Abstract

Primary cardiomyopathy is an important cause of mortality in children and adults. Apart from inherited disorders of myocardial contractile and structural proteins, several defects of energy metabolism may cause cardiomyopathy. Most of the energy required for myocardial contraction is derived from aerobic metabolism. Faulty aerobic metabolism involving the heart may be due to defects of mitochondrial oxidative phosphorylation or to defects of fatty acid oxidation. Considerable advances have been made in the last 10 years in understanding the biochemical and molecular characteristics of mitochondrial disorders. Several point mutations or large-scale rearrangements of mitochondrial DNA have been identified in patients with cardiomyopathy, either as part of complex multisystem syndromes or as the main clinical feature. Inborn errors of fatty acid oxidation are reported with increasing frequency as a cause of metabolic dysfunction, myopathy, cardiomyopathy, and sudden death in childhood. Advances in biochemical and molecular genetic techniques have considerably improved our understanding of the metabolic disorders causing cardiomyopathy, providing new tools for classification and diagnosis of candidate patients. The present review focuses on defects of mitochondrial oxidative metabolism associated with cardiomyopathy.

Keywords: Cardiomyopathy; Mitochondrial DNA; Respiratory chain; $\beta$-Oxidation; Oxidative metabolism

1. Introduction

Oxidative metabolism has the fundamental function of transducing energy into ATP by the oxidation of fuel molecules. In the resting state and during prolonged exercise the energy demands of skeletal muscle rely upon the aerobic metabolism of energy compounds [1,2]. The aerobic oxidation of energy substrates takes place within mitochondria through the enzyme activities of a number of metabolic pathways. Oxidation of fatty acids provides most of the energy required by the heart, and it is carried out through the sequential activities of the $\beta$-oxidation pathway, the Krebs cycle and the respiratory chain. In recent years, knowledge has increased on the role of defects of mitochondrial metabolism in the pathogenesis of syndromic and non-syndromic (i.e., isolated) cardiomyopathy.

Intensive investigation on patients and kindreds has substantially expanded our understanding of the biochemical and molecular bases of primary cardiomyopathy. The current classification includes two major categories: (a) disorders of myocardial contractile and structural proteins, and (b) disorders of cardiac energy metabolism [3,4]. Disorders of myocardial contractile and structural proteins include familial hypertrophic cardiomyopathy (usually inherited as an autosomal dominant trait) and (X-linked) dystrophinopathies. Disorders of cardiac energy metabolism include defects of mitochondrial oxidative phosphorylation (OXPHOS) and defects of fatty acid $\beta$-oxidation. The contribution of glycolysis to the energy required by myocardial contraction is limited, and the degree of cardiac involvement in glycogenoses, such as acid maltase deficiency, is likely to be the consequence of glycogen storage, together with the ensuing disruption of
myocardial fibers, more than the effect of the metabolic block itself [5].

Recent advances in biochemical and molecular genetic techniques have also contributed to improving our understanding of the metabolic disorders causing cardiomyopathy, by providing new tools for classification and diagnosis of candidate patients. In particular, defects of OXPHOS and β-oxidation are now recognized as major metabolic causes of cardiomyopathy and will be extensively discussed in this review.

2. Cardiomyopathy in disorders of mitochondrial oxidative phosphorylation (OXPHOS)

Mitochondrial respiration is carried out by five multimeric enzymes, the respiratory complexes, embedded in the inner mitochondrial membrane. The complexes catalyze a series of redox reactions carried out by the reducing equivalents generated by the degradation of energy substrates [6]. The energy liberated in the redox reactions sustains the formation of a proton electrochemical gradient across the mitochondrial inner membrane. The potential energy stored in the electrochemical gradient is in turn utilized by the ATP-synthase (complex V) to phosphorylate ADP to ATP (Fig. 1). From a genetic point of view, the mitochondrial respiratory chain is unique, as it is under the dual genetic control of the nuclear DNA (nDNA) and the mitochondrial DNA (mtDNA). Several protein subunits of the respiratory chain complexes are encoded by nDNA genes, synthesized in the cytoplasm, and imported into mitochondria. Human mtDNA is a circular chromosome of 16,569 base-pairs composed of double-stranded DNA. The two strands of mtDNA can be separated in alkaline cesium chloride gradients, according to a buoyant density difference due to a strand bias in the G + T content; the heavy (H) strand has a greater density than the light (L) strand [7] (Fig. 2). mtDNA contains 37 genes encoding the RNA components of the mitochondrial translation system (i.e., 22 transfer RNAs [tRNAs] as well as 12S and 16S ribosomal RNA [rRNAs]) and messenger RNAs (mRNAs) specifying 13 polypeptides that belong to four of the five complexes of the respiratory chain. Only complex II is entirely encoded by nDNA. The only non-coding region of mtDNA is the displacement loop (D-loop), an important area of interaction of mtDNA with nuclear-encoded proteins regulating mtDNA housekeeping functions. The organization of mtDNA is extremely compact because all of the coding sequences are contiguous to each other, with no introns.

**Fig. 1. Schematic representation of the mitochondrial respiratory chain.**

<table>
<thead>
<tr>
<th>Enzyme Complex</th>
<th>mtDNA-encoded subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (NADH dehydrogenase)</td>
<td>7 (ND1,2,3,4,4L,5,6)</td>
</tr>
<tr>
<td>II (Succinate dehydrogenase)</td>
<td>0</td>
</tr>
<tr>
<td>III (CoQH2-cytochrome c reductase)</td>
<td>1 (Cytochrome b)</td>
</tr>
<tr>
<td>IV (Cytochrome c oxidase)</td>
<td>3 (Cytochrome oxidase I, II, III)</td>
</tr>
<tr>
<td>V (ATP synthase)</td>
<td>2 (ATPase 6,8)</td>
</tr>
</tbody>
</table>
Unique genetic features differentiate mtDNA from nDNA: (1) The mitochondrial genome is maternally inherited, because during fertilization mitochondria are contributed only by the oocyte. As a consequence, affected mothers will transmit the disease to all of their offspring, but only daughters will transmit the disease to subsequent generations. (2) Mitochondria are polyplloid: a single cell contains hundreds of mitochondria, each containing 2–10 mtDNA molecules. Therefore, thousands copies of mtDNA will determine the mitochondrial genotype of each cell. (3) At cell division, mtDNAs are randomly distributed to daughter cells: if progenitor cells are heteroplasmic (i.e., they contain both normal and mutated mtDNA species), the relative proportion of different populations of mtDNA can vary considerably at each subsequent generation. This ‘mitotic segregation’ increases the variability of the mitochondrial genotypes and contributes to the different degrees of mtDNA heteroplasmy in tissues from the same patient. (4) Polyploidy and mitotic segregation determine to a large extent the phenotypic expression of mtDNA mutations. When mutated mtDNA genomes accumulate above a critical threshold, they are no longer complemented by the coexisting wild-type mtDNA species, and will be phenotypically expressed as a biochemical dysfunction of the cell (threshold effect). The extent of dysfunction will depend upon the aerobic energy requirements of each tissue. This explains why tissues with high energy demands, such as skeletal muscle, brain and heart, are more frequently affected by OXPHOS defects. In addition, these tissues are composed of highly differentiated, post-mitotic cells, in which no selection against cells containing defective mitochondria can occur. Thus, accumulation of mutated mtDNAs will progress in these organs until reaching the threshold of phenotypic expression. In practice, all mtDNA mutations behave as ‘recessive-like’ traits: a high proportion of mutant mtDNA (typically >60%) must be present, even in the most sensitive tissues, to produce a clinically relevant damage. The high threshold may depend upon the intrinsic pathogenic potential of the mutations, and also the remarkable ‘functional reserve’ of the respiratory chain.

The clinical presentation of mitochondrial disorders is extremely heterogeneous, ranging from isolated myopathies, to encephalomyopathies, cardiomyopathies or complex multisystem syndromes, in which several tissues are involved (Table 1). Most patients show a variable combination of abnormalities of the central nervous system and skeletal muscle. However, signs of cardiac involvement are frequent, either in association with neuromuscular symptoms or, less frequently, as the main clinical feature. We will focus on disorders associated with mutations of the mitochondrial genome and respiratory chain defects associated with cardiomyopathy. The latter is usually hypertrophic, but dilated forms have been described. Onset
can be at any age and severity is extremely variable. Detailed reviews on the neurological manifestations of OXPHOS defects are available elsewhere [8,9].

The observation of ragged red fibers (RRF) in skeletal muscle is probably the single most important clue to a mitochondrial disorder. The typical ragged-red appearance of muscle fibers is due to the accumulation of abnormal mitochondria of increased size under the sarcolemmal membrane. RRF are also frequently associated with fibers that appear non-reactive during histochemical staining for respiratory complex IV (cytochrome c oxidase). Diagnosis is often confirmed by biochemical assay of the respiratory chain activities and by molecular genetic analysis of mtDNA. However, neither clinical nor morphological signs are sufficient for a complete classification of mitochondrial cardiomyopathies, because the former are heterogeneous and the latter may be lacking in patients with biochemically and genetically proven mitochondrial disorders. Moreover, the same biochemical defect can be associated with completely different phenotypes. Molecular genetics has provided new clues to the establishment of the etiological classification of these disorders. However, when mtDNA mutations are absent, classification is based on biochemical findings only. Accordingly, mitochondrial cardiomyopathies can be divided into: (1) genetically defined defects of mtDNA, and (2) biochemically defined defects of the respiratory chain.

2.1. Genetically defined defects of mtDNA

Mutations of mtDNA, classified according to their molecular nature and pattern of transmission, comprise: (a) large scale re-arrangements of mtDNA, (b) point mutations of mtDNA, and (c) mtDNA lesions transmitted as Mendelian traits.

2.1.1. Large-scale re-arrangements of mtDNA

Large-scale re-arrangements of mtDNA either as single mtDNA deletions (mtDNA\(^{D^+}\)) or, more rarely, partial duplications, are always heteroplasmic (i.e., they coexist with variable amounts of wild-type mtDNA). Clinical presentations include Kearns-Sayre syndrome (KSS), sporadic chronic progressive external ophthalmoplegia (CPEO) and the rare Pearson’s bone marrow–pancreas syndrome [10–13]. KSS is characterized by the association of CPEO, pigmentary retinopathy and onset before age 20; cerebellar ataxia, short stature, hearing loss, and increased CSF proteins are frequent additional symptoms. These patients often develop a left anterior hemiblock, either alone or combined with a right bundle branch block, Mobitz type II atrioventricular block and complete heart block [14]. Because of the progressive deterioration of heart conduction, prophylactic placement of a pacemaker is mandatory in these patients; disease prognosis is poor. Although cardiomyopathy is unusual in KSS, dilated cardiomyopathy leading to heart failure has occasionally been reported. Thrombosis of the left atrial appendage leading to cardioembolic stroke has recently been described in one KSS case [15–18].

Single mtDNA\(^{D^+}\) can be detected by Southern blot analysis in almost all patients with KSS. Deleted mtDNA are widely distributed in different tissues, including the heart [19–22]. Since KSS is sporadic and the mtDNA deletion is the same in all tissues, this disorder is likely due to the clonal amplification of a single somatic mutation.

Most deletions range from 1.3 to 7.6 kb in length and include both mRNA and tRNA genes. Functional regions of mtDNA\(^{D^+}\) are flanked by direct repeats of variable length [23,24], suggesting that the molecular mechanism leading to deletions may be the slippage and mispairing of the single mtDNA strands during replication [25].

2.1.1.1. Cellular and molecular pathogenesis of large-scale re-arrangements of mtDNA. Since several tRNA genes are encompassed by deletions, mtDNA\(^{D^+}\) are unable to translate their own genes into proteins. Experiments of in situ hybridization and immunostaining of muscle have demonstrated that RRF contain high proportions of mtDNA\(^{D^+}\) molecules and that mtDNA translation does not occur [26,27]. Translation can still be possible through the complementation of deleted genomes by wild-type mtDNA within the same organelle. This hypothesis has been confirmed by studies performed with cybrids obtained by introducing variable amounts of wild-type mtDNA into \(\rho^0\) cells (cells deprived of mtDNA). These experiments demonstrated that translation of mtDNA in cultured cells drops dramatically when the relative proportion of mtDNA\(^{D^+}\) reaches 60% or beyond [28].

2.1.2. Point mutations of mtDNA

Several point mutations of mtDNA (pm-mtDNAs) have been reported and associated with well-defined clinical phenotypes. Virtually all pm-mtDNAs are maternally inherited. They may involve tRNA, mRNA and rRNA genes. In consideration of the high mutation rate of mtDNA, the pathogenicity of a point mutation is suggested by the fulfillment of the following criteria: (1) the mutation should involve a highly conserved nucleotide; (2) the mutation should strictly segregate with the clinical phenotype; and (3) the degree of mtDNA heteroplasmy (if present) should correlate well with the severity of the clinical and biochemical phenotype. Most pm-mtDNAs involve tRNA genes, are associated with RRF and with variable reduction of OXPHOS enzymes activities in skeletal muscle, most frequently complexes I and IV. By contrast, RRF are rarely observed in patients carrying point mutations in genes encoding subunits of the respiratory chain. A detailed description of the clinical features and syndromes associated with pm-mtDNAs is beyond the scope of this review; we will focus on those mutations that are associated with cardiomyopathy (Table 2).
Table 2
Point mutations of mtDNA associated with cardiomyopathy

<table>
<thead>
<tr>
<th>mtDNA gene</th>
<th>Mutation</th>
<th>Associated clinical features</th>
<th>Cardiomyopathy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNALeu(UUR)</td>
<td>A3243G</td>
<td>MELAS</td>
<td>HCM</td>
<td>[30–34,45]</td>
</tr>
<tr>
<td></td>
<td>A3260G</td>
<td>Diabetes, deafness, renal failure</td>
<td>HCM</td>
<td>[51–53]</td>
</tr>
<tr>
<td></td>
<td>C3303T</td>
<td>Myopathy</td>
<td>HCM, FICM</td>
<td>[56]</td>
</tr>
<tr>
<td>tRNAtaurine</td>
<td>A4300G</td>
<td>HCM</td>
<td></td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>A4269G</td>
<td>Multisystem</td>
<td>DCM</td>
<td>[57,58]</td>
</tr>
<tr>
<td></td>
<td>A4317G</td>
<td>Multisystem</td>
<td>FICM</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>A4320G</td>
<td>Encephalopathy</td>
<td>HCM</td>
<td>[62]</td>
</tr>
<tr>
<td>RNAcytosine</td>
<td>A8344G</td>
<td>MERRF</td>
<td>HCM</td>
<td>[64–67]</td>
</tr>
<tr>
<td></td>
<td>G8363A</td>
<td>Multisystem, hearing loss</td>
<td>HCM</td>
<td>[69]</td>
</tr>
<tr>
<td>tRNAGlu</td>
<td>T9997C</td>
<td>Bowel dysmotility</td>
<td>HCM</td>
<td>[70]</td>
</tr>
<tr>
<td>ATPase</td>
<td>T8893G</td>
<td>Leigh syndrome, NARP</td>
<td>HCM</td>
<td>[71–73]</td>
</tr>
</tbody>
</table>

HCM = hypertrophic cardiomyopathy; FICM = fatal infantile cardiomyopathy; DCM = dilated cardiomyopathy; NARP and MERRF, see text.

2.1.2.1. **Point mutations in the tRNALeu(UUR) gene.** The tRNALeu(UUR) gene is considered a mutational hot spot in the mitochondrial genome, because several pm-mtDNAs have been localized in this gene and associated with different phenotypes [29].

**Mutation A3243G** This is a frequent pm-mtDNA, usually associated with the MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes) [30]. MELAS is characterized by the following signs: (1) stroke-like episodes, with CT- or MRI-documented focal brain lesions, usually localized in the parieto-occipital lobes, and (2) lactic acidosis and/or presence of RRF in the muscle biopsy. Dementia, headache, recurrent vomiting, focal or generalized seizures and deafness are additional frequent signs [31]. However, the genotype to phenotype correlation of the A3243G mutation is rather loose, because patients with CPEO, myopathy alone, or even maternally inherited diabetes mellitus and deafness may have this mutation [32–34]. Moreover, considerable genetic heterogeneity is associated with the MELAS syndrome. Thus, MELAS-like phenotypes have been described with several other pm-mtDNAs, including nucleotide positions (np) 3251, 3252, 3271 and 3291 in the tRNALeu(UUR) [35–38], np 9957 in the cytochrome c oxidase III gene [39], and np 1642 in the tRNA gene for valine [40].

Signs of cardiac involvement, usually in the form of hypertrophic cardiomyopathy, have been reported with increased frequency in patients with MELAS, even in cases with a partial phenotype [41–44]. Hypertrophic cardiomyopathy has also been reported in association with diabetes mellitus, renal failure and sensorineural deafness in a large pedigree with the A3243G mutation [45]. Ventricular dysfunction in these patients has been observed by echocardiography, a simple investigation that should be performed in any candidate patient [46].

The pathogenesis of stroke-like episodes in the MELAS syndrome is still poorly understood. A primary mitochondrial dysfunction involving cerebral vessels has been hypothesized [47,48], based on the observation that the histochemical reaction to succinate dehydrogenase (SDH), which correlates with the number of mitochondria, shows SDH-reactive deposits in the walls of intramuscular blood vessels. This finding has been interpreted as a secondary response of blood vessel mitochondria to a respiratory deficiency caused by the MELAS mutation. Moreover, electron microscopy examination has disclosed increased numbers of mitochondria in smooth muscle cells [49]. Abnormal mitochondria of increased number and size have also been observed in the endothelium by electron microscopy examination of endomyocardial biopsy specimens [50].

**Mutation A3260G** In 1991, we described a large Italian pedigree with a cardiac syndrome dubbed as MIMyCa (Maternally Inherited Myopathy and Cardiomyopathy) [51]. As typically observed in mitochondrial disorders, the clinical presentation ranged from asymptomatic to severely affected subjects. The phenotype was characterized by proximal muscle weakness, exercise intolerance, increased blood lactate production at rest and during exercise, and impaired cardiac ejection fraction. Severe hypertrophic cardiomyopathy was associated with the Wolff-Parkinson-White syndrome in the most compromised patient. Maximum oxygen consumption, a physiological index of aerobic metabolism, measured by a standardized incremental exercise test, ranged from 76 to 100% of the predicted values in symptom-free relatives and from 42 to 62% in clinically affected subjects. The latter failed to increase their cardiac ejection fraction, as shown by radioisotopic angiocardiology performed at rest and during exercise. Biochemically, the activities of the respiratory chain complexes I and IV were markedly reduced in clinically affected subjects. Molecular genetic studies revealed the presence of a heteroplasmic A3260G transition in the tRNALeu(UUR) gene. The percentage of mutant mtDNA in muscle tissue correlated well with the activities of respiratory chain enzymes in vitro, and with oxygen consumption in vivo. A second unrelated family with the same syn-
drome was subsequently reported [52]. It is worth noting that a family with the same mutation but with a MELAS phenotype without cardiomyopathy has been recently reported from Japan, suggesting that both MELAS and MIMyca might be part of a spectrum of expression of the 3260 mutation [53]. The pathogenicity of the 3260 mutation was confirmed in our family by means of an ex vivo system based on transformant cybrids obtained by fusing cytoplasts derived from a A3260G-positive patient with tumor cells deprived of mtDNA (p56 cells) [54,55]. The resulting ‘transmitochondrial cybrid’ line contains the nuclear gene complement of the host p56 cell, ruling out the effect of the proband’s nuclear gene background. Interestingly, clones harboring nearly 100% mutant mtDNA showed significantly reduced activities of complex I and IV, low oxygen consumption, high lactate production, and low mitochondrial translation rate, compared to clones containing 100% normal mtDNA [55].

**Mutation C3303T** This mutation has been described in a family with maternal members of a large pedigree. Some patients were affected by fatal infantile cardiomyopathy, others suffered sudden death, and still others showed more chronic disease, characterized by cardiomyopathy and mitochondrial myopathy [56].

**2.1.2.2. Point mutations in the tRNAIsoleucine gene.** Several mutations have been reported within the tRNAle gene sequence and associated with cardiomyopathy, alone or as the main feature.

**Mutation A4269G** This mutation was described in a patient who died at the age of 18 years from progressive heart failure. The clinical picture was characterized by the association of short stature, deafness, epilepsy and glomerulosclerosis. Dilated cardiomyopathy appeared later in the course of the disease [57,58]. The effect of the A4269G mutation was studied in transmitochondrial cybrids, with results similar to those observed in the A3260G mutation [59].

**Mutation A4300G** The mutation was described in a young man with hypertrophic cardiomyopathy. Several maternal relatives had mild clinical and laboratory signs of cardiac hypertrophy [60]. In contrast with other pm-mtDNAs, the heart was the only affected organ.

**Mutation A4317G** This mutation was described in a 1-year-old boy who died of intractable cardiac failure, anemia, metabolic acidosis, and increased muscle enzymes. At necropsy severe dilatation and hypertrophy of the left ventricle were found [61].

**Mutation A4320G** The mutation has been identified in a 7-month-old infant with intractable hypertrophic cardiomyopathy and encephalopathy [62].

**2.1.2.3. Point mutations in the tRNAlysine gene.** Mutations in the tRNAlys gene have been associated with the Myoclonus Epilepsy with Ragged Red Fibres syndrome (MERRF), a severe neuromuscular disorder belonging to the ‘progressive myoclonus epilepsies’. MERRF is characterized by myoclonus or myoclonus epilepsy, muscle weakness and wasting, ataxia, deafness and dementia [63]. Most patients present a heteroplasmic A8344G transition [64]. A second mutation associated with MERRF or a MERRF/MELAS overlap has been found in the same gene (T8356C) [65,66]. More recently, a T7512C transition in the tRNAser(UCA) was found in a family with a MELAS/MERRF overlap syndrome [67]. Heart involvement is not frequent in MERRF [68]. However, a G8363A transition within the tRNAlys gene has been recently reported in association with maternally inherited cardiomyopathy and hearing loss in two unrelated families [69].

**2.1.2.4. Point mutations in the tRNAgly gene.** A single pedigree with maternally inherited non-obstructive cardiomyopathy presented a heteroplasmic T9997C transition within the tRNAgly gene. Several members of the same pedigree showed also intestinal dysmotility leading to pseudo-obstruction [70].

**2.1.2.5. Point mutations in mtDNA genes encoding subunits of the respiratory chain.** Point mutations in the ATPase 6 gene Heteroplasmic T8993G or T8993C mutations within the ATPase 6 gene are associated with maternally inherited Neuropathy, Ataxia, Retinitis Pigmentosa complex (NARP) [71,72]. The same mutation can affect patients with maternally-inherited Leigh’s syndrome (MILS) [73]. NARP and MILS may coexist in the same pedigree. MILS infants show psychomotor delay, cerebellar and pyramidal signs, dystonia, seizures, respiratory irregularities and incoordination of ocular movements. MRI of the brain shows symmetric lesions in the brainstem, thalamus and posterior columns of the spinal chord, as observed in infants with Leigh syndrome [74,75]. Impairment of ATP synthesis, as well as instability and altered assembly of the ATPase complex, have been reported in T8993G-positive patients [76,77]. Hypertrophic cardiomyopathy was reported in Leigh syndrome long before the ‘mtDNA era’ [78], and just recently confirmed in association with the T8993G mutation [79].

Several point mutations involving mtDNA genes encoding protein subunits of the respiratory chain have been reported and associated with Leber’s Hereditary Optic Neuropathy (LHON). LHON can occasionally be associated with cardiac conduction abnormalities (pre-excitation syndrome) [80,81].

**2.1.2.6. Cellular and molecular pathogenesis of point mutations of mtDNA.** The relationship between phenotypes, biochemical defects and molecular genetic findings is still poorly understood. Point mutations involving genes encoding subunits of the respiratory chain (i.e., NARP and LHON mutations) are not associated with RRF and, biochemically, should impair the activity of single enzyme complexes, but evidence for this is still limited. On the
other hand, patients with point mutations involving tRNA genes have RRF, and biochemical studies in muscle showed multiple defects of the respiratory chain, most frequently complexes I and IV. Mutations in tRNA genes are thought to impair mtDNA translation due to reduced availability of functional tRNAs. As reported for large-scale re-arrangements, the effect of point mutations has been studied in vitro using human cell lines deprived of mtDNA (p<sub>0</sub> cells) in which variable amounts of mutant mtDNA were introduced. Studies on such transmitochondrial cybrids have indeed confirmed that MERRF, MELAS and MIMyCa mutations cause a severe reduction of mitochondrial protein synthesis and respiration when the percentage of mutant mtDNA is above 80–90% [55,82–84].

2.1.3. mtDNA lesions transmitted as Mendelian traits

Mutations in nuclear genes involved in mtDNA housekeeping functions are proposed to be responsible for mtDNA abnormalities inherited as Mendelian traits.

Multiple deletions of mtDNA were first described in several Italian pedigrees affected with Autosomal Dominant Chronic External Ophthalmoplegia (AD-CPEO) [85]. The disease is characterized by proximal muscles weakness, wasting, ataxia, cataracts, vestibular areflexia and sensory-motor peripheral neuropathy. Southern blot analysis demonstrated multiple heteroplasmic deletions of mtDNA in muscle. AD-CPEO patients do not show signs of cardiac involvement. However, idiopathic dilated cardiomyopathy has been reported in a mother and son presenting multiple mtDNA deletions in both skeletal muscle and heart [86]. Multiple deletions were also found in a sporadic 41-year-old man with hypertrophic cardiomyopathy and mitochondrial myopathy with RRF, but no CPEO [87]. Autosomal recessive severe intractable cardiomyopathy and CPEO associated with multiple mtDNA deletions were recently described in two families from Saudi Arabia [88].

2.2. Biochemically defined defects of the respiratory chain

Although the majority of the protein subunits of the respiratory chain are encoded by nuclear DNA, no mutations in nuclear genes have been reported, with the exception of a point mutation in the gene encoding the flavoprotein subunit of succinate dehydrogenase. This mutation was found in two siblings with Leigh syndrome associated with deficiency of complex II [89].

Respiratory chain defects are extremely heterogeneous in their clinical features, but cardiomyopathy has been frequently reported. Several patients with cardiomyopathy associated with respiratory chain defects have been described in the literature, and were recently reviewed by Guenthard and co-workers [90–108]. Cardiomyopathy may be the main clinical feature, but it is usually part of complex syndromes characterized by metabolic acidosis, failure to thrive, and variable involvement of the central nervous system and skeletal muscle. Signs of cardiac involvement are often detected in early infancy. As in the case of pm-mtDNAs, cardiomyopathy associated with respiratory chain defects is usually hypertrophic and shows a rapid downhill course. In these patients, heart failure is the main cause of death in infancy. In most cases, the biochemical defect affects complex I or complex IV of the respiratory chain. Endomyocardial biopsy is the method of choice for early detection of OXPHOS defects in hypertrophic cardiomyopathy [109].

2.3. Mitochondria, cardiomyopathy and the aging process

Free radicals, normal by-products of OXPHOS, are harmful compounds [110]. Their accumulation may lead to damage of membranes, proteins and DNA. Scavenging enzymes such as superoxide dismutase as well as other molecules (i.e., glutathione, vitamins E, Q, C, coenzyme Q and carotenoids) limit the consequences of oxidant leakage. For instance, knockout mice lacking the gene encoding the mitochondrial manganese superoxide dismutase (MnSOD) develop metabolic acidosis and die a few days after birth of severe heart failure due to dilated cardiomyopathy [111]. Tissue damage mediated by oxygen radicals has been implicated in the pathogenesis of several neurodegenerative disorders, atherosclerosis and aging [112]. Several lines of evidence demonstrate a decline of mitochondrial enzyme activities with age, particularly of complex I [113]. Moreover, lack of an efficient repair mechanism and absence of histones make mtDNA more susceptible to the toxic effects of free radicals. Accumulation of mtDNA mutations are proposed to cause progressive impairment of mitochondrial respiration. Deletions of mtDNA accumulate with age in the human brain, skeletal muscle and heart [114,115]. However, the amount of deleted mtDNA seems to be too little to determine a significant reduction of OXPHOS. A recent study failed to detect OXPHOS defects in idiopathic dilated cardiomyopathy [116]. Therefore, whether low-percentage mtDNA deletions are the cause or the consequence of cardiomyopathy is still an open question [117].

3. Cardiomyopathy and defects of lipid metabolism

Fatty acids are the main source of chemical energy for myocardial contraction. To be oxidized, long-chain fatty acids activated as acyl-CoA esters need to cross the inner mitochondrial membrane. Since the latter is impermeable to acyl-CoA esters, acyl groups are transferred into mitochondria after esterification with carnitine. Translocation of fatty acids across the inner mitochondrial membrane is carried out by the concerted actions of three enzymes: i.e., the two carnitine palmitoyltransferases, CPT I and CPT II, and by carnitine-acylcarnitine translocase (CT). CPT I is located on the inner side of the outer mitochondrial mem-
brane while CPT II resides on the inner side of the inner mitochondrial membrane. Once in the mitochondrial matrix, fatty acids enter the β-oxidation pathway. The latter is composed of four sequential reactions (Fig. 3) [118–120]: (1) dehydrogenation of acyl-CoAs—this reaction is carried out by specific flavin-dependent dehydrogenases acting on short-, medium-, long- and very-long-chain acyl-CoAs (SCAD, MCAD, LCAD and VLCAD); (2) hydration of 2-enoyl-CoA; (3) dehydrogenation of 3-hydroxyacyl-CoA—this reaction is carried out on substrates of different length by two NAD-dependent dehydrogenases (i.e., the short-chain and long-chain 3-hydroxyacyl-CoA dehydrogenases); (4) conversion of 3-ketoacyl-CoA esters to acetyl-CoA plus a fatty acyl-CoA shortened by two carbon atoms. Recently, two novel membrane-bound enzymes of β-oxidation have been characterized: the very-long-chain acyl-CoA dehydrogenase (VLCAD) and a mitochondrial trifunctional protein (MTP) retaining the activities of a long-chain 2-enoyl-CoA hydratase, a long-chain 3-hydroxyacyl-CoA dehydrogenase and a long-chain 3-ketoacyl-CoA thiolase [121,122]. These discoveries suggest the existence of two β-oxidation pathways, one located in the mitochondrial membrane and the other in the mitochondrial matrix. The former is responsible for the oxidation of long-chain fatty acids, the latter for the oxidation of medium- and short-chain acyl-CoAs [123]. The electrons produced by FAD-dependent dehydrogenases are passed to coenzyme Q via the electron-transferring flavoprotein (ETF) and its dehydrogenase (ETF-coenzyme Q oxidoreductase, ETF-QO) while electrons resulting from NAD-dependent activities are transferred to complex I of the respiratory chain (Fig. 3).

The clinical features of inborn errors of fatty acid oxidation may vary according to the specific enzyme defect. Onset of the disease is sometimes at birth, and usually within the first years of life. Pediatric patients typically present acute episodes of liver dysfunction, metabolic acidosis, and hypoketotic hypoglycemia, triggered by fasting. Cardiac involvement, more frequently in the form of hypertrophic cardiomyopathy, may take a rapid downhill course, leading to overt heart failure, or may evolve progressively. Sudden death has also been reported. The disease is usually more severe, and with earlier onset, in patients with defects of long-chain fatty acid oxidation. This can be explained by the toxic action of long-chain carnitine esters, which may also cause severe cardiac arrhythmias [124]. Skeletal muscle involvement can lead to chronic progressive myopathy, particularly in juvenile-adult patients, or to acute episodes of paroxysmal myoglobinuria. In other cases, a subclinical myopathy is revealed by the presence of lipid storage, mainly in type I muscle fibers [118].

The diagnosis of defects of fatty acid oxidation requires several laboratory tests, including: (a) screening for hypoglycemia, metabolic acidosis and ketone body production, (b) plasma free and esterified carnitine profile, (c) analysis of the excretion of urinary organic acids (by gas chromatography–mass spectrometry), (d) carnitine assay in skeletal muscle, and (e) assay of β-oxidation enzyme activities on biopsied tissues.

![Fig. 3. Schematic representation of fatty acid β-oxidation. SCAD = short-chain acyl-CoA dehydrogenase; MCAD = medium-chain acyl-CoA dehydrogenase; LCAD = long-chain acyl-CoA dehydrogenase; VLCAD = very-long-chain acyl-CoA dehydrogenase; MTP = mitochondrial trifunctional protein; ETF = electron transfer flavoprotein; ETF-QO = electron transfer flavoprotein dehydrogenase.](image-url)
Since the clinical manifestations are heterogenous, and may involve muscle, heart and liver in variable combination, these disorders are classified according to the underlying biochemical defect (Table 3). We shall focus on defects of $\beta$-oxidation associated with cardiomyopathy. Other reviews provide for a detailed description of the clinical and biochemical features of $\beta$-oxidation defects [118–120].

3.1. Defects of fatty acids transport

3.1.1. Defects of fatty acids transport

Carnitine has a fundamental role in the transport of long-chain fatty acids across the mitochondrial membrane. About 25% of carnitine is synthesized in the liver and kidney while the remaining 75% comes with the diet [125]. Carnitine concentration is several-fold higher in the heart and skeletal muscle, implying that carnitine must be actively concentrated from blood. High-affinity receptors for carnitine have been identified in fibroblasts, and in skeletal and heart muscle [126].

The main clinical feature of primary CD is dilatative cardiomyopathy, leading to death if left untreated [127–130]. Hypoketotic hypoglycemia, generalized hypotonia and myopathy are frequent additional signs. However, clinical manifestations can vary, even in the same family [118,131]. Early diagnosis is mandatory because the disease responds rapidly and dramatically to carnitine supplementation (2–6 g per day). Diagnostic criteria include: (a) detection of very low levels of total and free carnitine in plasma and muscle, (b) absence of abnormal organic acids in 24-hour urine samples, and (3) impaired carnitine uptake in cultured fibroblasts.

A defective carnitine transport has been implicated in the pathogenesis of primary CD [132–134]. Patients’ cultured fibroblasts show negligible uptake of carnitine, while heterozygotes show $K_m$ values for carnitine transport similar to those of controls and a 50% reduction of $V_{max}$. Affected subjects are proposed to lack a functionally active high-affinity carnitine receptor [131–135]. Results on carnitine uptake clearly suggest autosomal recessive inheritance. However, autosomal dominant hypertrophic cardiomyopathy associated with muscle carnitine deficiency has been reported in a single pedigree. Carnitine uptake was not investigated [136].

3.1.2. Carnitine-acylcarnitine translocase deficiency (CT)

Carnitine-acylcarnitine translocase is an inner mitochondrial membrane protein that allows the transfer of carnitine and its esters across the inner mitochondrial membrane. CT deficiency is characterized by muscle weakness, hypoketotic hypoglycemia and cardiomyopathy. Free carnitine is very low in plasma, together with high levels of long-chain acylcarnitines. CT activity in cultured fibroblasts is markedly impaired [137,138].

3.1.3. Carnitine palmitoyltransferase deficiencies (CPT)

CPT is responsible for the transport of long-chain fatty acids across the inner mitochondrial membrane. CPT I and CPT II activities are almost certainly carried out by two different enzymes [139], and distinct defects of CPT I or CPT II have been described [140].

CPT I deficiency is characterized by recurrent attacks of hypoketotic hypoglycemia. No muscle or cardiac involvement has been reported. CPT I activity is reduced in liver and cultured fibroblasts, while CPT I and II activities are normal in skeletal muscle, supporting the existence of tissue-specific isoforms.

CPT II deficiency has been associated with three differ-

<table>
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<tr>
<th>Table 3</th>
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<tbody>
<tr>
<td>Classification of disorders of fatty acid oxidation</td>
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</table>

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<tr>
<th>Defects:</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Fatty acid transport</td>
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<tr>
<td>Primary carnitine deficiency</td>
<td>[127–135]</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase</td>
<td>(CPT-I, CPT-II) [140]</td>
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<tr>
<td>Carnitine-acylcarnitine translocase</td>
<td>[137,138]</td>
</tr>
<tr>
<td>(2) $\beta$-Oxidation enzymes</td>
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<tr>
<td>Short-chain acyl-CoA dehydrogenase</td>
<td>(SCAD) [168]</td>
</tr>
<tr>
<td>Medium-chain acyl-CoA dehydrogenase</td>
<td>(MCAD) [169]</td>
</tr>
<tr>
<td>Long-chain acyl-CoA dehydrogenase</td>
<td>(LCAD) [170]</td>
</tr>
<tr>
<td>Very-long-chain acyl-CoA dehydrogenase</td>
<td>(VLCAD) [147–156]</td>
</tr>
<tr>
<td>Short-chain 3-hydroxyacyl-CoA dehydrogenase</td>
<td>(SCHAD) [171]</td>
</tr>
<tr>
<td>Long-chain 3-hydroxyacyl-CoA dehydrogenase</td>
<td>(LCHAD) [160]</td>
</tr>
<tr>
<td>Mitochondrial trifunctional protein</td>
<td>(MTP) [164,165]</td>
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<tr>
<td>(3) Transferring flavoproteins (multiple acyl-CoA dehydrogenation defects)</td>
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<tr>
<td>Electron-transfer flavoprotein</td>
<td>(ETF) [167]</td>
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<tr>
<td>Electron-transfer flavoprotein-coenzyme Q oxidoreductase (ETF-QO)</td>
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<tr>
<td>Riboflavin responsive defects</td>
<td>[172]</td>
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ent phenotypes: (a) A muscular form, without heart involvement, characterized by recurrent episodes of myoglobinuria. Attacks are triggered by exercise, fasting, fever and other stress conditions. The transmission is autosomal recessive, with a typical predominance in young males. (b) A severe infantile form characterized by liver, muscle and heart involvement. (c) A fatal neonatal form characterized by hypoketotic hypoglycemia, cardiomyopathy and congenital anomalies.

Several point mutations have been identified in the CPT II gene [140]. A C → T transition causing a Ser → Leu substitution is the most frequent missense mutation, accounting for the majority of patients affected by adult-onset recurrent myoglobinuria [141,142]. Other less frequent missense mutations have been reported in patients with the muscular or hepatocardiomuscular form, while null mutations are responsible for the lethal neonatal presentation [143–146]

3.2. Defects of β-oxidation enzymes

Several genetic defects of the enzymes involved in the β-oxidation pathway have been reported. Defects of acyl-CoA dehydrogenases are probably the most frequent. The possible clinical manifestations include hypoglycemia, myopathy, cardiomyopathy, and sudden death. In particular, cardiac involvement has been reported in LCAD, VLCAD and MTP deficiencies (Table 3).

3.2.1. Long-chain (LCAD) and very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency

The VLCAD activity has only recently been discovered [121]. This may explain why several cases previously attributed to LCAD deficiency failed to show either molecular abnormalities in the LCAD gene or low LCAD immunoreactivity; it is likely that they were affected by VLCAD deficiency [147,148]. Disease onset is usually in early childhood. The clinical manifestations common to all cases include hypoketotic hypoglycemia and dicarboxylic aciduria. However, the clinical course may vary from severe, frequently lethal hypertrophic cardiomyopathy to milder phenotypes characterized by recurrent metabolic attacks [149,150]. Interestingly, fibroblasts from patients with cardiomyopathy accumulate long-chain acylcarnitine esters at a higher rate than fibroblasts from patients with less severe phenotypes [151]. Several mutations associated with VLCAD deficiency have been reported [152–156].

Cardiac positron emission tomography (PET) with [11C]palmitate and [13C]acetate as metabolic tracers has been proposed as a non-invasive method to investigate the myocardial involvement and to follow the effect of therapy in patients with defects of long-chain fatty acid oxidation. A decreased rate of long-chain fatty acid oxidation in the heart was suggested by the prolonged rate of palmitate clearance from the patients’ myocardium, compared to control subjects [157].

3.2.2. Mitochondrial trifunctional protein (MTP) deficiency

As already mentioned, the MTP complex contains three different enzyme activities: long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), 2-enoyl-CoA hydratase (both located in the four α-subunits), and long-chain 3-ketoacyl-CoA thiolase (located on the four β-subunits of the enzyme complex). Patients, classified according to the specific defective activity, can show an isolated LCHAD deficiency or a multienzyme deficiency. Both forms are associated with a typical hydroxyaciduria and hepatic dysfunction. Myopathy, cardiomyopathy leading to heart failure, and myoglobinuria can be associated features. Improvement of cardiac dysfunction has been reported after treatment with medium-chain triglycerides [158–160]. A missense mutation in the α-subunit of LCHAD has been identified [161–163]. The clinical presentation of patients with the multienzyme deficiency are similar to those of isolated LCHAD deficiency. All the known mutations responsible for MTP deficiency impair the formation of the whole enzyme complex [164–166].

3.3. Defects of electron-transferring flavoproteins

ETF and its dehydrogenase (ETF-QO) are mitochondrial flavoproteins which transfer electrons generated by the activity of acyl-CoA dehydrogenases to coenzyme Q, a lipoidal quinone serving as a shuttle molecule in the respiratory chain. Deficiency in either ETF or ETF-QO has been identified in multiple acyl-CoA dehydrogenase deficiency (also known as glutaric aciduria type II). These patients excrete massive amounts of several organic acids in their urine due to reduced oxidation of acyl-CoAs of different length. Infants affected by the most severe form of the disease present hypoglycemia, metabolic acidosis, hypotonia, hepatomegaly and multiple congenital anomalies. The syndrome leads to death in a few weeks. However, patients surviving longer (up to a few months of age) may develop cardiomyopathy [167].

4. Treatment of OXPHOS and β-oxidation defects

The outstanding advances in the molecular understanding of mitochondrial disorders have not been paralleled by comparable improvements in results of therapies for these diseases. Treatments investigated so far share the same goal of improving energy production by acting on the impaired metabolic pathway. However, several factors complicate the therapeutic approach to mitochondrial disorders, including our still limited understanding of the pathogenetic potential of each mtDNA mutation, the complex nDNA-mtDNA interrelationships and control mechanisms exerted on the respiratory chain, and the consequences of a remarkable genetic heterogeneity.
Apart from supportive therapy and correction of metabolic acidosis, specific treatments are lacking for both mitochondrial and \( \beta \)-oxidation defects. Several compounds, vitamins, coenzymes and metabolic intermediates have been tested in patients with OHIDPOS defects [173,174]. Quinone compounds, after being reduced to hydroxyquinones, facilitate the bypass of impaired respiratory chain complexes. Anecdotal reports have claimed that coenzyme Q\(_{10}\) is effective by reducing lactic acid in blood and cerebrospinal fluid, but preliminary data were not confirmed by further studies using CoQ\(_{10}\) and multiple vitamins. The experience with idebenone, menadione (Vitamin K\(_3\)) and phylloquinone (Vitamin K\(_1\)), even though partially successful, is too limited to draw definite conclusions. Riboflavin (vitamin B\(_2\)) has been used with success in a few patients with complex I deficiency. The rationale for its use is that riboflavin is a precursor of FMN and FAD, co-factors of the respiratory chain complexes I and II, respectively. Dichloroacetate (DCA) is a drug able to reduce lactic acid by stimulation of its oxidation to acetylCoA. A short DCA treatment improved blood lactate levels as well as brain oxidative metabolism measured by proton MRI spectroscopy in patients with mitochondrial disorders. On the basis of initial reports, more detailed investigations on DCA in patients with lactic acidosis associated with respiratory chain defects are awaited. Among \( \beta \)-oxidation defects, primary carnitine deficiency responds dramatically to carnitine, usually 2–6 g per day. Early diagnosis of the disease is mandatory since muscle and cardiac function recover rapidly and completely under carnitine supplementation [118].

Speculations can be made on the possibility of transferring genes to cells with mutations of mtDNA. Common approaches require the introduction of a gene into defective cells via viral or liposome-mediated gene transfer. However, transformation of mitochondria requires an appropriate transfection vector. It has been demonstrated that double-stranded DNA coupled to a mitochondrial leader peptide is able to enter the mitochondrial matrix using the existing protein import machinery [175].

5. Future perspectives

The identification of numerous mutations of human mtDNA associated with disease has revolutionized our concepts of and approach to mitochondrial disorders [176]. On the other hand, the establishment of a rational diagnostic strategy and pathogenetic interpretation of mtDNA abnormalities is based on the knowledge of the principles dictating mtDNA genetics, expression and control. A better understanding of mitochondrial biogenesis is also important for a genetic approach to therapy. For instance, an unknown mechanism controls importation from the cytoplasmic compartment of some RNA components of the mtDNA replication/transcription apparatus. The elucidation of such a mechanism will be of great importance for designing a therapeutic strategy based on RNA or DNA targeting into mitochondria.

A better understanding of the fundamental mechanisms of mitochondrial biogenesis is also important to explain numerous puzzling questions, such as the influence of nuclear genes in the clinical expression of mtDNA mutations, the importance of mitochondrial abnormalities in conditions having a major social impact, such as diabetes mellitus or ‘idiopathic’ cardiomyopathies, and the progressive failure of organs that occurs in ageing.

Furthermore, studies on the tissue distribution of mutations, on factors which influence their accumulation in tissues, and on their complementation by normal genomes are essential if genetic counseling, prenatal diagnosis and prognostic assessment of mitochondrial disorders are to become rational processes.

Finally, it is expected that molecular dissection of the human mitochondrial proteome will help identify the nuclear genes responsible for the many mitochondrial phenotypes that are not due to mtDNA mutations. The availability of genes controlling mitochondrial DNA replication, gene expression, and, possibly, repair will allow the creation of recombinant animal or cellular models of mitochondrial disorders, to test rational therapeutic strategies.

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