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Autosomal disorders of mitochondrial DNA maintenance

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Abstract

Mitochondrial DNA (mtDNA) is maternally inherited. After birth, secondary mtDNA defects can arise. MtDNA depletion is a reduction in the amount of mtDNA in particular tissues. Multiple deletions of mtDNA accumulate as somatic mutations in mainly postmitotic tissues. These disorders of mtDNA maintenance frequently show Mendelian inheritance. Positional cloning has identified several genes involved in the control of mtDNA stability.

Recessive mutations in the genes ECGF1, dGK, TK2, SUCLA2 and POLG cause mtDNA depletion syndromes (MDS). Generally, MDS has infantile onset tissue specific features. Mutations in the genes ECGF1, ANT1, C10orf2 and POLG are associated with multiple mtDNA deletions. The nature of these mutations is dominant in ANT1, C10orf2 and POLG and recessive in ECGF1, C10orf2 and POLG. Mutations in these genes frequently cause progressive external ophthalmoplegia (PEO). However, clinical heterogeneity results in different neurological syndromes with considerable overlap. The most common features are PEO, neuropathy, myopathy, ataxia, epilepsy and hepatopathy.

Key words : Mitochondrial DNA ; depletion ; multiple mtDNA deletions ; progressive external opthalmoplegia ; sensory ataxic neuropathy ; Alpers' syndrome ; MNGIE.

Introduction

Oxidative phosphorylation (OXPHOS) is the most important mitochondrial function and implies the formation of ATP by the respiratory chain enzymes (RC). Only a minority of the subunits of the RC multienzyme complexes is encoded by mitochondrial DNA (mtDNA). Genes on different chromosomes in the cell nucleus encode all the other RC subunits. Furthermore, autosomal inherited mitochondrial disorders include defects of assemblers of RC complexes (Zhu *et al.*, 1998 ; Vogel *et al.*, 2005), defects at transcriptional, translational or post-translational level of RC subunits encoded by mtDNA (Jacobs and Turnbull, 2005), defects leading to secondary RC chain dysfunction (Rotig *et al.*, 1997), defects of metabolic pathways of RC co-factors (e.g. coenzyme Q10 ; Sobreira *et al.*, 1997) and defective factors affecting mitochondrial biogenesis (Shoubridge, 2001). This review is confined to clinical features of autosomal inherited factors controlling mtDNA stability.

MtDNA shows maternal inheritance but in mammals it needs lifelong maintenance in postmitotic cells and in cells with a very low mitotic index, e.g. hepatocytes. Defective mtDNA maintenance can be due to acquired disease like toxicity of antiviral drugs in AIDS patients, which block mtDNA synthesis because of the reverse transcriptase properties of polymerase gamma (Arnaudo *et al.*, 1991 ; Lewis and Dalakas, 1995). Here, we only present the autosomal inherited mtDNA maintenance disorders. Both autosomal dominant and autosomal recessive inheritance modes are associated with secondary mtDNA abnormalities. These include mtDNA depletion, which is a significant reduction of the normal amount of mtDNA present in a particular tissue, and the accumulation of multiple deleted mtDNA species. One could speculate on the occurrence of other mtDNA abnormalities such as the accumulation of multiple point mutations (Kujoth *et al.*, 2005), but these are difficult to demonstrate. There is a hypothesis that both mtDNA depletion and the accumulation of multiple mtDNA deletions might result from impaired mtDNA replication, repair and recombination or from a combination of these mechanisms. In several instances this could be related to disturbed metabolism of mitochondrial nucleotides (Fig. 1) (Van Goethem *et al.*, 2003c).

mtDNA depletion syndromes (MDS ; MIM 251880)

Depletion of mtDNA shows autosomal recessive inheritance and is mostly encountered in infants. Clinical presentation is heterogeneous, including muscle weakness, hepatopathy, encephalopathy, cardiomyopathy, renal disease and lactic acidosis (Moraes *et al.*, 1991 ; Vu *et al.*, 1998). In most MDS patients the underlying genetic defect remains unknown though autosomal recessive inheritance is often suspected. Recently, positional

cloning techniques have identified mutations in different nuclear genes associated with MDS.

These genes include *ECGF1* (Nishino *et al.*, 1999), *dGK*/*DGUOK* (Mandel *et al.*, 2001), *TK2* (Saada *et al.*, 2001), *POLG* (Naviaux and Nguyen, 2004) and *SUCLA2* (Elpeleg *et al.*, 2005).

ECGF1 (MIM 131222) encodes thymidine phosphorylase, a multifunctional protein and mutations are typically encountered in Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE). MNGIE patients can also have multiple mtDNA deletions in muscle, and therefor it is discussed below (Hirano *et al.*, 1994).

dGK or *DGUOK* (MIM 601465) encodes deoxyguanosinekinase and mutations in this gene cause combined severe encephalopathy and hepatopathy with onset in the first six months of age and death in the first year of life. Patients have severe failure to thrive, lactic acidosis and hypoglycemia (Mandel *et al.*, 2001).

TK2 (MIM 188250) encodes Thymidine kinase. *TK2* mutations cause myopathy combined with a variable encephalopathy (Saada *et al.*, 2001).

SUCLA2 encodes Succinyl-CoA Synthase (MIM 603921). Mutations in *SUCLA2* cause early infantile onset encephalomyopathy. Psychomotor development is severely retarded and there are generalized seizures, hearing loss and marked muscle hypotonia (Elpeleg *et al.*, 2005).

POLG (MIM 174763) encodes polymerase gamma and *POLG* mutations have an extremely variable phenotype (see section on multiple mtDNA deletion disorders). One clinical phenotype associated with *POLG* mutations is Alpers' syndrome (Naviaux and Nguyen, 2004 ; Ferrari *et al.*, 2005). Alpers' syndrome is a hepatocerebral syndrome with onset age from 0-25 years. Clinical features include seizures, which are refractory, mixed-type, often focal (epilepsia partialis continua) and myoclonic. Psychomotor regression is often episodic with partial recovery. Early liver disease can lead to cirrhosis. EEG and VEP are abnormal, while ERG is normal. Neuropathology shows frequently patchy cerebral cortical involvement with macroscopic thinning of a granular and discolored cortical ribbon (Harding, 1990).

Disorders characterized by multiple deletions of mtDNA

Multiple mtDNA deletions are present in many isolated and familial patients. Inheritance can be autosomal dominant (Zeviani *et al.*, 1989), recessive (Bohlega *et al.*, 1996), or digenic (Van Goethem *et al.*, 2003a). Many patients present with progressive external ophthalmoplegia (PEO) (Zeviani *et al.*, 1989) but many other phenotypes occur. These include cardiomyopathy (Ozawa *et al.*, 1990 ; Suomalainen *et al.*, 1992), recurrent myoglobinuria (Ohno *et al.*, 1991), ataxia plus ketoacidotic coma (Cormier *et al.*, 1991), multiple symmetric lipomatosis (Klopstock *et al.*, 1994), and myoclonus epilepsy with ragged red fibers (MERRF) (Blumenthal *et al.*, 1998). Positional cloning studies revealed that mutations in the genes *ECGF1* (Nishino *et al.*, 1999), *ANT1* (Kaukonen *et al.*, 2000), *C10orf2* (Spelbrink *et al.*, 2001) and *POLG* (Van Goethem *et al.*, 2001) are associated with multiple mtDNA deletions. The nature of these mutations is dominant in *ANT1* (Kaukonen *et al.*, 2000), *C10orf2* (Spelbrink *et al.*, 2001) and *POLG* (Van Goethem *et al.*, 2001) and recessive in *ECGF1* (Nishino *et al.*, 1999), *C10orf2* (Li *et al.*, 2001) and *POLG* (Van Goethem *et al.*, 2001). One reported patient has a recessive *POLG* and a *C10orf2* mutation, suggestive of a digenic disorder (Van Goethem *et al.*, 2003a).

ECGF1 (MIM 131222) is located on chromosome 22q13,32-qter and encodes thymidine phosphorylase (TP), a multifunctional protein which drives the mitochondrial specific thymidine salvage pathway (Fig. 1). It is suggested that increased thymidine disturbs mitochondrial nucleoside and nucleotide pools. Inheritance is recessive. DNA analysis on muscle biopsy specimens shows mtDNA depletion or multiple deletions, or a combination of both. In plasma, thymidine concentrations are increased and leukocyte thymidine phosphorylase activity is decreased. Patients suffer from Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE). Clinical features include a combination of progressive external ophthalmoplegia, neuropathy and leukodystrophy. Gastroparesis and intestinal dysmotility, presenting with intestinal pseudoobstructions or chronic diarrhea are characteristic. Patients often also have cachexia and hearing loss. Contrary to the rather homogeneous combination of these disease features, onset age is quite variable, ranging from 4 to 40 years (Hirano *et al.*, 1994).

POLG (MIM 174763 ; locus 15q25) encodes DNA polymerase gamma, which is the only DNA polymerase active in mammalian mitochondria. Polymerase gamma is similar to family A polymerases and contains 3 exonuclease motifs (I, II, III) and 3 polymerase motifs (A, B, C) (Ropp and Copeland, 1996). Both exonuclease and polymerase functions are essential for mtDNA maintenance in human cultured cells (Spelbrink *et al.*, 2000). Mutations occur both in the exonuclease and the polymerase domain as well as in the spacer domain between these two functional regions (http ://dir-apps.niehs.nih.gov/polg/).

POLG mutations were first identified in familial progressive external ophthalmoplegia (PEO), both in dominant and recessive forms (Van Goethem *et al.*, 2001). Most mutations resulting in adPEO cluster around polymerase motif B. This form of adPEO shows high penetrance. AdPEO due to *POLG* mutations is a systemic disorder and

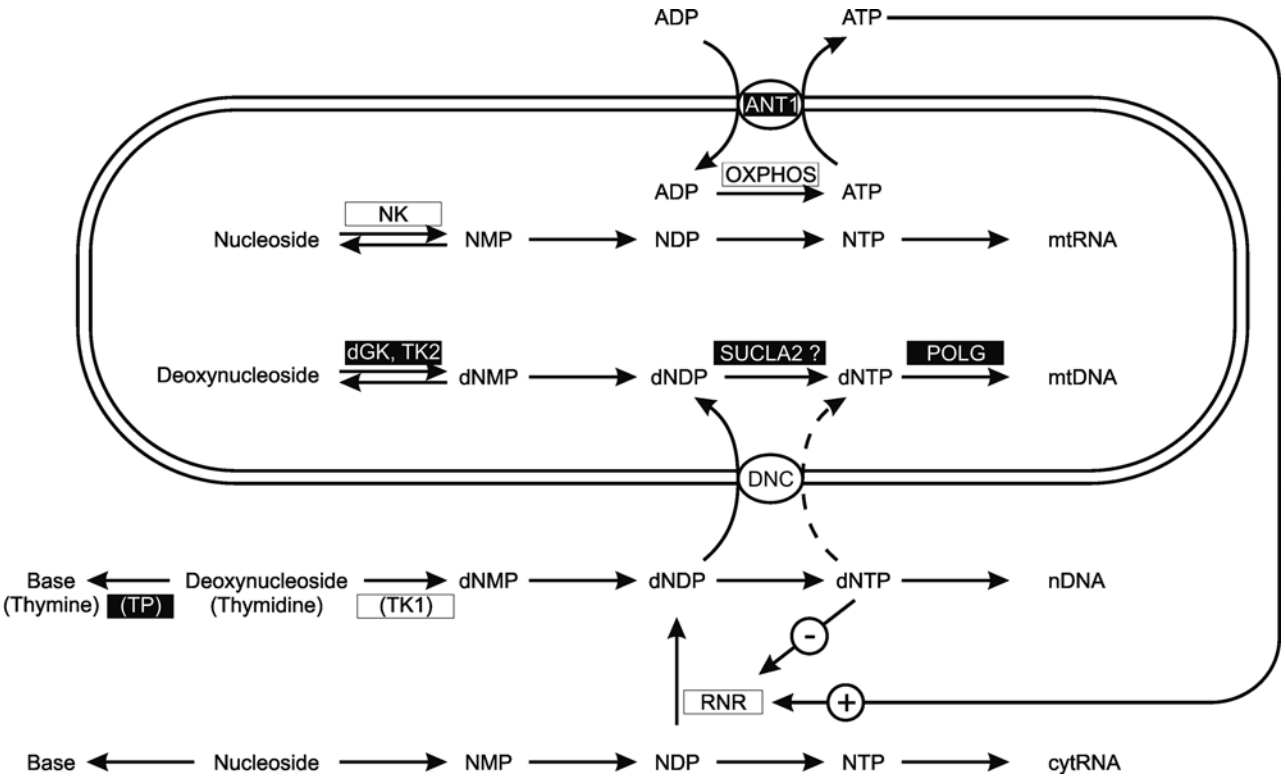

FIG. 1. — Schematic representation of mitochondrial nucleotide metabolism. A double line delineates the mitochondrial compartment. Enzymes are boxed. Primary defective enzymes or proteins involved in disorders of mtDNA maintenance are in black boxes. Only one deoxynucleoside (thymidine) out of four is metabolized by the enzymes between parentheses. ANT1= adenine nucleotide translocator 1. OXPHOS = oxidative phosphorylation. NK = nucleotide kinase. dGK = deoxyguanosine kinase. TK = thymidine kinase. POLG = polymerase gamma. DNC = deoxynucleotidecarrier. TP = thymidine phosphorylase. RNR = ribonucleotide reductase. SUCLA2 = succinyl-CoA synthase. dN = deoxynucleotide. nDNA = nuclear DNA. cytRNA = all extramitochondrial RNA molecules (mRNA, tRNA, rRNA). + denotes the strong allosteric activation of ribonucleotide reductase by ATP ; - denotes strong allosteric inhibition of RNR by dATP. Adapted and modified from Van Goethem *et al.*, 2003c.

usually PEO is the first sign, with adult onset age (25-45 years). There is progressive muscle weakness and death follows from respiratory muscle weakness leading to restrictive pulmonary disease. Associated features include sensory neuropathy (Van Goethem *et al.*, 1997), ataxia, L-dopa responsive Parkinson's disease, cataracts, presbyacusis, premature menopause, and amenorrhoea. Amenorrhoea is often the first sign in females (Luoma *et al.*, 2004). Muscle biopsy shows multiple mtDNA deletions (Van Goethem *et al.*, 1997).

Recessive *POLG* mutations were encountered in familial (Van Goethem *et al.*, 2001 ; Lamantea *et al.*, 2002 ; Van Goethem *et al.*, 2003e) and isolated patients with PEO (Van Goethem *et al.*, 2003b ; Agostino *et al.*, 2003 ; Di Fonzo *et al.*, 2003). Compared to adPEO, arPEO on the average has more prominent associated features which often are the presenting signs. Many patients have complaints from axonal neuropathy, which can be sensorimotor, sensory or sensory ataxic. Sensory ataxia often is a presenting feature and can precede PEO by several decades (Van Goethem *et al.*, 2003b). Other features include tremor, sensorineural hearing loss, CNS features and gastroparesis and intestinal pseudo-obstructions mimicking MNGIE (Van Goethem *et al.*, 2003e ; Mancuso *et al.*, 2003). In some patients muscle biopsy is entirely normal, including light microscopy, electron microscopy, biochemical analysis of respiratory chain enzymes and diagnostic Southern analysis of mtDNA (Van Goethem *et al.*, 2003b).

Recently *POLG* mutations were also identified in patients who had no PEO and no other muscle involvement. Several patients presented with ataxia combined with CNS features (Van Goethem *et al.*, 2004 ; Winterthun *et al.*, 2005 ; Hakonen *et al.*, 2005). One patient had features of MERRF (myoclonus, epilepsy and ragged red fibers) (Van Goethem *et al.*, 2003d). CNS features in these patients include myoclonus, seizures (status epilepticus, epilepsia partialis continua), cognitive decline, nystagmus, and dysarthria (Van Goethem *et al.*, 2004). Patients can develop severe hepatotoxicity when epilepsy is treated with sodium valproate (Van Goethem *et al.*, 2004 ; Van Goethem *et al.*, 2003d). Brain MRI sometimes shows lesions in the thalamus and basal ganglia, and cerebellar white matter lesions (Fig. 2 ; Van Goethem *et al.*, 2004). Recessive *POLG* mutations cause systemic

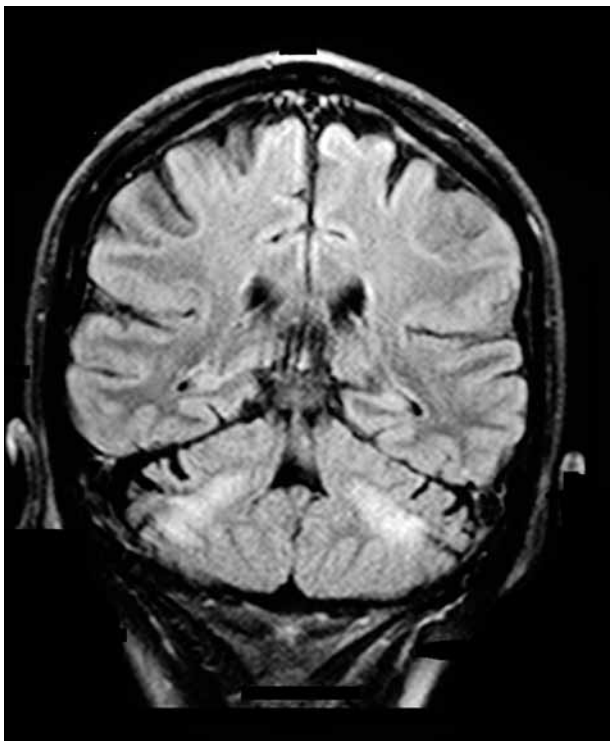

FIG. 2. — Brain MRI of patient, homozygous for recessive *POLG* A467T mutation. Note symmetric cerebellar white matter lesions on T2-weighted image.

disease and associated abnormalities outside the nervous system include mild cardiomyopathy, gastrointestinal dysmotility, weight loss, hepatic failure, cataracts, primary amenorrhoea and early menopause (Van Goethem *et al.*, 2004 ; Luoma *et al.*, 2004). In muscle, long PCR demonstrates multiple mtDNA deletions whereas Southern analysis can be normal (Van Goethem *et al.*, 2004 ; Van Goethem *et al.*, 2003d). In infantile hepatocerebral syndromes like Alpers' syndrome, recessive *POLG* mutations are associated with mtDNA depletion, as discussed above. Although some authors distinguish more than a dozen distinct mitochondrial syndromes caused by recessive *POLG* mutations, we suggest that patients are on a continuous spectrum with overlapping phenotypes.

Clinical clues are involvement of postmitotic tissues (muscle, CNS, peripheral and autonomic nerves, heart, ova, and ocular lens fibers) or tissues with low mitotic index (liver), and progressive disease course. There appears to be a 'core phenotype' of combined organ involvement with muscle, nerve, CNS and liver being most frequently affected (Van Goethem *et al.*, 2004).

C10orf2 (MIM 606075) encodes the mitochondrial protein twinkle. Twinkle is functional in hexameric form and colocalizes with mtDNA in mt 'nucleoids' (Spelbrink *et al.*, 2001). It functions as a mtDNA helicase (DNA helicases mediate DNA replication, repair, recombination and transcription and mutations in known human DNA helicases result in enhanced DNA recombination (Brosh, Jr. *et al.*, 2000)).

Dominant *C10orf2* / twinkle mutations cause adPEO (Spelbrink *et al.*, 2001). These mutations are less frequent cause of adPEO than dominant *POLG* mutations but more frequent than *ANT1* mutations.

C10orf2 / twinkle mutations cause a combination of PEO and moderate myopathy, but little multisystemic involvement (Suomalainen *et al.*, 1997 ; Lewis *et al.*, 2002). Some patients have L-dopa responsive Parkinson's disease (Van Goethem *et al.*, 2003a). Duplication of *C10orf2* occurred in one Finnish PEO family whereas several PEO families have point mutations in *C10orf2* (Spelbrink *et al.*, 2001). In muscle, multiple mtDNA deletions are evident by PCR techniques but not allways by Southern blotting (Deschauer *et al.*, 2003).

Recessive *C10orf2* / twinkle mutations cause Infantile Onset SpinoCerebellar Ataxia (IOSCA ; MIM 271245) (Nikali *et al.*, 2005). IOSCA is a Finnish infantile onset form of SCA. Onset usually is between 9-18 months. Patients suffer mainly from a combination of ataxia, athetosis, areflexia, and muscle hypotonia. In a late stage they also have hypacusis, ophthalmoplegia, and optic atrophy. Hypergonadotropic hypogonadism occurs in girls. Early death is attributed to status epilepticus (Koskinen *et al.*, 1994b). Morphologic studies reveal sensory axonal neuropathy and progressive atrophy of the cerebellum, brain stem and spinal cord (Koskinen *et al.*, 1994a).

ANT1 mutations cause adPEO (Kaukonen *et al.*, 2000). *ANT1* (MIM 103220) encodes the heart- and muscle- specific isoform of the adenine nucleotide translocator. This protein regulates the adenine nucleotide concentrations in the cytoplasm and in the mitochondrial matrix (Fig. 1). Altogether, *ANT1* mutations are a rare cause of PEO.
The clinical phenotype is confined to late onset PEO and a rather mild myopathy (Kaukonen *et al.*, 2000 ; Napoli *et al.*, 2001).

Conclusions

Both mtDNA depletion and multiple deletions might result from impaired mtDNA synthesis.

Pathology is confined to postmitotic tissues and liver. MtDNA depletion syndromes have an infantile onset age and a more severe clinical phenotype. Disorders characterized by multiple mtDNA deletions have a juvenile or adult onset age. In the latter disorders, dominant mutations cause PEO and myopathy with variable associated features. On the other hand, recessive mutations show phenotypic heterogeneity as is particularly evident in case of recessive *POLG* mutations.

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