Reversible valproate hepatotoxicity due to mutations in mitochondrial DNA polymerase γ (*POLG1*)

R McFarland,[1,2] G Hudson,[2] R W Taylor,[2] S H Green,[3] S Hodges,[1] P J McKiernan,[3] P F Chinnery,[1,2] V Ramesh[1]

[1] Newcastle upon Tyne NHS Hospitals Trust, Newcastle upon Tyne, UK; [2] Newcastle University, Newcastle upon Tyne, UK; [3] Birmingham Children's Hospital, Birmingham, UK

Correspondence to: Dr Robert McFarland, Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, 4th Floor, The Medical School, Framlington Place, Newcastle University, Newcastle NE2 4HH, UK; robert.mcfarland@ncl.ac.uk

Parental/guardian informed consent was obtained for publication of the person's details in this report.

Accepted 26 September 2007

ABSTRACT

We report the case of a 2-year-old boy with seizures who developed hepatic failure shortly after commencing sodium valproate. Unexpectedly, liver function returned to normal on stopping the drug. Sequencing of the mitochondrial polymerase γ gene (*POLG1*) revealed four heterozygous substitutions, two of which have been identified in cases of Alpers-Huttenlocher disease.

In 1931 Alpers first described post mortem findings of ''progressive degeneration of gray matter'' in an infant with a rapidly progressive neurodegenerative illness.[1] However, it was Huttenlocher who later recognised the clinical syndrome of psychomotor retardation, intractable epilepsy and liver failure.[2] Explosive onset of seizures (generalised, focal and myoclonic) usually occurs between the ages of 1 and 3 years and patients frequently present in status epilepticus or with epilepsia partialis continua. In many cases the onset of this intractable epilepsy heralds an inexorable and rapid decline resulting in death within months. In addition to the pre-terminal hepatic failure, there are a number of other associated clinical features including developmental delay and regression, hypotonia, cortical blindness, ataxia and in older patients an axonal sensory neuropathy. Although the molecular aetiology of this disease was initially unclear, it has now become apparent that almost all infants and young children with Alpers-Huttenlocher disease have a disorder of oxidative phosphorylation secondary to depletion of mitochondrial DNA (mtDNA). Mutations in three genes (*POLG1*, *DGOUK* and *MPV17*) are responsible for most cases of hepatocerebral mtDNA depletion, but interestingly, only mutations in *POLG1* have been associated with Alpers-Huttenlocher disease, epilepsy not being a feature of mutations in either *DGOUK* or *MPV17*.[3 4] Administration of the anti-convulsant drug sodium valproate has been associated with a fatal hepatopathy,[5] and it has been suggested that young children with Alpers-Huttenlocher disease may be at increased risk of this complication.[6] In these patients discontinuation of sodium valproate has not been associated with an improved clinical course in this fatal condition. Although the mutations identified in our patient have previously been reported as part of a study of the phenotypic presentation of *POLG* mutations,[7] this is the first description of the case and discussion of the important clinical points it raises regarding the investigation and treatment of children with liver failure precipitated by sodium valproate treatment.

CASE HISTORY

A previously well, developmentally normal 2-year-old boy presented with new onset epilepsy following minor head trauma. A CT scan of his brain performed following his head injury was normal, although EEG showed sharp and slow wave focus in the right posterior quadrant. Following an unsuccessful trial of carbamazepine, he was placed on a gradually increasing regimen of sodium valproate, reaching a maximum dose of 25 mg/kg/day. Almost 2 months after commencing this drug he became unwell with persistent vomiting and encephalopathy and was admitted to hospital. His GCS on admission was 3 and his blood sugar unrecordable. He had deranged liver function tests, prolonged clotting, elevated ammonia, and a high plasma lactate (14.8 mmol/l). Sodium valproate was stopped and supportive therapy instituted. He regained normal consciousness after several hours, although plasma lactate remained elevated at 7.9 mmol/l. Brain MRI scan showed abnormal white matter signal in the occipital and medial temporal lobes bilaterally (fig 1A), findings which persisted on a follow-up scan 15 months later (fig 1B). Hepatic dysfunction progressed (table 1), but the child was considered unsuitable for liver transplantation at this time because of a presumptive diagnosis of Alpers-Huttenlocher disease. With conservative management and vitamin K supplementation, his liver function returned to normal over a 6-month period. His epilepsy is currently treated with levetiracetam and seizures are infrequent. In view of the high lactate, seizures and hepatic dysfunction, mitochondrial disease was considered and a muscle biopsy was performed. Blood DNA was investigated for mutations in *POLG1*.

METHODS

Standard histological and histochemical (including sequential cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH)) analyses were performed on frozen sections (10 μm) of skeletal muscle biopsy obtained from the patient's left quadriceps. The activities of individual respiratory chain complexes and the matrix marker citrate synthase were determined as previously described.[8]

Total genomic DNA was extracted from several tissues by standard procedures. Rearrangements of mtDNA were investigated by Southern blot analysis and long-range PCR of skeletal muscle DNA. Sequencing of the entire coding region and adjacent intronic regions of *POLG1* was performed on blood DNA using a fluorescent chain terminating sequencing kit (Applied Biosciences, Foster

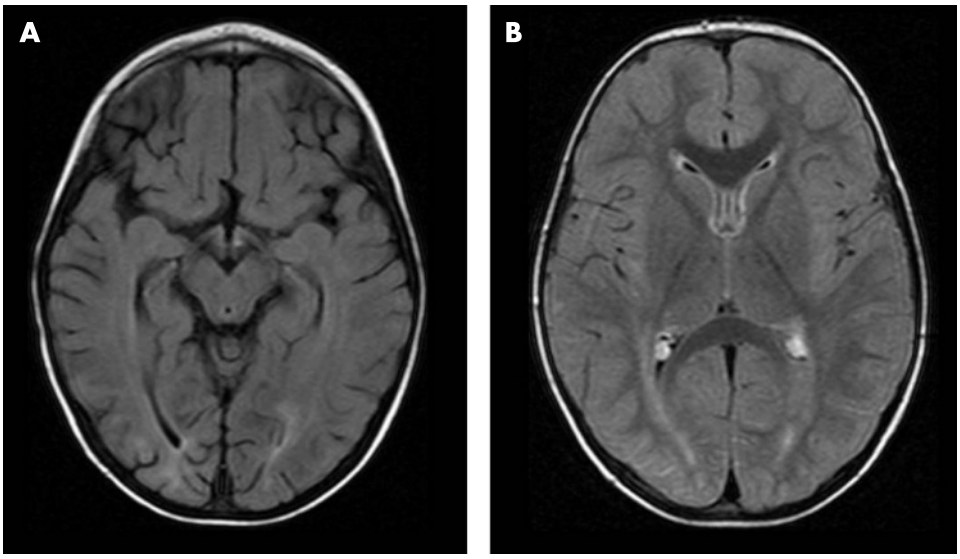

Figure 1 (A) Brain MRI scan demonstrating high signal intensity within occipital and temporal lobes. (B) Follow-up MRI scan demonstrating persistent high signal change 15 months later.

City, CA) and a fluorescent DNA analyser (primers and conditions available online, Applied Biosciences 3100). The sequence obtained was compared with the GenBank reference (accession number: BC050559) and substitutions were confirmed by reverse sequencing.

RESULTS

Muscle biopsy revealed no histochemical or biochemical abnormalities and both Southern blot and long-range PCR were normal. Sequencing of *POLG1* demonstrated four heterozygous substitutions, A467T, E1143G, Q879H and T885S (fig 2). Sequencing of parental DNA confirmed that the patient had inherited the A467T substitution in *cis* with T885S and in *trans* with Q879H and E1143G (fig 2).

DISCUSSION

Sodium valproate is a successful and frequently used first line therapy for a variety of different epileptic seizures and syndromes. Its use in very young patients with neurodevelopmental delay and epilepsia partialis continua has been questioned on the grounds that it may precipitate a neurometabolic decompensation in those with Alpers-Huttenlocher disease, leading to irreversible liver failure.[6]

Orthotopic liver transplantation has been attempted in patients with valproate-induced liver failure, some of whom have later been shown to have Alpers-Huttenlocher disease.[9 10] Although successful engraftment was achieved in most patients, they invariably died a short time later following progressive neurological deterioration. Consequently, valproate-induced liver failure has been considered a contraindication to orthotopic liver transplantation, and this was the case for our patient. Fortunately, his liver failure slowly reversed and 2 years on his epilepsy has been successfully managed without neurological decline. He has an unusual genotype with four different substitutions and this may have influenced the clinical course of his disease. The A467T mutation is known to lower DNA binding affinity and catalytic efficiency of *POLG1*,[11] but the role of the other substitutions is less clear. However, the E1143G mutation has been shown to partially rescue the deleterious effects of the W748S mutation (also associated with Alpers-Huttenlocher disease as well as ataxia and peripheral neuropathy), suggesting it may have a disease-modifying role.[12] The Q879H and T885S substitutions have not been reported in controls, but in the presence of two confirmed mutations it is difficult to be certain of their precise role in the disease pathogenesis. Both occur within the polymerase domain of *POLG1*, a region of the gene specifically affected in Alpers-Huttenlocher disease (http://tools.niehs.nih.gov/polg/index.cfm). Although neither amino acid substitution appears to be severe (amino acid remains hydrophilic), the Q879H substitution does affect a phylogentically conserved site (amino acid position 885 appears to be less well conserved) and is therefore likely to be contributing to disease.

This case illustrates a clinically important variation in the phenotype of Alpers-Huttenlocher disease, where liver failure has previously been considered a pre-terminal event and invariably associated with an inexorable neurological decline.[13] Sodium valproate played a key role in precipitating the liver failure in this case, but the mechanism for this drug effect remains elusive. Based on our observations, we recommend sequencing of *POLG1* in children with valproate-induced hepatic failure, particularly as identification of the E1143G

Table 1 Serial liver function tests of this patient over a 6-month period following presentation with encephalopathy

Investigation	Initial	+3 days	+7 days	+14 days	+21 days	+1 month	+2 months	+3 months	+6 months
APTT (24–39 s)	51	41	–	38	43	38	37	26	37
PT (11–13 s)	34	18	–	20	20	19	18	16	14
γ GT (0–70 U)	56	–	–	–	–	79	71	–	102
ALT (0–45 U)	249	311	247	218	423	128	64	38	39
SBR (0–19 U)	10	19	–	26	73	109	70	48	39
Albumin (34–50 g/l)	23	24	27	24	24	38	31	31	41
Ammonia (15–50 U)	218	42	53	66	58	45	–	58	–

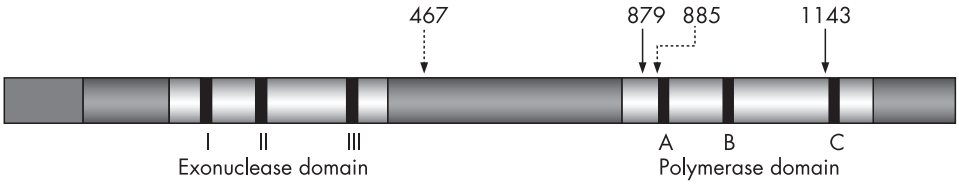

Figure 2 A schematic representation of *POLG1* showing the functional domains of this gene and the sites of mutation in our patient. The patient inherited four mutations in *POLG1*, two (A467G and T885S) from his mother (dotted line) and two (Q879H and E1143G) from his father (solid line).

mutation may indicate a more favourable outcome. Furthermore, we advise that, particularly in young children (<3 years old) with aggressive focal epilepsy, the *POLG1* gene should be sequenced prior to commencing sodium valproate therapy. In situations where this is not possible, then serum lactate, ammonia and liver function should be closely monitored.

Acknowledgements: The authors would like to thank Newcastle University, the Newcastle upon Tyne Hospitals Trust and Birmingham Children's Hospital for their continued support. RMcF is an MRC Clinician Scientist. PFC is a Wellcome Trust Senior Fellow.

Competing interests: None.

REFERENCES

1. **Alpers BJ.** Diffuse progressive degeneration of gray matter of cerebrum. *Arch Neurol Psychiatr* 1931;**25**:469–505.
2. **Huttenlocher PR,** Solitare GB, Adams G. Infantile diffuse cerebral degeneration with hepatic cirrhosis. *Arch Neurol* 1976;**33**(3):186–92.
3. **Mandel H,** Szargel R, Labay V, *et al*. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 2001;**29**(3):337–41.
4. **Spinazzola A,** Viscomi C, Fernandez-Vizarra E, *et al*. MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. *Nat Genet* 2006;**38**(5):570–5.
5. **Koenig SA,** Buesing D, Longin E, *et al*. Valproic acid-induced hepatopathy: nine new fatalities in Germany from 1994 to 2003. *Epilepsia* 2006;**47**(12):2027–31.
6. **Schwabe MJ,** Dobyns WB, Burke B, *et al*. Valproate-induced liver failure in one of two siblings with Alpers disease. *Pediatr Neurol* 1997;**16**(4):337–43.
7. **Horvath R,** Hudson G, Ferrari G, *et al*. Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene. *Brain* 2006;**129**(Pt 7):1674–84.
8. **Kirby DM,** Thorburn DR, Turnbull DM, *et al*. Biochemical assays of respiratory chain complex activity. *Methods Cell Biol* 2007;**80**:93–119.
9. **Kayihan N,** Nennesmo I, Ericzon BG, *et al*. Fatal deterioration of neurological disease after orthotopic liver transplantation for valproic acid-induced liver damage. *Pediatr Transplant* 2000;**4**(3):211–14.
10. **Delarue A,** Paut O, Guys JM, *et al*. Inappropriate liver transplantation in a child with Alpers-Huttenlocher syndrome misdiagnosed as valproate-induced acute liver failure. *Pediatr Transplant* 2000;**4**(1):67–71.
11. **Chan SS,** Longley MJ, Copeland WC. The common A467T mutation in the human mitochondrial DNA polymerase (POLG) compromises catalytic efficiency and interaction with the accessory subunit. *J Biol Chem* 2005;**280**(36):31341–6.
12. **Chan SS,** Longley MJ, Copeland WC. Modulation of the W748S mutation in DNA polymerase gamma by the E1143G polymorphism in mitochondrial disorders. *Hum Mol Genet* 2006;**15**(23):3473–83.
13. **Tzoulis C,** Engelsen BA, Telstad W, *et al*. The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases. *Brain* 2006;**129**(Pt 7):1685–92.