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Review

# Unsolved issues related to human mitochondrial diseases

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ABSTRACT

Human mitochondrial diseases, defined as the diseases due to a mitochondrial oxidative phosphorylation defect, represent a large group of very diverse diseases with respect to phenotype and genetic causes. They present with many unsolved issues, the comprehensive analysis of which is beyond the scope of this review. We here essentially focus on the mechanisms underlying the diversity of targeted tissues, which is an important component of the large panel of these diseases phenotypic expression. The reproducibility of genotype/phenotype expression, the presence of modifying factors, and the potential causes for the restricted pattern of tissular expression are reviewed.

Special emphasis is made on heteroplasmy, a specific feature of mitochondrial diseases, defined as the coexistence within the cell of mutant and wild type mitochondrial DNA molecules. Its existence permits unequal segregation during mitoses of the mitochondrial DNA populations and consequently heterogeneous tissue distribution of the mutation load. The observed tissue distributions of recurrent human mitochondrial DNA deleterious mutations are diverse but reproducible for a given mutation demonstrating that the segregation is not a random process. Its extent and mechanisms remain essentially unknown despite recent advances obtained in animal models.



## 1. Introduction

The definition of mitochondrial disease has long been debated and two main definitions still coexist: one restricts these diseases to those due to an alteration of the mitochondrial oxidative phosphorylation pathway (OXPHOS) [1] while the second one encompasses all the diseases due to the alteration of a mitochondrial component [2]. The second definition has at least two disadvantages with respect to the medical point of view. It puts together diseases with pathophysiological mechanisms that totally differ from those expected from an OXPHOS defect. Striking examples are the acute intoxication by ammonia due to *OTC* or *CPS* mutations or the defective cortisol and corticosterone biosynthesis due to *CYP11B1* mutations. Conversely it excludes diseases due to the alteration of a non-mitochondrial component that induces an OXPHOS defect. A well known example of that situation is the MNGIE (MyoNeuroGastroIntestinal Encephalopathy) syndrome due to mutations of *TYMP* encoding a cytosolic thymidine phosphorylase whose defect induces depletion of the mitochondrial DNA (mtDNA). In this review addressing the medical relevance of unsolved issues related to mitochondrial diseases, we define these diseases as those due to an OXPHOS defect.

Within that definition, mitochondrial diseases present with many unsolved issues such as the relative role of the diverse facets of the bioenergetic defect, the amplitude and potential compensation of the induced modification of reactive oxygen species metabolism and/or apoptosis balance, and the interplay between altered mitochondrial signaling and cellular compensatory responses. These aspects are most often addressed in cellular models, which however may lack relevance as cultured cells essentially rely on glycolysis for their energy production. Because of their direct impact on diagnosis, prognosis and/or therapeutic approaches of mitochondrial diseases this review will focus on the mechanisms underlying the extreme phenotypic diversity of mitochondrial

*Abbreviations:* OXPHOS, oxidative phosphorylation pathway; mtDNA, mitochondrial DNA.

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diseases, and especially heteroplasmy, i.e. the coexistence within the cell of mutant and wild type mtDNA molecules, which is frequent with deleterious mtDNA mutations.

## 2. The extreme phenotypic diversity of mitochondrial diseases is related to the diversity of the deleterious consequences of the causal genetic alterations

Signs/symptoms encountered in mitochondrial diseases are extremely diverse (Table 1). They may affect any organ. Furthermore within an organ or tissue they may involve different target cells (for example proximal tubular cells or glomerules in kidney) and/or imply different mechanisms (for example permanent muscle weakness with *TK2* mutations *vs* isolated intolerance to exercise with *MT-CYB* mutations or rapid progression to cirrhosis with *DGUOK* or *BCS1L* mutations *vs* recurrent accesses of liver insufficiency with full recovery with *UQCRB* mutations).

That extreme phenotypic diversity appears essentially related to the diversity of the causes. Indeed specific mutations most often reproducibly give identical or similar phenotype. This is commonly observed with mutations of nuclear DNA genes such as *TK2*, *TYMP* or *DGUOK* [3]. It is also encountered with mtDNA mutations. The three most recurrent mtDNA alterations (large size unique deletion, m.3243A>G MELAS and m.8344A>G MERRF mutation) have now been found in hundreds of patients. Their phenotypes share several common points but they also have unique features that often permit their recognition and, most importantly, indicate that they induce, at least partly, different mechanisms. The presence of lipomas for example is only observed with the m.8344A>G mutations and not with any other mtDNA-encoded tRNA genes. Although there always are overlapping or border cases, these are the minority and are over-represented in the literature.

Restricted tissue expression of the altered gene could explain part of the phenotypic diversity (see below). It cannot explain the diverse deleterious consequences within a same tissue. Our knowledge of the link between mutation and symptoms is clearly insufficient. Dissecting out that link requires on the one hand complete understanding of the symptoms, which may be very difficult when dealing with very complex organs such as brain, and comprehensive analysis of the mutation molecular and cellular consequences, which obviously can only be addressed in surrogate models, either cells derived from patients or genetically-modified animals.

## 3. The influence of modifying factors could be demonstrated in few instances

Modifying factors have been proposed to explain part of the phenotypic diversity observed with mitochondrial diseases. They cannot be demonstrated without sufficient recurrence of identical mutations as different mutations of the same gene may have significantly different impact. Such recurrence is rare in mitochondrial diseases because of the large genetic heterogeneity of these diseases.

The presence of modifying factors could however be demonstrated in diseases due to mutations of the *POLG* or *TK2* nuclear genes and of the mtDNA-encoded *ND1*, *ND4* and *ND6* genes causing Leber Hereditary Optic Neuropathy (LHON). They were proposed to underlie the absence of symptoms despite the presence of the mutations in LHON [4] or to significantly modify the presentation in the case of *POLG* or *TK2* [5,6].

The nature of these modifying factors would be essential to unravel as it would indicate the mechanisms allowing efficient modulation of the clinical consequences of the OXPHOS defect. The main candidate genetic modifiers have been the mtDNA haplogroup and nuclear genes encoding mtDNA replication factors [6,7]. Despite longstanding and intensive efforts in the case of LHON the modifying nuclear genes have however remained elusive [8–10]. The influence of the mtDNA haplogroup has been repeatedly proposed but has remained a likely hypothesis in the absence of reliable functional assay [11,12]. Only few attempts have been made to address the possible modulation of genetic OXPHOS defects. Among these it is interesting to note that several methods aiming at the increase of mtDNA expression through overexpression of translation factors [13] or induction of mitochondrial biogenesis [14,15] has shown promising efficacy.

**Table 1**
Diversity of signs/symptoms encountered in mitochondrial diseases.

| Organ | Signs/symptoms |
|---|---|
| Muscle | Intolerance to exercise, ocular myopathy, axial/limb weakness, myolysis |
| Brain | Cognitive defect, epilepsy, movement disorders, cerebellar syndrome, pseudo-strokes, leucodystrophy… |
| Peripheral nerve | Sensory neuronopathy, demyelinating neuropathy, axonal motor neuropathy… |
| Sensory organs | Pigmentary retinopathy, optic atrophy, deafness |
| Heart | Cardiomyopathy, conduction defects |
| Kidney | Proximal tubulopathy, glomerulopathy |
| Liver | Hepatic failure, cholestasis, cytolysis |
| Endocrine tissues | Diabetes, hypogonadism, growth hormone defect, parathyroid hormone defect… |
| Bone marrow | Sideroblastic anemia, pancytopenia |

## 4. Restricted tissue expression of an OXPHOS defect may have numerous potential causes

The restricted tissue expression of mitochondrial diseases can be ascribed to, at least, two different mechanisms. The first one is the restricted tissue expression of nuclear genes either the gene whose defect causes the disease and/or the genes involved in the cellular responses to the OXPHOS defect; the second mechanism is specific for heteroplasmic mtDNA mutation whose proportion may be heterogeneous between tissues. That latter phenomenon will be discussed in a specific chapter as it is both highly relevant to diseases and raises numerous unsolved issues.

Several genes responsible for a restricted pattern of symptoms are ubiquitously expressed. This is the case for example of *TK2* whose mutations induce an essentially isolated severe myopathy or for *GFM1* associated with hepatopathy. The steady-state expression of the gene in the target tissue does not seem to be a predictive factor of the tissue-specific expression of the disease. Indeed it was low in muscle for *TK2* [16] but high in liver for *GFM1* [17].

Restricted expression of gene(s) involved in the pathway targeted by the causal defect has also been proposed to play a role in the disease tissue-specific expression [17]. In that case it is the lack of appropriate compensation by these genes that would explain tissue sensitivity to the causal defect. That hypothesis has been supported by the observation of phenotypic improvement upon overexpression of candidate genes [13]. It is however lacking demonstration in human patients.

An alternative hypothesis for the restricted sensitivity of certain tissues to generalized defects is the presence of specific energy-demanding mechanisms within these tissues. That hypothesis has been proposed for the urinary loss of substrates normally actively reabsorbed in proximal tubules or the severe intolerance to exercise observed in several mitochondrial diseases. It could underlie the prominent neuronal dysfunction as ionic transport is together a highly energy-consuming process and the basis of neuronal life. However these diverse signs/symptoms do not directly correlate with the severity of the energy deprivation; in particular they can lack in apparently severe generalized OXPHOS defect. Our lack of