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Progressive external ophthalmoplegia and multiple mitochondrial DNA deletions

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Abstract

Progressive external ophthalmoplegia (PEO) with secondary accumulation of multiple deletions of mitochondrial DNA (mtDNA) clinically resembles disorders due to primary mutations of mtDNA but follows a Mendelian inheritance pattern. The disorder belongs to an interesting group of diseases in which both the nuclear and the mitochondrial genome are involved in the pathology. Both autosomal dominant (adPEO) and recessive (arPEO) variants of this disorder occur. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) patients may have multiple mtDNA deletions and/or depletion of mtDNA. Recent reports of mutations in Thymidine Phosphorylase in MNGIE, and of mutations in adenine nucleotide translocator (ANT1), Twinkle and mitochondrial DNA polymerase gamma (POLG) in adPEO, have lead to new insights in the pathogenesis of these disorders of mtDNA maintenance. We also identified POLG mutations in two families with arPEO, which underlines the crucial role of the mtDNA replication machinery for mtDNA maintenance.

Key words : Progressive external ophthalmoplegia ; mitochondrial myopathy ; mitochondrial DNA maintenance ; pathogenesis.

Disorders of oxidative phosphorylation are highly heterogeneous from both a clinical and a genetic point of view. The nuclear as well as the mitochondrial genome contain genes that are necessary for respiratory chain function. Consequently, different modes of inheritance are encountered in disorders of oxidative phosphorylation.

Progressive external ophthalmoplegia (PEO) is a typical syndrome associated with mutations of mitochondrial DNA (mtDNA) (Holt, Harding, and Morgan-Hughes 1988). Single large scale mtDNA deletions are present in PEO, that is confined to muscle pathology, as well as in the Kearns Sayre syndrome in which there is multiorgan involvement. These single mtDNA deletions occur in sporadic cases since they are de novo generated in the oocyte or during embryonic development. Maternal transmission of these deletions appears to be extremely rare. Multiple deletions of mtDNA have been reported in familial forms of PEO with both autosomal dominant (Zeviani *et al*., 1989) and recessive (Bohlega *et al*., 1996) inheritance. These deletions are generated de novo as somatic mutations in each affected subject. Accumulated deletions can be clonal, which suggests that deletion events are rare (Moslemi *et al*., 1999). Recently four nuclear genes have been identified that predispose to secondary mtDNA deletions in postmitotic tissues.

Autosomal dominant Progressive External Ophthalmoplegia (adPEO) is clinically characterized by ptosis, ophthalmoparesis and more generalised weakness of the skeletal muscles with exercise intolerance (Zeviani *et al*., 1990). Additional features vary among different families (Melberg *et al*., 1996, Servidei *et al*., 1991, Suomalainen *et al*., 1997). In a Belgian family we encountered dysphagia and clinical signs of predominantly sensory axonal peripheral neuropathy (Van Goethem *et al*., 1997). Three alcoholics also suffered from motor peripheral neuropathy. Sudden death at ages 52-66 years was due to respiratory insufficiency caused by weakness of the respiratory muscles and was commonly precipitated by a lower respiratory tract infection. The range of onset age in this family was 25-39 years. Muscle biopsy in 2 cases showed ragged red fibres and the accumulation of multiple mtDNA deletions. Electron microscopy demonstrated subsarcolemmal accumulation of numerous abnormally structured mitochondria with paracrystalline inclusions.

After exclusion of the known adPEO loci in this family (Van Goethem *et al*., 2000) we mapped a novel locus for adPEO to chromosome 15q22-q26 (Van Goethem *et al*., 2001). This candidate region includes the gene encoding the mitochondrial DNA polymerase gamma (POLG) that maps to 15q25. By direct sequencing of POLG we identified a heterozygous missense mutation in exon 18, substituting Tyr with Cys at codon 955 (Y955C).

POLG is most similar to family A type DNA polymerases with 3 exonuclease motifs I, II, and III, in a proofreading exonuclease domain and

3 polymerase motifs A, B and C, in the polymerase domain. Functional analysis of artificial POLG mutants in cultured human cells has provided evidence that POLG exo and pol functions are essential for mtDNA maintenance (Spelbrink *et al*., 2000). Y955C is located in pol motif B. Tyr at position 955 is extremely conserved in mtDNA polymerases of different species and in DNA polymerases of families A and B. A mutation of the corresponding Tyr in adenovirus-5 DNA polymerase effects a dramatic decrease in polymerase activity (of more than 90%) (Liu, Naismith, and Hay 2000).

In the adPEO patients the presence of the normal POLG allele prevents depletion of mtDNA. In postmitotic tissues however mtDNA is still constantly replicating and the mutated POLG may lead to accumulation of multiple deleted mtDNA molecules because of its ongoing activity with a 10-20 fold less efficient polymerisation of mtDNA and a consequently 10-20 fold increased risk of slippage-mispairing between small direct repeats, that occur normally as polymorphisms of the wild type mtDNA sequence. This is believed to be due to pausing or stalling of replication or to a slowed polymerisation rate of the defective POLG, which would leave the mtDNA in an single stranded configuration for a longer time. With time deleted mtDNA molecules have a replicative advantage over wild-type molecules since they are shorter and hence they are preferentially amplified.

We also sequenced POLG in 2 families with an inheritance pattern that is most compatible with autosomal recessive PEO (arPEO) (Van Goethem *et al*., 2001). In our family B the proband is a compound heterozygote for 2 missense mutations, which predict substitution of Ala with Thr at codon 476 (A467T) and of Leu with Arg at codon 304 (L304R). This family has a clinical onset at ages 16-25 years and in 1 case onset was with a generalised muscular dystrophy (Van Goethem *et al*., 1997). All three affected family members had severe psychiatric illness. 2 cases died at age 38-39 years due to respiratory insufficiency complicated by bronchopneumonia.

In another family with arPEO, family C, the clinical phenotype is still different with onset at ages 20-30 years with signs of predominantly sensory axonal peripheral neuropathy. Only a few decades later the core symptoms of PEO and generalised muscle weakness develop. These symptoms are preceded by dysarthria and disabling dysphagia and clinical differential diagnosis with oculopharyngeal muscular dystrophy is difficult. In this family both patients were compound heterozygotes for the A467T mutation and for another missense mutation predicting a substitution of Arg with Pro at codon 3 (R3P) (Van Goethem *et al*., 2001).

All these mutations in the Belgian arPEO families do not involve POLG exo or pol motifs but are evolutionary conserved residues in POLG in several species.

Further studies of POLG are required to elucidate the functional role of these mutations in PEO pathogenesis.

Recently several investigators have identified mutations in other nuclear genes that predispose to PEO and multiple mtDNA deletions.

Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE) is an autosomal recessively inherited disorder characterized by PEO, severe gastrointestinal dysmotility, peripheral neuropathy, cachexia, diffuse leukoencephalopathy on brain MRI and mitochondrial dysfunction (Hirano *et al*., 1994). Southern blot analysis of the patients' muscle often shows multiple mtDNA deletions and/or depletion but this is not always the case. MNGIE was mapped to the chromosome 22q13.32 region (Hirano *et al*., 1998). The disease is caused by mutations in the thymidine phosphorylase gene (TP) located in this region (Nishino, Spinazzola, and Hirano 1999). TP is a multifunctional enzyme which catalyses the breakdown of thymidine to be reutilised for dTTP synthesis in the thymidine salvage pathway. In MNGIE, TP mutations lead to a decrease in the enzyme activity down to 5% of controls. As TP regulates the dTTP levels of cells for DNA synthesis the mutated TP leads to aberrant extracellullar thymidine pools with much higher plasma levels of thymidine than in normal individuals. This could affect the balance of the intramitochondrial nucleoside and nucleotide pools and hence lead to generation of mtDNA deletions and to depletion of mtDNA by impairment of mtDNA replication.

In Italian adPEO patients Kaukonen *et al*. identified mutations of the adenine nucleotide translocator 1 (ANT1), which is the heart and muscle-specific isoform of the ADP/ATP transporter (Kaukonen *et al*., 2000). The ANT1 gene is located on chromosome 4q35. ANT1 determines the rate of ADP/ATP flux between the mitochondrion and the cytosol. It is possible that in patients with mutations of ANT1 intramitochondrial ATP depletion leads to the occurrence of multiple mtDNA deletions. An alternative explanation would be that the mtDNA deletions as in MNGIE originate from disturbed dNTP pools.

In a Finnish family adPEO has been mapped to chromosome 10q24 (Suomalainen *et al*., 1995). Later the gene was localised to a 7 cM critical interval (Li *et al*., 1999). A search for plausible candidate genes in this region lead to the discovery of *C10orf2* (Spelbrink *et al*., 2001). This gene encodes Twinkle, a novel mitochondrial protein with structural similarity to phage T7 gene 4 primase/helicase and other hexameric ring-helicases. Twinkle co-localizes with mtDNA in mitochondrial nucleoids. It has been proposed that the different coding-region adPEO mutations in Twinkle also

cause mitochondrial dNTP imbalance thereby affecting mtDNA replication and/or repair.

Conclusion

The last three years have provided knowledge of the first genes that are involved in mtDNA maintenance. The common defective mechanism that leads to the development of multiple mtDNA deletions in mutations of ANT1, TP or C10orf2 may be an imbalance in the mitochondrial deoxynucleotide pool. This may cause stalling of the mitochondrial DNA polymerase gamma. Our finding of mutations in the mtDNA polymerase gamma both in families with adPEO and in families with arPEO confirms the earlier hypothesis that the mtDNA replication machinery is involved in at least some cases of PEO with mtDNA deletions inherited as a Mendelian trait.

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