A polymorphic polymerase

As implied by Greek etymology, polymerases make multiple (poly) identical parts (meroi) of a template. Specifically, polymerase γ (POLG) is in charge of replicating the mitochondrial DNA (mtDNA) faithfully and steadily, so that mitochondria have adequate numbers of mtDNAs to maintain the structure and function of the respiratory chain. Two other terms with the same root apply to POLG: polymeric and polymorphic.

Polymeric refers to the fact that POLG is made of three subunits, one catalytic subunit, POLG proper, with polymerase (i.e. replicating) and exonuclease (i.e. proofreading) activities, and two smaller identical accessory subunits, POLG2, which are responsible for processive DNA synthesis and tight binding of the POLG complex to DNA (Yakubovskaya et al., 2006).

Polymorphic refers to the extraordinary spectrum of clinical phenotypes that have been associated with mutations in the POLG gene and are the subject of two papers in this issue of Brain. Both papers are based on large cohorts of patients, 26 in an Italian-Norwegian study (Tzoulis et al., 2006) and 38 in a collaborative study involving 6 European countries (Horvath et al., 2006). Although one of them focuses on two relatively common POLG mutations (A467T and W748S) whereas the other considers all polymorphisms, in fact 89 of them, both papers come to similar conclusions of great clinical interest.

To put the POLG story in some historical perspective, in 1999, the first nuclear mutations associated with progressive external ophthalmoplegia (PEO) and multiple mtDNA deletions in muscle (a plausible consequence of defective mtDNA replication) identified the first mutations in POLG. This chase had yielded two additional genes responsible for autosomal dominant PEO, ANT1 (Kaukonen et al., 2000) and PEO1 [formerly known as Twinkle (Spelbrink et al., 2001)]. Interestingly, however, POLG mutations could cause both autosomal dominant and autosomal recessive PEO (Van Goethem et al., 2001), the first of many unconventional features associated with changes in this gene. Soon, clinical heterogeneity became apparent, including ataxia, peripheral neuropathy, parkinsonism, psychiatric symptoms, myoclonus epilepsy mimicking MERRF and gastrointestinal symptoms mimicking MNGIE. In most patients, these protein manifestations were associated with PEO, which offered a valuable diagnostic clue, but they were also seen in patients without PEO, resulting in diagnostic riddles (Van Goethem et al., 2003, 2005).

The extreme example of clinical heterogeneity was offered by Alpers syndrome, a severe hepatocerebral disease of infancy associated with mtDNA depletion (a logical consequence of impaired mtDNA replication), first attributed to POLG mutations by Naviaux and Nguyen (Naviaux and Nguyen, 2004), then confirmed by others (Davidzon et al., 2005; Ferrari et al., 2005).

One of the strengths of the two papers in this issue of the journal is that both sets of authors reasonably 'synthesize' the astounding variety of clinical phenotypes by proposing a continuum of multisystemic symptoms and signs varying in onset from infancy to adulthood and in severity from fulminating hepatocerebral syndrome to slowly progressive PEO and ataxia.

Even considering only patients harbouring the A467T and W748S mutations, either as homozygotes or as compound heterozygotes, Tzoulis and co-workers encountered virtually the whole spectrum of symptoms and signs described above. Of special clinical interest are the following observations: (i) onset was commonly in the teens; (ii) epilepsy was the most frequent presenting symptom, often associated with migrainous headache and resulting in intractable status epilepticus; (iii) axonal sensorimotor neuropathy was present in all but one patient; (iv) PEO was frequent but not invariably present and usually appeared after age 20; (v) valproic acid triggered liver insufficiency in 7 out of 8 patients; (vii) one heterozygous mother (harbouring the W748S mutation) developed epilepsy at age 55 and had peripheral neuropathy and parkinsonism, clear evidence of manifesting heterozygosity. Although both mutations affect the 'linker region' between the polymerase and the exonuclease motifs, compound heterozygous patients were more severely affected than both types of homozygous patients and died younger, an interesting but unexplained observation.

As reported in this issue of Brain (page 1674), Horvath and co-workers took a more expansive approach: they included in their study 38 newly diagnosed patients with both dominant and recessive mutations anywhere in the POLG gene, classified them clinically, tried to glean genotype/phenotype correlations, considered the modifying effects of additional nucleotide changes in POLG or other genes, and drew an algorithm to help clinicians find their way from a morass of clinical presentations to POLG mutations. Let us consider these aspects one by one.
As already mentioned, despite the variety of mutations harboured by these patients, their clinical spectrum was not very different from that described by Tzoulis and co-workers (page 1685), ranging from a fatal childhood hepatopathy to a mild clinical syndrome affecting single organs and presenting in adult life. One difference was a higher proportion of patients with mild and adult-onset syndromes. Another was the occurrence of diabetes mellitus and cardiomyopathy, which were not seen in patients with A467T and W784S mutations. However, both groups shared the most common problems, myopathy, ataxia, PEO, sensorimotor peripheral neuropathy and epilepsy.

Regarding genotype/phenotype correlation, it was noted that most patients with severe, childhood-onset presentations had at least one mutation in the linker region and the other in the polymerase domain, whereas disorders starting in adolescence or adult life tended to have mutations in the exonuclease domain. The frequent occurrence of the A467T mutation in childhood-onset cases was confirmed, as was the observation that compound heterozygous patients for this mutation are more severely affected than homozygotes. The authors add a wise cautionary note that the conventional and convenient subdivision of the POLG gene into three distinct regions does not necessarily imply a strict biochemical correlation, as illustrated by the deleterious effects of mutations in the linker region on POLG catalytic efficiency and interaction with the POLG2 subunit (Chan et al., 2005).

A peculiar feature of patients harbouring POLG mutations is that they often have more than the one dominant or two recessive pathogenic mutations required by Mendelian genetics. Thus, although the E1143G change in the polymerase region is innocent enough to be tolerated in homozygosity by normal individuals, its association with the G517V mutation aggravated the clinical expression, as had been observed in another family with the A889T mutation (Hisama et al., 2005). In fact, more than two recessive changes were found in 11 patients, and as many as four in one child, raising two interesting questions: (i) which are the pathogenic mutations? And (ii) can additional changes act synergistically and worsen the phenotype? Interestingly, 9 out of 10 children with hepatencephalopathy presenting in the first year of life were boys, suggesting a gender bias. This agrees with findings in one series of patients with Alpers syndrome (Ferrari et al., 2005), where 7 out of 8 patients were boys, but not with a smaller series (Davidzon et al., 2005), in which 3 out of 4 patients were girls. The deleterious effect of valproic acid in precipitating liver failure in patients with POLG mutations was confirmed in this study.

Given the bewildering variety of clinical presentations due to POLG mutations, the diagnostic algorithm proposed by Horvath and co-workers is a useful tool, albeit with some limitations. The top boxes delineating clinical criteria are no great help in choosing candidates, but this reflects a central message of both papers, that the phenotypic spectrum associated with POLG mutations is extremely wide and still unfolding. After ‘classical Alpers syndrome’ is identified, screening for the A467T mutation in blood is useful but may not be much of a shortcut, as only 6 out of the 8 patients with Alpers syndrome described by Ferrari et al. (2005) and none of the 4 patients reported by Davidzon et al. (2005) harboured this common mutation. In considering the muscle biopsy, the importance of looking for multiple mtDNA deletions and mtDNA depletion not just by Southern blot but also by more sensitive techniques is correctly emphasized. Which calls attention to one of the few flaws of both papers, the relatively little attention paid to the mtDNA abnormalities in the patients described: it would have been interesting if Table 4 in Tzoulis’ paper and Table 1 in Horvath’s paper had included the abundance of mtDNA deletions or the degree of mtDNA depletion in muscle (or liver in patients with Alpers syndrome). But this is nit picking, as, importantly, both papers call to the attention of neurologists and paediatricians a ‘hot gene’, which, when mutated, can cause havoc with almost all functions of the nervous system and at almost all ages.

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