# CHAPTER 5

**Summary, conclusions and future perspectives**

# SUMMARY

The scope of my PhD thesis was to discovery new genes, mutations of which cause mitochondrial disorders, and to try to understand their role in pathological and physiological conditions.

In order to achieve these aims, I started my project studying families presenting with peculiar clinical phenotypes transmitted as autosomal recessive traits, and associated with OXPHOS defects. Thank to a collaboration with groups specialized in linkage analysis, it was possible to identify candidate loci in the affected subjects. Then I examined the disease loci using public databases (i.e. NCBI, UCSC and Ensembl), searching for information about sub-cellular localization, tissue distribution or association with known diseases of the proteins included in the region of interest. Moreover I used different bioinformatics tools able to predict the mitochondrial localization of given amino acid sequences (i.e. Mitoprot, TargetP, Wolf-Psort, Predotar, Mitopred, Maestro) (Calvo et al. 2006). With all these data, I created a list of candidate genes that were sequenced in probands and parents.

After identification of a change in a DNA sequence and in order to demonstrate its pathogenetic role, I followed a step-wise approach to demonstrate that:

1)       This change segregates within the family that means it is present in the homozygous state in the affected subject, in the heterozygous state in the parents, while all the other non-affected

members of the family are heterozygous or homozygous for the wt allele.

2)      It is absent in the general population or has a frequency less than 1% (otherwise it has to be considered a Single Nucleotide Polymorphism).

3)      It is a non-sense mutation or causes a frame-shift that alters the amino acid product or it is pathogenic in disease models (cultured cells, yeast systems…)

After the identification of the gene mutated in the screened families, the first characterization of the corresponding proteins was the evaluation of the sub-cellular distribution, in order to demonstrate the supposed mitochondrial localization.

I prepared constructs containing the full-length cDNAs of interest carrying on their C-terminus a HA tag, and inserted it into a eukaryotic-expression plasmid vector. The recombinant plasmids were transfected in COS7 cells for transient expression and in HeLa cells for stable expression. Then on these cells it was possible to perform immunofluorescence studies and experiments of Western blot analysis on different sub-cellular fractionations, using an anti-HA antibody.

To demonstrate active mitochondrial import, I performed an "in-vitro" assay on freshly isolated mitochondria that could show whether a given protein is imported into mitochondria through the $\Delta\Psi-$dependent import machinery and if the protein is cleaved, by mitochondrial processing peptidases that recognize an N-terminal presequence.

Another interesting point in the characterization of these proteins was the assessment of the tissue distribution through the evaluation of the level of their transcripts. This issue was investigated performing Northern blot or quantitative real-time PCR.

The last and more complicated task is to understand the pathogenetic role of these proteins. In fact these genes have been identified by reverse genetics in affected families. As a consequence often the alterations at the molecular or cellular levels due to mutations are clear and known, while the exact physiological function of the corresponding proteins is obscure and difficult to be assessed.

The presence of a specific mitochondrial alteration, for instance the defect in one complex of the MRC, as well as the identification of putative functional domains in the protein can help to formulate hypothesis about its role in normal conditions.

Moreover the creation of disease models, that can be cellular, yeast or animal models, allows to study in vivo the pathogenesis of these disorders.

Besides the identification of new disease genes, the main goal of my thesis, I was involved in the characterization of a protein (MR-1), mutations of which cause a movement disorder Parossistic Non-Kinesigenic Dyskinesia (PNKD). Two of the three alternatively spliced forms of MR-1 are located into mitochondria, and they are the isoforms containing the disease mutations; as a consequence PNKD could be considered a mitochondrial disorder.

The identification of new mitochondrial proteins is important to complete our knowledge on the human mitochondrial proteome. The gain insight on the functions of these new disease genes/proteins is a necessary step in order to better define the role of mitochondria in human diseases and to identify new possible specific therapeutic strategies.

**FASTKD2**

Analyzing a bedouin multi-consanguineous family with 2 affected siblings displaying cognitive and neurological regression, asymmetric brain atrophy, hemiplegia, and severe COX deficiency in muscle, I found a mutated gene, *FASTKD2*, on chromosome 2.

The identified nucleotide change segregated with the disease; it determines the creation of a TGA stop codon, hence predicting the synthesis of a prematurely truncated protein. Moreover using quantitative Real-Time PCR I detected a clear reduction (about 25% of control value, unpublished data) of FASTKD2 mRNA extracted from patient fibroblasts that could be due to an increase degradation of mutated species through mRNA decay mechanism.

FASTKD2 transcripts are present in all the analyzed tissues, with higher levels in brain and liver. The corresponding protein is localized in the mitochondrial inner compartment and it is not involved in cytochrome c oxidase assembly. This protein may have a role in apoptotic processes, in particular it could be a pro-apoptotic protein: indeed the protein has a putative BH3 domain, present in several proteins linked to apoptosis; over-expression of FASTKD2 causes apoptosis in transfected cells; staurosporine-treated mutant fibroblasts are morphologically different from controls and more resistant to induced apoptosis.

**SDHAF1**

After homozygosity mapping and candidate gene analysis, I identified a gene that was mutated in children with progressive

leukoencephalopathy associated with defective SCoQR (complex II), a key-enzyme of both TCA cycle and mitochondrial respiratory chain. Complex II holoenzyme in both patients muscle and fibroblasts was markedly reduced. The 115 amino acid gene product doesn't show any functional domain but belongs to the LYR-motif protein family. The LYR motif is common to several small subunits of complex I and is likely related to the binding of Fe-S catalytic centers

I found two different missense mutations segregating in the two pedigrees, absent in 800 healthy controls and 20 patients with defects of complex II and other clinical presentations. The mutant aminoacid residues are inside (R55P) or close to (G57R) LYR motif and both of them are conserved from humans to yeast, so that complementation assay in the latter organism was feasible for this gene.

Disruption of the ortholog gene, or expression of its variants corresponding to the human mutant ones, caused complex II deficiency and failure of OXPHOS-dependent growth in *Saccharomyces cerevisiae*. Complex II activity and amount were also restored in mutant fibroblasts, proportionally with the expression of recombinant wild-type gene. We termed the disease protein SDHAF1, for SDH Assembly Factor 1.

Using a tagged recombinant protein, I demonstrated that SDHAF1 is located in mitochondrial matrix. I then proved by *in-vitro* import assay that the SDHAF1 protein is translocated by the protonmotive-dependent transport system into the mitochondria without cleavage of the N-terminal mitochondrial targeting sequence.

My results demonstrate that (1) mutations in *SDHAF1* cause an isolated Complex II defect associated with a specific, progressive

leukoencephalopathic syndrome; (2) SDHAF1 is a mitochondrial matrix protein; (3) the SDHAF1 product is the first assembly factor specific to Complex II, since in both yeast and humans, its structural or functional disruption is associated with dramatic reduction of enzymatic activity and amount of CII.


## MR-1 (PNKD)

*MR-1* is a gene with three alternatively spliced forms: a long isoform (MR-1L), a medium isoform (MR-1M) and a small isoform (MR-1S).

Mutations in *MR-1* gene were discovered in kindreds with Paroxysmal Non Kinesigenic Dyskinesia (PNKD). In literature only 2 mutations (Ala to Val in positions 7 and 9) in *MR-1* gene were reported; these changes are in the N-terminal region common to isoforms MR-1L and MR-1S. In our paper we described a PNKD patient with a new mutation (A33P) localized in the same region.

Confocal immunofluorescence microscopy indicates that tagged MR-1L and MR-1S reside in mitochondria while MR-1M has a diffuse distribution throughout Endoplasmic Reticulum, Golgi apparatus and cell membrane. Active mitochondrial import was demonstrated in vitro for MR-1L and MR-1S.

I demonstrated that the mutations reported so far in PNKD patients, as well the new mutation reported by us, are in the mitochondrial targeting signal (MTS); however these changes seem to not affect the mitochondrial import. Noteworthy, after MTS cleavage, the mature form of patient PNKD protein is identical to mature wt product. Hence PNKD could be due to a novel mechanism based on a deleterious action of the MTS.

## DISCUSSION and CONCLUSIONS

The first report of a mitochondrial disease was done 50 years ago by Ernster et al. (Ernster et al. 1959) and for a long period these diagnosis have mainly been done by measuring enzymatic activities in muscle mitochondria. Then in 1988 the papers by Wallace et al. and Holt et al. showed the direct link between alterations of mtDNA and pathologic conditions. In the following years, the genetic basis of many mitochondrial disorders has been identified; because of the dual genetic control of OXPHOS system, the mutations can alter either mtDNA or nuclear DNA genes.

Even if mtDNA codes for essential subunits of MRC and ATP synthase, these are only 13, while the majority of OXPHOS components are nuclear encoded as well as many proteins required for import, assembly, protein synthesis and mtDNA maintenance. More than 1400 proteins are predicted to be located into mitochondria (Calvo et al. 2006) and hence to take part in one of the mitochondrial metabolic pathways. Each mutation affecting one of these proteins could be responsible for a mitochondrial disorder.

Taking also into account the different size of mitochondrial and nuclear genomes, it's obvious that the knowledge about mtDNA mutations is more detailed while the relationship between nuclear DNA and mitochondrial diseases need to be further investigated. A clear genetic cause is identified only in ≈50% of subjects with a diagnosis of mitochondrial disorders, the remaining being defined on the basis of morphological and/or biochemical defects. Most of the patients lacking a genetic diagnosis have probably mutations in

nuclear genes related to the OXPHOS system or to other crucial mitochondrial functions; for this reason it is important to search continuously for new genes responsible for these disorders.

## FASTKD2

Even if the mechanistic link between the gene and COX-deficiency is not demonstrated, the presence of a drastic mutation (a stop mutation predicting the synthesis of a truncated protein) in affected children is a strong evidence that *FASTKD2* is the causative gene for this disease. However, how it affects cytochrome c oxidase is not clear.

Because mutations in nuclear genes coding for assembly factors of COX, such as SURF-1, are known causes of complex IV deficiency, I verified if there was an aberrant assembly of COX in patients' samples and I demonstrated that this was not the case. Being FASTKD2 a putative kinase, for the presence of a Fas-activated Serine-Threonine Kinase Domain, it is possible to hypothesize that it is involved in the regulation, through phosphorylation, of subunits of complex IV. A recent paper by the group of Manfredi (Acin-Perez et al. 2009) demonstrated that phosphorylation of mitochondrial proteins, including specific subunits of cytochrome c oxidase, can be carried out by specific protein kinase (i.e. PKA) through a cyclic AMP signalling cascade contained within mitochondria, thus regulating OXPHOS activity. Interestingly this phosphorylation doesn't affect the steady-state protein level of COX (we observed the same phenomenon in FASTKD2 patient muscle) but probably modify the enzyme kinetics. This complex pathway has a role in the regulation of OXPHOS sensing nutritional availability through $CO_2$ generated by

TCA cycle; this results at the end in optimizing ATP generation and in minimizing ROS production. A defective regulation due to mutations affecting the kinase activity of a protein involved in this fined tuned process would have probably a damaging effect.

Otherwise the biochemical defect of COX could be a secondary effect due to other alterations of mitochondria. Furthermore, there is no direct evidence of COX-deficiency in brain; therefore, it is plausible that the encephalopathy could be due to other mitochondrial dysfunction rather than respiratory chain deficiency. In particular I showed that there was some evidence that the apoptotic process could be altered. However, different points still need to be elucidated.

The protein has a single known domain that is typical of FASTK, a protein that was demonstrated to have an anti-apoptotic function (Li et al. 2004). This protein is tethered to the outer mitochondrial membrane and released into cytosol after an apoptotic stimulus (especially fas ligand), were it can interact with other proteins (i.e. TIA-1) and exerts its protective effect. Instead, FASTKD2 has a different localization; it is a protein of the inner mitochondrial compartment (IMC). If it was involved in the same pathway of FASTK, there would be a specific step leading to the release of FASTKD2 from the IMC. A similar scenario is known in the case of another protein linked to this type of cell death, AIFM1; it is an IM protein that is cleaved in a specific position by a peptidase of the inter membrane space after an apoptotic stimulus and is then translocated to the cytosol and nucleus (Otera et al. 2005).

Another difference between FASTKD2 and FASTK is the role in favouring or preventing apoptosis. Whereas FASTK is known as a

survival protein, my data on FASTKD2 support the idea that it is a pro-apoptotic protein. In fact patient fibroblasts, lacking the full-length protein, are more resistant to induced apoptosis while over-expression of the protein led to cellular death.

However these opposite functions are frequent in proteins that share a BH3 domain; in addition to FASTK and FASTKD2, both anti-apoptotic (bcl-2, bcl-x) and pro-apoptotic (bax, bad, bak) proteins belong to this family.

The absence of a specific antibody to detect the endogenous level limited the evaluation of tissue distribution to the measurement of transcript levels in different mouse samples, which gave the higher value in the brain.

Unfortunately, patient cultured fibroblasts do not show COX-deficiency, therefore, I could not perform in vitro rescue of the biochemical phenotype with wild-type FASTKD2. Moreover we lost contact with the family and patient fibroblasts were inclined to senescence, possibly altering some of their features (for instance the response to apoptotic stimuli; Wang 1995, Seluanov et al. 2001).

Homologous genes exist only in mammals and birds, but not in lower eukaryotes, thus, models in "user-friendly" organisms is not possible. For these reason we asked to a specialized company to create a FASTKD2 knockout mouse model that we hope will give some answers to the many questions opened by my initial observations. I expect for this model to be available before the end of this year.

## SDHAF1

Mitochondrial complex II (cII) has a succinate dehydrogenase (SDH) activity, responsible for the oxidation of succinate to fumarate in TCA cycle and the possibility to transfer produced electrons to ubiquinone (Coenzyme Q) thus entering the respiratory chain.

cII deficiency are the cause of 8% (22/280) of OXPHOS biochemical defects system found in children with mitochondrial disease at Neurological Institute "Besta"; nevertheless no mutations in structural cII subunits were found in these 22 subjects. Moreover only four mutations (all in SDHA) have been reported till now in literature in patients with mitochondrial disorders (i.e. Leigh syndrome).

In two patients with leukoencephalopathy and severe, isolated defect of SDH-SCoQR in muscle and fibroblasts (as well in Turkish patients with the same phenotype) I found mutations in a new gene, now termed SDHAF1.

In my work, I have clearly demonstrated that SDHAF1 is a mitochondrial matrix protein, and is physically separated from SDH, which is a mitochondrial inner membrane protein. There are other arguments to conclude that SDHAF1 is not part of cII: the active form of cII crystallized from porcine heart mitochondria is composed of 4 subunits that do not comprise SDHAF1; in SDHAF1-lacking systems the SDH Km for succinate is the same as that found in wild-type cells. I think that these results are persuasive enough to conclude that SDHAF1 is an ancillary factor for the assembly/stabilization, but not a structural component of SDH.

While the structure of cII is well known, few information is available on how its four subunits are assembled into a functional holoenzyme.

The presence of a LYR signature in SDHAF1 suggests a role for this factor in assembly of FeS centres, present in SDHB subunit. However the mechanistic action of this new LYR protein in the assembly/stability of complex II, had to be addressed and it is necessary to evaluate if SDHAF1 acts on a specific subunit (namely SDHB) or has a more generalized effect.

The data I have been collected, do not demonstrate if SDHAF1 is involved in de novo assembly, stabilization, or both. A detailed assessment of the two potential role of SDHAF1 implies the availability of tools and technologies that require an ad hoc project, warranted for future work.

The discovery of new protein possibly involved in this process will help us to better understand it. After our publication on SDHAF1, another protein (SDH5) which takes place on cII assembly was reported (Hao et al. 2009). SDH5 is responsible of the flavination of SDHA subunit. Information from these (and eventually other yet to be discovered) cII assembly factors will help us to shed light on this issue.

Mutations affecting the structural subunits SDHB, SDHC and SDHD have been found in inherited neuro-endocrine tumours. A link between SDH and cancer has been hypothesized: accumulation of succinate in the cytosol is supposed to be an event leading to cellular reprogramming and switching from mitochondrial respiration to aerobic glycolysis.

Interestingly mutations in the recently discovered assembly factor SDH5 were found in patients with paragangliomas (Hao et al. 2009),

pointing out to the relationship between SDH and tumours. I have screened for SDHAF1 mutations some patients with PGL and reduced cII activity, but I haven't found anything.

The absence of SDHAF1 mutations in 20 individual with different clinical presentations, including Leigh syndrome, neonatal fatal lactic acidosis, or progressive myopathy, and in PGL subjects support the hypothesis that SDHAF1 mutations are specifically associated with severe complex II deficiency determining progressive, diffuse leuko-dystrophy. However it is necessary to extend the number of analysis to confirm this observation.


## MR-1 (PNKD)

Even if now the official name from HGNC (HUGO Gene Nomenclature Committee) for this gene is PNKD, I prefer to use the previous name MR-1 because it is easier to distinguish the three (long MR-1L, medium MR-1M and short MR-1S) isoforms and above all because only two of the three isoforms are associated to Paroxysmal Non-Kinesigenic Dyskinesia (PNKD).

All the known mutations responsible for PNKD, the two common A7V and A9V changes as well as the new mutation A33P reported in our paper, are in the N-terminal region common to isoforms MR-1L and MR-1S, but not MR-1M. I demonstrated that MR-1L and MR-1S are located into mitochondria, while MR-1M shows a diffuse localization.

My results differ from those reported by Lee et al. (2004), who attributed MR-1M to mitochondria, and MR1-S and MR-1L to the cytosol and plasma membrane. However, it should be noted that their

results were obtained only by IF detection using protein isoforms fused with the green fluorescent protein (GFP). GFP is a big protein of 27kDa that may give mislocalization (Lisembee et al. 2003). On the contrary I used tagged proteins, with a small HA tag of 9 amino acids and moreover my results were based on experiments of immunofluorescence, Western Blot on sub-cellular fractionations, and mitochondrial import. Noteworthy the last "in organello" assay was performed using a non-tagged protein.

However the most interesting aspect of our paper is the observation that the three mutations associated with PNKD are all contained within the mitochondrial target sequence (MTS) peptide. Obviously the first thing I analyzed was the import process; but I couldn't see any difference between wt and mutant proteins. After MTS cleavage, the mature proteins resulting from wt and mutant species are identical. As a consequence it is difficult to suppose an alteration of the catalytic activity of this protein to be the cause of PNKD.

All these data support the hypothesis that the mutant MTS could have a direct deleterious effect. This gain of toxic function is in agreement with the dominant inheritance of the disease while a defect in the enzymatic activity is usually associated with a recessive transmission.

The existence of this pathogenetic mechanism of disease is difficult to be demonstrated. It should be necessary to obtain an antibody directed against the MTS and to prove that this short peptide is retained inside the mitochondria, where it can produce a toxic effect. Other possibilities such as the use of N-terminal HA tagged proteins are advised against them because the tag could alter the recognition of the

MTS by the TOM/TIM system; the same is true for a plasmid expressing only the MTS with a C-terminal HA tag.

Another interesting suggestion comes from the tissue-distribution data; one mitochondrial isoform, namely MR-1L, is expressed only in the brain, while MR-1S is ubiquitous. Being PNKD a neurological disorder, it is possible that alterations in mitochondrial import or a toxic damage (especially of MR-1L) are selectively present in neurons, whereas cell types used for my experiment (HeLa and COS7 cells) are less sensitive to the detrimental effect of mutant MTS.

No other mutations in patients affected by PNKD have been so far identified in regions of the protein outside the MTS; this strongly suggests that mutant MTS could cause the disease. This is, to the best of my knowledge, a new pathogenic mechanism, and it is plausible that mutations in MTS of other mitochondrial proteins, irrespective for their function, could act in the same damaging way.

# FUTURE PERSPECTIVES

Mitochondrial diseases encompass a large fraction of human genetic disorders, mainly encephalo- and myopathies. Their prevalence is nowadays considered at least 1:5000 live births.

However their diagnosis is often difficult because of the heterogeneous clinical presentations of these disorders. Moreover, genetically specified disorders account for about 50% of all mitochondrial disease patients, the remaining 50% being defined as mitochondrial on the basis of morphological and/or biochemical alterations. Taking into account that the complete sequencing of mtDNA is feasible and today performed in many specialized laboratories, most of the genetically unclassified cases are likely due to mutations in nuclear genes related to the OXPHOS system rather than in mtDNA. For this reason there is a continuous need to search for new genes associated with mitochondrial phenotype.

This is important, first of all, for the sake of the patients, in order to obtain a molecular genetics diagnosis, which can be very useful for genetic counselling. Hence, the first translational consequence of my discoveries of new genes is the possibility to extend the mutational screening to larger cohort of patients, according to their clinical and biochemical phenotype. This will eventually allow assessing the frequency of mutations in these genes and their relevance in mitochondrial disorders.

However, the function of many newly discovered factors involved in mitochondrial dysfunctions is unknown and there is a general lack of understanding of many mechanisms leading to disease.

For these reasons my studies will go ahead with the hope to increase the knowledge about the functions of these newly identified mitochondrial proteins.

In order to reach my purpose, I will use animal models, which can help in further characterizing the physiopathology of the disorder and can possibly give suggestions about the protein function or the metabolic pathways mainly altered under disease conditions.

A mouse knockout model for FASTKD2 has been generated (it will be available in our lab before the end of the year) and will be investigated, in order to verify whether and to what extent it recapitulates the human encephalomyopathy caused by FASTKD2 mutations. If the biochemical COX defect will be reproduced in the KO mice, this model will be useful to assess the relationship between phosphorylation of mitochondrial proteins and modulation of OXPHOS activity (Hopper et al. 2006, Acin-Perez et al. 2009). Moreover the model will allow me to gain insight into the evaluation of the function of FASTKD2 in the apoptotic process that I have shown to be altered in patient fibroblasts.

These experiments are fundamental to identify the physiologic role of FASTKD2 and the candidate substrates for the predicted Kinase activity of FASTKD2. The investigation and experimental plan will proceed on the basis of these results.

Concerning the SDHAF1 project, we created yeast models either lacking the ortholog of SDHAF1 or carrying the ortholog gene with mutations corresponding to those found in patients. We will rely on this easy, "user-friendly" model to better characterize the influence of

SDHAF1 in the assembly of complex II, to eventually identify interaction of this protein with specific cII subunits or other factors, and to evaluate the stability of assembled complex. The results of these studies would allow us to learn some aspects concerning the assembly of complex II, a process with very few details already known.

Even if the demonstration of a direct link between mutations in MR-1 MTS and the pathogenesis of PNKD is not easy, I would like to clarify at least some aspects of the deleterious mechanism we have hypothesized. The evaluation of the mitochondrial import after transfection of recombinant HA-tagged MR-1 isoforms in neuronal cell lines could indicate me if there is an alteration of this process, selectively present in these cell types. Another experiment I would like to try, in order to show a toxic effect of the mutant MTS, is the cell transfection with a bicistonic vector, containing. two independent open reading frames (ORF): one corresponding to the MTS and one corresponding to a control protein such as the GFP. In this way it should be possible to distinguish transfected cells and to look at their features (for instance viability, mitochondrial membrane potential, ATP or ROS production...).

It's important to note that effective therapy is missing in mitochondrial disorders. This is due both to the lack of complete understanding of their pathogenesis and to the heterogeneity and complexity of these defects. There are some promising approaches but they are still in the first steps of evaluation (Koene & Smeitink. 2009).

The knowledge of the pathogenic mechanism is truly important to develop specific therapeutic strategies. For instance in the case of PNKD, taking into account the results of our study, an hypothetical future therapy (also gene therapy) should not be addressed to restore the enzymatic activity of mutant protein but, instead, it should act on scavenging the toxic mutant MTS.

Because of the central role of OXPHOS in metabolism, many OXPHOS defects are related to abnormal metabolic consequences. Hence one of the most often used therapies in mitochondrial diseases is a metabolic manipulation through supplementation of drugs, vitamins, coenzymes, free-radical scavengers, substrates or precursors for specific mitochondrial pathways.

For instance, riboflavin supplementation gave clinical improvements in few cases with complex I (Bernsen et al. 1993) or complex II deficiency (Pinard et al. 1999, Bugiani et al. 2006). The latter study was carried on in our Institute on three complex II-deficient children. After treatment two patients with leukoencephalopathy showed stabilization or an improvement of their neurological condition. Addition of riboflavin to growth medium of cultured fibroblasts increased complex II activity in patients. I found mutations in SDHAF1 in only one of these subjects. Riboflavin supplementation was repeated in cultured fibroblasts from her cousin and from the other child carrying the same mutation, reported in our study. While in the first sample we confirmed the beneficial effect of riboflavin, in the

latter there was no difference in enzymatic activity before and after treatment.

I would like to further investigate this aspect repeating the controversial "in vitro" assays and extending them to fibroblasts with the other mutation I identified and possibly other cII defective subjects with or without mutations in this gene. This could be of great interest in the case of a confirmation of a role for riboflavin in the treatment of patients with cII deficiency in general or according to their genetically defined status.

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# ABBREVIATIONS

ADP: Adenosine diphosphate

AIF: Apoptosis inducing factor

ATP: Adenosine triphosphate

COX: Cytochrome c oxidase

CSF: Cerebro-spinal fluid

DNA: Deoxyribonucleic acid

FAD: flavin adenine dinucleotide

$FADH_2$: reduced flavin adenine dinucleotide

FASTKD2: Fas activated serine-threonine kinase domain 2

IM: Inner membrane

IMS: Inter membrane space

LHON: Leber hereditary optic neuropathy

MR-1: Myofibrillogenesis regulator 1

MRC: Mitochondrial respiratory chain

mtDNA: Mitochondrial DNA

MTS: Mitochondrial targeting signal

$NAD^+$: oxidized nicotinamide adenine dinucleotide

NADH: reduced nicotinamide adenine dinucleotide

OM: Outer membrane

OMIM: Online Mendelian Inheritance in Man

OXPHOS: Oxydative Phosphorylation

PDHc: Pyruvate dehydrogenase complex

PNKD: Paroxysmal non kinesigenic Dyskinesia

ROS: Reactive oxygen species

RRF: Ragged red fibers

SDH: Succinate dehydrogenase

SDHAF1: SDH assembly factor 1

TCA: Tricarboxylic acid

TIM: Translocases of inner membrane

TOM: Translocases of outer membrane

## WEB RESOURCES

- ClustalW, http://www.ebi.ac.uk/Tools/clustalw/index.html
- Ensembl, http://www.ensembl.org
- GeneCards, http://www.genecards.org/index.shtml
- Mitoprot, http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html
- NCBI database, http://www.ncbi.nlm.nih.gov
- Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
- Predotar, http://urgi.versailles.inra.fr/predotar/predotar.html
- Psort II, http://psort.ims.u-tokyo.ac.jp/form2.html
- TargetP, http://www.cbs.dtu.dk/services/TargetP
- TMHMM, http://www.cbs.dtu.dk/services/TMHMM/
- TMpred, http://www.ch.embnet.org/software/TMPRED_form.html
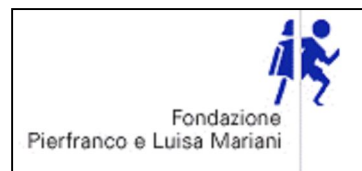- Wolf-PSORT, http://www.wolfpsort.org

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