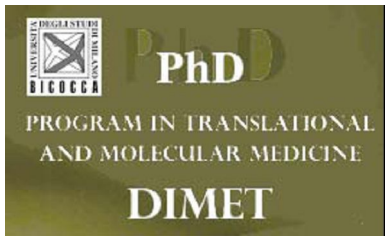

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Identification and characterization of nuclear genes responsible for human mitochondrial disorders: *FASTKD2*, responsible for a neurological disease associated with COX deficiency and *SDHAF1*, encoding a complex II assembly factor, mutated in SDH-defective leukoencephalopathy

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Chapter 5: Summary, conclusions and future perspectives

CHAPTER 1

General introduction: Mitochondria and mitochondrial medicine

Mitochondria

Mitochondria are intracellular organelles, ubiquitously found in eukaryotes. They originated from a primitive, bacteria-like organism that colonized primitive eukaryotic cells and created an endosymbiotic relationship with them (Margulis et al. 1976). Mitochondria, thanks to their capacity to support aerobic respiration and to generate adenosine triphosphate (ATP), became the principal intracellular source of energy. However during evolution, they lost their independence and most of their original bacterial proteome, relying on the import of cytosolic protein for several functions (Lister et al. 2005). In addition to their primary function, the production of ATP by oxidative phosphorylation (OXPHOS), mitochondria have a primary role in different metabolic pathways: the tricarboxylic acid cycle (TCA) and β-oxidation, for the metabolism of carbohydrates and fats, the porphyrin (Hamza 2006), lipid and steroid hormone (Jefcoate et al. 2002) synthesis. Moreover mitochondria have been shown to be involved in cell signalling, in particular they play an important role in the apoptosis (Schapira 2006, Letai et al. 2008), and in calcium homeostasis (Rimessi et al. 2008).

Mitochondria have a double-membrane structure (Fig.1a). They are present in hundreds/thousands copies in every cell, the amount depending on the cell type. Although traditionally considered as isolated organelles, recent findings indicate that they form a complex network (Fig.1b), with frequent fissions and fusions (Margineantu et al. 2002). Nevertheless they can be divided into 4 compartments: the

outer membrane (OM), the inter-membrane space (IMS), the inner membrane (IM) and the mitochondrial matrix. The OM is a porous membrane that allows passage of small substances between the cytosol and the IMS. The IM contains the complexes of the mitochondrial respiratory chain (MRC) and functions also as a barrier to ionic diffusion, allowing the creation of the proton gradient necessary to produce ATP. The matrix hosts several enzymes involved in different pathways (i.e. TCA cycle and β -oxidation) and the mitochondrial deoxyribonucleic acid (mtDNA).

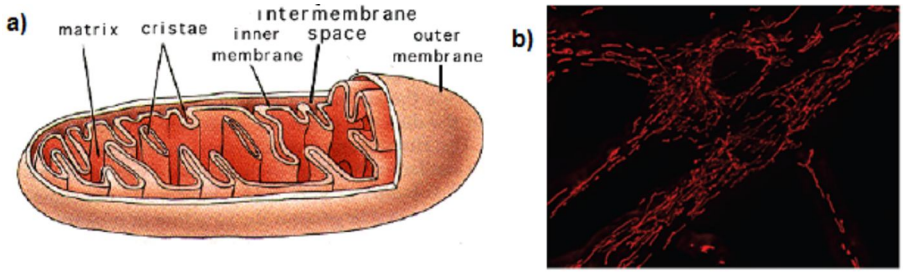

Figure 1. Mitochondrial structure

a) Schematic representation of the double membrane structure of mitochondria. b) Confocal image of fibroblasts stained with Mitotracker Red. This image demonstrates the reticular pattern of interconnecting mitochondria that result from the dynamic processes of fission and fusion.

Mitochondrial genetics

Mitochondria contain their genome, a circular double-strand DNA of 16569 base pairs. MtDNA contains no introns and small non-coding regions; it codes for 13 protein of the OXPHOS system and for 2 ribosomal and 22 transfer RNAs, necessary for the mitochondrial protein synthesis (Fig.2). However mitochondria depend upon the nucleus for supplying of all the other OXPHOS proteins as well as the

enzymes necessary for replication, repair, transcription, translation, maintenance and many structural proteins.

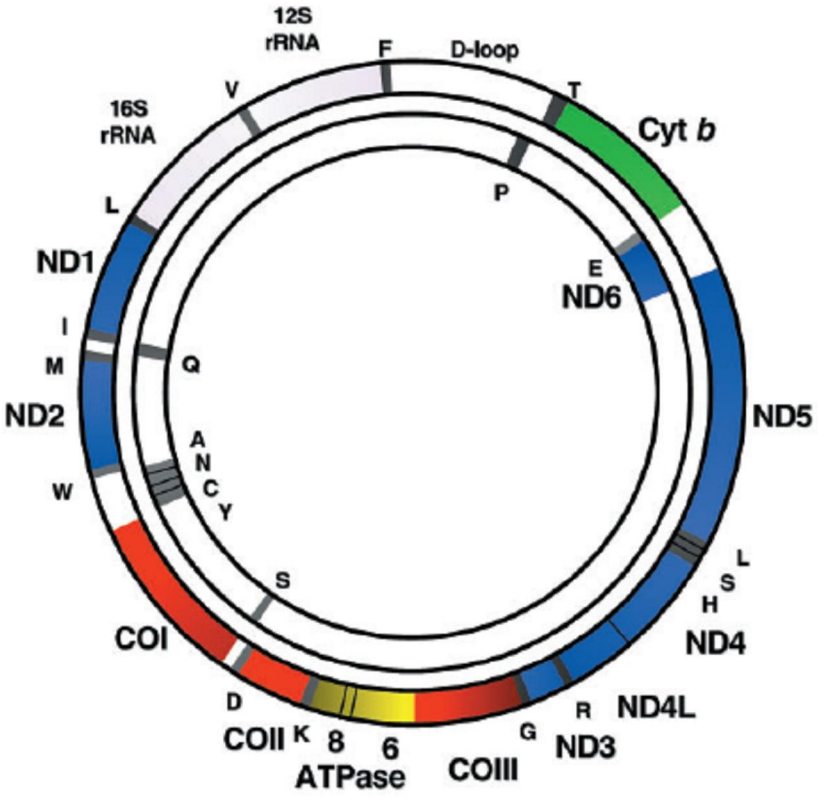

Figure 2. Mitochondrial DNA

Genes coding for OXPHOS proteins: complex I genes = blue; complex III cytb (cytochrome b) gene = green; complex IV genes = red; complex V genes = yellow. Genes coding for RNAs: tuna genes = grey; reran genes = purple. COI = complex I; COII = complex II; COIII = complex III.

The replication of mtDNA is a continuous process, independent from nuclear DNA replication, and requires several nuclear encoded factors such as DNA polymerase gamma (POLG), mitochondrial transcription factor A (mtTFA), mt single-strand binding protein (mtSSB) and enzymes important for the supply of deoxynucleotides, such as

thymidine kinase 2 (TK2) and deoxy-guanosine kinase (dGUOK). MtDNA is transcribed polycistronically and translated by mitochondrial ribosomes.

MtDNA is transmitted through the maternal line. This means that only the mother transmits her oocyte mtDNA to all of her offspring, and her daughters transmit their mtDNA to the next generation. A single case of paternal inheritance has been published (Schwartz et al. 2002) but, despite several studies, further cases of paternal transmission have never been described. The dogma of the maternal inheritance of the mtDNA remains true in practice for genetic counselling and for evolutionary studies.

Each human cell has thousands of mitochondria and within each single mitochondrion are present multiple copies (2 to 10) of mtDNA. Usually all these copies are identical, a status known as homoplasmy. However it is possible that errors occur during replication or repair of mtDNA, leading to the formation of a mutant mtDNA molecule. The rate of mutations in mtDNA is greater than those of genomic DNA probably because of the lack of histones and the presence of high level of free oxygen radicals. Mutant mtDNA could coexist with wt mtDNA in the same cell. This coexistence is called heteroplasmy. This is a dynamic phenomenon and the proportion of mutated vs. wt mtDNA (level of heteroplasmy) can change among cells and tissues, because at every cell division mitochondria are randomly segregated. A bottleneck effect is active in primordial female germinal cells (primary oogonia) that drastically reduces the number of segregating units responsible for the transmission of mtDNA characters to the subsequent generation.

Clinical presentations are usually associated with heteroplasmic mutations. Only when mutated gene copies accumulate over a certain "threshold", the deleterious effects of the mutation will not be counterbalanced by the co-existing wild-type mtDNA, and will be expressed phenotypically as a cellular dysfunction and disease. This threshold is tissue-specific, different for different mutations and in different individuals.

Nevertheless there are diseases caused by homoplasmic mutation, such as Leber's hereditary optic neuropathy (LHON), the first disorder associated to mitochondrial DNA mutation (Wallace et al. 1988).

Recent findings have shown that various mtDNA maintenance proteins specifically co-localize with mtDNA in intra-mitochondrial foci designated nucleoids. Mammalian nucleoids are now considered as specialized mtDNA-containing structures within the mitochondrial network, organized as complexes with a variety of proteins that protect mtDNA from insults and provide the appropriate microenvironment for mtDNA maintenance and gene expression.

Oxidative Phosphorylation

The most important activity of mitochondria is the oxidative phosphorylation. (Zeviani & DiDonato 2004). It is carried out by a series of multi-heteromeric complexes, located in the mitochondrial inner membrane through sequential reactions of reduction and oxidation, which form the so called "cellular respiration" and constitute the mitochondrial respiratory chain (MRC). Following the dehydrogenation of the redox equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2), they

generate a flow of electrons that results in the extrusion of hydrogen ions from the matrix to the IMS (exploited by proton translocating complexes I, III and IV and generating a membrane potential of about 180 mV) and in the production of water from molecular oxygen (by complex IV). The energy generated during these reactions, corresponding to the proton gradient across the IM, can be utilized by Complex V, or ATP synthetase, to condensate inorganic phosphate Pi and adenosine diphosphate (ADP) to ATP, the energy "currency" of the cell (Fig. 3).

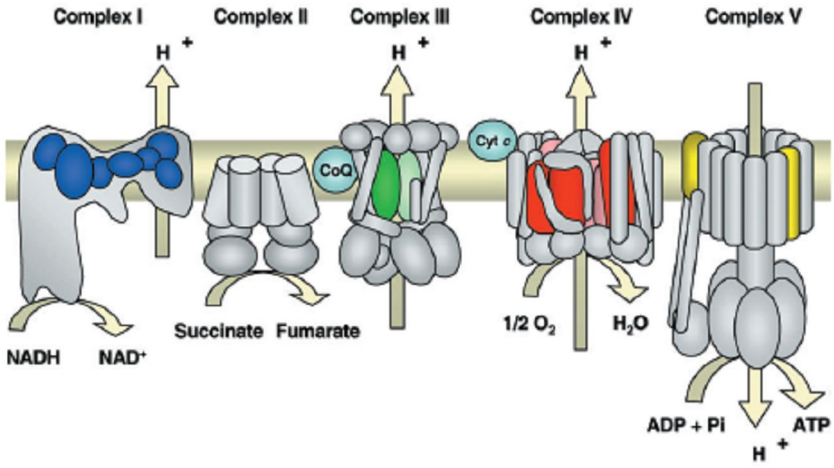

Figure 3. Mitochondrial OXPHOS system

Subunits encoded by mtDNA are shown in different colours in each complex

In addition to the above- mentioned complexes, two electron carriers (ubiquinone or CoQ, and cytochrome *c*) and a set of accessory complexes that can supply electrons take part in this process, including complex II (succinate-ubiquinone reductase), the electron-transfer flavoprotein-ubiquinone reductase (ETF-QR) and dihydroorotate dehydrogenase (DHODH).

In mammalian mitochondria Complex I (NADH-ubiquinone oxidoreductase) catalyzes the oxidation of NADH, derived from the oxidation of pyruvate, fatty acids, and amino acids, by ubiquinone. It is a macromolecular structure composed of ≈45 subunits with a total molecular mass of 1000kDa (Carroll et al. 2006). Seven subunits are encoded by mtDNA, the others by nuclear genes.

Complex II (Succinate-ubiquinone reductase) is composed of four subunits all encoded by nuclear genome. It catalyzes the oxidation of succinate to fumarate and transfers electrons to ubiquinone moieties. Complex III (ubiquinol-cytochrome c reductase) is made up of 11 subunits, of which all but one (cytochrome b) are encoded by nuclear DNA. Human cytochrome c oxidase (COX, complex IV) is composed of thirteen subunits: the three largest ones are encoded by mtDNA genes, while the remaining subunits are encoded by nuclear genes. ATP synthase (complex V) comprises an integral membrane component F0 and a peripheral moiety F1. All five subunits of F1 (a, b, c, d, e) and most F0 subunits of the ATP synthase are nuclear encoded. Only two F0 proteins (ATP6 and 8) are encoded by mitochondrial DNA (Boyer 1993).

Metabolic fuels feed reducing equivalents to the respiratory chain via glycolysis, fatty acid or amino acid oxidation. Pyruvate, which stands at the crossroads of glycolysis, gluconeogenesis and OXPHOS, is a key point in carbohydrate oxidation and feeds into the TCA cycle via the pyruvate dehydrogenase complex (PDHC) to generate acetyl-CoA. Fatty acids are oxidized within the mitochondrial matrix by β-oxidation, which again generates acetyl-CoA for the TCA cycle (Fig.4).

Reducing equivalents generated during the oxidation of the primary substrate or from the TCA cycle are transferred to OXPHOS as NADH (cI), or reduced flavins (entering at cII or cIII).

Mitochondria account for more than ninety percent of the energy utilized by our organism. When a failure in energy supply occurs, due to a defect in mitochondrial OXPHOS, the survival of the cell, and as a consequence, the life of our entire organism, is seriously at risk. Tissues and organs with the highest energy demand, including brain, skeletal muscles, and heart, are usually the most affected ones.

Reactive oxygen species (ROS)

The OXPHOS system is a finely tuned series of oxidative and reductive reactions. Any disturbance of this electron flow can lead to accumulation of intermediates which are potentially toxic. For instance, during respiration a small percentage (1-2%) of molecular oxygen is not fully reduced to water but instead is partially reduced to the superoxide anion, O_2^- (or hydrogen peroxide, H_2O_2), which can be converted to the highly reactive species hydroxyl ion (OH^-); together these oxygen derivatives are called ROS (Balaban et al. 2005). ROS are mainly generated at two sites in OXPHOS, cI and cIII. Although they can be toxic to the cell, they also play a role in diverse signalling pathways. To counteract the potential harmful effects of ROS, cells are equipped with an anti-oxidant machinery, including the mitochondrial manganese superoxide dismutase and glutathione peroxidase (Nelson et al. 2004, Zeviani & Lamantea 2006) and anti-oxidant substances (Fig.4).

Whether a cell experiences oxidative stress depends on the level of production of each oxidative species and of its removal, and on the effect of damage repair pathways. When these are out of balance, damage to mitochondrial respiratory complexes and mtDNA can arise leading to further mitochondrial dysfunction. Whereas ROS are often considered as a single entity, each individual ROS has its own mechanism of production and detoxification, and each has its own specificity in term of biological targets, consequently the pathological effects vary depending on the ROS involved.

Mitochondrial radical production seems to be a consequence of normal mitochondrial function. However, the changes that occur to mitochondrial activity in mtDNA related disease may significantly increase ROS production and thereby contribute to the pathophysiology of these disorders. An increase in ROS could arise by a number of mechanisms. Firstly, inhibition of the respiratory chain can lead to a reduction of its electron carrying components and a consequent increase in ROS production. Inhibition of ATP synthesis can also lead to reduction of respiratory carriers and an increase in mitochondrial membrane potential, which also favours radical production. MtDNA mutations that lead to incorrectly assembled complexes could increase superoxide formation by allowing greater interaction between oxygen and redox active respiratory chain components, such as the semiquinone radical. Finally, as Complexes I and III contain iron-sulphur cluster, the production of hydroxyl radical could be mediated by Fenton reaction (Lin et al. 2006).

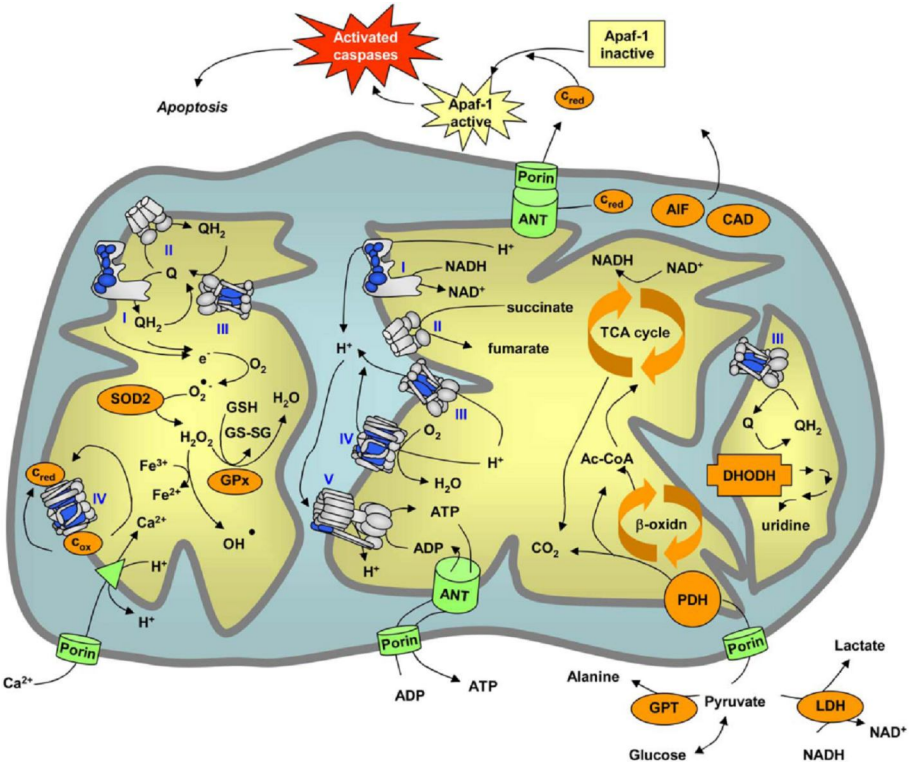

Figure 4. Mitochondrial energy metabolism

Schematic representation of mitochondrial energy metabolism and related pathways. I, II, III, IV, and V, complexes I-V. Complexes II, III, and IV are shown as functionally active dimers. b-oxidn, b-oxidation; PDH, pyruvate dehydrogenase complex; Ac-CoA, acetyl-CoA; DHODH, dihydroorotate dehydrogenase; GPT, glutamic pyruvic transaminase; LDH, lactate dehydrogenase; AIF, apoptosis inducing factor; CAD, caspase-activated DNase; Q, coenzyme Q (ubiquinone); QH2, reduced coenzyme Q (ubiquinol); c-red and c-ox, reduced and oxidized cytochrome c. MtDNA-encoded subunits are shown in blue, membrane transporters in green, other enzymes and pathways in orange. Different metabolic pathways are depicted in different areas of the mitochondrial matrix and inner membrane compartments purely for illustrative purposes. The left-hand area shows Ca2+ transport and the respiratory chain enzymes that are involved in the production of ROS. The middle area shows the main mitochondrial energy pathways. The right-hand area shows the action of DHODH.
Components of the mitochondrial apoptotic pathway (AIF, cytochrome c, CAD) are located in the intermembrane space and, upon pro-apoptotic signalling, are released in the cytosol. (from Smeitink et al. 2006)

Apoptosis

Mitochondria, as source of energy, are clearly fundamental to the life of eukaryotic cells. Moreover it has become clear that mitochondria also play a key role in the pathways to cell death and are the central stations for apoptotic signals (Zamzami et al. 1996, Kroemer 1997, Green 2005) (Fig.4). This role is not due only to a "loss of function" causing an energetic deficit. Rather, it is an active process that, after permeabilization of mitochondrial membranes, leads to the release of potentially toxic proteins and to alterations of the mitochondrial physiological vital functions (Liu et al. 1996).

After the induction of apoptosis, cytochrome c (cyt c) is released from mitochondria and interacts in the cytosol with Apaf1 and procaspase 9, creating a complex called apoptosome, which activates the caspase cascade (Yuan et al. 2003; Danial & Korsmeyer 2004). Cytochrome c is a heme protein normally involved in electron transfer between complexes III and IV of the respiratory chain (Wang. 2002, Green & Reed 1998, Kroemer & Reed 2000).

Apart from the caspase-dependent pathway, mitochondrial factors also initiate a caspase-independent apoptotic signalling cascade (Cregan et al. 2004, Hong et al. 2004). This pathway is initiated by the release of the mitochondrial protein, apoptosis-inducing factor (AIF). AIF is a flavoprotein with NADH oxidase activity normally present in the mitochondrial intermembrane space (Susin et al. 1999) or associated with the inner mitochondrial membrane (Arnoult et al. 2002). Upon apoptosis induction, AIF translocates from mitochondria to the cytosol and to the nucleus where induces chromatin condensation and DNA

fragmentation through interactions and activation of nucleases, for instance EndoG (Cande et al. 2002, Lipton & Bossy-Wetzel 2002).
Inactivation of the flavin adenine nucleotide (FAD) and mutations that destroy the FAD binding site do not affect the apoptogenic function of AIF (Miramar et al. 2001, Loeffler et al. 2001). Thus, the apoptogenic activity of AIF does not depend on its NADH oxidase activity. On the contrary, mutations of amino-acid residues required for DNA binding (but not for redox activity) abrogate the apoptogenic potential of AIF in vitro (Ye et al. 2002, Cheung et al. 06)
In general, OXPHOS dysfunction leads to dysregulation of apoptotic signalling, so that some cells activate apoptosis mechanisms, whereas other cells fail to die when they should, leading to failure of physiological functions and perhaps also favouring replicative senescence. The intramitochondrial signals influencing apoptosis may themselves be complex, depending on the status of the electrochemical proton gradient, ROS production, metabolite and ion concentrations, and ATP levels.

Mitochondrial import

Mitochondria import many hundreds of different proteins that are encoded by nuclear genes and synthesized as precursor on cytosolic ribosomes. These proteins are targeted to the mitochondria, translocated across the mitochondrial membranes and sorted to the different mitochondrial sub-compartments (Neupert 1997). Separate translocases in the OM (TOM complex) and in the IM (TIM complex) facilitate recognition of preproteins and transport through the two

membranes (Wiedemann et al. 2004). Several cytosolic chaperones are involved in guiding the preprotein toward mitochondrial surface.

Mitochondrial precursor proteins can be classified in two main groups. The first comprises preproteins that are directed to the matrix and some of the proteins of the IM and of the IMS, characterized by the presence of an N-terminal cleavable mitochondrial targeting sequence (MTS). The second group includes all OM proteins and many IMS and IM proteins, which carry various internal target signals and posses no cleavable extensions.

The insertion of the preprotein into the OM is facilitated by the TOM complex and represents the entry gate for practically all nuclear-encoded proteins that have to enter into the mitochondria. The TOM complex is composed of several receptors and a common insertion pore that guides preproteins through the OM. The presence of two or more binding sites with increasing affinity for the presequence is the driving force of this process.

After this step, there are three pathways that the precursor protein can follow:

1-Preproteins with a cleavable MTS are transferred to the TIM complex (TIM23) thank to the membrane potential. The matrix chaperone mtHsp70 induce the completion of the translocation into the matrix; this step is ATP-dependent. Then specific metalloproteases (MPP) remove the cleavable targeting sequence and the correct folding of the protein occurs by chaperons (i.e. mtHsp60).

2-Many hydrophobic proteins of the IM are inserted without passage through the matrix, by the use of both TOM and TIM complexes (TIM22) and with the involvement of IMS components.

3-OM proteins are integrated into the OM by the sorting and assembly machinery (SAM complex).

The real system of the import into mitochondria is more complex and both OM and IM contains multisubunit protein complexes involved in recognition of the preprotein, in translocation from TOM complex to the OM, the IMS or TIM complex, in assembling of the complexes and in facilitating folding in the matrix and processing of imported proteins. Moreover there are several examples of atypical mitochondrial import; for instance cytochrome c is translocated across the OM without mediation of TOM complex.

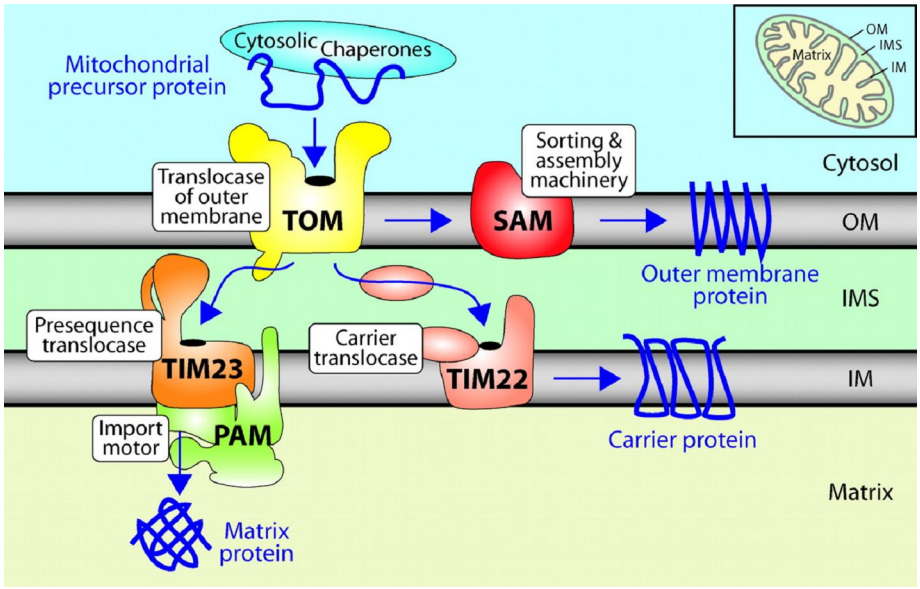

Figure 5. Protein import pathways (from Wiedemann et al. 2004)

Mitochondrial medicine

Mitochondria are the organelles responsible for the supply of energy to the cell through the OXPHOS system. For this reason any defect or alteration in this process lead to pathological consequences, mainly in tissues and organs that have a highly energetic request. The consequences of an impairment of the OXPHOS system are decrease of ATP production and increase of reactive oxygen species (ROS), plus their associated up- and downstream effects.

More than twenty years ago, the first evidences of direct link between mitochondria and disease arose; in 1988 Wallace et al. found a mtDNA mutation causing Leber's hereditary optic neuropathy (Wallace et al. 1988) and Holt et al. identified deletions in mtDNA causing myopathy (Holt et al. 1988).

After these papers, there has been a series of reports about the clinical, biochemical and genetic characterizations of mitochondrial diseases; moreover an involvement of mitochondria has become evident in many other clinical conditions including neurodegenerative disorders (Swerdlow 2009), cancer (Carew & Huang. 2002) and ageing (Reddy 2008). All these considerations lead to the use of the term "mitochondrial medicine" to underline the central role of the mitochondrion not only in the classical mitochondrial diseases but also in a wide and increasing range of illness.

Mitochondrial disorders

Whereas in the past the term "mitochondrial disorders" was used to any alterations affecting mitochondria, it is currently referred to genetic defects of OXPHOS (Zeviani & Lamantea 2006).

Because OXPHOS is necessary for nearly all cells, any organ can be affected in mitochondrial disease. In addition, given the complexity of mitochondrial genetics and biochemistry, mitochondrial inherited diseases may present with a vast range of symptoms, severity, age of onset and outcome (DiMauro & Davidzon 2005). The maxim “any tissue, any symptom, any age” well resume all these concepts (Munich et al. 1996). Combined, genetic defects of OXPHOS have an incidence at approximately 1:5000 (Schaefer et al. 2004, Thornburn et al. 2004, Uusimaa et al. 2007, Mancuso et al. 2007), or even more (Cree et al. 2009) and are therefore not to be considered “so rare” diseases.

The clinical manifestations of mitochondrial disorders are extremely heterogeneous. They range from lesions in single tissues, such as the optic nerve in Leber’s hereditary optic neuropathy (LHON), to more diffuse lesions including myopathies, encephalomyopathies, cardiopathies and hepatopathies, to complex multisystem syndromes with onset ranging from neonatal to adult life.

Tissue specificity, due to variable metabolic thresholds for the different OXPHOS complexes in each tissue, may limit the systemic effect of metabolic changes. Moreover there are to be considered many contributing factors including tissue-specific expression of OXPHOS genes, different metabolic needs, and tissue dependent segregation of heteroplasmy.

Nevertheless mitochondrial disease commonly presents with a combination of muscle and brain involvement; tissues that are post-mitotic and have high metabolic requests.

The spectrum of mitochondrial diseases in children is wide, usually different from that found in adults, and new clinical features are continuously reported (Debray et al. 2008).
Some presentations alone are strong indications of a mitochondrial involvement, but there are often additional albeit not specific symptoms to be taken into account (Tab.1).

Common neurological manifestations of mitochondrial disease include seizures, migraine, stroke-like episodes, neuropathy and dystonia. Often, mitochondrial disease is only considered when such features occur in conjunction with other symptoms, such as deafness, visual impairment and/or diabetes. However multi-organ involvement, a hallmark of mitochondrial disease, may not be evident, above all at initial presentations.
Patients, particularly children, with evident signs of respiratory chain dysfunction could undergo extensive investigations and analysis, without the identification of the biochemical or genetic cause for their mitochondrial disease. Clinical scoring systems exist and allow such children to be classified as probably, possible or unlikely mitochondrial disease (Bernier et al. 2002, Haas 2007); but this diagnostic classification is of limited utility, especially in genetic counselling.

Table 1. Clinical and biochemical findings of mitochondrial disease in children (adapted from Debray et al. 2008)

(A) Signs and symptoms highly suggestive of a mitochondrial disease	(B) Signs and symptoms compatible with mitochondrial disease
Neurologic Episodic or progressive mental regression. Episodic neurological symptoms of unknown cause Cerebral stroke-like episode with nonvascular distribution of lesions Unexplained brainstem dysfunction (oculomotor changes, altered level of consciousness, hypothermia or hyperthermia, hypotension or hypertension) Brainstem involvement in MRI (Leigh syndrome-like) **Muscular** Myopathy with presence of ragged red fibres **Cardiovascular** Unexplained hypertrophic cardiomyopathy Arrhythmia of unknown cause: heart block, and others **Ophthalmologic** External ophthalmoplegia with or without ptosis Sudden or insidious optic neuropathy **Gastroenterologic** Unexplained liver failure (especially if valproate-related) Severe intestinal dysmotility, chronic pseudoobstruction **Clinical biochemistry** Persistent elevation of blood lactate Episodes of acidosis, ketosis or hyperlactatemia, exceeding the expected physiological concentration; postprandial ketosis Characteristic MRS spectra (lactate)	**General** Failure to thrive; short stature Fatigue **Neurologic** Progressive or static developmental delay; encephalopathy Cerebral atrophy Seizures, especially myoclonic Peripheral (usually axonal) neuropathy; unexplained spinal muscular atrophy Cerebellar ataxia Extrapyrimidal movement disorders Hypotonia or progressive spasticity Leukodystrophy Exercise intolerance with or without rhabdomyolyis Migraine **Other** Ophthalmologic (optic atrophy, cataracts), pigmentary retinal degeneration Sensorineural hearing loss; aminoglycoside-induced deafness Sideroblastic anemia Dermatological (hypertrichosis, pili torti, subcutaneous lipomas) Endocrine (hypoparathyroidism, glucose intolerance, diabetes) Dilated cardiomyopathy Recurrent vomiting Unexplained liver disease (fatty liver, hepatocellular lysis, cirrhosis) Pancreatic insufficiency Renal (tubular acidosis; renal Fanconi syndrome; unexplained glomerulopathy; nephrotic syndrome)

In general, childhood presentations of mitochondrial disease tend to be more severe than those with adult onset and frequently involve many different organ systems. Hepatic dysfunction and haemopoietic stem cell failure are uncommon features of adult-onset mitochondrial disease, but are seen more often in children. Renal disease also appears to be a more prominent clinical feature of paediatric mitochondrial disorders (Bourdon et al. 2007, DeLonlay et al. 2001).
Adult patients can be affected by isolated myopathy resulting only in fatigue, muscle weakness and/or exercise intolerance, but muscle symptoms can also be associated with involvement of the central and peripheral nervous systems, manifesting symptoms, which may include ataxia (i.e. motor incoordination), sensory-neural hearing loss, seizures, polyneuropathy, retinopathy, and, more rarely, movement disorders and cognitive deterioration.

A hallmark of mitochondrial dysfunction is lactic acidosis; acidosis is derived from the reduction to lactate of pyruvate, which accumulates for the block of respiration. Hyperlactatemia however may be absent in clear mitochondrial disease or present only during stress. Liquoral or cerebrospinal fluid (CSF) lactate level is a more reliable diagnostic marker than blood, above all in patients with brain involvement (Brown et al. 1988, Finsterer. 2001). Proton magnetic resonance spectroscopy (H^+MRS) allows non-invasive detection of elevated cerebral lactate and other relevant compounds (Haas et al. 2008).

Several morphological and biochemical features characterize many of these syndromes albeit there isn’t an always valid rule. Histological

and histochemical analysis of muscle biopsy remains a very important step for the detection of mitochondrial disease, especially in adult patients. One of the best-known morphological alterations is the transformation of scattered muscle fibres into “ragged red fibres” (RRF). RRFs are characterized by the accumulation of abnormal mitochondria under the sarcolemmal membrane. The same aggregations can also be observed using succinate dehydrogenase (SDH) assay, that can be even more useful because complex II (subunits of which are encoded only by nuclear genome) is completely unaffected by abnormalities of mtDNA. This assay can be combined with the cytochrome c oxidase (COX, respiratory complex IV) reaction; in mitochondrial disorders a common finding is the presence of muscle fibres that stain negative to this histochemical reaction. Moreover a mosaic pattern of COX activity is indicative of a heteroplasmic mtDNA mutation, the intensity of the stain depending on the mutation load between different fibres.
However, these typical “mitochondrial” alterations may be absent in otherwise demonstrated mitochondrial disorders. This is the case of LHON or Neuropathy Ataxia and Retinits Pigmentosa (NARP), and it is also true in many paediatric cases.

In patients with a highly probable diagnosis of mitochondrial disease, spectrophotometric assays of the respiratory chain complexes can be carried out on small samples of frozen tissue (usually muscle biopsy) or cultured skin fibroblasts. Skin biopsy is minimally invasive and can give indication about the biochemical defect in about 50% cases (Depaepe et al. 2006, Janssen et al. 2007); if fibroblast analysis is not

informative, muscle (or liver) biopsy is needed. It's important to consider that respiratory chain defects may be tissue-specific (due to different heteroplasmy of mtDNA mutations and tissue-specific levels of nuclear gene products (Antonicka et al. 2006). As a consequence, the study of clinically affected tissues, if possible, is strongly preferred because provides the highest probability of informative results.

The molecular diagnosis of the mitochondrial disease is complex. Because of its dual genetic control, OXPHOS disorders can be due to mutations in mtDNA or nuclear DNA genes (Zeviani & DiDonato 2004). Nuclear genetic defects are easily investigated in freshly extracted DNA from peripheral white blood cells. Blood could be less useful for detecting mtDNA mutations, because the level of heteroplasmy could be too low to be detected. Skeletal muscle is the tissue of choice for molecular genetic analysis of mtDNA. This is because skeletal muscle is often an affected tissue, samples may be already available for enzymatic assays, and for some mutations the levels of heteroplasmy in skeletal muscle reflect those in other affected post-mitotic tissues such as the brain (Oldfors et al. 1995).

mtDNA mutations

Even if the proteins encoded by mtDNA are essential, they comprise only a small fraction of the total number of protein involved in a functional OXPHOS system. About 10-25% of mitochondrial diseases are caused by mutations in mtDNA (Mancuso et al. 2007, Mcfarland et al. 2004).

When the mtDNA should be analyzed, it is important to take into consideration the following differences from nuclear DNA:
1) The mitochondrial genome is maternally inherited; 2) Mitochondria are polyploidy and the mtDNA genotype can be composed of a single mtDNA species (homoplasmy) or two different genotypes can co-exist in variable proportion (heteroplasmy); 3) A threshold effect modulates the phenotypic expression of a mtDNA-associated symptoms.

Mutations of mtDNA can be classified into large-scale rearrangements (deletions or duplications) and point mutations. Both groups have been associated with well-defined clinical syndromes. While large-scale rearrangements are usually sporadic, point mutations are usually maternally inherited.

Large-scale rearrangements of mtDNA

Single, large-scale rearrangements of mtDNA comprise single partial deletions, or partial duplications. The size of deletions can vary from few bases to several kilobases and be located in any part of the molecule. The most common deletion is 5Kb long, and affects a region containing tRNAs and protein-coding genes. Usually deletions encompass several genes and are invariably heteroplasmic.
Rearranged molecules, lacking a portion of the mitochondrial genome, can be detected as an independent mtDNA species (single mtDNA deletion) or joined to a wild-type molecule or a mixture of the two rearrangements co-exists in the same cell (Zeviani et al. 1988; Poulton et al. 1989).

Three main clinical phenotypes are associated with these mutations: **Kearns–Sayre syndrome (KSS, OMIM**#530000**), sporadic progressive external ophthalmoplegia (PEO) and Pearson's syndrome (OMIM**#557000).

KSS is a usually sporadic disorder characterized by chronic progressive external ophthalmoplegia, onset before age of 20 years; and pigmentary retinopathy. Patients with this disease always show RRFs in muscle biopsy (Mita et al. 1989).

Single deletions/duplications can also be present in patients with milder phenotypes such as **PEO**, characterized by late-onset progressive external ophtalmoplegia, proximal myopathy and exercise intolerance. In KSS and PEO, diabetes mellitus and hearing loss are frequent additional features, which may occasionally precede, even by years, the onset of neuromuscular symptoms (Shoffner et al. 1989).

Finally, large-scale single deletions/duplications of mtDNA may cause **Pearson's bone-marrow–pancreas syndrome**, a rare disorder of early infancy characterized by connatal sideroblastic pancytopenia and, less frequently, severe exocrine pancreatic insufficiency with malabsorption (Rotig et al. 1990).

Some patients have duplication of mtDNA, which although might not be pathogenic themselves, could be an intermediate step toward the generations of deletions.

<u>mtDNA point mutations</u>

These mutations can be substitutions of single bases or micro-insertions/micro-deletions in the mtDNA molecule. They can be localized into transfer RNAs (tRNA), ribosomal RNAs, (rRNA), or

genes encoding OXPHOS subunits. Unlike mtDNA rearrangements, mtDNA point mutations are transmitted maternally. They may be heteroplasmic or homoplasmic (DiMauro & Schon 2003).

Nowadays sequencing of the entire mitochondrial genome is feasible and useful in excluding mtDNA involvement prior to investigating candidate nuclear genes. However, in consideration of the highly polymorphic status of mtDNA, it is often difficult to decide which variants are polymorphisms and which are pathogenic mutations. Some criteria to assess the pathogenic role of mtDNA mutations were suggested (DiMauro & Schon 2001): the mutation must not be a known neutral polymorphism; the base change must affect an evolutionarily conserved and functionally important site; deleterious mutations are usually heteroplasmic; the degree of heteroplasmy in different family members has to be concordant with the severity of symptoms.

The exceptions to these rules are frequent and homoplasmic mutations are an example, which don't fulfil these criteria. Functional evidences are always recommended but necessary in these cases to assess the real pathogenicity.

The clinical expression associated to mtDNA mutations is wide; the phenotypes more frequent are the following:

- **Leber's Hereditary Optic Neuropathy** (**LHON**, OMIM#535000) is a juvenile-onset disease affecting mostly males. It is characterized by acute loss of central vision due to rapidly progressive optic atrophy. This partial or complete, usually permanent loss of vision, is the only consistent manifestation of the disease which, more rarely,

may also include alterations in cardiac rhythm. The muscle biopsy does not show evidence of ragged-red fibres and is not recommended for the diagnosis of the disease. LHON is considered the most common disease caused by mtDNA mutations. Three mutations (G3460A, G11778A or T14484C) of mtDNA, in the genes encoding subunits ND1, ND4, and ND6 of complex I, respectively, account for about 95% of cases(Man et al. 2003). Other mutations, all present in complex I mtDNA genes, have been identified (Valentino et al. 2002, 2004). All these mutations are usually homoplasmic or in high mutant heteroplasmic proportions.

- **Neurogenic muscle weakness, ataxia, retinitis pigmentosa** (**NARP**, OMIM#551500) can also include, besides the symptoms that give the name to this disorder, epilepsy, and sometimes mental deterioration. Symptoms usually appear in adulthood. Ragged-red fibres are absent in the muscle biopsy. The disease is associated with mutation T8993G in the gene encoding subunit 6 of mitochondrial ATPase (complex V of the respiratory chain). In patients presenting a milder NARP phenotype, a transition T->C in the same position has also been described.

- **Leigh syndrome** (**MILS**, maternally inherited Leigh syndrome **OMIM**#256000). The same T8993G mutation when present in >90% of heteroplasmy, leads to the more severe, earlier onset Leigh syndrome or Subacute Necrotizing Encephalomyelopathy. Affected infants show severe psychomotor delay, cerebellar and pyramidal signs, dystonia, seizures, respiratory abnormalities, incoordination of ocular movements, and recurrent vomiting.

- **Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes** (**MELAS**, OMIM#540000) is defined by the following symptoms: 1) stroke-like episodes caused by focal cerebral lesions, often localized in the parieto-occipital regions of the brain; 2) lactic acidosis or abnormal lactic levels in blood (and cerebro-spinal fluid, CSF); 3) "ragged-red" fibres in the muscle biopsy. Other signs involving the central nervous system are mental deterioration, recurrent migraine with "cerebral" vomiting, focal or generalized epilepsy and neurosensorial deafness. The disease is transmitted maternally and the onset varies from early childhood to young adulthood. MELAS syndrome is typically associated with mutation A3243G in the gene encoding $tRNA^{Leu}$ (UUR). Other point mutations associated with MELAS have been reported, although they are much rarer than the A3243G (Taylor & Turnbull 2005).

- **Myoclonus Epilepsy with Ragged-Red Fibers** (**MERRF**, OMIM#545000) is characterized by myoclonus, epilepsy, muscle weakness, motor incoordination (ataxia) and sometimes, mental deterioration. Clinical manifestations can vary greatly even within the same family. This phenotypic variability is attributed to the level of heteroplasmy and to the tissue distribution of the mutation. The major part of affected families carries an A8344G transition in the gene encoding $tRNA^{Lys}$. Numerous other point mutations of mtDNA have been associated with different clinical phenotypes in single patients or in a few families (Zeviani & Di Donato 2004).

Nuclear gene mutations

In addition to mtDNA, nuclear genes can also be responsible for a wide spectrum of mitochondrial disorders. Nuclear genes are responsible for the greater number of components of OXPHOS system and are also required for the import of proteins into the mitochondrion, for the assembly and many other functions necessary to maintain a functional OXPHOS system. In addition, mtDNA replication, transcription and translation are absolutely dependent on nuclear encoded proteins.

Accordingly, a classification can be proposed for these defects, including:

1. Disorders due to defects in nuclear gene encoding structural components of the OXPHOS complexes

2. Disorders due to defects in nuclear gene encoding assembly factors of the OXPHOS complexes

3. Disorders due to gene defects affecting mtDNA maintenance, replication and expression.

4. Defects of genes encoding factors involved in the biosynthesis of lipids and cofactors

5. Defects of proteins involved in mitochondrial biogenesis or factors indirectly related to OXPHOS

(1) Deficiencies of respiratory chain components

Although 72 of the 85 subunits of the OXPHOS system are encoded by nuclear DNA, mutations of these genes have only rarely been described. This could imply that such mutations are highly deleterious and probably embriolethal. Mutations that have been described in fact

are usually associated to a neonatal or early-onset, although occasional patients with a late onset of disease have been reported. On the other hand the screening of the nuclear-encoded subunits of respiratory chain has not always been done in a systematic manner, especially for complex I, and only recently the number of reports regarding mutations in structural OXPHOS component is grown up.

The mutations in nuclear encoded subunits identified so far, are mainly abnormalities of complex I found in patients with- infancy or childhood-onset, even if also mutations in structural subunits of complex II and IV were described.

Defects of Complex I subunits

Complex I is a macromolecular structure composed of ≈45 subunits in mammals (Carrol et al. 2006). The human nuclear genes coding for Complex I subunits are known and have been sequenced, but for many of them the exact function and structure are still obscure. Complex I deficiency is the most common cause of respiratory chain disease (Dimauro & Schon 2003). The clinical presentation is a progressive neurological disorder, often Leigh syndrome, occasionally complicated by cardiomyopathy, or multisystem involvement.

Complex I deficiency with autosomal recessive inheritance results from mutation in nuclear-encoded genes, including NDUFV1 (OMIM 161015), NDUFV2 (OMIM 600532), NDUFS1 (OMIM 157655), NDUFS2 (OMIM 602985), NDUFS3 (OMIM 603846), NDUFS4 (OMIM 602694), NDUFS6 (OMIM 603848), NDUFS7 (OMIM 601825), NDUFS8 (OMIM 602141), NDUFA2 (OMIM 602137), NDUFA11 (OMIM 612638), NDUFAF3 (OMIM 612911).

Defects of Complex II subunits

Mitochondrial disease involving Complex II is rare, representing 2-8% cases of respiratory chain deficiency (Munich et al. 2001, Ghezzi et al. 2009). Clinically, Leigh syndrome is the most common presentation, but myopathy, encephalopathy, and isolated cardiomyopathy have also been reported. Moreover different mutations in cII subunits (SDHB, SDHC and SDHD) have been associated to tumoral forms, such as paragangliomas and phaeochromocytomas.

Defects of Complex III subunits

To date, no mutations in nuclear genes encoding complex III subunits have been reported, but a complex rearrangement of the human QP-C gene (7 modified amino acids and addition of a 14-aminoacid-long segment at the C terminus) (Haut et al. 2003). The QP-C gene encodes the ubiquinone-binding protein, also known as subunit VII, of complex III. Surprisingly, although the rearrangement resulted in severe complex III deficiency in the liver, the patients had no permanent liver dysfunction but only metabolic crises after fasting.

Defects of Complex IV subunits

While different mutations in mitochondrial encoded subunits of cIV have been described, there is a single case reporting a mutation in nuclear encoded subunit (Massa et al. 2008). This mutation affects the subunit COX6B1 and is associated to a combination of early-onset

leukodystrophic encephalopathy, myopathy and growth retardation associated with COX deficiency of unknown cause.

Complex V

No pathogenic mutations involving nuclear encoded structural subunits have yet been found.

(2) Respiratory chain complex assembly deficiencies

Complex I

Mutations in assembly factors are a frequent cause of isolated complex I deficiency (Ogilvie et al. 2005) and other mutations and new genes are regularly being identified in the last years (Saada et al. 2008, Pagliarini et al. 2008) They comprise complex I assembly genes B17.2L (609653), HRPAP20 (611776), C20ORF7 (612360), C8ORF38 (OMIM#252010, Ogilvie et al. 2005, Pagliarini et al. 2008). More than half of the patients with cI deficiency lack mutations in any known cI subunit, suggesting that yet unidentified genes necessary for assembly or stability of cI are mutated in the remaining cases (Janssen et al. 2006). Mutations in cI assembly cause a wide range of clinical disorders, ranging from lethal neonatal disease to adult-onset neurodegenerative disorders. Phenotypes include macrocephaly with progressive leukodystrophy, nonspecific encephalopathy, cardiomyopathy, myopathy, liver disease, Leigh syndrome, Leber hereditary optic neuropathy (Loeffen et al. 2000, Pitkanen et al. 1996, Robinson 1998).

Complex III

Complex III deficiencies are reported, associated to mutations in the BCS1L gene. These mutations have been shown in infantile cases of complex III deficiency associated with neonatal proximal tubulopathy, hepatic involvement and encephalopathy (DeLonlay et al. 2001, Zeviani et al. 2003) and in GRACILE syndrome comprising growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death (Visapaa et al. 2002).

Complex IV

Defects of genes encoding assembly factors are also the main cause of complex IV deficiency. Almost all the nuclear-gene defects of COX identified are due to mutations in assembly factors of the enzyme, including SURF1 (Tiranti et al. 1998), SCO1 (Valnot et al. 2000a), SCO2 (Papadopoulou et al. 1999), COX10 (Valnot et al. 2000b), COX15 (Antonicka et al. 2003) and possibly LRPPRC (Mootha et al. 2003), and it is estimated that complex IV alone requires over 20 factors (Devenish et al 2000, Fontanesi et al. 2006). These autosomal recessive COX deficiencies usually present in early life with Leigh syndrome, myopathy, encephalopathy, lactic acidosis and a rapidly progressive course with early death. Recently one mutation in a mitochondrial translational activator required for efficient translation of COX subunit I, TACO1, has been found in affected members of a family with slowly progressive Leigh syndrome and characterized by reduction of complex IV biochemical activity and amount.

Complex V

Few cases of complex V deficiency have been attributed to mutations in the assembly factor ATP12 (Demeirleir et al. 2004). Complex V deficiency seems to be an early presenting disease with lactic acidosis immediately after birth, dysmorphic features, and methyl glutaconic aciduria that are the major indications for the diagnosis (Sperl et al. 2006). Last year, another gene, TMEM70, was found mutated in individuals with isolated deficiency of ATP synthase, mostly of Gipsy ethnic origin (Cizkova et al. 2008); the prevalent mutation can result in either severe or milder phenotypes.

(3) Disorders due to gene defects altering the mtDNA maintenance

MtDNA remains dependent upon nuclear genome for the production of proteins involved in its replication, transcription, translation, repair and maintenance.

Replication of mtDNA requires a small set of proteins: i.e. the DNA polymerase gamma (POLG), Twinkle helicase (PEO1), mitochondrial single-stranded DNA binding protein (mtSSB), and a supply of deoxy-nucleotides triphosphate (dNTPs). Structural defects of the DNA-processive enzymes are often associated with mtDNA mutagenesis and multiple mtDNA deletions (qualitative alterations), whereas defects affecting the dNTP pool usually cause mtDNA depletion, that mean reduction of mtDNA copy number/cell (quantitative alterations).

Ant1. In humans, the mitochondrial ANT exist in three different isoforms (ANT1, 2 and 3), different for tissue distribution and kinetic properties. The ANT1 protein is a homodimer highly expressed in heart and skeletal muscle and located in the IMM, where it is the most

abundant protein. It exchanges ATP and ADP in and out of the mitochondrial matrix. ANT1 mutations are responsible for a relatively benign, slowly progressive, and extremely rare form of autosomal dominant form of PEO. Only one patient with recessive mutation of ANT1 has been reported so far (Palmieri et al. 2005). He presented with hypertrophic cardiomyopathy, mild myopathy with exercise intolerance, RRF and lactic acidosis, but no ophthalmoplegia. mtDNA multiple deletions were found in muscle.

Twinkle (PEO1). Twinkle is a helicase involved in mtDNA replication. Mutations in PEO1, the gene that encodes Twinkle, are associated with clinical presentations of variable severity, ranging from late-onset 'pure' PEO to PEO 'plus' syndromes, with proximal muscle and facial weakness, mild ataxia and peripheral neuropathy. A recessive PEO1 mutation causes infantile onset spino-cerebellar ataxia (IOSCA) (Nikali et al. 2005), a disease characterized by a combination of ataxia, athetosis, muscle hypotonia and severe epilepsy. Other features such as ophthalmoplegia, hearing loss and optic atrophy appear later in the disease course. These patients show mtDNA depletion in brain and liver.

POLG1. The mtDNA polymerase, pol-γ, is the key enzyme for mtDNA replication and repair. It is a heterotrimer constituted by a catalytic subunit (pol-γA, encoded by POLG1) and two moieties of an accessory subunit (pol-γB, encoded by POLG2), which function as a DNA binding factor, increasing the processivity of the polymerase holoenzyme. Mutations affecting POLG1 are one of the most frequent

causes of mitochondrial disease. More than 100 mutations in pol-γ have been reported. This gene is the most frequent cause of autosomal dominant PEO (adPEO). In adPEO due to POLG1 mutations, typical features are severe dysphagia and dysphonia, and occasionally, a movement disorder including parkinsonism, cerebellar dysfunction or chorea (Luoma et al. 2004). There is a correlation between the type of mutation and the severity of the disease. Several recessive mutations of POLG1 are also reported in PEO patients with multiple mtDNA deletions (Agostino et al. 2003); in different recessive syndromes such as SANDO (Van Goethem et al. 2003) or SCAE; in infantile Alpers-Huttenlocher syndrome or Hepatopathic Poliodystrophy (Naviaux et al. 2004, Ferrari et al. 2005).

Only one heterozygous dominant mutation has been identified in POLG2 in a 60-year-old woman with adult-onset PEO, cardiac conduction defect and increased CK (Longley et al. 2006).

Albeit mtDNA depletion can be due to POLG1 (Naviaux et al. 2004) or Twinkle (Sarzi et al. 2007) mutations, as in the case of AHS (Alpers-Huttenlocher syndrome), mtDNA depletion syndromes (MDS) are usually caused by mutations in different factors that control the mitochondrial or cytosolic supply of dNTPs, the precursors used by DNA polymerase for DNA replication and repair. The mutagenic effects of dNTP pool imbalance have been known for several decades with respect to the nuclear genome. In the last ten years, six genes coding for enzyme directly involved in dNTP synthesis have been recognized to cause severe MDS.

Thymidine Phosphorylase (TP or TYMP) TP is an enzyme involved in the catabolism of the pyrimidine nucleosides. Defects of TP are thought to produce an excess of dTTP, resulting in the imbalance of dNTP pools that can affect both the rate and fidelity of mtDNA replication. This is reflected by the presence of multiple deletions, accumulation of point mutations, and partial depletion of mtDNA, mainly in muscle. TP mutations are the cause of mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE), a disease characterized by ophthalmoparesis, peripheral neuropathy, leukoencephalopathy, and gastrointestinal symptoms such as intestinal dysmotility.

Thymidine kinase 2 (TK2) TK2 is a deoxyribonucleoside kinase that phosphorylates thymidine, deoxycytidine, and deoxyuridine. The clinical spectrum associated to its mutations varies from a severe muscle weakness with marked dystrophic alterations, encephalopathy and seizures (Galbiati et al. 2006) to a milder myopathic phenotype with longer survival, no motor regression and in some patients proximal tubulopathy (Saada et al. 2001).

p53 controlled RR (p53R2 or RRM2B) Ribonucleotide reductase is the major regulator of dNTP *de novo* synthesis, and defects of its small inducible subunit p53R2 may cause infantile multisystem disorders, juvenile-onset MNGIE or adult-onset myopathy, with renal proximal tubulopathy and nephrocalcinosis (Bornstein et al. 2008, Kollberg et al. 2009). A heterozygous non-sense mutation in p53R2 was found in

a large family with autosomal dominant PEO and multiple mtDNA deletions (Tyynismaa et al. 2009)

Deoxy-guanosine kinase (*dGK or dGUOK)* In mammalian cells, the phosphorylation of purine deoxyribonucleosides is mediated predominantly by two deoxyribonucleoside kinases: cytosolic deoxycytidine kinase (dCK) and mitochondrial deoxyguanosine kinase (dGK). Mutations in dGK gene are associated to a hepatocerebral form of mtDNA depletion. Symptoms include persistent vomiting, failure to thrive, hypotonia and hypoglycaemia associated with progressive neurological symptoms. The liver dysfunction is usually progressive, evolving from microvescicular steatosis into cirrhosis and chronic liver failure (Saada et al. 2001, Freisinger et al. 2006).

Succinyl-CoA synthetase (*SUCLA2 and SUCLG1)* Succinyl-CoA synthetase (SCS) is composed of an invariant alpha subunit and a beta subunit that determines whether the enzyme is GTP-specific (G-SCS) or ATP-specific (A-SCS) (Sanadi et al. 1954). Thus, the two enzymes, SCS-G and SCS-A, catalyze a similar reaction by using different phosphate donors (Johnson et al. 1998*a*, 1998*b*). The activity of both SCS-G and SCS-A is present in mitochondria from rat liver, kidney, and heart, but SCS-A, the ADP-forming enzyme, predominates in the brain (Lambeth et al. 2004).

The *SUCLA2* gene encodes the β subunit of the ADP-forming succinyl-CoA synthetase (SCS-A) (Allen & Ottaway 1986; Weitzman et al. 1986). This mitochondrial matrix enzyme catalyzes the

formation of succinate and ATP from succinyl-CoA and ADP in the tricarboxylic acid (TCA) cycle in a reversible manner. Mutations in SUCLA2 cause encephalomyopathic mitochondrial DNA depletion and mild methylmalonic aciduria, (Elpeleg et al. 2005, Carrozzo et al. 2007).

The *SUCLG1* (also reported as *SUCLA1*) gene encodes the alpha subunit of SCS. The first 27 amino acids of SUCLG1 are a mitochondrial targeting sequence. Mutations in this gene were found in a severe form associated with combined muscle and liver mtDNA depletion, dysmorphic features, neonatal lactic acidosis and death in the first days of life (Ostergaard et al. 2007).

Only in a fraction of the reported cases of MDS, a causative mutation has been found. It is likely that other genes of dNTP regulation or mtDNA maintenance will be found.

For instance a peculiar form of hepato-cerebral MDS is due to mutation in MPV17 gene, which codes for a protein with still unknown function (Spinazzola et al. 2006).

Transcription of mtDNA requires a small number of nucleus-encoded proteins including a single RNA polymerase (POLRMT), auxiliary factors necessary for promoter recognition (TFB1M, TFB2M) and promoter activation (TFAM), and a transcription termination factor (mTERF) (Scarpulla 2008). No mutation affecting these transcriptional factors has yet been described.

Mitochondrial DNA translation or protein synthesis is carried out in the mitochondrial matrix by a machinery, which is composed of tRNAs and rRNAs synthesized in situ from the corresponding mitochondrial genes and a number of proteins encoded by nuclear DNA and imported into mitochondria. It is a four-step process involving nuclear encoded translation initiation (IF2, IF3), elongation (EF-Tu, EF-Ts, EF-G1 and EF-G2), termination (RF1) and ribosome recycling factors (Koenen et al. 2004).

Genetic diseases due to defective mitochondrial protein synthesis have so far been associated with mutations in eight nuclear genes.

A homozygous mutation in the gene encoding the mitochondrial ribosomal protein subunit 16 (*MRPS16*) was found in a patient with dysmorphic features, hypotonia and intractable lactic acidosis. (Miller et al. 2004). The gene encoding pseudouridine synthase 1 (*PUS1*), which converts uridine in pseudouridine in several positions of tRNAs is mutated in the myopathy, lactic acidosis and sideroblastic anaemia (MLASA) syndrome (Bykhovskaya et al. 2004). Mutations in the gene (*EFG1*) encoding mitochondrial elongation factor, EF-G1, were identified in two patients with severe lactic acidosis, postnatal liver failure and a generalized deficiency of mitochondrial protein synthesis (Koenen et al. 2004). The same homozygous missense mutation in *TSFM*, a gene encoding the mitochondrial translation elongation factor, EF-Ts, can cause encephalomyopathy or hypertrophic cardiomyopathy in unrelated infant patients (Smeitink et al. 2006b). Only one homozygous mutation was identified in the mitochondrial elongation factor Tu (EF-Tu) in a patient with a severe infantile macrocystic leucodystrophy with micropolygyria (Valente et al.

2007). Three additional genes have recently been linked to mtDNA translation defects: mutations in *DARS2* (Scheper et al. 2007) which encodes the mitochondrial aspartyl tRNA synthetase, were identified in several different families with leucoencephalopathy affecting the brain stem and spinal cord and high level of lactate; one mutation in *RARS2* (Edvardson et al. 2007), the gene encoding mitochondrial arginine tRNA (tRNA) synthetase, has been associated with severe infantile encephalopathy; finally mutation in the *MRPS22* (Saada et al. 2007) gene which encodes a mitochondrial ribosomal protein has been associated with antenatal hydrops, hypotonia, cardiomyopathy and tubulopathy.

However the clinical expression of mutations in these genes remains to be further analysed, as well as the search of new candidate genes affecting mitochondrial protein synthesis.

(4) Defects of genes encoding factors involved in the biosynthesis of lipids and cofactors

Defects of the membrane lipid milieu

Except for cytochrome *c*, which is located in the intermembrane space, all components of the respiratory chain are embedded in the lipid milieu of the inner mitochondrial membrane, which is composed predominantly of cardiolipin. Cardiolipin is not merely a scaffold but is essential for proper functioning of several mitochondrial OXPHOS complexes and several mitochondrial carrier proteins (Jiang et al. 2000, Gohil et al. 2004). This is the reason why defects in cardiolipin could cause OXPHOS dysfunction and hence mitochondrial disease. In fact there is an example on this regard, the Barth syndrome

(mitochondrial myopathy, cardiomyopathy, growth retardation, and leukopenia) (Barth et al 1999). The mutated gene in this syndrome, *TAZ* (or *G4.5)*, encodes an acyl–coenzyme A synthetase (tafazzin) that must have an important role in cardiolipin synthesis, because cardiolipin concentrations are markedly decreased in skeletal and cardiac muscle and in platelets from affected patients (Schlame et al. 2000).

Coenzyme Q deficiency

CoQ or ubiquinone is a lipophilic component of the electron-transport chain, which transfers electrons from Complex I or II, and from the oxidation of fatty acids and branched-chain amino acids, via flavin-linked dehydrogenases to Complex III. The CoQ also plays a role as an antioxidant and as a membrane stabilizer. Although the biochemical and molecular bases remain undefined, primary CoQ deficiency is a potentially important cause of recurrent myoglobinuria or ataxia or both.

Mutations in the CoQ10 biosynthetic genes, *COQ2* (Quinzii et al. 2006), *PDSS1* (Mollet et al. 2007) and *PDSS2* (Lopez et al. 2006) were reported in patients with severe infantile mitochondrial syndromes and tissue CoQ10 deficiency (Rotig et al. 2007), whereas the molecular genetic defect of adult-onset CoQ10 deficiency remains undefined (Quinzii et al. 2007).

Mutations in *APTX* (Quinzii et al. 2006) and *ADCK3* (Lagier-Tourenne et al. 2008) were recently found in patients with ataxia and low levels of CoQ in muscle biopsies, supporting the hypothesis that

the ataxic form is a genetically heterogeneous disease in which CoQ10 deficiency can be secondary (Le ber et al. 2007).

FeS protein defects

The OXPHOS complexes need to be equipped with cofactors such as copper, heme or iron-sulphur (FeS) clusters that are necessary for their electron transport capacity. A whole range of complex-specific chaperones, assembly factors and enzymes involved in the biosynthesis and incorporation of prosthetic groups are necessary for the assembly of intact and enzymatically functional complexes.

Mitochondrial FeS cluster assembly machinery is indispensable for life owing to their function in the maturation of all cellular FeS proteins (Lill & Mühlenhoff, 2006). Alterations in this process have obviously deleterious consequences; for instance ABC7, an iron mitochondrial exporter, which controls the generation of cytosolic iron-sulphur proteins, is responsible for X-linked sideroblastic anaemia and ataxia; frataxin, a mitochondrial protein which is responsible for Friedreich's ataxia, is also involved in iron handling, heme synthesis and iron-sulphur protein maintenance (see also the relative paragraph in the chapter on neurodegenerative mitochondriopathies).

Copper

A defect of copper internalization into mitochondria is caused by mutations of *ATP7B* (ATPase copper transporting beta polypeptide) gene on chromosome 13, which are associated with Wilson's disease. The pathogenesis of Wilson's disease may involve either direct

damage to copper containing enzymes, such as cytochrome c oxidase, or more generic oxidative damage to the cell owing to the accumulation of copper (see the relative paragraph in the chapter on neurodegenerative mitochondriopathies).

(5) Defects of proteins involved in mitochondrial biogenesis or factors indirectly related to OXPHOS

Fission and fusion defects

Mitochondria are not static and isolated organelles but form a complex network. Mitochondrial fusion and fission require conserved protein machineries at the outer and inner membranes that mediate membrane mixing and division events. The proteins that regulate mitochondrial dynamics are now associated with a broad range of cellular functions: they play roles in maintaining the (1) integrity of mitochondria, (2) electrical and biochemical connectivity, (3) turnover of mitochondria, and (4) segregation and protection of mitochondrial DNA (Okamoto et al. 2005).

GTPases of the dynamin family have a crucial role in both fission and fusion. Mgm1 is a GTPase and member of the dynamin protein family and OPA1 is its human homologue. Both regulate the morphology of the inner mitochondrial membrane. Mutations in this gene lead to autosomal dominant optic atrophy (OPA1). OPA1 protein is also implicated in apoptosis (Frezza et al. 2006) and oxidative phosphorylation (Zanna et al. 2008), and multiple mtDNA deletions are found in muscle of some patients (Amati-Bonneau et al. 2008, Hudson et al. 2008), suggesting that the OPA1 protein also influences mtDNA maintenance.

MFN2 gene, encoding another dynamin-like enzyme regulating the fission-fusion dynamics, mitofusin 2, is responsible for mitochondrial disorders, autosomal dominant Charcot-Marie-Tooth neuropathy type 2A and 4A (CMT2A, CMT4A) (Zuchner et al. 2004, Zuchner et al. 2005).

Interestingly mutation in the *DLP1* gene, which encodes a dynamin-like protein implicated in mitochondrial and peroxisomal fission, causes morphologically striking defects in both organelles and severe clinical symptoms of systemic lactic acidosis, increased plasma very long chain fatty acids, and microcephaly. Respiratory chain function was normal in patient homogenates, suggesting that the sub-compartment localization is important in determining the function for mitochondrial proteins (Waterham et al. 2007).

Defects of mitochondrial protein import

Two known mitochondrial diseases are clearly attributable to abnormal protein import.

X-linked Mohr–Tranebjaerg syndrome is characterized by deafness, followed by progressive neurological troubles, including dystonia and optic atrophy (Roesch et al. 2002); this disorder is due to mutations in *TIMM8A*, encoding DDP1 (deafness–dystonia protein1), a component of the import machinery for mitochondrial carrier proteins.

Mutation of *DNAJC19*, encoding a putative mitochondrial import protein, causes autosomal recessive dilated cardiomyopathy with ataxia (Davey et al. 2006).

Aging

Aging is associated with a general loss of functions at the level of the whole organism that has origins in cellular deterioration. Most cellular components, including mitochondria, require continuous recycling and regeneration throughout the lifespan. Mitochondria are particularly susceptive to damage over time as they are the major bioenergetic machinery and source of oxidative stress in cells. Effective control of mitochondrial biogenesis and turnover, therefore, becomes critical for the maintenance of energy production, the prevention of endogenous oxidative stress and the promotion of healthy aging (López-Lluch et al. 2008)

In aging, mitochondrial dysfunction is caused by an accumulation of mtDNA mutations and by an increase in ROS production. In studies of aging and mtDNA, researchers found that several tissues from old individuals have lower mitochondrial function than those from younger individuals (Cooper et al. 1992). Both mtDNA point mutations and deletions are highly prevalent in aged cells, and 8-hydroxy-2-deoxyguanosine appears to be highly prevalent in aged tissues (Kujoth et al. 2006). From aging studies in mouse models, it appears that mitochondrially generated ROS are a critical factor in aging (Trifunovic et al. 2004, Kujoth et al. 2005). If mitochondrial ROS production can be controlled, it may be possible to delay aging extending a healthy lifespan. Several aging transgenic mice studies revealed that mitochondrially targeted antioxidants play an important role in decreasing free radical production and oxidative damage and aging phenotypes (Van Remmen et al. 2004, Ran et al. 2004, Schriner

et al. 2005). Overall, these aging mice studies suggest that mtDNA mutations are critically involved in producing aging phenotypes, and further, mitochondrially generated free radicals are important factors in determining aging and longevity.

Neurodegenerative mitochondriopathies

It has been established that aging is a major risk factor for developing several neurodegenerative diseases (Beal et al. 2005, Reddy 2007). However, the precise connection between aging and neurodegenerative diseases is unclear. Several studies and reviews have reported that age-related ROS produced by mitochondria are a factor in the development and progression of late-onset neurodegenerative diseases (Swerdlow & Khan 2004, Wallace 2005, Schapira 2006, Reddy and Beal 2008); in addition abnormalities of mtDNA and OXPHOS activity have been identified in different neurodegenerative diseases. Whether they represent primary or secondary defects because of other factors not directly related to pathogenesis, is sometimes hard to be distinguished; neurodegeneration versus mitochondrial dysfunction can be considered two sides of the same coin (Zeviani & Carelli 2007).

In the case of autosomal dominant, recessive, or matrilineal disorders, the primary event can be obvious. With sporadic disorders it is typically less clear. Regardless of whether mitochondrial dysfunction is a primary cause of neurodegeneration, a mediator of neurodegeneration, or an epiphenomenon, someone proposes defining

neurodegenerative diseases with recognized mitochondrial dysfunction as “neurodegenerative mitochondriopathies” (Swerdlow et al. 2007).

Alzheimer’s disease

Alzheimer’s disease (AD) is the most common neurodegenerative disease. The typical course is progressive cognitive decline with remarkable amnesia. Risk increases dramatically with advancing age, and among those over 85 years old nearly half are affected (Evans et al. 1989). As the disease progresses, neurodegeneration becomes increasingly pervasive.

AD is often divided into early versus late onset forms, and autosomal dominant versus non autosomal dominant forms. Autosomal dominant AD, with rare exceptions, typically presents before the age of 65. Mutations in the *amyloid-β protein precursor* (*AβPP*), *presenilin 1* (*PS1*), and *presenilin 2* (*PS2*) genes cause autosomal dominant AD and appear to alter processing of AβPP towards amyloid-β (Aβ) derivative (Scheuner et al. 1996). Aβ is the major constituent of amyloid plaques found in the brains of elderly individuals with and without AD. Accordingly, an “amyloid cascade hypothesis” proposes AD is primarily a consequence of abnormal AβPP processing to particular Aβ variants, which then gain a toxic function (Hardy et al. 1992). Brain mitochondria are clearly altered in persons with AD. There is agreement that human AD brains contain reduced numbers of normal mitochondria (De la monte et al. 2000). Amiloid (Aβ) can inhibit OXPHOS in mitochondria (Pereira et al. 1998). COX activity

is reduced in AD platelets, fibroblasts and large parts of the brain (Parker et al. 1990, Kish et al. 1992, Swerdlow et al. 2002).

Cybrid studies suggest mtDNA is at least partly responsible for reduced COX activity in AD (Swerdlow et al. 1997). In cybrid cells obtained from platelets of AD patients, thus containing AD subject mitochondria/mtDNA, was reported a reduced COX activity. AD cybrid cell lines also overproduce free radicals and Aβ (Swerdlow et al 2007). No mtDNA mutation has consistently been found in AD, hence the COX deficiency could represent secondary damage from, for instance, free radical generation.

Parkinson's disease

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and its prevalence rises with age. 1-3% of those over 80 are affected (Tanner et al. 1996). Neuron loss is particularly profound in the substantia nigra, although early neurodegeneration also occurs in other discrete brainstem nuclei. Surviving nigral neurons may contain intracytoplasmic inclusions called Lewy bodies. The presence of nigral Lewy bodies in conjunction with substantia nigra pars compacta neuron loss establishes the histologic diagnosis. The classic clinical signs of resting tremor, bradykinesia, rigidity, and postural instability are associated with loss of substantia nigra dopaminergic neurons. Like AD, PD is clinically classified into early and late onset variants and Mendelian (familial) versus non-Mendelian variants. The percentage of Mendelian cases declines with advancing age, while the percentage of non-Mendelian cases increases.

MRC complex I activity was shown to be reduced in persons with idiopathic PD (Parker et al. 1989, Bindoff et al. 1989). Activity is reduced in multiple tissues, including substantia nigra, frontal cortex, platelets, muscle, and fibroblasts and so it is probably a systemic event (Parker et al. 2008). It was hypothesized that because mtDNA contributes so importantly to complex I structure and function, and because mtDNA abnormalities can produce sporadic disease, alterations in mtDNA were an important cause of PD (Parker et al. 1989). This hypothesis was tested using cybrids, and multiple groups report that PD cybrid cell lines have reduced complex I activity (Swerdlow et al. 1996, Esteves et al. 2008). In addition to reduced complex I activity, these cell lines have increased reactive oxygen species production, reduced mitochondrial calcium storage, and cytoplasmic α-synuclein aggregations. The true mtDNA alterations that account for this are unknown. Mitochondrial haplogroup and polymorphism association studies suggest mtDNA variation could alter PD risk (Van der Walt et al. 2003, Ghezzi et al. 2005, Pyle et al. 2005). Also environmental factors are important in PD pathogenesis; pesticide and toxins leading to parkinsonism inducing dopaminergic cell death in people and animal models, are associated to complex I inhibition (Seaton et al. 1997, Hatcher et al. 2008).

A ‘MitoPark’ mouse, in which mtDNA is lost in dopaminergic neurons, develops slowly progressive parkinsonism with accumulation of intracellular inclusion bodies (Ekstrand et al. 2007).

A variety of autosomal dominant and recessive forms of genetically inherited PD are now defined. Mitochondrial dysfunction is implicated in several variants. One cause of autosomal dominant PD is

mutation of the *α-synuclein* gene (Polymeropoulos et al. 1997), and Lewy bodies consist largely of fibrillar α-synuclein. (Lee et al. 2002). Other genes implicated in Mendelian PD encode proteins that localize to mitochondria or influence mitochondrial physiology. Examples of such proteins include parkin, DJ1, PINK1, and LRRK2 (Tan et al. 2007). This convergence upon mitochondria suggests at the very least that mitochondria partecipate in a common pathway of neurodegeneration.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is characterized by progressive weakness that arises from upper and lower motor neuron degeneration (Mitchell et al. 2007). ALS is more common in people over 50 years of age and in men. It is relatively rare, with a prevalence that is below 1% of the population. Similar to AD and PD, both sporadic and Mendelian forms exist, and with increasing age the proportion of those with Mendelian inheritance decreases. The most studied Mendelian form of ALS is caused by mutation of the *superoxide dismutase 1* (*SOD1*) gene. *SOD1* mutation accounts for approximately 2% of ALS cases. Perturbations of mitochondrial ultrastructure in ALS were revealed several decades ago (Hirano et al. 1984). Cytoplasmic inclusions (Bunina bodies) that may represent mitochondria containing autophagic vacuoles are observed in ALS motor neurons (Hart et al. 1977). Cybrids from ALS patients showed a significant reduction in complex I activity and non-significant trends towards reduced complex III and IV activities (Swerdlow et al. 1996). A sporadic ALS reduction in complex I activity, with mtDNA

perturbation, was found in ALS muscle biopsies (Wiederman et al. 1998).

Progressive supranuclear palsy and multiple system atrophy

After PD, progressive supranuclear palsy (PSP) is the most common neurodegenerative movement disorder. It presents most frequently in the seventh decade with prominent postural instability and an increasingly hypokinetic syndrome.

Data indicative of mitochondrial alterations in PSP patients are reported. Muscle mitochondria have decreased ATP production (Dimonte et al. 1994). Ketoglutarate dehydrogenase and aconitase activities are reduced in the cerebellum (Park et al. 2001). Both muscle and brain appear bioenergetically compromised when studied using phospho-magnetic resonance spectroscopy (Martinelli et al. 2000). PSP cybrids in which platelet mitochondria from PSP subjects were transferred to human neuroblastoma cells, showed reduced complex I activity and increased free radical production (Swerdlow et al. 2000), as reported also in PD.

Huntington's disease

Huntington's disease (HD) is a strictly autosomal dominant, hyperkinetic neurodegenerative movement disorder. Neuropsychiatric symptoms and signs are also important clinical features (Vonsattel et al. 1998). HD is caused by a CAG repeat expansion in one copy of the *Huntingtin* gene, which encodes a protein called huntingtin (The Huntington's Disease Collaborative Research Group. 1993).

Polyglutamine extension appears to confer a toxic gain-of function to huntingtin protein.

Evidence of mitochondrial dysfunction, particularly a complex II defect, in the pathogenesis of Huntington's disease has been accumulated over the last 30 years and further provided by studies carried out in yeast strains (Solans et al. 2006) and cultured striatal neurons (Fukui et al. 2007). Huntingtin has been shown to physically associate with mitochondrial membranes and interfere with mitochondrial calcium handling (Panov et al. 2002). Huntingtin has been proposed to interfere with mitochondrial biogenesis by disrupting peroxisome proliferator activated receptor γ coactivator 1 α (PGC1α), a transcription co-activator that facilitates mitochondrial biogenesis (Cui et al. 2006). Therefore activation of PGC1α may be considered as a new therapeutic target in Huntington's disease

Friedreich's Ataxia

Friedreich's ataxia (FA) is a relatively common autosomal recessive genetic disorder (Pandolfo et al. 2008). It presents as progressive sensory loss, weakness, and dyscoordination. Sensory neurons with cell bodies in dorsal root ganglia and which project through the spinal cord dorsal columns degenerate initially, and neurodegeneration later spreads to the spinocerebellar tracts, cerebellar purkinje cells, and corticospinal tracts. Symptomatic onset most commonly occurs during the second decade.

Causal mutations reside in the *FXN* gene (Campuzano et al. 1996). Often mutant alleles contain an expansion of an intronic GAA repeat segment. Point mutations within exons have been found, and alleles

with point mutations are occasionally paired with GAA expansions in individuals with late onset Friedreich's ataxia. *FXN* encodes frataxin, a mitochondrially targeted protein that is an iron chaperone and plays a role in mitochondrial iron handling (Pandolfo 2006). Frataxin contributes to iron-sulfur cluster and heme synthesis. Both iron-sulfur clusters and heme are required MRC constituents.

Wilson's disease

Wilson's disease, also called Hepatolenticular Degeneration, is a neuropsychiatric and hypokinetic movement disorder (Das et al. 2006). The age of onset is variable, but it usually occurs before the sixth decade. Neurodegeneration classically appears as necrosis within the basal ganglia, but can also involve the brainstem, thalamus, cerebellum, and cerebral cortex.

Wilson's disease is inherited as an autosomal recessive disorder, with mutations occurring within the *ATPase copper transporting beta polypeptide (ATP7B)* gene (Tanzi et al. 1993). The ATP7B protein localizes into mitochondria (Lutsenko et al. 1998). MCR dysfunction has been demonstrated in liver tissue from Wilson's disease patients (Gu et al. 2000). Recessive mutation of *ATP7B* leads to perturbed mitochondrial copper homeostasis and, as a consequence, impaired mitochondrial function. Oxidative stress, reduced COX activity, and activation of intrinsic apoptotic cascades may represent common features of copper-mediated mitochondrial toxicity (Rossi et al. 2004).

Hereditary spastic paraplegia

An autosomal recessive form of hereditary spastic paraplegia is due to mutations in the *SPG7* gene, which encodes paraplegin, a mitochondrial protein similar to yeast metalloproteases (Casari et al. 1998). Impairment of the respiratory chain is suggested by the presence of ragged-red fibers and fibers deficient in cytochrome *c* oxidase in muscle from affected patients.

Mitochondria and cancer

Mitochondrial defects have been suspected to play an important role in the development and progression of cancer. Since the initial publications by Warburg over half a century ago (Warburg et al. 1930, Warburg 1956), a number of cancer-related mitochondrial defects have been identified and described in the literature. These defects include altered expression and activity of respiratory chain subunits and glycolytic enzymes, decreased oxidation of NADH-linked substrates, and mitochondrial DNA (mtDNA) mutations (Kroemer 2006). Whereas there are many reports of these phenomena, the exact mechanisms responsible for the initiation and evolution of mtDNA mutations, and their roles in the development of cancer and disease progression still remain to be elucidated.

It’s a common observation that cancer cells tend to synthesize ATP mainly through aerobic glycolysis, the so-called “Warburg effect”. This metabolic state is associated with high glucose uptake and local acidification because of lactate production.

Several mechanisms have been proposed to explain this phenomenon, including the up-regulation of rate-limiting steps of glycolysis, the accumulation of mutations in the mitochondrial genome, the hypoxia induced switch from mitochondrial respiration to glycolysis or the metabolic reprogramming resulting from the loss-of-function of enzymes. In specific cases, mitochondrial enzymes can act as tumour-suppressor proteins whose mutations indirectly favour aerobic glycolysis. Mutation in genes encoding mitochondrial proteins such as succinate dehydrogenase (SDH subunits B, C or D) and fumarate dehydrogenase is an oncogenic event, causing phaeochromocytomas (in the case of SDH mutations) and leiomyosarcomas or renal carcinomas (in the case of fumarate dehydrogenase mutations). The loss of function of these deyhdrogenases results in the accumulation of fumarate and succinate in the cytosol, respectively. This eventually favours the activation of the transcription factor hypoxia-inducible factor (HIF) and hence a general reprogramming of the metabolism towards aerobic glycolysis (King et al. 2006).

Aerobic glycolysis in cancer is not just a historical reminiscence or a biochemical curiosity. In fact, the Warburg effect is actually the basis for the widespread application of positron emission tomography in which a glucose analogue tracer (2-18fluoro-2-deoxy-D-glucose) is used to differentiate between normal and tumour tissue.

A possible explanation to the switch from mitochondrial respiration to glycolysis could be the fact that cancer cells accumulate defects in the mitochondrial genome, leading to deficient mitochondrial respiration and ATP generation (Brandon et al. 2006, Chatterjee et al. 2006). Another important feature to be taken into account is that cancer cells

are generally more active than normal cells in metabolic ROS generation and are constantly under oxidative stress (Hileman et al. 2001). It is possible that certain mtDNA mutations may be caused by endogenous ROS in cancer cells. Mutations in mtDNA could in turn cause further increases of ROS production, leading to additional mutations and oxidative stress.

In summary, the presence of mtDNA mutations in cancer cells is concordant with the intrinsic susceptibility of mtDNA to damage and constitutive oxidative stress. Alterations in OXPHOS activity and mtDNA abnormalities appear to be a general feature of malignant cells. In fact mutations and deletions in mtDNA and abnormal expression of mtDNA-encoded proteins have been observed in various tumors (Tan et al. 2002).

Finally, given the importance of mitochondria in apoptosis, all these alterations can lead to an enhanced resistance of cancer cells to apoptotic signalling cascade. The mitochondrial outer membrane (MOM) permeabilization is a necessary step in this pathway (Zamzami et al. 1998): usually pro-apoptotic proteins (cytochrome c, apoptosis inducing factor (AIF), endonuclease G, and smac/DIABLO) reside in the mitochondria but after an apoptotic stimulus are released into the cytosol. A block of MOM permeabilization is associated to development of tumours for instance the switch from pre-neoplasia to overt neoplasia in haematological cancers (Fontenay et al. 2006), even if the mechanistic link between aerobic glycolysis and MOM permeabilization resistance is not clear yet.

Treatment of the mitochondrial diseases

There are many strategies to treat mitochondrial disease albeit an effective therapy is missing, to date. They are based on genetic or metabolic interventions, involving either correction of the affected gene or attenuation of the negative up- or downstream effects caused by the defective enzyme complex/complexes. These include (i) preventing transmission of mtDNA and mitochondrial nDNA gene defects, (ii) gene therapy (replacement or repair), (iii) altering the balance between wild-type and mutated mtDNA (i.e. exercise training), (iv) controlled regulation of specific transcriptional regulators and (v) metabolic manipulation (radical oxygen scavenging, mitochondrial calcium homeostasis, and uncoupling proteins).

Table 2. Recent strategies for mitochondrial therapy

Preventing transmission of mtDNA	Gene therapy	Altering the balance between mutated and wt mtDNA	Controlled regulation of transcription regulators	Metabolic manipulation
Oocyte donation	Allotopic expression	Mutation repair (Zinc finger binding proteins)	Sirtuins	Preventing oxygen damage (scavenging, allotropic expression)
Mutation analysis of chorionic villi	Correction of translational defects (tRNA)	Restriction endonucleases		Calcium modulation
Preimplantation genetic diagnosis	Expression of nuclear encoded ANT-1 and TFAM	Peptide nucleic acid oligomers		Uncoupling proteins
		Stimulating satellite cells		Nutritional intervention

A recent and comprehensive review on this topic, was written by Koene et al. (2009), with the last new approaches, some of which have yet to be explored in humans (Tab.2).

An intervention used in patients with mitochondrial disease is the metabolic therapy.

Metabolic acidosis resulting from increased lactate production, a common feature in mitochondrial defects, can be treated, at least in the short-term, buffering with antiacids such as sodium bicarbonate. Some drugs, which stimulates pyruvate oxidation (i.e. dichloroacetate), has also been used to lower lactate concentration, but adverse effects preclude its widespread clinical use (Barshop et al. 2004).

In the rare mitochondrial disorder, MNGIE, due to mutations in *TP* gene (Hirano et al. 2005), the problem is the accumulation of dangerous nucleoside precursors, thymidine. A therapeutical symptomatic approach is to try to decrease the blood level of these substances through transfusions or by binding them to a water-soluble compound facilitating urinary excretion. Dietary supplementation of cofactors and vitamins is also widely used in the treatment of OXPHOS disease, but there is little evidence, apart from anecdotal reports, to support their use.

Creatine is the substrate for the synthesis of phosphocreatine, the most abundant energy storage compound in muscle, heart and brain. Different trials in patients with neuromuscular disorders gave opposite results regarding the efficacy of this substance. However considering the absence of adverse effects, creatinine supplementation may be

warranted in patients with muscle weakness even before the confirmation of its efficacy.

Ubiquinone (CoQ) and its shorter chain analogue idebenone, could theoretically be effective for improving OXPHOS or, alternatively, as antioxidants or ROS scavengers. Although CoQ has shown some preliminary promising results in Parkinson's disease and Friedreich's ataxia (Rustin et al. 1999), there have been no large-scale studies to determine the effectiveness of CoQ in these disorders. This treatment is most clearly effective in the small number of patients who have a specific CoQ deficiency (Salviati et al. 2005).

Since defects of OXPHOS result in the increased production of free radicals, the use of antioxidants has some sound basis. This is the reason of the supplementation with vitamins like tocopherols (Kir et al. 2005). The above reported CoQ and N-acetylcysteine reduce free radical production in cybrids (Mattiazzi et al. 2004). More recently, antioxidant compounds, which are directly targeted to mitochondria have been shown to be effective in cultured cell models of OXPHOS disease, but has yet to be tested in a clinical trial (James et al. 2005, Koopman et al. 2005).

Scope of the thesis

My researches during the DIMET project have been focused on the discovery of new genes responsible for mitochondrial disorders and the characterization of their role.

Recent epidemiological studies show that mitochondrial disorders have an incidence of 1:5000. These disorders are very heterogeneous and hence the diagnosis is difficult. Moreover mitochondrial dysfunctions are now clearly related to a wide range of disease conditions (i.e. neurodegeneration and cancer).

The majority of the inherited mitochondrial disorders, especially those with onset in infancy or childhood, are due to nuclear genes encoding proteins targeted to mitochondria. While identification of mutations in mitochondrial DNA has become relatively easy thank to the feasibility to perform the complete sequence analysis of mtDNA, the analysis of genomic DNA is more complicate and therefore the number of nuclear genes associated with mitochondrial diseases is still small.

Genome-wide analysis in families with autosomal recessive mitochondrial disorders could help to identify a genomic region to be further investigated. However, about one half/one third of the components of the mitochondrial proteome have yet to be identified, and this lack of information makes the search of candidate genes more difficult.

By linkage analysis or homozygosity mapping and prioritization of candidate genes, I studied subjects from multiconsanguineos families characterized by clinical pictures compatible with mitochondrial disorders.

In chapter 2, there is the report regarding the discovery of a nonsense mutation in two brothers displaying asymmetric brain atrophy, psychomotor regression and severe complex IV deficiency. The mutated gene codes for a mitochondrial predicted kinase that may have a role in apoptosis.

Using the same procedure, I take part in a project, which leads to the identification of the first assembly factor for complex II of the OXPHOS system (Chapter 3). Two different mutations were found in two pedigrees, with affected children characterized by acute psychomotor regression followed by spastic quadriparesis and/or dystonia. The pathogenic role of the mutations was confirmed in cellular and yeast models.

Finally, in chapter 4, there is the characterization of a protein, MR-1, already known and responsible for a movement disorder (PNKD, Paroxysmal non kinesigenic Dyskinesia). The mutant isoforms were erroneously localized into cytosol or membranes, whereas I demonstrated that they are mitochondrial and that the mutations reported so far in PNKD patients (and a new mutation identify in our study) are in the mitochondrial targeting signal (MTS). Hence PNKD could be considered a mitochondrial disease, due to a novel mechanism based on a deleterious action of the MTS

REFERENCES

Agostino A, Valletta L, Chinnery PF et al. (2003) Mutations of ANT1, Twinkle, and POLG1 in sporadic progressive external ophthalmoplegia (PEO). Neurology 60: 1354–6.

Allen DA &, Ottaway JH. (1986) Succinate thiokinase in pigeon breast muscle mitochondria. FEBS Lett. 194(1):171-5.

Amati-Bonneau P, Valentino ML, Reynier P, Gallardo ME, Bornstein B, Boissière A, et al. (2008) OPA1 mutations induce mitochondrial DNA instability and optic atrophy 'plus' phenotypes. Brain. 131(Pt 2):338-51.

Antonicka H, Mattman A, Carlson CG, Glerum DM., Hoffbuhr KC, Leary SC, Kennaway NG, and Shoubridge EA (2003). Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. Am J Hum Genet 72, 101-114.

Antonicka H, Sasarman F, Kennaway NG, Shoubridge EA. (2006) The molecular basis for tissue specificity of the oxidative phosphorylation deficiencies in patients with mutations in the mitochondrial translation factor EFG1. Hum Mol Genet 15:1835–46.

Arnoult D, Parone P, Martinou JC, Antonsson B, Estaquier J, Ameisen JC. (2002) Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome c release in response to several proapoptotic stimuli. J Cell Biol.159(6):923-9.

Balaban RS, Nemoto S, Finkel T. (2005) Mitochondria, oxidants, and aging. Cell 120: 483–95.

Barshop BA, Naviaux RK, McGowan KA, Levine F, Nyhan WL, Loupis-Geller A, Haas RH. (2004) Chronic treatment of mitochondrial disease patients with dichloroacetate. Mol Genet Metab. 83(1-2):138-49.

Barth PG, Wanders RJ, Vreken P, Janssen EA, Lam J, Baas F. (1999) X-linked cardioskeletal myopathy and neutropenia (Barth syndrome) (MIM 302060). J Inherit Metab Dis. 22(4):555-67.

Beal MF. (2005). Mitochondria take center stage in aging and neurodegeneration. Annals of Neurology 58: 495–505.

Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. (2002) Diagnostic criteria for respiratory chain disorders in adults and children. Neurology 59: 1406–11.

Bindoff LA, Birch-Machin M, Cartlidge NE, Parker WD Jr., Turnbull DM (1989) Mitochondrial function in Parkinson's disease. Lancet 2: 49.

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.

Bourdon A, Minai L, Serre V, Jais JP, Sarzi E, Aubert,S, Chretien D, de Lonlay P, Paquis-Flucklinger V, Arakawa H, et al. (2007). Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. Nat Genet 39, 776-780.

Boyer PD (1993). The binding change mechanism for ATP synthase -some probabilities and possibilities. Biochim Biophys Acta 1140, 215-250.

Brandon M, Baldi P, Wallace DC. (2006) Mitochondrial mutations in cancer. Oncogene 25(34):4647-62.

Brown RE, Bhuvaneswaran C, Brewster M. (1988) Effects of peroxidized polyunsaturated fatty acids on mitochondrial function and structure: pathogenetic implications for Reye's syndrome. Ann Clin Lab Sci. 18(4):337-43.

Bykhovskaya Y, Mengesha E, Wang D et al. (2004) Phenotype of non-syndromic deafness associated with the mitochondrial A1555G mutation is modulated by mitochondrial RNA modifying enzymes MTO1 and GTPBP3. Mol Genet Metab 83: 199–206.

Campuzano V, Montermini L, Molto M D, et al. (1996) Friedreich's ataxia: Autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science, 271, 1423–1427.

Candé C, Cecconi F, Dessen P, Kroemer G. (2002) Apoptosis-inducing factor (AIF): key to the conserved caspase-independent pathways of cell death? J Cell Sci. 115(Pt 24):4727-34.

Carew JS, and Huang P (2002). Mitochondrial defects in cancer. Mol Cancer 1, 9.

Carroll J, Fearnley IM, Skehel JM, Shannon RJ, Hirst J, and Walker JE (2006). Bovine Complex I Is a Complex of 45 Different Subunits. J. Biol. Chem. 281, 32724-32727.

Carrozzo R, Dionisi-Vici C, Steuerwald U et al. (2007) SUCLA2 mutations are associated with mild methylmalonic aciduria, Leigh-like encephalomyopathy, dystonia and deafness. Brain 13: 862–74.

Casari G, De Fusco M, Ciarmatori S, Zeviani M, Mora M, Fernandez P, De Michele G, Filla A, Cocozza S, Marconi R, Dürr A, Fontaine B, Ballabio A. (1998) Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. Cell. 93(6):973-83.

Chatterjee A, Mambo E, Sidransky D. (2006) Mitochondrial DNA mutations in human cancer. Oncogene 25(34):4663-74.

Cheung EC, Joza N, Steenaart NA, McClellan KA, Neuspiel M, McNamara S, MacLaurin JG, Rippstein P, Park DS, Shore GC, McBride HM, Penninger JM, Slack RS. (2006) Dissociating the dual roles of apoptosis-inducing factor in maintaining mitochondrial structure and apoptosis. EMBO J. 25(17):4061-73.

Cízková A, Stránecký V, Mayr JA, Tesarová M, Havlícková V, Paul J, Ivánek R, Kuss AW, Hansíková H, Kaplanová V, Vrbacký M, Hartmannová H, Nosková L, Honzík T, Drahota Z, Magner M, Hejzlarová K, Sperl W, Zeman J, Houstek J, Kmoch S. (2008) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. Nat Genet. 40(11):1288-90.

Coenen MJ, Antonicka H, Ugalde C, et al. (2004) Mutant mitochondrial elongation factor G1 and combined oxidative phosphorylation deficiency. N Engl J Med 351:2080–2086.

Cooper J M, Mann V M, Schapira AH. (1992). Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: Effect of ageing. Journal of the Neurological Sciences, 113, 91–98.

Cree LM, Samuels DC, Chinnery PF. (2009) The inheritance of pathogenic mitochondrial DNA mutations. Biochim Biophys Acta. Mar 19. [Epub ahead of print]

Cregan SP, Dawson VL, Slack RS. (2004) Role of AIF in caspase-dependent and caspase-independent cell death. Oncogene.23(16):2785-96.

Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D (2006) Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 127, 59-69.

Danial NN& Korsmeyer SJ. (2004) Cell death: critical control points. Cell 116(2):205-19.

Das SK, Ray K (2006) Wilson's disease: an update. Nat Clin Pract Neurol 2, 482-493.

Davey KM, Parboosingh JS, McLeod DR, Chan A, Casey R, Ferreira P, Snyder FF, Bridge PJ, Bernier FP. (2006) Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. J Med Genet. 43(5):385-93.

de la Monte SM, Luong T, Neely TR, Robinson D, Wands JR (2000) Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's disease. Lab Invest 80, 1323-1335.

Debray FG, Lambert M, Mitchell GA. (2008) Disorders of mitochondrial function. Curr Opin Pediatr.20(4):471-82.

de Lonlay P, Valnot I, Barrientos A, Gorbatyuk M, Tzagoloff A, Taanman JW, Benayoun E, Chretien D, Kadhom N, Lombes A, et al. (2001). A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. Nat Genet 29, 57-60.

De Meirleir L, Seneca S, Lissens W, De Clercq I, Eyskens F, Gerlo E, Smet J, and Van Coster R (2004). Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12. J Med Genet 41, 120-124.

de Paepe B, Smet J, Leroy JG, Seneca S, George E, Matthys D, van Maldergem L, Scalais E, Lissens W, de Meirleir L, Meulemans A, van Coster R. (2006) Diagnostic

value of immunostaining in cultured skin fibroblasts from patients with oxidative phosphorylation defects. Pediatr Res. 59(1):2-6.

Devenish RJ, Prescott M, Roucou X, Nagley P. (2000) Insights into ATP synthase assembly and function through the molecular genetic manipulation of subunits of the yeast mitochondrial enzyme complex. Biochim Biophys Acta. 1458(2-3):428-42.

Dimauro S, & Davidzon G. (2005) Mitochondrial DNA and disease. Ann Med 37: 222–32.

DiMauro S, & Schon EA. (2001) Mitochondrial DNA mutations in human disease. Am J Med Genet 106: 18–26.

DiMauro S, and Schon EA (2003). Mitochondrial respiratory-chain diseases. N Engl J Med 348, 2656-2668.

Di Monte DA, Harati Y, Jankovic J, Sandy MS, Jewell SA, Langston JW (1994) Muscle mitochondrial ATP production in progressive supranuclear palsy. J Neurochem 62, 1631-1634.

Edvardson S, Shaag A, Kolesnikova O et al. (2007) Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. Am J Hum Genet 81: 857–62.

Ekstrand MI, Terzioglu M, Galter D, Zhu S, Hofstetter C, Lindqvist E, Thams S, Bergstrand A, Hansson FS, Trifunovic A, et al. (2007). Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proc Natl Acad Sci U S A 104, 1325-1330.

Elpeleg O, Miller C, Hershkovitz E et al. (2005) Deficiency of the ADP-forming succinyl-CoA synthase activity is associated with encephalomyopathy and mitochondrial DNA depletion. Am J Hum Genet 76: 1081–6.

Esteves AR, Domingues AF, Ferreira IL, Januario C, Swerdlow RH, Oliveira CR, Cardoso SM (2008) Mitochondrial function in Parkinson's disease cybrids containing an nt2 neuron-like nuclear background. Mitochondrion 8, 219-228.

Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, Chown MJ, Hebert LE, Hennekens CH, Taylor JO (1989) Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. JAMA 262, 2551-2556.

Ferrari G, Lamantea E, Donati A et al. (2005) Infantile hepatocerebralsyndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. Brain 128: 723–31.

Finsterer J. (2001) Cerebrospinal-fluid lactate in adult mitochondriopathy with and without encephalopathy. Acta Med Austriaca.;28(5):152-5.

Fontanesi F, Soto IC, Horn D, Barrientos A. (2006) Assembly of mitochondrial cytochrome c-oxidase, a complicated and highly regulated cellular process. Am J Physiol Cell Physiol. 291(6):C1129-47.

Fontenay M, Cathelin S, Amiot M, Gyan E, Solary E. (2006). Oncogene 25: 4757–4767.

Freisinger P, Futterer N, Lankes E, et al. (2006) Hepatocerebral mitochondrial DANN depletion syndrome caused by deoxyguanosine kinase (DGUOK) mutations. Arch Neurol 63:1129–1134.

Frezza C, Cipolat S, Martins de Brito O, et al. (2006) OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell 126:177–189.

Fukui H, Diaz F, Garcia S, and Moraes CT (2007). Cytochrome c oxidase deficiency in neurons decreases both oxidative stress and amyloid formation in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 104, 14163-14168.

Galbiati S, Bordoni A, Papadimitriou D, Toscano A, Rodolico C, Katsarou E, et al. (2006) New mutations in TK2 gene associated with mitochondrial DNA depletion. Pediatr Neurol 34 (3): 177-185.

Ghezzi D, Marelli C, Achilli A, Goldwurm S, Pezzoli G, Barone P, Pellecchia MT, Stanzione P, Brusa L, Bentivoglio AR, Bonuccelli U, Petrozzi L, Abbruzzese G, Marchese R, Cortelli P, Grimaldi D, Martinelli P, Ferrarese C, Garavaglia B, Sangiorgi S, Carelli V, Torroni A, Albanese A, Zeviani M. (2005) Mitochondrial DNA haplogroup K is associated with a lower risk of Parkinson's disease in Italians. Eur J Hum Genet. 13(6):748-52.

Ghezzi D, Goffrini P, Uziel G, Horvath R, Klopstock T, Lochmüller H, D'Adamo P, Gasparini P, Strom TM, Prokisch H, Invernizzi F, Ferrero I, Zeviani M. (2009) *SDHAF1*, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy. Nature Genetics 41, 654–656.

Gohil VM, Hayes P, Matsuyama S, Schägger H, Schlame M, Greenberg ML. (2004) Cardiolipin biosynthesis and mitochondrial respiratory chain function are interdependent.J Biol Chem. 279(41):42612-8.

Green DR, & Reed JC. (1998) Mitochondria and apoptosis. Science 281(5381): 1309-12.

Green DR. (2005) Apoptotic pathways: ten minutes to dead. Cell 121(5):671-4.

Gu M, Cooper JM, Butler P, Walker AP, Mistry PK, Dooley JS, Schapira AH. (2000) Oxidative-phosphorylation defects in liver of patients with Wilson's disease. Lancet 356(9228):469-74.

Haas RH. (2007) The evidence basis for coenzyme Q therapy in oxidative phosphorylation disease. Mitochondrion 7 Suppl:S136-45.

Haas RH, Parikh S, Falk MJ, Saneto RP, Wolf NI, Darin N, Wong LJ, Cohen BH, Naviaux RK. Mitochondrial Medicine Society's Committee on Diagnosis. (2008) The in-depth evaluation of suspected mitochondrial disease. Mol Genet Metab. 94(1):16-37.

Hamza I. (2006) Intracellular trafficking of porphyrins. ACS Chem. Biol. 1(10):627-9.

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256, 184-185.

Hart MN, Cancilla PA, Frommes S, Hirano A (1977) Anterior horn cell degeneration and Bunina-type inclusions associated with dementia. *Acta* Neuropathol 38, 225-228.

Hatcher JM, Pennell KD, Miller GW. (2008) Parkinson's disease and pesticides: a toxicological perspective.Trends Pharmacol Sci. 29(6):322-9.

Haut S, Brivet M, Touati G, Rustin P, Lebon S, Garcia-Cazorla A, Saudubray JM, Boutron A, Legrand A, Slama A. (2003) A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis. Hum Genet. 113(2):118-22.

Hileman EA Achanta G, Huang P. (2001) Superoxide dismutase: an emerging target for cancer therapeutics. Expert Opin Ther Targets 5(6):697-710.

Hirano A, Nakano I, Kurland LT, Mulder DW, Holley PW, Saccomanno G (1984) Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 43, 471-480.

Hirano M, Lagier-Tourenne C, Valentino ML, Martí R, Nishigaki Y. (2005) Thymidine phosphorylase mutations cause instability of mitochondrial DNA. Gene 354:152-6.

Holt IJ, Harding AE, and Morgan-Hughes JA (1988) Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature 331, 717-719.

Hong SJ, Dawson TM, Dawson VL. (2004) Nuclear and mitochondrial conversations in cell death: PARP-1 and AIF signaling. Trends Pharmacol Sci.25(5):259-64.

Hudson G, Amati-Bonneau P, Blakely EL et al. Mutation of OPA1 causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance. Brain 2008; 131: 329–37.

James AM, Cochemé HM, Smith RA, Murphy MP. (2005) Interactions of mitochondria-targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species. Implications for the use of exogenous ubiquinones as therapies and experimental tools. J Biol Chem. 280(22):21295-312.

Janssen RJ, Nijtmans LG, van den Heuvel LP, and Smeitink JA (2006). Mitochondrial complex I: structure, function and pathology. J Inherit Metab Dis 29, 499-515.

Janssen AJ, Trijbels FJ, Sengers RC, Smeitink JA, van den Heuvel LP, Wintjes LT, Stoltenborg-Hogenkamp BJ, Rodenburg RJ. (2007) Spectrophotometric assay for complex I of the respiratory chain in tissue samples and cultured fibroblasts. Clin Chem. 53(4):729-34.

Jefcoate C. (2002) High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. J Clin Invest. 110(7):881-90.

Jiang F, Ryan MT, Schlame M, Zhao M, Gu Z, Klingenberg M, Pfanner N, Greenberg ML. (2000) Absence of cardiolipin in the crd1 null mutant results in

decreased mitochondrial membrane potential and reduced mitochondrial function. J Biol Chem.275(29):22387-94.

Johnson JD, Mehus JG, Tews K, Milavetz BI, Lambeth DO. (1998a) Genetic evidence for the expression of ATP- and GTP-specific succinyl-CoA synthetases in multicellular eucaryotes. J Biol Chem. 273(42):27580-6.

Johnson JD, Muhonen WW, Lambeth DO. (1998b) Characterization of the ATP- and GTP-specific succinyl-CoA synthetases in pigeon. The enzymes incorporate the same alpha-subunit. J Biol Chem. 273(42):27573-9.

King A, Selak MA, Gottlieb E. (2006) Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 25: 4675–4682.

Kir HM, Dillioglugil MO, Tugay M, Eraldemir C, Ozdoğan HK. (2005) Effects of vitamins E, A and D on MDA, GSH, NO levels and SOD activities in 5/6 nephrectomized rats. Am J Nephrol.25(5):441-6.

Kish SJ, Bergeron C, Rajput A, Dozic S, Mastrogiacomo F, Chang LJ, Wilson JM, DiStefano LM, Nobrega JN (1992) Brain cytochrome oxidase in Alzheimer's disease. J Neurochem 59, 776-779.

Koene S. & Smeitink J. (2009) Mitochondrial medicine: entering the era of treatment. Journal of Internal Medicine 265; 193–209.

Kollberg G, Darin N, Benan K, Moslemi AR, Lindal S, Tulinius M, Oldfors A, and Holme E (2009). A novel homozygous RRM2B missense mutation in association with severe mtDNA depletion. Neuromuscul Disord. 2009 Feb;19(2):147-50.

Koopman WJ, Verkaart S, Visch HJ, van der Westhuizen FH, Murphy MP, van den Heuvel LW, Smeitink JA, Willems PH. (2005) Inhibition of complex I of the electron transport chain causes O2-. -mediated mitochondrial outgrowth. Am J Physiol Cell Physiol. 288(6):C1440-50.

Kroemer G, & Reed JC (2000). Mitochondrial control of cell death. Nat Med 6, 513-519.

Kroemer G. (1997) Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution. Cell Death Differ. 4(6):443-56.

Kroemer G. (2006). Mitochondria in cancer. Oncogene, 25, 4630-4632.

Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, et al. (2005). Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 309, 481-484.

Kujoth, G. C., Leeuwenburgh, C., & Prolla, T. A. (2006). Mitochondrial DNA mutations and apoptosis in mammalian aging. Cancer Research, 66, 7386–7389.

Lagier-Tourenne C, Tazir M, López LC, Quinzii CM, Assoum M, Drouot N, Busso C, Makri S, Ali-Pacha L, Benhassine T, Anheim M, Lynch DR, Thibault C, Plewniak F, Bianchetti L, Tranchant C, Poch O, DiMauro S, Mandel JL, Barros MH, Hirano M, Koenig M. (2008) ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q10 deficiency. Am J Hum Genet. 82(3):661-72.

Lambeth DO, Tews KN, Adkins S, Frohlich D, Milavetz BI. (2004) Expression of two succinyl-CoA synthetases with different nucleotide specificities in mammalian tissues. J Biol Chem. 279(35):36621-4.

Le Ber I, Dubourg O, Benoist JF, et al. (2007) Muscle coenzyme Q10 deficiencies in ataxia with oculomotor apraxia 1. Neurology 68:295–297.

Lee HJ, Shin SY, Choi C, Lee YH, Lee SJ (2002) Formation and removal of alphasynuclein aggregates in cells exposed to mitochondrial inhibitors. J Biol Chem 277, 5411-5417.

Letai AG. (2008) Diagnosing and exploiting cancer's addiction to blocks in apoptosis.Nat Rev Cancer. 8(2):121-32.

Lill R, & Mühlenhoff U.(2006) Iron-sulfur protein biogenesis in eukaryotes: components and mechanisms. Annu Rev Cell Dev Biol.22:457-86.

Lin MT, Beal MF. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795.

Lipton SA, & Bossy-Wetzel E. (2002) Dueling activities of AIF in cell death versus survival: DNA binding and redox activity. Cell. 18;111(2):147-50.

Lister et al. 2005Lister R, Hulett JM, Lithgow T, Whelan J. (2005) Protein import into mitochondria: origins and functions today. Mol Membr Biol 22: 87–100.

Liu X, Kim CN, Yang J, Jemmerson R, Wang X. (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 12;86(1):147-57.

Loeffen JL, Smeitink JA, Trijbels JM et al. (2000) Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. Hum Mutat 15: 123–34.

Loeffler M, Daugas E, Susin SA, Zamzami N, Metivier D, Nieminen AL, Brothers G, Penninger JM, Kroemer G. (2001) Dominant cell death induction by extramitochondrially targeted apoptosis-inducing factor. FASEB J. 2001 Mar;15(3):758-67.

Longley MJ, Clark S, Yu Wai MC et al. (2006) Mutant POLG2 disrupts DNA polymerase gamma subunits and causes progressive external ophthalmoplegia. Am J Hum Genet 78: 1026–34.

Lopez LC, Schuelke M, Quinzii CM, et al. (2006) Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. Am J Hum Genet 79:1125–1129.

López-Lluch G, Irusta PM, Navas P, de Cabo R. (2008) Mitochondrial biogenesis and healthy aging. Exp Gerontol. 43(9):813-9.

Luoma P, Melberg A, Rinne JO et al. (2004) Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. Lancet 364: 875–82.

Lutsenko S, Cooper MJ. (1998) Localization of the Wilson's disease protein product to mitochondria. Proc Natl Acad Sci U S A. 26;95(11):6004-9.

Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. (2003) The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 72: 333–9.

Mancuso C, Scapagini G, Currò D, Giuffrida Stella AM, De Marco C, Butterfield DA, Calabrese V. (2007) Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. Front Biosci. 12:1107-23.

Margineantu DH, Gregory Cox W, Sundell L, Sherwood SW, Beechem JM, Capaldi RA. (2002) Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. Mitochondrion 1:425–35.

Margulis L. (1976) Genetic and evolutionary consequences of symbiosis. Exp Parasitol 39: 277–349.

Martinelli P, Scaglione C, Lodi R, Iotti S, Barbiroli B (2000) Deficit of brain and skeletal muscle bioenergetics in progressive supranuclear palsy shown in vivo by phosphorus magnetic resonance spectroscopy. Mov Disord 15, 889-893.

Massa V, Fernandez-Vizarra E, Alshahwan S, Bakhsh E, Goffrini P, Ferrero I, Mereghetti P, D'Adamo P, Gasparini P, and Zeviani M (2008). Severe infantile encephalomyopathy caused by a mutation in COX6B1, a nucleus-encoded subunit of cytochrome c oxidase. Am J Hum Genet 82, 1281-1289.

Mattiazzi M, Vijayvergiya C, Gajewski CD, DeVivo DC, Lenaz G, Wiedmann M, Manfredi G. (2004) The mtDNA T8993G (NARP) mutation results in an impairment of oxidative phosphorylation that can be improved by antioxidants. Hum Mol Genet. 13(8):869-79.

McFarland R, Kirby DM, Fowler KJ et al. (2004) De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency. Ann Neuro; 55: 58–64.

Miller C, Saada A, Shaul N, et al. (2004) Defective mitochondrial translation caused by a ribosomal protein (MRPS16) mutation. Ann Neurol 56:734–738.

Miramar MD, Costantini P, Ravagnan L, Saraiva LM, Haouzi D, Brothers G et al. (2001). NADH oxidase activity of mitochondrial apoptosis-inducing factor. J. Biol. Chem. 276, 16391–16398.

Mita S, Schmidt B, Schon EA, Di Mauro S , Bonilla E. (1989) Detection of ‘deleted’ mitochondrial genomes in cytochrome-c oxidase-deficient muscle fibres of a patient with Kearns-Sayre syndrome. *Proc Natl Acad Sci USA* 86, pp. 9509–9513.

Mitchell JD, Borasio GD (2007) Amyotrophic lateral sclerosis. Lancet 369, 2031-2041.

Mollet J, Giurgea I, Schlemmer D, et al. (2007) Prenyldiphosphate synthase, subunit 1 (PDSS1) and OH-benzoate polyprenyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders. J Clin Invest 117:765–772.

Mootha VK, Lepage P, Miller K et al. Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. (2003) Proc Natl Acad Sci USA 100: 605–10.

Munich A, Rotig A, Chretien D, Saudubray JM, Cormier V, Rustin P (1996) Clinical presentation, andlaboratory investigations in respiratory chain deficiency. Eur J Pediatr 155:262–264.

Munnich A et al. (2001) in: Scriver C.R., Beaudet A.L., Sly W.S., Valle D. (Eds.), The Metabolic and Molecular Bases of Inherited Disease, 8th edn., McGraw-Hill Medical Publishing Division, New York, 2261–2274.

Naviaux RK, & Nguyen KV (2004). POLG mutations associated with Alpers' syndrome and mitochondrial DNA depletion. Ann Neurol 55, 706-712.

Nelson KK, Melendez JA. (2004) Mitochondrial redox control of matrix metalloproteinases. Free Radic Biol Med 37:768–84.

Neupert W. (1997) Protein import into mitochondria. Annual Review of Biochemistry Vol. 66: 863-917.

Nikali K, Suomalainen A, Saharinen J, Kuokkanen M, Spelbrink JN, Lönnqvist T, Peltonen L. (2005) Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. Human Molecular Genetics 14(20):2981-2990.

Ogilvie I, Kennaway NG, and Shoubridge EA (2005). A molecular chaperone for mitochondrial complex I assembly is mutated in a progressive encephalopathy. J. Clin. Invest. 115, 2784-2792.

Okamoto K & Shaw JM. (2005) Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annual Review of Genetics 39: 503-536.

Oldfors A, Holme E, Tulinius M, Larsson NG. (1995) Tissue distribution and disease manifestations of the tRNA(Lys) A–>G(8344) mitochondrial DNA mutation in a case of myoclonus epilepsy and ragged red fibres. Acta Neuropathol 90: 328–33.

Ostergaard E, Christensen E, Kristensen E et al. (2007) Deficiency of the alpha subunit of succinate-coenzyme A ligase causes fatal infantile lactic acidosis with mitochondrial DNA depletion. Am J Hum Genet 81: 383–7.

Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, et al. (2008). A mitochondrial protein compendium elucidates complex I disease biology. Cell 134, 112-123.

Palmieri L, Alberio S, Pisano I, Lodi T et al. (2005) Complete loss-of-function of the heart / muscle-specific adenine nucleotide translocator is associated with mitochondrial myopathy and cardiomyopathy. Hum Mol Genet 14: 3079–88.

Pandolfo M (2006) Iron and Friedreich ataxia. J Neural Transm [Suppl] 70: 143–146.

Pandolfo M (2008) Friedreich ataxia. Arch Neurol 65, 1296-1303.

Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci 5, 731-736.

Papadopoulou LC, Sue CM, Davidson MM, Tanji K, Nishino I, Sadlock JE, Krishna S, Walker W, Selby J, Glerum DM, et al. (1999). Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. Nat Genet 23, 333-337.

Park LC, Albers DS, Xu H, Lindsay JG, Beal MF, Gibson GE (2001) Mitochondrial impairment in the cerebellum of the patients with progressive supranuclear palsy. J Neurosci Res 66, 1028-1034.

Parker WD, Jr., Boyson SJ, Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol 26, 719-723.

Parker WD, Jr., Filley CM, Parks JK (1990) Cytochrome oxidase deficiency in Alzheimer's disease. Neurology 40, 1302-1303.

Parker WD, Jr., Parks JK, Swerdlow RH (2008) Complex I deficiency in Parkinson's disease frontal cortex. Brain Res 1189, 215-218.

Pereira C, Santos MS, Oliveira C (1998) Mitochondrial function impairment induced by amyloid beta-peptide on PC12 cells. Neuroreport 9, 1749-1755.

Pitkanen S, & Robinson BH. (1996) Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. J Clin Invest 98, 345-351.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 276(5321):2045-7.

Poulton J, Gardiner RM. (1989) Non-invasive diagnosis of mitochondrial myopathy. Lancet 1: 961.

Pyle A, Foltynie T, Tiangyou W, Lambert C, Keers SM, Allcock LM, Davison J, Lewis SJ, Perry RH, Barker R, et al. (2005). Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD. Ann Neurol 57, 564-567.

Quinzii C, Naini A, Salviati L, et al. (2006) A mutation in para-hydroxybenzoatepolyprenyl transferase (COQ2) causes primary coenzyme Q10 deficiency. Am J Hum Genet 78:345–349.

Quinzii CM, Dimauro S, Hirano M. (2007) Human coenzyme Q10 deficiency. Neurochem Res 32: 723–7.

Ran Q, Liang H, Gu M, Qi W, Walter CA, Roberts LJ, Herman B, Richardson A, Van Remmen H. (2004) Transgenic mice overexpressing glutathione peroxidase 4 are protected against oxidative stress-induced apoptosis. J Biol Chem, 279(53), 55137-46.

Reddy PH. (2008) Mitochondrial medicine for aging and neurodegenerative diseases. Neuromol. Med. 10: 291–315.

Reddy PH & Beal MF. (2008) Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. Trends Mol. Med. 14: 45–53.

Reddy PH. (2007) Mitochondrial dysfunction in aging and Alzheimer's disease: strategies to protect neurons, Antioxid. Redox Signal. 9: 1647–1658.

Robinson BH. (1998). Human complex I deficiency: clinical spectrum and involvement of oxygen free radicals in the pathogenicity of the defect. Biochim Biophys Acta 1364, 271-286.

Roesch K, Curran SP, Tranebjaerg L, Koehler CM. (2002) Human deafness dystonia syndrome is caused by a defect in assembly of the DDP1/TIMM8a–TIMM13 complex. Human Molecular Genetics 11 (5) 477-486.

Rimessi A, Giorgi C, Pinton P, Rizzuto R. (2008) The versatility of mitochondrial calcium signals: From stimulation of cell metabolism to induction of cell death. Biochimica et Biophysica Acta 1777: 808–816.

Rossi L, Lombardo MF, Ciriolo MR, Rotilio G (2004) Mitochondrial dysfunction in neurodegenerative diseases associated with copper imbalance. Neurochem Res 29, 493-504.

Rötig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, Schmitz J, et al. (1990) Pearson's marrow-pancreas syndrome: A multisystem mitochondrial disorder in infancy. J Clin Invest 86: 1601–1608.

Rotig A, Mollet J, Rio M, Munnich A. (2007) Infantile and pediatric quinone deficiency diseases. Mitochondrion 7 (Suppl 1):S112–S121.

Rustin P, von Kleist-Retzow JC, Chantrel-Groussard K, Sidi D, Munnich A, Rotig A. (1999) Effect of idebenone on cardiomyopathy in Friedreich ataxia: a preliminary study. Lancet 354: 477–479.

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.

Saada A, Shaag A, Arnon S et al. (2007) Antenatal mitochondrial disease caused by mitochondrial ribosomal protein (MRPS22) mutation. Med Genet 44: 784–6.

Salviati L, Sacconi S, Murer L, Zacchello G, Franceschini L, Laverda AM, Basso G, Quinzii C, Angelini C, Hirano M, Naini AB, Navas P, DiMauro S, Montini G. (2005) Infantile encephalomyopathy and nephropathy with CoQ10 deficiency: A CoQ10-responsive condition. NEUROLOGY 65: 606-608.

Sanadi DR, Langley M, White F. (1954) α-Ketoglutaric Dehydrogeanase. The role of thioctic acid. JBC 234, I.

Sarzi E, Goffart S, Serre V, Chretien D, Slama A, Munnich A, Spelbrink JN, and Rotig A (2007). Twinkle helicase (PEO1) gene mutation causes mitochondrial DNA depletion. Ann Neurol 62, 579-587.

Scarpulla RC. (2008) Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol Rev 88: 611–38.

Schaefer AM, Taylor RW, Turnbull DM, and Chinnery PF (2004). The epidemiology of mitochondrial disorders--past, present and future. Biochim Biophys Acta 1659, 115-120.

Schapira AH. (2006) Mitochondrial disease. Lancet 368:70–82.

Scheper GC, van der Klok T, van Andel RJ et al. (2007) Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. Nat Genet 39: 534–9.

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 2, 864-870.

Schlame M, Rua D, Greenberg ML. (2000) The biosynthesis and functional role of cardiolipin. Prog. Lipid. Res. 39: 257–288.

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, et al. (2005) Science 308: 1909–1911.

Schwartz M, Vissing J. (2002) Paternal inheritance of mitochondrial DNA. N Engl J Med 347: 576–80.

Seaton TA., Cooper JM.,Schapira AH. (1997). Free radicals scavengers protect dopaminergic cell lines from apoptosis induced by complex I inhibitors. Brain Res. 777**:** 110–118.

Shoffner JM, Lott MT, Voljavec AS, Soueidan SA, Costigan DA, Wallace DC. (1989) Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. Proc Natl Acad Sci USA 86: 7952–7956.

Smeitink JA, Zeviani M, Turnbull DM, Jacobs HT. (2006a) Mitochondrial medicine: a metabolic perspective on the pathology of oxidative phosphorylation disorders. Cell Metab 3: 9–13.

Smeitink JA, Elpeleg O, Antonicka H et al. (2006b) Distinct clinical phenotypes associated with a mutation in the mitochondrial translation elongation factor EFTs. Am J Hum Genet 79:869–77.

Solans A, Zambrano A, Rodriguez M, Barrientos A. (2006) Cytotoxicity of a mutant huntingtin fragment in yeast involves early alterations in mitochondrial OXPHOS complexes II and III. Hum Mol Genet 15: 3063–3081.

Sperl W, Jesina P, Zeman J, Mayr JA, DeMeirleir L, VanCoster R, et al. (2006) Deficiency of mitochondrial ATP synthase of nuclear genetic origin..Neuromuscular Disorders 16: 821–829.

Spinazzola A, Viscomi C, Fernandez-Vizarra E et al. (2006) MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. Nat Genet 38: 570–5.

Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM et al. (1999). Molecular characterization of mitochondrial apoptosis-inducing factor. Nature, 397, 441–446.

Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, Bennett JP, Jr., Davis RE, Parker WD, Jr. (1996) Origin and functional consequences of the complex I defect in Parkinson's disease. Ann Neurol 40, 663-671.

Swerdlow RH, Parks JK, Cassarino DS, Maguire DJ, Maguire RS, Bennett JP, Jr., Davis RE, Parker WD, Jr. (1997) Cybrids in Alzheimer's disease: a cellular model of the disease? Neurology 49, 918-925.

Swerdlow RH (2000) Role of Mitochondria in Parkinson's Disease. In Chesselet MF, ed. Molecular Mechanisms of Neurodegenerative Diseases. New Jersey, Humana Press Inc, 233-270.

Swerdlow RH, Kish SJ (2002) Mitochondria in Alzheimer's disease. Int Rev Neurobiol 53, 341-385.

Swerdlow RH, Khan SM (2004) A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. Med Hypotheses 63, 8-20.

Swerdlow RH (2007) Mitochondria in cybrids containing mtDNA from persons with mitochondriopathies. J Neurosci Res 85, 3416-3428.

Swerdlow RH, Khan SM (2009) The Alzheimer's disease mitochondrial cascade hypothesis: an update. Exp Neurol 218(2):308-15.

Tan DJ, Bai RK, Wong LJ. (2002) Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer Res, 62:972-976.

Tan EK, Skipper LM (2007) Pathogenic mutations in Parkinson disease. Hum Mutat 28, 641-653.

Tanner CM, Goldman SM (1996) Epidemiology of Parkinson's disease. Neurol Clin 14, 317-335.

Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Parano E, Pavone L, Brzustowicz LM, et al. (1993) The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat Genet 5, 344-350.

Taylor RW, & Turnbull DM (2005). Mitochondrial DNA mutations in human disease. Nat Rev Genet 6, 389-402.

The Huntington's Disease Collaborative Research Group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72, 971-983.

Thorburn DR, Sugiana C, Salemi R et al. (2004) Biochemical and molecular diagnosis of mitochondrial respiratory chain disorders. Biochim Biophys Acta 1659: 121–8.

Tiranti V, Hoertnagel K, Carrozzo R, Galimberti C, Munaro M, Granatiero M, Zelante L, Gasparini P, Marzella R, Rocchi M, et al. (1998). Mutations of SURF-1

in Leigh disease associated with cytochrome c oxidase deficiency. Am J Hum Genet 63, 1609-1621.

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, et al. (2004). Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429, 417-423.

Tyynismaa H, Ylikallio E, Patel M, Molnar MJ, Haller RG, Suomalainen A. (2009) A heterozygous truncating mutation in RRM2B causes autosomal-dominant progressive external ophthalmoplegia with multiple mtDNA deletions. Am J Hum Genet. 85(2): 290-5.

Uusimaa J, Moilanen JS, Vainionpaa L et al. (2007) Prevalence, segregation, and phenotype of the mitochondrial DNA 3243A>G mutation in children. Ann Neurol 62: 278–87.

Valente L, Tiranti V, Marsano RM et al. (2007) Infantile encephalopathy and defective mitochondrial DNA translation in patients with mutations of mitochondrial elongation factors EFG1 and EFTu. Am J Hum Genet 80: 44–58.

Valentino ML, Avoni P, Barboni P et al. (2002) Mitochondrial DNA nucleotide changes C14482G and C14482A in the ND6 gene are pathogenic for Leber's hereditary optic neuropathy. Ann Neurol 51: 774-778.

Valentino ML, Barboni P, Ghelli A, Bucchi L, Rengo C, Achilli A, Torroni A, Lugaresi A, Lodi R, Barbiroli B, Dotti M, Federico A, Baruzzi A, Carelli V.(2004) The ND1 gene of complex I is a mutational hot spot for Leber's hereditary optic neuropathy. Ann Neurol. 56(5):631-41.

Valnot I, Osmond S, Gigarel N, Mehaye B, Amiel J, Cormier-Daire V, Munnich A, Bonnefont JP, Rustin P, and Rotig A (2000a). Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. Am J Hum Genet 67, 1104-1109.

Valnot I, von Kleist-Retzow JC, Barrientos A, Gorbatyuk M, Taanman JW, Mehaye B, Rustin P, Tzagoloff A, Munnich A, and Rotig A (2000b). A mutation in the human heme A:farnesyltransferase gene (COX10) causes cytochrome c oxidase deficiency. Hum Mol Genet 9, 1245-1249.

Van Goethem G, Schwartz M, Lofgren A, Dermaut B, Van Broeckhoven C, Vissing J. (2003) Novel POLG mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. Eur J Hum Genet 11: 547–9.

Van Remmen H, Qi W, Sabia M, Freeman G, Estlack L, Yang H, et al. (2004). Multiple deficiencies in antioxidant enzymes in mice result in a compound increase in sensitivity to oxidative stress. Free Radical Biology and Medicine, 36, 1625–1634.

van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Haines JL, Koller WC, Lyons K, et al. (2003). Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet 72, 804-811.

Visapaa I, Fellman V, Vesa J, Dasvarma A, Hutton JL, Kumar V, Payne GS, Makarow M, Van Coster R, Taylor RW, et al. (2002). GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. Am J Hum Genet 71, 863-876.

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57, 369-384.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ, and Nikoskelainen EK (1988). Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242, 1427-1430.

Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dan for evolutionary medicine, Annu. Rev. Genet. 39: 359-407.

Wang, J.C. (2002). Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol 3, 430-440.

Warburg O, Wind F, and Neglers E (1930). On the metabolism of tumors in the body. (London.: Arnold Constable & Co.).

Warburg O. (1956). On respiratory impairment in cancer cells. Science 124, 269-270.

Waterham HR, Koster J, van Roermund CW, et al.(2007) A lethal defect of mitochondrial and peroxisomal fission. N Engl J Med 2007; 356:1736–1741.

Weitzman PD, Jenkins T, Else AJ, Holt RA. (1986) Occurrence of two distinct succinate thiokinases in animal tissues. FEBS Lett. 199(1):57-60.

Wiedemann N, Frazier AE, Pfanner N. (2004) The protein import machinery of mitochondria. JBC 279 (15): 14473-14476.

Wiedemann FR, Winkler K, Kuznetsov AV, Bartels C, Vielhaber S, Feistner H, Kunz WS (1998) Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. J Neurol Sci 156, 65-72.

Ye H, Cande C, Stephanoou NC, Jiang S, Gurbuxani S, Larochette N et al. (2002). DNA binding is required for the apoptogenic action of apoptosis inducing factor. Nat. Struct. Biol. 9, 680–684.

Yuan J, Murrell GA, Trickett A, Wang MX. (2003) Involvement of cytochrome c release and caspase-3 activation in the oxidative stress-induced apoptosis in human tendon fibroblasts. Biochim Biophys Acta. 1641(1):35-41.

Zamzami N, Susin SA, Marchetti P, Hirsch T, Gómez-Monterrey I, Castedo M, Kroemer G. (1996).Mitochondrial control of nuclear apoptosis. J. Exp. Med. 183, 1533-1544.

Zamzami N, Brenner C, Marzo I, Susin SA, Kroemer G. (1998). Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. Oncogene 16: 2265–2282.

Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S et al. (2008). OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. Brain, 131, 352–367.

Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, and Rowland LP (1988). Deletions of mitochondrial DNA in Kearns-Sayre syndrome. Neurology 38, 1339-1346.

Zeviani M, Spinazzola A, Carelli V. (2003) Nuclear genes in mitochondrial disorders. Curr Opin Genet Dev 2003; 13: 262–70.

Zeviani M & DiDonato S. (2004) Mitochondrial disorders. Brain 10: 2153–72.

Zeviani M & Lamantea E. (2006) Genetic Disorders of the Mitochondrial OXPHOS System. SCIENCE & MEDICINE vol. 10(3):154-167.

Zeviani M & Carelli V. (2007) Mitochondrial disorders. Current Opinion in Neurology 20: 564–571.

Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, et al. (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot–Marie–Tooth neuropathy type 2A. Nat Genet 36: 449–51.

Züchner S, Noureddine M, Kennerson M, Verhoeven K, Claeys K, De Jonghe P, Merory J, Oliveira SA, Speer MC, Stenger JE, Walizada G, Zhu D, Pericak-Vance MA, Nicholson G, Timmerman V, Vance JM. (2005) Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. Nat Genet. 37(3):289-94.