One complex world of mitochondrial parkinsonism

Rare neurological diseases are back in the spotlight (Rohn, 2013). Technological advances linked to massively parallel ‘second generation’ sequencing have catalysed an upsurge of interest in monogenic disorders (Singleton, 2011), leading to an unexpected and unprecedented explosion in disease gene discovery (over 20/month in the early part of 2013, of which Brain has published its fair share). These advances are clearly important for the families and patients concerned, providing a clear diagnosis culminating years, and sometimes decades of investigation. This new knowledge enables reliable prognostic and genetic counselling, and disease prevention through prenatal diagnosis. However, there is now additional excitement based on the realization that, in many instances, discovering a new gene responsible for an esoteric familial disorder can have much greater significance, casting light on a disease mechanism relevant for the common diseases seen in general neurological practice.

These observations have not escaped the attention of the pharmaceutical industry and Governments (Rohn, 2013). Both now recognize the importance of the common phenotypes seen as part of a rare disease. The precision offered by a molecular diagnosis allows the assembly of near homogenous patient cohorts. When deeply phenotyped over time, these cohorts provide an ideal test bed for novel treatments targeting the known mechanism. If successful, this provides the rationale for much more expensive randomized controlled trials manipulating the same biochemical pathway in patients with a more common disorder sharing some of the clinical characteristics of the rare disease. Although discovered in the ‘pre-exome’ era, mutations in POLG raise just this possibility. POLG mutations are a rare cause of neurological disease, but the tantalizing observation of L-DOPA responsive parkinsonism in some patients (Luoma et al., 2004) rekindled interest in the role of the mitochondria in idiopathic Parkinson’s disease, and raised the possibility of testing new mitochondria-enhancing antiparkinsonian drugs in POLG patients.

In humans, POLG is found on chromosome 15q25 and codes for the only DNA polymerase found in mitochondria, polymerase gamma (Copeland, 2010). The first pathogenic mutations in POLG were described in 2001 in several Belgian families with adult-onset autosomal chronic progressive external ophthalmoplegia (Van Goethem et al., 2001): two had a clear dominant disorder, and one appeared to be recessive, but both were associated with the presence of multiple deletions of mitochondrial DNA in skeletal muscle biopsies. Subsequent candidate gene analysis revealed recessive POLG mutations in the majority of children with the often fatal Alpers-Huttenlocher syndrome (Naviaux and Nguyen, 2004), a totally different disease presenting in infancy with encephalopathy and liver failure, the latter often precipitated by sodium valproate. However, in Alpers-Huttenlocher syndrome, the POLG mutations were associated with loss (or depletion) of mitochondrial DNA in affected tissues. Even more curiously, recessive POLG mutations were then seen in adults presenting with sensory and cerebellar ataxia, leading to overlapping acronyms, including sensory ataxic neuropathy with dysarthria and ophthalmoplegia (SANDO, Van Goethem et al., 2003); and mitochondrial recessive ataxia syndrome (MIRAS, Hakonen et al., 2005), both linked to multiple deletions of mitochondrial DNA. As the number of patients increased, the boundaries between these apparently distinct syndromes became increasingly blurred, revealing a spectrum of POLG phenotypes presenting from ‘the cradle to the grave’ (Horvath et al., 2006). It remains unclear why often the very same POLG mutations can cause very different phenotypes. This topic was the subject of a recent paper in Brain from Rita Horvath et al. (Neeve et al., 2012).

Shortly after the first description, Anu Suomalainen and colleagues noted the surprisingly high frequency of parkinsonian features in the elderly relatives of patients with classical autosomal dominant progressive external ophthalmoplegia (Luoma et al., 2004). The parkinsonism segregated with POLG mutations in these, and other families (Hudson et al., 2007). At around the same time, several laboratories described mitochondrial defects in the substantia nigra neurons of post-mortem brains from patients with idiopathic Parkinson’s disease, linked to the high levels of somatic mitochondrial DNA deletions (Bender et al., 2006; Kraytsberg et al., 2006). Thus, the circumstantial evidence suggested that mitochondrial DNA mutations in single cells contributed to the pathogenesis of parkinsonism—the idea being that de novo mitochondrial DNA deletions were generated within neurons during life, and that these ‘clonally expanded’, eventually replacing wild-type (normal) mitochondrial DNA within the cell. The mitochondrial DNA deletions removed genes that were critically important for intra-mitochondrial protein synthesis, leading to a defect of mitochondrial respiration and bioenergetic failure. In the short term, the biochemical defect would lead to neuronal dysfunction, but in the longer term, this was thought to cause neurodegeneration in the substantia nigra pars compacta, the
primary pathological target in idiopathic Parkinson’s disease. Interestingly, subsequent work showed that the mitochondrial DNA deletions found in the substantia nigra pars compacta of POLG patients were very similar to those found in idiopathic Parkinson’s disease, and even healthy aged brains, albeit at higher levels in Parkinson’s disease (Reeve et al., 2008). This implicates a common mechanism that was enhanced by a particular genetic background. Three papers in the current issue of *Brain* provide observations in support of this hypothesis.

Two papers submitted at almost the same time were based on observations in human brains (Palin et al., 2013; Tzoulis et al., 2013). Eino Palin and colleagues from Helsinki studied post-mortem tissue from Finns with two different autosomal disorders known to cause secondary mitochondrial DNA mutations (so-called disorders of mitochondrial DNA maintenance) (Palin et al., 2013). All of the patients with POLG and C10orf2 (Twinkle) mutations showed a significant loss of dopaminergic neurons in the substantia nigra, which was most severe in the POLG cases. They did not observe α-synuclein positive inclusions or Lewy bodies, considered the hallmark of idiopathic Parkinson’s disease, nor any tau-positive neurites. Immunochemistry revealed a profound and preferential loss of respiratory chain complex I subunits in mesencephalic neurons in all of the cases studied, whether or not there was clinical Parkinsonism.

In a more extensive study of POLG cases, Charalampous (Haris) Tzoulis et al. (2013) carried out pre-mortem (n = 5) and post-mortem (n = 6) assessments of 11 Norwegians. Like Palin et al. (2013) they also saw extensive nigrostriatal degeneration, but paradoxically, their patients showed no parkinsonian symptoms or signs. How can we resolve these conflicting data? One possible explanation is that the Norwegian patients were studied at a less advanced stage, and had sufficient functional reserve to compensate for the neuronal loss in the substantia nigra pars compacta. Alternatively, as Tzoulis et al. (2013) speculate, cerebellar and thalamic dysfunction could have masked the extrapyramidal features in their patients. If the Finnish patients were at a more advanced stage of the disease, these compensatory mechanisms would no longer be effective, thus revealing an underlying parkinsonian syndrome. This complicated mechanism could explain why the complex I defect in the substantia nigra pars compacta did not directly correlate with the parkinsonian syndrome.

Together, these two studies demonstrate both the strengths and weaknesses of a clinico-pathological approach: despite being directly relevant to the human condition, observations in each brain only provide a single snap-shot in time. This can be misleading because the disease is being studied long after the first horse has bolted, when the primary mechanism may no longer be relevant. For some tissues and organs it is possible to take biopsies early in the clinical course—perhaps even before symptoms develop. This can be enormously revealing and, for example, is one reason why we know so much about the pathogenesis of muscular dystrophies. However, this is not an option for diseases that primarily affect the brain. Under these circumstances, an animal model of the disease can be enormously valuable.

At the opposite end of Europe, Perier et al. (2013) were studying a transgenic mouse transmitting a POLG mutation (D257A) predicted to affect the proof-reading capacity of polymerase gamma. They observed a time-dependent accumulation of mitochondrial DNA deletions in several tissues, including the brain. Interestingly, only a few substantia nigra neurons showed a defect of cytochrome c oxidase (COX, respiratory chain complex IV) activity, and these contained very high levels of deleted mitochondrial DNA (~60%). Neurons containing lower levels of deleted mitochondrial DNA (<60%) had an increased mitochondrial DNA content, enhanced mitochondrial respiration, greater striatal dopamine levels, and were apparently resistant to mitochondrial neurotoxins. Although the mice did not completely recapitulate the human disorder, these findings support the existence of endogenous compensatory mechanisms in mammalian neurons, perhaps explaining the late presentation of parkinsonian features in only a small proportion of POLG patients.

A similar compensatory mechanism has been postulated in the skeletal muscle of patients with primary inherited mitochondrial DNA diseases, with some support from serial muscle biopsy data in humans (Durham et al., 2006). Here, the mitochondria proliferate in response to the accumulating genetic defect (the so-called ‘sick mitochondrion hypothesis’), leading to the formation of ragged-red fibres due to the sub-sarcolemmal accumulation of mitochondria. In the short term, this will increase the mitochondrial DNA content, with a corresponding increase in the wild-type compensating for the mutation and preventing a biochemical defect from developing. However, in the long term, this mechanism leads to the inevitable amplification of mutant species, the loss of wild-type genomes, and the subsequent respiratory chain defect (Chinnery and Samuels, 1999). In keeping with this, Tzoulis et al. (2013) observed reduction in mitochondrial DNA content in human substantia nigra pars compacta neurons, perhaps because these neurons had crossed the rubicon from compensation to decompensation, explaining the cell loss. It will be fascinating to see whether substantia nigra pars compacta neurons in aged POLG D257A mice undergo a similar fate.

Only by combining the data from these three different studies are we starting to make headway in understanding the complex pathogenesis of the parkinsonism due to POLG mutations, and particularly how this evolves over time—but several questions remain. Although there may be common mechanisms underpinning the parkinsonism in different disorders of mitochondrial DNA maintenance (e.g. due to POLG and C10orf2 mutations), we cannot be sure of this at present, and even within the same gene, dominant and recessive alleles could behave differently. These genotypic differences could explain some of the discrepancies between the POLG cases reported in *Brain* (Palin et al., 2013; Tzoulis et al., 2013). Mechanistically, it would be extremely interesting to study complex I immunocytochemically in the *POLG* D257A mice. Both Palin and Tzoulis observed a selective defect in respiratory chain complex I abundance in the human substantia nigra pars compacta. The complex I defect is understandable, because complex I genes account for ~40% of the coding region of mitochondrial DNA and are usually disrupted by mitochondrial DNA deletions. However, the deletions also often involve transfer RNA genes that can affect the synthesis of other mitochondrial DNA-encoded respiratory chain subunits (complex III or IV, COX). It is not clear why these were spared in both human studies, particularly given the observations in
idiopathic Parkinson’s disease where COX defects are a frequent finding (Bender et al., 2006; Kraysberg et al., 2006). However, if complex I is particularly sensitive to mitochondrial DNA deletions in the POLG257A mice, then it is likely that the mitochondrial deficit will be much more extensive than that documented by Perier et al. (2013) using COX histochimistry. If correct, this would add weight to their important observation that compensatory mechanisms are at play, and that these prevent neuronal loss.

The abnormalities of this one respiratory chain complex (complex one, NADH:ubiquinone oxidoreductase, EC 1.6.5.3) hark back to much earlier documentation of complex I defects in the brains of patients with idiopathic Parkinson’s disease (Schapira et al., 1990), and of drug-induced parkinsonism caused by the complex I inhibitors: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1984) and pesticides including rotenone (Betarbet et al., 2000). Complex I is emerging as a common mechanistic theme in parkinsonism, with substantia nigra pars compacta neurons being particularly vulnerable to defects in this specific enzyme—but there is still dissent, even in the papers discussed here. The severe and universal defect of complex I observed by Palin et al. (2013) prompted them to conclude that the biochemical defect was not the driving force behind the parkinsonism. Similar observations by Tzoulis et al. (2013) led to entirely the opposite conclusion, that the complex defect was a key early feature preceding the neuronal loss. Weighing up all of the evidence, my own view is that the complex I defect is likely to be a critical step in the pathogenesis of the dopaminergic neuronal loss, but more extensive studies are required to provide definitive proof—and POLG257A and other mice (Trifunovic et al., 2004) provide excellent models to address this issue.

Understanding this level of detail will be critically important if we are to realise the potential offered by rare diseases like POLG disorders. Is the complex I defect key? Can we enhance the compensatory mechanisms? And when would be the best time to intervene? A combined approach studying patients and animal models with POLG mutations will hopefully address these issues, leading to clinical trials using designer therapies monitored by sensitive biomarkers, such as the DAT scan shown by Tzoulis et al. (2013). This will hopefully demonstrate proof-of-principle, and motivate clinicians and the pharmaceutical industry to explore mitochondrial enhancers in idiopathic Parkinson’s disease.

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References

Adrenoleukodystrophy and the mitochondrial connection: clues for supplementing Lorenzo’s oil

The 1992 American film ‘Lorenzo’s Oil’ became a euphemism for finding cures for devastating neurological diseases. Lorenzo’s disease was adrenoleukodystrophy, which is caused by defects in peroxisomal fatty acid beta-oxidation. The effect results in the accumulation of very-long chain fatty acids in several organs, particularly the adrenal cortex, testis and the nervous system. Myelinated tissues are most severely affected, underlying the chief clinical symptoms, which can vary from vegetative state in early childhood to paraparesis in adulthood (Kemp et al., 2012). The genetic cause of adrenoleukodystrophy was identified as recessive mutations in the X-linked ABCD1 gene (Mosser et al., 1993), which codes for a peroxisomal membrane transporter responsible for shuttling very long chain fatty acids into peroxisomes. Although mitochondria are major sites of lipid catabolism, these very long chain fatty acids (≥22 carbons) cannot be metabolized in mitochondria, and need to gain access to peroxisomes to be degraded. Interestingly, not all males with adrenoleukodystrophy develop CNS demyelination and consequent neuroinflammation, suggesting that modifying factors (genetic, epigenetic and environmental) play a role in the CNS symptoms of adrenoleukodystrophy.

Although the pathophysiology of adrenoleukodystrophy is not fully understood, individuals with adrenoleukodystrophy show high levels of saturated very long chain fatty acids—mostly cerotic acid (C26:0)—in affected tissues. Free saturated fatty acids are known inducers of apoptosis and this effect increases with chain length (Artwohl et al., 2009). Accordingly, exposure of oligodendrocytes and astocytes to C22:0, C24:0 and C26:0 (but not C16:0) fatty acids caused cell death within 24h. Likewise, treatment of neural cells with C26:0 was toxic to mitochondria leading to deregulation of intracellular calcium (Hein et al., 2008).

Abcd1 knockout mice have a phenotype that resembles adrenoleukodystrophy and show oxidative damage in the spinal cord months before neuropathological signs appear. More recently, mitochondrial degeneration was found to be a prominent feature in the pathology of this mouse model and this mitochondrial dysfunction was associated with increased oxidative stress (Lopez-Erauskin et al., 2012).

Despite the movie’s relatively happy ending, subsequent studies showed that Lorenzo’s oil, a mixture of unsaturated fatty acids that inhibit elongation of saturated fatty acids, was beneficial mostly to presymptomatic patients with only minor effects if administered after disease onset (Berger and Gartner, 2006). Therefore, adrenoleukodystrophy continues to be a devastating disease without a cure or even effective treatment if not detected early on. Consequently, the search for novel treatments is still active.

Because of the observed mitochondrial and oxidative abnormalities, Morató et al. (2013) reason in the present issue that activating pathways associated with the preservation of mitochondrial biogenesis and antioxidant defences could be beneficial to mouse models of the disease. Activators of this pathway have been shown to improve health in models of mitochondrial diseases (Wenz et al., 2008) as well as of neurodegenerative conditions such as Huntington’s disease (Johri et al., 2012). Among the various candidates to activate this pathway, pioglitazone stands out as an approved treatment as well as a drug capable of crossing the blood–brain barrier. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) and—to a lesser extent—PPARα, modulating expression of genes involved in the control of glucose and lipid metabolism. Although PPARγ is not a specific activator of mitochondrial biogenesis, it increases transcription of some genes coding for mitochondrial proteins together with the PPAR co-activator PGC-1α. Morató et al. (2013) treated Abcd1−/− mice at 12 months of age with pioglitazone corresponding to a dose of 9 mg/kg/day per mouse. They also employed a similar regimen to a more severe model of the disease (Abcd1−/Abcd2−/− mice).

The reduction in transcript levels for PGC-1α, the transcription factors NRF1, TFAM and PPARγ as well as downstream mitochondrial protein-coding genes observed in Abcd1−/− spinal cords were markedly improved with the pioglitazone treatment (Fig. 1).