Review

Molecular genetic and clinical aspects of mitochondrial disorders in childhood

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Received 6 November 2006; received in revised form 17 January 2007; accepted 2 February 2007

Abstract

Mitochondrial OXPHOS disorders are caused by mutations in mitochondrial or nuclear genes, which directly or indirectly affect mitochondrial oxidative phosphorylation (OXPHOS). Primary mtDNA abnormalities in children are due to rearrangements (deletions or duplications) and point mutations or insertions. Mutations in the nuclear-encoded polypeptide subunits of OXPHOS result in complex I and II deficiency, whereas mutations in the nuclear proteins involved in the assembly of OXPHOS subunits cause defects in complexes I, III, IV, and V. Here, we review recent progress in the identification of mitochondrial and nuclear gene defects and the associated clinical manifestations of these disorders in childhood.

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Keywords: mtDNA; OXPHOS; Mitochondrial disorders; Mutation

1. Introduction

The discovery, in 1988, that mitochondrial DNA (mtDNA) mutations cause disease in humans (Holt et al., 1988; Lestienne and Ponsot, 1988; Zeviani et al., 1988) opened a new research field involving these disorders. Since then, more than 100 pathogenic mtDNA point mutations and numerous mtDNA rearrangements have been discovered, but important questions concerning the relationship between these mutations and their phenotypic expression remain to be resolved.

Mutations in mtDNA only explain about 20% of mitochondrial OXPHOS disorders in childhood (Darin et al., 2001). The list of nuclear gene mutations involved in OXPHOS diseases are increasing continuously and may for example involve structural subunits of the respiratory chain, assembly factors, translation factors as well proteins important for the maintenance of mtDNA.

The mitochondrial OXPHOS disorders probably constitute the most common group of neurometabolic diseases in childhood (Darin et al., 2001). The clinical features of these disorders are sometimes characteristic but never specific for any genetic defect. The most commonly affected organs are those with a high energy demand such as skeletal muscle and/or the central nervous system, which explain why the term “mitochondrial encephalomyopathies” (DiMauro et al., 1999) has been applied. However, virtually any organ and tissue can be affected. Heterogeneity in the distribution of mutated mtDNA and the tissue-specific expression of nuclear genes are two plausible explanations for the widely varying clinical phenotypes (Larsson and Clayton, 1995).

The major function performed by mitochondria in aerobically active cells is the synthesis of ATP (Fang et al., 1994). The process of ATP synthesis is mediated by the OXPHOS system. The OXPHOS system, located in the mitochondrial inner membrane, is composed of five multisubunit enzyme complexes (Fig. 1). Four of them are NADH-dehydrogenase (NADH–ubiquinone oxidoreductase, complex I), succinate dehydrogenase...
(succinate–ubiquinone oxidoreductase, complex II), cytochrome bc1 complex (ubiquinol–cytochrome c oxidoreductase, complex III), and cytochrome c oxidase (complex IV). Substrates feed reducing equivalents to the respiratory chain at different points. Electrons are passed down the chain and protons are pumped across the inner mitochondrial membrane, building up a gradient, which is used to drive ATP production through the reentry of protons via ATP synthase (complex V) (Janssen et al., 2004). Mitochondria also participate in the generation of reactive oxygen species, and the initiation of apoptosis may be an additional mechanism for pathology (Wallace et al., 1998).

Human mtDNA is a 16.6 kb double-stranded circular molecule encoding for 13 enzyme subunits involved in the respiratory chain (Fig. 2) (Anderson et al., 1981). They include seven subunits of complex I, cytochrome b of complex III, three subunits of complex IV, and two subunits of complex V. The mtDNA also encodes for two rRNAs and 22 tRNAs necessary for mitochondrial protein synthesis. The replication of mtDNA requires several nuclear-encoded factors such as mtRNA polymerase, mitochondrial transcription factor A (mtTFA), DNA polymerase gamma (POLG), deoxyguanosine kinase (DGUK), and thymidine kinase 2 (TK2) (Larsson and Clayton, 1995; Larsson and Luft, 1999; Kollberg et al., 2005). mtDNA is more vulnerable to mutation than nuclear DNA and the mutation rate is much higher in mtDNA. This can be explained by the lack of histones in mtDNA and the continuous exposure of the molecule to reactive oxygen species. In pathological conditions, there is often a mixture of wild-type and mutated mtDNA, so-called heteroplasmy. mtDNA mutations are functionally recessive and the proportion of mtDNA with a pathogenic mutation must reach a certain level (threshold level) for the phenotypic expression of the mutation. mtDNA is almost exclusively maternally inherited and many mtDNA disorders therefore display a maternal mode of inheritance.

In this article, we review recent progress in the identification of mitochondrial and nuclear gene defects in association with OXPHOS disorders. The genetics and the clinical manifestations of these disorders will be discussed.
2. Nuclear gene mutations affecting OXPHOS

Nuclear DNA encodes the majority of the approximately 85 structural subunits of the OXPHOS enzyme complexes but there are at least nearly 800 other proteins expressed in the mitochondria that are also synthesized by nuclear genes (Cotter et al., 2004). These proteins may for example be involved in the assembly of the enzyme complexes, maintenance of mtDNA, mitochondrial translation or mitochondrial dynamics. These proteins are synthesized in the cytoplasm, usually as precursors containing an N-terminal extension, which serves as a leader peptide that precisely addresses the protein to mitochondria.

2.1. Mutations in the nuclear-encoded polypeptide subunits of OXPHOS

2.1.1. Complex I

Reduced nicotinamide adenine dinucleotide (NADH)–ubiquinone oxidoreductase (complex I) catalyzes the transfer of electrons from NADH to ubiquinone and couples this to proton pumping out of the mitochondrial matrix and into the intermembrane space (Fig. 1). Complex I is the largest enzyme-complex of the respiratory chain and contains at least 43 structural subunits, seven of which are encoded by mtDNA (ND1–ND6, ND4L) (Anderson et al., 1981). Complex I has an L-shaped configuration and can be fragmented into three different fractions. The largest hydrophobic membrane fraction (HP) attaches the complex to the mitochondrial inner membrane while the matrix component is cleaved into the flavoprotein (FP) and the iron–sulphur protein fraction (IP) (Galante and Hatefi, 1979). The complete function of complex I is not yet known. Electron transfer through complex I starts in the FP fraction. The presence of an iron–sulphur cluster in the IP fraction facilitates further electron transport to the HP fraction, which is probably involved in ubiquinone binding and proton translocation (Ohnishi et al., 1998).

Isolated complex I deficiency is the most commonly identified biochemical defect in mitochondrial OXPHOS disorders (Darin et al., 2001), but it is probably under-diagnosed, since both lactate levels and muscle morphology may be normal (Kirby et al., 1999; Loeffen et al., 2000). As in other mitochondrial OXPHOS disorders, the clinical features are extremely variable. The most common clinical phenotype in childhood is probably Leigh syndrome (LS, MIM 25600) (Morris et al., 1996). In overall terms, LS is characterized neuropathologically by neuronal loss, status spongiosus, and astrocytosis affecting the cerebral cortex (Harding et al., 1995) and clinically by early-onset psychomotor retardation (often episodic), refractory seizures (often myoclonic or with epilepsy partialis continua), cortical blindness and liver disease (Darin et al., 2001).

Nuclear mutations are probably involved in 90–95% of children with complex I deficiency (Triepels et al., 2001) but it was not until 1998 that the first mutations were identified in the NDUFS4 and NDUFS8 genes by the group in Nijmegen. (Loeffen et al., 1998; van den Heuvel and Smeitink, 2001) An increasing number of pathogenic mutations in genes encoding different structural subunits of complex I have since then been identified. However, the genetic cause remains unclear in about 60% of patients with complex I deficiency. (Triepels et al., 2001). Pathogenic mutations have been identified in numerous structural subunits associated with different clinical phenotypes (Table 1) and in B17.2L, an assembly factor for complex I, in a child with progressive encephalopathy (Ogilvie et al., 2005).

2.1.2. Complex II

Succinate–ubiquinone oxidoreductase (SQR) consists of four nuclear-encoded subunits and is part of both the respiratory chain and the Krebs cycle. It catalyzes the oxidation of succinate to fumarate and transfers the electrons to the ubiquinone pool of the respiratory chain (Fig. 1). Subunits A and B form the succinate dehydrogenase (SDH), while subunits C and D anchor the enzyme to the membrane (Rustin et al., 2002). Mutations in the SDH A, encoding the Fp subunit, have been found in occasional children presenting with LS and complex II deficiency (Bourgeron et al., 1995; Parfait et al., 2000), but also in two sisters with a late onset neurodegenerative disease (Birch-Machin, 2000). In contrast, subunits B, C, and D appear to act as tumor suppressor genes and mutations have been identified in familial paraganglioma and familial as well as in appar-
ently sporadic pheochromocytoma (Gimm et al., 2000; Astuti et al., 2001; Baysal, 2003).

2.2. Mutations in the proteins involved in the assembly of OXPHOS subunits

2.2.1. Complex III

Ubiquinol cytochrome c reductase (complex III) catalyzes the transfer of electrons from the succinate and nicotinamide-linked dehydrogenases to cytochrome c and it couples this reaction to proton pumping across the inner mitochondrial membrane. Complex III is made up of 11 subunits, of which all but one (cytochrome b) are encoded by nuclear DNA. Pathogenic mutations have not been found in any of the nuclear-encoded subunits. **BCS1L** is an assembly factor for complex III in human (Petruzzella et al., 1999). Mutations in this gene have been identified in children with complex III deficiency and features of tubulopathy, encephalopathy, and liver failure (de Lonlay et al., 2001) and in the GRACLE (growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death) syndrome (Visapaa et al., 2002).

2.2.2. Complex IV

Cytochrome c oxidase (COX or Complex IV) is the terminal enzyme of the respiratory chain. It catalyzes the transfer of electrons from reduced cytochrome c to molecular oxygen and it couples this reaction to proton pumping across the inner mitochondrial membrane. The COX enzyme complex is composed of 13 structural subunits, three of which are encoded by genes of the mtDNA and form the catalytic core of the enzyme. The other 10 subunits are products of nuclear genes that are translated on cytoplasmic ribosomes and transferred to the mitochondria by different modes of transport. Even though a handful of mutations have been identified in each of the three mtDNA-encoded COX subunits (Adams et al., 1997; Jaksch et al., 1998), a large number of proteins are involved in the assembly and maintenance of the COX complex. In fact, more than 30 different genetic complementation groups for COX assembly have been identified in the yeast *Saccharomyces cerevisiae* (McEwen et al., 1986; Tzagoloff et al., 1990). Many of the identified accessory proteins in yeast have their counterparts in humans and an increasing number of these proteins have been associated with disease.

The SURF1 protein is a putative assembly or maintenance factor for COX (Tiranti et al., 1999), while SCO1 and SCO2 are believed to be involved in copper delivery for the copper centers of COX (Glerum et al., 1996). The **COX10** gene encodes heme A:farnesyltransferase, which is required for the biogenesis of functional COX in yeast (Nobrega et al., 1990), while the COX15 protein is involved in the synthesis of heme A, the heme prosthetic group of COX (Barros et al., 2001). The **SURF1** gene, which is located on chromosome 9q34, has been found to be the most frequently involved gene in LS with COX deficiency (Zhu et al., 1998; Tiranti et al., 1999; Sue et al., 2000; Moslemi et al., 2003). Mutations in the **SCO2** gene, located on chromosome 22q13, were initially identified in 5 unrelated infants with fatal cardioencephalopathy (Papadopoulou et al., 1999; Jaksch et al., 2000). Recently, however, three additional infants have been identified with a different phenotype of delayed infantile onset Leigh-like syndrome, neurogenic muscular atrophy, and hypertrophic cardiomyopathy (Jaksch et al., 2001). Compared with mutations in the **SURF1** gene, **SCO2** mutations appear to be a rarer cause of COX-deficiency, judging by the few cases reported in the literature (Darin et al., 2003).

The other identified genetic defects of putative assembly or maintenance factors for COX appear to be even less frequent. Mutations in the **SCO1** gene, localized on chromosome 17p13.1, have only been found in two siblings with neonatal-onset hepatic failure and encephalopathy (Valnot et al., 2001).

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**Table 1**

Nuclear genes involved in complex I deficiency

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Clinical phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDUFS1 (IP)</td>
<td>Leukodystrophy, Leigh-like with hepatomegaly, growth retardation and anemia</td>
<td>Bénit et al. (2001)</td>
</tr>
<tr>
<td>NDUFS2 (IP)</td>
<td>Leigh</td>
<td>Loeffen et al. (2001)</td>
</tr>
<tr>
<td>NDUFS3 (IP)</td>
<td>Leigh</td>
<td>Bénit et al. (2004)</td>
</tr>
<tr>
<td>NDUFS6 (IP)</td>
<td>Leigh</td>
<td>Procaccio et al., 2004</td>
</tr>
<tr>
<td>NDUFS7 (HP)</td>
<td>Leigh</td>
<td>Triepels et al. (1999)</td>
</tr>
<tr>
<td>NDUFS8 (IP)</td>
<td>Leigh</td>
<td>Schuelke et al. (1999)</td>
</tr>
<tr>
<td>NDUFV1 (FP)</td>
<td>Macrocephaly, Leukodystrophy and myoclonic epilepsy</td>
<td></td>
</tr>
<tr>
<td>NDUFV2 (FP)</td>
<td>Early onset hypertrophic cardiomyopathy and encephalopathy</td>
<td></td>
</tr>
</tbody>
</table>

et al., 2000a), while a mutation in the COX10 gene, located on chromosome 17p13.1–q11.1, has only been identified in three consanguineous siblings with mitochondrial encephalopathy and tubulopathy (Valnot et al., 2000b). The latest contribution to the literature, pathogenic mutations in the COX 15 gene, located on chromosome 10q22, has been identified in a single patient with fatal infantile hypertrophic cardiomyopathy (Antonicka et al., 2003).

2.2.3. Complex V

ATP synthase or ATPase (complex V) couples proton flow from the intermembrane space back to the matrix by the conversion of ADP and inorganic phosphate to ATP. ATP synthase is made up of an integral membrane component, F0, composed of at least seven subunits, and a peripheral moiety F1, composed of six subunits, which uses the proton gradient to convert ADP to ATP (and vice versa). Nuclear DNA encodes all the subunits of the two subcomplexes, except for ATPase 6 and 8 of the F0 segment (Elston et al., 1998; Kinosita et al., 2004). No pathogenic mutations have been identified in any of the nuclear subunits. Several assembly genes have been studied in yeast and their human analogs have been described (Wang et al., 2001; Ackerman, 2002). Pathogenic mutations have been identified in one of these genes, ATP12, in two unrelated cases with reduced complex V activity. One case had a malformation syndrome resembling COFS (cerebro-oculofaciokondro) except for the absence of microphthalmia and cataracts, and the other presented with severe lethal neonatal lactic acidosis (De Meirleir et al., 2004).

2.3. Mutations in proteins involved in mitochondrial translation

In addition to mitochondrial tRNAs and rRNAs, several nuclear-encoded factors, such as pseudouridine synthase 1 (PUS1), elongation factor G1 (EFG1), the mitochondrial elongation factor Tu (EFTu), and ribosomal protein S16 (MRPS16), are involved in the mitochondrial translation machinery. Mutations in these genes have recently been linked to a new class of OXPHOS disorders characterized by reduced activity in all the mtDNA-encoded respiratory chain complexes and a mitochondrial translation defect. Mutation in the PUS1 gene has been associated with mitochondrial myopathy and sideroblastic anemia (MLASA), which is a rare autosomal recessive disease in children and adults. Affected individuals express progressive exercise intolerance during childhood and the onset of sideroblastic anemia with basilar lactic acidemia and mitochondrial myopathy in adolescence (Casas and Fischel-Ghodsian, 2004). Mutation in the EFG1 gene has been associated with prenatal onset of encephalopathy and hepatopathy and early onset LS (Coenen et al., 2004; Valente et al., 2007). Mutations in the EFTu had been described in a patient with severe infantile macrocystic leukodystrophy with micropolygyria (Valente et al., 2007). A nonsense mutation in MRPS16 has been associated in a child with facial dysmorphic features, limb edema, agenesis of the corpus callosum, increased liver transaminases, lactic acidosis and death at 3 years of age. The patient had complexes I and IV and a mitochondrial translation defect.

2.4. Mutations in proteins involved in mtDNA maintenance

All the factors responsible for mtDNA replication are encoded in nuclear DNA. Defects in the cross-talk between the two genomes are thus due to primary mutations in nuclear DNA which cause pathogenic secondary alterations in mtDNA, either multiple mtDNA deletions and/or a reduced copy number of mtDNA (mt DNA depletion).

The mtDNA depletion syndromes are the most common disorders due to defects in intergenomic communication in childhood. This group of autosomal recessive diseases display a very variable phenotype, which may involve muscle, liver, brain, heart, and other organs (Macmillan and Shoubridge, 1996; Vu et al., 1998), including the syndromes of Leigh (Absalon et al., 2001) and Alpers (Naviau et al., 1999). These disorders may well be under-diagnosed in childhood and broad-based suspicion is warranted, since they may present prenatally with dysmorphic features (Macmillan and Shoubridge, 1996) and with non-specific symptoms in infancy, such as vomiting, failure to thrive, and developmental delay, before multiorgan involvement occurs. (Tsao et al., 2000). Muscle biopsy typically shows ragged red fibers while biochemical investigations often show COX deficiency, sometimes associated with decreased activity in other respiratory chain complexes (Vu et al., 1998). Mutations have recently been identified in the deoxyguanosine kinase gene (DGUK) and MPV17 associated with a hepatoocular form and the thymidine kinase gene (TK2) described in a myopathic variant (Mandel et al., 2001; Saada et al., 2001; Spinazzola et al., 2006). In the vast majority of cases, however, the genetic cause remains unclear (Carrozzo et al., 2003).

POLG1 encodes the catalytic subunit of mitochondrial DNA polymerase that is the only known polymerase for mtDNA (Clayton, 1982). POLG1 is probably the most frequently involved nuclear gene giving rise to mitochondrial diseases. The pathogenesis involves mtDNA depletion and/or the accumulation of multiple mtDNA deletions in clinically affected tissues (Kollberg et al., 2006). There is a broad clinical spectrum, from fatal encephalopathy and liver failure in early childhood to mild ataxia or external ophthalmoplegia in late adulthood. In fact, the phenotypic spectrum overlaps. In adults, pathogenic mutations in POLG1 have been associated with autosomal dominant and recessive forms of progressive external ophthalmoplegia (PEO) (Van Goethem et al., 2001), sensory ataxic neuropathy, dystarthis and ophthalmoparesis (SANDO) (Van Goethem et al., 2003), recessive ataxic syndromes, and...
Parkinsonism (Luoma et al., 2004; Davidzon et al., 2006). The clinical phenotype in the recessive variant is sometimes more severe with onset in childhood of PEO (Van Goethem et al., 2003), MERRF-like features (Van Goethem et al., 2003), or ataxic syndromes (Kollberg et al., 2006; Tzoulis et al., 2006). In children, homozygous or compound heterozygous mutations have frequently been associated with Alpers syndrome (MIM 203700) with liver involvement, also called Alpers-Huttenlocher syndrome (Brandon et al., 2005; Davidzon et al., 2005; Navaiaux and Nguyen, 2005). Characteristic clinical features include early-onset psychomotor regression, with a relapsing and remitting course frequently exacerbated by infections, ataxia, refractory seizures with myoclonias and status epilepticus, stroke-like episodes, and frequently liver failure. Patients run a high risk of deterioration if they are exposed to valporate (Horvath et al., 2006; Kollberg et al., 2006; Tzoulis et al., 2006).

Multiple mtDNA deletions, mtDNA depletion, and site-specific point mutations have also been described in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) (Hirano et al., 1994; Papadimitriou et al., 1998; Nishigaki et al., 2003). In this disorder, pathogenic mutations have been identified in the thymidine phosphorylase (TP) gene, located on chromosome 22q13.32–qter (Nishino et al., 1999). MNGIE is an autosomal recessive disease, characterized by PEO, gastrointestinal dysmotility, thin body habitus, peripheral neuropathy, myopathy, leukoencephalopathy, and lactic acidosis. Although this is predominantly a disease of adults, childhood-onset variants have also been described (Teitelbaum et al., 2002).

2.5. Mutations in genes involved in the fusion and fission of mitochondrial membranes

The mitochondria are dynamic organelles that constantly fuse and divide. The balance of these opposing processes regulates the morphology of the mitochondrial network (Chen and Chan, 2005). Mutations in the two pro-fusion genes, Mitofusin 2 (MFN2) and optic atrophy type 1 (OPA1), have recently been identified in human diseases. Pathogenic mutations in MFN2 have been identified in the first intron of the frataxin gene, and is associated with a decreased frataxin protein (Campuzano et al., 1997). This nuclear-encoded protein is localized in the mitochondrial matrix and although the exact role of frataxin is not known, mitochondrial respiratory chain deficiency, oxidative stress and mitochondrial iron accumulation have been shown to be important components of the disease mechanism (Rotig et al., 1997). This knowledge has led to the initiation of therapy with antioxidants, which has been shown to be effective against the hypertrophic cardiomyopathy in this disease (Rustin et al., 1999).

2.6. Other defects in nuclear-encoded proteins affecting the OXPHOS system

2.6.1. Friedreich’s ataxia (FA)

FA (MIM 229300) is a neurodegenerative disorder, which usually starts with progressive ataxia between 5 and 16 years of age leading to wheel-chair dependency by 25 years of age on average. It is characterized by combined involvement of peripheral nerves, cerebellar tracts, pyramidal tracts, and posterior columns. Skeletal deformities and cardiac involvement are common and involvement of optic pathways and diabetes mellitus are sometimes associated (Harding, 1981). FRDA results from a triplet expansion in the first intron of the frataxin gene, and is associated with a decreased frataxin protein (Campuzano et al., 1996; Campuzano et al., 1997). This nuclear-encoded protein is localized in the mitochondrial matrix and although the exact role of frataxin is not known, mitochondrial respiratory chain deficiency, oxidative stress and mitochondrial iron accumulation have been shown to be important components of the disease mechanism (Rotig et al., 1997). This knowledge has led to the initiation of therapy with antioxidants, which has been shown to be effective against the hypertrophic cardiomyopathy in this disease (Rustin et al., 1999).

2.6.2. Hereditary spastic paraplegia (HSP)

HSP constitute a group of disorders with variable age of onset and inheritance, characterized by progressive weakness and spasticity of the lower limbs due to degeneration of corticospinal axons (Fink, 2003). Mutations in the paraplegin gene, a mitochondrial metalloprotease localized to chromosome 16q24.3 have been associated with an autosomal recessive HSP (HSP7) (Casari et al., 1998). A complex I–III deficiency of the respiratory chain has been found in this disorder and may reflect excess free radical-mediated damage, judged by the similarities in this aspect to FA and the SOD2 knockout mouse (Wilkinson et al., 2004). Mutations in the gene encoding mitochondrial chaperonin Hsp60, on chromosome 2q24–34, have been identified in a French family with an autosomal dominant form (Hansen et al., 2002). Recently, respiratory chain defects have also been identified in several other HSPs, indicating that mitochondrial dysfunction might be an important pathogenic mechanism in these disorders (Piemonte et al., 2001; McDermott et al., 2003).

3. Primary mtDNA abnormalities affecting OXPHOS

Primary mtDNA abnormalities in children are due to mtDNA rearrangements (deletions or duplications) and point mutations or insertions. Single mtDNA deletions are usually sporadic and females with mtDNA deletions usually do not transmit the deletion to their children. mtDNA point mutations show usually maternally mode of inheritance with males and females equally affected but sporadic mutations occur as well.
3.1. Point mutations

Disorders associated with mtDNA point mutations in children may be due to either point mutations/insertions in tRNA, rRNA or protein coding genes (DiMauro and Moraes, 1993; Wallace, 1994; Larsson and Clayton, 1995). Mutations in tRNA genes cause impaired protein synthesis affecting all polypeptides encoded by mtDNA. More than 100 different pathogenic tRNA mutations have been reported in adults and children in association with different endocrine, neurological, cardiological, and neuromuscular disorders (Chinnery and Turnbull, 2000; Brandfonbrener et al., 2005).

The earliest discovered pathogenic tRNA mutations were the tRNA Leu(UUR) A3243G and (tRNA Leu(UUR) A3243G (Goto et al., 1990; Shoffner et al., 1990). These genes have been shown to be particular ‘hotspots’ for mutations in mtDNA. The tRNA Leu(UUR) A3243G was first described in association with myoclonus epilepsy and ragged red fibers (MERRF, MIM 545000). The most characteristic symptom of MERRF is myoclonus epilepsy, generalized convulsions, cerebellar ataxia, myopathy, mental deterioration, and lactic acidosis (Kishi et al., 1988). Clinical manifestation of the tRNA Leu(UUR) A3243G may be quite variable (Silvestri et al., 1992; Hammers et al., 1993). The mutation changes a conserved nucleotide in the TψC arm, which is involved in the interaction of the tRNA with the ribosomal surface (Shoffner et al., 1990). PCR analysis of single muscle fibers has demonstrated that high levels of the mutation, >95%, cause dysfunction of complex IV in muscle fibers (Moslemi et al., 1998). The clinical manifestations of the tRNA Leu(UUR) A3243G mutation may vary to an even greater extent than the tRNA Leu(UUR) A3243G but is typically found in association with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS, MIM 540000). MELAS is characterized by short stature, seizure, lactic acidosis and recurrent cerebral insults causing hemiplegia, hemianopia or cortical blindness (Hirano and Pavlakis, 1994). Onset of the disease is in childhood or later in life. In adults, the A3243G mutation may in addition manifest as maternally inherited diabetes-deafness and hypertrophic cardiomyopathy (Reardon et al., 1992; van den Ouweland et al., 1992; Manouvrier et al., 1995).

Pathogenic mutations in mitochondrial rRNA have been reported in association with maternally inherited hearing loss (Fischel-Ghodsian, 1999; Zhao et al., 2004).

mtDNA encodes 13 enzyme subunits involved in the respiratory chain complexes (I, III, IV, and V). Mutations in these genes lead to impaired function of the affected enzyme complex.

Complex I, the largest of the OXPHOS complexes, consist of 43 subunits, seven of which are encoded by mtDNA (ND1–ND6 and ND4L). Mutations in these genes are associated with Leber’s hereditary optic neuropathy (LHON, MIM 535000), which predominantly affects males in the late twenties, is characterized by acute or subacute visual loss with maternal inheritance. The majority (approximately 90%) of LHON patients have pathogenic mutations in either one of the G11778A in ND4, A3460G in ND1 or 14484 in ND6 genes (Wallace et al., 1988; Poulton et al., 1991; Brandon et al., 2005). Even though pathology of LHON in general is limited to the optic system, in rare case, complication by Leigh-like encephalopathy or more frequently a multiple sclerosis-like syndrome may occur (Harding et al., 1992; Vannoppenbosch et al., 2000; Funalot et al., 2002).

Although only one subunit of complex III, cytochrome $b$, is encoded by mtDNA, several mutations in this gene have been associated with various OXPHOS disorders such as LHON, mitochondrial encephalomyopathy, hypertrophic cardiomyopathy, and exercise intolerance (Johns and Neufeld, 1991; Andreu et al., 1999; Keightley et al., 2000).

Complex IV, the terminal step of the respiratory chain, consists of 13 enzyme–subunits, of which three are mitochondrially encoded (COXI, II and III). COXI, II, and III constitute the catalytic core of the complex and pathogenic mutations in these subunits have been associated with Leigh-like syndrome, mitochondrial myopathy, rhabdomyolysis and myopathy with exercise intolerance (Rahman et al., 1999; Tiranti et al., 2000; McFarland et al., 2004).

Pathogenic mutations in the ATPase 6 gene leading to partial complex V deficiency have been described in various neurological diseases. Among these mutations the T8993G mutation, converting a conserved leucine into arginine (L155R), is the most common. This mutation causes neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP, MIM 551500) (Holt et al., 1990), when the mutant load is high. The same mutation, at levels close to homoplasy, causes MILS (Tatuch et al., 1992). A second mutation at the same position (T8993C), replacing leucine to proline (L155P) is usually associated with milder clinical phenotypes (de Vries et al., 1993). Other mutations in ATPase 6 gene, e.g. T9176G (L217P), T9185C (L220P) and T9191C (L222P) have also been described in association with LS and familial bilateral striatal necrosis (FBSN) (Thyagarajan et al., 1995; Wilson et al., 2000; Moslemi et al., 2005) (Dionisi-Vici et al., 1998; Carrozzo et al., 2000).

3.2. mtDNA rearrangements

Single mtDNA deletions in children are associated with multisystem disorders. In infants mtDNA deletions have been associated with Pearson’s syndrome (PS, MIM 557000), which is characterized by sideroblastic anemia, hepatic, renal, and exocrine pancreatic dysfunction (Rotig et al., 1990; Smith et al., 1995). In addition to mtDNA deletions several cases with PS with mtDNA duplications have been described (Smith et al., 1995; Muraki et al., 2001; Jacobs et al., 2004). Infants with PS usually die in the first year of age. The survivors may develop Kearns–Sayre syndrome (KSS, MIM 530000) later on in childhood (Larsson et al., 1990). KSS is usually a disease with onset in adolescence, clinically associated with heart block, growth failure,
mental retardation, ataxia, progressive external ophtalmoplegia, and pigmentary degeneration of the retina (Zeviani et al., 1988; Oldfors et al., 1990). Muscle biopsy in KSS usually shows ragged red fibers with deficient COX activity. In addition to PS and KSS, mtDNA deletions may occasionally be the cause of severe to fatal dilated cardiomyopathy (Marin-Garcia et al., 1996; Moslemi et al., 2000; Ruppert and Maisch, 2000) and atypical multisystem presentations have also been described (Tulinius et al., 1995).

In summary, OXPHOS related disorders are relatively frequent causes of disease in childhood with a broad spectrum of clinical presentations. They are caused by mutations in mitochondrial or nuclear genes, which directly or indirectly affect mitochondrial oxidative phosphorylation (OXPHOS). The recent progresses in DNA research presented in this article have broadened the understanding of the pathogenesis behind these disorders and have facilitated prenatal diagnosis.

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Please cite this article in press as: Moslemi, A.-R., Darin, N., Molecular genetic and clinical aspects of mitochondrial ... Mitochondrion (2007), doi:10.1016/j.mito.2007.02.002


