Dysfunctional mitochondrial maintenance: what breaks the circle of life?

Despite being recognized as the first Mendelian-inherited mitochondrial disorders (Zeviani et al., 1990), it has taken over a quarter of a century to realize that disorders of mitochondrial DNA maintenance form a large proportion of the patients seen in adult neurogenetics clinics. Most conditions were initially identified in families presenting with the ‘classical’ mitochondrial phenotype of late-onset chronic progressive external ophthalmoplegia and bilateral ptosis. However, several papers published in Brain in the last 2 years have shown that the field may have been barking up the wrong (phenotypic) tree. The clinical manifestations associated with disorders of mitochondrial DNA maintenance are proving to be much more heterogeneous than was initially understood; and, interestingly, one of the more recently implicated nuclear genetic defects, OPA1, is a major player in the stability of the mitochondrial network. This principle comes to the fore yet again in this issue of Brain, strongly suggesting that the burden of human disease associated with the accumulation of somatic mitochondrial DNA abnormalities and disturbed mitochondrial dynamics will expand further, whilst providing valuable mechanistic insight of much broader pathophysiological relevance.

First defined at the molecular level in 2001 (Van Goethem et al., 2001), large pedigrees with autosomal dominant chronic progressive external ophthalmoplegia due to mutations in POLG are well established and now feature routinely in neurology and ophthalmology textbooks. POLG codes for the only DNA polymerase present within mitochondria, polymerase γ, and mutations in this nuclear gene lead to point mutations and the accumulation of mitochondrial DNA deletions throughout life (Chan and Copeland, 2009). A combination of clonal expansion and recurrent mutation drive the level of these mutations upwards within individual cells (see Box 1 for a detailed explanation). When the percentage level exceeds a critical threshold, the cells express a biochemical defect that can affect one or more components of the respiratory chain, leading to a defect of ATP synthesis and the generation of reactive oxygen species, both of which have been implicated in the pathophysiology of this group of disorders. Interestingly, mice with POLG mutations also accumulate mutations of mitochondrial DNA; and they age prematurely (Trifunovic et al., 2004; Kujoth et al., 2005). But the role of the secondary mutations and reactive oxygen species production remains contentious in the ageing process (Trifunovic et al., 2005; Ameur et al., 2011).

Other proteins directly involved in mitochondrial DNA replication include the mitochondrial DNA helicase Twinkle (PEO1: Spelbrink et al., 2001), the accessory polymerase subunit p55 (POLG2: Longley et al., 2006) and the adenine nucleotide translocase (ANT1). Further culprits are indirectly involved by maintaining the pool of nucleoside mitochondrial DNA ‘building blocks’ (TK2, DGUOK, RRM2B, TYMP: Nishino et al., 1999; Mandel et al., 2001; Saada et al., 2001; Bourdon et al., 2007). Mutations in all these genes were mapped in families with well-documented histochemical or biochemical ‘mitochondrial’ phenotypes; and were associated with secondary mitochondrial DNA deletions, point mutations, or the loss of mitochondrial DNA (deletion), through relatively easily understood mechanisms. However, these secondary mitochondrial DNA defects have also been described in a number of other neurogenetic diseases, not thought to be primary disorders of the respiratory chain.

Two papers previously published in Brain (Amati-Bonneau et al., 2008; Hudson et al., 2008) paved the way for the subsequent definitive clinical description of ‘dominant optic atrophy plus’ (DOA+) due to mutations in OPA1 (Yu-Wai-Man et al., 2010). The initial description emphasized extra-ocular features in ~20% of patients harbouring OPA1 mutations with deafness, peripheral neuropathy, cerebellar ataxia, proximal myopathy and chronic progressive external ophthalmoplegia; however more detailed pheno-typing suggests that most, if not all, OPA1 patients have evidence of multi-system neurological dysfunction (Yu-Wai-Man et al., 2010; Baker et al., 2011). Thus, although progressive visual failure due to optic nerve degeneration remains a cardinal feature of the disease, the disorder should really be considered as a classical multi-system mitochondrial disorder. Patients with OPA1 mutations harbour cytochrome c oxidase-negative fibres and multiple deletions of mitochondrial DNA in muscle, even in the absence of clinically obvious extra-ocular features. Therefore, the question remains: are these secondary mutations involved in the pathogenesis, or do they represent collateral damage, an epiphenomenon secondary to mitochondrial dysfunction? Part of the problem relates to the inherent difficulty in gaining access to retinal ganglion cells from affected individuals, but studies in two opa1 mouse models [one first described in Brain (Alavi et al., 2007)] indicate that the multiple deletions of mitochondrial DNA are not primarily responsible for the optic neuropathy (Alavi et al., 2009;
Yu-Wai-Man et al., 2009). On the other hand, the presence of cytochrome c oxidase-negative fibres and multiple mitochondrial DNA deletions in skeletal muscle do correlate with the severe multi-system disorder, implicating a pathogenic role (Yu-Wai-Man et al., 2012). Thus, clonal expansion appears to be important for the extra-ocular features. But how does this occur? The family now described by Cécile Rouzier and colleagues (Rouzier et al., 2012) adds weight to an emerging mechanism.

Rouzier et al. (2012) describe a Tunisian family with a multi-system neurological disorder characterized by optic atrophy, an axonal sensorimotor neuropathy and cerebellar ataxia. Skeletal muscle biopsies revealed abnormally high levels of cytochrome c oxidase-deficient fibres and multiple mitochondrial DNA deletions. Having excluded known mitochondrial DNA maintenance genes, the authors sequenced MFN2, which is known to cause the axonal form of Charcot–Marie–Tooth disease (CMT2A), and the form of Charcot–Marie–Tooth with associated optic atrophy (hereditary motor and sensory neuropathy, HMSN type VI). Identifying a novel heterozygous mutation in the GTPase domain, they provide evidence to support pathogenicity, including the rather intriguing finding that the missense mutation reduces the level of the Mfn2 protein. Subsequent work in fibroblast cultures showed that the MFN2 mutation causes mitochondrial fragmentation. Thus, the clinical, histochemical and morphological abnormalities are strikingly similar to those observed in OPA1 patients, pointing towards a common mechanism driven by mutations in the highly conserved GTPase domain, the ‘business end’ of these two evolutionarily closely related dynamin membrane proteins, with OPA1 and MFN2 operating in tandem to mediate mitochondrial fusion.

In broad terms, there are two possible explanations. Rouzier et al. (2012) explore the hypothesis that the observed defect of mitochondrial fusion leads to an increased mitochondrial DNA mutation rate, and provide some supporting evidence that the ability to repair stress-induced mitochondrial DNA damage is compromised. However, the other possibility is that, rather than increasing the number of de novo mutations, the mitochondrial fragmentation simply accelerates the accumulation of pre-existing age-associated somatic mitochondrial DNA mutations. Modelling studies based on population genetic theory provide support for the latter mechanism, because any partitioning of the mitochondrial pool will reduce the ‘effective population size’ of mitochondrial DNA molecules, thus accelerating segregation (Elson et al., 2001; Payne et al., 2011). A detailed catalogue of the secondary mutations will help to separate these two possibilities, particularly if the mutational spectra in patients harbouring MFN2 and OPA1 mutations resemble those seen in much older normal healthy individuals.

Given the shared mechanism, why are the phenotypes of OPA1 and MFN2 not identical? Rouzier et al. (2012) point out that, unlike OPA1, their patients do not have chronic progressive external ophthalmoplegia. It is, however, early days. Chronic progressive external ophthalmoplegia is a late feature of DOA+ due to OPA1 mutations, and only one of the MFN2 patients was over 50 years of age. Quantitative assessment of extra-ocular motility may provide an early clue of latent progressive external ophthalmoplegia. Moreover, even within one MFN2 family, the phenotype is highly variable, with subclinical disease in mutation carrier, mirroring recent findings in OPA1 (Baker et al., 2011). Opa1 mouse studies show that numerous cellular and molecular mechanisms can be involved, probably because these large complicated proteins can have different functions in particular contexts (Alavi et al., 2009; Yu-Wai-Man et al., 2009). It is, therefore, entirely understandable that there will be phenotypic differences—but our prediction is that the distinction will blur as more patients are studied.

The accelerated clonal expansion of acquired somatic mitochondrial DNA mutations is emerging as a common mechanism leading to cellular dysfunction and disease in several different contexts—from the single-gene neurogenetic diseases discussed above, through to sporadic neurological and neuromuscular disorders including idiopathic Parkinson’s disease, multiple sclerosis and sporadic inclusion body myositis (Moslemi et al., 1997; Bender et al., 2006; Mahad et al., 2009). Recent work also implicates the same mechanism in the neurological complications of anti-HIV drug toxicity (Payne et al., 2011), with broader implications for the healthy ageing process. Families with rare monogenic disorders are therefore still teaching us about fundamental disease mechanisms that turn out to have much broader relevance; and with the vast potential unleashed by the widespread use of whole exome and whole genome sequencing, their time is now.

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Box 1 How do mitochondrial DNA mutations ‘clonally expand’ to cause a biochemical cellular defect?

Mitochondrial DNA is a high copy number genome, with up to 100 000 molecules in an individual cell. Unlike nuclear DNA, mitochondrial DNA is continuously being destroyed and copied independently of the cell cycle (i.e. relaxed replication). By chance, a single mitochondrial DNA mutation may arise in a cell and, instead of being destroyed, it may be copied more than once. At any one time, relaxed replication could shift the level of the mutation up or down through ‘random drift’. However, for mitochondrial DNA deletions, there is some evidence that the small size of the deleted molecule allows them to replicate more frequently (Diaz et al., 2002), driving mutation levels upward. Occasionally, the original mutation repopulates the entire cell, eventually reaching high enough levels to cause a biochemical defect. This process is referred to as clonal expansion, and can be observed both in mitochondrial disorders and normal ageing. In the latter situation, the clonal expansion of somatic mitochondrial DNA mutations is a normal age-related biological phenomenon accounting for the small percentage of cytochrome c oxidase-negative fibres observed in muscle biopsies taken from healthy individuals over the age of 60 years.
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