mtDNA Defects in Degenerative Disorders

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Abstract

Major new advances in the genetic and biochemical characterization of mitochondrial myopathies are discussed, within a general presentation of this important new area of human pathology. Mitochondrial disorders can be due to mutations in either nuclear or mitochondrial genes involved in the synthesis of individual respiratory chain subunits. During the past few years mitochondrial syndrome have been associated with mitochondrial DNA (mt DNA) alterations (mutations) such as deletions, duplications, point mutations and depletions. The phenotypic expression of the mitochondrial DNA mutations depends on the interplay among the relative amount of mutated vs wild-type genomes, ie, the degree of mitochondrial heteroplasmy and its tissue and cell distribution, the reliance of the affected tissues on aerobic energy supply, the age and gender of the individual, and other still poorly understood factors including individual "nuclear genetic background" and environmental factors. The clinical presentations of mitochondrial syndromes vary quite widely: from 'pure' encephalopathies to 'pure' myopathies and syndromes involving only non-neurological systems (e.g. Pearson's pancreas-bone marrow syndrome, diabetes mellitus, cardiomyopathies). In addition a major theory regarding the mechanism of neuronal degeneration in several movement disorders is that mitochondrial defects may play a role: biochemical studies in Parkinson's disease, Huntington's disease, multiple system atrophy, and idiopathic dystonia have shown defects in enzymes of oxidative phosphorylation. As some mitochondrial pathologies offer the possibility of therapy, past clinical trials and future perspective in gene therapy will be discussed.

Key words: mitochondrial-DNA, oxidative phosphorylation, human disease, base substitution, rearrangements.

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Mitochondrial diseases are an heterogeneous group of pathologies in which, using morphological, genetical and biochemical criteria, a primary mitochondrial disfunction has been suspected or demonstrated. Clinically these are progressive pathologies in which the muscle can be involved alone (mitochondrial myopathies) or with other systems, brain especially (encefalomyopathies). Mitochondria are the only intracellular organelle that have proteins encoded by two genomes, nuclear (nDNA) and mitochondrial DNA (mtDNA).

Most of the mitochondrial proteins are encoded by the nuclear genome, and the mitochondrial DNA (a closed circular double-stranded molecule of 16,5 Kb) encodes only 13 polypeptides that are subunits of the mitochondrial energy-generating pathway; it encodes also 22 tRNA and 2 rRNA. An association between specific clinical fenotypes and some mt-DNA mutations has been demonstrated. On the contrary, nuclear DNA defects have been demonstrated only in few patients, making the understanding of the mitochondrial diseases caused by nDNA defects very difficult.

According to the recent mitochondrial molecular biology contributes, mitochondrial diseases have been divided in different types caused by (Tab 1):

1) mitochondrial DNA defects,
2) defects of the nuclear genes encoding proteins expressed in mitochondria,
3) nuclear gene defects that modify the expression of proteins regulating the communication between nucleus and mitochondria.

In the nuclear forms we distinguish:

a) pathologies in which a tissue-specific enzyme is involved, where the alterate expression of genes have a different tissue distribution (for example the fatal infan-

Table 1. Schematic representation of the etiology of mitochondrial diseases.

Mitochondrial diseases		
nuclear DNA	tissue-specific gene	Fatal infantile myopathy (COX deficiency)
	non tissue-specific gene	Leigh syndrome Pyruvate dehydrogenase complex (PDHC) deficiency COX deficiencies
	protein import gene	Methylmalonic acidemia Congenital myopathy
	nDNA: mtDNA communication	Multiple mtDNA deletions mtDNA depletion
mitochondrial DNA	point mutations of protein encoding mtDNA genes (mit-)	Leber's optic hereditary neuroretinopathy (LHON) Ataxia-retinitis pigmentosa-dementia complex
	point mutations of mtDNA-tRNA genes (syn-)	MERRF MELAS MERRF-MELAS MIMyCa
	large-scale rearrangements of mtDNA (ρ-)	Kearns-Sayre syndrome Chronic progressive external ophthalmoplegia (CPEO) Pearson's syndrome

tile cytochrome-C oxidase deficiency, due to a selective loss of COX subunit VII)

b) pathologies caused by a nuclear gene defect that has a multisystemic expression (Pyruvate-dehydrogenase deficiency and Leigh Syndrome)

c) gene defects modifying the intramitochondrial transport of proteins synthesized in the cytoplasm. The clinical entities showing an alteration of the communication machinery between nucleus and cytoplasm are worthy of a special consideration: we can consider in this group the autosomal dominant mitochondrial myopathy with multiple mtDNA deletions and the mtDNA depletion.

Among the mtDNA defects correlated with specific syndromes encephalomyophatic forms have been identified in association with the presence of ragged-red fibers in the muscle, and others without specific muscular alterations.

The first group includes myoclonus epilepsy with ragged-red fibers (MERRF), mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes (MELAS), Kearns-Sayre Syndrome and chronic progressive external ophtalmoplegia (CPEO).

The second group includes the Leber's hereditary optic neuropathy (LHON) and the entities showing ataxia, retinitis pigmentosa and dementia.

Among the mtDNA mutations we consider: large deletions (single or multiple), point mutations in mtDNA-tRNA genes and point mutations in enzymatic subunits of the mitochondrial respiratory chain. For the last two groups a maternal transmission has been demonstrated.

Genetics of mitocondrial diseases

Primary defects in mitochondrial function are involved in over 100 diseases. The field in dramatic expansion reflects the growth of knowledge in three different areas:

1) characterization of the mitochondrial structure and function.
2) elucidation of the steps involved in mitochondrial biosynthesis.
3) discovery of specific mitochondrial DNA and of its mutations and deletions.

Secondary defects of mitochondria (mtDNA mutations and impaired oxidation) include:

1) aging and age-related degenerative diseases (Parkinson, Alzheimer, Huntington and Down Syndrome)
2) amyotrophic lateral sclerosis
3) cardiomyopathies
4) atherosclerosis
5) Diabetes mellitus

We point out furthermore the relations between mitochondrial pathology and "malignant migraine" or multiple sclerosis.

The biochemical classification of the mitochondrial myopathies includes almost 120 forms, and the most important are:

- Kerns Sayre Syndrome (KSS)
- myoclonus epilepsy with ragged-red fibers (MERRF)
- Leber's hereditary optic neuropathy (LHON)
- mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes (MELAS)
- Leigh disease
- chronic progressive external ophthalmoplegia (CPEO)
- Alper's Syndrome

To better understand why some organs are more affected than others, we must remember that tissues with a high demand for ATP and oxidative turnover are preferentially affected in different combinations. In some syndromes, endocrine glands are involved with signs of diabetes, hypoparathyroidism and short stature. Common feature in this group of inborn metabolic errors is the involvement of specific enzymes in the pathway of aerobic energy production in the mitochondria.

The birth of Mitochondrial medicine: Luft disease [36]: the first patient found to have a mitochondrial disease was a 30year-old-woman who developed the first symptoms at the age of 7. Abnormal heat production, enormous compensatory perspiration, metabolism increase with extremely high caloric intake (> 3000 Kcal per day) at a stable body weight. General weakness, basal metabolic rate of + 180% (normal thyroid function). Biochemical studies showed a bad coupler mitochondrial respiration. Maximal rate of respiration in the presence of substrate alone without addition of ADP+ Pi, but almost normal phosphorylating efficiency in the presence of ADP and Pi. High ATPase activity, only slightly stimulated by 2,4-dinitrophenol; energy leak above the level of the phosphorylating system; high levels of cytochrome oxidase; high content of RNA in a muscle homogenate; morphologically, large accumulations of mitochondria and vast paracryrstalline inclusions were present.

The history of the mt DNA and of its mutations (1988) is characterized by these steps:

- Nass (1963) [45] clearly showed for the first time the presence of DNA in mitochondria
- Schatz (1964) [57] isolated DNA from purified yeast mitochondria
- Anderson (1981) [2] completed the sequence of human mtDNA
- Holt (1988) [29] described the association of different sporadic human encephalomyopathies with large deletions of mtDNA.
- Wallace (1988) [70] described a G to A transition mutation at nucleotide pair 11778 of the mtDNA in patients with LHON disease.
- Shoffner (1990) [60] described the point mutation in the tRNA lys gene of the mtDNA in MERRF Syndrome.
- Goto (1990) [25] described a point mutation of the tRNA leuUUR gene in MELAS.

A special feature of these tRNA mutations is that they have indistinguishable consequences at the biochemical level, producing partial defects in the mtDNA-dependent respiratory complexes.

- Moraes (1989) [42] described large-scale deletions ranging from 1,3 to 7,6 Kb in the sporadic adult-onset CPEO and in 100% of patients with KSS. In some instances, evolution from tissue specific to a multisystemic disorder could be observed, and probably can be explained by an increase in the mutated mtDNA fraction with age [9].
- Zeviani (1991) [74] described families with disturbances in interactions between nucleus and mitochondria (CPEO-like Syndrome). This disease has an autosomal dominant inheritance that implies mutation in an unknown nucleus encoded gene.
- Poulton (1992) [53] and Yuzaki (1989) [73] described families with a syndrome like adult CPEO, with multiple mtDNA deletions and with an autosomal recessive inheritance.
- Arnaudo (1990) [4] showed how in AIDS patients undergoing long-term treatment with azidothymidine (AZT) developed destructive mitochondrial myopathy with RRF and mtDNA depletion.

Mitochondrial genetics

The human mtDNA is a closed circular molecule of 16,569 nucleotide pairs, each mitocondria containing from two to 100 molecules and each cell containing from 100 to 1000 mitocondria; for this reason these are the most represented genes of the Human genome.

In the normal people this copies are identical, each of them contains genes encoding 13 proteins (subunits of the mitochondrial energy generating pathway), 22 transfer RNA and a small (12S) and a large (16S) ribosomal RNA (rRNA). Complex I is made by more than 40 polypeptides, 7 encoded from mtDNA (Fig 1);
Complex II involves 4 nuclear polypeptides,
Complex III includes 11 polypeptides, one from mtDNA,
Complex IV includes 13 polypeptides, 3 (COX I, COX II, COX III) of them from mtDNA.

An essential intruder

All animal and vegetable cells, besides the principal genetic system of the nucleus, contain a second genetic system localized in the mitochondria. These are the energetic machineries of the cell, specialized in energy production from respiration.

1,5 billion years ago, our ancient progenitors were not provided of aerobic metabolism and they acquired the capability of using oxygen by the inclusion of organisms able to do this.

During their long evolution ancient bacteria became more and more dependent on the host cell for all their functions, and this was due to the progressive transfer of most of their genes to the nucleus. The remaining DNA (mtDNA, mitochondrial DNA) is very small (3000 times smaller than the smallest human chromosome); each mitochondria contains many copies of it and each cell contain hundreds of mitochondria [62].

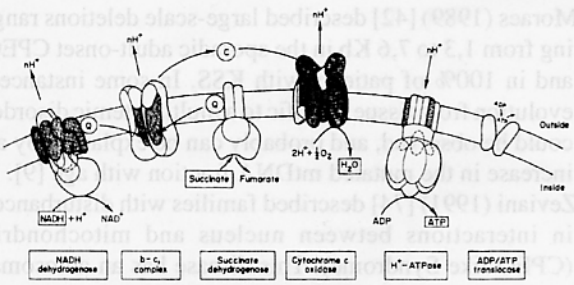

Figure 1. Mitochondrial Respiratory Chain map. The enzymatic subunits codified by the mitochondrial DNA

A great tendency to mutate

mtDNA is replicated and maintained in the supercoiled form by DNA polymerases, helicases, etc. codified by the nucleus.

Human mtDNA has a particular tendency to mutate, modifying the nucleotidic sequence (Fig. 2).

This tendency to mutate is 10 times higher than in the nuclear genes. Besides the mutations occurring as a result of replication mistakes, a great number of them are caused by chemical agents on DNA. We know that cellular DNA undergoes the oxidative damage caused by the derivatives of oxygen produced by aerobic metabolism. This kind of damage is 10 times more frequent in mtDNA than in nuclear DNA, because during normal aerobic respiration mitochondria use more than 90% of cellular oxygen, producing water and other "exhaust" products such as hydrogen peroxide, singlet oxygen, oxygen anion (free radicals) which can become mutagens if not promptly inactivated (Fig 3) [28]. There's also the damage produced by the alkylation (hydrocarbon's chains addition). DNA repairing systems are inefficient in mitochondria; moreover, mtDNA is not protected, as nuclear DNA, by histones or similar proteins.

Between two individuals selected at random, their mtDNA differ on the average for 50 of the 16560 base pair, that is to say for the 0,3% of the total (polymorphism) [62].

Germ-Line Cells Mutations

If the sequence alterations that frequently involve the mtDNA occur in germ-line cells, they can be transmitted to the offspring. This is an exclusively matrilinear transmission, in fact the oocyte, on its own, brings the mtDNA to the zygote during the fertilization [23]. For this reason each individual inherits his mtDNA exclusively from his mother, and so on. Hence most of the polymorphic mutations that exist in the general population today occurred long time ago and have long since segregated to homoplasmy. By contrast, severe deleterious mutations are rapidly eliminated by selection in the form of genetic disease, and the individuals harbouring such mutations are quickly brought to the attention of the clinician.

Therefore, mtDNA mutation that cause severe disease are generally new heteroplasmic mutations.

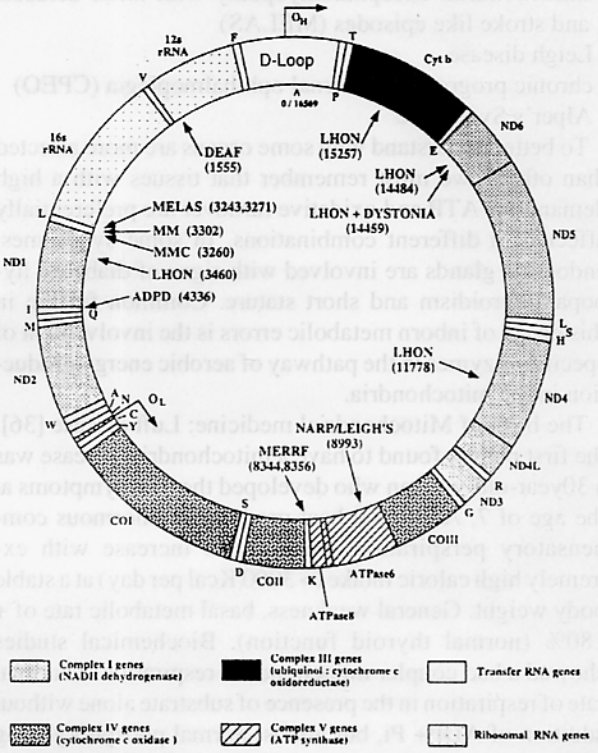

Figure 2. Human mitochondrial DNA showing locations of gene mutation. OH: Heavy Chain origin of replication; OL: Light Chain origin of replication; PH: Heavy chain Promoter; PL: Low Chain Promoter; F, T, P, E, L, S, H, R, G, K, S, D, Y, C, N, A, W, Q, I, M, L, V: t-RNA genes indicated by specific aminoacids; ADPD: Alzheimer disease and Parkinson disease; DEAF: deafness; LDYT: Leber Dystonia; LHON: Leber Hereditary Optic Neuropathy; MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke like episodes; MERRF: Myoclonic Epilepsy and Ragged Red Fibers; MM: Mitochondrial Myopathy; NARP: Neurogenic muscle weakness, Ataxia, Retinitis Pigmentosa. The capital letter following the number indicating mutation site represents the pathogenic nucleotide.

Somatic mtDNA mutations

The capacity for oxidative phosphorylation has been shown to decline with age in several postmitotic somatic tissues [68]. This decline is paralleled by the age-related accumulation of somatic mtDNA mutations, both as deletions and point mutations. Assuming that the physiological effects of somatic and inherited mutations are additive, the milder the inherited mitochondrial defect, the more somatic damage is necessary before symptoms appear (Fig 3). On the other hand, in individuals inheriting more severe

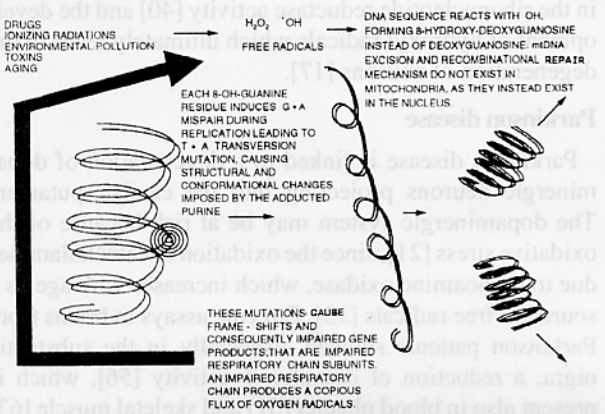

Figure 3. Free radicals theory. The higher steady state of oxidative damage in mtDNA than in nuclear DNA is due to a copious flux of oxygen radicals, inefficient repair and the nakedness of mtDNA. The oxidative damage can be accumulated even in short period of a toxic exposure or progressively during aging. Several point mutations found in mtDNA of patients with mitochondrial myopathy could be originated from the oxygen damage of mtDNA. Conformational changes in the DNA helix by the adducted purine would promote deletion of mtDNA which is common in degenerative neuro-muscular disease.

mitochondrial defects, less somatic damage is necessary for clinical symptoms to appear.

Therefore, in the case of the numerous mtDNA somatic mutations it is the quantitative difference and distribution of tissues which affects health, whereas in the case of germ cells it is the qualitative nature of the mutation which is important for the clinical phenotype (Fig 2).

Mildly deleterious mutations

These mutations generally cause diseases which appear in young adults. Most mutations causing Leber hereditary optic neuropathy (LHON) fall into this category [71]. The effects of LHON are acute or subacute vision loss with bilateral central scotoma and deterioration of the optic nerve; the onset is at the beginning of adult age. At least thirteen mutations, which alter the aminoacid structure, have been observed, distributed in various enzymatic subunits of the respiratory chain. These mutations fall into two categories: four of which are deemed to be primary mutations because they frequently cause blindness and they are enough, on their own, to cause the disease. Nine others are deemed to be secondary because they cannot, on their own, cause blindness, although they have often been observed in LHON patients and this suggests that they contribute to the pathology.

Severely deleterious mutations

Severe mtDNA mutations generally result in childhood diseases. The more they approach homoplasmy, the more they are lethal at an earlier age. The mutant offspring rapidly die out because of their reduced ability to adapt to the environment. Consequently, each family is a new heteroplasmic mutation. A ND6 mutation at the nucleotide 14459 converts a mildly conserved alanine to a valine. When the mutation approaches homoplasmy, it causes childhood dystonia associated with a degeneration of the basal ganglia. Individuals with a lower percentage of mutant mtDNA show LHON.

A couple of mutations causing a change in the aminoacid sequence occur in the ATPase 6 gene at the nucleotide 8993. These mutations convert a highly conserved leucine to an arginine or a proline [30]. Both mutations cause a broad range of clinical symptomatologies ranging from mild retinitis pigmentosa to mental retardation, macular degeneration, olivopontocerebellar atrophy and the lethal form of the Leigh syndrome (MILS) [59].

Disease threshold and tissue distribution

The cellular energetic threshold is that degree of inhibition of the respiratory complexes below which the overall efficiency of oxidative phosphorylation is impaired. For example, the respiratory capacity of heteroplasmic cells of the MERRF and MELAS mutations [12, 11] remain near normal values until around 90% of the mtDNA are mutant. Above the 90% level, oxidative phosphorylation drops rapidly, reaching near zero above a level of 95% mutant mtDNA.

Various organs and tissues rely on mitochondrial energy in different measures (in decreasing order: central nervous system, heart, skeletal muscle, kidneys, endocrine systems and liver). Therefore, a decreasing cellular capacity of energy production corresponds to an increasingly severe alteration in the functioning of the organs.

The aging process and the mitochondria

The capacity for oxidative phosphorylation declines with age because of the accumulation of defective mtDNA and defective nuclear DNA. With age, a 5-kb common deletion of mtDNA accumulates in the human brain and skeletal muscle. In the human heart, a mtDNA deletion (especially a 3.6 kb deletion) has been shown to accumulate after 35 years of age [14] and the number of COX negative skeletal fibers increase over the years [68].

The imbalance between oxidation and antioxidation of the aging heart tissue may lead to free radicals-mediated lipid peroxidation, including that of LDL (low density lipoprotein). Aldehyde-modified apolipoprotein B (product of the lipid hydroperoxidation) alters the receptor affinity and, consequently, accumulates [65, 24]. Such an accumulation can cause the appearance of atherosclerotic plaques [66].

Diabetes mellitus

Non-insulin-dependent diabetes mellitus (NIDDM) is an age-related disease which also shows features typical of a degenerative disorder. There is evidence showing that alterations of mtDNA occur in two of the tissues more involved in diabetes, pancreatic islets and skeletal muscle, both of which require a high metabolic and oxygen level. Some diabetogenic agents (interleukin 1, beta and gamma interferon, tumour necrosis factor alpha, alloxan and streptozotocin) produce breaks of mtDNA in the pancreatic islets, which are eventually followed by cellular death [31]. It is important to keep in mind that patients with KSS, CPEO and MELAS present an incidence of diabetes several times higher than the general population [49]. The earlier the onset of mitochondrial myopathy in these conditions, the more frequent is its association with insulin-dependent diabetes [20].

Recently, a systemic 10.4 kb mtDNA deletion was found in a family with maternally transmitted diabetes and sensor-neural deafness. Afterward, an A-to-G transition at nucleotide pair 3243, a conserved position in the mitochondrial gene for tRNA for UUR leucine, was found in families with diabetes [69].

Dilated cardiomyopathy

Deletions of the mtDNA in the heart muscle have been found in some patients [27]. Most of these cardiomyopathies are familial. Multiple deletions have been found in a family and this suggests the presence of a nuclear gene defect which may lead to this kind of deletions [67].

Central Nervous System (CNS)

The CNS obtains its energy almost exclusively from oxidative phosphorylation and, therefore, consumes a large amount of oxygen. Some imbalances between oxidation and antioxidation in the CNS tissues could cause a decrease in the efficiency of this system. This could decrease ATP availability to cellular functions in the central nervous system such as calcium and sodium/potassium pumps, ATP regulated potassium channels, exocytosis and various phosphorylation processes [15]. There is increasing evidence that glutamate may be a major mediator of oxidative stress in the CNS, mainly through the activation of ionotropic receptors, distinguished by the specific agonists. In fact, the activation of glutamate receptors due to these agonists in the tissue culture causes a neural degeneration [10]. The processes involve the receptor-mediated flow of sodium and calcium; such a flow leads to a series of occurrences which range from the starting of the arachidonic acid cascade and of proteases to the stimulation of nitric oxide synthase. The depolarization increases ATP consumption due to the sodium potassium ATPase and increases the oxidative phosphorylation with the terminal production of superoxide radicals. These radicals increase the release of glutamate and inhibit its inactivation, thus promoting noxious events [72]. The nitric oxide released in the above mentioned process interferes with numerous events such as oxidative phosphorylation with a decrease in the ribonucleotide reductase activity [40] and the development of hydroxyl radicals which ultimately leads to the degeneration of neurons [17].

Parkinson disease

Parkinson disease is linked to a degeneration of dopaminergic neurons projecting into the caudate-putamen. The dopaminergic system may be at risk because of the oxidative stress [21], since the oxidation of catecholamines due to monoamine oxidase, which increases with age, is a source of free radicals [13]. Enzyme assays in brains from Parkinson patients showed, especially in the substantia nigra, a reduction of complex 1 activity [56], which is present also in blood platelet [51] and skeletal muscle [63] mitochondria. Moreover, some mtDNA encoded subunits of complex 1 appeared to decrease in the nigrostriatal brain region of Parkinson patients [10].

Finally, trials show that Parkinson disease may be induced by a specific toxin of the substantia nigra, 1-methyl-4-phenil-1, 2, 3, 6-tetrahydropyridine (MPTP), which is a strong inhibitor of NADH-coenzyme Q reductase (complex 1) and generator of oxygen radicals.

Amyotrophic lateral sclerosis

ALS is associated with a progressive degeneration of the motor neurons in the brain stem and spinal cord. In about 10% of the patients ALS is inherited as autosomal dominant trait with a high penetrance after the sixth decade [43]. There are some studies suggesting that the oxidative stress and activation of glutamate-mediated ionic channels may be involved in the amyotrophic lateral sclerosis [10]. Eleven different mutations which alter the aminoacid sequence of the gene encoding one form of the superoxide dismutase (SOD 1) - this gene is responsible for the degradation of the toxic superoxide anion O2 - have been observed in families suffering from a autosomal dominant form of ALS [54]. These data tend to suggest that oxidative stress is a typical joint cause of ALS.

Alzheimer disease

Alzheimer disease is an age-related dementia, characterized pathologically by neurofibrillary tangles, senile plaques and amyloid deposits in the CNS. Mitochondrial functions in this disease are the following: oxidative phosporylation is not efficiently linked in homogenates of cortex taken from patients [64]; noticeable reduction has been observed in pyruvate dehydrogenase in frontal an occipital cortex [58] and in the complex 1 activity in blood platelet mitochondria [50] and some particular mutations of mtDNA have been found in brain sections [34].

The 4336G ADPD mutation (Alzheimer disease, Parkinson disease) was observed in 0.7% of the general population, but in patients suffering from late-onset AD and Parkinson disease its incidence is 5.2%. This alters the mildly conserved nucleotide at the junction between the aminoacid acceptor and TYC stem [37].

Additional harmful mutations are 3397 ADPD (methionine changed into valine in ND1 gene). The 4336G AD may be a risk factor for Alzheimer disease, but other factors such as nuclear gene mutations, environmental stress, additional inherited mtDNA mutations and the accumulation of somatic mtDNA mutations, may interact causing the brain tissues to go beyond the balance threshold.

Huntington disease

Is an autosomal inherited disorder, characterized by disturbances of the movement and progressive dementia; intrastriatal injection of a glutamate receptor agonist reproduced several aspects of the neuropathology of HD, indicating some disfunction in the disposition of excitatory amino acids. The levels of glutamate in cerebrospinal fluid were reported to be elevated in HD. A Complex IV defect in the caudate, but not in the cortex, was found in brains from patients with HD [6], and also a Complex I defect in blood platelet mitochondria [52].

These and other data favour the possible involvement in HD of oxidative stress, perhaps in combination with disfunction of glutamate metabolism.

Mitochondrial encephalomyopathies therapy

Phenotypical heterogeneity and relatively low incidence of the mitochondrial pathology are the objective handicaps to a rational evaluation of therapeutic efficacy of pharmacological approaches.

Concerning the first aspect, clinical heterogeneity is linked to the genetical base of the forms caused by mitochondrial DNA damage.

However, we must stress that syndromic groups defined in the past by molecular biology have been then confirmed, suggesting that a predictability and a reproducibility of some clinical phenotypes exist, knowing biochemical and genetical bases of these disorders.

Consequently, an exact identification of patients with the different forms (PEO, PEO plus, KSS, MERRF etc.) can allow the study of groups of patients with the same pathology.

Concerning the second aspect, there are not epidemiological studies which allow to find the real incidence of mitochondrial pathology, while the increasing knowledge in this field tend to extend the limits even beyond the specific neurological interest.

General measures

There are some general measures that guide the management of patients with a metabolic disfunction involving the capability of producing energy for the cell.

Avoidance of extremes of temperatures, excessive muscular exercises and glucose intolerance, maintenance of an adequate hydratation.

Avoidance of drugs inhibiting the function of the respiratory chain (phenytoin, barbiturates) and mitochondrial protein synthesis (Cloramphenycol, tetracyclines).

Steroids are potentially lethal drugs: two not diabetic patients with KSS had a mortal form of lactic acidosis after prednisone administration [16].

We have to point out how in patients with MELAS the administration of steroids resulted in a resolution of the clinical symptoms correlated with stroke-like episodes [26, 41].

About the next pharmacological approaches, most of them concern small groups of patients in open-studies or have an anecdotal character.

The main result is the control of lactacidemia, obtained with high doses of thiamine (900 mg/die) in three patients with KSS [35], with α-ketoglutarate in seven patients with PEO plus [55], with folic acid in a patient with KSS [1] and with dichloroacetate in a patient with MELAS [18].

The use of L-5-hydroxytryptophan and carbidopa resolved myoclonus and a central neurogenic hypoventilation in a patient with ataxia and progressive myoclonus [22], such an improvement in muscular weakness has been obtained using high doses of menadione and ascorbate in a patient with Complex III deficiency [3].

Coenzyme Q10 therapy

Coenzyme Q10 is first of all the moving carrier of electrons between flavoproteins (Complex I and II) and cytochromes (Complex III) ; it acts also to stabilize the mitochondrial membrane and to protect from the damage of the free-radicals [32, 33].

The first indication of a therapeutic effect of the CoQ10 regard a systemic mitochondrial form with myoglobinuria due to a primitive deficit of CoQ10 [48]. This pathology is often difficult to detect, because its diagnosis is based (besides the classic morphological and biochemical criteria) on analytic measurement of the CoQ10 concentration in affected tissues. CoQ10 is the first treatment for this pathology.

The same Author reported in two different studies [47, 46], an improvement in pyruvate and lactate serum levels, in instrumental parameters (defects of the atrio-ventricular conduction, alteration of the central sensitive conduction, measured with sensitive evoked potentials) and a clinical improvement (weakness and cerebellar ataxia) in seven patients with KSS after a treatment with CoQ10 for 3-14 months at the dosage of 120-150 mg/die.

The same conclusions were reported in a study on three patients with KSS and on four with PEO in which a 12 months therapy with 120 mg/die of CoQ10 induced a decrease of lactate levels basal and after muscular exercise, an improvement of muscular strength in three cases and of the ataxia in one [7].

These results yielded to a multicenter trial completed by 44 patients with mitochondrial encephalomyopathy using doses of 2 mg/kg/die and made of a 6 months "open" part to select the therapy responders and of a three months" blind" part [8].

In the whole population of studied patients, lactic acidosis, global and segmental muscle strength and some

platelet mitochondrial respiratory chain enzymes improved in a statistically significative manner.

To confirm this conclusion, CoQ10 was shown to have a protective effect against 2,4 dinitrophenol, an uncoupler of the oxidative phosphorilation, in myogenic tissue cultures of patients with PEO [39]. Desnuelle [19] and Shoffner [61] et al. described dramatic improvement in other patients with KSS treated with CoQ10. In both cases the treatment with CoQ 10 alone or in addition with succinate was determinant in the resolution of acute respiratory insufficiency.

Zierz et al. [75] confirmed the utility of prolonged therapy with CoQ10 in a patient with a severe muscular deficit of CoQ10, while pointed out the absence of significative improvements in KSS patients with normal muscular levels.

The attempt to document a change of the oxidative phosphorilation parameters using in vivo research methods, such as Spectroscopic Magnetic Resonance for 31P, obtained apparently controversial results. After a prolonged treatment, a patient with mitochondrial encephalomyopathy and cytocrome C oxidase deficiency showed an improvement in lactate and piruvate serum levels in basal conditions and after aerobic work, to which corresponds a decrease of the recovery time of the ratio between phosphocreatine and inorganic phosphate, which is an index of the in vivo tissue respiratory functionality [44].

This data was confirmed in two other patients treated for ten months in a trial in which, moreover, an increase in oxygen consumption during exercise was pointed out [5].

On the contrary, an heterogeneous group of 16 patients (6 of them more than 60 years old) treated for two months with CoQ10 variably associated with menadione, ascorbate, riboflavin and niacine, was not able to prove significative changes of lactate serum levels and of the spectroscopic parameters, while in some patients with diabetes there was a decrease of insulin use. In this study the shortness of the cycles of treatment may justify the negative results [38].

The whole acquired data indicate that the CoQ10 is a drug with therapeutic efficacy in mitochondrial myopathies; this efficacy is observed at high dosage (more than 120 mg/die); maximum effects come out at least after a three months treatment. The CoQ10 improves important parameters of cellular aerobic metabolism inducing an increased tolerance to stress and a reduction of hypostenia; it prevents the accumulation of the free radicals oxidative damage that occurs with aging in physiological conditions and that is extraordinary increased in case of primary mitochondrial disfunction.

For this reason therapy must start as soon as possible and continued all life long, to prevent the accumulation of irreversible damages.

When these alterations have been established, with a consequent cellular death and a substitutive fibrosis (ptosis and ophtalmoparesis, damage to myocardic conduction tissues, neuronal damage), CoQ10 is generally unusefull.

In conclusion, the high dosage therapy with CoQ10 is the rational approach to the treatment of patients with primary defects of the mitochondrial electronic transport chain and in the prevention of associated multisystemic degenerative damages.

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