Defining the Mitochondrial POLG-Related Spinocerebellar Ataxia and Epilepsy in Norway

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Mitochondria are the major producers of cellular energy and failure of this process is associated with a range of devastating diseases that affect both children and adults. In addition to causing disease, mitochondrial dysfunction is also implicated in basic cellular processes such as apoptosis, the ageing process and the development of cancer. In addition to their essential role in energy metabolism, mitochondria have their own DNA (mtDNA), a small, maternally inherited genome that encodes 13 subunits of the respiratory chain. The remaining respiratory chain subunits and all other mitochondrial proteins, including those involved with mtDNA replication and homeostasis, are encoded by nuclear genes.

DNA-polymerase γ (pol γ) is the enzyme that replicates and repairs mitochondrial DNA (mtDNA). The enzyme comprises one catalytic subunit (pol γA), and two accessory subunits (pol γB). Pol γA consists of a polymerase (replicating) domain and an exonuclease (proof-reading) domain, separated by a large linker region to which the accessory subunits bind.[1] These proteins are encoded by genes found on the chromosomes. Initially, mutations in the Pol γA gene were identified in families with autosomally inherited, progressive external ophthalmoplegia (PEO).[1] Subsequently, more than 130 pathogenic mutations in Pol γA have been described causing a wide spectrum of neurological syndromes ranging from adult onset PEO / myopathy to severe infantile encephalopathies including Alper's syndrome, parkinsonism and the syndrome of mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), also known as mitochondrial recessive ataxic syndrome (MIRAS),[1,2] that is the focus of this article.

As with all competitive science, our studies were performed on a backdrop of intense activity focussed around nuclear genes causing mitochondrial disease, particularly polymerase gamma. Our work on MSCAE started in 2003 while studying several patients with the phenotype of progressive ataxia, PEO and epilepsy, a combination that strongly suggested a mitochondrial aetiology. Initially, however, while skeletal muscle histochemistry showed scattered COX deficient fibres in some, both biochemical studies and standard genetic studies of mtDNA failed to uncover a defect; respiratory chain activities were normal and both sequencing and Southern blotting of mtDNA showed no evidence of mutation. Help solving this puzzle was provided by colleagues in Newcastle, UK, who were at that time developing a real-time technique for detecting mtDNA rearrangements. With their help, we were able to show that our patients had multiple mtDNA deletions in their muscle. With this confirmation of mitochondrial involvement, we refocused our efforts on elucidating the genetic cause and chose Pol γA as the first candidate. This work, which was also a collaboration with colleagues from Milan, Italy, identified two Pol γA mutations, c.1399G>A (p.A467T) and 2243G>C (p.W748S), in all patients.[2] Further, we established that these mutations were present at high levels in the population of Western Norway with frequencies of 1:100 for each.

Despite the intense focus surrounding polymerase gamma at this time, including the finding that it was responsible for different diseases, including Alper's, parkinsonism and complex forms of ataxia, we were still surprised when we began identifying large numbers of patients with the same two mutations identified in our first study. Patients with ataxia and aggressive epilepsy were referred to us from colleagues both in neurology and paediatrics. In a relatively short time, we collected 26 patients from 20 families all carrying the c.1399G>A or/and c.2243G>C Pol γA mutations, either in the homozygous state or in combination. With such a large material we were able to make a full description of the clinical spectrum and natural history of the syndrome and correlate clinical features with the genotype.[3] This study led us to the discovery that genotype had a major impact on survival; patients who were homozygous for one or other of the mutations had significantly better survival than those who were compound heterozygous in trans (1399G>A/2243G>C). As yet, we are unable to explain the poorer prognosis seen in compound heterozygotes, but have confirmed that the result also holds true when including all patients reported with these two mutations. Our analysis

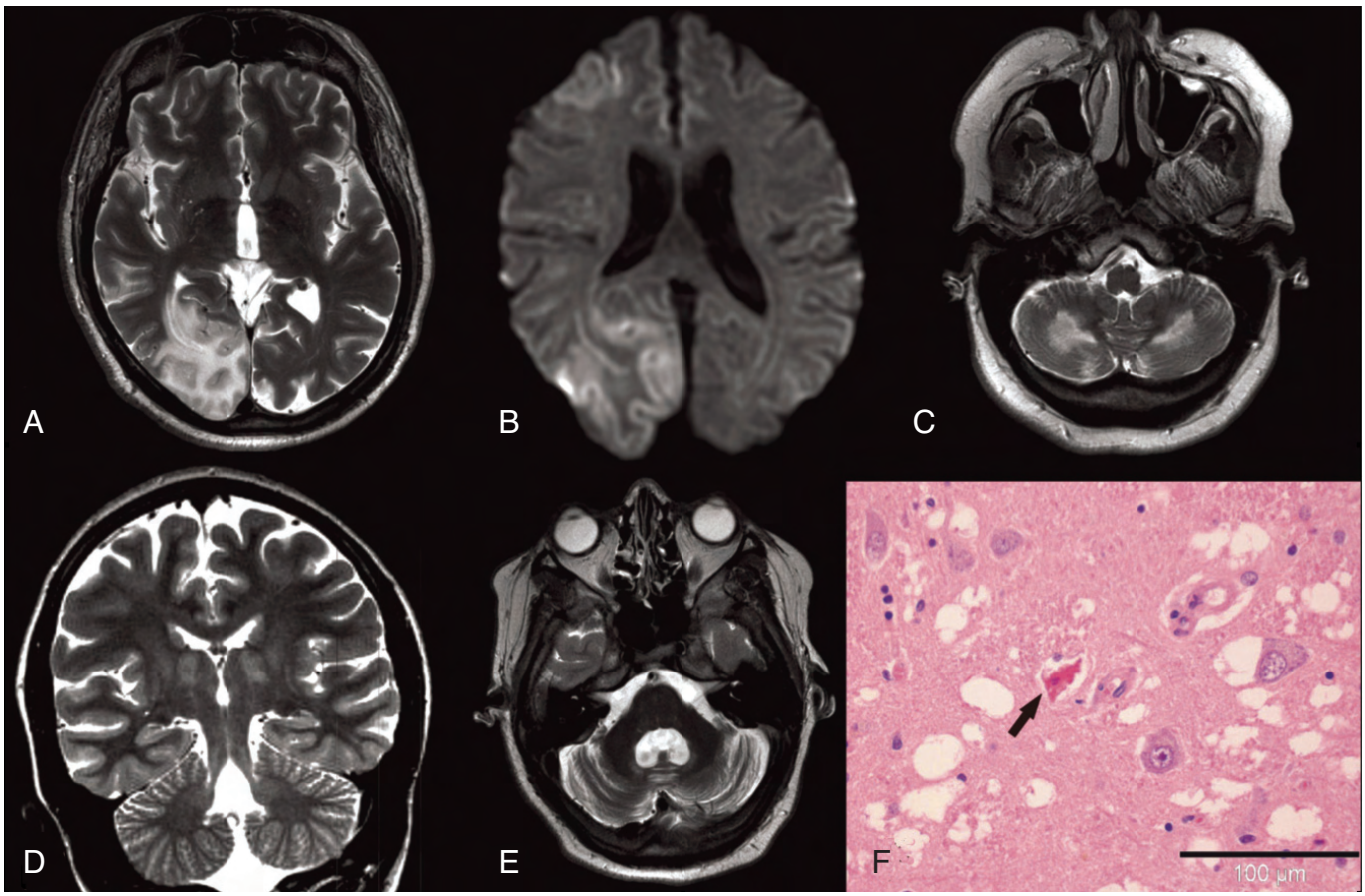

Figure. Representative cerebral MRI and histopathological findings in MSCAE.
A. axial T2 weighted MRI showing a large right occipital stroke-like lesion. B. Diffusion weighted image (b=1000) of the same lesion showing restricted water diffusion in the cortex (confirmed by ADC measurements). C. axial image showing high T2 signal intensity in the cerebellar white matter. D. coronal MRI showing high T2 signal lesions of the thalami. E. axial T2 weighted image depicting atrophy of the cerebellum and dentate nuclei and high T2 signal in the latter. F. hematoxylin and eosin staining of the thalamus with a single necrotic eosinophilic neuron surrounded by normal looking neighbouring cells.

also showed that sodium valproate was highly toxic in this group of patients, leading to fatal hepatic necrosis in several; we suspect, moreover, that the long established link between mitochondrial disease and this anticonvulsant is indeed entirely due to its' specific toxicity for these patients.

Since other groups were also reporting significant numbers of patients with Pol γA mutations and ataxia, we were able to compare their clinical findings with ours. One interesting difference, particularly between patients from Finland and our cohort, was the frequency and destructiveness of the epilepsy occurring in Norwegian patients. Together with our colleague, Bernt Engelsen, we analysed the type and course of the epilepsy and found that our patients developed a specific syndromic epilepsy with a predilection for the occipital lobes. Irrespective of genotype, our patients developed an epileptic syndrome showing initial features of occipital lobe epilepsy.[4] Subsequently, the epilepsy becomes generalised and patients experience simple and complex partial seizures, myoclonus and both epilepsia partialis continua and frequent convulsive status epilepticus. Prognosis was extremely poor for those who developed convulsive status epilepticus and despite heroic treatment, most died and those surviving were usually severely damaged.[4]

The latest phase of our work has focussed on describing the neuroradiological features of the syndrome and elucidating the cellular mechanisms underlying neuronal dysfunction and death. Using a combination of MRI and histopathological investigation we have explored what is happening at the cellular level, focussing particularly on the central nervous system since this is the major site of disease in MSCAE.[5] Our MRI studies identified a specific pattern of brain involvement; findings could be classified into three groups, progressive cerebral and cerebellar atrophy, chronic focal signal changes affecting the thalamus, cerebellar white matter and medulla oblongata, and hyperacute, rapidly evolving stroke-like cortical lesions. The latter occurred during acute episodes of decompensation with rapidly progressive encephalopathy and convulsive status epilepticus. By studying MRI taken very early in lesion evolution, we found that stroke-lesions in MSCAE showed restricted water diffusion suggesting intracellular water sequestration i.e. cytotoxic cerebral oedema. As we did not find evidence of cerebral ischaemia, the cytotoxic oedema was attributed to primary neuronal energy failure caused by respiratory chain dysfunction due to mtDNA damage (Figure 1A-E).

The occurrence of energy deficiency in neurons was further supported by histopathological findings including selective neuronal loss and eosinophilic necrosis (Figure 1F) in the cerebral cortex and thalamus, and cortical laminar necrosis.[5]

Our studies have confirmed Pol γA as a major cause of syndromic mitochondrial disease in adults. We have defined the phenotype and, in particular, the occipital epilepsy and shown that genotype is an important prognostic factor. Lastly, we have confirmed that the hitherto assumed role of energy failure in disease evolution. In the future we hope to elaborate further the cellular consequences of Pol γA mutation in both cellular and animal models in order to identify possible treatments. ◆

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