Underlying role of mitochondrial mutagenesis in the pathogenesis of a disease and current approaches for translational research

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Abstract

Mitochondrial diseases have been extensively investigated over the last three decades but many questions regarding their underlying aetiologies remain unanswered. Mitochondrial dysfunction is not only responsible for a range of neurological and myopathy diseases, but is also considered pivotal in a broader spectrum of common diseases such as epilepsy, autism and bipolar disorder. These disorders are a challenge to diagnose and treat as their aetiology might be multifactorial. In this review, the focus is placed on potential mechanisms capable of introducing defects in mitochondria resulting in disease. Special attention is given to the influence of xenobiotics on mitochondria; environmental factors inducing mutations or epigenetic changes in the mitochondrial genome can alter its expression and impair the whole cell's functionality. Specifically, we suggest that environmental agents can cause damage by generating abasic sites in mitochondrial DNA, which consequently lead to mutagenesis. Abasic sites are observed in DNA after spontaneous loss of a nucleic base (*e.g.*, "apurinic sites" after loss of purines, adenine or guanine) or through base excision repair; if left unrepaired, they can produce mutagenic DNA lesions. Moreover, we describe current approaches for handling mitochondrial diseases, as well as available prenatal diagnostic tests towards eliminating these maternally-inherited diseases. Undoubtedly, more research is required, as current therapeutic approaches mostly employ palliative therapies rather than targeting primary mechanisms or prophylactic approaches. More effort is needed into further unravelling the relationship between xenobiotics and mitochondria as the extent of influence in mitochondrial pathogenesis is increasingly recognised.

Keywords Abasic sites; Aetiology; Disease; Mitochondria; Mutations; Xenobiotics

Introduction to mitochondria: their role, genome and origin

In addition to causing disease by inducing irreversible alterations to nuclear DNA, environmental factors can also influence other cellular components. It is now recognized that epigenetic influences to mitochondria can give rise to disease pathogenesis. Mitochondria are organelles found in every eukaryotic cell and are commonly referred to as the "powerhouse of the cell", as they generate 90% of the cell's adenosine triphosphate (ATP), which is then used for releasing stored energy required for maintenance of cellular integrity (1). However, mitochondria also have unique, discriminant roles that extend beyond their primary role as ATP generators; maintaining control of the cell cycle and growth, regulating cell signalling, cell death and cellular metabolism are some of the additional functions of mitochondria (2).

Mitochondria are the only cell components having their own genome, other than nucleus. The human mitochondrial DNA (mtDNA) is a single, circular chromosome consisting of ~16.500 base pairs (bp) and encodes 37 genes: 13 for subunits of respiratory complexes, 22 for mitochondrial tRNA and 2 for rRNA (3,4). In contrast to the nuclear DNA (nDNA) of eukaryotic cells, packaged in chromatin (5), mtDNA is packaged into bacterial nucleoid-like structures called mitochondrial nucleoids, with the help of the "mitochondrial transcription factor A" (TFAM) (6,7). TFAM molecules have a similar role with the histones in the nucleus, transforming mtDNA into a more compact, U-like shape (8). However, research on the exact structure and size of these nucleoids is still ongoing and under a lot of debate (9). Each mitochondrion contains 2-10 copies of mtDNA and each mammalian cell contains 100-1.000 of these organelles, depending on the energy requirements of each cell type; thus, there are finally 1.000-10.000 copies of mtDNA within each individual cell (10).

The replication process of the mitochondrial genome does not occur in a single occasion during a cell cycle, as happens with the nDNA (*i.e.*, stringent replication); mtDNA

is degraded and replaced continuously through a random process called relaxed replication (11,12). This process can also take place in non-dividing cells, such as skeletal muscle and brain, leading to the replication of some chromosomes, whereas some others may not replicate at all; however, even if relaxed replication happens randomly, there is still control on the copy number, so that every cell will end up with a constant number of mtDNA molecules (11). The nucleus' contribution is also critical for the mitochondria's genome expression, such as replication, transcription or DNA repair, as many necessary proteins are encoded by nuclear genes (13). For instance, the required proteins for replication and transcription are polymerase gamma (pol γ) (14) and TFAM (15); both are encoded in the cell's nucleus.

For many years it was believed that pol γ replicated mtDNA *via* a bidirectional mechanism, the "strand-displacement mechanism", where the external strand ("leading strand") is unravelled replicating the two-thirds of the mtDNA in one direction; then the synthesis of the internal strand ("lagging strand") follows (16). During the replication of the leading strand, the lagging strand was thought to be coated by proteins (17). There is now recent data suggesting the "bootlace mechanism" where instead of proteins in the protective role, there is RNA complementary to the lagging strand; this way DNA breakage is minimised to only a few damaged bases, which could then be replaced by using "bootlace" RNA as a repair template (18). The source of this RNA sequence has not yet been established but it is hypothesised to be either product of a primase or a transcript "threaded" directly onto the lagging strand (19).

Furthermore, although mtDNA significantly resembles the bacterial genome, the origin of the mitochondria within a cell has been controversial for many decades. There is marked support for the endosymbiotic theory (20), according to which mitochondria were initially separate, autonomous prokaryotic organisms before they entered an amitochondriate

eukaryotic cell, implementing in this way oxidative mechanisms (which were not previously present) and forming the final structure of eukaryotic cells (21,22). On the other hand, many others support a different theory, which is based on the possibility that "this organelle originated at essentially the same time as the nuclear component of the eukaryotic cell" (23,24) . Overall, most of the evidence leans towards the first hypothesis.

Mitochondria also have a distinct inheritance pattern as it is non-Mendelian, which means that all genetic information comes exclusively from one parent, the mother (25). Although both sperm and egg cells include mitochondria, the ones from the sperm (located in the tail of the sperm cell) are broken down immediately after fertilisation and as a result, if a mother harbours a mutation in her mtDNA, she is more likely to pass it down to her offspring (26). However, segregation of mitochondria appears to be greatly affected, not only by random events but also by the dynamics (fusion, fission, movement and mitophagy) of these organelles (4,27) and hence, there is a variance in the severity of the mutation in the descendants, who can eventually become heteroplasmic (different copies of mtDNA within a cell) or homoplasmic (uniform copies of mtDNA within a cell) for a mtDNA mutation. It is well-accepted that the existence of a single mutation is not enough for the development of a pathogenic phenotype; conversely, the mutation level has to "exceed a critical threshold", before clinical features occur (10,12). After the observation that siblings from the same family can often have different levels of mutated mtDNA, the theory of mitochondrial "genetic bottleneck" came to the fore, in order to describe the restriction in the number of mtDNA transmitted between generations (28,29). This restriction is responsible not only for the variety of mutated levels between siblings, but also for the quick reversion from heteroplasmy to homoplasmy in just a small number of generations (30).

Introduction to mitochondrial diseases: occurrence and potential causes

Even though the mitochondrial genome is considerably shorter in comparison to nDNA (3×10^9 bp), it is still extremely important for the maintenance of stable respiratory functions of each individual cell, and therefore of a whole organism. Despite the smaller number of genes in mitochondria, the mutation rate is 10 to 16 times higher than nuclear mutations (10). In reality, none of the mitochondrial genes control an individual's physical appearance (height, eye/hair colour, etc.), but when these contain mutations the chances of a disease occurrence are quite high, leading to a variety of mild or extremely severe disorders, known as mitochondrial diseases. Mitochondrial dysfunction can also lead to the increased production of reactive oxygen species (ROS), which sequentially contributes to retrograde redox signalling from the mitochondrion to the cytosol and nucleus; hence mitochondria can have a great impact on the cell's replication and function (31,32). Recent studies have focused extensively on the signalling (cross-talk) between mitochondria and nucleus, and the importance of these "bidirectional interactions" in cellular mechanisms and responses to exogenous agents (such as environmental stressors)~~,~~ has been made clear (33,34).

Mitochondrial disease compromises cell energy production and this can limit energy for the cell, affecting the function of all organs and especially those with high energy demands such as brain, muscle, liver, heart, kidney, etc. (Figure 1) (4,35,36). The causes of mitochondrial diseases may vary and result from the accumulation of different factors (12). Most of the disorders are often due to mutations in mtDNA or in nuclear genes that encode mitochondrial proteins (37). The later is the result of the majority of mitochondrial proteins (~900) that are encoded in the nucleus, synthesized within the cytosol and then imported into the mitochondria (4); thus these nuclear genes [named "nuclear-mitochondrial genes" (38)] can equally affect the mitochondria's function.

Mutations in a stem cell's mitochondria are also thought to be responsible for the initiation and recurrence of many diseases. Recently, it has been observed that accumulated mtDNA mutations occurring in human colonic crypt stem cells can expand and cause serious defects in their differentiated progeny (39-41). Colonic stem cells were chosen for pilot studies, as they reside at the base of the colon crypts and produce other cells that migrate towards the top. Ultimately, the majority of the cells within the crypt originate from these stem cells, which makes it easier to identify potential mutations by studying any progeny cell without the need of isolating individual stem cells (39). Hence, it is likely that mtDNA mutations in these stem cells will be easily transferred to daughter cells throughout relaxed replication and if they finally affect an important base in a structural or RNA gene, then they can cause biochemical defects as a consequence (40). Further experiments have shown that through fission, the mutated colonic crypts can also expand to create two mutated daughter crypts, which inevitably results in the spread of any mutation across the colon tissue throughout an individual's lifetime (40). It is thought that probably only stem cells have the ability to accumulate mitochondrial mutations to a detectable proportion, as these mutations take many years to expand and stem cells have the sufficient life-span for that (42). However, it is fortunate that this spreading of mutations happens fairly slowly, as the estimated time for crypt fission to occur in humans can vary from 17 to 30 years (40,43).

An additional potential cause of mitochondrial disease is the existence of defects in the final pathway of mitochondrial energy metabolism, *i.e.* oxidative phosphorylation (OXPHOS) (36), where increased production of mitochondrial ROS can change gene expression, induce cancer cell proliferation or even lead to cell apoptosis or necrosis (32). Mitochondrial disorders may be also attributable to dysfunction of mitochondrial proteins (44), inheritance (29,45), inefficiency of DNA-repair mechanisms during mtDNA replication (10) and various environmental influences [such as UV exposure (46), smoking, alcohol

consumption (47), pesticide exposure (48), use of antiretroviral drugs (49), exposure to industrial toxins (50) and other chemicals (51)].

Epigenetic changes caused by environmental factors on mtDNA, such as methylation and hydroxymethylation, can alter mtDNA expression and subsequently lead to further alterations of nuclear DNA too; the resulting modified genome is capable of causing a variety of diseases (52,53). In this review, we mainly focus on environmental contaminants leading to mitochondrial mutagenesis and also shed some light on potential mechanisms under which mitochondria become pathogenic. Some reviews have already been conducted on a number of environmental chemicals that introduce toxicity to mitochondria and their genome (51,54,55); however, the precise mechanisms of toxicity are not fully understood. Surprisingly, chemical contaminants were found to be responsible for both decreases and increases of mtDNA copies, which was attributed to the competing effects between ROS-induced damage and simultaneous biogenesis respectively; moreover, many of them seem to cause different extents of damage in mtDNA and nDNA in a dose-related fashion (54).

Exogenous contaminants in mitochondrial toxicity: the contradiction

Recent evidence links pharmaceuticals or environmental pollutants to mitochondrial toxicity and consequently disease. As might be expected, pharmaceuticals have been better studied in comparison to environmental contaminants, leaving many question marks with regards to the latter's mechanism and degree of influence. Specific drugs have now been identified as previously-undiscovered mitochondrial toxicants leading to disease; adriamycin for example, -an anti-cancer chemotherapy drug- has been found to affect primarily the mitochondrion by generating ROS and inhibiting ATP production, leading thus to cardiomyopathy (56). Likewise, numerous environmental contaminants, such as silver nanoparticles -used as bactericidal agents in water- have been repeatedly blamed for "poisoning" mitochondria.

Silver nanoparticles have antibacterial properties by which they disrupt bacterial cell walls and deposit on their membrane; considering mitochondrial similarities to bacteria, there is a high chance that silver nanoparticles have the exact same effects on mitochondria as well, causing impairment of their function (57,58).

On the other hand, the belief that environment adversely affects mitochondria and their genome, has recently been questioned as it contradicts some experimental evidence suggesting that endogenous mechanisms have a far greater impact in mitochondria than any environmental contaminant. By using mtDNA sequencing they concluded that errors during mtDNA replication and lack of repair mechanisms are responsible for causing more mitochondrial mutations than any exogenous factor (59). For example, after studying lung cancer and melanomas, they found no evidence of the mutational signatures coming from tobacco smoke or ultraviolet light exposure respectively; also, in breast cancer cases, *BRCA1* and *BRCA2* mutations also showed no influence on mtDNA, in contrast to the nDNA. The same study also supports, contrary to conventional wisdom, that most mtDNA mutations act as "passenger mutations" having no incredible effect on cancer development and spreading (59). However, even though this study incorporated a large number of subjects (1675 cancer biopsies across 31 tumour types), its limitation was the focus on only one disease, cancer. It is thus possible that this finding is valid in the presence of cancer, but invalid in other diseases, such as Parkinson's disease, epilepsy, cardiovascular disease or other mitochondrial diseases. Uncontrollable genome proliferation, including mtDNA, is a common characteristic of all cancer cells and it could be a reflection of observations during sequencing. Moreover, the study makes clear that "the *majority* of the mutations were introduced from errors during DNA duplication", implying that there are more mutations, even if these represent the minority, that could be caused by other factors.

It is evident that there is still a lack of conclusive evidence on whether endogenous, exogenous or even a combination of both, can affect mitochondrial function and in what extent.

Mitochondrial heterogeneity and vulnerability

The same environmental or pharmacological exposures can lead to different disease prevalence among individuals, as a result of differing genetic background (54,60). For instance, tobacco use and heavy alcohol consumption increase the risk of blindness in specific mitochondrial diseased people (47). Similarly, use of common aminoglycoside antibiotics can cause deafness in people with certain mtDNA mutations, even when prescribed in low doses, clearly showing a gene-environment interaction (61). Furthermore, each mitochondrion exhibits similar heterogeneity not only from tissue to tissue (*e.g.*, heart mitochondria *versus* kidney mitochondria *versus* skeletal muscle mitochondria), but also within individual cells of the same tissue (*e.g.*, intermyofibrillar *versus* subsarcolemmal within skeletal muscle), which renders it even harder to conclude in general assumptions; this variability leads consequently to different protein composition and tissue function, which could potentially be the reason why certain mtDNA mutations occur in certain tissues (60) (Figure 2). In contrast, there are numerous factors that can undoubtedly cause damage to mitochondria and their genome regardless of the aforementioned heterogeneity. Firstly, mtDNA can get adversely affected by its proximity to the electron transfer chain (29), where high levels of ROS are produced, as well as by its histone-free packaging (5) and insufficiency of repair mechanisms (10), compared to the nuclear DNA. Secondly, it is possible for various contaminants, such as lipophilic compounds (*e.g.*, polycyclic aromatic compounds and alkylating agents), heavy metals (*e.g.*, mercury , cadmium , lead) or even other organic chemicals (*e.g.*, paraquat and 1-methyl-4-phenylpyridinium) to accumulate in the phospholipid membranes of mitochondria; this renders them capable of causing

dysfunctionality in the organelles (54). Finally, another potential reason for mitochondrial impairment is the contribution of cytochrome P450 systems (CYPs) in the metabolism of xenobiotic compounds (62). CYP enzymes interact with the initial incoming compound (*e.g.*, "parent" drug) to generate its metabolites; likewise, they can bioactivate inert chemicals accumulated in mitochondrial membranes, such as environmental toxicants, and transform them to their toxic metabolite form (62,63). At the same time, however, we have to keep in mind that apart from their vulnerability to the above-mentioned factors, mitochondria are also equipped with protective mechanisms helping them to overcome severe damages or reactive compounds. The high copy number, dynamic processes, such as fusion and fission, mitophagy of heavily damaged mitochondria and proteins bound to the mtDNA, such as TFAM, are potentially some of the shields of mitochondria (54,64).

Bioactivation of xenobiotics is triggered when a single chemical enters the organism, primarily through the routes of inhalation, ingestion or dermal absorption (65). Biotransformation describes the metabolic process of xenobiotics, occurs in three separate phases and is catalysed by a variety of different enzymes, predominantly CYPs (62,66). For many years, it was believed that xenobiotic metabolism solely indicated the deactivation of parent compounds, but it was then found that it can also cause activation of previously inert chemicals, producing highly reactive, electrophile molecules, *i.e.*, bioactivation (67). These reactive intermediates can bind covalently to DNA, RNA or proteins and produce adducts, or even interact with the tissue oxygen and produce ROS, which also lead to mutations and damage (66,68,69). DNA adducts resulting from the reaction between the active electrophiles and the two purine bases, adenine and guanine, can lead to two products: a stable DNA adduct or a depurinating DNA adduct. When a stable adduct is formed, the electrophile compound remains covalently bonded to DNA, while depurinating adducts are spontaneously released from DNA after breakage of the glycosidic bond, creating "apurinic sites of DNA"

which are responsible for further mutagenesis (69). Just as these reactive electrophile might react with nDNA, there is a good possibility that they might also covalently bind to bases in mtDNA (Figure 3).

Potential mechanism for mitochondrial mutagenesis: abasic sites in mtDNA

Precursor CYP enzymes are coded in the nucleus and depending on their "targeting sequence", which is controlled by the amino-terminal part of the peptide, they target either the endoplasmic reticulum (ER) or the mitochondria (62). The ones that are destined to become mitochondrial P450s (only 7 out of 57 genes encode mitochondrial P450s), are imported into the mitochondrial matrix and processed to become mature; finally, the mature derivatives get incorporated into the inner membrane of the mitochondrion, with their catalytic domain exposed to the matrix area (62). At the same time, various toxicants (70-76) accumulate in the phospholipid mitochondrial membranes and as a result they get easily bioactivated by CYP enzymes. To summarise, this is one of the proposed mechanisms for the activation of relatively non-reactive chemicals in mitochondria, which would lead inevitably to mitochondrial mutagenesis (Figure 3). An alternative hypothesis is that the chemical/compound is initially metabolised in the ER by microsomal P450s and then the resulting metabolites penetrate the mitochondrial membranes to give rise to mutations (54). This penetration is absolutely feasible as the outer membrane contains porins, which make it easily permeable to various molecules, while on the other hand, the inner membrane is less permeable to big molecules but still contains transporters which allow endogenous compounds and possibly xenobiotics to enter the matrix (77). Either way, it is likely that these activated toxicants will lead to abasic site formation in mtDNA. However, despite the current knowledge on mtDNA and its role in mutagenesis, understanding of many pathways remains limited and is often extrapolated from our knowledge of nuclear DNA and mechanisms so far.

Specific mitochondrial diseases

Mitochondrial defects are responsible for a number of diseases including Kearns-Sayre syndrome (KSS), Pearson's syndrome and chronic progressive external ophthalmoplegia (CPEO), which are all due to large-scale rearrangements of mtDNA (single deletions, duplications or a mixture of the two). Others are due to point mutations in mtDNA and these include MELAS (mitochondrial neuro-gastro-intestinal encephalomyopathy), MERRF (myoclonic epilepsy with ragged red fibres), NARP (neuropathy ataxia and retinitis pigmentosa), MILS (maternally inherited Leigh syndrome), LHON (Leber's hereditary optic neuropathy), diabetes mellitus and deafness (36,78). There are also a number of mitochondrial diseases triggered by nuclear mutations such as dominant optic atrophy (mutation in *OPA1*), Freidreich's ataxia (mutation in *FRDA1*), hereditary spastic paraplegia (mutation in *SPG7*), Leigh syndrome (mutation in both *SURF1* and *mtDNA ATP6*), Wilson's disease (*ATP7B*), Barth syndrome (*TAZ*) and many more (36,79). Other clinical pathologies linked with mitochondrial dysfunction include: neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Huntington's disease (80), and even schizophrenia, bipolar disorder, epilepsy, stroke, cardiovascular disease and chronic fatigue syndrome (81). Finally, there is also an accumulating body of evidence to support the involvement of mitochondria with senescence (12), cancer (82) and autism (83). Although it is still relatively difficult to distinguish a mitochondrial disease from symptoms alone, it is feasible to set up a tentative connection between specific mutations and diseases (35,84).

In general, the prevalence of mitochondrial diseases depends on many factors, such as the mutation rate and inheritance pattern. mtDNA deletions are infrequently inherited from mother to child (mostly sporadic/de novo), less common than point mutations and therefore are rare. Other mtDNA and nDNA mutations are more likely to be maternally transmitted and cause significant disease (85,86). It is also possible that ethnic differences contribute to the

prevalence of mtDNA mutations. To illustrate this, different mtDNA mutation rates were found in two separate studies; T14484C mutation, causing LHON disease, was mainly observed in French-Canadian families, while A3243G mutation, causing MELAS syndrome, was predominant among specific subgroups of Finnish families (85). It has been hypothesized that this difference is due to the geographical, cultural and linguistic isolation of the Finnish population and the "founder effect", which suggests that these families may share the same maternal lineage; in other words, have been derived from the same woman (87).

Understanding (dys-)functionality in isolated mitochondria

When a xenobiotic enters the human body, it not only causes mitochondrial dysfunction, but may also induce mutations or damage to more than one organelle or macromolecule at the same time. For this reason, it is of great importance to investigate mitochondria separately from the rest of cell, in order to determine the toxicity of chemicals solely in mitochondria (88). To date, there are already several approaches which can be used to explore these semi-autonomous organelles in intact cells, in isolation or even *in vivo* (88,89). All of the techniques, with either isolated mitochondria or intact cells and living organisms have their strengths and weaknesses, which are given in more detail in previous reviews (54,89).

Isolating mitochondria from the rest of the cell aids the investigation of specific mitochondrial processes, such as respiration, as there is no incoming interference from the cytosol. Diagnosing a disease only from a single defect (*e.g.*, ATP reduction) or a type of mutation (*e.g.*, deletion, point mutation, etc.) remains considerably difficult though (60). Many mitochondrial disorders, even when caused by the same mutation, have diverse pathophysiological characteristics, which makes it really hard to distinguish. For instance, some mtDNA mutations demonstrate high tissue-specificity, others affect different tissues and organs in different individuals and with differing ages of onset, depending on the

individuals' genetic background, and others can cause different extents of deficiency in different individuals (54). Diseases caused by point mutations in mtDNA, such as LHON and MELAS syndromes, manifest clinically as neuropathy and myopathy/encephalopathy, causing mainly sight loss and stroke-like episodes respectively. Similarly, KSS and CPEO syndromes, both caused by deletions of mtDNA, are mostly presented as ataxia/neuropathy/opthalmoplegia and bilateral ptosis/opthalmoplegia respectively (36). It is thus obvious that not all point mutations or deletions cause the same disease severity or symptoms. Clinical manifestations may vary depending on the mutation load as well. During a study on patients with the 3243A>G mutation in the $tRNA^{Leu(UUR)}$ gene, individuals with 50% mutation load in their muscle cells were found with inefficient oxygen intake during exercise and abnormal morphology of muscle fibres, whereas when the mutation load exceeded 65%, they could develop diabetes mellitus and hearing loss (90). Thus, it is difficult to create a clear link between clinical phenotype and mtDNA mutations, apart from tentative clinical-correlations. Establishing the exact causes of mitochondrial pathogenesis is still undergoing research, with only 50% of severe mitochondrial diseases identified to date (60).

In terms of diagnosing mitochondrial diseases clinically, there is a combination of tests including family history, which can be run if a mitochondrial disorder is suspected. Some of these tests may include examination of clinical features, sequencing of mtDNA/nDNA for potential mutations, muscle biopsy, blood or urine tests, brain MRI (magnetic resonance imaging), ophthalmology and audiology tests, spectroscopy, metabolic screening in cerebrospinal fluid (CSF) etc. (84,91,92). However, despite the advances in the field, many mitochondrial disorders are still poorly recognised and diagnosed due to their complexity and diversity of symptoms. It is also established that diagnosis of these diseases is easier in adults than in children, as the first are more likely to carry "easily defined" mtDNA

mutations, while the latter carry mostly nuclear DNA mutations, where the "classic" symptoms of a mitochondrial disease are not present (91).

Future scope for translational research (current approaches)

Despite establishing over twenty years ago that mitochondrial disease is responsible for a wide range of conditions (around 1 in 5,000 individuals is affected from these diseases), there are no effective treatments (60,93). Clinicians and scientists try their best to help these patients by relieving their symptoms and maximizing the organs' functionality, either by supportive medicine and dietary supplementation or by corrective surgery, even though the benefits of the latter are sometimes temporary (37,94). Resistance exercise training has positive effects as well, as it improves oxidative capacity and induces mitochondrial biogenesis in skeletal muscles of individuals with mtDNA deletions (95). Due to lack of effective treatments it is of crucial importance, that high-risk women undergo a series of prenatal tests. Genetic counselling, followed by chorionic villous sampling (CVS), amniocentesis or preimplantation genetic diagnosis (PGD) when necessary, is provided to future mothers as they can get informed about the different types of mitochondrial mutations (*e.g.*, deletions and point mutations of mtDNA) and the underlying risks of transmitting them to their descendants; thus they can then take a decision to either continue with their pregnancy or have a termination. Despite the advances in prenatal testing, it is still challenging to interpret the results due to mtDNA heteroplasmy and the complexity of these genetics (93). In case of high levels of heteroplasmy or homoplasmic mtDNA mutations, the only reproductive options are adoption, ovum donation or mitochondrial donation, which is described in details below (96).

Future research in mitochondria diseases is likely to focus in four main areas:

- Altering the mtDNA heteroplasmy by stimulation of the satellite cells, which are precursors of muscle cells, able to proliferate and regenerate cells with no mutant mtDNA (97); or by preventing the replication of mutant mtDNA with the help of peptide nucleic acids (PNAs) complementary to the mutated mtDNA sequence, allowing thus propagation of the wild-type (98);
- Selective elimination of mutant mtDNA by a restriction enzyme, capable of targeting only the undesirable-mutated sequence, without thus affecting the wild-type mtDNA (99) or the import of a normal tRNA for the repair of respiratory deficiencies (100);
- Replacement of a defective protein, such as a respiratory-chain complex, with a similar protein from another organism could also be considered as a therapy of mitochondrial disorders, as it has already been effective *in vitro* where a yeast enzyme, imported in human cells, restored the activity of Complex I enzyme (96);
- Mitochondrial donation is an additional technique for dealing mitochondrial disorders, as it can prevent transmittance of the mutation from mother to child, which is critical considering the lack of successful treatments. This method is an *in vitro* fertilization (IVF) technique, where nuclear DNA from a patient woman is transferred into an enucleated donor oocyte or zygote, without the "carryover" of the mutated mtDNA (96). However, this approach has raised many ethical concerns and is not yet widely accepted.

Conclusion

Normal mitochondrial physiology is integral to healthy wellbeing. After decades of research in interpreting mitochondrion function, there is currently no treatment against mitochondrial diseases, which reflects the complexity of dysfunction when it occurs. Environmental factors are now thought to be a potential aetiology to some mitochondrial diseases. Understanding

the extent of genotoxic and/or epigenetic influences, will enable us to move towards novel research techniques, develop diagnostic tests and perhaps influence lifestyle changes.

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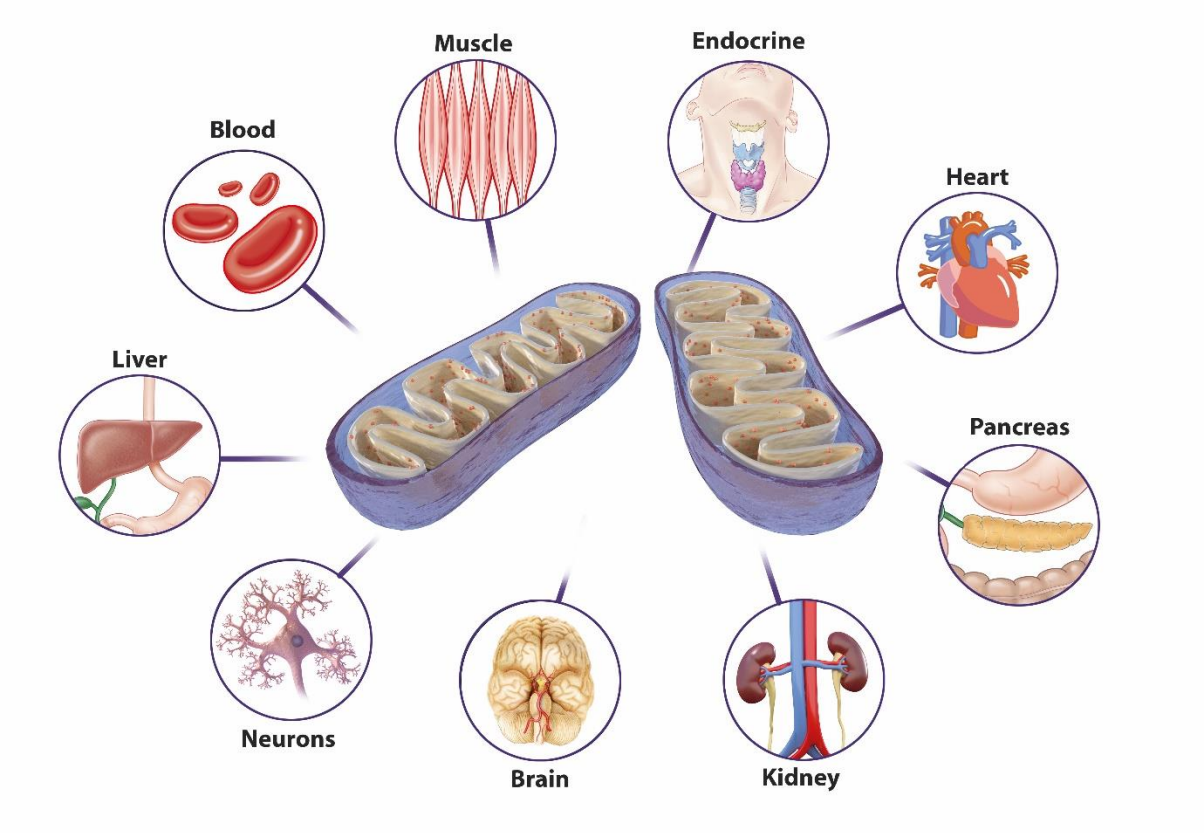

Fig 1: Schematic of the most-affected organs in mitochondrial diseases

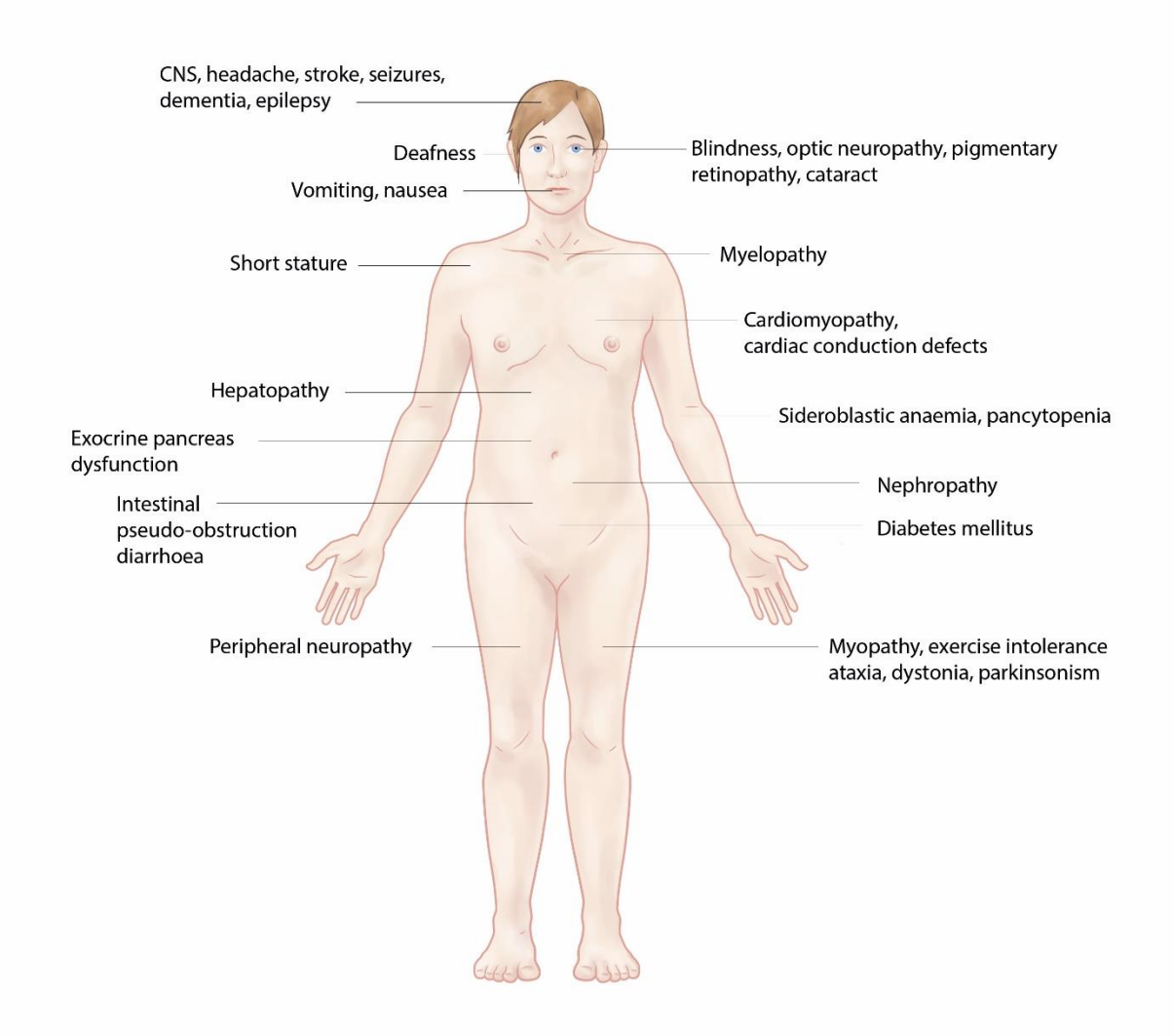

Fig 2: Phenotypic manifestations of mitochondrial diseases

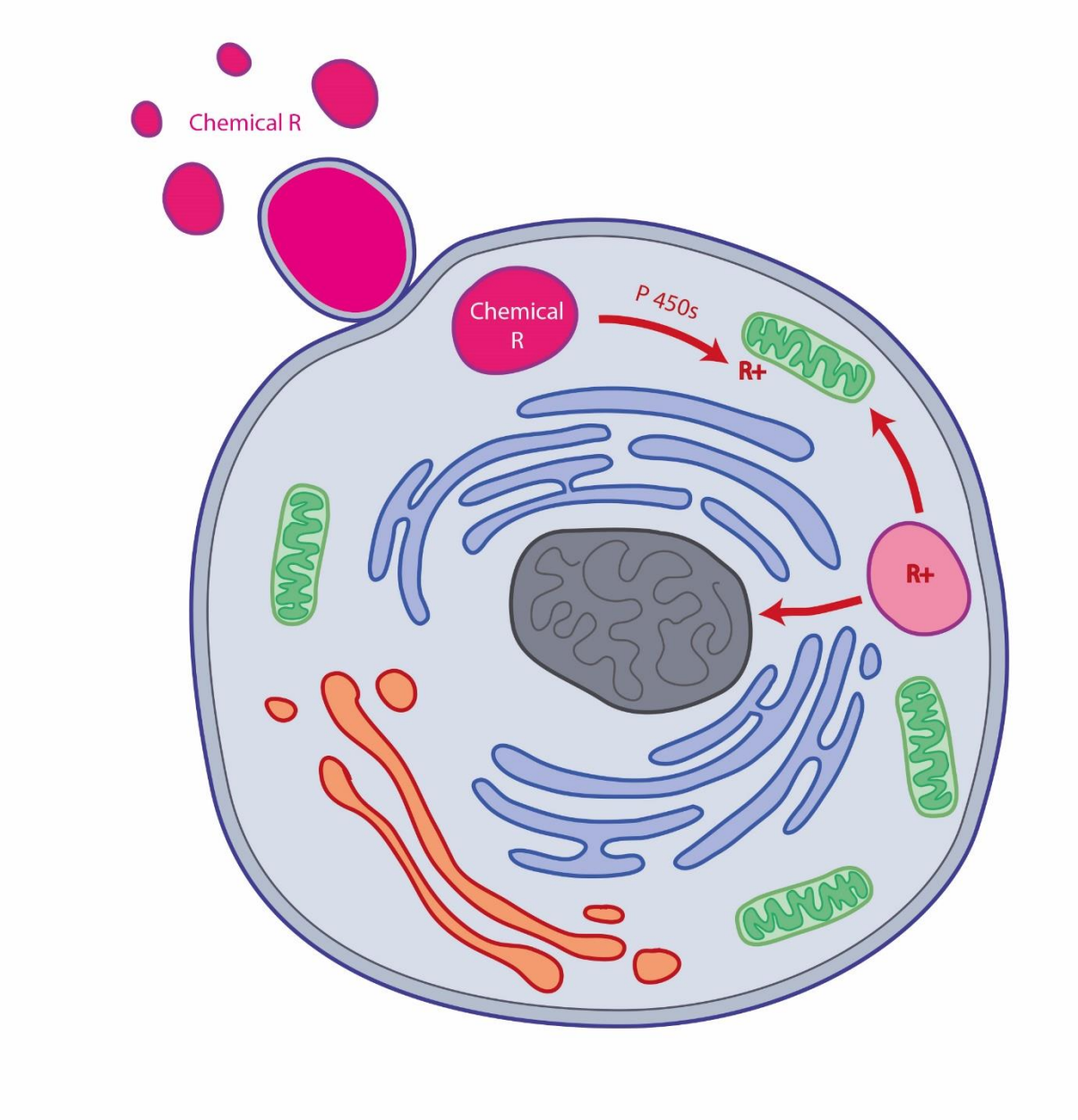

Fig 3: Chemical (R) penetrating a cell's membrane; there it can get bioactivated by different enzymes and predominantly cytochrome P450 enzyme to form its electrophilic compound (R+), which is highly reactive. The toxic derivative can then interact with nuclear and mitochondrial DNA, forming thus apurinic sites