EDITORIAL

# Disentangling (Epi)Genetic and Environmental Contributions to the Mitochondrial 3243A>G Mutation Phenotype

## Phenotypic Destiny in Mitochondrial Disease?

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**Mitochondrial diseases** are a group of heterogeneous disorders caused by inherited mutations in the mitochondrial genome (mtDNA) and nuclear genome. Typically, mutations in the mtDNA are maternally inherited and cause respiratory chain defects and account for a substantial fraction of childhood and adult neurometabolic disease, with an estimated prevalence of 1:5000 (0.02%).[1] The most common mtDNA mutation is the mitochondrial 3243A>G mutation (m.3243A>G) in the *MTTL1* gene (OMIM 590050), which encodes the transfer RNA $tRNA^{Leu(UUR)}$.[1] This mutation is associated with multiple clinical and psychiatric manifestations, including diabetes, deafness, exercise intolerance, myopathy, cardiomyopathy, lactic acidosis, ophthalmoplegia, and neurological symptoms such as seizures, dementia, and myoclonus.[2,3] In the most severe cases, m.3243A>G causes mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, which is associated with disability and early death.[2,3] Within families, affected individuals vary widely in terms of age at onset—ranging from less than 1 year to more than 50 years of age—spectrum of clinical manifestations, and disease progression. In fact, some individuals are asymptomatic despite carrying equivalent mtDNA mutation levels in blood and/or urine.[2,3] The origin of such broad phenotypic variability has been a 2-decade conundrum in mitochondrial medicine. How can patients carrying an identical mtDNA mutation exhibit such broad differences in symptoms, age at onset, and disease course? And to what extent is the disease phenotype genetically determined and environmentally modulated?

Related article

In this issue of *JAMA Neurology*, Maeda et al[4] studied 2 pairs of monozygotic twins with m.3243A>G who developed MELAS syndrome. Their compelling case studies suggest that genetic factors play a major role in determining the clinical phenotype. In the first twin pair, both patients developed diabetes and deafness in their 30s, experienced a sudden cardiac arrest within a year of each other, subsequently developed congestive heart failure, and died at 53 and 54 years of age.[4] In the second twin pair studied, both patients presented with hearing loss and with parietotemporal stroke-like episodes within a year of each other.[4] Differences were also present between the twin pairs. In the first twin pair, 1 sibling had a unilateral occipital lesion and a cerebral infarction causing sudden death, whereas his brother had bitemporal lesions, developed akinetic mutism, and died of congestive heart failure. In the second twin pair, cerebral lesions were on opposite sides of the brain, and 1 patient developed hallucinations whereas his brother did not. Nevertheless, given the shared genetic composition in both individuals, this striking similarity in phenotypic signature suggests that the disease course is in large part determined by genetic factors.

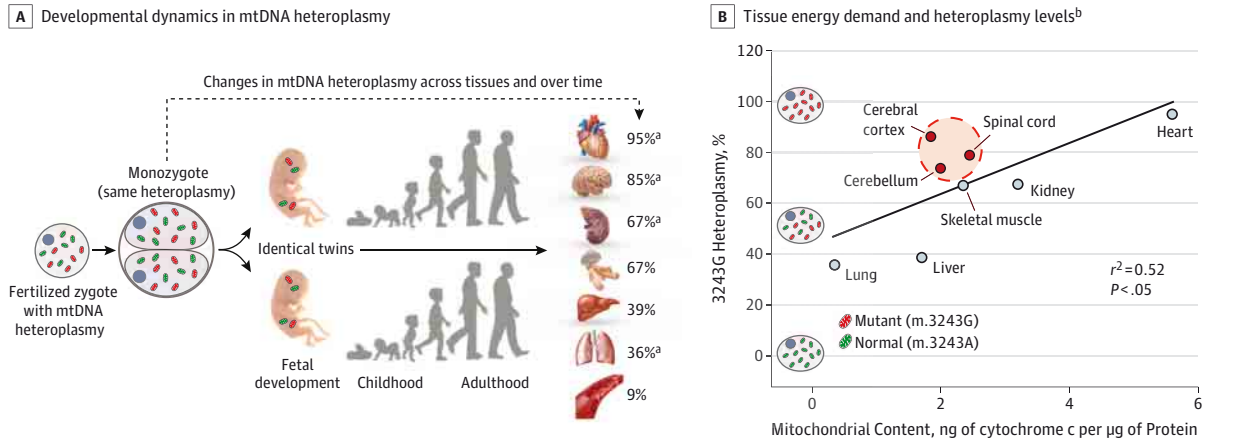
This report[4] is supported by a previous monozygotic twin study by Spyropoulos et al,[5] who also studied identical twins with an mtDNA mutation (m.14487T>C) in the *MTND6* gene. Both brothers similarly experienced tonic-clonic seizures beginning 3 years apart, at 18 years and 21 years, which caused myoclonus of the right hand, altered color vision, bilateral ptosis, and transient white-matter lesions of the brain (albeit of different regions). The clinical pictures differed in the sensory numbness of the hand, hemiparesthesia, and frequency of seizures. Thus, both studies demonstrate that identical sets of mtDNA and nuclear DNA genes can produce strikingly similar clinical phenotypes.[4,5]

Whether the phenotype of m.3243A>G is established prior to organ formation at the zygote stage is an interesting question raised by Maeda et al,[4] with important implications regarding the modifiability of the disease process. If indeed the mitochondrial disease process is "predestined" based on mtDNA heteroplasmy and nuclear DNA of the embryo, then this would cast somewhat of a shadow on the potential of prophylactic and therapeutic interventions to have meaningful clinical effectiveness. This would also suggest that modifiers of disease risk known to influence other conditions, including behavioral, dietary, and psychosocial factors, would be irrelevant to the pathogenesis of MELAS syndrome caused by m.3243A>G.

However, there is evidence against a purely genetically determined course of mitochondrial disease. Animal models of mtDNA diseases indicate that diet, exercise, and environmental factors, such as hypoxia, can alter the course of mitochondrial disease, suggesting some modifiability.[6] Likewise, Leber hereditary optic neuropathy (LHON) caused by mtDNA mutations appears to be exacerbated by smoking and possibly by alcohol consumption.[7] Estrogen levels may also modify its pathogenesis, although more work is needed in this area.

Another intriguing issue raised in the article by Maeda et al[4] relates to tissue-specific segregation of m.3243A>G. If heteroplasmy was determined solely by the initial proportion of mutant and normal mtDNA molecules in the oocyte, then all tissues would be expected to have equal heteroplasmy levels.

Figure. Life Span Perspective and Variable Mitochondrial DNA Heteroplasmy Across Tissues




A, The single-cell zygote contains a certain percentage of mutant and normal mitochondrial DNA (mtDNA) molecules, termed *mtDNA heteroplasmy*. In monozygotic twins, this mutation load is distributed equally into the blastocysts, leading to early embryos of equal heteroplasmy levels prior to organ formation. The article by Maeda et al[4] shows near equal mtDNA mitochondrial 3243A>G mutation (m.3243A>G) heteroplasmy between most tissues of a pair of adult twins, suggesting that genetics and initial heteroplasmy levels play a determinant role in dictating later-life heteroplasmy and clinical phenotype. The report[4] also documents substantial heteroplasmy differences across tissues, indicating that tissue-specific factors affect m.3243A>G expansion across the life span. B, Association between the average mtDNA m.3243A>G heteroplasmy levels in the 3 autopsied patients studied in Maeda et al[4] and mitochondrial content across mouse tissues, representing energy demand.[8] This association suggests that nongenetic factors are involved in the segregation of mutant and normal mtDNA molecules during development. Central nervous system tissues are indicated by the light red circle.

[a] Tissues differing by greater than 10% in heteroplasmy levels between monozygotic twins.

[b] Correlation coefficient excluding central nervous system tissues: $r^2 = 0.91$; $P < .05$.

However, the extensive characterization of m.3243A>G across 24 different tissues illustrates heteroplasmic levels ranging from 5% in the blood to 99% in the heart (**Figure**, A). Although declines in the m.3243A>G load have been consistently observed in blood,[9] whether differences in tissue levels arise during embryogenesis, early postnatal development, or even later is not fully understood.

At least 2 factors could account for tissue differences in mtDNA heteroplasmy. One possible factor is the existence of mechanisms enabling selective degradation via mitophagy of dysfunctional mitochondria, leading to the removal of mutant mtDNA molecules. Activation of this process can effectively reduce the mtDNA mutation load in heteroplasmic cultured cells.[10] However, this is obviously an imperfect process in vivo because mtDNA mutations persist to pathological levels in the germline and in the human population. A second potential determinant of tissue segregation is the converse process of mitochondrial biogenesis, which drives the expansion of mitochondrial content in energy-demanding tissues. Mitochondria with mutant mtDNA produce signals that stimulate compensatory upregulation of biogenesis in affected cells, leading to their hyperproliferation relative to normal mitochondria. If this led to selective amplification of mitochondria carrying a mutant mtDNA molecule, then this would promote the progressive accumulation of mtDNA mutations over time. These explanations beg the question: why do either or both processes occur in some tissues more than in others?

An additional and often underappreciated point is that mitochondria are not all created equal. Across tissues, they exhibit profound differences in protein and lipid composition[8] and functional specialization.[11] The content of mitochondria also differs substantially between tissues by as much as an order of magnitude.[8] Comparing tissue differences in mitochondrial content with heteroplasmy levels across the 3 autopsied patients reported by Maeda et al[4] reveals a significant positive association (Figure, B). This suggests that tissue-specific energy demand may accelerate or promote expansion of mtDNA defects. Because every tissue contains the same genetic material, the corollary is that the expansion of mtDNA defects within cells is likely influenced by nongenetic factors.

The maintenance of stable or even decreasing levels of mtDNA heteroplasmy may in part depend on the balance between selective degradation and biogenesis. In tissues with high energy demand, biogenesis may counterbalance mitophagy, leading to the expansion of mtDNA defects. On the other hand, in cell types with little energy demand, mitophagy may predominate and thus exert more stringent mitochondrial quality control, which would override biogenesis. This may account for the substantially lower mtDNA heteroplasmy in the blood and lung (low energy demand) compared with the heart and brain (high energy demand) and could possibly explain the decrease in m.3243A>G heteroplasmy in the blood over time.[9]

It is difficult to consider tissue-specific differences and relative contribution of genetic and environmental factors without touching on epigenetics. Epigenetic (from the Greek *epi*, meaning "on top of" [the genes]) modifications regulate the behavior of genes, principally within the nucleus but also possibly those of the mtDNA.[12] The significance of genes and of the mutation they harbor derives from the defective gene product (RNA or protein) they yield; m.3243A>G yields a defec-

tive transfer RNA, which secondarily affects the synthesis of all mtDNA-encoded proteins, and respiratory chain dysfunction. Increasing levels of this mutation cause profound changes in nuclear gene expression, including modulation of the epigenetic machinery that lays down and removes posttranslational modifications on the DNA—that is, the epigenome.[13] Notably, identical twins show substantial similarity in their epigenome and DNA cytosine methylation in particular at birth, suggesting a high degree of epigenetic heritability. However, epigenetic differences at birth are subject to in utero environmental factors and maternal stress levels, and epigenetic divergences in monozygotic twins increase over the lifetime under environmental influences.[14] Whether epigenetic factors affect the onset and progression of mitochondrial disease is a pressing question that remains to be established. Future studies aiming to disentangle genetic and environmental contributions to mitonuclear crosstalk should consider the potential role of epigenetic regulation of gene behavior.

It should also be noted that although they represent a powerful design, twin studies and the conclusions they afford on heritability are not without limitations. The proportion of genetic heritability for complex traits varies depending on environmental factors. This counterintuitive notion is exemplified by the observation that the heritable genetic contribution for intellectual quotient is greater in more affluent families than in those of lower socioeconomic status.[15] Clinically, the relative effects of genetic and environmental factors might therefore differ as a function of sociocultural context and other behavioral factors and may differ for different mutations. Definite proof would require large-scale longitudinal studies (eg, Kaufmann et al,[2] Nesbitt et al,[3] and Kirkman et al[7]) with sufficient statistical power to test interaction effects among relevant genetic, biological, and behavioral variables, along with experimental preclinical studies.

In summary, beyond the self-evident point that genetic identity may largely determine the clinical phenotype, the article by Maeda et al[4] on m.3243A>G-carrying monozygotic twins showing strikingly similar disease trajectories and overlapping symptoms underscores 2 other important points. First, the nuclear background must interact with the mtDNA defect in a bidirectional manner, such that the clinical expression of a given mtDNA defect is contingent on the behavior of nuclear genes. Whether epigenetic remodeling of nuclear or mitochondrial gene expression is involved in this crosstalk remains to be established. Second is the postulate that the phenotype of m.3243A>G disease is "predestined," being determined prior to organ formation. Although of obvious appeal from a genetic perspective, more evidence is required to ascertain this point from longitudinal natural history studies involving sufficient numbers of molecularly defined patients with well-characterized environmental exposures.

Achieving this will require multicenter international collaboration and the partnership of neurology with other disciplines to leverage available tools to map the biological embedding of environmental exposures. Nevertheless, the compelling evidence of clinical concordance in monozygotic twins[4,5] forces us to reflect on the influence of environmental and behavioral factors in mitochondrial disease progression. Elucidating these questions should help to better predict disease progression and hopefully inform the design of interventions that will produce maximal effect on the clinical trajectory of mitochondrial disorders.

## ARTICLE INFORMATION

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