Abstract

Oxidative phosphorylation – ATP synthesis by the oxygen-consuming respiratory chain (RC) – supplies most organs and tissues with a readily usable energy source, and is already fully functioning at birth. This means that, in theory, RC deficiency can give rise to any symptom in any organ or tissue at any age and with any mode of inheritance, due to the two-fold genetic origin of RC components (nuclear DNA and mitochondrial DNA). It has long been erroneously believed that RC disorders originate from mutations of mtDNA as, for some time, only mutations or deletions of mtDNA could be identified. However, the number of disease-causing mutations in nuclear genes is now steadily growing. These genes not only encode the various subunits of each complex, but also the ancillary proteins involved in the different stages of holoenzyme biogenesis, including transcription, translation, chaperoning, addition of prosthetic groups and assembly of proteins, as well as the various enzymes involved in mtDNA metabolism.

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1. Mitochondrial respiratory chain

The mitochondrial respiratory chain (RC) catalyzes the oxidation of fuel molecules by oxygen, and the concomitant energy transduction into adenosine triphosphate (ATP) via five complexes embedded in the inner mitochondrial membrane [1] (Fig. 1). Complex I (CI, NADH-coenzyme Q reductase) carries reducing equivalents from NADH to coenzyme Q (CoQ, ubiquinone) and comprises more than 40 different polypeptides. Complex II (CII, succinate-CoQ reductase) carries reducing equivalents from FADH2 to CoQ and comprises four polypeptides, including the FAD-dependent succinate dehydrogenase (SDH) and iron-sulphur proteins. Complex III (CIII, reduced CoQ-cytochrome c reductase) carries electrons from CoQ to cytochrome c, and has 11 subunits, while complex IV (CIV, cytochrome c oxidase or COX), the terminal oxidase of the respiratory chain, catalyzes the transfer of reducing equivalents from cytochrome c to molecular oxygen. It is composed of two cytochromes (a and a3), two copper atoms and 13 different protein subunits.
ubiquinone.

Fig. 1. The mitochondrial respiratory chain. CI–CV: complexes I to V. Q. 

During the oxidation process, electrons are transferred to oxygen via the energy-transducing complexes of the respiratory chain: CI, CIII and CIV for NADH-producing substrates; CII, CIII and CIV for succinate; and CIII and CIV for FADH2 derived from the β-oxidation pathway via electron transfer flavoprotein (ETF) and the ETF–CoQ oxidoreductase system. CoQ, a highly hydrophobic quinone, and cytochrome c, a low-molecular-weight haemoprotein, act as ‘shuttles’ between complexes. The free energy generated from the redox reactions is converted into a transmembrane proton gradient. Protons are pumped through CI, CIII and CIV of the respiratory chain, which creates a charge differential. Complex V (ATP synthase) allows protons to flow back into the mitochondrial matrix and uses the released energy to synthesize ATP. Three ATP molecules are produced from the oxidized NADH.

2. Mitochondrial genetics

Mitochondrial RC is made up of about 100 different proteins, only 13 of which are encoded by mitochondrial genes; the rest are encoded by nuclear genes. All of the RC complexes, except CII, have a double genetic origin, and one to seven subunits of the complexes are mitochondrially encoded. In addition, several hundreds of nuclear genes are needed for various RC functions. The result is that the number of mitochondrial proteins represents more than 3% of all cellular proteins.

2.1. Mitochondrial DNA

Human mitochondrial DNA (mtDNA) is a 16569 base-pair, closed circular molecule [2]. Mitochondrion makes a large reticular network and contains several molecules of mtDNA. Each molecule contains 37 genes encoding one large and one small ribosomal RNA (12S rRNA and 16S rRNA, respectively), 22 transfer RNAs (tRNA) and 13 key respiratory chain subunits [3]. ND1–ND6 are subunits of CI, cytochrome b is the only mitochondrially encoded subunit of CIII, COXI–COXIII are subunits of CIV, and ATP6 and ATP8 are subunits of ATPase (complex V).

The mitochondrion has independent replication, transcription and translation systems. The mitochondrial genome is replicated in two times. Replication starts at the heavy-strand replication origin (OH) and extends clockwise around the mtDNA. When the light-strand replication origin (OL) is exposed as a single strand, the second strand is then replicated in the opposite direction, starting from OL [4]. Thus, replication is bidirectional, but asynchronous. Recently, a new model of mtDNA replication has been proposed in mammals. Replication of mtDNA arises from multiple origins and proceeds via a strand-coupled mechanism [5]. The two mtDNA strands are transcribed from specific promoters into polycistrionic RNA, which is further processed into ribosomal RNAs (rRNA), transfer RNAs (tRNA) and messenger RNAs (mRNA). Mitochondrial mRNAs are translated within the mitochondrial matrix using nuclear-encoded machinery, but following a specific genetic code that is different from the nuclear one.

During cell division, mitochondria are randomly partitioned into daughter cells (mitotic segregation). Usually, all mtDNA molecules are identical but, occasionally, a mixture of wild-type and mutant mtDNA is encountered. This is called ‘heteroplasmy’, whereas ‘homoplasmy’ refers to the occurrence of only one type of mtDNA. In heteroplastic cells, however, the mtDNA genotype can shift during cell replication. Consequently, some lineages drift toward wild-type mtDNA and become homoplasmic, while others remain heteroplasmic.

The mitochondrial genome is maternally transmitted. The mother transmits her mtDNA to all her progeny, males and females, and her daughters, in turn, will transmit their mtDNA to the next generation. Theoretically, males never transmit their mtDNA.

2.2. Nuclear genes

2.2.1. Genes encoding respiratory chain subunits

The majority of RC proteins are encoded by nuclear genes. The nuclear-encoded proteins are translated in cytosol and transported across mitochondrial membranes. These nuclear genes are spread out across all human chromosomes on both autosomes and sexual chromosomes. For example, 33 genes of CI subunits have been mapped to various autosomes, one to the X chromosome and seven to mtDNA. Several of these nuclear genes have one or more pseudogenes (non-expressed copies) that can complicate mutation screening in patients.

2.2.2. Genes involved in respiratory chain assembly

The large number of RC proteins and their double genetic origin indicate tightly regulated communication between mitochondria and nuclear compartments. Therefore, in addition to the structural components of the RC, a large number of nuclear-encoded proteins are involved in the assembly and maintenance of complexes. Most of these genes were first identified in yeast, a model organism for mitochondrial function and dysfunction. Indeed, analysis of yeast mutations resulting in abnormal RC assembly has led to the identification of many of the nuclear products involved in protein folding, stabilization, quality control, membrane translocation and cofactor
addition [6]. To date, at least 350 such genes are known in yeast.

2.2.3. Genes involved in mtDNA metabolism and maintenance

Mitochondria possess specific replication, transcription and repair mechanisms. All of the proteins involved in these mechanisms are encoded by nuclear genes, translated in the cytosol and then translocated to mitochondria. Only the two rRNAs (12S rRNA and 16S rRNA) and the 22 tRNAs are mitochondrially encoded. Thus far, over 100 genes in yeast are known to result in mtDNA loss when defective [7,8]. The proteins involved in mammalian mtDNA maintenance are those directly involved in mtDNA processing, such as DNA polymerase γ (POLG), Twinkle helicase and mitochondrial transcription factor (TFAM). It has long been claimed that mitochondria have no repair mechanisms, but recent evidence suggests that specific DNA repair processes are present in these organelles [8].

2.2.4. Genes involved in mitochondrial protein translation

Mitochondrial translation requires both mitochondrial and nuclear genes. Mitochondrial DNA encodes rRNA and tRNA, whereas nuclear genes encode ribosomal proteins, aminoacyl tRNA synthetases, tRNA modification enzymes, and elongation and termination factors. Altogether, several hundreds of proteins are involved in the translation of the 13 proteins encoded by the mitochondrial genome, emphasizing the considerable investment required to maintain the mitochondrial genetic system [9].

In addition, a large number of other nuclear genes encode proteins that are not directly related to RC assembly or mtDNA maintenance, but which may interact with them. Mutations in these genes can, therefore, give rise to abnormal RC. Among them are chaperones, proteases, proteins involved in mitochondrial inheritance or morphology, antioxidant enzymes, and various carriers of iron, phosphate and citrate. As it is commonly assumed that mitochondria contain roughly 1000 different proteins [10], their genes are all possible candidates for mitochondrial disorders.

3. Mitochondrial respiratory chain disorders

Oxidative phosphorylation is an ubiquitous metabolic pathway that supplies most organs and tissues with energy. Consequently, RC deficiency can theoretically give rise to any symptom in any organ or tissue and at any age with any mode of inheritance, due to the twofold genetic origin of respiratory enzymes (nuclear DNA and mtDNA).

These disorders are characterized by a vast clinical heterogeneity, suggesting high genetic heterogeneity. Yet, although the common feature is RC deficiency, the conditions are due to different enzyme or protein deficiencies. Moreover, the age of onset is highly variable (ranging from the neonatal period to late adulthood), and the deficiency can result in isolated organ deficiency or multivisceral involvement. In the past few years, it has become increasingly clear that genetic defects of oxidative phosphorylation account for a wide variety of clinical symptoms in childhood [11]. In general, the diagnosis of RC deficiency is difficult to make on the basis of a single initial symptom, but becomes easier when two or more seemingly unrelated symptoms are observed.

RC deficiency can be associated with diabetes. Mitochondrial diabetes classically begins during adulthood. Most cases of mitochondrial diabetes are maternally inherited and caused by mtDNA mutations (see below). Nevertheless, some patients with unidentified mutations have also developed diabetes in the course of their disease.

3.1. Metabolic screening

The current screening for RC deficiency includes determinations of plasma lactate, pyruvate, and ketone bodies and their molar ratios as indexes of oxidation/reduction status in cytoplasm and mitochondria, respectively. Persistent hyperlactataemia (> 2.5 mM), with elevated lactate/pyruvate (L/P, > 20) and ketone body molar ratios, is highly suggestive of RC deficiency (particularly in the postabsorptive period). In addition, an investigation of redox status in plasma can help to differentiate the various forms of congenital lactic acidosis, based on L/P and ketone body molar ratios in vivo. Indeed, impairment of oxidative phosphorylation usually results in L/P ratios > 20 and ketone body ratios > 2, whereas a defect of the pyruvate dehydrogenase (PDH) complex results in low L/P ratios (< 10). When basal screening tests are inconclusive, other tests need to be carried out [11,12].

When screening tests are negative, RC deficiency may be misdiagnosed. For this reason, the investigation of patients at risk of RC deficiency should include systematic screening of all possible target organs and tissues, regardless of the onset symptom, as multiple-organ involvement is an important diagnostic clue to RC deficiency.

3.2. Biochemical defects

Diagnostic tests include polarographic and spectrophotometric studies, each provides an independent clue towards the diagnosis of RC deficiency.

Polarographic studies consist in measurement of oxygen consumption by mitochondria-enriched fractions, using a Clarke electrode in the presence of various oxidative substrates (malate + pyruvate, malate + glutamate, succinate, palmitate, etc.) [12]. Measurement of oxygen consumption using intact or detergent-permeabilized circulating lymphocytes (isolated from 10 mL of blood on a Percoll cushion) or cultured cells (lymphoblastoid cell lines, skin fibroblasts) is also feasible, and represents a noninvasive and easily reproducible diagnostic test. The only limitation of these techniques is the absolute requirement for fresh material: no polarographic studies are possible using frozen material.

Spectrophotometric studies involve isolated or combined respiratory enzyme assays, using specific electron donors and acceptors. These do not require isolation of mitochondrial fractions and can be carried out on tissue homogenates. For this reason, the amount of material required for enzyme assays (1–20 mg) is very small and can easily be carried out using needle biopsies of liver, kidney or endomyocardial biopsies or from
a pellet of lymphocytes or cultured skin fibroblasts. Samples should be immediately frozen and kept dry in liquid nitrogen (or at −80 °C) [12].

The question of which tissue should be investigated merits particular attention. In principle, the relevant tissue is the one which clinically expresses the disease. Whatever the affected organ, it is mandatory to take skin-biopsy patients (even post-mortem) for subsequent investigations using cultured fibroblasts. CI, CIV and CI+CIV deficiencies are comparable in frequency (around 25%). A variety of neuromuscular and non-neuromuscular symptoms may be observed, although trunk hypotonia, growth retardation, cardiomyopathy, encephalopathy and liver failure are the most frequent symptoms. However, so far, no clear correlation between type of RC deficiency and clinical presentation has been observed. Similar clinical presentations of mitochondrial dysfunction can result from various RC deficiencies or gene mutations, thereby hampering easy classification of these diseases. For example, Leigh syndrome, a subacute necrotizing encephalomyopathy resulting in a devastating encephalopathy, characterized by recurrent attacks of psychomotor regression, with pyramidal and extrapyramidal symptoms, leukodystrophy and brain-stem dysfunction, may be associated with deficiency of any of the RC complexes and be the result of mutations in either mitochondrial or nuclear genes. Moreover, the mutant genes can encode structural proteins of RC complexes, proteins involved in the assembly of these complexes or proteins involved in the process of mitochondrial translation. On the other hand, in most cases, mutations of a specific gene lead to a relatively homogeneous clinical presentation. Thus, elucidating the genetic bases of RC is essential for both the genetic diagnosis of patients and our fundamental knowledge of these disorders, a prerequisite of any therapy-based research.

4. Genetics of mitochondrial respiratory chain disorders

Mitochondrial RC disorders are genetically heterogeneous. Indeed, the RC is made up of around 100 polypeptides encoded by as many different genes. These genes are either nuclear or mitochondrial. In addition, the biogenesis and assembly of all these polypeptides require several dozens of nuclear genes, some of which are found only in humans. Mitochondrial RC disorders can result from a mutation of any one of these hundreds of genes. Thus, all modes of inheritance may be encountered in mitochondrial RC disorders: sporadic cases due to mtDNA mutations or nuclear gene mutations; maternal transmission in the case of mtDNA mutations; and autosomal recessive, dominant or X-linked inheritance. Unfortunately, in only a few patients (at least 20% in our series) have the disease-causing mutations been identified.

4.1. Mitochondrial DNA mutations

Pathological alterations of mtDNA fall into three major categories: point mutations; deletion-duplications; and copy-number mutations (depletions). In most cases, mtDNA mutations are heteroplasmic as both normal and mutant mtDNA are present. In cells harbouring both mutant and wild-type molecules, the phenotype is a reflection of the proportion of mutant mtDNA molecules and the extent to which the cell type relies on mitochondrial function. Point mutations include amino-acid substitutions and protein-synthesis mutations (tRNA, rRNA).

4.1.1. Mitochondrial protein-synthesis point mutations

The A3243G mutation in the tRNA\textsuperscript{Lys} gene is responsible for the mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome [13]. MELAS is characterized by onset in childhood with intermittent hemicranial headache, vomiting, proximal limb weakness, recurrent neurological deficit resembling stroke (hemiparesis, cortical blindness, hemianopsia), lactic acidosis and, occasionally, ragged red fibres on muscle biopsy. Computed tomography (CT) brain imaging shows low-density areas (usually posterior) that may involve both white and gray matter, but which do not always correlate with clinical symptoms or vascular territories. The pathogenesis of stroke-like episodes in MELAS has been ascribed to either cerebral blood flow disruption or acute metabolic decompensation in biochemically deficient areas of the brain.

The A8344G missense mutation in the mt tRNA\textsuperscript{Lys} gene accounts for 80% of cases of myoclonus epilepsy with ragged red fibres (MERRF) [14]. This disease is characterized by encephalomyopathy with myoclonus, ataxia, hearing loss, muscle weakness and generalized seizures. Syndromic forms of maternally transmitted diabetes represent a frequent clinical presentation of A3243G mutation in the tRNA\textsuperscript{Lys} gene, which is also known to account for MELAS [3,15–17]. Sensorineural hearing loss, cardiomyopathy, vision failure, headache and renal disease are frequently associated with diabetes, and the hearing loss usually develops after the onset of diabetes. It is generally assumed that 1.5% of cases of diabetes in Europe are due to mtDNA mutation and perhaps as much as 5% of cases in East Asia [18]. The A3243G mutation is the most frequent mitochondrial mutation associated with diabetes, although other mutations have been reported [3].

Also, several other mutations of either tRNA or rRNA genes have been reported in non-related families [3].

4.1.2. Mutations in protein-coding genes

The most frequent mutations in mitochondrial genes encoding structural proteins have been reported in Leber hereditary optic neuropathy (LHON) and in neurogenic, ataxia and retinitis pigmentosa (NARP)/Leigh syndrome [3]. These mutations are recurrent mutations, as numerous non-related patients harbour these mutations. Other mutations have also been described in a number of genes, but these are most often restricted to a specific patient or family.

NARP, and variable sensory neuropathy, seizures and mental retardation, are due to an amino-acid change in the ATPase6 gene (T8993G) [19].

LHON is associated with rapid bilateral central vision loss due to optic-nerve death. Cardiac dysrhythmia is frequently associated with the disease, but no evidence of skeletal muscle
pathology or gross structural mitochondrial abnormality has been documented. The median age for vision loss is 20–24 years, but may occur at any time between adolescence and late adulthood. Expression among maternally related individuals is variable, and there is a bias towards affecting males. To date, the disease has been associated with primary mutations (G11778A, T14484C, G15257A), although several other missense mutations—called ‘secondary mutations’—in the mtDNA can act autonomously or in association with each other to cause the disease [3].

CI deficiency is the most frequent cause of mitochondrial disorders, having been found in > 30% of patients. Trunk hypotonia, antenatal and postnatal growth retardation, encephalopathy and liver failure are the main clinical features [20,21]. Systematic sequencing of the mitochondrial CI genes has shown that around 20% of CI-deficient patients harboured point mutations in one of these genes — at least, in our series.

In contrast, CIII deficiency is a relatively rare cause of respiratory enzyme dysfunction [30]. Indeed, in our experience, of all RC enzyme-deficient patients, only 7% had a CIII deficiency [22]. However, the clinical presentation of CII-deficient patients is highly heterogeneous, including myopathy, encephalomyopathy, multiorgan disorders, cardiomyopathy, tubulopathy and intrauterine growth retardation [22,23]. This complex contains 11 subunits, and only one — cytochrome b (cyt b) — is of mitochondrial origin. So far, 12 cyt b mutations have been described in association with various clinical presentations. Interestingly, in most patients (8/12), the predominating presenting feature was severe exercise intolerance, sometimes including muscle weakness and/or myoglobinuria [24]. Two other patients presented with cardiomyopathy, another patient had encephalomyopathy, and a further patient had MELAS and an akinetic rigid syndrome.

Cytochrome c oxidase (COX) deficiency is one of the most frequent causes of RC disorder in childhood and is clinically heterogeneous, with phenotypes such as encephalomyopathy, Leigh syndrome [25], fatal and benign infantile myopathy, hepatic failure [26] and myoglobinuria [27]. In fact, mtDNA mutations have been identified in patients with various clinical presentations. These mutations have been described in the three mitochondrial COX genes (COXI, COXII, COXIII) [3], and have always been associated with single pedigrees and are, therefore, private mutations. Most patients with mtDNA COX mutations have muscle-related or neuromuscular symptoms. However, mutations of COXI have also been reported in two cases of acquired sideroblastic anaemia [28].

Most of these mutations are maternally inherited and heteroplasmic, and associated with a striking variety of clinical phenotypes, depending on the proportion of mutant mtDNA inherited among the maternal relatives. Within one particular pedigree, clinical presentations can range from migraines and attention-deficit disorders to the full MELAS syndrome. Maternal relatives of patients are generally healthy as long as they have no more than 85% mutant mtDNA. Once the percentage of mutant mtDNA rises beyond this level, there are increasingly serious consequences for clinical phenotype, thus highlighting the sharp threshold in protein-synthesis mutations.

4.1.3. Large-scale mtDNA rearrangements

The second category of mtDNA diseases is deletion of the mitochondrial genome. Although the size and position of the deletion differs markedly among patients, they usually encompass several encoding and tRNA genes. They are usually sporadic, heteroplasmic and unique, and frequently occur between directly repeated sequences, suggesting that they are caused by de novo rearrangements arising during oogenesis or early development.

The Kearns-Sayre syndrome (KSS) is a multisystem disorder characterized by a non-variable triad: onset before age 20; progressive external ophthalmoplegia; and pigmentary retinal degeneration, plus at least one of the following: complete heart block, cerebrospinal fluid (CSF) protein > 100 mg/dL and/or cerebellar ataxia. Large-scale heteroplasmic mtDNA deletions are frequently detected in skeletal muscle [29].

Pearson syndrome comprises refractory sideroblastic anaemia, with variable neutropenia and thrombocytopenia, vacuolization of marrow precursors and exocrine pancreatic dysfunction. Severe transfusion-dependent macrocytic anaemia begins in early infancy (before age 1 year) and is fatal by 3 years of age in 62% of cases. The patients who survive spontaneously recover from their myelodysplasia, but usually develop KSS. Large-scale heteroplasmic mtDNA deletions are present in all tissues, with the ratio of normal-to-deleted genomes being related to expression of the disease [30].

Progressive external ophthalmoplegia (PEO) is a mitochondrial myopathy with progressive muscle weakness and external ophthalmoplegia. Ataxia, episodic ketoacidotic coma and early death have also been reported. Large-scale mtDNA deletions are found in the skeletal muscle of the patients [31].

Few patients with syndromic diabetes also present with mtDNA deletions. In most cases, the deletion is maternally inherited and always heteroplasmic. Diabetes is frequently associated with deafness, but may also be part of a multiorgan disorder [32–35]. It is worthwhile noting that the most common deletion (4977 bp) found in 30% of patients harbouring a unique deletion, flanked by 13 bp direct repeats, has been described in both Pearson syndrome and KSS, and subsequently also reported in PEO. Thus, no correlation can be found between clinical presentation and the nature or extent of the rearrangements. Finding progressive organ involvement should prompt the suspicion of a diagnosis of mtDNA rearrangement, and lead to long-range polymerase chain reaction (PCR) or Southern blot analyses on total DNA.

Quantitative (depletion) and qualitative (multiple deletion) mtDNA anomalies may also be due to mutations of nuclear genes involved in mtDNA maintenance. In such cases, the disease is inherited as an autosomal dominant or recessive trait (see below).

4.2. Nuclear DNA mutations

The number of disease-causing mutations in nuclear genes is steadily growing, and these mutations probably underlie the vast majority of RC deficiencies. It should be borne in mind that mtDNA deletions and mutations account for no more than 15–20% of cases — at least, among paediatric patients. Thus, in
most cases, nuclear gene defects are those most likely responsible for RC deficiency.

Indeed, proper RC functioning requires not only the presence of various subunits of each complex, but also ancillary proteins at different stages of holoenzyme biogenesis, including transcription, translation, chaperoning, addition of prosthetic groups and assembly of proteins, as well as various enzymes involved in mtDNA metabolism.

4.2.2. Genes involved in RC assembly

The various nuclear genes encoding RC subunits have all been identified and mapped, and mutations of some of these genes have been found in patients. Interestingly, most of the mutations encountered have been in CI genes whereas, despite the concerted efforts of various teams of researchers, very few mutations in other genes encoding other complex subunits have been found.

The first mutation in a gene encoding an RC subunit was reported in 1995 in two sisters with Leigh syndrome and CII deficiency. The pathogenic mutation was in the SDHA gene encoding the flavoprotein of CII [36]. Mutations in the same gene were subsequently reported in another patient who also presented with Leigh syndrome [37,38]. However, only a few patients with CII deficiency have been molecularly characterized, despite systematic study of the four genes encoding the CII subunits, suggesting that these deficiencies could be due to mutations in assembly proteins. Nevertheless, mutations of the three other genes encoding subunits B, C and D of CII have been reported in hereditary paraganglioma and phaeochromocytoma, suggesting that such ‘housekeeping’ genes may be involved in carcinogenesis [39]. It is hypothesized that SDH mutations cause an accumulation of succinate and reactive oxygen species (ROS), which could act as downstream signalling molecules to activate hypoxia-inducing pathways [39].

The pioneering work by Smeitink et al. in the Netherlands identified the first molecular bases of CI deficiencies [21]. This complex is the largest RC complex, and consists of seven mitochondrial-encoded and at least 35 nuclear-encoded proteins. CI deficiency is one of the most common causes of mitochondrial disease. Screening of the various structural CI genes by gene analyses, mutations in the CI deficiency have been identified in humans by homology with their yeast counterparts. Also, searching for disease-causing genes in patients has led to the identification of new human genes involved in these mechanisms.

CI, the largest RC complex, has several assembly factors (NDUFAF1, NDUFAF2, C6orf66, C8orf38 and C20orf7) that...
Fig. 3. Mitochondrial DNA replication and translation of proteins encoded by mtDNA. The gene mutations resulting in mitochondrial diseases and multiple respiratory chain deficiencies are shown in the black ovals.

Using different approaches in patients with COX deficiency such as gene-mapping, functional complementation or candidate gene studies, several assembly genes have been identified as disease-causing. SURF1 represents a major gene for Leigh syndrome associated with COX deficiency, with 25–75% of Leigh–COX patients having SURF1 mutations [49]. It has also recently been shown that SURF1 in bacteria is a heme-binding protein that may be involved in heme insertion in cytochrome oxidase [50]. COX10 encodes heme A:farnesyltransferase, catalyzing the first step in the conversion of protoheme to the heme A prosthetic group required for cytochrome c oxidase activity, while COX15 allows the hydroxylation of heme O to form heme A. However, few patients present with mutations of these two genes, thereby hindering any genotype–phenotype correlation. COX15 mutations lead to cardiomyopathy or Leigh syndrome [51–53], whereas COX10 mutations are associated with tubulopathy and leukodystrophy [54,55]. Mutations in SCO1 [56] and SCO2 genes [57], both of which are involved in mitochondrial copper maturation and synthesis of subunit II of COX [58], also give rise to hepatopathy and ketoacidotic coma (SCO1) and cardiomyopathy (SCO2). Mutations in LRPPRC cause Leigh syndrome, French-Canadian type [59]. The LRPPRC protein is thought to be involved in the translation or stability of the mRNA of subunits I and III of COX [60]. FASTKD2 mutations, reported in only one kindred, cause encephalomyopathy and convulsions [61]. Although the gene is not directly involved in the assembly of CIV, it may play a role in the regulation of mitochondrial apoptosis (Fig. 2). TACO1 is a recently identified gene of COX deficiency that encodes a translational activator of the COX1 subunit encoded by mtDNA. Mutations of this gene result in late-onset Leigh syndrome [62].

Most complex V deficiencies are associated with mtDNA mutations (ATP6 and ATP8 gene mutations), and only two complex V nuclear mutations have been reported. The ATP12 gene encodes a protein required for assembly of the alpha and beta subunits, and mutations of this gene, reported in one patient, resulted in dysmorphic features, neurological involvement and methylglycine conformation [63]. Recently, a large kindred of Gipsy origin with isolated complex V deficiency and neonatal encephalocardiomyopathy were reported to present with mutations in TMEM70, a gene encoding a transmembrane mitochondrial protein of yet unknown function involved in the assembly of complex V [64] (Fig. 2).

Several proteins of the RC are iron-sulphur proteins, and deficiencies in the assembly of the iron-sulphur cluster have been reported to result in dysfunction of CI, CII and CIII, which contain iron-sulphur proteins. Indeed, Friedreich’s ataxia, one of the most common forms of autosomal recessive ataxia, associated with hypertrophic cardiomyopathy and diabetes in 10% of cases, is due to a mutation of frataxin, a mitochondrial protein involved in iron-sulphur protein biogenesis [65]. Mutations on the iron-sulphur cluster scaffold protein (ISCU), which interacts with frataxin in iron-sulphur cluster biosynthesis, lead to myopathy, with exercise intolerance and myoglobinuria [66,67].

Electron transfer along the RC depends on a quinone pool synthesized in the mitochondria. Deficiencies in several enzymes or proteins (PDSS1, PDSS2, COQ2, COQ9, CABC1) involved in this biosynthesis pathway are reportedly associated with various clinical presentations [68–70] (Fig. 2).

4.2.3. Genes involved in mtDNA stability

Mitochondrial DNA is packaged into protein–DNA complexes called ‘nucleoids’. Nucleoids in cultured mammalian cells contain 5–10 mtDNA molecules and appear to be tethered to the inner mitochondrial membrane. Although their protein composition remains controversial, it is now well established.
that nucleoids contain the mtDNA replisome [71]. Crucial proteins or enzymes involved in either mtDNA replication (mtDNA polymerase γ, mtSSB, Twinkle helicase) or transcription (TFAM) are also components of nucleoids. In theory, defects in any of the proteins involved in mtDNA replication can affect mtDNA copy number. Such replication is also highly dependent on mitochondrial deoxyribonucleotide triphosphate (dNTP) supply, suggesting that mutations in several genes involved in mitochondrial dNTP synthesis may, therefore, result in mtDNA anomalies (Fig. 3).

Autosomal-dominant external ophthalmoplegia (adPEO) is associated with multiple mtDNA deletions [72], which are restricted to muscle tissue. With an onset in adulthood, adPEO includes progressive weakness of the extraocular muscles as a cardinal feature; patients have ptosis and limited eye movements, with additional features varying from one family to another. Most cases of adPEO associated with multiple mtDNA deletions are due to mutations in \( \text{POLG1} \) (mtDNA polymerase γ) [73], \( \text{POLG2} \), \( \text{ANT1} \) (mitochondrial ADP/ATP translocator) [75], \( \text{PEO1} \) (Twinkle helicase) [76] and \( \text{OPA1} \), a dynamin-related GTPase involved in mitochondrial fusion [77] (Fig. 3). In rare cases, the disease is autosomal-recessive.

Mitochondrial DNA depletion syndrome (MDS) was initially described as congenital myopathy or hepatopathy [78]. Since then, however, many patients have demonstrated different clinical presentations, including hepatocerebral, myopathic and encephalomyopathic forms [79,80]. In such patients, there is a marked (usually tissue-specific) deficiency in mtDNA levels, with a residual amount of mtDNA that is often < 10% of normal values. Such depletion leads to deficiencies of multiple RC complexes, while nuclear-encoded components such as CII are mostly expressed normally. Depletion is related to abnormal mtDNA replication, especially defects in dNTP supply and replication mechanisms. Mutations in \( \text{POLG} \) and \( \text{PEO1} \) encoding the Twinkle helicase, two replication factors, are associated with severe and often fatal hepatocerebral forms [81] and/or Alpers’ syndrome [82], characterized by psychomotor retardation, intractable epilepsy and liver failure in infants and young children. Hepatocerebral forms are also associated with the \( \text{DGUOK} \) gene [83], which encodes the mitochondrial deoxyguanosine kinase involved in the salvage pathway of dNTP for mtDNA synthesis, or the \( \text{MPV17} \) gene, which encodes a protein of unknown function [84]. The \( \text{TK2} \) gene encodes mitochondrial thymidine kinase, which is also involved in mitochondrial dNTP salvage. Mutations of this gene are associated with severe infantile myopathy [85] and, recently, with motor neuron disease resembling spinal muscular atrophy [86], encephalopathy or seizures, cardiomyopathy or dystrophic changes in muscle [87]. Synthesis of mtDNA requires not only sufficient mitochondrial dNTP pools, but also balanced cytosolic dNTP synthesis. Indeed, mutations of \( \text{RRM2B} \) encoding a small subunit of cytosolic ribonucleotide reductase, the enzyme that catalyzes dNDP synthesis from NDP, results in severe muscle mtDNA depletion and neonatal trunk hypotonia, with hyperlactataemia [88]. Mutations of two subunits of the succinyl-CoA synthase \( (\text{SUCLA2, SUCLG1}) \) have been reported in patients with mild mtDNA depletion. \( \text{SUCLA2} \) patients present with psychomotor retardation, muscle hypotonia, hearing impairment and seizures [89], whereas those with \( \text{SUCLG1} \) mutations have encephalomyopathy and methylmalonic aciduria [90]. Although both these mutations lead to methylmalonic aciduria, the pathogenesis of this condition is poorly understood.

Mitochondrial neurogastrointestinal encephalopathy (MNGIE) syndrome is a multisystem disorder clinically characterized by onset between the second and fifth decades of life, ptosis, PEO, gastrointestinal dysmotility, diffuse leukoencephalopathy, peripheral neuropathy and myopathy. Patients may have multiple mtDNA deletions and/or mtDNA depletion. The disease-causing gene \( (\text{TP}) \) encodes thymidine phosphorylase [91]. Mutations of this gene can affect the balance of the intramitochondrial dNTP pool, and lead to mtDNA deletions and depletion [80].

Interestingly, it is becoming more and more evident that mutations of a same gene can be associated with various clinical presentations, and result in either quantitative or qualitative mtDNA anomalies. In addition, the inheritance of the associated diseases may be either dominant or recessive. Indeed, \( \text{POLG} \) and \( \text{PEO1} \) mutations can lead to adPEO with multiple mtDNA depletion or a fatal recessive hepatocerebral form with severe mtDNA depletion. On the other hand, \( \text{RRM2B} \) mutations, which usually cause severe muscle mtDNA depletion in neonates, have been recently associated with adPEO, with multiple mtDNA deletions [92], and with MNGIE syndrome in adult patients [93].

4.2.4. Genes involved in translation of mtDNA-encoded proteins

Mitochondria contain separate protein-synthesis mechanisms allowing the synthesis of polypeptides encoded by mtDNA. The process not only requires tRNA and rRNA encoded by mtDNA, but also hundreds of nuclear genes encoding ribosomal proteins, aminoacyl-tRNA synthetases, tRNA modification enzymes, rRNA base-modification enzymes, and elongation and termination factors [9] (Fig. 3). Mutations in several of these factors have been reported in patients with multiple RC deficiencies and various clinical presentations, such as myopathy and sideroblastic anaemia (\( \text{PUS1} \)) [94], leukoencephalopathy (\( \text{DARS2} \)) [95], pontocerebellar hypoplasia (\( \text{RARS2} \)) [96], encephalomyopathy (\( \text{TSFM} \)) [97], hypertrophic cardiomyopathy (\( \text{TSFM} \)) [97], hepatocerebral atrophy (\( \text{ETF}\)) [98], infantile encephalopathy (\( \text{ETF}\)) [99], fatal neonatal lactic acidosis (\( \text{MRPS16} \)) [100], multisystem involvement (\( \text{MRPS22} \)) [101] and hepatic failure (\( \text{TRMU} \)) [102]. Considering the large number of patients with multiple RC deficiencies without mtDNA anomalies, it may be hypothesized that such cases may be related to abnormal translation due to nuclear gene deficiency.

5. Genetic counseling and prenatal diagnosis

Prenatal diagnoses of mitochondrial disorders fall in two categories, depending on the nature of the identified mutation. If the disease is related to a nuclear gene mutation, mutation screening using a sample of chorionic villi at 10 weeks of gestation offers early and reliable prenatal diagnosis. Identification of an
mtDNA mutation in the proband should always prompt examination and testing of the patient’s maternal relatives for the mutation. In cases of maternal inheritance of mtDNA mutations (or deletions), there is no risk for the progeny of an affected male. The risk is high, however, for the progeny of carrier females. In this case, prenatal diagnosis based on chorionic villi or amniotic cells represents a rational approach to the prevention of these serious disorders. Nevertheless, prevention is currently hampered by our incomplete knowledge of the actual proportion of mutant mtDNA, its relationship to disease severity, and its random tissue distribution and selection in the affected population during development, which may also be related to various metabolic activities. According to the data so far, having a percentage of mutant mtDNA < 30% or > 80% is predictive of a reasonable chance of a good or bad prognosis, respectively [103]. Any results in between these limits would be of even less certain predictive value. But whatever the results, any studies aimed at prenatal diagnosis or predictive genetic guidelines require careful validation, as the proportions of mutant mtDNA may change not only between fetal life and infancy, but also throughout adulthood.

6. Conclusion

The increasing number of abnormal mitochondrial functions and genes leading to RC deficiency continues to shed light on the clinical heterogeneity of mitochondrial disorders. The identification of the disease-causing genes is not only important for genetic counselling and prenatal diagnosis, but also for a greater understanding of the pathophysiology of these disorders. However, the ever-increasing numbers of genes involved in mitochondrial functions and possibly mitochondrial diseases require the development of large-scale, high-throughput technologies that can increase our insight into these highly complicated pathologies.

Conflict of interest

The author has nothing to declare.

References


