

## Mitochondrial DNA-related disorders: emphasis on mechanisms and heterogeneity

Umut CAGIN<sup>1\*</sup>, Jose Antonio ENRIQUEZ<sup>1,2</sup>

<sup>1</sup>Cardiovascular Development and Repair Department, National Center for Cardiovascular Research (CNIC), Madrid, Spain

<sup>2</sup>Department of Biochemistry and Molecular and Cellular Biology, Faculty of Science, University of Zaragoza, Zaragoza, Spain

Received: 06.05.2015

Accepted/Published Online: 06.08.2015

Printed: 31.12.2015

**Abstract:** Mitochondrial diseases are a heterogeneous group of disorders that are currently the focus of intense research. The many cell functions performed by mitochondria include ATP production, calcium homeostasis, and apoptosis. One of the unique properties of mitochondria is the existence of a separate mitochondrial genome (mitochondrial DNA, mtDNA) found in varying copy numbers and containing 37 genes, 13 of them encoding proteins. All 13 mitochondrially encoded proteins form part of oxidative phosphorylation complexes through combination with approximately 100 nuclear DNA-encoded proteins. Coregulation of nDNA and mtDNA is therefore essential for mitochondrial function, and this coregulation contributes to the heterogeneity and complexity observed in mitochondrial disorders. In recent times, significant advances have been made in our understanding of mtDNA-related disorders. A comprehensive review of these studies will benefit both current and new researchers and clinicians involved in the field. This review examines the major types of mtDNA-related defects and their pathogenic mechanisms, with a special emphasis on the heterogeneity of mitochondrial disorders. Potential treatment strategies specialized for each of the disorders, including the hormone melatonin and the recent advances in gene therapy, related to their potential applications for the management of the primary mtDNA disorders are also discussed.

**Key words:** Mitochondria, oxidative phosphorylation, mitochondrial DNA, mitochondrial DNA disorders, mitochondrial DNA mutations

### 1. Introduction

Mitochondria are the organelles with the main responsibility for energy production in cells, generated in the form of ATP by oxidative phosphorylation (OXPHOS). Mitochondria are additionally involved in many other important processes, including apoptosis and calcium homeostasis (Pozzan et al., 2000; Newmeyer and Ferguson-Miller, 2003). Furthermore, mitochondria are the main site of the production of reactive oxygen species (ROS). The tricarboxylic acid (TCA) cycle, which in addition to its contribution to ATP production also generates several important metabolic intermediates, occurs in mitochondria (Boveris et al., 1972).

Mitochondria contain their own genetic material, called mitochondrial DNA (mtDNA), a 16,569-bp-long, double-stranded, circular molecule containing just 37 genes. Only 13 of these genes encode proteins, all of them contributing to the formation of OXPHOS complexes through association with nuclear DNA (nDNA)-encoded proteins. The remaining genes encode 22 tRNAs and 2 rRNAs (Anderson et al., 1981). The copy number of mtDNA varies depending on the cell type and energy demand of the tissue.

Replication of mtDNA is dependent on proteins encoded by nDNA and imported to mitochondria. Therefore, mtDNA-related diseases can be caused not only by mutations in mtDNA but also by defects in nDNA-encoded factors required for mtDNA replication (Figure 1). This dual-dependence of mtDNA integrity is largely responsible for the heterogeneous nature of mtDNA-related diseases.

The history of research on mtDNA-related diseases is relatively short, with the first disease-causing mtDNA mutations reported in 1988 (Holt et al., 1988; Wallace et al., 1988). These two ground-breaking studies paved the way to the discovery and characterization of a large number of mtDNA mutations, and at least 300 pathogenic mtDNA mutations have now been identified ([www.mitomap.org](http://www.mitomap.org)). Due to the high degree of heterogeneity in mtDNA-related diseases, it is very hard to quantify their prevalence. Studies have been conducted to define the severity of mtDNA mutations in specific populations; however, a worldwide study in this field is missing. A study based in northeastern England reported that mtDNA-related diseases occurred at a frequency of 1 in 10,000 in working-age adults with a further 1 in 6000 people (below retirement age) at

\* Correspondence: [ucagin@cnic.es](mailto:ucagin@cnic.es)

risk of developing these diseases (Schaefer et al., 2008). Subsequent reports have suggested that the frequency of mtDNA mutations is much higher (Greaves et al., 2012). Recent advances in molecular biology and sequencing techniques will inevitably lead to the characterization of new mtDNA mutations, which will help provide a more accurate estimate of the prevalence of these diseases, and thereby a better understanding of the impact of mtDNA-related diseases on society.

The mechanisms underlying mtDNA-related disorders can explain why they are associated with such a high degree of clinical heterogeneity. The mechanistic details of mtDNA dysfunction need to be investigated in depth in order to design novel therapeutic strategies. This review examines the state of the art regarding these mechanisms and reviews some recent therapeutic advances in the field.

## 2. Features of mtDNA genetics

### 2.1. The mtDNA molecule at a glance

The publication of the first sequence of human mtDNA in 1981 marked a milestone in mitochondrial research (Anderson et al., 1981). Our current understanding of the mtDNA sequence incorporates subsequent revisions (Andrews et al., 1999). The mtDNA molecule contains no introns and only a few noncoding bases (Anderson et al., 1981). The molecule is formed by a guanine-rich heavy strand (H) and a cytosine-rich light strand (L). The only noncoding region in mtDNA is called the D-loop (displacement loop) and it contains control elements required for mtDNA transcription and replication (Shadel and Clayton, 1997). Unlike the nuclear genome, which is found in 2 copies of 23 chromosomes ( $2n = 46$ ), mtDNA can occur in multiple copy numbers. The maintenance of mtDNA copy number is discussed in a separate section.

mtDNA exists in protein-linked structures called nucleoids, compact macrocomplexes formed and stabilized principally through the action of mitochondrial transcription factor A (TFAM) (Kaufman et al., 2007). mtDNA nucleoid-associated proteins are a subject of intense research but as of yet there is no consensus on their characteristics or number (Hensen et al., 2014). Further advances in this area will provide a clearer picture on the role of nucleoid structure in mtDNA maintenance.

Maintenance and homeostasis of mtDNA is determined by various processes specialized for their function. In the following sections we review some of the key features of mtDNA in order to provide a better understanding of mtDNA-related diseases and their mechanisms. These features should be taken into account during diagnosis and treatment of mitochondrial disease patients.

### 2.2. Heteroplasmy and threshold

MtDNA accumulates mutations at a fast rate, leading to mitochondrial dysfunction. The inherent ROS formation

associated with the OXPHOS system might be the primary cause of the accumulation of mutations in mtDNA (Brown et al., 1979). Nonmutated and mutated mtDNA copies can coexist in the same cell and even in the same mitochondria, a condition called heteroplasmy. MtDNA mutations can also be found in a homoplasmic state, when no nonmutated mtDNA copies are present. In the heteroplasmic state, the proportion of mutated mtDNA copies needs to pass a certain threshold in order to cause a pathological defect detectable by biochemical tests. However, there is no clear correlation between the heteroplasmy level and the clinical severity of disease (Wong, 2007), and the threshold level can vary depending on the mutation type, adding another level of complexity to the treatment of mtDNA-related disorders. A recent study showed that small changes in mtDNA heteroplasmy cause significant changes in the nDNA transcriptome (Picard et al., 2014). This transcriptional reprogramming upon mitochondrial dysfunction is also known as retrograde signaling and is activated by changes in ATP/AMP, NADH/NAD<sup>+</sup>, ROS, cytosolic Ca<sup>2+</sup>, and membrane potential. These changes are interpreted by the cells as signals of mitochondrial dysfunction. Retrograde signaling further causes changes in expression of genes involved in various signaling pathways (e.g., mTOR, Myc, NFκB), mitochondrial transcription, capacity, or biogenesis and usually causes a metabolic switch towards glycolysis. The interconnected complex mechanism of this phenomenon is the subject of a recent review by our group (Cagin and Enriquez, 2015).

### 2.3. Random segregation, mtDNA bottleneck, and clonal expansion

Mitochondrial segregation during cell division has been suggested to be random, which need to be investigated in deeper detail (Lombes et al., 2014). This suggests that the segregation of mitochondria carrying mutant and wild-type mtDNA will result in one daughter cell having more mutant mtDNA and the other having more wild-type mtDNA. Random mitochondrial segregation would result in the production of daughter cells with varying proportions of putatively mutant mtDNA. If the mutant mtDNA proportion exceeds a certain threshold level, a biochemical defect will be evident. In contrast, in fast-dividing cells this random segregation can result in rapid loss of mutant mtDNA, as has been observed in blood samples (Rahman et al., 2001). Furthermore, evidence from multiple studies suggests that a bottleneck during development results in rapid segregation of heteroplasmic mtDNA, providing another route to homoplasmy (Upholt and Dawid, 1977; Olivo et al., 1983; Holt et al., 1989; Vilkkilä et al., 1990; Blok et al., 1997; Cree et al., 2008b). However, the developmental phase at which the bottleneck occurs is not clearly defined and is debated.

A mosaic pattern of mtDNA is observed in COX-deficient muscle fibers of patients with mitochondrial dysfunction. The suggested mechanism responsible for this is called clonal expansion, and it is due to random replication of mtDNA resulting in cells with differing proportions of mutant to wild-type mtDNA. This will result in loss of mutant mtDNA in some cells, providing protection for those specific cells. However, the cells that accumulate mutant mtDNA will over time develop biochemical defects. Clonal expansion also happens in postmitotic cells, explaining the accumulation of mutant mtDNA with aging in muscle cells and neurons (Greaves et al., 2012). The rapidly aging world population has generated great interest in the involvement of mitochondria in aging, and the mechanisms connecting mtDNA mechanisms to aging are central to understanding the exact roles of mitochondria in healthy aging.

2.4. Maternal inheritance

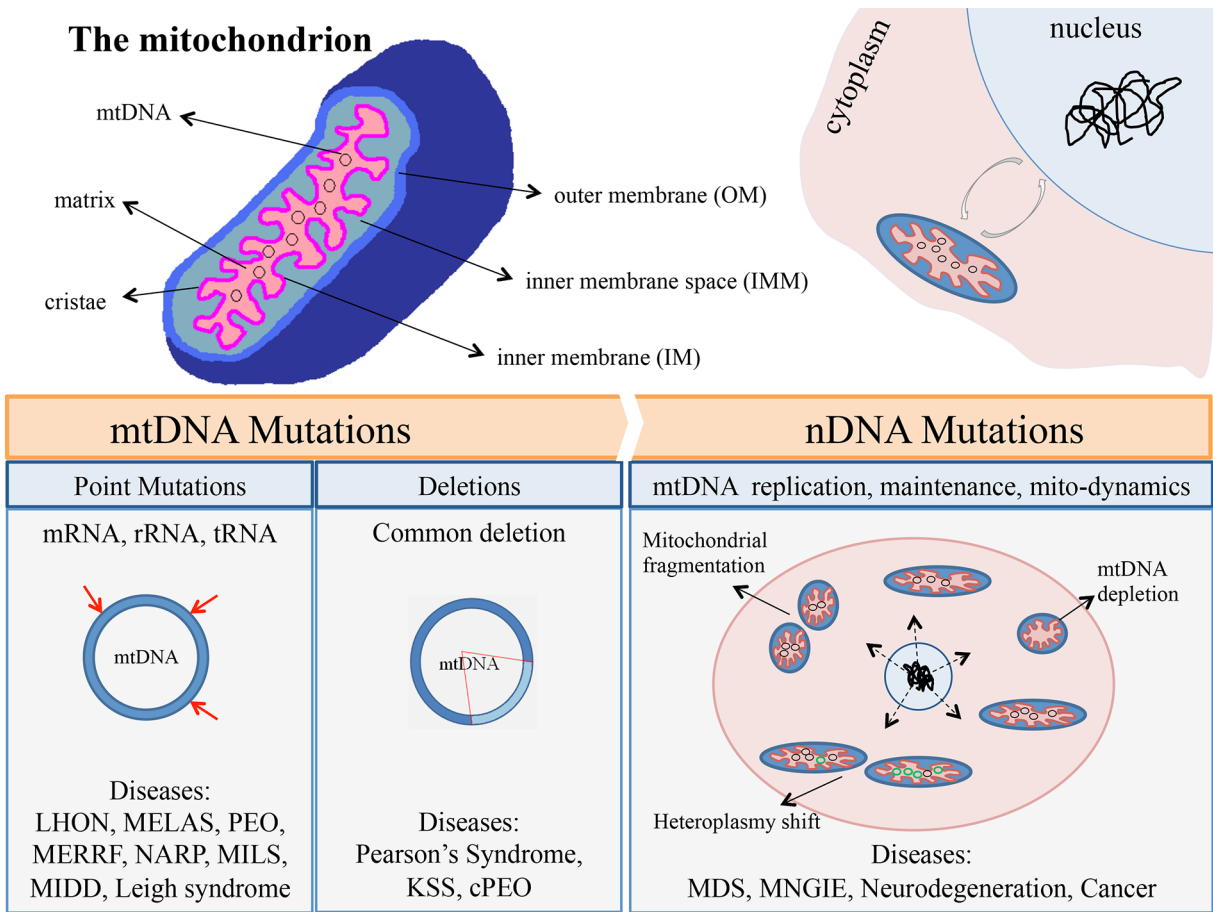
Unlike nDNA, mtDNA is transmitted to offspring uniparentally, in most animals from the mother (Giles et

al., 1980). Maternal inheritance is widely accepted among researchers; however, some published data suggest that a limited degree of paternal inheritance can occur. For example, some studies have suggested paternal inheritance of a ND2 gene mutation (Schwartz and Vissing, 2002). The existence of a low level of paternal inheritance has been supported by subsequent studies and might account for some rare familial mtDNA diseases with no identified cause (Danan et al., 1999; Marchington et al., 2002; Filosto et al., 2003; Taylor et al., 2003b; Schwartz and Vissing, 2004). However, maternal inheritance is far more likely and should be examined as the more likely option to track the familial history of the mutation.

3. Types and mechanisms of mtDNA dysfunction

3.1. Primary mutations

MtDNA point mutations and deletions can be classified as primary mtDNA disorders (Figure 1). MtDNA point mutations can occur in mt-tRNA, mt-rRNA, or mt-mRNA. The clinical symptoms of mtDNA point mutations



**Figure 1.** nDNA and mtDNA mutations associated with mtDNA-related diseases. Mitochondria are surrounded by two membranes, as shown in the upper left image. MtDNA is located within the matrix. A continuously cross-talk between mitochondria and nuclei (upper right panel) is required for mitochondrial function. Mutations both in mtDNA and nDNA can result in mtDNA-related diseases. Schematic representations of types of nDNA and mtDNA mutations that result in mtDNA-related diseases are listed in the lower panels.

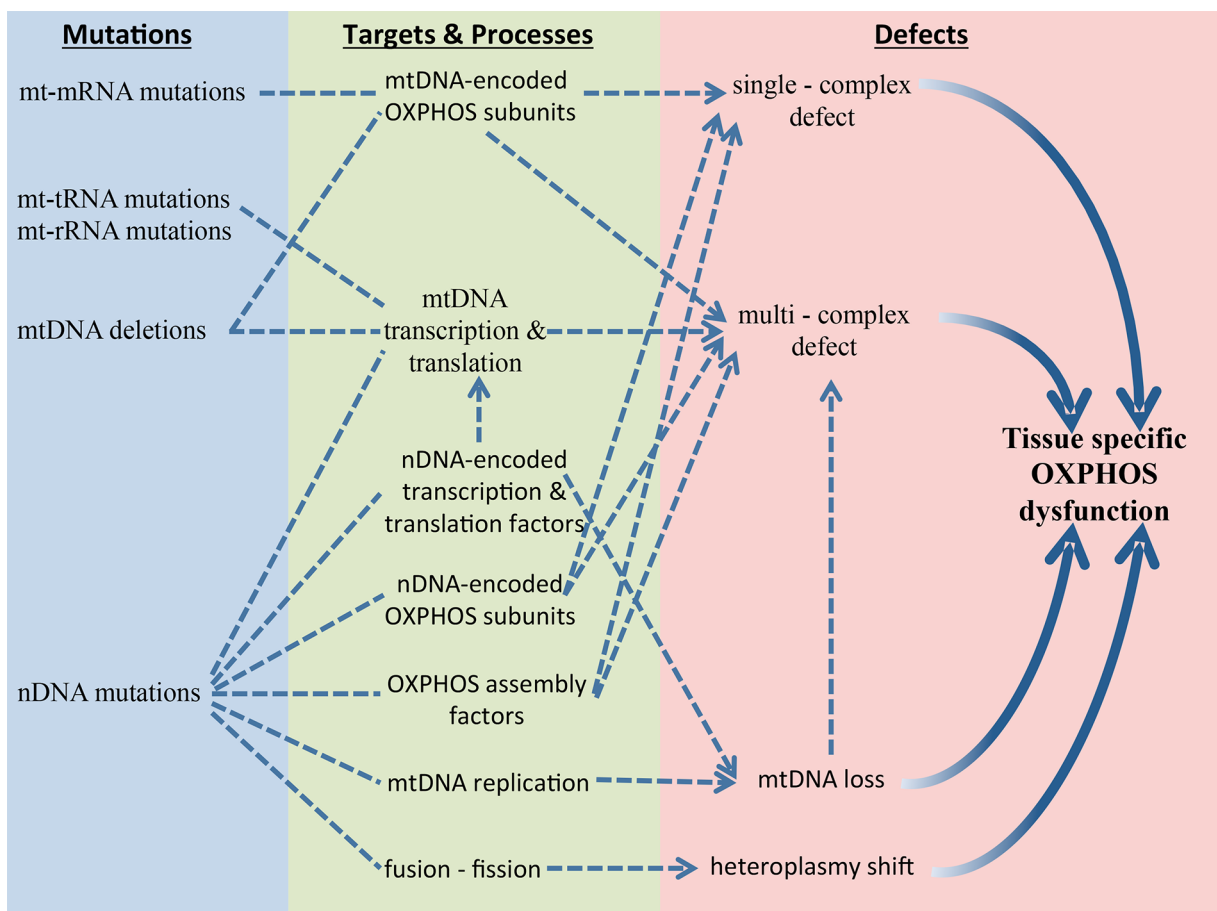
are heterogeneous, and in some cases the same mutation can result in different symptoms. Most point mutations that result in a clinical phenotype occur in mt-tRNA and usually result in an overall reduction in mitochondrial protein synthesis (Mariotti et al., 1994). In contrast, mt-mRNA mutations usually (but not always) result in specific respiratory complex defects (Figure 2). The disease-causing heteroplasmy threshold can differ depending on the type of mutation, with mtDNA deletions usually having lower disease-causing threshold than mt-tRNA mutations (approximately 60% vs. 80%; Kirino et al., 2004; Taylor and Turnbull, 2005).

### 3.2. Point mutations

In general, point mutations are heteroplasmic and highly recessive. However, there are reports of homoplasmic mtDNA point mutations affecting a single tissue and characterized by incomplete penetrance (McFarland et al., 2002; Taylor et al., 2003a; Temperley et al., 2003; McFarland et al., 2004; McFarland et al., 2007; Yang et al.,

2009). The three most common homoplasmic mutations, causing Leber's hereditary optic neuropathy (LHON), are m.11778G > A, m.3460G > A, and m.14484T > C (McFarland et al., 2007).

Here we focus on the three most common heteroplasmic mutations observed in patients. The commonest mt-tRNA mutation is the m.3243A > G mutation on mt-tRNA<sup>Leu(UUR)</sup>, first characterized in a subgroup of patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (Goto et al., 1990). More recent studies reported that this mutation mostly results in maternally inherited diabetes and deafness (MIDD) (Nesbitt et al., 2013). The same study showed that this mutation can result in progressive external ophthalmoplegia (PEO) and that some affected individuals have overlapping symptoms (i.e. MELAS + MIDD, MELAS + PEO, MIDD + PEO). Furthermore, 9% of carriers show no clinical phenotype, and the high levels of heterogeneity makes diagnosis of the disease very challenging (Nesbitt et al., 2013).



**Figure 2.** Mechanisms of nDNA and mtDNA mutations associated with mtDNA-related diseases: mutations resulting in mtDNA-related diseases can either occur on mtDNA or nuclear genome (left, blue panel). These mutations have multiple targets, which can affect various processes (middle, green panel). These can result in a single-complex or multiple-complex defect or mtDNA loss or a shift in heteroplasmy; in all cases, OXPHOS activity is disrupted (right, red panel).

The second most common heteroplasmic point mutation is also on a tRNA gene. The m.8444A > G substitution in mt-tRNA<sup>Lys</sup> (Shoffner et al., 1990) mostly results in myoclonic epilepsy with ragged red fibers (MERFF) and is also linked to some cases of a MIDD-like syndrome (Santorelli et al., 1996a). Comparison of the m.32343A > G and m.8444A > G mutations highlights the clinical heterogeneity of mtDNA-related diseases, although both cause OXPHOS defects through a decreased protein synthesis rate (Yasukawa et al., 2000).

Clinically heterogeneous disease states also result from an amino acid substitution (Leu 156 Arg) in ATP synthase subunit 6, caused by the m.8993T > G mutation (Santorelli et al., 1996b). This mutation is mainly linked to two diseases: NARP (neuropathy, ataxia, retinitis pigmentosa) and MILS (maternally inherited Leigh's syndrome). Interestingly, the mutation causes NARP at 90%–95% heteroplasmy and causes MILS at higher heteroplasmy rates (Tatuch et al., 1992).

### 3.3. Deletions

MtDNA deletions result from defects in mtDNA replication, maintenance, and repair (Kaukonen, 2000; Spelbrink et al., 2001). MtDNA deletions mostly occur between origins of replication ( $O_H$  and  $O_L$ ) and regions flanked by tandem repeat sequences (Schon et al., 1989; Krishnan et al., 2008). Deletions are normally sporadic, occur in the germline, and are not transmitted to offspring (Chinnery et al., 2004). The size of large-scale mtDNA deletions varies, but many patients have a ~5-kb 'common-deletion' that spans the ATPase8 and ND5 genes as represented in Figure 1 (Larsson and Holme, 1992).

The main determinants of clinical severity of a deletion seem to be its abundance and tissue distribution, rather than the size and location of the deletion itself (Tuppen et al., 2010). However, recent studies also suggest a correlation among deletion size, heteroplasmy, and clinical severity (Grady et al., 2014).

MtDNA deletions are frequently associated with three syndromes: Pearson syndrome, Kearns–Sayre syndrome (KSS), and chronic progressive external ophthalmoplegia (cPEO) (Zeviani et al., 1988; Moraes et al., 1989; Rotig et al., 1990). Moreover, many studies have shown that mtDNA deletions accumulate with aging and are mainly linked to nervous system disorders (Cortopassi et al., 1992; Bender et al., 2006; Kraytsberg et al., 2006). Increased levels of mtDNA deletions have been found in the brains of elderly individuals and in heart tissue affected by coronary atherosclerosis (Corral-Debrinski et al., 1992a, 1992b).

### 3.4. mtDNA copy number maintenance

In addition to direct mutations in mtDNA, mtDNA dysfunction can also be caused by mutations in nDNA (Figure 1). MtDNA-related nuclear genes that cause disease mainly affect mtDNA replication and copy number

maintenance, and they fall into two categories: those that directly affect the mtDNA replication fork and mutations in genes that are involved in the supply of nucleotides for mtDNA replication (such as mitochondrial thymidine kinase TK2, the deoxyguanosine kinase dGK, and adenine nucleotide transporter ANT1) (Copeland, 2008). The effects of these mutations are sometimes called secondary mtDNA defects. mtDNA copy number is regulated in accordance with the energy demand of the cell. Cells under a high energy demand, like muscle cells and neurons, thus need to maintain high numbers of healthy and functional mtDNA. This homeostasis can be disrupted either by increased replication of mtDNA or by targeted degradation of damaged mtDNA molecules (Szkarczyk et al., 2014). There is some supporting evidence that oxidative stress can increase mtDNA degradation (Shokolenko et al., 2009; Furda et al., 2012).

Copy number maintenance is central to healthy aging. mtDNA copy number decreases with aging in different tissues, including pancreatic islets, skeletal muscle, cerebral cortex, and heart muscle (Frahm et al., 2005; Short et al., 2005; Cree et al., 2008a). Similar observations have been reported in model organisms, such as an age-related decrease in mtDNA copy number in the rat CNS (McInerney et al., 2009). However, the impact of mitochondrial dysfunction on aging varies between species such as mice and humans (Greaves et al., 2011). One study of two well-known mtDNA-related diseases, MELAS and MERRE, showed an aging-related reduction in mtDNA levels in leukocytes (Liu et al., 2006). Mutations in the OPA1 gene, whose function is to induce mitochondrial fragmentation, result in dominant optic atrophy (Alavi and Fuhrmann, 2013). There are conflicting results concerning whether this mutation results in mtDNA loss or mtDNA proliferation (Iommarini et al., 2012; Sitarz et al., 2012). Further work is needed to determine the mechanisms of mtDNA replication and their involvement in disease progression.

The factors needed for mtDNA replication are completely encoded by nDNA. This adds another level of complexity to the maintenance of a healthy mtDNA population. MtDNA polymerase  $\gamma$  (Pol  $\gamma$ ), a heterodimeric protein consisting of a catalytic subunit (PolgA) and a processivity subunit (polgB), is the exclusive mediator of mtDNA polymerase activity (Carrodeguas et al., 2001). Mitochondrial DNA helicase, also called Twinkle, is responsible for 5'-3' mtDNA helicase activity, and mitochondrial single-strand binding protein (mtSSB) is responsible for the stabilization of the single strand once the double strand is unwound (Spelbrink et al., 2001). Together with TFAM these proteins form the mitochondrial replisome, which can be reconstituted in vitro (Korhonen et al., 2004). The mechanism of mtDNA replication is



not fully defined. There are mainly two models: strand displacement and strand coupling (Clayton, 1982; Holt et al., 2000). Both models have been critically appraised, but a detailed description of this area is beyond the scope of this review (Bogenhagen and Clayton, 2003b, 2003a; Holt and Jacobs, 2003). Pol  $\gamma$  is one of the most widely studied nuclear genes involved in mtDNA disorders because of its high error rate of 1:50,000 bp (Longley et al., 2001). More than 150 mutations in Pol  $\gamma$  have been identified in patients with mtDNA disorders (Human DNA Pol Gamma Mutation Database, <http://tools.niehs.nih.gov/polg/>). These mutations can be found in all domains of Pol  $\gamma$  and can cause PEO, Alpers syndrome (Naviaux and Nguyen, 2004), ataxia neuropathy (Van Goethem et al., 2004), and male infertility (Rovio et al., 2001). A detailed overview of Pol  $\gamma$  is available in the Human DNA Pol Gamma Mutation Database (<http://tools.niehs.nih.gov/polg/>).

### 3.5. mtDNA depletion syndrome

Loss of mtDNA in tissues is usually identified as mtDNA depletion syndrome (MDS). General clinical symptoms include muscle weakness, progressive encephalopathy, liver failure, and disturbed function of the skeletal muscle, kidneys, and brain (Rahman and Poulton, 2009). MDS was first characterized as an autosomal recessive disorder (Moraes et al., 1991) and is widely reported in childhood respiratory deficiency syndromes (Sarzi et al., 2007).

The genetic alterations leading to MDS commonly affect the mtDNA replication components (Pol  $\gamma$ , Twinkle, TFAM) (Spelbrink et al., 2001), but they can also involve genes required for the supply of sufficient dNTPs for mtDNA replication. dNTPs are supplied to mitochondria either by active transport of cytosolic dNTPs or through a salvage pathway that mainly requires TK2 (thymidine kinase 2) (Suomalainen and Isohanni, 2010). Mutations in TK2 have been reported as the most common cause of MDS (Saada et al., 2001). MDS can also be triggered by mutations in dGK (Mandel et al., 2001) or ribonucleotide reductase (Bornstein et al., 2008), and another enzyme important for maintaining the dNTP pool, TP (thymidine phosphorylase), causes the mtDNA-related disease MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) (Nishino et al., 1999).

## 4. Clinical, biochemical features, and diagnosis of mtDNA-related diseases

### 4.1. Clinical assessment and examination

The diagnosis of mtDNA-related diseases is quite complex, which should be extensive. The age of onset of mtDNA-related diseases is variable and is determined primarily by the severity of the defect and the mutation load and to a lesser extent by environmental and nuclear genetic modifier factors. It is common to classify mtDNA-related diseases into two groups: 'early infancy and childhood' vs.

'late childhood and adulthood'. Leigh syndrome, mtDNA depletion syndromes, KSS, and Pearson syndrome fall into the first group, while mitochondrial encephalopathy, MELAS, cPEO, neuropathy, NARP, LHON, and MERRF are usually observed in late childhood and adulthood (Tuppen et al., 2010). The high degree of tissue heterogeneity is another hallmark of mtDNA-related diseases. Neurological, gastrointestinal, cardiac, respiratory, endocrinal, ophthalmological, hematological, renal, muscular system, and liver failure are involved in mtDNA-related diseases (Vafai and Mootha, 2012).

In order to assess the clinical situation of a patient, the clinician should carry out various tests on the possible organ systems that could be involved in the disease. One group of these tests is based on imaging. Cardiac failure can be investigated by electrocardiography (ECG), electromyography (EMG) can reveal muscular defects, and computed tomography (CT) and magnetic resonance imaging (MRI) findings can help to evaluate the patients' condition (Menezes et al., 2014). Certain blood-based biochemical tests have shown promise as diagnostic tools for mitochondrial disorders. Measuring the level of lactate, pyruvate, creatine kinase, total blood count, thyroid and liver function, bone chemistry, and blood glucose levels can be informative (Naviaux, 2004). For example, it is common to observe high lactate and pyruvate levels in patients with mitochondrial disorders as a result of a compensatory mechanism to overcome ATP deficit; however, this is not always observed in LHON and KSS. Organic acids and amino acid profiles measured by chromatography, mass spectrometry, or HPLC can aid the observations. Although a clear correlation is missing, an increase in alanine, proline, glycine, sarcosine, and tyrosine can be observed in mtDNA-related diseases. In addition, novel biomarker discoveries are expected to help towards the diagnosis of mitochondrial disorders, thereby decreasing the diagnostic work currently required. So far, cytokine FGF-21 (fibroblast growth factor-21) has been found to be a biomarker for muscle-specific mitochondrial diseases (Suomalainen et al., 2011).

### 4.2. Histopathology

Regardless of the underlying mutation, all mtDNA-related disorders result in defects in oxidative phosphorylation as shown in Figure 2 (Betts et al., 2004). However, tissue heterogeneity and specificity remain the major reasons for the need of detailed analysis of mitochondrial function in different tissues, if possible. In order to carry out a more extensive assessment, a muscle biopsy is needed since this tissue mostly, but not always, shows a defect in most of the mtDNA-related diseases (Janssen et al., 2003). Effective communication with the patient is needed at this stage in order to obtain a muscle biopsy sample together with a fibroblast sample, both of which can be

used for subsequent analysis. One of the most established techniques is the staining of the muscle fibers with Gomori trichrome, which results in ragged red fibers (RRFs), which reflect the abnormal subsarcolemmal accumulation of mitochondria (Tuppen et al., 2010). The most common and penetrant phenotype is COX (cytochrome c oxidase) deficiency, first reported in chronic PEO patients' fibers (Johnson et al., 1983). COX/SDH (succinate dehydrogenase) immunohistochemical double-staining is commonly used to classify normal versus deficient cells in mitochondrial diseases. SDH staining shows a normal pattern in disorders caused only by mtDNA defects but not by nDNA (so-called primary mtDNA mutations), since all subunits of complex II are encoded by nDNA. Therefore, SDH staining can be mainly used to identify complex II defects. COX staining can result in a mosaic pattern, or in some cases, global and uniform decrease is observed. Mosaic COX deficiency highlights heteroplasmy differences; on the other hand, uniform COX defects are related to nDNA mutations mainly encoding COX subunits and assembly factors (e.g., SURF1, SCO2) (Zhu et al., 1998). It should also be noted that COX deficiency may be observed upon aging since there is an accumulation of mtDNA mutations by age (Brierley et al., 1998). Such cases should be classified in the context of aging rather than specific mtDNA-related diseases. Finally, more analysis can be made by measuring mitochondrial ultrastructure using electron microscopy and respiratory chain complex activities from isolated mitochondria; however, these also depend on the availability of the materials and resources. A more detailed explanation of clinical symptoms and histopathological findings was reviewed elsewhere (Taylor and Turnbull, 2005; Greaves et al., 2012).

#### 4.3. DNA-based diagnosis

The detailed investigation of the family history of the patient may reveal the genetic defect of the individual and help to differentiate whether it is mtDNA or nDNA related, since mtDNA has a maternal inheritance. In detail, observation of mtDNA deletions usually reflects a defect in mtDNA maintenance genes (e.g., PolG, PEO1, OPA1) and mtDNA depletion reflects defective mtDNA replication related to the replication genes (PolG, mtSSBP, Twinkle, Tfam) or genes involved in the supply of the dNTP pool (TK2, dGK). In some cases, the clinical phenotypes may reflect a common mutation causing well-defined diseases such as MELAS (m.3243A>G) or MERRF (m.8344A>G). In such cases, a direct sequencing of the genetic location or RFLP can be applied. Additionally, the heteroplasmy levels of these mutations should be determined either by RFLP or TaqMan real-time PCR analysis. Furthermore, long-range PCR or real-time PCR can be used to detect the mtDNA deletions (He et al., 2002). These primary

mtDNA defects were discussed in the earlier sections of this study. Furthermore, mtDNA depletion, caused by nDNA-encoded gene mutations (for details, see Section 3), can be detected by real-time PCR. There are many cases where the disease-causing mutation on mtDNA has not been defined before. Direct sequencing of the whole mtDNA can provide much information. However, care should be taken while choosing the candidate mutation and a pathogenic disease causing mutation. The criteria for this were published by DiMauro and Schon and we refer their work for this purpose (DiMauro and Schon, 2001).

The improvements in sequencing technologies allowed the usage of next-generation sequencing (NGS) as a new way to diagnose mitochondrial diseases. Recent figures show that NGS allowed not only to assign already characterized mutations to patients but also to discover novel mutations (Menezes et al., 2014). Whole-genome sequencing (WGS) is still very expensive to be used on a daily basis, but whole-exome sequencing (WES) has been used commonly in recent years. Although intronic defects can be linked to some diseases, most genetic diseases are caused by mutations present in exons (coding regions), which correspond to less than 2% of the whole genome. By using WES, all of the variants in exons can be detected in a single experiment rather than in several sequencing runs. The difficulty in this is to assign a variant as a disease-causing variant. Here, we refer to a recent review published by Suomalainen's group for details and criteria of variant identification (Carroll et al., 2014). A more targeted approach for mitochondrial diseases can be taken by using MitoExome, which has been proven to detect both known and novel mutations causing mitochondrial diseases. Independent studies have shown that this methodology can be applied successfully (Calvo et al., 2012; Vasta et al., 2012; Lieber et al., 2013). All these advances in sequencing resulted in characterizing around 50 novel mutations, although recent reports highlight that more improvements are needed. Two independent studies carried out at hospitals in Japan and the United States show that for around 50% of the WES cases, the functional pathogenic (disease-causing) variant could not be determined (Lieber et al., 2013; Ohtake et al., 2014). Better comparative analyses will be made possible by including more sequencing data of more cases (especially control for nonmitochondrial diseases). More reliable sequencing reactions are also needed together with efficient bioinformatics tools. In any case, assigning a highly probable variant (mutation) as a disease-causing variant remains a challenge. Therefore, functional studies on cells (depending on the availability) should be performed before making a decision about a causative DNA variant (Danhauser et al., 2011).

## 5. Potential treatment strategies

### 5.1. Treatments to improve quality of life

Improving the quality of life of the patients should be the primary goal to be achieved right after the diagnosis. It has been shown that exercise therapy can help this; however, it neither stops nor prevents the occurrence of the disease. Aerobic training proved to improve OXPHOS activity and exercise capacity of patients with mitochondrial myopathy (Jeppesen et al., 2006). Furthermore, endurance exercise improved mitochondrial myopathy symptoms in a mouse model of the disease (Wenz et al., 2009).

Controlled and optimized diet can also help to improve quality of life. A ketogenic diet, rich in fat and low in glucose, results in high amounts of mitochondrial beta-oxidation and ketone body production, which has been shown to slow the progression of mitochondrial myopathy in mice (Ahola-Erkkila et al., 2010). Other multiple studies have shown various phenotypically beneficial effects such as reduction in epileptic seizures, improved brain energy metabolism, inhibition of ROS production, and increased neuroglia interaction and ATP concentrations (Khan et al., 2015). Furthermore, a ketogenic diet was found to decrease the level of deleted mtDNA copies, causing a shift in heteroplasmy, in cultured human cells (Santra et al., 2004).

Most treatment strategies for mtDNA-related diseases follow the guidelines published by the Wellcome Trust Centre for Mitochondrial Research in Newcastle, UK (<http://www.newcastle-mitochondria.com/service/patient-care-guidelines/>). The discovery of mtDNA mutations and progress in our understanding of disease mechanisms have paved the way toward potential cures for mtDNA-related diseases. Experimental animal models have been very informative in defining and tailoring treatment strategies. Animal models of mtDNA-related disorders are described in a number of valuable and detailed reviews (Oliveira et al., 2010; Palladino, 2010; Dogan and Trifunovic, 2011; Dunn et al., 2012).

### 5.2. Pharmacological treatments

Potential therapeutic strategies are focused on the positive effects of pharmacological and biochemical agents observed in cellular and animal models. One of the frequently observed outcomes of mtDNA-related disorders is a change (usually an increase) in the level of oxidative stress. Mitochondria are a major producer of cellular ROS and multiple mtDNA mutations have been shown to result in increased ROS. The neurodegeneration phenotype observed in LHON patients was found to be caused by increased level of ROS. Creation of cybrid lines harboring 11,778 and 3460 mutations and subsequently differentiating them into neurons resulted in increased ROS (more in 3460 mutations); however, undifferentiated cells did not show any difference (Wong et al., 2002).

Two NARP-causing mutations at the same location (m8993T>G and m8993T>C) are reported to cause increased ROS production and SOD activity (Baracca et al., 2007). It is worth further emphasizing that although these two mutations occur at the same location, the level of increase in ROS is different. Furthermore, increased ROS production was observed in different tissues (heart and brain) in a heteroplasmic mouse model harboring the commonly observed m3243A>G tRNA mutation (Li et al., 2008). In view of the focus of this special issue on 'Melatonin and Mitochondria Interact in Diseases', we detail here some of the recent advances on the potentially beneficial effects of melatonin, and specifically mtDNA-related disorders.

The hormone melatonin, secreted by the pineal gland, is a potent free-radical scavenger and regulates redox-active enzymes (Galano et al., 2011). Melatonin is also regarded as a possible mitochondrial protector and can restore mitochondrial function in a mouse model of Alzheimer disease involving a transgenic mutant form of APP (amyloid precursor protein) (Dragicevic et al., 2011). More information related to the roles of melatonin in neurodegenerative process can be found in the literature (Pandi-Perumal et al., 2013). A recent study was carried out on NARP cybrids harboring 98% heteroplasmy in the m.8993T > G mutation, which is associated with a defect in F1F0-ATP synthase. Melatonin has been reported to have the potential to rescue heart function in patients after myocardial ischemia/reperfusion (Huang et al., 2013), a conclusion supported by similar studies (Peng et al., 2012). As explained earlier, the ~5-kb 'common-deletion' is widely observed in patients with mtDNA-related diseases. The increased mitochondrial ROS (mROS) production associated with the common deletion can be rescued by melatonin, preventing mROS-mediated depolarization of membrane potential (Jou et al., 2007).

Furthermore, the lifespan-extending effect of rapamycin (Bonawitz et al., 2007; Pan et al., 2012) has been shown to have a mtDNA origin (Villa-Cuesta et al., 2014). This further highlights the complex dual control of mitochondrial homeostasis. Potential dietary therapeutic approaches have also explored the potential of alpha-ketoglutarate/aspartate supplements (Sgarbi et al., 2009). Continuing research in this area will provide additional or complementary therapeutic opportunities.

Similar approaches have been used to study the potential benefits other metabolites and vitamins, etc. Frequently cocktails of various cofactors and vitamins are being used. The choice of these agents is usually made if the molecule under question is defective, less produced, or mutated or if its transport is being impaired. Here we summarize most of the molecules used for the treatment of mitochondrial diseases. Coenzyme Q10 (CoQ10, ubiquinone) is part of the electron transport chain and it is also a ROS



scavenger (Ernster and Dallner, 1995). CoQ10 deficiency is commonly observed in various different mitochondrial diseases and CoQ10 supplementation has been overall successful (Quinzii and Hirano, 2010). Riboflavin (water-soluble vitamin B, B<sub>2</sub>) is a precursor of flavoprotein, which has key roles for complex I, complex II, the Krebs cycle, and fatty acid oxidation. Therefore, riboflavin is usually supplied in complex I and complex II diseases (Bernsen et al., 1993; Bugiani et al., 2006). Creatine is found at high concentrations in energy-demanding tissues such as the muscle and brain. It can be classified as an energy-boosting compound since it forms phosphocreatine, which is a source of high-energy phosphate. It has been used in combination with CoQ10 (Dimauro and Rustin, 2009). L-Arginine, which is a semiessential amino acid, has been successfully used for treatment of MELAS (Koga et al., 2002). Carnitine, which has a key role in fatty acid metabolism, is used to increase free carnitine levels and is usually used together with CoQ10 (Marriage et al., 2004). Dichloroacetate (DCA) is a lactic acid-lowering molecule that can be targeted to patients with increased lactate or lactic acidosis. However, a double-blinded MELAS study showed toxic effects of DCA, which resulted in decline of its use (Kaufmann et al., 2006). Idebenone is an analogue of CoQ and has been used in LHON patients with some degree of success (Carelli et al., 2011). Vitamins B1, C, and E and alpha-lipoic acid, dimethylglycine, and whey-based glycine are other molecules in use. For a detailed review of each of these molecules we refer to the very valuable work of others (Parikh et al., 2009; Kanabus et al., 2014). Compounds undergoing clinical trials between 1997 and 2012 were also recently reviewed (Kerr, 2013).

There are other pharmacological agents present on the verge of clinical trials for the treatment of mitochondrial diseases. One group of such agents with promising outcomes are focused on increasing mitochondrial biogenesis via PGC1 $\alpha$ , PPARs, NRF1/2, YY1, and ERRs (Andreux et al., 2013). Bezafibrate, which is a synthetic ligand of PPAR $\alpha$ , has been proposed to be a new pharmacological agent supported by several positive results obtained in mouse models (Kanabus et al., 2014). A clinical trial of bezafibrate in mitochondrial disorders is missing, although promising observations were made in a clinical trial of a fatty acid oxidation disorder called carnitine palmitoyl transferase 2 (CPT2) deficiency (Bonfont et al., 2010). Another candidate targeting mitochondrial biogenesis is resveratrol, which is an activator of sirtuin SIRT1. Until now, resveratrol has only been included in a clinical trial of healthy elderly individuals (rather than mitochondrial disease patients), and increased mitochondrial function was observed (Libri et al., 2012). Finally, AICAR, which is an AMPK activator, was tested in human complex I-deficient fibroblasts and promising

results were obtained, such as increased ATP levels and decreased ROS (Golubitzky et al., 2011). In summary, very detailed clinical trials are needed in order to determine the benefits of using mitochondrial biogenesis activators as new therapeutic agents.

In addition to ROS, other toxic substances such as hydrogen sulfide (H<sub>2</sub>S) are also the target of some potential treatments. It has been shown that NAC and metronidazole treatment is useful for ethylmalonic encephalopathy (Viscomi et al., 2010). Furthermore, targeting mitochondrial dynamics, autophagy, and mitochondrial membrane lipids are other possibilities that need to be investigated at the preclinical level (Andreux et al., 2013).

### 5.3. Gene therapy

Current treatment strategies are aimed at ameliorating symptoms and reducing the risk of complications. Cure and prevention of mtDNA-related diseases, however, requires more effective treatments. The primary goal for an effective gene therapy of mtDNA-related diseases is to alter the level of heteroplasmy, either by targeted degradation of mutant mtDNA or by blocking replication (Taylor et al., 1997).

The earliest gene therapy trials, started in the late 1990s, tested the potential of mitochondria-targeted peptide nucleic acid (PNA) oligomers, which are DNA-like molecules with an aminoglycine backbone instead of phosphate ribose groups. In vitro assays showed that this technique could prevent replication of mutant mtDNA copies; however, cell line studies were less successful (Chinnery et al., 1999).

The next approach tried was to target mitochondrial genes with endonucleases. The m.8993T > G mutation is known to generate a specific SmaI endonuclease cleavage site. Therefore, delivery of SmaI to mitochondria containing the mutant mtDNA has the potential to deplete mutant mtDNA copies and result in biochemical rescue (Tanaka et al., 2002). Further advances in this field have been made possible by the use of chimeric zinc finger methylases (Minczuk et al., 2006). Use of specific zinc finger peptides (ZFPs) to target the m.8993T > G mutation resulted in reduction of heteroplasmy over a 30-day period (Minczuk et al., 2008). More recently, the development of transcription activator-like effectors (TALEs) and TALE nucleases (TALENs) has produced very promising results for mtDNA gene therapy (Boch et al., 2009; Hockemeyer et al., 2011). The use of TALENs resulted in reductions in heteroplasmy from 70% to 30% without affecting mtDNA copy number (Bacman et al., 2012). Supporting results have also been reported in a cybrid cell model of the common deletion (Gammage et al., 2014). Detailed analysis of these improvements are needed, and these techniques and their potential uses were critically evaluated in recent reviews

(Kaufmann et al., 2013; Moraes et al., 2014). Furthermore, it has been reported recently that the transmission of mtDNA mutations can be prevented in the germline by the use of TALENs to eliminate mutated mtDNA copies (Reddy et al., 2015). The same group also adapted the 'MitoTALENs' technology, so they can be used by viral delivery vectors (Hashimoto et al., 2015). The common expectation in the field is to start testing this technology on patients who are waiting to be treated.

#### 5.4. Genetic counseling

One of the most ideal strategies is to prevent mtDNA-related disease before it occurs. Therefore, genetic counseling and prenatal diagnosis have started to be applied. Informing parents about the real risk of having a child with a mitochondrial disease should be done by professionals.

Mechanism of transmission and segregation of mtDNA species (mutated and nonmutated) in the germline is not fully understood; therefore, prenatal genetic diagnosis resulted in limited success (Poulton et al., 2009). Although it is not possible to estimate heteroplasmy levels in individual tissues, and therefore not possible to predict clinical outcomes, prenatal genetic diagnosis has reportedly been applied successfully in several cases (Bouchet et al., 2006). Due to these uncertainties, parents are aiming to have offspring without any mutated mtDNA copies by the help of preimplantation genetic diagnosis (PGD) (Hellebrekers et al., 2012).

#### 6. Concluding remarks

Mitochondria are central to many processes in the cell, and disturbances to ATP metabolism trigger a wide range of symptoms. Increasing evidence has implicated mtDNA

defects in numerous diseases such as LHON, MELAS, PEO, NARP, MILS, KSS, and Pearson syndrome and also in broader diseases such as neurodegeneration and cancer. A healthy mtDNA population is vital for the maintenance of efficient ATP production. However, further detailed research into the mechanisms of mtDNA-related diseases is required to develop effective diagnostic and treatment options. Recent advances in NGS discussed here will be particularly useful for effective diagnosis of patients. More detailed clinical trials incorporating mtDNA-related targets promise optimized pharmacological treatments in the coming years. Recent improvements in gene therapy are very promising, especially the use of TALENs, which has been successfully reported for the treatment of LHON and NARP. The first clinical trials of gene therapy are expected to start in the next few years. Global, multidisciplinary, and collaborative work on mtDNA-related diseases will be fundamental for the discovery of effective diagnostic and therapeutic strategies.

#### Acknowledgments

We would like to thank Dr Manuel José Gómez Rodríguez for the useful suggestions on the manuscript. This study was supported by grants from the Ministerio de Economía y Competitividad (SAF2012-1207 & CSD2007-00020), the Comunidad de Madrid (CAM/API1009), the EU (Mitochondrial European Educational Training, MEET: European Commission Seventh Framework Programme, FP7-PEOPLE-2012-ITN MARIE CURIE, grant agreement No. 317433), and the Instituto de Salud Carlos III (FIS grant PI11-00078). The CNIC is supported by the Ministerio de Economía y Competitividad and the Pro-CNIC Foundation.

#### References

- Ahola-Erkila S, Carroll CJ, Peltola-Mjosund K, Tulkki V, Mattila I, Seppanen-Laakso T, Oresic M, Tynismaa H, Suomalainen A (2010). Ketogenic diet slows down mitochondrial myopathy progression in mice. *Hum Mol Genet* 19: 1974–1984.
- Alavi MV, Fuhrmann N (2013). Dominant optic atrophy, OPA1, and mitochondrial quality control: understanding mitochondrial network dynamics. *Mol Neurodegener* 8: 32.
- Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F et al. (1981). Sequence and organization of the human mitochondrial genome. *Nature* 290: 457–465.
- Andreux PA, Houtkooper RH, Auwerx J (2013). Pharmacological approaches to restore mitochondrial function. *Nat Rev Drug Discov* 12: 465–483.
- Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 23: 147.
- Bacman SR, Williams SL, Duan D, Moraes CT (2012). Manipulation of mtDNA heteroplasmy in all striated muscles of newborn mice by AAV9-mediated delivery of a mitochondria-targeted restriction endonuclease. *Gene Ther* 19: 1101–1106.
- Baracca A, Sgarbi G, Mattiazzi M, Casalea G, Pagnotta E, Valentino ML, Moggio M, Lenaz G, Carelli V, Solaini G (2007). Biochemical phenotypes associated with the mitochondrial ATP6 gene mutations at nt8993. *Biochim Biophys Acta* 1767: 913–919.

- Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T et al. (2006). High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* 38: 515–517.
- Bernsen PL, Gabreels FJ, Ruitenbeek W, Hamburger HL (1993). Treatment of complex I deficiency with riboflavin. *J Neurol Sci* 118: 181–187.
- Betts J, Lightowlers RN, Turnbull DM (2004). Neuropathological aspects of mitochondrial DNA disease. *Neurochem Res* 29: 505–511.
- Blok RB, Gook DA, Thorburn DR, Dahl HH (1997). Skewed segregation of the mtDNA nt 8993 (T→G) mutation in human oocytes. *Am J Hum Genet* 60: 1495–1501.
- Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* 326: 1509–1512.
- Bogenhagen DE, Clayton DA (2003a). Concluding remarks: the mitochondrial DNA replication bubble has not burst. *Trends Biochem Sci* 28: 404–405.
- Bogenhagen DE, Clayton DA (2003b). The mitochondrial DNA replication bubble has not burst. *Trends Biochem Sci* 28: 357–360.
- Bonawitz ND, Chatenay-Lapointe M, Pan Y, Shadel GS (2007). Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell Metab* 5: 265–277.
- Bonnefont JP, Bastin J, Laforet P, Aubey F, Mogenet A, Romano S, Ricquier D, Gobin-Limballe S, Vassault A, Behin A et al. (2010). Long-term follow-up of bezafibrate treatment in patients with the myopathic form of carnitine palmitoyltransferase 2 deficiency. *Clin Pharmacol Ther* 88: 101–108.
- Bornstein B, Area E, Flanigan KM, Ganesh J, Jayakar P, Swoboda KJ, Coku J, Naini A, Shanske S, Tanji K et al. (2008). Mitochondrial DNA depletion syndrome due to mutations in the RRM2B gene. *Neuromuscul Disord* 18: 453–459.
- Bouchet C, Steffann J, Corcos J, Monnot S, Paquis V, Rotig A, Lebon S, Levy P, Royer G, Giurgea I et al. (2006). Prenatal diagnosis of myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome: contribution to understanding mitochondrial DNA segregation during human embryofetal development. *J Med Genet* 43: 788–792.
- Boveris A, Oshino N, Chance B (1972). The cellular production of hydrogen peroxide. *Biochem J* 128: 617–630.
- Brierley EJ, Johnson MA, Lightowlers RN, James OF, Turnbull DM (1998). Role of mitochondrial DNA mutations in human aging: implications for the central nervous system and muscle. *Ann Neurol* 43: 217–223.
- Brown WM, George M Jr, Wilson AC (1979). Rapid evolution of animal mitochondrial DNA. *P Natl Acad Sci USA* 76: 1967–1971.
- Bugiani M, Lamantea E, Invernizzi F, Moroni I, Bizzi A, Zeviani M, Uziel G (2006). Effects of riboflavin in children with complex II deficiency. *Brain Dev* 28: 576–581.
- Cagin U, Enriquez JA (2015). The complex crosstalk between mitochondria and the nucleus: what goes in between? *Int J Biochem Cell Biol* 63: 10–15.
- Calvo SE, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, Laskowski A, Garone C, Liu S, Jaffe DB et al. (2012). Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci Transl Med* 4: 118ra110.
- Carelli V, La Morgia C, Valentino ML, Rizzo G, Carbonelli M, De Negri AM, Sadun F, Carta A, Guerriero S, Simonelli F et al. (2011). Idebenone treatment in Leber's hereditary optic neuropathy. *Brain* 134: e188.
- Carrodegua JA, Theis K, Bogenhagen DE, Kisker C (2001). Crystal structure and deletion analysis show that the accessory subunit of mammalian DNA polymerase gamma, Pol gamma B, functions as a homodimer. *Mol Cell* 7: 43–54.
- Carroll CJ, Brilhante V, Suomalainen A (2014). Next-generation sequencing for mitochondrial disorders. *Br J Pharmacol* 171: 1837–1853.
- Chinnery PF, DiMauro S, Shanske S, Schon EA, Zeviani M, Mariotti C, Carrara F, Lombes A, Laforet P, Ogier H et al. (2004). Risk of developing a mitochondrial DNA deletion disorder. *Lancet* 364: 592–596.
- Chinnery PF, Taylor RW, Diekert K, Lill R, Turnbull DM, Lightowlers RN (1999). Peptide nucleic acid delivery to human mitochondria. *Gene Ther* 6: 1919–1928.
- Clayton DA (1982). Replication of animal mitochondrial DNA. *Cell* 28: 693–705.
- Copeland WC (2008). Inherited mitochondrial diseases of DNA replication. *Annu Rev Med* 59: 131–146.
- Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC (1992a). Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nat Genet* 2: 324–329.
- Corral-Debrinski M, Shoffner JM, Lott MT, Wallace DC (1992b). Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. *Mutat Res* 275: 169–180.
- Cortopassi GA, Shibata D, Soong NW, Arnheim N (1992). A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. *P Natl Acad Sci USA* 89: 7370–7374.
- Cree LM, Patel SK, Pyle A, Lynn S, Turnbull DM, Chinnery PF, Walker M (2008a). Age-related decline in mitochondrial DNA copy number in isolated human pancreatic islets. *Diabetologia* 51: 1440–1443.
- Cree LM, Samuels DC, de Sousa Lopes SC, Rajasimha HK, Wonnapijit P, Mann JR, Dahl HH, Chinnery PF (2008b). A reduction of mitochondrial DNA molecules during embryogenesis explains the rapid segregation of genotypes. *Nat Genet* 40: 249–254.

- Danan C, Sternberg D, Van Steirteghem A, Cazeneuve C, Duquesnoy P, Besmond C, Goossens M, Lissens W, Amselem S (1999). Evaluation of parental mitochondrial inheritance in neonates born after intracytoplasmic sperm injection. *Am J Hum Genet* 65: 463–473.
- Danhauser K, Iuso A, Haack TB, Freisinger P, Brockmann K, Mayr JA, Meitinger T, Prokisch H (2011). Cellular rescue-assay aids verification of causative DNA-variants in mitochondrial complex I deficiency. *Mol Genet Metab* 103: 161–166.
- DiMauro S, Rustin P (2009). A critical approach to the therapy of mitochondrial respiratory chain and oxidative phosphorylation diseases. *Biochim Biophys Acta* 1792: 1159–1167.
- DiMauro S, Schon EA (2001). Mitochondrial DNA mutations in human disease. *Am J Med Genet* 106: 18–26.
- Dogan SA, Trifunovic A (2011). Modelling mitochondrial dysfunction in mice. *Physiol Res* 60 (Suppl 1): S61–70.
- Dragicevic N, Copes N, O'Neal-Moffitt G, Jin J, Buzzeo R, Mamcarz M, Tan J, Cao C, Olcese JM, Arendash GW et al. (2011). Melatonin treatment restores mitochondrial function in Alzheimer's mice: a mitochondrial protective role of melatonin membrane receptor signaling. *J Pineal Res* 51: 75–86.
- Dunn DA, Cannon MV, Irwin MH, Pinkert CA (2012). Animal models of human mitochondrial DNA mutations. *Biochim Biophys Acta* 1820: 601–607.
- Ernster L, Dallner G (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1271: 195–204.
- Filosto M, Mancuso M, Vives-Bauza C, Vila MR, Shanske S, Hirano M, Andreu AL, DiMauro S (2003). Lack of paternal inheritance of muscle mitochondrial DNA in sporadic mitochondrial myopathies. *Ann Neurol* 54: 524–526.
- Frahm T, Mohamed SA, Bruse P, Gemund C, Oehmichen M, Meissner C (2005). Lack of age-related increase of mitochondrial DNA amount in brain, skeletal muscle and human heart. *Mech Ageing Dev* 126: 1192–1200.
- Furda AM, Marrangoni AM, Lokshin A, Van Houten B (2012). Oxidants and not alkylating agents induce rapid mtDNA loss and mitochondrial dysfunction. *DNA Repair (Amst)* 11: 684–692.
- Galano A, Tan DX, Reiter RJ (2011). Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J Pineal Res* 51: 1–16.
- Gammage PA, Rorbach J, Vincent AI, Rebar EJ, Minczuk M (2014). Mitochondrially targeted ZFNs for selective degradation of pathogenic mitochondrial genomes bearing large-scale deletions or point mutations. *EMBO Mol Med* 6: 458–466.
- Giles RE, Blanc H, Cann HM, Wallace DC (1980). Maternal inheritance of human mitochondrial DNA. *P Natl Acad Sci USA* 77: 6715–6719.
- Golubitzky A, Dan P, Weissman S, Link G, Wikstrom JD, Saada A (2011). Screening for active small molecules in mitochondrial complex I deficient patient's fibroblasts, reveals AICAR as the most beneficial compound. *PLoS One* 6: e26883.
- Goto Y, Nonaka I, Horai S (1990). A mutation in the tRNA(Leu) (UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 348: 651–653.
- Grady JP, Campbell G, Ratnaik T, Blakely EL, Falkous G, Nesbitt V, Schaefer AM, McNally RJ, Gorman GS, Taylor RW et al. (2014). Disease progression in patients with single, large-scale mitochondrial DNA deletions. *Brain* 137: 323–334.
- Greaves LC, Barron MJ, Campbell-Shiel G, Kirkwood TB, Turnbull DM (2011). Differences in the accumulation of mitochondrial defects with age in mice and humans. *Mech Ageing Dev* 132: 588–591.
- Greaves LC, Reeve AK, Taylor RW, Turnbull DM (2012). Mitochondrial DNA and disease. *J Pathol* 226: 274–286.
- Hashimoto M, Bacman SR, Peralta S, Falk MJ, Chomyn A, Chan DC, Williams SL, Moraes CT (2015). MitoTALEN: A general approach to reduce mutant mtDNA loads and restore oxidative phosphorylation function in mitochondrial diseases. *Mol Ther* (in press).
- He L, Chinnery PF, Durham SE, Blakely EL, Wardell TM, Borthwick GM, Taylor RW, Turnbull DM (2002). Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR. *Nucleic Acids Res* 30: e68.
- Hellebrekers DM, Wolfe R, Hendrickx AT, de Coo IF, de Die CE, Geraedts JP, Chinnery PF, Smeets HJ (2012). PGD and heteroplasmic mitochondrial DNA point mutations: a systematic review estimating the chance of healthy offspring. *Hum Reprod Update* 18: 341–349.
- Hensen F, Cansiz S, Gerhold JM, Spelbrink JN (2014). To be or not to be a nucleoid protein: a comparison of mass-spectrometry based approaches in the identification of potential mtDNA-nucleoid associated proteins. *Biochimie* 100: 219–226.
- Hockemeyer D, Wang H, Kiani S, Lai CS, Gao Q, Cassidy JP, Cost GJ, Zhang L, Santiago Y, Miller JC et al. (2011). Genetic engineering of human pluripotent cells using TALE nucleases. *Nat Biotechnol* 29: 731–734.
- Holt IJ, Harding AE, Morgan-Hughes JA (1988). Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 331: 717–719.
- Holt IJ, Jacobs HT (2003). Response: the mitochondrial DNA replication bubble has not burst. *Trends Biochem Sci* 28: 355–356.
- Holt IJ, Lorimer HE, Jacobs HT (2000). Coupled leading- and lagging-strand synthesis of mammalian mitochondrial DNA. *Cell* 100: 515–524.
- Holt IJ, Miller DH, Harding AE (1989). Genetic heterogeneity and mitochondrial DNA heteroplasmy in Leber's hereditary optic neuropathy. *J Med Genet* 26: 739–743.
- Huang WY, Jou MJ, Peng TI (2013). mtDNA T8993G mutation-induced F1F0-ATP synthase defect augments mitochondrial dysfunction associated with hypoxia/reoxygenation: the protective role of melatonin. *PLoS One* 8: e81546.

- Iommarini L, Maresca A, Caporali L, Valentino ML, Liguori R, Giordano C, Carelli V (2012). Revisiting the issue of mitochondrial DNA content in optic mitochondrial pathies. *Neurology* 79: 1517–1519.
- Janssen AJ, Smeitink JA, van den Heuvel LP (2003). Some practical aspects of providing a diagnostic service for respiratory chain defects. *Ann Clin Biochem* 40: 3–8.
- Jeppesen TD, Schwartz M, Olsen DB, Wibrand F, Krag T, Duno M, Hauerslev S, Vissing J (2006). Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy. *Brain* 129: 3402–3412.
- Johnson MA, Turnbull DM, Dick DJ, Sherratt HS (1983). A partial deficiency of cytochrome c oxidase in chronic progressive external ophthalmoplegia. *J Neurol Sci* 60: 31–53.
- Jou MJ, Peng TI, Yu PZ, Jou SB, Reiter RJ, Chen JY, Wu HY, Chen CC, Hsu LF (2007). Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. *J Pineal Res* 43: 389–403.
- Kanabus M, Heales SJ, Rahman S (2014). Development of pharmacological strategies for mitochondrial disorders. *Br J Pharmacol* 171: 1798–1817.
- Kaufman BA, Durisic N, Mativetsky JM, Costantino S, Hancock MA, Grutter P, Shoubridge EA (2007). The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. *Mol Biol Cell* 18: 3225–3236.
- Kaufmann KB, Buning H, Galy A, Schambach A, Grez M (2013). Gene therapy on the move. *EMBO Mol Med* 5: 1642–1661.
- Kaufmann P, Engelstad K, Wei Y, Jung S, Sano MC, Shungu DC, Millar WS, Hong X, Gooch CL, Mao X et al. (2006). Dichloroacetate causes toxic neuropathy in MELAS: a randomized, controlled clinical trial. *Neurology* 66: 324–330.
- Kerr DS (2013). Review of clinical trials for mitochondrial disorders: 1997–2012. *Neurotherapeutics* 10: 307–319.
- Khan NA, Govindaraj P, Meena AK, Thangaraj K (2015). Mitochondrial disorders: challenges in diagnosis & treatment. *Indian J Med Res* 141: 13–26.
- Kirino Y, Yasukawa T, Ohta S, Akira S, Ishihara K, Watanabe K, Suzuki T (2004). Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. *P Natl Acad Sci USA* 101: 15070–15075.
- Koga Y, Ishibashi M, Ueki I, Yatsuga S, Fukiyama R, Akita Y, Matsuishi T (2002). Effects of L-arginine on the acute phase of strokes in three patients with MELAS. *Neurology* 58: 827–828.
- Korhonen JA, Pham XH, Pellegrini M, Falkenberg M (2004). Reconstitution of a minimal mtDNA replisome in vitro. *EMBO J* 23: 2423–2429.
- Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, Khrapko K (2006). Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat Genet* 38: 518–520.
- Krishnan KJ, Reeve AK, Samuels DC, Chinnery PF, Blackwood JK, Taylor RW, Wanrooij S, Spelbrink JN, Lightowlers RN, Turnbull DM (2008). What causes mitochondrial DNA deletions in human cells? *Nat Genet* 40: 275–279.
- Larsson NG, Holme E (1992). Multiple short direct repeats associated with single mtDNA deletions. *Biochim Biophys Acta* 1139: 311–314.
- Li J, Zhou K, Meng X, Wu Q, Li S, Liu Y, Wang J (2008). Increased ROS generation and SOD activity in heteroplasmic tissues of transmittochondrial mice with A3243G mitochondrial DNA mutation. *Genet Mol Res* 7: 1054–1062.
- Libri V, Brown AP, Gambarota G, Haddad J, Shields GS, Dawes H, Pinato DJ, Hoffman E, Elliot PJ, Vlasuk GP et al. (2012). A pilot randomized, placebo controlled, double blind phase I trial of the novel SIRT1 activator SRT2104 in elderly volunteers. *PLoS One* 7: e51395.
- Lieber DS, Calvo SE, Shanahan K, Slate NG, Liu S, Hershan SG, Gold NB, Chapman BA, Thorburn DR, Berry GT et al. (2013). Targeted exome sequencing of suspected mitochondrial disorders. *Neurology* 80: 1762–1770.
- Liu CS, Cheng WL, Lee CE, Ma YS, Lin CY, Huang CC, Wei YH (2006). Alteration in the copy number of mitochondrial DNA in leukocytes of patients with mitochondrial encephalomyopathies. *Acta Neurol Scand* 113: 334–341.
- Lombes A, Aure K, Bellanne-Chantelot C, Gilleron M, Jardel C (2014). Unsolved issues related to human mitochondrial diseases. *Biochimie* 100: 171–176.
- Longley MJ, Nguyen D, Kunkel TA, Copeland WC (2001). The fidelity of human DNA polymerase gamma with and without exonucleolytic proofreading and the p55 accessory subunit. *J Biol Chem* 276: 38555–38562.
- Mandel H, Szargel R, Labay V, Elpeleg O, Saada A, Shalata A, Anbinder Y, Berkowitz D, Hartman C, Barak M et al. (2001). The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 29: 337–341.
- Marchington DR, Scott Brown MS, Lamb VK, van Golde RJ, Kremer JA, Tuerlings JH, Mariman EC, Balen AH, Poulton J (2002). No evidence for paternal mtDNA transmission to offspring or extra-embryonic tissues after ICSI. *Mol Hum Reprod* 8: 1046–1049.
- Mariotti C, Tiranti V, Carrara F, Dallapiccola B, DiDonato S, Zeviani M (1994). Defective respiratory capacity and mitochondrial protein synthesis in transformant cybrids harboring the tRNA<sup>(Leu(UUR))</sup> mutation associated with maternally inherited myopathy and cardiomyopathy. *J Clin Invest* 93: 1102–1107.
- Marriage BJ, Clandinin MT, Macdonald IM, Glerum DM (2004). Cofactor treatment improves ATP synthetic capacity in patients with oxidative phosphorylation disorders. *Mol Genet Metab* 81: 263–272.
- McFarland R, Chinnery PF, Blakely EL, Schaefer AM, Morris AA, Foster SM, Tuppen HA, Ramesh V, Dorman PJ, Turnbull DM et al. (2007). Homoplasmy, heteroplasmy, and mitochondrial dystonia. *Neurology* 69: 911–916.



- McFarland R, Clark KM, Morris AA, Taylor RW, Macphail S, Lightowlers RN, Turnbull DM (2002). Multiple neonatal deaths due to a homoplasmic mitochondrial DNA mutation. *Nat Genet* 30: 145–146.
- McFarland R, Schaefer AM, Gardner JL, Lynn S, Hayes CM, Barron MJ, Walker M, Chinnery PF, Taylor RW, Turnbull DM (2004). Familial myopathy: new insights into the T14709C mitochondrial tRNA mutation. *Ann Neurol* 55: 478–484.
- McInerney SC, Brown AL, Smith DW (2009). Region-specific changes in mitochondrial D-loop in aged rat CNS. *Mech Ageing Dev* 130: 343–349.
- Menezes MJ, Riley LG, Christodoulou J (2014). Mitochondrial respiratory chain disorders in childhood: insights into diagnosis and management in the new era of genomic medicine. *Biochim Biophys Acta* 1840: 1368–1379.
- Minczuk M, Papworth MA, Kolasinska P, Murphy MP, Klug A (2006). Sequence-specific modification of mitochondrial DNA using a chimeric zinc finger methylase. *P Natl Acad Sci USA* 103: 19689–19694.
- Minczuk M, Papworth MA, Miller JC, Murphy MP, Klug A (2008). Development of a single-chain, quasi-dimeric zinc-finger nuclease for the selective degradation of mutated human mitochondrial DNA. *Nucleic Acids Res* 36: 3926–3938.
- Moraes CT, Bacman SR, Williams SL (2014). Manipulating mitochondrial genomes in the clinic: playing by different rules. *Trends Cell Biol* 24: 209–211.
- Moraes CT, DiMauro S, Zeviani M, Lombes A, Shanske S, Miranda AF, Nakase H, Bonilla E, Werneck LC, Servidei S et al. (1989). Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. *N Engl J Med* 320: 1293–1299.
- Moraes CT, Shanske S, Tritschler HJ, Aprille JR, Andreetta F, Bonilla E, Schon EA, DiMauro S (1991). mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 48: 492–501.
- Naviaux RK (2004). Developing a systematic approach to the diagnosis and classification of mitochondrial disease. *Mitochondrion* 4: 351–361.
- Naviaux RK, Nguyen KV (2004). POLG mutations associated with Alpers' syndrome and mitochondrial DNA depletion. *Ann Neurol* 55: 706–712.
- Nesbitt V, Pitceathly RD, Turnbull DM, Taylor RW, Sweeney MG, Mudanohwo EE, Rahman S, Hanna MG, McFarland R (2013). The UK MRC Mitochondrial Disease Patient Cohort Study: clinical phenotypes associated with the m.3243A>G mutation—implications for diagnosis and management. *J Neurol Neurosurg Psychiatry* 84: 936–938.
- Newmeyer DD, Ferguson-Miller S (2003). Mitochondria: releasing power for life and unleashing the machineries of death. *Cell* 112: 481–490.
- Nishino I, Spinazzola A, Hirano M (1999). Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* 283: 689–692.
- Ohtake A, Murayama K, Mori M, Harashima H, Yamazaki T, Tamaru S, Yamashita Y, Kishita Y, Nakachi Y, Kohda M et al. (2014). Diagnosis and molecular basis of mitochondrial respiratory chain disorders: exome sequencing for disease gene identification. *Biochim Biophys Acta* 1840: 1355–1359.
- Oliveira MT, Garesse R, Kaguni LS (2010). Animal models of mitochondrial DNA transactions in disease and ageing. *Exp Gerontol* 45: 489–502.
- Olivo PD, Van de Walle MJ, Laipis PJ, Hauswirth WW (1983). Nucleotide sequence evidence for rapid genotypic shifts in the bovine mitochondrial DNA D-loop. *Nature* 306: 400–402.
- Palladino MJ (2010). Modeling mitochondrial encephalomyopathy in *Drosophila*. *Neurobiol Dis* 40: 40–45.
- Pan Y, Nishida Y, Wang M, Verdin E (2012). Metabolic regulation, mitochondria and the life-prolonging effect of rapamycin: a mini-review. *Gerontology* 58: 524–530.
- Pandi-Perumal SR, BaHammam AS, Brown GM, Spence DW, Bharti VK, Kaur C, Hardeland R, Cardinali DP (2013). Melatonin antioxidative defense: therapeutical implications for aging and neurodegenerative processes. *Neurotox Res* 23: 267–300.
- Parikh S, Saneto R, Falk MJ, Anselm I, Cohen BH, Haas R, Medicine Society TM (2009). A modern approach to the treatment of mitochondrial disease. *Curr Treat Options Neurol* 11: 414–430.
- Peng TI, Hsiao CW, Reiter RJ, Tanaka M, Lai YK, Jou MJ (2012). mtDNA T8993G mutation-induced mitochondrial complex V inhibition augments cardiolipin-dependent alterations in mitochondrial dynamics during oxidative, Ca<sup>2+</sup>, and lipid insults in NARP cybrids: a potential therapeutic target for melatonin. *J Pineal Res* 52: 93–106.
- Picard M, Zhang J, Hancock S, Derbeneva O, Golhar R, Golik P, O'Hearn S, Levy S, Potluri P, Lvova M et al. (2014). Progressive increase in mtDNA 3243A>G heteroplasmy causes abrupt transcriptional reprogramming. *P Natl Acad Sci USA* 111: E4033–4042.
- Poulton J, Kennedy S, Oakeshott P, Wells D (2009). Preventing transmission of maternally inherited mitochondrial DNA diseases. *BMJ* 338: b94.
- Pozzan T, Magalhaes P, Rizzuto R (2000). The comeback of mitochondria to calcium signalling. *Cell Calcium* 28: 279–283.
- Quinzii CM, Hirano M (2010). Coenzyme Q and mitochondrial disease. *Dev Disabil Res Rev* 16: 183–188.
- Rahman S, Poulton J (2009). Diagnosis of mitochondrial DNA depletion syndromes. *Arch Dis Child* 94: 3–5.
- Rahman S, Poulton J, Marchington D, Suomalainen A (2001). Decrease of 3243 A->G mtDNA mutation from blood in MELAS syndrome: a longitudinal study. *Am J Hum Genet* 68: 238–240.
- Reddy P, Ocampo A, Suzuki K, Luo J, Bacman SR, Williams SL, Sugawara A, Okamura D, Tsunekawa Y, Wu J et al. (2015). Selective elimination of mitochondrial mutations in the germline by genome editing. *Cell* 161: 459–469.



- Rotig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, Schmitz J, Rustin P, Fischer A, Saudubray JM et al. (1990). Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. *J Clin Invest* 86: 1601–1608.
- Rovio AT, Marchington DR, Donat S, Schuppe HC, Abel J, Fritsche E, Elliott DJ, Laippala P, Ahola AL, McNay D et al. (2001). Mutations at the mitochondrial DNA polymerase (POLG) locus associated with male infertility. *Nat Genet* 29: 261–262.
- Saada A, Shaag A, Mandel H, Nevo Y, Eriksson S, Elpeleg O (2001). Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. *Nat Genet* 29: 342–344.
- Santorelli FM, Mak SC, El-Schahawi M, Casali C, Shanske S, Baram TZ, Madrid RE, DiMauro S (1996a). Maternally inherited cardiomyopathy and hearing loss associated with a novel mutation in the mitochondrial tRNA(Lys) gene (G8363A). *Am J Hum Genet* 58: 933–939.
- Santorelli FM, Mak SC, Vazquez-Memije ME, Shanske S, Kranz-Eble P, Jain KD, Bluestone DL, De Vivo DC, DiMauro S (1996b). Clinical heterogeneity associated with the mitochondrial DNA T8993C point mutation. *Pediatr Res* 39: 914–917.
- Santra S, Gilkerson RW, Davidson M, Schon EA (2004). Ketogenic treatment reduces deleted mitochondrial DNAs in cultured human cells. *Ann Neurol* 56: 662–669.
- Sarzi E, Bourdon A, Chretien D, Zarhrate M, Corcos J, Slama A, Cormier-Daire V, de Lonlay P, Munnich A, Rotig A (2007). Mitochondrial DNA depletion is a prevalent cause of multiple respiratory chain deficiency in childhood. *J Pediatr* 150: 531–534.e6.
- Schaefer AM, McFarland R, Blakely EL, He L, Whittaker RG, Taylor RW, Chinnery PF, Turnbull DM (2008). Prevalence of mitochondrial DNA disease in adults. *Ann Neurol* 63: 35–39.
- Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, DiMauro S (1989). A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. *Science* 244: 346–349.
- Schwartz M, Vissing J (2002). Paternal inheritance of mitochondrial DNA. *N Engl J Med* 347: 576–580.
- Schwartz M, Vissing J (2004). No evidence for paternal inheritance of mtDNA in patients with sporadic mtDNA mutations. *J Neurol Sci* 218: 99–101.
- Sgarbi G, Casalena GA, Baracca A, Lenaz G, DiMauro S, Solaini G (2009). Human NARP mitochondrial mutation metabolism corrected with alpha-ketoglutarate/aspartate: a potential new therapy. *Arch Neurol* 66: 951–957.
- Shadel GS, Clayton DA (1997). Mitochondrial DNA maintenance in vertebrates. *Annu Rev Biochem* 66: 409–435.
- Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC (1990). Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA<sup>Lys</sup> mutation. *Cell* 61: 931–937.
- Shokolenko I, Venediktova N, Bochkareva A, Wilson GL, Alexeyev MF (2009). Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Res* 37: 2539–2548.
- Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005). Decline in skeletal muscle mitochondrial function with aging in humans. *P Natl Acad Sci USA* 102: 5618–5623.
- Sitarz KS, Almind GJ, Horvath R, Czermin B, Gronskov K, Pyle A, Taylor RW, Larsen M, Chinnery PF, Yu-Wai-Man P (2012). OPA1 mutations induce mtDNA proliferation in leukocytes of patients with dominant optic atrophy. *Neurology* 79: 1515–1517.
- Spelbrink JN, Li FY, Tiranti V, Nikali K, Yuan QP, Tariq M, Wanrooij S, Garrido N, Comi G, Morandi L et al. (2001). Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet* 28: 223–231.
- Suomalainen A, Elo JM, Pietilainen KH, Hakonen AH, Sevastianova K, Korpela M, Isohanni P, Marjavaara SK, Tyni T, Kiuru-Enari S et al. (2011). FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. *Lancet Neurol* 10: 806–818.
- Suomalainen A, Isohanni P (2010). Mitochondrial DNA depletion syndromes--many genes, common mechanisms. *Neuromuscul Disord* 20: 429–437.
- Szklarczyk R, Nooteboom M, Osiewicz HD (2014). Control of mitochondrial integrity in ageing and disease. *Philos T R Soc Lond B* 369: 20130439.
- Tanaka M, Borgeld HJ, Zhang J, Muramatsu S, Gong JS, Yoneda M, Maruyama W, Naoi M, Ibi T, Sahashi K et al. (2002). Gene therapy for mitochondrial disease by delivering restriction endonuclease SmaI into mitochondria. *J Biomed Sci* 9: 534–541.
- Tatuch Y, Christodoulou J, Feigenbaum A, Clarke JT, Wherret J, Smith C, Rudd N, Petrova-Benedict R, Robinson BH (1992). Heteroplasmic mtDNA mutation (T---G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 50: 852–858.
- Taylor RW, Chinnery PF, Turnbull DM, Lightowlers RN (1997). Selective inhibition of mutant human mitochondrial DNA replication in vitro by peptide nucleic acids. *Nat Genet* 15: 212–215.
- Taylor RW, Giordano C, Davidson MM, d'Amati G, Bain H, Hayes CM, Leonard H, Barron MJ, Casali C, Santorelli FM et al. (2003a). A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy. *J Am Coll Cardiol* 41: 1786–1796.
- Taylor RW, McDonnell MT, Blakely EL, Chinnery PF, Taylor GA, Howell N, Zeviani M, Briem E, Carrara F, Turnbull DM (2003b). Genotypes from patients indicate no paternal mitochondrial DNA contribution. *Ann Neurol* 54: 521–524.
- Taylor RW, Turnbull DM (2005). Mitochondrial DNA mutations in human disease. *Nat Rev Genet* 6: 389–402.
- Temperley RJ, Seneca SH, Tonska K, Bartnik E, Bindoff LA, Lightowlers RN, Chrzanowska-Lightowlers ZM (2003). Investigation of a pathogenic mtDNA microdeletion reveals a translation-dependent deadenylation decay pathway in human mitochondria. *Hum Mol Genet* 12: 2341–2348.

- Tuppen HA, Blakely EL, Turnbull DM, Taylor RW (2010). Mitochondrial DNA mutations and human disease. *Biochim Biophys Acta* 1797: 113–128.
- Upholt WB, Dawid IB (1977). Mapping of mitochondrial DNA of individual sheep and goats: rapid evolution in the D loop region. *Cell* 11: 571–583.
- Vafai SB, Mootha VK (2012). Mitochondrial disorders as windows into an ancient organelle. *Nature* 491: 374–383.
- Van Goethem G, Luoma P, Rantamaki M, Al Memar A, Kaakkola S, Hackman P, Krahe R, Lofgren A, Martin JJ, De Jonghe P et al. (2004). POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement. *Neurology* 63: 1251–1257.
- Vasta V, Merritt JL 2nd, Saneto RP, Hahn SH (2012). Next-generation sequencing for mitochondrial diseases: a wide diagnostic spectrum. *Pediatr Int* 54: 585–601.
- Vilkkii J, Savontaus ML, Nikoskelainen EK (1990). Segregation of mitochondrial genomes in a heteroplasmic lineage with Leber hereditary optic neuroretinopathy. *Am J Hum Genet* 47: 95–100.
- Villa-Cuesta E, Holmbeck MA, Rand DM (2014). Rapamycin increases mitochondrial efficiency by mtDNA-dependent reprogramming of mitochondrial metabolism in *Drosophila*. *J Cell Sci* 127: 2282–2290.
- Viscomi C, Burlina AB, Dweikat I, Savoiardo M, Lamperti C, Hildebrandt T, Tiranti V, Zeviani M (2010). Combined treatment with oral metronidazole and N-acetylcysteine is effective in ethylmalonic encephalopathy. *Nat Med* 16: 869–871.
- Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ 2nd, Nikoskelainen EK (1988). Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 242: 1427–1430.
- Wenz T, Diaz F, Hernandez D, Moraes CT (2009). Endurance exercise is protective for mice with mitochondrial myopathy. *J Appl Physiol* (1985) 106: 1712–1719.
- Wong A, Cavelier L, Collins-Schramm HE, Seldin MF, McGrogan M, Savontaus ML, Cortopassi GA (2002). Differentiation-specific effects of LHON mutations introduced into neuronal NT2 cells. *Hum Mol Genet* 11: 431–438.
- Wong LJ (2007). Diagnostic challenges of mitochondrial DNA disorders. *Mitochondrion* 7: 45–52.
- Yang J, Zhu Y, Tong Y, Chen L, Liu L, Zhang Z, Wang X, Huang D, Qiu W, Zhuang S et al. (2009). Confirmation of the mitochondrial ND1 gene mutation G3635A as a primary LHON mutation. *Biochem Biophys Res Commun* 386: 50–54.
- Yasukawa T, Suzuki T, Ishii N, Ueda T, Ohta S, Watanabe K (2000). Defect in modification at the anticodon wobble nucleotide of mitochondrial tRNA<sup>Lys</sup> with the MERRF encephalomyopathy pathogenic mutation. *FEBS Lett* 467: 175–178.
- Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP (1988). Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 38: 1339–1346.
- Zhu Z, Yao J, Johns T, Fu K, De Bie I, Macmillan C, Cuthbert AP, Newbold RF, Wang J, Chevrette M et al. (1998). SURF1, encoding a factor involved in the biogenesis of cytochrome c oxidase, is mutated in Leigh syndrome. *Nat Genet* 20: 337–343.