A variable neurodegenerative phenotype with polymerase γ mutation

Deficiency of mitochondrial DNA polymerase γ (POLG), which is responsible for mtDNA replication and repair, causes mitochondrial diseases including autosomal dominant progressive external ophthalmoplegia (PEO),[1] childhood hepato-encephalopathy (Alpers–Huttenlocher syndrome), adult-onset spinocerebellar ataxia, and sensory nerve degeneration with dysarthria and ophthalmoparesis (SANDO).[2 3]

CASE REPORTS

Two siblings of non-consanguineous healthy Vietnamese parents were normal until childhood (fig 1A,B).

Patient II.2, the 27-year-old male, developed from the age of 15 progressive cerebellar ataxia, neuropathy, restless legs syndrome, on two occasions a transient left hemihypesthesia and four focal myoclonic epileptic seizures and received lamotrigine. He became wheelchair-bound at 22 because of severe ataxia. By 25, he developed dysphagia and weight loss. Muscle strength was preserved. No external ophthalmoplegia or cognitive deficits occurred. After discontinuing lamotrigine, he exhibited grand maux, requiring combination treatment. He became anxious and complained of migrainous headaches and abdominal pain. Soon after, he died after prolonged status epilepticus.

Lactate-ischaemia ergometry at age 20 was unremarkable. In serum, lactate and CK (244 U/l) were mildly elevated. In CSF, protein was elevated to 138–146 mg/dl, and lactate was normal. Liver enzymes, echocardiography and abdominal ultrasound were unremarkable. Electroneurography showed a sensori-axonal neuropathy. Visually evoked potentials (VEP) and vestibulogram were pathological; funduscopy and electroretinogram were normal. EEG was generally slowed with occipital 2–5/s theta/delta waves. MRI revealed mild frontoparietal leucoencephalopathy, mild cerebellar atrophy and T2-hyperintensities in the cerebellar peduncles (fig 1A). MR spectroscopy was normal. Muscle biopsy revealed no ragged red and/or COX negative fibres. Screening for mtDNA deletions in fibroblasts was negative.

Patient II.3, the 23-year-old sister, exhibited from 6 years progressively focal motor and generalised tonic-clonic seizures, delayed psychomotor development, sensori-axonal neuropathy and mild tetraparesis, and from 18 years a cerebellar syndrome. No external ophthalmoplegia occurred. For 4 years on primidone and valproate, no seizures and no hepatopathy were noted. At age 23, she developed refractory status epilepticus, remained comatose with intermittent focal seizures and developed intestinal pseudoobstruction short before death.

Blood tests revealed normal lactate and liver function. CSF analysis showed high protein (264 mg/dl) and lactate levels, and VEP were prolonged. MRI showed very mild leucoencephalopathy, infratentorial atrophy and T2-hyperintensities in the cerebellar hemispheres (arrow), and during status epilepticus transient cortical T2-hyperintensities bilaterally (fig 1A).

Both patients carried the POLG1 missense mutations, c.2284G>A (A862T) and c.2890C>T (R964C) compound heterozygously. The parents and the unaffected brother were heterozygous (fig 1B).

To validate pathogenicity, the two mutations were introduced in the equivalent position of *MIP1* gene, the POLG1 orthologue of yeast *Saccharomyces cerevisiae*. *MIP1* A665, corresponding to POLG1 A862, was converted to T665. *MIP1* Q766, corresponding to POLG1 R964, was first converted to R766 (“humanised”) and then to C766. *Petite* (*MIP1* 2.2% (SD 0.2)) and erythromycin-resistant (Ery^R) mutant (*MIP1* 1.6 (SD 0.4)$\times 10^{-7}$) frequencies served as a measure for mtDNA deletions and mtDNA point mutations respectively.

The mip^{A665T} mutant was unable to complement the *Δmip1* oxidative growth defect and to maintain mtDNA (*petites* 100% (0.0)*). The $mip1^{R766C}$ allele complemented the oxidative growth defect. However, the $mip1^{R766C}$ mutant showed a twofold increase in *petite* (3.8% (0.3)*) and Ery^R mutant (3.9 (0.4)$\times 10^{-7}$*) frequencies compared with the humanised one. The $mip^{A665T}/mip1^{R766C}$ heterozygous strain showed a fivefold *petite* (4.7% (0.2)*) and an 11-fold Ery^R mutant (6.8 (1.1)$\times 10^{-7}$*) frequency increase compared with homozygous wildtype (*petites* 0.9% (0.2), EryR mutants 0.6 (0.1)$\times 10^{-7}$). The asterisk indicates significance ($p<0.01$) compared with *MIP1* or *MIP1/MIP1* in a double tailed z test.

DISCUSSION

Two siblings with phenotypically variable presentation carried compound heterozygous POLG1 polymerase domain mutations, c.2890C>T (R964C) in exon 18 and c.2284G>A (A862T) in exon 16 (fig 1C), which change highly conserved amino acids.

Both mutations have been recently reported in a compound heterozygous subject affected by juvenile onset ataxia-neuropathy syndrome.[3] Moreover, the R964C mutation was detected homozygously in a patient developing lactic acidosis after anti-HIV therapy.[4] Recombinant POLG showed only 14% residual activity, and mtDNA copies were decreased in lymphoblastoid cells after supplementation with stavudine. Furthermore, this mutation is near the polymerase B motive, which is involved in dNTP-binding. It was not present in 26 patients and 100 Thai control samples. We did not detect both mutations in 100 German samples.

In yeast the substitution equivalent to A862T of POLG1 renders Mip1 polymerase unable to maintain mtDNA in vivo. The substitution equivalent to R964C slightly increases mtDNA deletions and point mutations. Yeast carrying both mutations shows a fivefold increase in mtDNA deletions and an 11-fold increase in mtDNA point mutability. We previously showed that an increase in mtDNA deletions was not coupled with a significant increase in mtDNA point mutability in strains carrying MIP1 recessive mutations in heterozygous compounds.[5] The observation that in yeast the point mutability prevails on the extended one could account for what was observed in patients lacking mtDNA deletions.

Disease onset was infantile with epilepsy and mental retardation in the female and juvenile with predominant ataxia in the male. No external ophthalmoplegia and—very unusually for recessive POLG deficiencies—no liver involvement even after valproic acid therapy occurred. In the brother no muscle pathology was detected. The phenotype was a combination of symptoms typical for Alpers syndrome (therapy-resistant epilepsy, stroke-like episodes, but without hepatopathy and blindness) and SANDO (sensori-axonal neuropathy, dysarthria but no ophthalmoparesis). The variability raises the possibility of influential epigenetic factors.

The dramatic deterioration after status epilepticus stresses the importance of early anticonvulsant therapy escalation even if

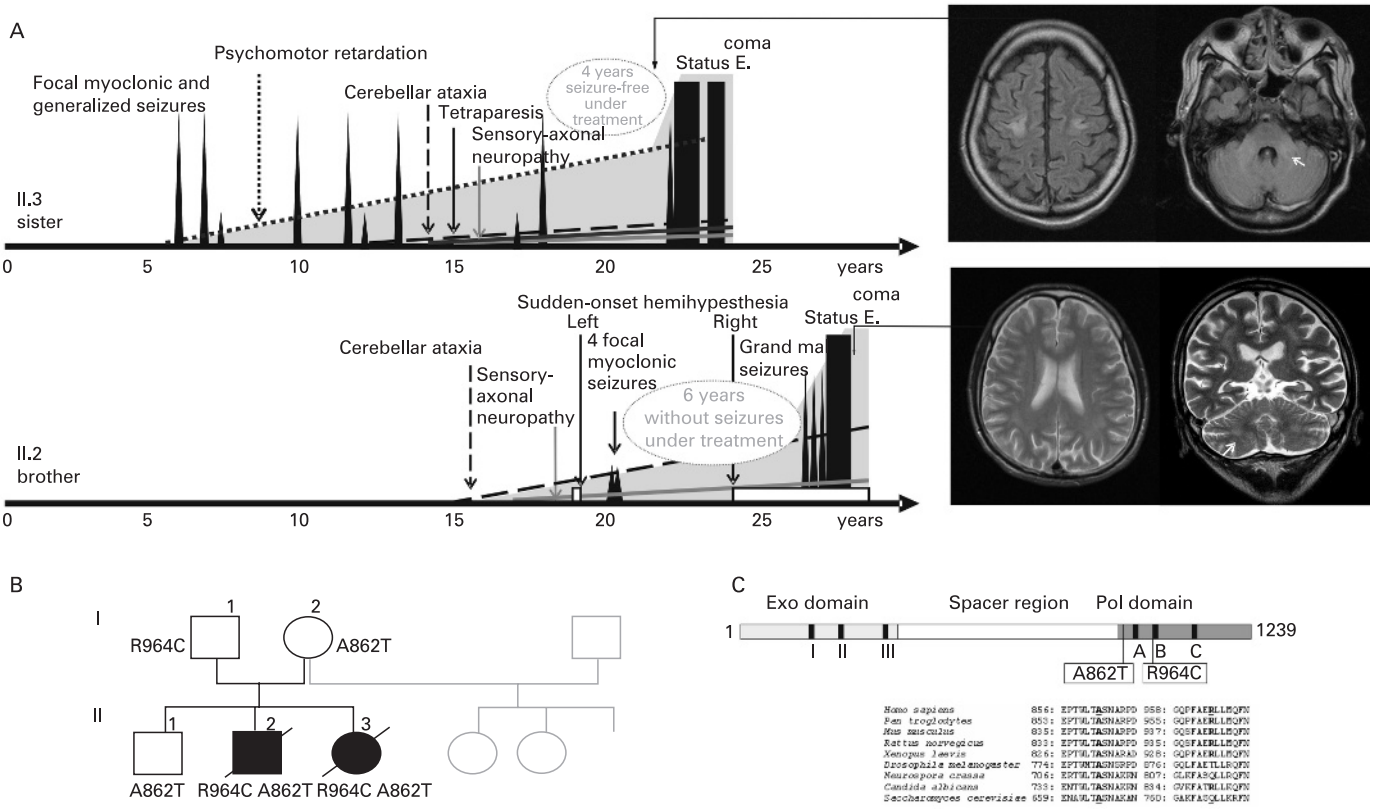

Figure 1 (A) Clinical course of the sister (II.3) and the brother (II.2) with MRI of the sister revealing cortical lesions and cerebellar T2-hyperintensities (arrow) and the brother's MRI showing leucoencephalopathy and cerebellar T2-hyperintensities. (B) Family pedigree with polymerase γ 1 (POLG1) mutations. Squares represent males, circles females, filled symbols affected and slashed symbols deceased individuals. (C) Mutant residues in POLG1 and amino-acid alignment showing evolutionary conservation of altered residues.

seizures are focal and rare. Both patients eventually received coenzyme Q10 and creatine, but therapeutic effects remain uncertain.

Concerning MRI in POLG disease, there are reports of cerebrocortical and cerebellar atrophy, leucoencephalopathy, cortical hyperintensities and focal lesions, partially reversible, in thalami, basal ganglia, occipital poles and in cerebellar hemispheres.[2] Interestingly, both siblings displayed symmetrical cerebellar T2-hyperintensities. Leucoencephalopathy was mild. Cerebellar atrophy was more prominent in the female, not correlating with severity of ataxia. Her transient precentral cortical T2-hyperintensities most likely represent postictal vasogenic oedema.

Carrier frequency studies suggest that the disease is underdiagnosed as having an A467T mutation frequency of 0.69% in British and 0.19% in German controls.[2] Taken together, our cases highlight the importance of genetic testing for POLG disorders also in the absence of external ophthalmoplegia, typical muscle pathology, valproate toxicity and hepatic pathology.

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Acknowledgements: We thank J Schäfer, Department of Neurology, University of Dresden, for the mtDNA testing in fibroblasts and discussion of the case, and S DiMauro, Neurological Institute of New York Columbia University, for discussion of the case.

Funding: Department of Neurology, Charité University Medicine Berlin, Helmholtz Gemeinschaft für Forschungseinrichtung. Telethon-Italy no GGP07019.

Competing interests: None.

Patient consent: Obtained from the patient's family.

Provenance and peer review: Not commissioned; externally peer reviewed.

Received 22 October 2008
Revised 30 December 2008
Accepted 17 January 2009

J Neurol Neurosurg Psychiatry 2009;**80**:1181–1182.
doi:10.1136/jnnp.2008.166066

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doi: 10.1136/jnnp.2008.166066