The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases

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We studied 26 patients belonging to 20 families with a disorder caused by mutations in the POLG gene. The patients were homozygous for 1399 G/A or 2243 G/C (giving the amino acid changes A467T and W748S, respectively) or compound heterozygotes for these two mutations. Irrespective of genotype, the patients exhibited a progressive neurological disorder usually starting in their teens and characterized by epilepsy, headache, ataxia, neuropathy, myoclonus and late onset ophthalmoplegia. However, major differences in survival were seen depending on genotype, with compound heterozygotes having a significantly shorter survival time than patients homozygous either for the A467T or W748S (p = 0.006). Epilepsy occurred in 22 of the 26 patients and in the majority of these there was an occipital EEG focus. Episodes of both generalized and focal motor status epilepticus were common and highly resistant to treatment, even with generalized anaesthesia. Status epilepticus was the recorded cause of death in 9 of 11 patients. Liver failure was the sole cause of death in two patients and evolved terminally in six others, all but one of whom were being treated with sodium valproate. Two patients underwent liver transplantation, but only one survived. Delayed psychomotor development and subsequent cognitive decline also occurs. This study demonstrates the clinical spectrum of a disorder that combines features of Alpers’ syndrome and a later onset mitochondrial spinocerebellar ataxia with epilepsy and headache. Patients with this disorder are at high risk of death from status epilepticus and from liver failure, if exposed to sodium valproate. Each mutation appears capable of producing a disorder that is recessively inherited, although we also find evidence in one patient suggesting that heterozygotes may manifest. Compound heterozygotes have a significantly more severe phenotype raising the possibility of a dominant negative effect.

Keywords: mitochondrial; POLG; ataxia; hepatic; Alpers

Abbreviations: mtDNA = mitochondrial DNA; PEO = progressive external ophthalmoplegia


Introduction

Disease due to defects in mitochondrial DNA (mtDNA) may be primary, that is, due to a mutation affecting this genome, or arise secondarily from a defect in a nuclear encoded protein involved in mtDNA homeostasis. Nuclear gene defects affecting mtDNA homeostasis give rise to multiple different mtDNA deletions or a quantitative loss of this genome called mtDNA depletion. Nuclear genes giving rise to multiple mtDNA deletion include the mtDNA polymerase (POLG) (Van Goethem et al., 2001), the adenine nucleotide transporter (1) (Kaukonen et al., 2000) and Twinkle (Spelbrink et al., 2001), a mitochondrial helicase.

The human mitochondrial DNA polymerase is thought to be a heterotrimer consisting of a catalytic subunit (POLG), containing the polymerase and exonuclease activities, and
two accessory subunits (POLG2) thought to be important for processivity (Yakubovskaya et al., 2005). Mutations in the POLG gene cause autosomal dominant and recessive progressive external ophthalmoplegia (PEO) (Lamantia et al., 2002), Alpers' syndrome (Naviaux and Nguyen, 2004; Ferrari et al., 2005), a sensory ataxic neuropathy, dystarthritis and ophthalmopaesiasis (SANDO) (Van Goethem et al., 2003a), parkinsonism (Luoma et al., 2004) and a mitochondrial recessive ataxic syndrome (Van Goethem et al., 2004; Hakonen et al., 2005; Winterthun et al., 2005). While the majority of mutations cause PEO, two mutations, the 1399G/A and 2243G/C giving the amino acid substitutions A467T and W748S, respectively, cause a recessive mitochondrial ataxic syndrome. These amino acid substitutions do not affect the polymerase or exonuclease regions of POLG, but the intervening 'spacer' region.

The A467T mutation is found in different populations and, in combination with other missense mutations, causes recessively inherited PEO. The A467T and W748S, alone or in combination, also cause more complex ataxic syndromes (Van Goethem et al., 2003b; Van Goethem et al., 2004; Hakonen et al., 2005; Winterthun et al., 2005) and Alpers' syndrome (Naviaux and Nguyen, 2004; Ferrari et al., 2005). Recently, the W748S mutation was shown to be a common cause of ataxia in Northern Europe (Hakonen et al., 2005) and we have shown that the combined carrier frequency for the A467T and W748S in the Norwegian population might be as high as 1 : 50 (Winterthun et al., 2005).

This study describes a large series of patients with the A467T and W748S mutations who present with a progressive and often fatal neurological disorder that has features of both mitochondrial spinocerebellar ataxia and Alpers' syndrome.

Patients and methods
We investigated 26 patients (Table 1) of whom 22 came from Western Norway, two from Central Norway and one from Northern Norway. One patient came from Southern Italy. Data on six patients (1a, 2a, 3b, 4b, 13 and 16, Table 1) have been reported earlier but have included to provide a complete review of the clinical syndrome and allow comparison between genotypes. Patients were assessed by at least one of the neurologists involved in this study. For deceased individuals, the case notes were obtained and data analysed. The clinical features of all patients are shown in Table 1. One obligate carrier is also included (27 g). She is heterozygous for the W748S and the mother of a compound heterozygote male (7 g) with progressive ataxia. Screening, both by DHLPC and DNA sequencing, of her whole POLG gene shows no other mutation, but did identify several recognized polymorphisms that were all heterozygous [IVS12 –22T>G; IVS20 –36A>G/–11T>C; IVS143G (3428 A>G); IVS22 –19T>G; 3370 Gdel]. She developed epilepsy at the age of 55 years and when examined at age 72 she had combined sensory and cerebellar ataxia, ptosis, mild limitation of eye movement and features of parkinsonism (with asymmetrical involvement showing bradykinesia, tremor and rigidity).

Onset
Age of onset is variable, ranging from 2 to 36 years (mean 14.5 years) and is similar for all three genotypes (Table 2). If patient 27 is included, the age range is further extended, i.e. 2–55 (mean 16.0 years), however, the majority start in their teens. The first manifestation in 13 patients was epilepsy, associated in three cases with headache. In seven patients, headaches were the first feature and a diagnosis of migraine was considered in six. One child presented with speech delay and four patients presented with an insidious, slowly progressive unsteadiness.

Course
The course is relentless and in several patients rapidly fulminant. Epilepsy, when present, is an early manifestation. Ataxia and peripheral neuropathy develop in the great majority, being absent only in those with the shortest disease duration. Nystagmus is found, particularly in those with ataxia, and appears to worsen after seizures, as does the myoclonus that occurs in 18 of 26 patients. External ophthalmoplegia, when present, develops after the age of 20, with a mean time of onset after presentation of 13.8 years (range 1–30).

Disease morbidity and mortality are closely correlated to the severity of the epilepsy with nine patients dying in
HA = headache, not otherwise classified. MLH = migraine-like headache based either on ongoing clinical evaluation or taken from the patient’s notes. PGU = progressive gait unsteadiness. SD = speech delay. # = Patient 15 was recorded as clumsy when first seen but not investigated further. Epi = epilepsy. Epilepsy is recorded as present (+) or absent (−) and (v) if continuous visual phenomena are present. If the patient has suffered episodes of status epilepticus (status +) and whether these included focal motor activity (fm). Patients with neuropathy are shown with (+) if confirmed electrophysiologically, (±) if only shown on clinical examination. CNF = the finding of cytochrome oxidase negative fibres on muscle biopsy. NAD = no abnormality detected. ND = not done. Ptosis (P/E O (+)) means either or both, and the age first recorded, (−) = not present. Pt i.d. = patient identification number and a letter indicates those to whom they are related. The patients are divided into those homozygous either for the A467T/A467T or W748S/W748S. Kaplan–Meier survival analysis shows that compound heterozygous patients do significantly worse than those who are homozygous either for the A467T/A467T or W748S/W748S. Comparing disease duration (age of onset to age of death or Table 2 Statistical comparison of the three genotypes

<table>
<thead>
<tr>
<th>Sex</th>
<th>A467T/A467T</th>
<th>A467T/W748S</th>
<th>W748S/W748S</th>
<th>P</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>3 (60%)</td>
<td>3 (42.9%)</td>
<td>9 (69.2%)</td>
<td>0.51</td>
<td>15 (60%)</td>
</tr>
<tr>
<td>Male</td>
<td>2 (40%)</td>
<td>4 (57.1%)</td>
<td>4 (30.8%)</td>
<td>0.067</td>
<td>10 (40%)</td>
</tr>
<tr>
<td>Mean age onset</td>
<td>12.4</td>
<td>19.7</td>
<td>12.5</td>
<td>(9.5, 19.3)</td>
<td>(5.5, 19.3)</td>
</tr>
<tr>
<td>Median survival</td>
<td>50</td>
<td>6</td>
<td>26</td>
<td>0.006</td>
<td>42</td>
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Gender, age of onset and survival were analysed for each of the three genotypes; Group (1) A467T/A467T, Group (2) A467T/W748S, Group (3) W748S/W748S. No difference in sex distribution (Fisher exact test) or age of onset (one-way analysis of variance) is seen, but genotype has a significant effect on survival (log-rank test) with the compound heterozygotes having the shortest time from onset to death (graphically represented in Fig. 1A).
current age) showed that compound heterozygotes have a poorer survival rate ($P = 0.006$) (Table 2, Fig. 1A).

**Epilepsy**

Epilepsy is a major feature of this disorder. Both partial and secondary generalized (tonic–clonic) seizures are seen, the commonest being simple partial motor seizures, often affecting an upper limb and neck and evolving into epilepsia partialis continua. In eight patients, epilepsy was preceded or accompanied by visual phenomena consisting of coloured lights, scotomata or ‘blurred vision’. These visual symptoms persist postictally and are, in most cases, correlated with occipital changes on MRI, most often occurring on the right side, but also seen on the left and bilaterally (Table 4). The majority of our patients have an occipital focus on their EEG, although other focal abnormalities are also found and the EEG picture varies between patients and over time in the same individual.

Simple partial motor status epilepticus affecting an upper limb and neck is seen in all three genotypes and interestingly most often affects the left side. These patients have occipital EEG changes and may develop infarct-like changes on MRI (Fig. 2). Both the focal motor and generalized epileptic activity were highly resistant to treatment even with high dose combinations of antiepileptic drugs and, in the case of generalized status epilepticus, treatment with general anaesthesia. Status epilepticus was the recorded cause of death in nine patients (Table 3).

One patient (26) had facial myokymia and later developed asymptomatic, 2 Hz palatal myoclonus. A biopsy from the small intestine was performed to exclude Whipple’s disease. MRI showed high signal lesions (with expansion) of the inferior olives (Fig. 2). She also suffered mild intermittent asymmetrical jerking of the upper limbs and while these movements were synchronous with the facial movements, there were no corresponding EEG changes with either her facial, palatal or limb movements.

**Headache**

In ten patients, headache either was the presenting feature or (in three) started at the same time as the epilepsy. In six, the headaches were suspected to be migraine and in several were preceded and accompanied by transitory visual phenomena such as flashes and scotomata.

**Ataxia**

Ataxia is an early feature even when it is not the cause of presentation. Four patients presented with ataxic symptoms and in a fifth these were documented, but no further investigation performed. The ataxia is due to a combination of cerebellar and sensory deficits, it is slowly progressive, leading to moderately severe disability and wheelchair dependence in surviving patients.

**Peripheral neuropathy**

All patients have a peripheral neuropathy. In all but three, in whom only clinical evidence exists, neurophysiological investigation shows this to be a predominantly axonal sensorimotor neuropathy.
Liver involvement (Table 3)

Liver failure was the major cause of death in two patients (6, 8) and recorded together with status epilepticus in a third (9c). One patient underwent unsuccessful liver transplantation (6). In five patients (11d, 12d, 13, 15, 19f), liver failure was present terminally, in two associated with multiorgan failure (15, 19f). In seven of eight cases, hospital admission had been precipitated by status epilepticus and liver failure appeared after the introduction of sodium valproate. One patient (6) developed liver failure without preceding status epilepticus and another (26) developed liver failure, not in association with a terminal illness, 4 months after starting sodium valproate. Patient 26 later underwent successful liver transplantation. Progressive ataxia and asthenia now dominate her condition, but her seizures appear well controlled and she continues to use sodium valproate together with other anticonvulsants.

Delayed psychomotor development and cognitive decline

Delayed psychomotor development with late onset of talking and walking was reported in five patients, but was clinically documented only in one. Mild cognitive abnormalities were clinically suspected in eight patients. In four a mild cognitive impairment was confirmed by neuropsychological examination.

Clinical investigations

Cerebral MRI reveals high signal lesions in the majority that underwent investigation (Table 4). These occur most often in the occipital lobes (14 of 21 examined), deep cerebellar nuclei (6 of 21), thalamus (9 of 21) and basal ganglia (Fig. 2). Two patients had bilateral lesions in the inferior olivary nuclei.
and one of these had the clinical correlate of palatal myoclonus. The lesions were most evident on FLAIR or T2 weighted sequences. In one patient in whom sequential MRI series were recorded, lesions evolved in the left occipital and right motor and prefrontal cortex (Fig. 3). During this period she had continuous simple partial motor seizure activity involving the left arm as well as an unformed visual disturbance.

Table 4

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<tr>
<th>Pt I.D.</th>
<th>Occipital cortex</th>
<th>Deep cerebellar structures</th>
<th>Thalamus</th>
<th>Inferior olives</th>
<th>Cerebellar atrophy</th>
<th>Other</th>
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<td><strong>Total</strong></td>
<td><strong>14</strong></td>
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<td><strong>9</strong></td>
<td><strong>2</strong></td>
<td><strong>4</strong></td>
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The patient identification number (Pt I.D.) is the same as in Tables 1 and 3. Unless stated the investigation referred to is MRI. In several patients, the lesions are found only on the left side. Examples of lesions are given in Fig. 2.

Fig. 2. Cerebral MRI images showing the different radiological features seen in selected patients. (A) Atrophy of the superior cerebellar vermis in Patient 22 (T1W). (B) Bilateral high signal intensity lesions in the thalamus of Patient 23 (T2W). (C) Bilateral high signal intensity lesions in the olivary nuclei in Patient 26 (PDW). (D) and (E) High signal intensity lesions in the insula bilaterally and in the left occipital pole of Patient 10 (FLAIR). (F) High signal intensity lesion in the left occipital cortex of Patient 22 (FLAIR).
Pyramidal tract disturbance is notably absent; however, except for one patient (7) with mild spasticity in the legs and neither optic atrophy nor cardiomyopathy are seen. Comparison with other reports shows that spasticity and cardiomyopathy do occur in patients homozygous for the W748S (Hakonen et al., 2005). Epilepsy is also not a feature of Friedreich’s ataxia. Primary mtDNA disease can produce all the features seen in our patients and in several cases where Southern blotting was normal, we did sequence the whole mitochondrial genome without finding a mutation. We showed earlier that it is important to use sensitive techniques for the demonstration of multiple mtDNA deletions (Winterthun et al., 2005) and, with our current findings, we feel that recognition of the clinical features will focus the investigations on POLG and make it unnecessary to investigate mtDNA.

All but one of our patients has two affected alleles and a clear recessive pattern of inheritance. Patient 27 is the mother of a compound heterozygous male patient (A467T/W748S) and she is heterozygous for the 2243G/C that gives the W748S. She developed epilepsy at the age of 55, has an axonal peripheral neuropathy, ataxia, mild ptosis/PEO and features of mild parkinsonism (unilateral bradykinesia, tremor and rigidity). Parkinson’s disease has been described with POLG mutations (Luoma et al., 2004) although not the two described in our patients. Sequencing of the whole gene shows no other mutation, but several polymorphisms including the E1143G. The observation that this patient may indeed be a manifesting heterozygote is intriguing and warrants further studies, particularly in light of the high frequency of these mutations in Scandinavia and Finland (Hakonen et al., 2005; Winterthun et al., 2005).

Several of our patients developed liver failure. The combination of cerebral and hepatic disease occurs in the infantile disorder Alpers’ syndrome and recent work has shown that this too is caused by POLG mutations (Naviaux and Nguyen, 2004; Davidzon et al., 2005; Ferrari et al., 2005). The majority of our patients developed liver failure after receiving sodium valproate, a feature that has been reported previously (Van Goethem et al., 2004; Ferrari et al., 2005). Statistical comparison shows a highly significant association between liver disease and valproate use (P < 0.001), but no difference between the two groups exposed to this drug (A467T/W748S and W748S/W748S). Two patients developed liver failure not associated with intercurrent illness while the remainder did so in association with status epilepticus and/or multiorgan failure. Preliminary post mortem findings suggest that the pathological changes are similar in both suggesting that the disorder we describe is an overlap between the infantile disorder Alpers’ and an adult disorder without consistent liver involvement.

One of the most interesting findings of our study is the effect of genotype on survival. Compound heterozygous patients have a significantly poorer survival (P = 0.006) than do those homozygous for either the A467T or W748S (Table 2, Fig. 1A). If we include data taken from other studies

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**Fig. 3** Evolving MRI changes in Patient 22 with simple partial motor seizures and visual symptoms (T2-weighted MRI). The images on the right were taken 5 months before those on the left. Those on the right show no abnormalities, while those on the left show hyperintense lesions in the right motor prefrontal cortex (A) and left occipital cortex (B).

Extensive blood tests revealed no consistent abnormalities, particularly no evidence of diabetes. Lactate was not consistently elevated, but occasional patients had elevated levels. CSF analysis performed in four patients was normal in one while in the remaining three elevated protein concentration was detected (0.61–1.73).

**Discussion**

The syndrome we describe appears clinically consistent irrespective of which of the two POLG mutations is responsible. Epilepsy is the commonest presentation and often occurs in combination with headaches that have features of migraine. Typically these patients are young individuals presenting with their first seizure having a history of migraine like headaches and clinical findings suggestive of a sensory neuropathy. Less often, patients presented with a slowly progressive ataxia due to both sensory and cerebellar dysfunction combined with myoclonus. The age of onset ranges from 2 to 36, with most starting in their teens, thus both paediatric and adult neurologists must be able to recognize it. A similar spectrum of clinical features is described in a series of Finnish patients homozygous for the W748S mutation (Hakonen et al., 2005), although these lacked any evidence of hepatic involvement. Interestingly for a mitochondrial disease, diabetes mellitus is not found.

A differential diagnosis for this disorder is Friedreich’s ataxia and indeed this was considered in several patients.
looking at patients with these same two mutations (Van Goethem et al., 2004; Hakonen et al., 2005), the poorer survival of the compound heterozygotes becomes even more significant ($P = 0.0008$; Fig. 1B). Liver involvement also appears to be more severe in compound heterozygotes (Table 3).

How can we explain these results? Both mutations affect the spacer region of the POLG protein. In vitro studies of the A467T suggest that it leads to conformational changes in the catalytic subunit and disrupts binding of the accessory subunit, POLG2 (Chan et al., 2005). Currently, it is not known how the W748S affects function. The finding that compound heterozygotes have a poorer outcome than homozygotes is often taken to indicate the loss of protein–protein interaction, in this case suggesting interaction between two catalytic subunits. If the holoenzyme contains one catalytic (POLG) and two accessory (POLG2) subunits (Yakubovskaya et al., 2005), our findings raise the possibility of a quaternary interaction between catalytic subunits in different heterotrimers. This has major implications for our understanding of how the polymerase functions and raises the possibility that there is more than one holoenzyme involved in the replication process.

Supplementary material

Supplementary material is available at Brain online.

Acknowledgements

This work has been supported by Helse Vest (C.T, S.W, L.A.B), Odd Fellow Ordnen (L.A.B), Fondazione Telethon-Italy (grant no. GGP030039), Fondazione Pierfranco e Luisa Mariani and EUMITOCOMBAT network grant from the European Union Framework Program 6 (G.F, M.Z). We would like to thank Professor Helge Boman for helpful discussions.

References


