deleterious
Evolutionary constraints	None	Low	Moderate	High
Maintenance in population (>5 generations)	Yes	Yes	No	No
Number of associated haplotypes	Few	Few	Many	Many
Ethic correlation	Yes	Yes	No	No
Heteroplasmy	No	No	Yes/no	Yes
Clinical symptoms	Aging	Stereotyped disease	Variable disease	Multisystem disease
Onset of debilitating symptoms	Old age	Late middle age	Young adult	Childhood
Progression due to somatic mtDNA mutations	Gradual	Slow	Moderate	Rapid

tational phylogenies by parsimony analysis (PAUP) and by calculating interhaplotype and interpopulation genetic distances (2, 37, 40–44). Such studies have revealed an ancient mtDNA polymorphism, the gain/loss of a *Dde* I restriction site at np 10394, which subdivides the mtDNAs of all continents and thus predates the radiation of the races. Most African mtDNAs, including the oldest lineages, have the *Dde* I site, while a limited number of Africans lack the site (2). European, Asian, and Native American mtDNAs are approximately equally subdivided by this ancient polymorphism (37, 41–43). Beyond this common variant, most mtDNAs from Africa, Europe, Asia, and America contain continent-specific restriction site variants. For Africa, about 75% of mtDNAs are uniquely defined by the *Hpa* I morph 3 restriction site at np 3592 (2, 35, 38). In Europe, over 68% of the mtDNAs are contained within four European-specific mtDNA lineages defined by novel restriction site variants. The first European lineage lacks the *Dde* I site at np 10394 and is defined by an *Alu* I site loss at np 7025. The remaining three lineages have the *Dde* I np 10394 site. Among these, the second European lineage is delineated by a set of five restriction site changes, a *Dde* I site loss at np 1715, an *Alu* I site gain at np 10028, a *Hae* II site loss at np 4529, an *Ava* II site gain at np 8249, and a *Bam*HI/*Mbo* I site gain at np 16389. The third lineage is defined by two site losses, *Bst*NI at np 13704 and *Rsa* I at np 16065. The fourth lineage is delineated by a combined *Hae* II np 9052/*Hha* I np 9053 site loss (44). The Asian mtDNAs are distributed among a wide variety of lineages, all of which are subdivided into two major groups by the *Dde* I site at np 10394 and an adjacent *Alu* I site at np 10397 (37, 43). Important Asian-specific sublineages which have the *Dde* I and *Alu* I sites are haplogroup C, defined by a combined *Hin*cII site loss at np 13259 (45) and an *Alu* I site gain at np 13262, and haplogroup D, defined by an *Alu* I site loss at np 5176 (2, 43). Key Asian sublineages which lack the *Dde* I and *Alu* I sites include haplogroup F, delineated by a combined *Hpa* I/*Hin*cII site loss at np 12406 (*Hpa* I morph 1) plus a *Hae* III site gain at np 16517 (36, 37); haplogroup A, defined by a *Hae* III site gain at np 663; and haplogroup B (formerly D), defined by a 9-np intergenic deletion between *MTTK* and *MTCO2* (np 8271 and 8281) (2, 46) and a *Hae* III site at np 16517 (30, 37, 41). The haplogroup B 9-np deletion appears to have originated in southeastern China and spread along the Asian coast, ultimately migrating out onto the Pacific Islands, where its frequency increases towards fixation (37, 47, 48).

Single founding haplotypes of only four Asian haplogroups (A, B, C, and D) appear to have successfully migrated from Asia to the Americas with the first immigrants, the Paleoindians. Virtually all modern Native American mtDNA variation radiates from these four founding haplotypes (7, 40, 41, 45). Three of the haplogroups (A, C, and D) are also found at high frequencies in eastern Siberia, with their proportion becoming progressively enriched from south to northeast. The fourth haplogroup (B) has not been observed in Siberia (42). Comparison of the mtDNA variants in Native Americans versus Asians plus Siberians indicates that the continents share the founding haplotypes of A, B, C, and D, but all additional American mtDNA variation is specific to the Americas (41, 42). This implies that the American intrahaplogroup sequence variation originated after Native Americans and Siberians became separated, and thus is proportional to the time since those haplogroups arrived in the Americas. The sequence diversities of American haplogroups A, C, and D range from 0.053 to 0.096, but the diversity of haplogroup B is only 0.024 (41). Thus it seems that the Asian population expanded northeast relatively rapidly, with haplogroups A, C, and D becoming progressively enriched until individuals carrying only the founding haplotypes crossed the land bridge. The Siberian and American populations then became separated as the land bridge submerged, leaving the Siberian and American mtDNAs to radiate independently of each other. Much later, the founding haplotype of haplogroup B arrived, possibly by the coastal-maritime route. Descendants of haplogroup B subsequently dispersed among the A, C, and D populations of North, Central, and South America.

To estimate the times of these migrations, we determined the sequence evolution rate of Native American mtDNAs from the Chibcha speakers of Panama, for which independent estimates of their age are available. From this tribe, the mtDNA sequence divergence rate was found to be 2.2–2.9% per million years, indicating that haplogroups A, C, and D arrived between 22,000 and 29,000 years before the present and haplogroup B arrived between 8000 and 11,000 years before the present (49).

Later Asian immigrants founded the Na-Dene and Eskaleut linguistic groups. The Na-Dene of northwestern Canada and Alaska contain only mtDNAs of haplogroup A, with one-third of the mtDNAs having undergone a distinctive *Rsa* I site loss at np 16329. The overall diversity of the Na-Dene haplogroup A is 0.021, suggesting that they arrived shortly after the haplogroup B immigrants (40).

While evolutionarily interesting, these ethnic-specific variants appear to be of little importance to individual fitness and longevity. However, they are extremely numerous. Therefore, special care must be exercised to avoid accidentally attributing a pathological role to an ethnic-specific variant. An effective method for distinguishing a recent disease mutation from background polymorphisms in sequenced patient mtDNAs is to determine the phylogenetic relationship between the patient's haplotype and the world mtDNA phylogeny. This defines the control mtDNAs which are most closely related to the patient mtDNAs. A sequence variant found in the patient that is not present in the related controls must then be a recent mutation and hence a possible cause of the disease. The value of this approach is demonstrated in our recent article describing the identification of a mtDNA mutation for dystonia, *MTND6**-LDYT14459A (50).

Mildly Deleterious mtDNA Mutations

While many of the ethnic-specific mtDNA base substitutions do not change known genetic functions, others, such as the Asian *Hpa* I morph 1 variant at np 12406, alter moderately conserved amino acids (36). Since these variants have become established in their respective ethnic groups by drift, they must not substantially reduce reproductive fitness. However, it is possible that some of these variants cause subclinical reductions in oxidative phosphorylation capacity which, when exacerbated by the accumulation of somatic mtDNA mutations, can lead to organ failure in old age.

Evidence in support of this hypothesis comes from the extensive data on the mtDNA mutations that cause Leber hereditary optic neuropathy (LHON). LHON is a maternally inherited form of late-onset acute or subacute bilateral central vision loss leading to scotoma and blindness. Sixteen mtDNA mutations have been observed in association with LHON, and these mutations vary in their predilection to cause blindness and in whether the mutation is ancient or recent.

Current evidence suggests that 5 of the 16 LHON mutations are primary causes of LHON, though these 5 differ in their propensity for causing the disease. Listed in order from lowest to highest disease-causing potential, the primary mutations are *MTCYB**LHON15257A (51, 52), *MTND6**LHON14484C (53, 54), *MTND1**LHON3460A(55–57), *MTND5**-LHON11778A (58), and *MTND6**LDYT-14459A (50). Three additional mutants may also be primary, but require further characterization. These are *MTND5**-LHON13730A (57), *MTCO2**LHON-9438A, and *MTCO3**LHON9804A (59). Eight other mutations have been found at increased frequency among LHON patients (1–3). Generally, however, these occur in conjunction with one of the primary mutations, and they are assumed to be secondary variants which may act

synergistically to increase the severity of the oxidative phosphorylation defect and the probability of blindness (1–3). Of these synergistic mutations, the four most important are *MTND5**LHON13708A (52, 60), *MTND1**LHON3394C (61), *MTCO1**LHON7444A (62), and *MTND1**LHON4160 (63).

The criteria used for ranking the primary mutations from mild to severe include their clinical manifestations, association with specific mtDNA lineages, cooccurrence with secondary LHON mutations, heteroplasmy, amino acid conservation, penetrance, and the potential for spontaneous visual recovery (64–67). The two mildest primary LHON mutations fit into the category of mildly deleterious mutations (Table 1). The mildest of these is *MTCYB**LHON15257A, which is generally associated with LHON, but has also been found in 0.3% of the general population (61). This mutation has been observed on the same mtDNA lineage, usually together with the *MTND5**LHON13708A and *MTND6**LHON14484C mutations, in all but one case (68); is consistently homoplasmic; changes a highly conserved aspartate to an asparagine; has a penetrance in males of 72%; and has a probability of visual recovery of 28% (67). The second LHON mutation, *MTND6**LHON14484C, is significantly more severe. This mutation is also present in a significant percentage of LHON patients, but it has not been found in the general population. It is commonly associated with specific mtDNA lineages, often in association with the *MTND5**LHON13708A, *MTCYB**LHON15257A, or *MTND1**LHON3394C mutations; has been homoplasmic in every case but one (54); changes a weakly conserved methionine to a valine (Table 2); has a penetrance in males of 82%; and has a visual recovery rate of 37% (67). The remaining three primary LHON mutations are more severe (64, 67). Mutations *MTND1**LHON3460A and *MTND4**LHON11778A are moderately deleterious, being associated with LHON but occurring on a variety of different mtDNA lineages. Mutation *MTND6**LDYT14459A is severely deleterious, resulting in childhood-onset dystonia as well as LHON.

The secondary LHON mutations are also relevant to the mildly deleterious mutations. Comparison of the four most important mutations (*MTND5**LHON13708A, *MTND1**LHON3394C, *MTCO1**LHON7444A, and *MTND1**LHON4160) again reveals that they make a spectrum of contributions to the disease process. All of these mutations are consistently homoplasmic and associated with specific mtDNA lineages. The *MTND5**LHON13708A mutation changes a moderately conserved alanine to a threonine but is found in 4% of the normal population as well as in LHON patients (52, 60, 61). The *MTND1**LHON3394C mutation changes a highly conserved histidine to a tyrosine but is found in 1% of the normal population (61). The *MTCO1**LHON7444A mutation converts the termination codon of *MTCO1* to lysine, extending the polypeptide by three charged amino acids and is also found in 1% of the general population (62). Finally, the *MTND1**LHON4160C mutation converts a highly conserved leucine to a proline and has been observed, together with the primary *MTND6**LHON14484C mutation (63), in a family with LHON and pediatric neurodegenerative disease (63, 69). Thus, the *MTND1**LHON4160C mutation may be interacting with the *MTND6**LHON14484C mutation to give a more severe clinical phenotype.

Table 2. Comparisons between *MTND6* LHON mutations and wild-type sequences of various species

Mutant or species	Amino acid sequence: Around conserved residue 72	Amino acid sequence: Around weakly conserved residue 64
	72	64
LHON + dystonia (U.S.)	Y T T V*M A I	V M M
LHON	Y T T A M A I	V V† M
Human	Y T T A M A I	V M M
Cow	Y T T A M A T	V M M
Mouse	Y T T A M A T	V L M
Rat	Y T T A M A T	V L M
Xenopus laevis	Y S A A R A K	V L M
Sea urchin (*Strongylocentrotus purpuratus*)	Y S S A I S –	V L M
Chicken	Y S V S L A A	V L M
Quail (*Coturnix japonica*)	Y S V S L A A	V L M
Fruit fly (*Drosophila yakuba*)	Y V T S L A S	V L M
Caenorhabditis elegans	Y F S S L S K	V F I
Ascaris suum	Y F S S L S K	V F I

**MTND6* np 14459 mutation. This has been found in 1 LHON + dystonia (U.S.) family and in 0/348 controls (including 38 haplogroup D).

†*MTND6* np 14484 mutation. This has been found in (*i*) >15 LHON families; (*ii*) 1 LHON + dystonia (Australia) family, which also harbors an np 4160 mutation; and (*iii*) 0/250 controls.

Since severe LHON genotypes such as *MTND6**LHON14484C + *MTND1**LHON4160C (63) and *MTND6**LDYT14459A (50) cause early-onset neurodegenerative disease, it follows that much milder mtDNA mutations might predispose individuals to late-onset neurodegenerative diseases. The most common late-onset clinical presentations are the movement disorders [e.g., Parkinson disease (PD)] and dementias [e.g., Alzheimer disease (AD)]. This hypothesis is supported by the repeated reports of oxidative phosphorylation deficiency in tissues from AD and PD patients (70–75) and the recent discovery that a relatively rare European mtDNA tRNA^{Gln} polymorphism at np 4336 (*MTTQ**ADPD4336G) is found in 0.7% of the general population but in 5.2% of late-onset AD and PD patients (76). *MTTQ**ADPD4336G alters a moderately conserved nucleotide at the junction between the amino acid acceptor and TΨC stems, and it defines a European mtDNA lineage in which AD and PD patients cluster. Moreover, patient mtDNAs in this cluster can harbor additional deleterious mtDNA mutations. These include *MTND1**ADPD3397G, which changes a highly conserved methionine to a valine in the ND1 gene and has arisen two independent times in AD and PD patients, and *MTRNR1**ADPD 956–963 ins 5C, an ≈5-np insertion at np 956–965 in the 12S rRNA (76). These observations suggest that the *MTTQ**ADPD4336G mutation may be a risk factor in developing AD and PD in certain individuals, but that other factors such as nuclear gene mutations, environmental stress, additional inherited mtDNA mutations, and the accumulation of somatic mtDNA mutations may interact to drop brain tissues below expression thresholds, resulting in clinical manifestations late in life.

A somewhat similar situation has been encountered for neurosensory hearing loss in a large Arab pedigree with sporadic deafness among maternal relatives. Sequence analysis of the mtDNA of this family revealed a homoplasmic *MTRNR1**DEAF1555G mutation in the 12S rRNA. Proof that this variant contributed to the deafness came from the discovery that unrelated individuals who experienced deafness after aminoglycoside exposure had the same nucleotide substitution. Thus, the rRNA mutation predisposes individuals to deafness, but clinical expression requires additional factors such as modifying nuclear genes or environmental challenges (77).

Moderately Deleterious mtDNA Mutations

As the severity of the mtDNA mutations increases, the frequency and severity of their clinical manifestations increase.

Since more severe mutations have reduced fitness, they are more rapidly eliminated by selection. Hence, severe mutations will be recent, appear on different mtDNA haplotypes, and often be heteroplasmic.

The LHON mutations *MTND1**LHON3460A and *MTND4**LHON11778A fall into the moderately deleterious category. The *MTND1**LHON3460A mutation consistently presents as LHON, has appeared on several mtDNA lineages, occasionally cooccurs with other LHON mutations, is generally homoplasmic, changes a moderately conserved alanine to a threonine, is expressed in 69% of males, and exhibits a 22% spontaneous recovery rate (55, 56, 66, 67). The *MTND4**LHON11778A mutation is by far the most common cause of LHON (58), and it must be more pathogenic, since one individual is reported to have lost his vision at age 37 and then developed cerebellar-extrapyramidal tremor and left-side rigidity associated with bilateral basal ganglial lesions at age 38 (78). This presentation is similar, though milder, than the neurodegenerative disease and dystonia seen in the more severe *MTND6**LHON14484C + *MTND1**LHON4160Cand*MTND6**LDYT14459A genotypes. Additional characteristics of the moderately deleterious *MTND4**LHON11778A mutation are that it has arisen in multiple different mtDNA lineages (79), is only occasionally found with other LHON mutations (66), is frequently heteroplasmic (80), converts a highly conserved arginine to a histidine, is about 82% penetrant in males, and shows only 4% spontaneous recovery (64, 65, 68).

A variety of other moderately deleterious mutations have been identified, mostly in the mtDNA tRNA genes (2). The best studied of these are *MTTK**MERRF8344A (13, 14) and *MTTL1**MELAS3243C (81, 82). The clinical syndrome commonly associated with the np 8344 mutation is myoclonic epilepsy and ragged red fibers (MERRF), while that associated with the np 3243 mutation is mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS). These mutations are consistently heteroplasmic and subject to replicative segregation, so that clinical manifestations vary markedly among maternal relatives. MERRF family phenotypes can range from normal, through hearing loss and mild myopathy, to uncontrolled myoclonic jerking and multiple system failure (13–15). MELAS family phenotypes can include individuals who are normal, have adult-onset diabetes and deafness (83), manifest cardiomyopathy (22), experience stroke-like episodes and myopathy, or have ocular myopathy (81). Occasionally, children harboring either mutation can present with Leigh syndrome, a lethal childhood disease involving bilateral basal ganglial degeneration (84). It is common in both mutations for individuals to be clinically normal during childhood, develop symptoms as adolescents or adults, and then get worse as they age.

The delayed onset and subsequent progression of these diseases has been extensively studied in the MERRF syndrome. In one large MERRF pedigree, eight maternal relatives were thoroughly examined and found to differ at the clinical, biochemical, and molecular levels. These differences could be explained by two variables: the patient's inherited percentage of mutant mtDNAs and the patient's age. For example, the proband with 95% mutant mtDNAs developed symptoms as a teenager which progressed through her twenties into uncontrollable myoclonus, mitochondrial myopathy, neurosensory hearing loss, progressive dementia, cardiomyopathy, and renal failure. Biochemically, she had severe skeletal muscle mitochondrial enzyme defects in complexes I and IV and a lymphocyte mitochondrial protein synthesis defect (13, 85). Her comparable-age cousin with 85% mutant mtDNAs was clinically and biochemically normal, while her 65-year-old aunt, also with 85% mutant mtDNAs, was as severely affected as the proband (13–15, 86).

These observations support the hypothesis that in pedigrees harboring moderately deleterious mutations, maternal relatives remain relatively unaffected until meiotic segregation increases the percentage of mutant mtDNAs to close to cellular and organ energetic thresholds. Then small environmental challenges or slight increases in the percentage of mutant mtDNAs, due to either mitotic segregation or somatic mutation, lower energy outputs below expression thresholds, resulting in the onset and subsequent progression of the disease (1, 3).

Severely Deleterious mtDNA Mutations

Individuals harboring moderately deleterious mtDNA mutations, even at high percentages of mutant mtDNAs, generally function reasonably well through childhood. However, even more deleterious mtDNA mutations have been reported which result in severe, often lethal, childhood disease. Such severely deleterious mutations greatly reduce reproductive fitness and the maternal lineages rapidly die out. Consequently, all independent families are new, heteroplasmic mutations which exhibit striking clinical variation along the maternal lineage due to rapid replicative segregation.

Among the LHON mutations, *MTND6**LDYT14459A is the most severe and fits into the severely deleterious category. This mutation was discovered in a large Hispanic family consisting of 78 family members, 22 of which were affected along the maternal lineage. The clinical manifestations of the maternal relatives varied from normal, through late-onset optic atrophy, to early-onset dystonia accompanied by bilateral basal ganglial degeneration. The proportion of affected individuals and the severity of the disease increased with each successive generation, suggesting the segregation of a heteroplasmic mtDNA mutation (87). Molecular analysis revealed that this family harbored a Native American mtDNA from haplogroup D, and that the only novel mutation was a heteroplasmic G to A transition at np 14459 which converted a conserved alanine to a valine (Table 2) (50). The severity of the *MTND6**LDYT14459A mutation is demonstrated by its associated childhood-onset striatal necrosis and dystonia and by the facts that it is a new mtDNA mutation in Native American clade D and not found in 310 controls, that it is heteroplasmic, and that when approaching homoplasmic it has a penetrance of 48% of maternal relatives manifesting dystonia, 10% LHON, and 3% LHON plus dystonia (87, 88) (Table 1).

While clinical and genetic criteria clearly rank *MTND6**LDYT14459A as the most severe LHON mutation, the amino acid substitution is relatively conservative, converting an alanine found in taxa as diverse as human and sea urchin to a valine (Table 2). This contrasts with the less severe *MTND4**LHON11778A mutation, which changes a highly conserved arginine to a histidine. However, the other primary LHON mutation in the *MTND6* gene, *MTND6**LHON14484C, is even less conserved than *MTND6**LDYT14459A, changing a methionine found in human and cow to a valine (Table 2). Since *MTND6**LHON14484C results only in LHON, while *MTND6**LDYT14459A also causes dystonia, the two *MTND6* mutations are internally consistent, and they imply that mutations in *MTND6* are much more prone to cause blindness than mutations in *MTND4*. MTND6 is one of the less constrained complex I subunits, yet both LHON mutations occur in the most evolutionarily conserved region of the polypeptide (89). Perhaps, MTND6 is uniquely important for the normal function of the human visual pathway and basal ganglia (50).

Severely deleterious missense mutations have also been discovered in the *MTATP6* gene (*MTATP6**NARP8993G or C). One of these heteroplasmic mutations converts a highly conserved leucine to an arginine, while the other converts the leucine to a proline (90, 91). These mutations cause a broad spectrum of clinical presentations ranging from mild peripheral retinitis pigmentosum, through olivoponto-

cerebellar atrophy and macular degeneration, to lethal infantile Leigh syndrome accompanied by basal ganglial lesions (92–95). Biochemical analysis and somatic cell genetic studies have shown that the *MTATP6**NARP8993G mutation blocks the proton channel of the ATP synthase, thus inhibiting ATP generation from oxidative phosphorylation (96, 97).

A number of severely deleterious mtDNA tRNA mutations have also been identified which cause severe childhood disease when approaching homoplasmic. A mutation in the $tRNA^{Leu}_{UUR}$ gene at np 3302 (*MTTL1**MM3302G) alters the nucleotide at the end of the amino acid acceptor stem (98, 99). This mutation causes severe childhood and young adult myopathy and has been proposed to block the muscle-specific processing of the $tRNA^{Leu}_{UUR}$ transcript (99). An adjacent mutation, *MTTL1**MMC3303T, results in lethal childhood cardiomyopathy (100). A mutation in the $tRNA^{Pro}$ gene, *MTTP**MM15990A, converts the proline anticodon (UGG) to serine (UGA), and manifested as classical mitochondrial myopathy in a 7-year-old patient who harbored 85% mutant mtDNA (101).

Conclusions

To date, most studies on mtDNA sequence variation have been motivated by questions about population diversity and origins or by efforts to identify mtDNA mutations that cause maternally inherited diseases. The former studies emphasized neutral mtDNA polymorphisms (e.g., African np 3592 *Hpa* I site), while the latter focused on moderately deleterious mtDNA mutants (e.g., *MTND4**-LHON11778A, *MTTK**MERRF8344G, and *MTTL1**MELAS3243G). However, these studies have overlooked two other important classes of deleterious mtDNA mutations: the mildly deleterious mutations which may contribute to the common degenerative diseases of old age, and the severely deleterious mutations which may be an important factor in complex pediatric diseases.

To identify additional pediatric mtDNA diseases, it will be necessary to view family histories from a new perspective. Generally, it is assumed that an affected child with "normal" parents has inherited an autosomal or X-linked recessive or new dominant nuclear mutation. However, an alternative explanation might be that the child has inherited a high percentage of mutant mtDNAs from a mother whose mtDNA genotype is below the expression threshold. Similarly, to identify mtDNA mutations associated with late-onset degenerative diseases, it will be necessary to evaluate patient and population distributions of mildly deleterious mtDNA variants, in addition to analyzing pedigrees based on the assumption of dominant inheritance with incomplete penetrance.

While the mtDNA paradigm has already permitted identification of a variety of mtDNA mutants associated with clinical phenotypes such as AD and PD (76), blindness (58), epilepsy (13, 14), and dystonia (50), there is one class of mtDNA mutation, the advantageous mutation, that has eluded detection. Mitochondrial oxidative phosphorylation serves three vital and interrelated functions: reoxidation of NADH and $FADH_2$, generation of energy in the form of ATP, and regulation of temperature by generating heat. The generation of ATP requires tight coupling of oxidative phosphorylation and might be optimal in the tropics. The generation of heat requires loose coupling and might be advantageous in the Arctic. Certain mtDNA mutations might affect coupling and be selected for or against as women migrated between the equator and the poles. Such selective forces would enrich for specific mtDNA variants through genomic hitchhiking, thus limiting diversity. Subsequent migration of individuals carrying these mtDNAs to new environments would alter the selective pressures and might provide another mechanism for mitochondrial insufficiency.

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