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MITOCHONDRIAL DISEASES PART II: MOUSE MODELS OF OXPHOS DEFICIENCIES CAUSED BY DEFECTS IN REGULATORY FACTORS AND OTHER COMPONENTS REQUIRED FOR MITOCHONDRIAL FUNCTION

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Abstract

Mitochondrial disorders are defined as defects that affect the oxidative phosphorylation system (OXPHOS). They are characterized by a heterogeneous array of clinical presentations due in part to a wide variety of factors required for proper function of the components of the OXPHOS system. There is no cure for these disorders owing our poor knowledge of the pathogenic mechanisms of disease. To understand the mechanisms of human disease numerous mouse models have been developed in recent years. Here we summarize the features of several mouse models of mitochondrial diseases directly related to those factors affecting mtDNA maintenance, replication, transcription, translation as well to other proteins that are involved in mitochondrial dynamics and quality control which affect mitochondrial OXPHOS function without been intrinsic components of the system. We discuss how these models have contributed to our understanding of mitochondrial diseases and their pathogenic mechanisms.

Keywords

Mitochondrial DNA; mitochondrial transcription; mouse models; mitochondrial dynamics; quality control; mitochondrial diseases

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1. Introduction

Mammalian mitochondrial DNA (mtDNA) is a double-stranded, circular molecule of about 16,5 Kb encoding for 37 genes: 13 genes for oxidative phosphorylation (OXPHOS) complexes subunits, 22 tRNAs and 2 rRNAs necessary for mitochondrial translation (Anderson, 1981). Mitochondrial DNA follows peculiar non-Mendelian genetic rules, including matrilinear transmission, polyploidy, threshold effect and mitotic segregation. Mitochondrial replication, transcription and translation occur in a semi-autonomous fashion, since all the proteins necessary for these processes are encoded by nuclear DNA and imported from the cytoplasm (Falkenberg, 2007). The intricate molecular machinery involved in the maintenance, transcription and translation of mtDNA includes several proteins found mutated in human neurological disorders that share a common feature the presence of mtDNA deletions and/or depletion (Koopman, 2012; Spinazzola, 2011). These proteins are either essential for mtDNA replication (POLγ and TWINKLE), transcription/translation (TFAM, TFB1M, mTERFs, POLRMT, LRPPRC and DARS2), for the correct maintenance of the mitochondrial dNTPs pool (TP, TK, ANT1, and RRM2B). In addition, proteins involved in mitochondrial dynamics (MFN1, MFN2, OPA1, and DRP1) and in protein quality control (CLPP, AFG3L2, PARAPLEGIN, OMA1, PHBs, OMI and PARL) are also essential for proper mitochondrial function. Defects in the factors aforementioned can cause mitochondrial diseases. We have described mouse models of mitochondrial diseases that are directly related to components of the OXPHOS system in Part I of this review miniseries. In this chapter, Part II of the review miniseries, we summarize the mouse models that recapitulate defects in mtDNA instability, transcription, translation, mitochondrial dynamics, quality control and others. We discuss how thesemousemodels have contributed to our knowledge of the specific function of these factors and their role in the pathogenic mechanisms of mitochondrial disorders. A summary of these mousemodels is shown in Fig. 1. Part III of the review miniseries discusses the different therapeutic strategies that have been tested in the mouse models described in Parts I and II.

1. Mouse models of proteins involved in mtDNA stability, replication, transcription and translation

The mammalian mtDNA genome must be replicated, transcribed and translated to assure a proper mitochondrial function. Regulation of these processes is very important for modulating the OXPHOS capacity in response to metabolic requirements or to pathological/stress conditions. All the components needed for replication and transcription of the mtDNA are encoded in the nuclear genome. For the translation of the mtDNA, 22 tRNAs and 2 rRNAs encoded in the mitochondrial DNA are required in addition to numerous proteins that are imported from the cytoplasm. The mtDNA replication seems to relay in a single DNA polymerase, polymeraseγ. The core machinery required for basal mtDNA transcription in mammals (mtRNA-polymerase, TFAM, mtTFB2) and a few proteins that regulate mtDNA transcription has been described (mtTERFS, LRPPRC). Although the regulation of the mitochondrial transcription is a not completely understood process, the creation of knockout mouse models have clearly contributed to the understanding of mitochondrial transcription in mammals (reviewed in {Peralta, 2012 #591}). Mouse models of proteins

involved in the maintenance of mitochondrial DNA, replication, transcription and translation are summarized in Table I.

1.1. Polg

Mammalian polymerase γ (POLγ) is a heterotrimer composed by a catalytic subunit, encoded by the *POLG* gene, with DNA-polymerase and 3′-5′ exonuclease activities, and two identical accessory subunits, encoded by the *POLG2* gene, conferring high processivity to the catalytic subunit by increasing the affinity of the heterotrimer for template DNA.

Mutations in human *POLG* gene have been associated with different clinical presentations, ranging from diverse infantile encephalopathies to late-onset myopathies, but sharing mtDNA instability as a common feature (for a recent review see (Milone and Massie, 2010)). Close to 250 pathogenic mutations have been described for the *POLG* gene (http://tools.niehs.nih.gov/polg/index.cfm) making it one of the most common causes of mitochondrial diseases (for review see (Copeland and Longley, 2014). Several mouse models have been generated modifying the murine *Polg* gene; however they do not fully resemble the clinical spectrum of patients. A summary of all *Polg* mouse models is available in Table I. Homozygous *Polg* knockout mice died *in utero* at E7.5–8.5 due to an almost complete lack of mtDNA, indicating that DNA-polymerase activity is essential for mtDNA replication during embryonic development (Hance, 2005).

One of the most common mutations of the human mitochondrial polymerase associated with Progressive External Ophthalmoplegia (PEO) is the Y955C mutation. This mutation is known to affect the DNA-polymerase activity by promoting enzyme stalling and errors in replication. Transgenic mice created by expressing the human $POLG^{Y955C}$ mutant form died prematurely and presented with massive cardiomegaly with bilateral atrial enlargement (Lewis, 2007). Tissue analyses revealed the presence of mtDNA depletion and mtDNA damage, most likely caused by increased oxidative stress. Additionally, proliferation of mitochondrial showing *cristae* disorganization was observed presumably as a compensatory mechanism (Lewis, 2007).

Another mutation in *Polg* that causes impairment of the 3′-5′ exonuclease activity was modeled by the transgenic overexpression of the mutant $Polg^{D181A}$ in heart, pancreatic β-cells and neurons. The mutant $Polg^{D181A}$ invariably induced an accumulation of mtDNA point mutations and large-scale deletions (Bensch, 2009; Kasahara, 2006; Zhang, 2000). When the transgene was expressed in the heart, mice developed a severe progressive cardiomegaly associated with a profound alteration of transcriptional profile of genes involved in cardiac remodeling; heart and muscle contractility; energy and lipid metabolisms; and stress response and apoptosis (Zhang, 2005). When targeted to neurons, $Polg^{D181A}$ overexpression induced mtDNA instability in multiple brain regions, such as cortex, basal ganglia, hippocampus and retinal cells (Kasahara, 2006; Kong, 2011). These transgenic mice were perfectly normal in terms of general health, longevity and fertility; without any gross alteration in brain or retinal structure; or functional motor or coordination impairment. However, after 20 weeks of age, mice started to show some signs of neuronal dysfunction, including distorted circadian rhythms, enhanced startle response, and altered secretion pattern of monoamines (i.e. serotonin, dopamine and noradrenaline), mirroring

some of the mood disorder symptoms found in PEO patients (Kasahara, 2006; Lamperti and Zeviani, 2009; Mancuso, 2004). The accumulation of mtDNA point mutations and deletions in the *Polg*^{D181A} mice caused a mild mitochondrial dysfunction in the retina, with altered electroretinogram patterns but no loss of retinal ganglion cells (RGCs) or retinal thinning were found (Kong, 2011). The same transgene was targeted to pancreatic islets in a mouse model of early-onset diabetes, characterized by increased β-cells apoptosis and insulin insufficiency, supporting the mitochondrial dysfunction theory for the etiology of age-dependent diabetes (Bensch, 2009).

Two knock-in mutants of *Polg* have been generated independently by inducing the amino acid substitution D257A in the proofreading 3′-5′ exonuclease domain two (Table 1) (Kujoth, 2005; Trifunovic, 2004). The lack of proofreading activity of the so-called "Mutator mice" caused the accumulation of mtDNA point mutations and non-canonical multiple deletions in different tissues, leading to a progressive OXPHOS deficiency (Edgar, 2009; Trifunovic, 2004). Despite the different age of onset, these mice showed a reduced lifespan and developed a premature aging phenotype (Fox, 2012; Kujoth, 2005; Trifunovic, 2004). Unexpectedly, the accumulation of mtDNA mutations did not induce a massive chronic oxidative stress and the progeroid phenotype was mainly caused by increased apoptosis (Kujoth, 2005; Trifunovic, 2005). Although it is well established that the Mutator mouse is characterized by having mtDNA instability, whether the premature aging phenotype is caused by the accumulation of mtDNA mutations or by the multiple deletions is still a matter of discussion (Bailey, 2009; Edgar, 2009; Vermulst, 2007; Vermulst, 2008; Williams, 2010). Nonetheless, the peculiar progeroid phenotype makes the Mutator mouse a unique model for the study of the mitochondrial contribution to aging.

Dopaminergic neurons of the Mutator mouse mirrored the accumulation of mtDNA deletions found in patients with Parkinson's disease and aged individuals (Perier, 2013). However, despite the occurrence of mtDNA deletions, the mitochondrial function of the Mutator mouse was generally comparable to controls, despite the presence of few cytochrome *c* oxidase (COX) negative neurons. Interestingly, the dopaminergic neurons displayed higher levels of mtDNA-deleted copies. In addition, a global compensatory response was activated in these neurons, inducing higher dopamine levels and resistance to neurotoxins (Perier, 2013). As the Mutator mouse aged (12 to 14 months), it displayed a dopaminergic dysfunction that was associated with impaired locomotor activity and rotarod performance, reduced body mass and metabolic alterations (Dai, 2013).

The decline of mitochondrial function in skeletal muscle of the Mutator mouse was associated with a profound sarcopenia and exercise intolerance, mainly due to the loss of type I muscle fibers (Hiona, 2010; Safdar, 2011). Muscle from older Mutator mice displayed higher mitochondrial fission and autophagy levels, which most likely contributed to the sarcopenic phenotype observed in premature aging and differed from the response observed in normally-aged muscle (Joseph, 2013). Moreover, mtDNA instability induced a significant remodeling of gastrocnemius transcriptional profile, with a striking activation of several genes involved in RNA synthesis and ribosomal maturation, whereas a down-regulation of genes involved in energy metabolism was found (Hiona, 2010).

The Mutator mouse also developed age-dependent macrocytic anemia that invariantly led to premature death (Chen, 2009; Trifunovic, 2004). Abnormal erythroid development and defective lymphopoiesis found in the Mutator mouse were transferred to wild type littermates by bone marrow transplantation, suggesting a possible involvement of mitochondrial defects in the pathogenesis of some age-related myelodysplastic cases of anemia (Chen, 2009). This peculiar phenotype seems to be related to the presence of dysfunctional hematopoietic and neural somatic stem cell progenitors, already from embryogenesis (Ahlqvist, 2012).

Recent work demonstrated that the Mutator mouse also showed profound changes in intestinal absorption of nutrients even at 3 to 7 months of age, with restricted absorption of dietary lipids due to a severe impairment of stem/progenitor cells (Fox, 2012).

1.2. Polg2

Mutations in *POLG2*, which are not as predominant as mutations in *POLG*, have been described in patients with early- and late-onset autosomal dominant progressive external ophthalmoplegia (adPEO) (Longley, 2006; Walter, 2010; Young, 2011). Presumably, defective POLG2 with impaired processivity activity causes POLγ to stall leading to accumulation of mutations during mtDNA replication. To better understand the role of this accessory subunit and how it causes pathogenesis, a mouse model carrying the ablation of *Polg2* has been developed (Table I) (Humble, 2013). Heterozygous $Polg2^{+/-}$ mice were undistinguishable from controls in terms of development, lifespan and mitochondrial function. On the contrary, homozygous deletion of *Polg2* resulted in embryonic lethality at E8.0–8.5 with loss of mtDNA and mtDNA-encoded proteins. Lipid accumulation, severe ultra-structural defects and *cristae* disorganization were detected in the mitochondria of the $Polg2^{-/-}$ embryos, indicating that POLG2 function is critical for mtDNA replication and mammalian embryogenesis (Humble, 2013).

1.3. Mitochondrial helicase: Twinkle

TWINKLE is a mitochondrial hexameric helicase belonging to the RecA superfamily that forms, along with POLγ and the single-stranded mtDNA binding protein (mtSSB), the minimal mtDNA replisome (Korhonen, 2004; Spelbrink, 2001; Tyynismaa, 2004). Mutations in *Twinkle* have been associated with adPEO (Spelbrink, 2001), with recessively inherited infantile onset spinocerebellar ataxia (IOSCA) (Nikali, 2005) and with mtDNA depletion syndrome (MDS) (Hakonen, 2007; Sarzi, 2007). The common feature of all these diseases is the presence of mtDNA instability (depletion/deletions) caused by an alteration of the replication machinery. Homozygous ablation of *Twinkle* was embryonic lethal demonstrating that this protein is essential for mouse embryonic development (Milenkovic, 2013). Tissue-specific (heart and skeletal muscle) ablation of *Twinkle* resulted in premature death at the age of 19 weeks. The mice developed a progressive cardiomyopathy, with a significant reduction in mtDNA levels and OXPHOS dysfunction in heart and skeletal muscle (Milenkovic, 2013) (Table I).

Two transgenic mice overexpressing different mutant forms of *Twinkle* ($Twinkle^{A360T}$ and the $Twinkle^{dup353–365}$) have been developed. These mice are commonly named the “Deletor

mice" (Tyynismaa, 2005). Both mutant mice developed the same pathological phenotype on their adulthood. Skeletal muscle from 1-year-old animals revealed signs of mitochondrial myopathy, including COX negative fibers, mitochondrial proliferation and abnormal fiber size, caused by the accumulation of deleted mtDNA molecules. Multiple deletions, mtDNA depletion and COX negative cells were also evident in brain of 18 month old animals (Tyynismaa, 2005). The study of the replication pattern of various tissues of the Deletor mouse provided molecular details on the generation and accumulation of mtDNA multiple deletions. The authors proposed that the pausing of the mutant TWINKLE in the replication fork induced the accumulation of mtDNA replication intermediates and transcriptional defects. The replication fork stalling may enhance the generation of mtDNA deleted molecules by activating a mtDNA repair system or by inducing a local depletion followed by an inappropriate repopulation (Goffart, 2009). The accumulation of mtDNA multiple deletions in the Deletor mouse was closely linked to a decline of OXPHOS function and metabolic alterations as demonstrated by the accumulation of COX negative cells, abnormal levels of phospholipids, ketone bodies and free amino acids in plasma and increased phosphatidylcholine and sphingomyelin in skeletal muscle (Ahola-Erkkilä, 2010). Gene expression profile of the Deletor mouse in skeletal muscle revealed a marked up-regulation of the amino acid starvation response pathway (Tyynismaa, 2010). In particular, the metabolic hormone fibroblast growth factor 21 (FGF21), secreted by liver and adipose tissue upon prolonged fasting, was upregulated in the Deletor mouse and it has been also found to be a reliable predictive biomarker for human mitochondrial myopathies (Suomalainen, 2011; Tyynismaa, 2010). By mimicking the phenotype of late-onset progressive mitochondrial myopathies, the Deletor mouse represents a valuable tool for the prognostic and development of therapeutic strategies and provided valuable insights in the understanding of metabolic adaptive responses.

A transgenic mouse overexpressing the mutant *Twinkle*$^{dup353–365}$ in dopaminergic neurons was developed to investigate the effects of accumulation of mtDNA deletions in Parkinson's disease (Table I) (Song, 2012). Transgenic mice were not significantly different from controls in terms of longevity and body weight. No overt signs of movement disorders were found, however, *Twinkle* mutants displayed increased levels of age-related mtDNA deletions, reduced number of dopaminergic neurons and abnormalities in rotarod performance. A mild reduction of mitochondrial basal respiration was found in association with reduced levels of Parkin and increased mitophagy, suggesting a possible involvement of Twinkle in the regulation of mitochondrial quality control in dopaminergic neurons.

1.4. Mitochondrial transcription factor Tfam

The mitochondrial transcription factor A or TFAM protein plays an essential dual function in the mtDNA transcription (Fisher and Clayton, 1988) and mtDNA maintenance (Ekstrand, 2004). TFAM belongs to the high-mobility-group (HMG) box domain protein family (Parisi and Clayton, 1991), which can bind, unwind and bend DNA without sequence specificity. TFAM is the main protein component of the nucleoids and is involved in mtDNA packaging (Bonawitz, 2006; Cámara, 2011). Whole body ablation of the *Tfam* gene in mice (*Tfam*$^{-/-}$) causes severe mtDNA depletion, leading to a severe respiratory chain deficiency and

embryonic lethality between E8.5 and E10.5 (Larsson, 1998). To overcome the embryonic lethality, several *Tfam* conditional KO mouse models have been generated (Table I).

Specific inactivation of *Tfam* in skeletal muscle (*Mlc-1f-Cre/LoxP system*) resulted in myopathy with ragged-red fibers (RRFs), and progressive decline of OXPHOS function (Wredenberg, 2002). As a consequence, a marked compensatory mitochondrial proliferation was evident in skeletal muscle.

Disruption of *Tfam* in mouse cardiomyocytes was generated by expressing *Cre* recombinase under the muscle creatine kinase promoter (*Mck-Cre*). This conditional KO animal showed progressive dilated cardiomyopathy with heart conduction block, observed at 2 weeks of age. Animals worsened with time and died prematurely at 10 to 14 weeks of age (Wang, 1999a). Contrary to what was observed in the *Mlc-1-f-Tfam* KO mice, in the *Mck-Tfam* KO mitochondrial proliferation was evident only at later stages of disease progression (8 weeks) and it was not sufficient to sustain ATP deficiency.

Deletion of *Tfam* in pancreatic β-cells (*Rip-Cre*) led to diabetes at 5 weeks and severe mtDNA depletion and OXPHOS deficiency at 7 to 9 weeks of age leading to decreased insulin release and reduced β -cell mass (Silva, 2000). This mouse model nicely reproduced the β-cell pathology of human mitochondrial diabetes form, that constitutes about 0.5–1% of all types of diabetes mellitus and clearly undelines the crucial role of the OXPHOS system in insulin secretion.

Ablation of *Tfam* specifically in forebrain neurons by the *CaMKIIa-Cre* caused mtDNA depletion starting at 2 months of age followed by reduced levels of mitochondrial transcripts and OXPHOS deficiency at 4 months of age (Sorensen, 2001). Postnatal disruption of the OXPHOS system in forebrain neurons induced a Mitochondrial Late-Onset Neurodegeneration process (MILON mice). The onset of the neurodegenerative disease was observed at 5 months of age and animals died thereafter due to massive neuronal death and gliosis in neocortex and hippocampus (Sorensen, 2001).

Loss of TFAM protein in the dopaminergic neurons using *Dat-Cre* led to a parkinsonism phenotype with adult onset, progressive motor impairment, presence of intraneuronal inclusions and dopaminergic neuronal death (Ekstrand, 2007).

Specific inactivation of *Tfam* in both white and brown adipose tissue (using the *aP2* promoter for *Cre* expression) results in decreased complex I and IV enzymatic activities (Vernochet, 2012). Complex II dependent respiratory function, citrate synthase activity and basal respiratory capacity were markedly increased presumably as compensatory mechanism for the bioenergetic defect. Interestingly, *Tfam* deletion in adipose tissue resulted in increased mitochondrial capacity and mice were protected against obesity and insulin resistance after ingestion of a high fat diet (Vernochet, 2012).

To understand how mtDNA copy number is regulated *in vivo*, Suomalainen's group developed a double transgenic mouse ubiquitously overexpressing the mouse *Twinkle* and the human *TFAM (hTFAM*) genes (Ylikallio, 2010). Double transgenic mice had increased COX negative fibers in heart and skeletal muscle and accumulated enlarged and abnormal

mitochondria in muscle. Molecular analysis revealed significantly higher mtDNA copy number in the double transgenic than in mice expressing single transgene particularly in muscle and heart. *Twinkle* appeared to regulate *de novo* mtDNA synthesis. Interestingly, the high mtDNA copy number in the *Twinkle/hTFAM* mouse was associated with an increase in size of the nucleoids, which led to defects in transcription, increased mtDNA deletions and subsequent defects of the respiratory chain (Ylikallio, 2010).

1.5. Mitochondrial transcription factor B1

Mitochondrial transcription factors B1 and B2 (TFB1M and TFB2M) regulate mtDNA transcription by forming heterodimers with the mitochondrial RNA polymerase (Falkenberg, 2002) and mitochondrial translation by exerting their rRNA methyltransferase activity (Cotney and Shadel, 2006). Null mice for *Tfb1m* were not viable and died at about E8.5 showing severe developmental delay whereas the conditional disruption of *Tfb1m* in heart and skeletal muscle produced severe cardiomyopathy and premature death by 24 weeks of age (Metodiev, 2009). Interestingly, no mitochondrial abnormalities were found in the skeletal muscle of the KO mice, whereas the heart of the *Tfb1m* KO displayed impaired OXPHOS function and increased mitochondrial biogenesis most likely to compensate for the bioenergetic defect. Mitochondrial protein translation was abolished in the KO mice as a consequence of impaired ribosomal assembly due to the low levels 12S rRNA and the lack of adenine dimethylation of the rRNA (Metodiev, 2009). This mouse model confirmed the function of TFB1M and its importance on ribosomal assembly and mitochondrial protein translation (Table I).

A gene variant of *Tfb1m* has been identified as one of the risk genes for development of Type II diabetes in humans (Koeck, 2011). To investigate the role of TFB1M in diabetes, a conditional mouse model harbouring the ablation of *Tfb1m* in pancreatic β-cells was created. Ablation of this gene in β-cells resulted in a progressive dysfunction of islets and development of diabetes resulting from the impaired mitochondrial function, which led to perturbations in insulin secretion. Among the pathological phenotypes are increased cell death of β-cells, inflammation and oxidative stress (Sharoyko, 2014). This mouse model underscores the importance of *Tfb1m* as a risk factor for diabetes.

1.6. Mitochondrial terminator factors-Mterf

The mitochondrial termination factor (MTERF) protein family is composed by 5 transcription termination factors, named MTERF1–5, which are implicated in the regulation of mitochondrial transcription and protein synthesis and in the coordination between transcription and replication (Bruni, 2012; Linder, 2005; Park, 2007; Roberti, 2009).

Park and collaborators generated the first KO mouse by inactivating of *Mterf3* in the germ line (Table I) (Park, 2007). Homozygous embryos presented an improper development and died between E8.5 and E10.5. The specific inactivation of *Mterf3* in heart and skeletal muscle produced a severe mitochondrial cardiomyopathy leading to premature death (Park, 2007). Shortly before death, these mice developed progressive bradycardia that later developed into an atrioventricular block (Andersson, 2011). Accumulation of aberrant mitochondria in the heart was associated with a severe OXPHOS impairment, characterized

by the progressive disassembly of respiratory complexes with the exception of complex II in the KO mice. The loss of MTERF3 did not affect mtDNA abundance, but increased transcription initiation on both mtDNA strands, leading to decreased expression of critical promoter-distal tRNA genes, which may possibly explain the bioenergetic dysfunction (Park, 2007).

Moraes group also observed an imbalance of mitochondrial tRNA levels in *Mterf2*$^{-/-}$ mice, although the loss of this factor caused an opposite effect on the steady-state levels of mitochondrial mRNAs by decreasing them (Wenz, 2009). *Mterf2*$^{-/-}$ mice were viable and presented normal basal metabolism and heat production, with only a modest tendency to weight and adipose tissue loss, which was more evident in females. These mice did not display an overt phenotype, but only a mild exercise intolerance and muscle weakness that worsened when feeding mice with a ketogenic diet. Under such metabolic stress conditions, *Mterf2*$^{-/-}$ mice developed memory and cognitive impairment concomitant with an imbalance of steady-state levels mitochondrial tRNAs levels, multiple OXPHOS deficiencies and lower ATP production in multiple tissues despite compensatory mitochondrial biogenesis (Wenz, 2009).

Ubiquitous inactivation of *Mterf4* is embryonic lethal (E8.5), whereas heterozygous mice were indistinguishable from wild type littermates. When *Mterf4* is conditionally deleted in heart and skeletal muscle, mice manifested weight loss and mitochondrial cardiomyopathy that led to a premature death (maximum lifespan 21 weeks) (Table I) (Cámara, 2011). The heart of the *Mterf4*$^{-/-}$ mice showed a drastic reduction of fully assembled OXPHOS complexes, bioenergetic defects and an activation of compensatory mitochondrial biogenesis. Furthermore, a massive increase in mitochondrial transcripts including several tRNAs was evident. Inactivation of *Mterf4* in murine heart revealed its involvement in the regulation of mitochondrial translation, instead of mitochondrial transcription as previously proposed (Cámara, 2011). In fact, despite the abundance of mitochondrial ribosomal proteins and 12S and 16S rRNA, the *in vitro* mitochondrial translation was severely impaired and mitoribosomes were not completely assembled due to the lack of the MTERF4/NSUN4 complex. MTERF4 forms a complex with the ribosomal RNA methyltransferase NSUN4 to control mitochondrial ribosomal biogenesis and mitochondrial translation (Cámara, 2011).

Unexpectedly and in contrast to *Mterf3 and Mterf4* KO mice, *Mterf1* KO animals were viable and had no OXPHOS defects (Terzioglu, 2013). Mitochondrial DNA replication, transcription and translation were not affected by the *Mterf1* ablation since mitochondrial rRNA, 12S rRNA, 16S rRNA, and various mRNAs levels were not altered. However, the *Mterf1* KO mice showed increased levels of rRNA antisense transcripts and decreased abundance of the light-strand promoter (LSP) proximal 7S transcript leading to the hypothesis that MTERF1 terminates the L-strand transcription at its binding site in $tRNA^{Leucine}$ and thereby prevents the elongation of the L-strand transcription complexes by interfering with transcription initiation at the LSP. Thus, in contrast to previous *in vitro* results that suggested that MTERF1 regulated heavy-strand transcription levels, loss of *Mterf1 in vivo* strongly suggests that this protein influences transcription initiation at the LSP (Terzioglu, 2013).

1.7. Mitochondrial DNA-directed RNA polymerase, *Polrmt*

POLRMT is a nuclear encoded RNA polymerase that is located in mitochondria and helps in the transcription of mitochondrial genes. It has been reported that a splice variant of POLRMT, spRNAP-IV, acts inside the nucleus by transcribing a subset of genes. To shed light on the function of POLRMT, a conditional KO mouse has been generated by targeting the deletion of exon 3 that disrupts the expression of both the mitochondrial and the splice variant (Kuhl, 2014). Ablation of the floxed *Polrmt* in skeletal muscle and heart using the creatine kinase Cre caused a drastic reduction of *de novo* synthesis of mitochondrial proteins. However, transcripts of nuclear genes that are targets of spRNAP-IV, did not show any differences between *Polrmt* KO mice and controls. Accordingly, no nuclear expression of spRNAP-IV or other isoforms was detected in several tissues demonstrating that *Polrmt* has exclusively a mitochondrial localization and function (Kuhl, 2014). Unfortunately, the phenotype of the *Polrmt* KO was not described in this brief communication.

1.8. Leucine-rich pentatricopeptide repeat-containing protein, *Lrpprc*

LRPPRC encodes for a mitochondrial protein involved in the translation and/or stability of the mtDNA-encoded transcripts (Harmel, 2013; Ruzzenente, 2012; Xu, 2004). LRPPRC belongs to the pentatricopeptide repeat (PPR) protein family, whose members are characterized by a repeated motif of 35 amino acids and implicated in RNA binding (Small and Peeters, 2000). Several independent studies demonstrated that LRPPRC is mainly localized into the mitochondrial matrix (Sasarman, 2010; Sterky, 2010; Xu, 2004), however it has also been reported that it is able to interact with the PGC-1α suggesting a possible nuclear function (Cooper, 2006). The founder recessive c.1119C>T mutation in the *LRPPRC* gene causes the French-Canadian variant of Leigh Syndrome (FCLS), a severe unusual form of infantile LS characterized by a profound complex IV defect in brain and liver (Debray, 2011; Merante, 1993; Mootha, 2003). Clinical presentations of this form include mild regression of psychomotor skills and fatal lactic acidosis leading to death between 3 and 10 years of age. The mechanism by which LRPPRC regulates the mtDNA expression is still controversial, however, it has been demonstrated that this protein forms a complex with the stem-loop interacting RNA binding protein (SLIRP), a protein involved in the maintenance of mtRNAs (Baughman, 2009).

The generation of KO mice has partially elucidated the protein function *in vivo*, providing multiple hints for the identification of the molecular mechanisms underlying the pathogenesis of FCLS. Constitutive ablation of *Lrpprc* in homozygosis induced a developmental delay in murine embryos starting from E8.5–10.5 and a reduced CIV activity that rapidly led to death before E12.5 (Ruzzenente, 2012; Xu, 2012). On the other hand, conditional KO of *Lrpprc* in heart and skeletal muscle induced a dramatic reduction in mice lifespan and a progressive mitochondrial cardiomyopathy with CIV deficiency and compensatory mitochondrial proliferation (Ruzzenente, 2012)(Table I). The selectivity of CIV deficiency found in both mutant mice and FCLS patients has been partially explained by the binding of LRPPRC to a specific segment of *Cox1* mRNA (Xu, 2012) and by a reduction on the translation of mtDNA-encoded CIV genes due to the destabilization of a RNA-dependent complex involved in the regulation of mitochondrial mRNAs stability (Ruzzenente, 2012; Xu, 2012).

Recent studies on the heart specific *Lrpprc* KO mouse revealed that the bioenergetic dysfunction observed was due to a defect on the assembly of the ATP synthase (CV) rather than to the CIV deficiency initially described. The assembly defect in CV led to altered mitochondrial *cristae* morphology, increased ROS production and increased mitochondrial membrane potential (Mourier, 2014).

1.9. Mitochondrial aspartyl-tRNA synthetase

The mitochondrial aspartyl-tRNA synthetase catalyzes the aminoacylation of its cognate tRNA. The aspartyl-tRNA synthetase is encoded by the *DARS2* gene and mutations on this gene are associated with leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) (Scheper, 2007). Patients with LBSL develop spasticity, progressive cerebellar ataxia, spinal cord dysfunction and cognitive deficit. Unexpectedly, fibroblasts and lymphoblast derived from LBSL patients did not uncover any OXPHOS defect (Scheper, 2007). It is possible that the mutant protein still retains residual activity and is able to sustain normal mitochondrial protein synthesis or perhaps this protein has other unknown functions or tissue specific effects. Ablation of *Dars2* in mouse caused embryonic lethality at E8.5 whereas heterozygous mice did not showed any phenotype even until 2 years of age, indicating that one copy of the gene is sufficient to maintain normal mitochondrial protein synthesis (Dogan, 2014). Conditional ablation of floxed *Dars2* in heart and skeletal muscle achieved by the creatine kinase Cre caused cardiomyopathy and premature death by 6–7 weeks of age (Table I). Both heart and muscle *Dars2* KO tissues showed impaired mitochondrial protein synthesis leading to OXPHOS deficiency. Interestingly, different adaptive responses to the genetic defect were observed in heart and muscle tissues independently of the OXPHOS defect. In heart, the ablation of *Dars2* increased mitochondrial biogenesis, UPR^{mt} (mitochondrial unfolded protein response), downregulation of autophagy and upregulation of the cytokine Fgf21. In contrast, muscle tissue seemed to have a higher capacity for perturbed protein homeostasis and did not elicit the compensatory mechanisms observed in heart (Dogan, 2014). Although the human disorder caused by mutations in DARS2 does not have a muscular or cardiac component in the pathology and progression of the disease, the heart and muscle conditional *Dars2* KO mouse is a good animal model to illustrate how different tissues react and adapt to the OXPHOS deficiency and to give insights into the pathological mechanisms of mitochondrial diseases.

1.10. Adenine nucleotide translocator isoform1 (Ant1)

The constant exchange between mitochondrial matrix generated ATP and cytosolic ADP, required for a correct energetic balance, is provided by the adenine nucleotide translocators (ANTs), a family of transmembrane proteins embedded in the inner mitochondrial membrane. Four different ANT isoforms are differentially expressed in human tissues. In particular, ANT1 is mainly expressed in high-energy demand tissues, such as heart, skeletal muscle and brain, and ANT3 is ubiquitously expressed (Li, 1989; Stepien, 1992). Mutations in *ANT1* have been found in patients affected by severe mitochondrial diseases associated with mtDNA instability, such as adPEO and autosomal recessive mitochondrial myopathy and cardiomyopathy (Korhonen, 2004; Napoli, 2001; Palmieri, 2005). The essential role played by *Ant1* in energy homeostasis has been demonstrated by its inactivation in a mouse

model (Graham, 1997). *Ant1*$^{-/-}$ mice developed a myopathy and hypertrophic cardiomyopathy at 8 months. Interestingly there were no neurological defects in retina and brain in these animals (Lee, 2009; Phillips, 2010) (Table II). Skeletal muscle and heart from *Ant1*$^{-/-}$ mice showed typical traits of mitochondrial myopathy, characterized by mitochondrial proliferation, RRFs, mosaicism of COX negative fibers and aberrant mitochondria. Similarly to many patients with severe mitochondrial diseases, these mice manifested metabolic alterations, such as accumulation of lactate, Krebs cycle intermediates and alanine in serum (Graham, 1997). The muscle and heart mitochondria of the *Ant1*$^{-/-}$ mouse also exhibited increased ROS production associated to the upregulation of cellular antioxidant machinery, suggesting chronic oxidative stress (Esposito, 1999). However, the most striking feature of *Ant1*$^{-/-}$ heart and muscle was the presence of mtDNA large-scale rearrangements, in particular the accumulation of multiple deletions in the mtDNA. Thus, the dNTP pool imbalance caused by *Ant1* dysfunction in combination with ROS-mediated DNA damage are likely to be responsible for the generation of deleted mtDNA species, which, in turn, could induce a severe decrease in the cellular respiratory capacity (Kaukonen, 2000; Spinazzola and Zeviani, 2005). Mutations in the *Ant1* gene cause PEO in humans, where the typical hallmark is ptosis, due to dysfunction of the extra ocular muscles (EOMs). However in the *Ant1*$^{-/-}$ mouse model no ocular motor defects or specific EOMs fatigability were observed. Interestingly, the levels of the isoform *Ant2* were increased in the EOMs of *Ant1*$^{-/-}$ mouse, suggesting that it may partially compensate for the loss of *Ant1* and be responsible for the lack of phenotype in this particular muscle (Yin, 2005). It remains to be clarified whether the lack of *Ant1* in the brain could be compensated by the overexpression of other isoforms and be the reason for the lack of neurological phenotype in the *Ant1*$^{-/-}$ model.

1.11. Thymidine Kinase 2 (Tk2)

The correct maintenance of mtDNA depends not only on the normal activity of the enzymes composing the mitochondrial replisome, but also on a balanced pool of dNTPs. Depending on the proliferative status of the cell, dNTPs may be either imported into the mitochondria from the cytosol, where *de novo* synthesis occurs, or synthesized from intermediates in the degradative pathway for nucleotides in the mitochondrial matrix (the salvage pathway). The first step for the salvage pathway synthesis of deoxypyrimidine nucleoside triphosphates is provided by the constitutively expressed mitochondrial thymidine kinase (TK2) (Wang and Eriksson, 2000; Wang, 1999b). TK2 activity is more relevant in post-mitotic tissues, whereas *de novo* synthesis and *Tk1*-mediated cytosolic salvage pathways are crucial in proliferating cells (Coppock and Pardee, 1987; Ferraro, 2005; Reichard, 1988). Mutations in *TK2* gene cause mitochondrial DNA depletion syndrome (MDS) and autosomic recessive Progressive External Ophthalmoplegia (arPEO) in humans (Götz, 2008; Mancuso, 2003; Oskoui, 2006; Saada, 2001; Tyynismaa, 2012; Wang, 2005). The clinical spectrum of *TK2* mutations is broad, ranging from severe fatal infantile myopathy with motor regression, spinal muscular atrophy, rigid spine syndrome to mild mitochondrial myopathy (Oskoui, 2006).

Two different *Tk2* deficient mice were developed (Akman, 2008; Zhou, 2008). One is a *Tk2* KO mouse generated by deleting exon 4 and part of exon 5, encoding for the substrate

binding domain of the enzyme active site (Zhou, 2008). The other is a knock-in mouse harboring the 378–379CG>AA mutation that induces the amino acid substitution H126N, corresponding to the human pathogenic mutation H121N (Table II) (Akman, 2008; Saada, 2001). Despite the genetic difference and the presence of some residual TK2 activity in the knock-in animals, these two *Tk2* deficient mice shared traits of isolated encephalopathy without major skeletal muscle alterations. Heterozygous mice were comparable to their littermate controls, whereas homozygous mice displayed a growth delay starting from P7–P10 and died prematurely. The most relevant symptoms were ataxic gait, coarse tremors, impaired motor coordination, abnormal limb clasping and generalized weakness (Akman, 2008; Bartesaghi, 2010; Zhou, 2008). Progressive mtDNA depletion was detected in multiple tissues and organs although to different degrees (Akman, 2008; Zhou, 2008). In brain, the most affected tissue, the mtDNA depletion was associated with an unbalanced pool of dNTPs and severe mitochondrial bioenergetic defect, characterized by loss of mtDNA-encoded OXPHOS subunits, CI and CIV deficiency and ATP reduction. *Tk2*$^{-/-}$ mice were also characterized by a severe and progressive hypothermia, due to the nearly complete absence of hypodermal fat layer (Zhou, 2008). Brown adipose tissue (BAT) from both mice presented several abnormalities, including morphological alterations, accumulation of lipid vesicles, mtDNA depletion and moderate bioenergetic dysfunction, whereas white adipose tissue (WAT) was only modestly affected. Adipokines and genes involved in the regulation of thermogenesis, such as *Ucp-1* and *Pgc-1α*, were down-regulated, suggesting an alteration in the endocrine properties of adipose tissues (Villarroya, 2011). In the liver of the *Tk2*$^{-/-}$ mice, mtDNA depletion induced mitochondrial proliferation, accumulation of fat vesicles and altered mitochondrial β-oxidation (Zhou, 2013). Increased levels of cholesterol, non-esterified fatty acids and long chain acylcarnitines were found in plasma of the *Tk2*$^{-/-}$ mice (Zhou, 2013). The main differences between patients and these animal models reside in the severe neurological symptoms and the lack of myopathic phenotype in mice. This can be partially explained by the fact that null mutations have never been found in patients, suggesting a possible incompatibility with human development (Bartesaghi, 2010). Moreover, since mouse brain mainly relies on *Tk2* activity for dNTP synthesis; it would be more severely affected by mtDNA depletion than any other tissue. However, a recent study identified a mild myopathic phenotype in *Tk2*$^{-/-}$ mice skeletal muscles and heart, most likely attributable to an altered pool of myogenic progenitor cells (Paredes, 2013), indicating that the *Tk2*$^{-/-}$ mice could represent a valuable tool for the study of MDS.

1.12. Thymidine Phosphorylase (Tp)

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive syndromic disease clinically defined by the occurrence of gastrointestinal dysmotility with intestinal pseudo-obstruction, peripheral neuropathy, ptosis, ophthalmoparesis and hearing loss (Lara, 2007; Nishino, 1999). Mutations in the *TYMP or TP* gene account for the vast majority of MNGIE cases and produce a severe defect in thymidine phosphorylase (TP) catalytic activity, causing a marked increase of thymidine and deoxyuridine in plasma and tissues of affected patients (Nishigaki, 2003; Nishino, 1999; Papadimitriou, 1998; Valentino, 2007). TP is a key enzyme of the nucleotide metabolism that in combination with uridine phosphorylase (UP) provides the reversible conversion of

pyrimidine deoxyriboside to pyrimidine base. Thus, TP regulates intracellular and plasma thymidine levels. In humans, these two enzymes do not share their substrates whereas mouse UP cleaves both thymidine and uridine (el Kouni, 1993).

Double knockout of $Tp^{-/-}Up^{-/-}$ mice were viable with similar growth rate and life span than the control animals although they showed hyperintense brain lesions in Magnetic Resonance Imaging and axonal swelling suggestive of a mitochondrial leukoencephalopathy (Table II) (Haraguchi, 2002). Ablation of *Tp* and *Up* genes produced increased levels of thymidine, uridine and deoxyuridine in plasma and in several tissues including brain, heart, kidney and skeletal muscle (López, 2009). The thymidine and uridine imbalance has been pointed responsible for the progressive mtDNA depletion and the mitochondrial defects found in the $Tp^{-/-}Up^{-/-}$ brain, partially resembling the molecular hallmark of MNGIE patients (López, 2009). Interestingly, the fact that muscle, liver and kidney did not show mtDNA depletion, having similar thymidine and uridine imbalance levels than brain raised the question of why is this phenotype is brain-specific (López, 2009). It is possible that either the short life span of mice when compared to humans do not allow for the accumulation of significant amounts of somatic mtDNA alterations in tissues other than brain or that post mitotic neurons are more dependent on the mitochondrial pyrimidine salvage pathway than mitotically active tissues.

1.13. Ribonucleotide reductase subunit 2B (Rrm2b)

Ribonucleotide reductase is a heterotetrameric enzyme composed of two large (R1) and two small (R2) subunits and is essential for dNTPs synthesis during the S phase of the cell cycle, providing the *de novo* conversion of ribonucleoside 5′-diphosphates into deoxyribonucleoside 5′-diphosphates (Parker, 1994). Bourdon and collaborators identified several mutations in the *RRM2B* gene in seven children affected by trunk hypotonia, lactic acidosis and renal tubulopathy, caused by severe mtDNA depletion (Bourdon, 2007). A growing number of *RRM2B* mutations have been found as causative of different mitochondrial disorders such as other cases of mtDNA depletion syndrome and renal tubolopathy (Acham-Roschitz, 2009; Bornstein, 2008; Kollberg, 2009), PEO (Fratter, 2011; Takata, 2011; Tyynismaa, 2009) and MNGIE (Shaibani, 2009), highlighting the importance of this gene in mtDNA maintenance. $Rrm2b^{-/-}$ mice displayed growth retardation and muscle atrophy and prematurely died of severe renal failure by 14 weeks (Bourdon, 2007; Kimura, 2003). Despite the lack of an overt OXPHOS deficiency, severe mtDNA depletion was found in skeletal muscle, kidney and liver, most likely caused by an alteration of the dNTP pool (Table II) (Bourdon, 2007; Kimura, 2003). The phenotype of the *Rrm2b* KO mouse resembled the one found in patients.

1.14. Mpv17

MPV17 gene encodes for a mitochondrial inner membrane protein involved mtDNA maintenance although its precise function remains unknown (Bottani, 2014; Spinazzola, 2006; Trott and Morano, 2004). Mutations in human *MPV17* gene cause a peculiar form of infantile hepatocerebral mtDNA depletion syndrome, characterized by severe hypoglycemic crises and progressive impairment of hepatic function that ultimately leads to liver cirrhosis (Spinazzola, 2006). A specific founder mutation in the *MPV17* gene (R50Q) is the cause of

the Navajo neurohepatopathy, an autosomal recessive syndrome prevalent in the Navajo population (Karadimas, 2006). Besides liver failure, the clinical presentation of this syndrome includes severe sensory and motor neuropathy, corneal ulcerations, leukoencephalopathy, and recurrent metabolic acidosis (Karadimas, 2006; Vu, 2001).

Knockout *Mpv17*$^{-/-}$ mouse developed a progressive kidney dysfunction leading to systemic hypertension at 2–3 months after birth (Table II) (Clozel, 1999; Weiher, 1990). Intriguingly, this renal phenotype apparently disappeared after few generations, and subsequent studies reported only a severe sensorineural hearing loss (Meyer zum Gottesberge and Felix, 2005; Meyer zum Gottesberge, 2012; Meyer zum Gottesberge, 1996). A reduction of mtDNA levels was found in multiple tissues, including skeletal muscle, kidney and brain. The most affected tissue was liver, in which the mtDNA depletion was associated with CI and CIV deficiency (Spinazzola, 2006). Interestingly, the *Mpv17* KO mice did not show an overt liver dysfunction but only subclinical mitochondrial hepatopathy and myopathy. Elevated AST, ALT, CK enzyme levels and slight lactic acidosis in blood and the presence of some COX negative fibers in skeletal muscle and hepatocytes were observed in KO mice. The administration of a ketogenic diet led to severe liver failure and cirrhosis in the *Mpv17*$^{-/-}$ mice (Bottani, 2014; Viscomi, 2009). Similar to what was found in other mouse models, the severe mtDNA depletion induced by *Mpv17* ablation triggered a functional compensation that involves an increase of mtDNA transcriptional events. This compensatory mechanism would account for decreased and unevenly distributed COX staining in the analyzed tissues (Viscomi, 2009 #228). Glomerular mtDNA depletion and increased mitochondrial ROS production were also detected in *Mpv17*$^{-/-}$ mice (Casalena, 2014; Viscomi, 2009).

2. Mouse models of proteins involved in mitochondrial dynamics

Mitochondria are dynamic organelles that are constantly undergoing fusion and fission events as part of their normal function to adapt to the cellular energetic demands and maintain the integrity of the mitochondrial network. In mammals, a series of proteins belonging to the dynamin related protein family are involved in mitochondrial fission and fusion (for review see (Hoppins, 2014)). Mitochondrial dynamics, when altered, can cause OXPHOS defects due to inability of the mitochondrial network to respond to the physiological requirements. Defects in mitochondrial dynamics are associated with neurodegenerative diseases. Mutations in *MFN2* and *OPA1* genes are responsible for type 2A Charcot-Marie-Tooth disease (CMT2A) and autosomal dominant optic atrophy (adOA) respectively (Alexander, 2000; Delettre, 2000; Züchner, 2004). Nonetheless, missense mutations in the GTPase domain of OPA1 have been associated with syndromic forms of adOA, characterized by the accumulation of mtDNA multiple deletions in skeletal muscle, unveiling a role of OPA1 in the correct maintenance of mtDNA (Amati-Bonneau, 2008; Hudson, 2008). It has been also reported a case of fatal infantile encephalopathy and lactic acidosis due to *DRP1* c.1184C>A mutation that causes a defective mitochondrial and peroxisomal fission (Waterham, 2007). Mouse models of proteins involved in mitochondrial network dynamics are summarized in Table III.

2.1. Mitofusins (Mfn1 and Mfn2)

Mitofusins are GTPases located in the outer mitochondrial membrane that are essential for mitochondrial fusion. Inactivation of both *Mfn1* and *Mfn2* in mouse resulted in embryonic lethality between E10.5–11.5, due to a severe developmental delay and placental defects (Chen, 2003; Chen, 2007). No overt phenotype was displayed when *Mfn1* or *Mfn2* were ablated specifically in skeletal muscle (Chen, 2010). However, double-KO mice were severely shrunken and died prematurely at 6–8 weeks of age. They displayed low blood glucose levels, reduced body temperature and lactic acidosis (Chen, 2010). The weight loss was mostly due to the presence of smaller skeletal muscles with subsarcolemmal accumulation of aberrant and fragmented mitochondria (Chen, 2010). Progressive mtDNA depletion, accumulation of point mutations and multiple deletions were found not only in young double KO mice, but also in aged $Mfn1^{-/-}/Mfn2^{+/-}$ animals (Chen, 2010; Vermulst, 2008). Interestingly, the lack of *Mfn1* is not compatible with the presence of an error-prone polymerase γ, since $Mfn1^{-/-}/Polg^{D257A/D257A}$ double mutant mice died *in utero* (Chen, 2010). These data support the idea of a major involvement of mitochondrial fusion machinery in the maintenance of mtDNA integrity and stability.

Complete *Mfn2* KO, produced by using Meox2-*Cre* that ubiquitously expresses Cre except in placenta, were born at expected Mendelian frequency, but 1/3 of them die at post-natal day P1 (P1). The *Mfn2* KO survivors showed severe defects in movement and balance, suggesting a major role of *Mfn2* in cerebellum development and function (Chen, 2007). The postnatal ablation of *Mfn2* specifically in cerebellum produced a fast and progressive reduction of cerebellar volume due to a specific degeneration of Purkinje cells (PC). When *Mfn2* was specifically disrupted in PCs after P7, mice displayed a pathological phenotype with an onset at 2 months of age characterized by shaky gait and poor rotarod performance due to progressive PC degeneration and axonal alterations (Chen, 2007).

The inactivation of *Mfn2* but not of *Mfn1* in cardiac muscle caused a hypertrophic cardiomyopathy with mitochondrial enlargement and increased left ventricle mass (Chen, 2012b; Papanicolaou, 2011). The lack of this phenotype in the heart specific *Mfn1* KO indicates that MFN1 and MFN2 have different functions, as there was not a compensatory overexpression of the non-targeted protein in either of the KO mice. Studies in Ca^{2+} handling revealed that MFN2, but not MFN1, plays an important role in the interaction between the mitochondria and the endoplasmic reticulum beside its role in mitochondrial fusion (Chen, 2012b).

The specific *Mfn2* disruption in kidney did not affect renal morphology, structure and tubular function, although a slight increase of apoptotic nephrons was observed (Gall, 2012). Primary cultures of *Mfn2* deficient proximal tubule epithelial cells displayed mitochondrial fragmentation and were more prone to apoptosis after ATP deprivation (Gall, 2012).

Liver-specific ablation of *Mfn2* led to glucose intolerance, enhanced hepatic gluconeogenesis and impaired response to insulin caused by an altered insulin-signaling pathway in both liver and muscle (Sebastian, 2012). OXPHOS deficiency, increased oxidative stress and endoplasmic reticulum stress were also identified in KO mice, indicating that *Mfn2* plays a role in coordinating insulin signaling and mitochondria/

endoplasmic reticulum crosstalk which appears to be essential for glucose homeostasis (Sebastian, 2012). Likewise, *Mfn2* specific deletion in skeletal muscle also resulted in alterations on the glucose homeostasis of this tissue (Sebastian, 2012).

The mouse models described above represent useful tools for a deeper understanding of the role that Mitofusins play in cell and tissue physiology but disappointingly, the *Mfn2* KO phenotype did not recapitulate the clinical features of CMT2A patients. To address this issue, two transgenic mice expressing mutant alleles of *Mfn2*, have been generated. The first one expressed the $Mfn2^{T105M}$ dominant mutant allele specifically in peripheral motor neurons (transgene expression driven by the HB9 promoter) (Detmer, 2008). Homozygous $Mfn2^{T105M}$ mice displayed a severe phenotype, mostly affecting hind limbs function. Interestingly, the forelimbs and the posterior musculature of the hind limbs were not affected, only the anterior musculature of the hind limbs became atrophic, causing muscle weakness similar to what was found in CMT2A patients (Detmer, 2008). As expected, sensory roots were normal, whereas motor neuron roots displayed a significant loss of axons (Detmer, 2008). The phenotype of the transgenic mouse presented incomplete penetrance, as is seen in CMT2A and other mitochondrial disorders.

The other transgenic *Mfn2* mouse, called MitoCharc, was obtained by expressing a human mutant $Mfn2^{R94Q}$ selectively in neurons (Cartoni, 2010). Two mutant strains have been characterized; one was heterozygous and expressed the mutant *Mfn2* mainly in dorsal root ganglia, whereas the other strain was homozygous and showed a preferential expression of the mutant allele in the spinal cord. Despite their different neurological phenotypes, these mice displayed gait defects and poor rotarod performance at 5 months of age. Homozygous animals presented a variable phenotype, with only some mice severely affected, suggesting a possible incomplete penetrance (Cartoni, 2010). Interestingly, no ataxic gait was found in these mice and their cerebellum development and layer organization were normal. Electron microscopy of motor neurons revealed an accumulation of enlarged mitochondria in small and medium myelinated axons. A combined CII plus CV deficiency accompanied by a severe decrease in ATP synthesis was detected in brain (Guillet, 2011). Overall, this age-dependent phenotype was compatible with a CMT2A late onset neuropathy (Cartoni, 2010).

2.2. Opa1

OPA1 is a GTPase required for mitochondrial inner membrane fusion. Mutations in *Opa1* produce autosomal dominant optic atrophy (adOA) in humans which is characterized by a progressive loss of vision and blindness with an onset in childhood (Carelli, 2004). To model the adOA phenotype, two different animal models of *Opa1* haploinsufficiency were generated. One mouse harbored the 1051C>T mutation which induced a Q285STOP in exon 8 ($Opa1^{Q285STOP}$); the other mouse harbored the 1065+5G>A which produced a mutant form of *Opa1* lacking exon 10 ($Opa1^{329–355del}$) (Alavi, 2007; Davies, 2007). These mice were generated after screening an N-ethyl-N-nitrosourea (ENU)-mutagenized murine DNA library for mutants harboring Opa1 mutations and displayed some peculiar symptoms of human DOA, such visual loss associated to optic nerve fibers demyelinization (for a review see (Williams, 2011)). In homozygosis, expression of both mutations in mice resulted in embryonic lethality with a block in development between E8.5 and E13.5. Heterozygous

mice for both mutant forms showed a reduction of about 50% of the levels of OPA1 in several tissues in agreement with gene dosage. Both transgenic mice developed visual loss and other neurological symptoms that progressively worsened at about 12 to 13 months of age (Alavi, 2007; Davies, 2007; Yu-Wai-Man, 2009). The major differences between these two Opa1 mutant mice were the severe loss of retinal ganglion cells (RGC) and thinning of the retinal nerve fiber layer that occurred in the *Opa1*$^{329–355del}$ but not in the *Opa1*^{Q285STOP} model in which retinal architecture and morphology were normal (Alavi, 2007; Davies, 2007). In addition, the *Opa1*$^{329–355del}$ showed gliosis, reduced number of large axons in the optic nerve, swollen or disorganized axons and the loss of myelin sheets, associated with activation of astroglia and microglia (Alavi, 2007; Nguyen, 2011). Conversely, the *Opa1*^{Q285STOP} mouse showed age-dependent axon demyelination, dendritic abnormalities and decreased glutamatergic synaptic density, suggesting that a functional impairment rather than a degeneration of RGCs was responsible for the vision loss (Williams, 2012).

In terms of extra-ocular phenotype, *Opa1*^{Q285STOP} mice presented subtle neuromuscular abnormalities, whereas *Opa1*$^{329–355del}$ mice showed a more severe syndrome with abnormal clutching reflex, resting tremor of the upper limbs and decreased rotarod performance (Alavi, 2009). However, skeletal muscle histology was normal and no mtDNA alterations (multiple deletions, depletion, proliferation) were found (Alavi, 2007; Davies, 2007). Abnormalities in the cardiac function were also described in the *Opa1*$^{329–355del}$ mice (Piquereau, 2012) and in heterozygous *Opa1*$^{+/-}$ mice (Chen, 2012a).

Another *Opa1* deficient mouse harboring an A>G transition in intron 19 that creates a cryptic splice acceptor site was described (Moore, 2010). This 6bp insertion is predicted to produce a mutant form of *Opa1* with the W616C amino acid substitution as well as the addition of amino acids leucine and arginine due to the translation of the intronic sequence (*Opa1*$^{W616C+insLR}$) (Moore, 2010). Homozygote animals died between E9.5 and E11.5. MEFs derived from homozygous embryos showed a fragmented mitochondrial network probably due to the cytosolic mislocalization of mutated OPA1 (Moore, 2010). Unfortunately, the phenotype of adult heterozygous animal carrying this mutation has not been reported.

Opa1 transgenic mouse carrying the recurrent *Opa1*^{delTTAG} micro-deletion, commonly found in adOA patients, was created. A multi-systemic disorder was displayed in heterozygous mice whereas homozygotes died in utero at E10.5 (Sarzi, 2012). In particular, these mice experienced visual failure, as documented by altered visual evoked potentials and loss of RGCs. The retinal phenotype was accompanied by deafness, encephalomyopathy, peripheral neuropathy, ataxia and cardiomyopathy. Additionally, premature axonal degeneration and demyelination were observed. These phenotypes were likely due to the OXPHOS defects, in particular CIV deficiency and supercomplex instability found in multiple tissues that may lead to increased levels of autophagy and mitophagy (Sarzi, 2012).

2.3. Drp1

Mitochondrial fission is regulated by a set of nuclear encoded proteins and it has been involved in the modulation of apoptosis, mitochondria quality control and embryogenesis (reviewed in (Sesaki, 2014). Mitochondrial fission is mainly controlled by Dynamin-related

protein 1 (Drp1), a cytosolic soluble protein which is recruited on the mitochondrial outer membrane through the interaction with the mitochondrial fission factor (Mff) and Fis1. Interestingly, a peculiar case of a newborn girl with a dominant-negative allele of *DRP1* has been described (Waterham, 2007). This patient died at 37 days of age and showed a complex neurological syndrome characterized by microcephaly, abnormal brain development, optic atrophy and severe lactic acidosis.

The complete loss of Drp1 in mice is embryonic lethal between E10.5 and E12.5, revealing the essential role of this protein during development (Ishihara, 2009). The mutant embryos were significantly smaller, showed defects in heart, liver and brain organogenesis and presented enlarged mitochondria, which were positive for COX staining. Because apoptotic cells were detected in the neuroepithelium of $Drp1^{-/-}$ embryos, a conditional KO for brain was created to investigate the role of Drp1 in neurons (Ishihara, 2009). Conditional KO mice were born at expected Mendelian frequency but died within a day. Analysis of the KO mice showed white matter hypoplasia in forebrain, brainstem and cerebellum. Moreover, apoptotic events and uneven mitochondria distribution in mature neurons indicated that Drp1-dependent mitochondrial fission is essential for brain development, in particular in the formation of a proper neuronal network and synapsis function (Ishihara, 2009). In contrast, heterozygous $Drp1^{+/-}$ mice were undistinguishable from controls in terms of lifespan, fertility, viability and brain development (Manczak, 2012).

The specific ablation of *Drp1* in heart resulted in embryonic lethality prompting the development of an inducible cardiac-specific conditional Drp1 KO mice, in which Drp1 expression was modulated by tamoxifen (Ikeda, 2014). After the tamoxifen treatment, Drp1 resulted downregulated and mice developed a progressive left ventricle hypertrophy with myocardial fibrosis and cardiac dysfunction, which led to premature death (13 weeks after tamoxifen injection). In *Drp1* defective heart, a block in mitophagy induced an increase in mitochondrial mass and mtDNA copy number, which was associated with the appearance of multiple OXPHOS defects and increased ROS production. Such mitochondrial and autophagy defects promoted apoptosis and necrosis in cardiomyocyte leading to the cardiac pathological phenotype. Interestingly, heterozygous KO mice did not show any overt phenotype under basal conditions but when fasting, the left ventricle function decreased more severely than in control mice. Heterozygous KO mice were more sensitive to ischemia/reperfusion injury, indicating that Drp1 and mitochondrial function are critical for cardiac response to stress (Ikeda, 2014).

3. Mouse models of proteins involved in mitochondrial protein quality control

Mitochondria count with a surveillance system to remove damage proteins, which is composed by several proteases that are located in different mitochondrial compartments. In the matrix, damaged/misfolded proteins are degraded by the ClpXP complex or by the Lon protease and proteins containing oxidized methionine can be repaired by the MsrA/MsrB system. In the mitochondrial inner membrane, the ATP-dependent metalloproteases belonging to the AAA^+ (ATPase associated with various cellular activities) superfamily, m-AAA and i-AAA regulate the degradation of most of the OXPHOS subunits and the

maturation of a ribosomal protein and the PARL protease regulates the degradation of OPA1. In the intermembrane space, the HtrA2 protease also regulates the degradation of OPA1. In the mitochondrial outer membrane, damaged proteins are degraded by the ubiquitin/26S -proteasome system. [reviewed in (Fischer, 2012)]. Although defects in the mitochondrial surveillance and quality control system are not considered *bona fide* mitochondrial diseases, they are associated with numeorus neurological disorders that are known to have impaired mitochondrial function [reviewed in (Martinelli and Rugarli, 2010; Rugarli and Langer, 2012)]. Therefore, we had included mice models with defects in the quality control machinary in this review and are summarized in Table IV.

3.1. Clpp protease

CLPP or caseinolytic peptidase P is a serine proteases found in the mitochondrial matrix. CLPP associates with CLPX, a chaperone with ATPase activity, to form an active ClpXP proteolytic complex. This complex has a barrel shape proteolytic chamber, formed by hexameric rings of CLPX binding to two heptameric rings of CLPP, where unfolded substrates are degraded to small peptides which are then further processed by other exopeptidases (Kim, 2001). Mutations in *CLPP* have been associated with Perrault syndrome's ovarian failure and sensorineural deafness (Jenkinson, 2013).

Disruption of the *Clpp* gene in mouse using the genetrap methodology caused growth delay, weight loss, decreased spontaneous activity, hearing deficit and decreased pre- and postnatal survival (Gispert, 2013). However, no overt mitochondrial dysfunction, oxidative stress or alterations in mitochondrial dynamics were observed in most tissues. Instead, increased levels of mtDNA were observed in tissues analyzed perhaps accounting for the lack of defective mitochondrial bioenergetics. Interestingly, the gonads were vulnerable to the ablation of *Clpp* and both male and female KO mice were infertile. In testis, spermatids and spermatozoa were missing in the seminiferous tubes. In ovaries, the folicullar layer was significantly reduced by increased cell loss (Gispert, 2013). The phenotype of the $Clpp^{-/-}$ mouse mimics the human hallmarks of ovarian failure and deafness in Perrault syndrome.

3.2. m-AAA protease

The m-AAA protease is oriented to the matrix side of the mitochondrial inner membrane and is involved in the degradation of damaged membrane proteins or non-assembled subunits of the respiratory chain. In mammals, the m-AAA protease is composed of paraplegin and AFG3L2, which can associate to form different isozymes: either a homo-oligomeric complex composed by AFG3L2 alone or a hetero-oligomeric complex containing paraplegin and AFG3L2.

3.2.1. Afg3l2

Mutations in *AFG3L2* have been associated with spinocerebelar ataxia type 28 (SCA28) and with spastic ataxia (SPAX5). SCA28 is a dominant form of spinocerebral ataxias whereas SPAX5 is a recessive early onset and rapid neurodegenerative form of spastic ataxia (Di Bella, 2010; Pierson, 2011). The first two mouse models with defects in the *Afg3l2* gene where characterized by Maltecca and collaborators (Maltecca, 2008). The first mouse model was the $Afg3l2^{par/par}$, in which the authors discovered that the spontaneous mutation

paralysé (*par*) that occurred in the C57BL/6 colony at the Pasteur Institute (Duchen, 1983) corresponded to a missense mutation in the *Afg3l2* gene. This missense mutation led to the amino acid change R389G in the highly conserved AAA^+ domain but did not affected the levels of expression of the protease (Maltecca, 2008). The second mouse model was the $Afg3l2^{Emv66/Emv66}$, that was created by the Emv66 provirus insertion within intron 14 of the *Afg3l2* gene (Maltecca, 2008). The viral insertion created a 4 base pair duplication that resulted in creation of a stop codon and the formation of a truncated protein. Neither the truncated nor the full length protease were detected in the $Afg3l2^{Emv66/Emv66}$ mouse. Interestingly, both $Afg3l2^{par/par}$ and $Afg3l2^{Emv66/Emv66}$ displayed the same phenotype characterized by a severe progressive paralysis starting at P7 with the hind limbs and by P12–P14 all limbs were completely immobile. Premature death occurred by P15. Both mouse models had impaired axonal development and delayed myelination, however, no neuronal loss was observed. Analysis of liver and muscle did not showed major abnormalities except for decreased fiber size, which was attributed to the axonal impairment. Decreased ATP levels and defects in OXPHOS were observed due to decreased assembly of complexes I and III in brain tissue of both $Afg3l2^{par/par}$ *and* $Afg3l2^{Emv66/Emv66}$ mice (Maltecca, 2008). Studies on mitochondrial protein synthesis rate *in organe*llo, using isolated brain mitochondria, revealed that in the $Afg3l2^{Emv66/Emv66}$ KO mouse the mitochondrial translation was severely impaired owing to defects in mitoribosome assembly (Almajan, 2012). Whether these protease is involved in mitoribosome assembly or stability remains to be determined.

Further analysis of cortical neurons in the $Afg3l2^{Emv66/Emv66}$ revealed the presence of hyperphosphorylated tau, which is associated with microtubule disorganization leading to the impaired axonal development found in this KO mouse (Kondadi, 2014). Heterozygous $Afg3l2^{+/Emv66}$ displayed a slow progressive decline in balance and motor coordination due to the degeneration of Purkinje cells containing dendritic hypertrophy and loss of cerebellar granular cells. Increased gliosis and oxidative stress were observed along with decreased ATP levels and defects in mitochondrial morphology and function. The mouse phenotype recapitulates pathology observed in SCA28 patients (Maltecca, 2009). The specific ablation of the floxed *Afg3l2* in PC cells using the L7-Cre transgenic mouse resulted in gait disturbances noticeable at 8 weeks of age. Analysis of 4 week old KO mice showed neuronal normal appearance, however there were signs of inflammation indicated by reactive microglia and astrocytes. As the $Afg3l2^{+/Emv66}$ KO mice aged, severe neurodegeneration of PC cells was observed and by 12 weeks of age all these neurons had disappeared. Mitochondrial morphology was altered and mitochondrial network appeared fragmented and about 10% of PC displayed severe COX deficiency (Maltecca, 2009).

Conditional deletion of Afg3l2 in neurons under the CaMKII promoter led to neuronal loss by 8 weeks of age in both cortex and hippocampus. No evidence of oxidative stress (protein carbonyls or increased SOD2 levels) was found in the 8 week neuron specific *Afg3l2* KO, however tau hyperphosphorylation was observed indicating increased ROS production that activates stress kinases PKA and ERK leading to tau phosphorylation (Kondadi, 2014). Unfortunately the full description of the phenotype of this neuron-specific *Afg3l2* KO mouse was not mentioned in the manuscript.

3.2.2. Paraplegin

Mutations in the *SPG7* gene, which encodes for paraplegin, have been associated with autosomal recessive form of hereditary spastic paraplegia (HSP) characterized by the retrograde degeneration of cortical motor neuron axons leading to a progressive weakness of the lower limbs (Harding, 1993). Paraplegin is located in the mitochondrial inner membrane, however, an alternative splicing form of *SPG7* creates paraplegin-2 which is located in the endoplasmic reticulum (Mancuso, 2012).

A mitochondrial specific paraplegin $Spg7^{-/-}$ mouse was created by the targeted deletion of exons 1 and 2 of *Spg7* by homologous recombination leading to the absence of the mRNA and paraplegin protein (Ferreirinha, 2004) The KO mouse appeared normal, however showed a progressive decrease in rotarod perfomance starting at 4 months of age. A late onset axonal swelling was observed at 1 year or age and axonal degeneration was evident 3 months later. The severity of the neuropatology in the $Spg7^{-/-}$ mouse was found from distal to proximal along the axons (retrograde degeneration). Mitochondrial morphological abnormalities were also observed along with the accumulation of neurofilaments and orgenelles in the axonal swollen regions suggesting an impairement of the retrograde axonal transport (Ferreirinha, 2004). This mouse model recapitulates nicely the axonal degeneration found in HSP patients.

Interestingly, the axonal degeneration of the $Spg7^{-/-}$ mouse was acelerated in the presence of the $Afg3l2^{/Emv66}$ allele (Martinelli, 2009). The $Spg7^{-/-}Afg3l2^{+/Emv66}$ mouse were less active and had imparied gait coordination particularly in the hind limbs at about 6 weeks of age. The phenotype worsened rapidly and by 9 weeks of age the $Spg7^{-/-}Afg3l2^{+/Emv66}$ mice had ataxic movements, kyphosis and severe weight loss eventually leading to premature death by 13−20 weeks of age. In addition to the axonal degeneration, there was also a cerebellar and hippocampal degeneration accompanied by gliosis. Mitochondrial abnormalities were also observed with OXPHOs defects and decreased mtDNA levels in the tissues affected (Martinelli, 2009).

3.3. Oma1

OMA1 is a zinc metalloprotease located in the inner mitochondrial membrane that was first identified in yeast as a protease with overlapping activities with the m-AAA proteases (Kaser, 2003) OMA1 cleaves OPA1 at S1 site leading to the loss of the long isoforms of OPA1 (L-OPA1) and resulting in the inactivation of OPA1 and cessation of inner membrane fusion (Ehses, 2009; Head, 2009).

Mice deficient in OMA1 ($Oma1^{-/-}$) developed normally, were fertile, life span was normal and no neuropathological abnormalities were observed. The KO miced did not have severe abnormalities except for increased body weight suggesting defects in metabolic homeostasis (Quiros, 2012). When challenged with a high fat diet, $Oma1^{-/-}$ animals showed diet-induced obesity. Significant alterations in lipid metabolism such as increased levels of triglycerides and hepatic steatosis was observed in the KO mice. Moreover, $Oma1^{-/-}$ mice exhibited impaired thermogenesis after cold stress. In contrast to control mice, $Oma1^{-/-}$ mice were unable to deplete lipid droplets from brown fat adipocytes after cold stress (Quiros, 2012).

Analysis of OPA1 processing revealed that it was clearly impaired in the *Oma1*$^{-/-}$ mice and this defect induced mitochondrial morphology alterations in MEFs, liver and brown adipose tissue upon stress. These results indicate that although OMA1 mediates the *in vivo* cleavage of OPA1, it is not essential for embryonic developmental and suggest an important and unexpected role of OMA1 in stress-induced OPA1 processing that is required to maintain metabolic homeostasis. Interestingly, OXPHOS function was not altered in *Oma1*$^{-/-}$ MEFs and tissues (Quiros, 2012). Because mitochondrial fragmentation has been associated with acute kidney injury, *Oma1*$^{-/-}$ mice has been used in an experimental model of acute kidney injury in order to assess whether OMA1-dependent OPA1 proteolysis plays a pathological role in *vivo* (Xiao, 2014). When mice were subjected to renal ischemia-reperfusion, several features observed in control animals after the injury, such as mitochondrial fragmentation, tubular damage and apoptosis were attenuated in the *Oma1*$^{-/-}$ mice. The OPA1 long isoform (L2) disappeared during renal ischemia-reperfusion in controls mice but was preserved in *Oma1*$^{-/-}$ mice. These results indicate that OMA1-mediated OPA1 proteolysis plays an important role in the disruption of mitochondrial dynamic in ischemic acute kidney injury (Xiao, 2014)

In addition to OMA1, the ATP dependent protease YME1L cleaves OPA1 at position S2, to balance mitochondrial fusion and fission (Griparic, 2007). *Yme1L* deficient MEFs showed fragmented mitochondria whereas double knockout for *Oma1* and *Yme1L* are able to maintain tubular mitochondria in the absence of the short OPA1 isoforms (Anand, 2014). These results indicate that OPA1 processing is dispensable for mitochondrial fusion and suggest a stimulatory role of small-OPA1 isoforms for mitochondrial fission. Unfortunatedly, no information of the phenotype of the *Yme1L* or the double KO mice was provided in the manuscript.

3.4. Prohibitins

Mitochondrial quality control involves also the prohibitin (PHB) complex, a ubiquitous protein complex located in the inner mitochondrial membrane, which is comprised by the two PHB family members, namely PHB1 and PHB2. Mammalian PHB1 has been identified as a potential tumor suppressor (McClung, 1989), although it has been subsequently found that such anti-proliferative activity was mediated by the 3′-UTR and not by the protein itself. PHB1 and PHB2 heterodimers form a ring-like structure within the mitochondrial inner membrane, which has been shown to regulate membrane protein degradation by the mitochondrial m-AAA protease (Steglich, 1999), to function as a chaperone (Nijtmans, 2000), and it is also involved in mtDNA maintenance (Kasashima, 2008) and mitochondrial dynamics (Merkwirth, 2008). Moreover, it has been recently demonstrated that PHB2 forms a complex with DNAJC19, which regulates mitochondrial *cristae* morphogenesis and cardiolipin acylation, similar to what occur in Tafazzin deficient models (Richter-Dennerlein, 2014). Interestingly, DNAJC19 and Tafazzin have been found mutated in two previously unlinked mitochondrial disorders, namely dilated cardiomyopathy with ataxia and Barth syndrome. Beside their role in controlling mitochondrial functions, prohibitins have been also linked to cell cycle progression, transcriptional regulation, apoptosis and cell signaling (Merkwirth and Langer, 2009).

Mouse models of PHB have been developed in the past years. Loss of prohibitin genes in *C. elegans* and mice results embryonic lethal, highlighting their essential functions during early phases of development (Artal-Sanz, 2003; He, 2008; Merkwirth, 2008).

The specific ablation of *Phb1* in liver induced a severe liver injury with signs of necrosis and inflammation starting at 3 weeks of age (Ko, 2010). Pre-neoplastic markers resulted highly expressed at 3 weeks and hepatic nodules were evident from 14 weeks and worsened to multifocal hepatocellular carcinoma, indicating that Phb exert a tumor suppressor function in hepatocytes. Ultra structural analysis of liver of the *Phb1* KO showed swollen mitochondria with aberrant morphology and lack of *cristae* organization (Ko, 2010).

Neuronal specific conditional KO of *Phb2* mice were born at expected Mendelian frequency and were indistinguishable from littermate controls until week 12 of age, when they started to show weight loss, cachexia and kyphosis (Merkwirth, 2012). Moreover, the *Phb2* KO mice displayed a progressive behavioral and cognitive impairment, due to a severe progressive neurodegeneration leading to premature death by 22 weeks of age. The neuron specific *Phb2* KO mice also displayed a late onset mitochondrial deficiency, characterized by altered mitochondrial morphology and Opa1 processing, mtDNA depletion and reduced oxygen consumption. Moreover, loss of Phb2 induced a massive activation MAP kinases leading to tau hyperphosphorylation and accumulation of aberrant filamentous structures in neurons (Merkwirth, 2012).

Prohibitins have been shown to be also essential for pancreatic function and survival, as conditional ablation of *Phb2* in β-cells induced alteration of insulin secretion and glucose homeostasis, loss of β-cells and severe diabetes (Supale, 2013). Similar to the other *Phb* defective models, mitochondrial fragmentation, aberrant OPA1 processing, mtDNA depletion and altered OXPHOS complexes activity were also evident in the β-cells specific KO mice (Supale, 2013).

To date, *PHBs* have never been found mutated in human pathologies, but these studies on mouse models clearly indicate their crucial function in cell proliferation, cell survival and energy metabolism, pointing these genes as possible candidates for diverse human diseases.

3.5. HtrA2/Omi

HTRA2, also known as OMI, is an ATP-independent serine protease that is located in the intermembrane space of the mitochondrion and is involved in the clearance of unfolded proteins. Mice with a defective enzymatic activity of the HtrA2, due to a missense mutation (mnd2 mice) or to the targeted deletion (HtrA2 KO mice) of the gene encoding for the HtrA2 protease, exhibited Parkinson's disease-like phenotype characterized by muscle wasting, neurodegeneration, involution of spleen and thymus and premature death by P30–P40 (Jones, 2003; Martins, 2004). The mnd2 mice were rescued by the transgenic expressing the human gene, *HTRA2*, specifically in the brain under the control of the neuron-specific enolase promoter (Kang, 2013) (Kang, 2007). The rescued mice did not show early lethality and no sign of neurodegeneration or any involvement of the spleen and thymus were found at 2 months of age. Interestingly, the rescued mice displayed peculiar signs of premature aging as osteoporosis, lordokyphosis, weight loss, hair loss, muscle

atrophy with and onset at about 21 weeks and death occurring at around 67 weeks. As the rescued mnd2 mice got older (60 weeks), it showed additional signs of aging as cardiomyopathy, splenomegaly, thymus atrophy and increased extramedullary hematopoiesis. Moreover, heart, skeletal muscle and duodenum contained several COX negative fibers and large number of mtDNA deletions (Kang, 2013). The accumulation of mtDNA deletions is considered to be a major contributor in premature aging (Kujoth, 2005). Autophagy was also increased in all tissues with the exception of the brain in 5 week old rescued mice, probably as a compensatory quality control mechanism. The reason why different tissues lead to such different phenotypes is not clear but shows a differential susceptibility to impaired mitochondrial quality control. Brain was the most vulnerable tissue to the absence of HrtA2 and the accumulation of damaged/unfolded proteins.

3.6. PARL

PARL (presenilin-ssociated rhomboid-like) is a rhomboid protease located in the mitochondrial inner membrane and appears to regulate the processing of OPA1. A *Parl*$^{-/-}$ mouse was created by the ubiquitous deletion of the floxed gene using the phosphoglycerate kinase promoter, PGK-Cre (Cipolat, 2006). The *Parl* KO mouse developed severe weight loss, cachexia, atrophy of thymus and spleen that resulted in premature death by 8–12 weeks of age. No abnormalities were found in the spinal motoneurons to explain the muscular degeneration however neuronal cell loss was observed int thalamus and striatum. Interestingly, ATP levels and OXPHOS function were not altered in muscle but increased apoptosis was observed in spleen and thymus (Cipolat, 2006).

4. Other models of OXPHOS deficiencies

4.1. Aif

The apoptosis-inducing factor (AIF) is a FAD-dependent mitochondrial protein that is not a subunit of complex I but it has been indirectly linked to its maintenance/assembly. *Aif* is drastically reduced in the *Harlequin* mice due to a mutation caused by a retroviral insertion (Klein, 2002) (Table V). The *Harlequin* mice displayed a dramatic reduction in CI activity and levels in cerebellum and retina (the most affected tissues), underscoring a possible involvement of this loss in the neurodegeneration observed in these mice (Vahsen, 2004). More direct evidence of the pathogenic role of AIF and its relationship with CI failure came by conditionally deleting the gene in the skeletal muscle and heart (Joza, 2005). The ablation of *Aif* led to progressive skeletal muscle atrophy and dilated cardiomyopathy around 9 weeks of age. CI activity was reduced about 80% although the biochemical defect was of late onset (5 months of age). Lactate levels were also increased in the heart, consistent with mitochondrial defects causing cardiomyopathies (Echaniz-Laguna, 2012; Wortmann, 2012). The *Aif* KO tissues displayed increased oxidative stress as confirmed by the increased amounts of lipid peroxidation markers.

Conditional deletion of *Aif* in the telencephalon resulted in embryonic lethality at E14 and mice showed reduced cortical thickness due to neuronal death. Morphological analysis of mitochondria, in cultured KO neurons, showed aberrant mitochondria with disorganized *cristae* that may explain the respiratory chain deficiency observed in *Aif* KO mice (Cheung,

2006). Recent observations have shown that a decreased OXPHOS capacity in muscle may lead to insulin resistance (Rabøl, 2006). Conditional ablation of *Aif* in muscle and liver provided an *in vivo* model to study this phenomenon. Authors found that *Aif* inactivation resulted in a down regulation of OXPHOS gene expression without major changes in mitochondrial morphology but leading to a combined respiratory chain complex deficiency (Pospisilik, 2007). Metabolic analysis in muscle and liver of KO mice showed increased glucose uptake, increased glycolysis and reduced lipid storage, even when they were fed with a high-fat diet. The same phenotype was observed in the systemic *Aif* KO mice.

Although *Aif* mouse models have offered the opportunity to study pathological consequences of CI defects *in vivo*, recent studies of patients carrying mutations in *Aif* did not revealed defects in CI (Ghezzi, 2010), questioning the link between *Aif* and CI deficiencies.

4.2. Mito-*Pst1*

A mouse model of mtDNA deletions/depletion was ingeniously created by producing mtDNA double strand breaks via the expression and target the restriction endonuclease *PstI* to the mitochondria (*mito-PstI*) (Table V). In the first study, *mito-PstI* was expressed in the skeletal muscle (Srivastava and Moraes, 2005). This transgenic mice developed COX deficiency and ragged red fibers due to a muscle specific mtDNA depletion and deletions (Srivastava and Moraes, 2005). Subsequently, an inducible *mito-PstI* transgene was engineered and expressed in the adult neurons using the tetracycline-regulated expression system (Fukui and Moraes, 2009). Animals were shortly induced for 3 days at 4 weeks of age and analyzed 10 weeks later. This transient expression of *mito-PstI* was shown to induce mtDNA deletions and depletions (Fukui and Moraes, 2009). Interestingly, mtDNA with large deletions accumulate faster than those with smaller deletions, implying a replicative advantage of smaller mtDNAs. Therefore, this study revealed that double strand breaks, DNA repair systems and replicative advantage are likely mechanisms underlying the generation of age-associated accumulation of mtDNA deletions in mammalian neurons. Long-term expression of *PstI* in the adult neurons led to an OXPHOS deficiency, mostly due to mtDNA depletion associated with a progressive neurodegenerative phenotype particularly affecting the striatum. (Pickrell, 2011a).

Expression *of mito-PstI* in dopaminergic neurons (DA) has provided a novel model to study the role of mitochondrial defects in the pathophysiology of Parkinson's disease. The *DA-mito-PstI* mice recapitulated most of the major features of PD and showed mtDNA depletion in the striatum (Pickrell, 2011b).

Transient systemic overexpression of *mito-Pst1* led to mtDNA depletion, muscle wasting and a decline in locomotor activity later in life (Wang, 2013). Mice showed a significant decline in muscle satellite cells suggesting that the systemic mitochondrial dysfunction plays an important role in age-related muscle wasting by preferentially affecting the myosatellite cell pool. Collectively, these studies indicate that the *mito-Pst1* mouse is a suitable model for the study of mitochondrial depletion syndromes.

4.3. Frataxin

FRATAXIN is a mitochondrial protein that is involved in the biosynthesis of the iron-sulfur clusters and is encoded by the *Fxn* gene (Martelli, 2007). Expansion of the GAA triplet in the first intron of the *Fnx* gene leads to transcriptional silencing and is the molecular cause of Friedreich's ataxia (FRDA), a syndrome presenting neurodegeneration, hypertrophyc cardiomyopathy and diabetes (Bayot, 2011). The three biochemical features found in patients are: (i) the presence of iron deposits in cardiomyocytes, (ii) decreased iron sulfur cluster-containing enzyme activities in heart homogenates (aconitase and respiratory chain complex I, II and CIII), and (iii) the presence of markers of oxidative damage in blood and urine (Perdomini, 2013). Several mouse models have been generated to study the pathophysiology of FRDA. Complete ablation of *Fxn* is not compatible with life in mouse (Cossee, 2000). Conditional knockout mouse models in heart (Puccio, 2001), neurons (Puccio, 2001; Simon, 2004), β-cells (Ristow, 2003) and liver (Martelli, 2012) reproduced most of the charateristic features of the human disease: hypertrophic cardiomyopathy, progressive spinocerebellar and sensory ataxia and progressive diabetes. However, the phenotypes in mice were more rapid and severe when compared to the FRDA patients, but have contributed to the understanding of the molecular mechanisms of disease. Interestingly, in contrast to what has been observed extensively in clinical studies, the mice did not show any oxidative damage. This difference could be explained because in mice the total deletion of *Fxn* happens in a certain tissue at a specific time, whereas FRDA is characterized by partial Frataxin deficiency in all cells throughout the lifetime.

A knock-in mouse carrying the GAA repeat expansion into the first intron of the mouse *Fxn* locus has been created to reproduce the genomic context of the disease (Miranda, 2002). Heterozygous knock-in, knock-out *Fxn* mice (KIKO) showed 25% residual frataxin compared to control animals, resemblesing the levels found in patients. However, no overt phenotype was detected but significant transcriptional changes were found in genes related to mitochondrial and amino acid metabolism, nucleic acid binding, signal transduction and stress response (Coppola, 2006).

Transgenic overexpression of the human *Fxn* containing GAA repeats in the first intron in mice reproduced a mild progressive phenotype, with locomotor defects and giant vacuoles in the large sensory neurons of the dorsal root ganglia (Al-Mahdawi, 2004; Al-Mahdawi, 2006). Contrary to the conditional KO models described above, slight signs of oxidative damage were reported in several tissues of this trangenic model (Al-Mahdawi, 2006).

4.4. Ethe1

ETHE1 encodes a ubiquitous mitochondrial sulfur dioxygenase (SDO) involved in the detoxification of hydrogen sulfide (H_2S) (Tiranti, 2009), which is produced by the catabolism of sulfurated amino acids in tissues and by anaerobic bacteria in the large intestine. In mammalian tissues, H_2S is a gasotransmitter acting as a signaling molecule, but at supraphysiological concentrations it is a potent inhibitor of COX (Dorman, 2002). Mutations in *ETHE1* gene in humans cause ethylmalonic encephalopathy (EE), a fatal autosomal recessive disorder characterized by early-onset encephalopathy, microangiopathy, chronic diarrhea, defective COX in muscle and brain, high concentration of C4 and C5

acylcarnitines in blood and high excretion of ethylmalonic acid in urine. Constitutive ablation of *Ethe1* gene in mouse caused growth arrest from P15, reduced motor activity, and early death, between 5 and 6 weeks of age (Tiranti, 2009). COX activity was severely impaired in brain and muscle in the *Ethe1*$^{-/-}$ mouse, in which very high levels of H_2S were observed. Thiosulfate levels in the plasma, a biomarker reflecting the accumulation of H_2S, were very elevated in *Ethe1*$^{-/-}$ mouse compared to controls, resembling the situation in patients. Also increased amounts of lactate and C4 and C5 acylcarnitines in blood, and increased levels of ethylmalonic acid in urine of were detected in the *Ethe1*$^{-/-}$ mouse. The phenotype of the *Ethe1*$^{-/-}$ mouse mimics all the hallmarks observed in the human ethylmalonic encephalopathy from the early onset and fatal course to the biochemical profile in blood and urine to the COX deficiency in brain and muscle tissue (Tiranti, 2009). *Ethe1* was conditionally deleted in muscle, brain and liver (Di Meo, 2011). Besides the COX defect specifically in brain and muscle, none of the three conditional mouse models showed the clinical and biological hallmarks of EE, at least during the first 3 months of life. Urine thiosulfate levels were similar between the 3 conditional mouse models and wild-type (Di Meo, 2011). This result indicated that the main pathogenic mechanism in the EE is the accumulation of the H_2S and it byproducts in the blood rather than the COX deficiency in the tissues.

Concluding remarks

Here we described those mouse models of different factors that affect the proper function of the electron transport chain. Interestingly, the majority of these models recapitulated human phenotypes associated with mitochondrial diseases and other disorders. Additionally, these mouse models represent great tools to study adaptive responses and compensatory mechanisms (mitochondrial biogenesis) to counteract the bioenergetics defect. Many of the knockout models have helped to understand the function of a particular gene. However, in many instances the creation of knock-in models may be more informative and relevant when trying to recapitulate the disease phenotype in animals. Nevertheless, all mouse models have had a great impact in our understanding of the pathological mechanisms and have advanced the field of mitochondrial diseases forward in relatively short time.

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Highlights

- Mitochondrial diseases can be caused by defects in factors that do not form part of the OXPHOS system but that can affect its function.
- Defects in proteins involved in mtDNA replication, transcription, translation and maintenance can cause mitochondrial diseases.
- Defects in proteins involved in mitochondrial dynamics and protein quality control also contribute to the development of defects in mitochondrial function.
- Mouse models of these factors have contributed to elucidate their function and the molecular bases of the disorders.

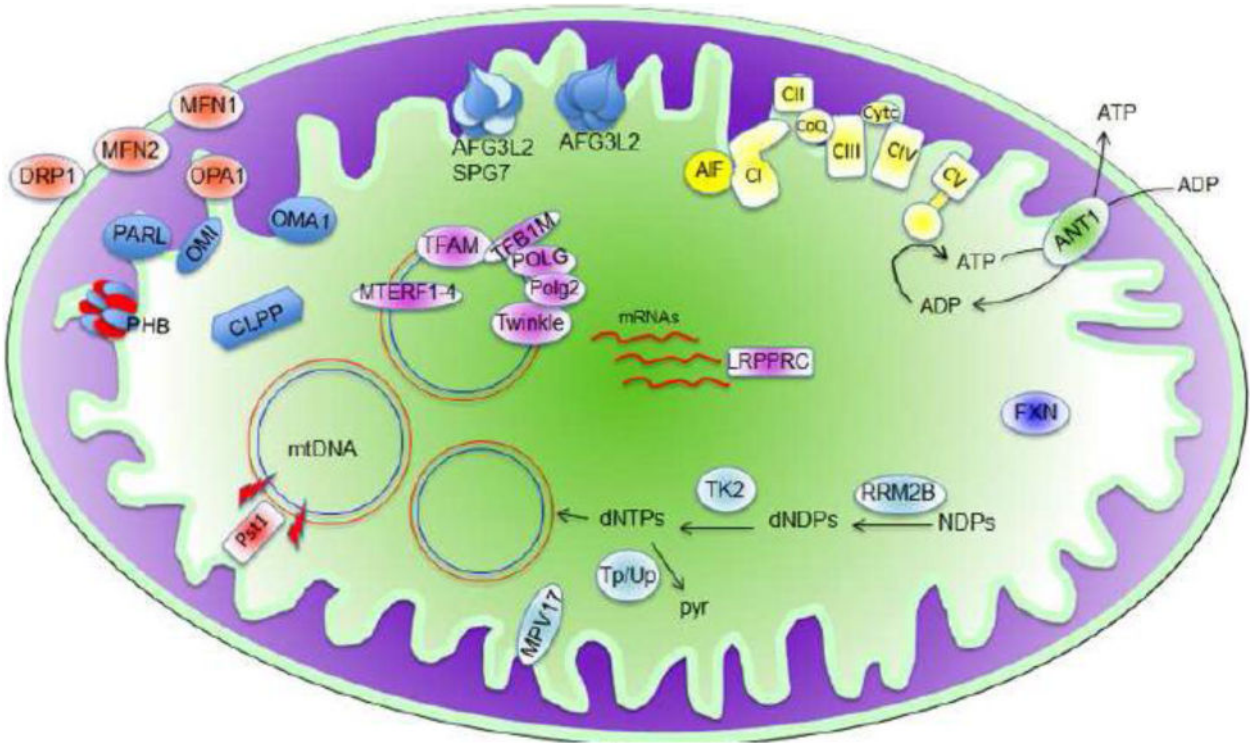

Figure 1. Mouse models of mitochondrial diseases

Mouse models that affect the oxidative phosphorylation system which is represented in light yellow in the mitochondrial inner membrane. Mouse models affecting mitochondrial dynamics are represented in light orange (MFN1, MFN2, OPA1 and DRP1). Mouse models of proteins involved in mtDNA replication, transcription and translation are represented in light pink (POLG, POLG2, TFAM, TFB1, TWINKLE, MTERFs and LRPPRC). Mouse models of proteins required for dNTPs synthesis, and mtDNA synthesis and stability are represented in light blue (RRM2B, TK2, TP, UP and MPV17). Mouse model for mtDNA deletion/depletion caused by double-strand brakes (red bolt) is represented in red (mito-Pst1). Mouse models of protein quality control are represented in blue (CLPP, PHB, PARL, OMI, OMA1, AFG3L2+SPG7, AFG3L2). Other mouse models (AIF1, ANT1 and FXN). NDPs: nucleotide diphosphates; dNDPs: deoxy-NDPs; dNTPs: deoxy nucleotide triphosphates; Pyr: pyrimidines; mtDNA: mitochondrial DNA.

Table I

Mouse models of proteins involved in mtDNA replication/transcription and translation

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Polg	mtDNA replication (catalytic subunit of Polγ)	Knockout: -Whole body (Homologous recombination)	Embryonic lethal (E7.5–8.5)	(Hance, 2005)
		Overexpression of human *POLG*^{Y955C} (associated with PEO)	Early death, cardiomegaly	(Lewis, 2007)
		Overexpression of *Polg*^{D181A} (lacking proof reading activity) in: -Heart -Pancreatic β-cells -Neurons	Cardiomegaly Early onset diabetes Neuronal dysfunction after 5m of age	(Zhang, 2000) (Bensch, 2009) (Kasahara, 2006) (Kong, 2011)
		Knock-in: -*Polg*^{D257A} (lacking proof reading activity)	Reduced lifespan and premature aging	(Kujoth, 2005; Trifunovic, 2004)
Polg2	mtDNA replication (accesory subunit of Polγ)	Knockout: -Whole body (Homologous recombination)	Embryonic lethal (E8.0–8.5)	(Humble, 2013)
Twinkle	mtDNA replication (Helicase)	Knockout: -Whole body (Homologous recombination)	Embryonic lethal	(Milenkovic, 2013)
		Conditional knockout: -Cardiac/ skeletal muscle (*Mck-Cre*)	Progressive cardiomyopathy and early death	(Milenkovic, 2013)
		Overexpression of *Twinkle*^{A360T} (Ubiquitous)	Late-onset myopathy	(Tyynismaa, 2005)
		Overexpression of *Twinkle* $^{dup353–365}$ (Ubiquitous)		
		Overexpression of *Twinkle*$^{dup353–365}$ in dopaminergic neurons (TH-promoter)	No overt phenotype. Dopaminergic neurodegeneration.	(Song, 2012)
Tfam	mtDNA maintenance and mtDNA transcription	Knockout: -Whole body (Homologous recombination)	Embryonic lethal (E8.5–10.5)	(Larsson, 1998)
		Conditional knockout: -Skeletal muscle (*Mlc-1f-Cre*)	Myopathy	(Wredenberg, 2002)
		-Cardiac muscle (*Mck-Cre*)	Cardiomyopathy. Early death (10–14 weeks)	(Freyer, 2010; Hansson, 2004; Wang, 1999a) (Li, 2000)
		-Pancreatic β-cells (*Rip-Cre*)	Diabetes	(Silva, 2000)
		-Forebrain neurons (*CaMKIIa-Cre*)	Late-onset neurodegeneration	(Dufour, 2008; Sorensen, 2001)
		-Dopaminergic neurons (*Dat-Cre*)	Progressive parkinsonism	(Ekstrand, 2007)
		-Adipose tissue (*aP2-Cre*)	Protection against obesity and insulin resistance	(Vernochet, 2012)
Tfam /Twinkle	mtDNA maintenance and replication	Overexpresion of: wt mouse *Twinkl*e and wt human *Tfam*	Partial OXPHOS deficiency heart and skeletal muscle	(Ylikallio, 2010)
Tfb1m	Mitochondrial transcription and translation	Knockout: Full body (β-actin)	Embryonic lethal (E8.5)	(Metodiev, 2009)
		Conditional knockout: -Heart and skeletal muscle (*Mck-Cre*)	Cardiomyopathy Mitochondrial protein synthesis defect No phenotype in skeletal muscle	(Metodiev, 2009)
		-Pancreatic β-cells (*Rip-Cre*)	Development of diabetes with progressive islet dysfunction	(Sharoyko, 2014)

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Mterf1	Transcription initiation at the light-strand promoter of the mtDNA	Knockout: -Whole body (Homologous recombination)	Viable, with normal oxidative phosphorylation capacity	(Terzioglu, 2013)
Mterf2	mtDNA Transcription (positive regulator)	Knockout: -Whole body (Homologous recombination)	Viable. Mild myopathy and memory defects. Decreased levels mitochondrial transcripts.	(Wenz, 2009)
Mterf3	Transcription terminator of the mtDNA	Knockout: -Whole body (Homologous recombination) -Heart and skeletal muscle (*Mck-Cre*)	Embryonic lethal (E8.5–E10.5) Severe cardiomyopathy and premature death	(Park, 2007) (Park, 2007) (Andersson, 2011)
Mterf4	Mitochondrial ribosomal biogenesis	Knockout: -Whole body (Homologous recombination) -Heart and skeletal muscle (*Mck-Cre*)	Embryonic lethal (Before E8.5) Severe cardiomyopathy and premature death	(Cámara, 2011) (Cámara, 2011)
Polrmt	Mitochondrial RNA polymerase	-Heart and skeletal muscle (*Mck-Cre*)	Not described	(Kuhl, 2014).
Lrpprc	Translation/stability of the mitochondrial mRNAs	Knockout: -Whole body (Gene trap)	Embryonic lethal (Before E12.5)	(Xu, 2012)
		Heart and skeletal muscle (*Mck-Cre*)	Cardiomyopathy and reduced life span	(Ruzzenente, 2012)
Dars2	Mitochondrial aspartyl-tRNA synthetase	Knockout: Whole body (Gene trap)	Embryonic lethal (E8.5)	(Dogan, 2014)
		Heart and skeletal muscle (*Mck-Cre*)	Cardiomyopathy and premature death by 6–7 weeks of age.	

Table II

Mouse models of proteins involved in the maintenance of mitochondrial dNTPs pool.

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Ant1	Adenine nucleotide translocase	Knockout: -Whole body (Homologous recombination)	Late-onset myopathy and hypertrophic cardiomyopathy	(Graham, 1997)
Tk2	Synthesis of deoxypyrimidine nucleoside triphosphates	Knockout: -Whole body (Homologous recombination) Knock-in: $Tk2^{378–379CG>AA}$ (pathogenic form)	Encephalopathy and premature death Encephalopathy and premature death	(Zhou, 2008) (Akman, 2008)
Tp/Up	Metabolism of nucleotides	Knockout: -Whole body (Homologous recombination)	Leuko-encephalopahty	(Haraguchi, 2002) (López, 2009)
Rrm2b	Synthesis of dNTPs	Knockout: -Whole body (Homologous recombination)	Premature death, muscle and renal atrophy	(Kimura, 2003)
Mpv17	Unknown function	Knockout: -Whole body (Homologous recombination)	Renal degeneration, hearing loss Mild hepatopathy and myopathy	(Clozel, 1999; Viscomi, 2009).

Table III

Mouse models of proteins involved in mitochondrial dynamics.

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Mnf1	Mitochondrial fusion	Knockout: -Whole body (Homologous recombination)	Embryonic lethal (E10.5–E11.5)	(Chen, 2003)
		Conditional Knockout: -Skeltal muscle (*Mlc*-Cre) -Heart (*Myh6*-Cre)	No overt phenotype No overt phenotype	(Chen, 2010) (Chen, 2012b)
Mnf2	Mitochondrial fusion	Knockout: -Whole body (Homologous recombination)	Embryonic lethal (E10.5–E11.5)	(Chen, 2003)
		Conditional Knockout: -Whole body except placenta (*Meox2*-Cre)	Cerebellum atrophy Lethal at P1-P17	(Chen, 2007)
		-Developing cerebellar primordia (*Wnt1*-Cre and *En1*-Cre)	Cerebellum atrophy Lethal at P1–P17	(Chen, 2007)
		- Adult cerebellum Purkinje cells (*L7*-Cre)	Motor impairment, Purkinje cells degeneration	(Chen, 2007)
		-Skeltal muscle (*Mlc*-Cre)	No overt phenotype	(Chen, 2010)
		-Heart (α-*Mhc*-Cre and *Myh6*-Cre)	Mild cardiac hypertrophy	(Papanicolaou, 2011) (Chen, 2012b)
		-Kidney (*Pax*-Cre)	Increased apoptotic nephrons	(Gall, 2012)
		-liver and skeletal muscle (*Alb*-Cre, *Mef2c*-Cre)	Altered insulin signaling in liver and muscle	(Sebastian, 2012)
		Expression of: -$Mfn2^{T105M}$ in motor neurons (associated with CMT2A)	Atrophy in the anterior muscles of the hind limbs	(Detmer, 2008)
		Expression of: -$Mfn2^{R94Q}$ in neurons (associated with CMT2A)	Locomotor impairment and gait defects	(Cartoni, 2010)
Mnf1/Mnf2	Mitochondrial fusion	Conditional Knockout: -Skeltal muscle (*Mlc*-Cre)	Muscle atrophy, premature dead (6–8 weeks)	(Chen, 2010)
Mnf1/Polg	Mitochondrial fusion/mtDNA replication	Knockout/knock-in -$Mnf1^{-/-}$- $Polg^{D257A}$ (Whole body)	Embryonic lethal	(Chen, 2010)
Opa1	Mitochondrial fusion	$Opa1^{Q285STOP}$ (ENU-mutagenesis) -Homozygous -Heterozygous $Opa1^{Q285STOP/}$	Embryonic lethal (E13.5) Visual loss Degeneration of the optic nerve, without loss of RGC	(Davies, 2007)
		$Opa1^{329–355del}$ (Deletion ENU- mutagenesis) -Homozygous -Heterozygous	Embryonic lethal (E8.5) Visual loss Degeneration of the optic nerve and loss of RGC. Tremor and abnormal clutching reflex	(Alavi, 2007) (Alavi, 2009)
		$Opa1^{W616C+insLR}$ (ENU-mutagenesis) -Homozygous	Embryonic lethal (E9.5–11.5)	(Moore, 2010)
		Knock-in: $Opa1^{delTTAG}$ -Homozygous -Heterozygous	Embryonic lethal (E10.5) Visual loss. Axonal and RGC degeneration. Deafness Cardiomyopathy	(Sarzi, 2012)
Drp1	Mitochondrial fission, mitophagy, apoptosis regulation	Whole body: exon 2 ablation	Embryonic lethal	(Ishihara, 2009)
		Conditional KO: – Brain (*Nestin*-Cre)	Premature death, brain development defects, white matter hypoplasia	(Ishihara, 2009)

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
		Tamoxifen-inducible conditional knock down: – Heart (*αMHC*-MerCreMer)	Premature death, left ventricular hypertrophy, cardiac dysfunction, increased sensitivity to ischemia/ reperfusion, OXPHOS defects, block in mitophagy	(Ikeda, 2014)

Table IV

Mouse models of proteins involved in mitochondrial quality control.

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Clpp	Mitochondrial matrix protease	Knockout: -Whole body (Homologous recombination)	Growth delay, weight loss, decreased spontaneous activity, hearing deficit and decreased pre- and postnatal survival. Infertility	(Gispert, 2013)
Agf3l2	m-AAA$_+$ protease, assembly of CI, CIII and CIV, mitochondrial protein synthesis, mitochondrial morphology and network maintenance	*Paralyze* Spontaneous mutation causing amino acid change R389G	Severe progressive paralysis with premature death by P15. Impaired axonal development and delayed myelination	(Maltecca, 2008)
		provirus Emv66 insertion		
		Conditional KO: -Purkinje cells (L7-Cre)	Gait disturbances, PC degeneration, mitochondrial fragmentation, impaired mitochondrial protein synthesis. OXPHOS defect (CI, CIII and CIV)	(Almajan, 2012)
		Conditional KO: -Forebrain (*CaMKIIa*-Cre)	Neurodegeneration, tau hyperphosphorylation	(Kondadi, 2014)
Spg7	Mitochondrial inner membrane metalloprotease	Knockout: -Whole body (Homologous recombination)	Axonal degeneration at 3 months and impaired rotarod performance at 4 months	(Ferreirinha, 2004).
		-Whole body (Homologous recombination) and *Afg3l2*$^{+/Emv66}$	Axonal degeneration, cerebellar and hippocampal degeneration accompanied by gliosis. Premature death by 13–20 weeks of age.	(Martinelli, 2009).
Oma1	Mitochondrial inner membrane protease involved in Opa1 processing	Knockout: -Whole body (Homologous recombination)	Viable. Diet-induced obesity and impaired thermogenesis. Increased tubular mitochondria in liver and brown adipose tissue.	(Quiros, 2012)
Phb1	Mitochondrial Chaperone, mtDNA maintenance, Opa1 processing	Knockout: -Whole body (gene-trap): exon 7 ablation	Embryonic lethal	(He, 2008)
		Conditional KO: -Liver (*Albumin*-Cre)	Liver injury, fibrosis, and hepatocellular carcinoma	(Ko, 2010)
Phb2	Mitochondrial Chaperone, mtDNA maintenance, Opa1 processing	Knockout: -Whole body (gene-replacement)	Embryonic lethal	(Merkwirth, 2008)
		Conditional KO: -Forebrain (*CaMKIIa*-Cre)	Progressive behavioral and cognitive impairment, neurodegeneration, mitochondrial deficiency	(Merkwirth, 2008)
		Conditional KO: -Pancreas β-cells (*Ins2-Cre*)	Diabetes, altered insulin secretion, glucose homeostasis, mitochondrial deficiency.	(Supale, 2013)
HtrA2/Omi	Mitochondrial intermembrane space Serine protease	Homozygous *mnd2* Spontaneous missense mutation Ser276Cys	Parkinson like phenotype: degeneration of striatal neurons, microglia activation and muscle wasting. Premature death by P30–P40	(Jones, 2003)
		Knockout: -Whole body (Homologous recombination) Knockout *HtrA2* mice expressing Human *HTRA2* in CNS (enolase-promoter)	Parkinson like phenotype: Neurodegeneration with loss of neurons in the striatum. Spleen and thymus involution. Premature death by P30. Rescue of the neurodeneration and premature death. Accelerated aging phenotypes, such as heart enlargement, and death by 12–17 months of age. Elevated levels of clonally expanded mtDNA deletions in tissues.	(Martins, 2004) (Kang, 2013)
Parl	Mitochondrial inner membrane protease involved in Opa1 processing	Knockout: -Whole body (PGK-Cre)	Severe weight loss, cachexia, atrophy of thymus and spleen. Premature death by 8–12 weeks of age. Neuronal death in thalamus and striatum.	(Cipolat, 2006).

Table V

Other mouse models of OXPHOS deficiency.

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Aif	Unknown function	Knockout: -Whole body (Retroviral insertion)	Neuronal degeneration, Reduction in CI activity	(Klein, 2002; Vahsen, 2004)
		Conditional Knockout: - Skeletal muscle and heart (*Mck*-Cre)	Skeletal muscle atrophy and dilated cardiomyopathy, oxidative stress, reduction in CI activity	(Joza, 2005)
		- Telencephalon (*Foxg1*-Cre)	Embrionic lethal (E14)	(Cheung, 2006)
		-Muscle (*Mck*-Cre)	Combined OXPHOS deficiency, insulin resistance	(Pospisilik, 2007)
		-Liver (*Alb*-Cre)	Combined OXPHOS deficiency, insulin resistance	(Pospisilik, 2007)
PstI	Mitochondria-targeted restriction endonuclease	Overexpression of *mito-PstI* -Skeletal muscle (*Actin-mito-PstI*)	mtDNA depletion and deletions, COX deficiency and RRG	(Srivastava and Moraes, 2005)
		Overexpression of *Pst1* -Forebrain neurons (Inducible *CaMKIIa-tTA*)	mtDNA depletion and deletions, limb 'clasping' neurological phenotype	(Fukui and Moraes, 2009)
		Overexpression of *Pst1* -Forebrain neurons (Inducible *CaMKIIa-tTA*)	mtDNA depletion and OXPHOS deficiency	(Pickrell, 2011a)
		Overexpression of *Pst1* -Dopaminergic neurons (DAT-*tTA*)	Neurodegeneration of the dopaminergic neurons, and striatal DA depletion.	(Pickrell, 2011b)
		Overexpression of *Pst1* -Whole body (*Rosa26-rtTA*)	Muscle wasting, decreased locomotor activity and mtDNA depletion	(Wang, 2013)
Fxn	Fe/S cluster biosynthesis	Knockout: -Whole body (Homologous recombination)	Embryonic lethal (E6.5)	(Cossee, 2000)
		Conditional Knockout: - Skeletal muscle and heart (*Mck*-Cre)	Cardiac hypertrophy Iron accumulation	(Puccio, 2001)
		- Neuron specific (*Nse*-Cre)	Progressive ataxia, cardiac hypertrophy	(Puccio, 2001)
		-Pancreatic β-cells (*Ins2-Cre*)	Diabetes and loss of β-cells mass	(Ristow, 2003)
		- DRG, spinal cord and cerebellum (*Prp*-Cre)	Cerebellar and sensory ataxia, loss of propioception	(Simon, 2004)
		-Liver (*Alb*-Cre)	Reduced survival, liver regeneration	(Martelli, 2012)
		Knock-in/Knockout: -$Fxn^{-/(GAA)_{230}}$ (KIKO)	No overt phenotype, mild heart fibrosis	(Miranda, 2002)
		Overexpression in knockout *Fxn* mice of: -Human *Fxn* carrying GAA repeats (YG8R and YG22R)	Mild progressive coordination phenotype, iron deposits. Vacuoles in large sensory neurons.	(Al-Mahdawi, 2004; Al-Mahdawi, 2006)

Gene	Mitochondrial function	Genetic Manipulation	Phenotype	References
Ethe1	Mitochondrial sulfur dioxygenase (SDO)	Knockout: -Whole body (Homologous recombination)	EE syndrome Growth arrest at postnatal day 15. Lethal at 5–6 weeks after birth. Reduced motor activity.	(Tiranti, 2009)
		Conditional Knockout: - Liver (*Alb*-Cre)	No overt phenotype	(Di Meo, 2011)
		- Muscle (*MyoD*-Cre)	No overt phenotype Isolated COX deficiency in muscle	
		-Brain (*Nestin*-Cre)	No overt phenotype Isolated COX deficiency in brain	

Abbreviations: adOA: Autosomal dominant optic atrophy; CMT2A: Charcot-Marie-Tooth type 2;CI: Complex I; DA: Dopamine; DRG: Dorsal root ganglia; EE: Ethylmalonic Encephalopathy FRDA: Friedreich ataxia; MCK: Muscle creatine kinase; NSE: Neuron specific enolase; OXPHOS: Oxidative phosphorylation system; PEO: Progressive External Ophthalmoplegia; RRF: Ragged red fibers; RGC: Retinal Ganglion Cells; Polγ: mtDNA polymerase