Mitochondrial diseases of nuclear origin

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Abstract
Mitochondrial disorders of nuclear DNA (nDNA) origin include oxidative phosphorylation (OXPHOS) disorders (such as Leigh syndrome, paraganglioma); defects in nuclear-encoded mitochondrial proteins for mtDNA integrity (progressive external ophthalmoplegia (PEO) and mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome; and mitochondrial disorders with secondary effects on the OXPHOS system (Friedreich ataxia and hereditary spastic paraplegia). OXPHOS is a main function of mitochondria, that is, the oxidation of substrates to generate ATP produced by the cell. The large majority of the OXPHOS subunits are encoded in the nucleus. In addition, the nucleus also encodes a large number of proteins that are involved in OXPHOS complex assembly and maintenance, mtDNA replication, transcription, translation, and repair. OXPHOS disorders are associated with a diverse array of multisystem diseases often referred to as mitochondrial encephalomyopathies because of the prominent involvement of the central nervous system and the skeletal muscle. Once believed to be rare, it is now clear that OXPHOS deficiencies are an important cause of a wide range of neuromuscular, cardiac and endocrine disorders, and even some cancers. The prevalence of these diseases has been estimated at 1:11 000. In children most oxidative phosphorylation disorders are of nuclear origin, transmitted as autosomal recessive traits, usually with severe phenotypes and a fatal outcome. Mendelian oxidative phosphorylation disorders have also been described in adults, most of which result in a progressive loss of the integrity of mtDNA. Therapy is scarce and palliative in disorders associated with nuclear OXPHOS genes.

Keywords
COX deficiency, Leigh syndrome, mitochondrial disorders, metabolic encephalomyopathies, OXPHOS

Definition

Mitochondrial disorders of nuclear DNA (nDNA) origin include oxidative phosphorylation (OXPHOS) disorders, defects in nuclear-encoded mitochondrial proteins for mtDNA integrity, and mitochondrial disorders with secondary effects on the OXPHOS system. The OXPHOS system is composed of the four enzyme complexes (complexes I-IV) that make up the mitochondrial respiratory chain, and the ATP synthase complex (complex V), which uses the energy generated by electron transport along the respiratory chain to produce ATP. The large majority of the OXPHOS subunits are encoded in the nucleus [Saraste *et al*., 1999; Shanske *et al*., 2001]. In addition to the 82 structural

components of the OXPHOS system, the nucleus also encodes a large number of proteins that have only been partially characterized and are involved in OXPHOS complex assembly and maintenance, mtDNA replication, transcription, translation, and repair.

Etiology and related clinical characteristics

Deficiencies in oxidative phosphorylation are associated with a diverse array of multisystem disorders that are often referred to as mitochondrial encephalomyopathies because of the prominent involvement of the nervous system and skeletal muscle. We will only consider nuclear mitochondrial disorders in OXPHOS related proteins and nuclear defects in nuclear-encoded mitochondrial proteins for mtDNA integrity. Defects in non-OXPHOS nuclear-encoded mitochondrial proteins, related to a number of neurodegenerative "mitochondrial" disorders with secondary effects on the OXPHOS system (such as hereditary spastic paraplegia and Friedreich ataxia), will not be part of this chapter.

Nuclear defects in OXPHOS related proteins

Complex I

A survey of the cDNAs encoding the most evolutionarily conserved nDNA subunits of complex I uncovered mutations in less than 50% of the patients [Triepels *et al*., 2001], suggesting that factors involved in the assembly or maintenance of the complex — which remain completely unknown in humans — are a frequent case in these disorders.

Complex I deficiency starts mostly at birth or early childhood, and includes a great variety of clinical presentations that complicate the diagnostic process in individual patients. In general, complex I failure will result in multisystem disorders with a fatal outcome [Loeffen *et al*., 2000; Robinson *et al*., 1998; Kirby *et al*., 1999]. The most affected tissues are usually those requiring a high-energy production, like brain, heart, kidney, and skeletal muscle. Ophthalmologic signs such as external ophthalmoplegia, ptosis, cataract, and retinopathy are frequently expressed [Robinson *et al*., 1998]. Leigh syndrome (LS) [Leigh, 1951] or Leigh-like disease are the most common phenotypes associated with an isolated complex I deficiency, representing up to 50% of total cases [Robinson *et al*., 1998; Rahman *et al*., 1996]. LS is an early-onset, fatal neurodegenerative disorder characterized pathologically by bilateral lesions in the brainstem, basal ganglia, thalamus and spinal cord, and characterized clinically by psychomotor retardation and brainstem or basal ganglia dysfunction. Other commonly observed phenotypes associated with complex I deficiency are cardiomyopathy (11%), fatal infantile lactic acidosis (11%), macrocephaly with progressive leukodystrophy (7%), and unspecified encephalopathy (21%) [Loeffen *et al*., 2000].

Mutations in nuclear subunits of complex I have been reported in a number of studies (see Loeffen *et al*., 2000 for a review) and comprise variants in *NDUFS4* (W15X, W96X, and R105X), *NDUFS7* (V122M), *NDUFS8* (P79L, R102H), *NDUFS1* (R241W, D252G, R557X, M707V), and *NDUFV1* (including R59X, Y204C, C206G, E214K, etc) among others.

Complex II

Complex II is the only respiratory chain complex whose subunits are entirely encoded by nuclear genes. It is composed of two soluble proteins, the flavoprotein (SDHA) and iron-sulfur (SDHB) subunits, which are anchored on the matrix side of the inner membrane by two membrane subunits [cybS (SDHC) and cybL (SDHD)]. Missense mutations in the *SDHA* gene have been found in two families with autosomal recessive LS [Bourgeron *et al*., 1995; Parfait *et al*., 2000] and in a family with a late-onset neurodegenerative disease characterized by optic atrophy, ataxia and myopathy [Birch-Machin *et al*., 2000]. Mutations in both *SDHC* and *SDHD* (on chromosome 1q21 and 11q23, respectively) encoding the large and small cyb subunits, have been reported in families with autosomal dominant hereditary paraganglioma (PGL), a disorder characterized by the presence of benign, highly vascularized tumors of parasympathetic ganglia in the head and neck [Baysal *et al*., 2000; Niemann and Muller, 2000]. About half of the PGLs are familial and these have been linked to missense or nonsense mutations in *SDHD*, and a mutation in *SDHC* [Niemann and Muller, 2000]. Germline mutations in *SDHB* and *SDHD* have also been reported in patients with familial pheochromocytoma, chromaffin cell tumors that usually arise in the adrenal medulla, and more rarely in sympathetic ganglia, with or without PGL [Niemann and Muller, 2000]. These studies clearly implicate genes encoding structural subunits of complex II as tumor suppressors, but the molecular basis for these effects remains undetermined.

Complex III

Complex III is composed of 11 polypeptide subunits, all but cytochrome b being encoded by nuclear genes. Complex III catalyzes the transfer of electrons from ubiquinone to cytochrome c. While a number of mutations have been reported in cytochrome b in patients with myopathy, with or without myoglobinuria [Andreu *et al*., 1999], no mutations have thus far been reported in the nuclear-encoded structural subunits [Valnot *et*

al., 1999]. Missense mutations in *BCS1-L* on chromosome 2q33, whose product is putatively involved in the assembly of the complex, were found in four Turkish families with early-onset tubulopathy, hepatopathy and encephalopathy [de Lonlay *et al*., 2001].

Complex IV

Complex IV (COX), the terminal enzyme in the mitochondrial respiratory chain, catalyzes the reduction of molecular oxygen by reduced cytochrome c. It is composed of 13 subunits, 10 of which are encoded by nuclear genes. Two of the nuclear subunits (VIa and VIIa) have tissue-specific isoforms. Although their cDNAs were cloned in the 80's based on analogy with their orthologs in yeast, mutations have not been identified in any of the 10 nuclear-encoded structural genes in patients with any of the above phenotypes [Jaksch *et al*., 1998]. Several gene defects have, however, been reported in factors important in the biogenesis of the COX complex. These include: i) the *SURF1* gene observed in the large majority of LS-COX patients [Pequignot *et al*., 2001]; ii) the *SCO2* gene — a mitochondrial copper chaperone which transports copper to complex IV— a rare cause of fatal, early-onset COX deficiency with hypertrophic cardiomyopathy and encephalopathy [Papadopoulou *et al*., 1999]; iii) the *SCO1* gene — encoding another protein implicated in mitochondrial copper delivery — has been reported in association with familial hepatopathy and ketoacidotic coma, but no cardiac symptoms [Valnot *et al*., 2000].

Patients with autosomal recessive COX deficiency can present with a number of different clinical phenotypes including classical LS, a French–Canadian form of LS, fatal infantile COX deficiency, hypertrophic cardiomyopathy and myopathy, and a reversible COX deficiency confined to skeletal muscle [Robinson, 2000].

Complex V

Complex V, the ATP synthase, is composed of 16 subunits. Subunits A6 and A6L are encoded by the ATP 6 and 8 mtDNA genes, respectively, and the other subunits are encoded in the nucleus. The complex consists of the membrane-spanning F_0 segment, responsible for proton translocation, and the F_1 stalk that extends into the matrix and contains the catalytic center. While mutations in *ATP6* have been described in a syndrome of neuropathy, ataxia and retinitis pigmentosa (NARP) [Holt *et al*., 1990] and maternally-inherited LS [Tatuch *et al*., 1992; Carrozzo *et al*., 2001], no mutations have yet been described in nuclear-encoded subunits. However, a still unknown gene defect affecting complex V assembly has been hypothesized in a syndrome consisting of cardiomegaly, hepatomegaly and lactic acidosis [Houstek *et al*., 1999].

Nuclear defects in mitochondrial proteins for mtDNA integrity

The factors involved in mammalian mtDNA maintenance are all encoded by nuclear genes, and transported into the mitochondria. They include those involved directly in DNA processing, such as the mtDNA polymerase *γ (POLG1)*, a helicase, a primase, and a ligase. Two human disease groups (progressive external ophthalmoplegia (PEO) and mitochondrial DNA depletion syndrome) result from these disturbed mtDNA maintenance mechanisms. These disorders show either quantitative loss of mtDNA, *i.e.* mitochondrial DNA depletion, or qualitative accumulation of multiple large-scale mtDNA deletions. Their inheritance is autosomal, indicating that a primary nuclear gene defect secondarily causes the mtDNA alteration [Hirano and Vu, 2000]. The most common is PEO syndrome, which is transmitted in most cases as an autosomal dominant trait (adPEO), or more rarely as an autosomal recessive trait (arPEO). In adPEO, linkage analysis detected pathogenic loci on chromosome 10q24 [Suomalainen *et al*., 1995], 4q34–35 [Kaukonen *et al*., 1999] and 15q22–q26 [Van Goethem *et al*., 2001], encoding the *ANT1*, *C10orf2*, and *POLG1* genes, respectively. There is at least one additional, still unidentified locus. PEO is characterized clinically by ophthalmoparesis and exercise intolerance with onset usually between 18 and 40 years of age [Suomalainen *et al*., 1995]. However, muscle weakness and peripheral neuropathy should also be considered as cardinal features. Cardiac involvement is so significant that the association between PEO and cardiomyopathy requires analyses for multiple large-scale mtDNA deletions and patients with multiple deletions should always receive careful cardiologic evaluation. Of particular interest is the clinical manifestation of affective disorders in patients or relatives. Psychiatric illnesses can be the expression of CNS involvement in the setting of multisystem clinical syndromes due to mtDNA defects.

The combination of arPEO, severe gastrointestinal dysmotility, peripheral neuropathy, cachexia, diffuse leukoencephalopathy on brain MRI, and mitochondrial dysfunction (histological, biochemical or genetic abnormalities of the mitochondria) identifies the mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome [Hirano and Vu, 2000]. The disease onset ranges from five months to 43

years of age. The MNGIE syndrome was mapped by linkage analysis into the chromosome 22q13.32-qter region, and afterwards shown to be caused by mutations in the thymidine phosphorylase (*TP*) gene [Nishino *et al*., 1999].

Diagnostic methods
Investigation of skeletal muscle biopsy requires histochemistry — to reveal ragged-red/cytochrome c oxidase fibers — and biochemistry, to show isolated or combined enzymatic defects of OXPHOS disorders. Southern blot analysis in skeletal muscle is required to unravel the presence of different degrees of multiple mtDNA deletions.

Genetic testing
Genetic testing using peripheral blood DNA is available in selected centers for the *SURF1*, *SCO2* and *SCO1*, *ANT1*, *C10orf2*, *POLG1* and *TP genes.*

Prevalence
Once thought to be very rare, it is now clear that OXPHOS deficiencies are an important cause of a wide range of neuromuscular, cardiac and endocrine disorders, and even some cancers. The prevalence of these diseases has been estimated at 1:11000 [DiMauro, 2001].
Isolated complex I deficiency is probably the most common enzyme defect [Loeffen *et al*., 2000] among the OXPHOS disorders and seems to follow an autosomal recessive mode of inheritance in about 70-80% of the cases [Loeffen *et al*., 2000; Bourgeron *et al*., 1995; Munnich *et al*., 1992].

Inheritance
If mutations in mtDNA are a frequent cause of OXPHOS defects in adults, in children most oxidative phosphorylation disorders are of nuclear origin, transmitted as autosomal recessive traits, usually with severe phenotypes and a fatal outcome. Mendelian oxidative phosphorylation disorders have also been described in adults, most of which result in a progressive loss of the integrity of mtDNA. Sporadic cases with PEO exist as well and might represent *de novo* mutations.

Genetic counseling and prenatal diagnosis
When genetic screening has identified a pathogenic mutation (see Genetic testing), genetic counseling might propose a safe prenatal diagnosis in chorionic villi. In sporadic cases, a *de novo* mutation should be considered.

Management
As for many mitochondrial disorders, therapy is scarce and palliative in disorders associated with nuclear OXPHOS genes. In advanced stages, surgical correction for a severe ptosis may be considered in patients with progressive ophthalmoparesis.

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