POLG1 manifestations in childhood

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ABSTRACT

Objective: Mitochondrial DNA polymerase γ (*POLG1*) mutations in children often manifest as Alpers syndrome, whereas in adults, a common manifestation is mitochondrial recessive ataxia syndrome (MIRAS) with severe epilepsy. Because some patients with MIRAS have presented with ataxia or epilepsy already in childhood, we searched for *POLG1* mutations in neurologic manifestations in childhood.

Methods: We investigated *POLG1* in 136 children, all clinically suspected to have mitochondrial disease, with one or more of the following: ataxia, axonal neuropathy, severe epilepsy without known epilepsy syndrome, epileptic encephalopathy, encephalohepatopathy, or neuropathologically verified Alpers syndrome.

Results: Seven patients had *POLG1* mutations, and all of them had severe encephalopathy with intractable epilepsy. Four patients had died after exposure to sodium valproate. Brain MRI showed parieto-occipital or thalamic hyperintense lesions, white matter abnormality, and atrophy. Muscle histology and mitochondrial biochemistry results were normal in all.

Conclusions: *POLG1* analysis should belong to the first-line DNA diagnostic tests for children with an encephalitis-like presentation evolving into epileptic encephalopathy with liver involvement (Alpers syndrome), even if brain MRI and morphology, respiratory chain activities, and the amount of mitochondrial DNA in the skeletal muscle are normal. *POLG1* analysis should precede valproate therapy in pediatric patients with a typical phenotype. However, *POLG1* is not a common cause of isolated epilepsy or ataxia in childhood. ***Neurology*® 2011;76:811–815**

GLOSSARY

MIRAS = mitochondrial recessive ataxia syndrome; **mtDNA** = mitochondrial DNA; **POLG** = polymerase gamma.

Mutations in the gene encoding the catalytic subunit of replicative mitochondrial DNA polymerase γ (*POLG1*) are a major cause of mitochondrial disease in patients of all ages. In childhood, *POLG1* mutations often manifest as severe epileptic encephalopathy with or without liver involvement (Alpers or Alpers-Huttenlocher syndrome).[1] The liver failure, often precipitated by valproate exposure, is commonly fatal.[2] In adults, the clinical presentation of *POLG1* mutations is variable, ranging from progressive external ophthalmoplegia, epilepsy, ataxia, polyneuropathy, and stroke-like lesions to migraine-like headache.[3-5] A common manifestation is mitochondrial recessive ataxia syndrome (MIRAS), an adult-onset disease with ataxia and axonal neuropathy, often accompanied by partial epilepsy with occipital lobe predilection. The most common *POLG1* mutations underlying MIRAS in the Western world lead to amino acid changes W748S + E1143G or A467T. In retrospect, patients with MIRAS reported childhood clumsiness, ataxia, migraine-like

Supplemental data at www.neurology.org

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headache, or epilepsy.[4,5] We studied here whether childhood epilepsy, ataxia, or sensory axonal neuropathy is explained by *POLG1* mutations. In addition, we searched for *POLG1* mutations in children with neuropathologically verified Alpers syndrome without liver involvement and in those with unknown encephalopathy with epilepsy.

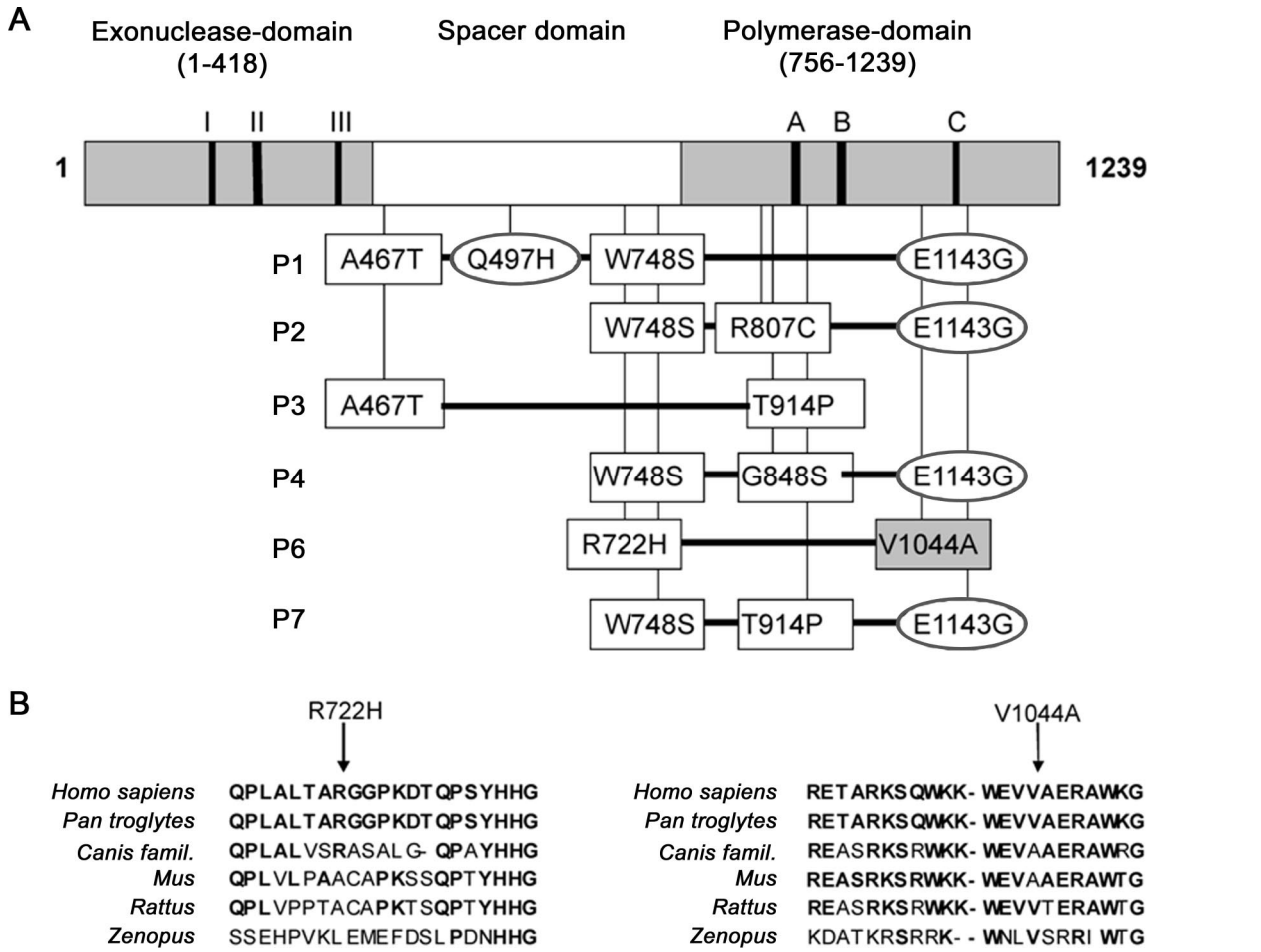
METHODS **Patients.** A total of 136 pediatric patients, aged from 1 month to 19 years, were investigated for *POLG1* mutations. Presenting symptoms were ataxia (n = 37), epilepsy (n = 31), neuropathologically verified Alpers syndrome without liver involvement (n = 6), hepatoencephalopathy (including Alpers-Huttenlocher syndrome, n = 8), epileptic encephalopathy (n = 41), neuropathy (n = 4), and other (n = 9). The patients with severe partial epilepsy were considered for a mitochondrial etiology due to elevation of transaminases, intolerance of valproate, onset with prolonged or recurrent status epilepticus, elevated lactate, and disease not fulfilling the criteria for known epilepsy syndromes.

Standard protocol approvals, registrations, and patient consents. This study was approved by the institutional ethics committee. All samples were taken after receipt of parents' informed consent.

Molecular methods. The entire *POLG1* coding region was sequenced in 105 patients and the exons 7, 13, and 18 including the common mutations (p.W748S, A467T, and Y955C) in 31 patients. The coding sequence and the intron–exon boundaries of *POLG1* were PCR-amplified and sequenced using the BigDye Terminator Ready Reaction Kit v.3.1 on an ABI 3730XL DNA analyzer (Applied Biosystems). Novel mutations were screened in 370 anonymous control DNA samples. The mitochondrial DNA (mtDNA) quantification by real-time PCR was performed, and the data were analyzed as described by Götz et al.,[6] using age-matched control DNA samples from snap-frozen muscle and liver.

RESULTS **Molecular analysis.** We identified compound heterozygous *POLG1* mutations in 7 patients

Figure 1 Molecular analysis



(A) *POLG1* mutations in Finnish children. Mutations are marked with a square, and the E1143G variant, which existed in all patients with a W748S mutation, and the Q497H variant are marked with a circle. The novel mutation is highlighted. (B) Multiple sequence alignment of the POLG protein with eukaryotic homologs, showing the conservation of arginine in site 722 and of valine in site 1044.

Table Summary of the findings in patients with *POLG1* mutations

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Onset of epilepsy	1 y	6 y	9 mo	11 mo	1 y 9 mo	2.5 y	2.5 y
Early motor development	N	N	D	N	D	D	D
Presenting features	V, LC, pSE	V, H, LC, pSE	V, SE	pSE	V, HP, SE	LC, pSE	V, pSE
EPC	+	+	+	+	+	–	+
Myoclonus	+	+	+	+	–	+	+
Ataxia	–	+	–	–	+	+	+
Hemiparesis	–	–	+	+	+	–	–
Ophthalmologic findings	–	VFD	–	LoV, OA	rLoV	–	–
Psychomotor regression	+	–	+	+	+	+	–
Valproate therapy	+	+	+	–	+	–	–
Liver involvement	+ (bV)	+	+	+ (wV)	+	S	–
Cause of death (age, y)	P (3)	LF (7)	LF (1.5)	P (11)	E, LF (3)	Alive (4)	Alive (3)
Lactate in blood[a]	2.8	2.6	1.3–4.6	1.0	NA	2.6	1.1
Lactate in CSF[b]	NA	NA	3.9	1.9	NA	NA	1.85
Protein in CSF[c]	1,200	NA	452	980	NA	148	347
Muscle histology	Mild fiber size variation	Steatosis	Steatosis	NA	Normal	Steatosis	NA
Muscle biochemistry	Normal	Activities normal, succ:cyt c low	Normal	Normal	NA	Normal	NA
Residual mtDNA	M 72%–76%	M 151%–163%	M 49%–63%	NA	NA	M 196%–227%; L 67%–84%	NA
Mutations	W748S+E1143G / R807C	W748S+Q497H+ E1143G / A467T	A467T / T914P	W748S+E1143G / G848S	NA, sister of P4	R722H / V1044A	W748S+E1143G / T914P

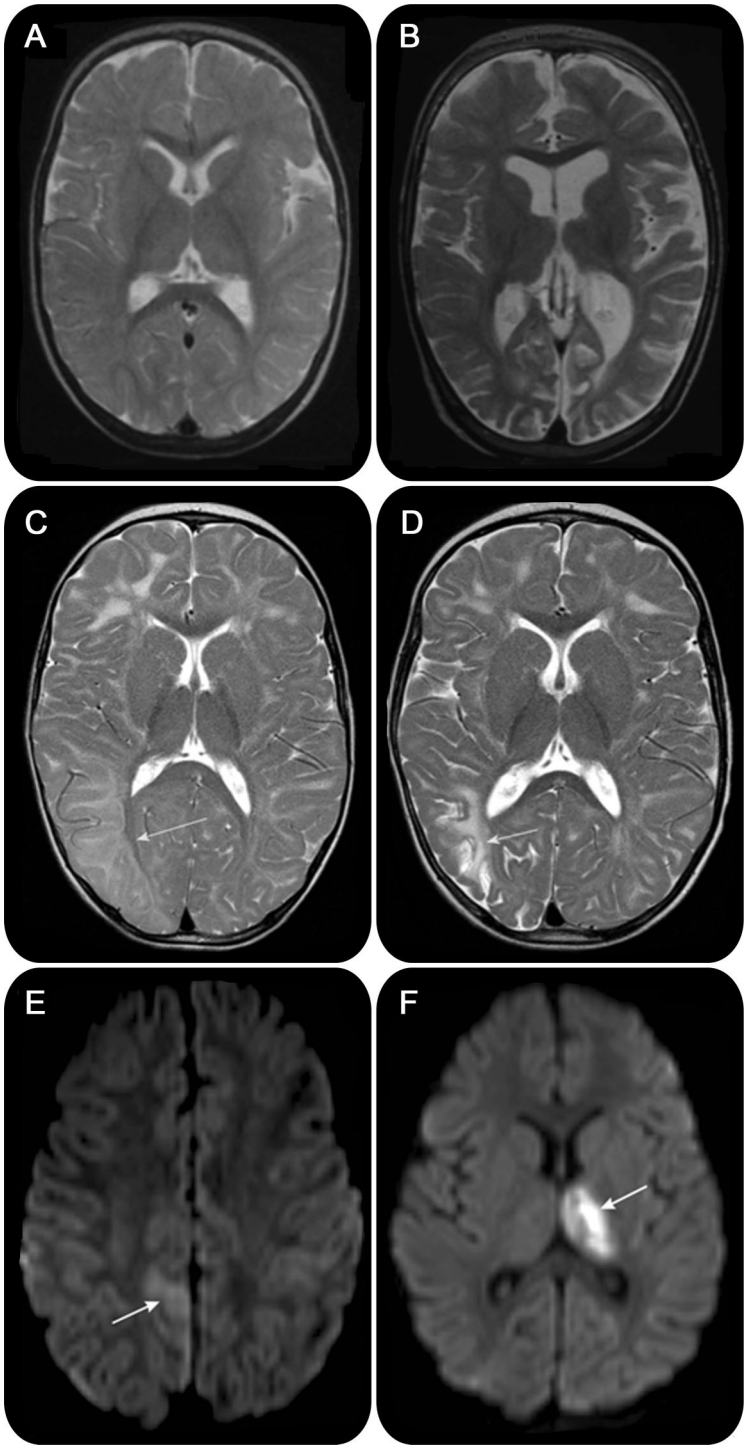
Abbreviations: bV = before valproate; D = delayed; E = epilepsy; EPC = epilepsia partialis continua; H = headache; HP = hemiparesis; L = liver; LC = lowered consciousness; LF = liver failure; LoV = loss of vision; M = muscle; mtDNA = mitochondrial DNA; N = normal; NA = not available; OA = optic atrophy; P = pneumonia; pSE = partial status epilepticus; rLoV = reversible loss of vision; S = subclinical; SE = status epilepticus; V = vomiting; VFD = visual field defect; wV = without valproate.
[a] Reference range: 0.6–2.4 mmol/L.
[b] Reference range: 0.6–2.7 mmol/L.
[c] Reference range: 150–300 mg/L.

from 6 families (figure 1A, table). One patient had a novel change, c.3131 T>C transversion (p.V1044A), in compound heterozygosity with a change leading to p.R722H. Four of 370 Finnish control individuals were heterozygous carriers of the R722H change (carrier frequency 1:95), but no one carried V1044A. In the crystal structure of POLG,[7] the R722 locates in the junction between spacer subdomains and the accessory subunit, in close vicinity to a common Alpers syndrome–associated pathogenic site A467 (figure e-1 on the *Neurology*® Web site at www.neurology.org), which fits with a pathogenic structural change. V1044 locates within the helix partially involved in binding of the single-stranded DNA template. Further analysis of R722H is found in appendix e-1.

Clinical presentation. The table illustrates the symptoms, signs, and findings of the children with *POLG1* mutations. All belonged to the disease groups encephalohepatopathy or encephalopathy with epilepsy. Disease onset was acute or subacute, with decreased consciousness, vomiting, and partial status epilepticus, often with preceding infection. All developed intractable epilepsy, 6 of them with predominantly clonic, psychomotor, and myoclonic seizures as well as development of epilepsia partialis continua, which was exceptionally therapy-resistant. All patients exposed to sodium valproate developed fatal liver disease. None of the patients showed significant changes in histologic or respiratory chain enzyme analysis in the muscle biopsy sample. mtDNA quantification showed normal to mildly decreased amounts of mtDNA in the muscle of all patients. Neuropathologic examination of P1 and P3 showed laminar necrosis in the cortex and neuronal loss in basal ganglia, thalamus, and hippocampi. Figure 2, A–F, presents the variability of MRI findings. One patient with an Alpers-like phenotype and novel mutations is described in appendix e-2.

DISCUSSION In our representative collection of 136 children with a variety of neurologic symptoms,

Figure 2 Brain imaging findings of patients 1, 6, and 7



T2-weighted MRI of patient 1 showed normal findings at the time of disease onset, when he presented with decreased consciousness and partial status epilepticus with epileptic discharges in the left parietal lobe (A), but 14 months later MRI showed severe cortical and hippocampal atrophy (not shown), more pronounced on the left hemisphere (B). (C) T2-weighted MRI of patient 6 displayed extensive white matter changes and an edematous lesion in the right temporo-occipital region (arrow) at the time of disease onset. (D) Seven months later, there was cortical laminar necrosis and atrophy at the site of the previous acute lesion (arrow). (E) Diffusion-weighted MRI of patient 7 at the time of the first status epilepticus showed restricted diffusion in the right parietal lobe (arrow). (F) Eight months later the lesion had disappeared, whereas a new lesion had appeared in the left thalamus (arrow), the side of her epileptic focus at that time.

we identified 7 children with compound heterozygous mutations in *POLG1*. All of these patients had Alpers or Alpers-like syndrome, with devastating intractable epilepsy and psychomotor regression with or without liver involvement. However, typical neuropathologic Alpers syndrome–like changes in children lacking liver involvement were not associated with *POLG1* mutations, suggesting that these findings are not *POLG1*-specific. Furthermore, isolated ataxia or epilepsy was not a typical manifesting symptom of *POLG1* disease. These results indicate that a childhood hepatoencephalopathy, but not isolated epilepsy or ataxia, is a clear indication to study *POLG1*.

Adults with *POLG1* defects often have normal muscle morphology and biochemistry,[3,4] whereas children usually show combined deficiency of respiratory chain enzymes.[8] Our results show that normal morphology, respiratory chain enzyme activities, and mtDNA amounts in skeletal muscle do not exclude *POLG1* defects in pediatric patients.

Typical brain MRI findings of *POLG1* disease include cortical and deep gray matter abnormalities with a predilection for the posterior parts of the brain or thalamus.[4,9] The serial MRI of our patients illustrated well the progressive and fluctuating nature of the disease: MRI results may be normal at the time of disease onset, and existing hyperintense (T2-weighted) posterior or thalamic lesions sometimes regressed[9] or disappeared. However, *POLG1* disease usually progressed to brain atrophy. Different from *POLG1* disease, our patients with neuropathologically verified Alpers disease and normal *POLG1* had a rapidly progressive encephalopathy, with MRI findings changing from normal to severe atrophy within weeks.

Most patients with MIRAS in Finland carry a homozygous *POLG1* change (p.W748S in cis with p.E1143G) due to a founder mutation.[4] It was surprising, however, that we identified a variety of different *POLG1* mutations in Finnish children, and no W748 + E1143G homozygotes were found, even among children with milder clinical presentation. This result suggests that the severe early-onset phenotypes require a combination of mutations affecting different functions of the polymerase. One patient (P2) had 4 different amino acid changes in POLG: A467T / W748S+Q497H+E1143G, both alleles typical for MIRAS. She presented with encephalitis-like onset and partial status epilepticus at 6 years, later than our other patients, followed by visual symptoms, tremor, and ataxia, symptoms that are common in patients with later onset.[4,5] Whether E1143G and Q497H polymorphic variants could have a modifying role for POLG function remains to

be studied. One of our patients with Alpers syndrome had a compound R807C change with W748S, which supports the pathogenic role of R807C, previously reported in an adult with ataxia-neuropathy syndrome.[10] Patient 6 had an Alpers-like phenotype and a novel compound heterozygous change, p.V1044A with R722H. R722H had previously been considered to be a polymorphic variant by us, but our current clinical and molecular data support a possible pathogenic role.

Our study shows that *POLG1* is not a common cause of isolated epilepsy or ataxia of childhood or of pure encephalopathy with Alpers-like neuropathologic changes. However, *POLG1* is a specific cause of Alpers encephalohepatopathy, even if radiologic, morphologic, and biochemical signs of mitochondriopathy are lacking. *POLG1* testing should precede valproate treatment of such patients. Even in genetic isolates, a variety of *POLG1* mutations may underlie the disorders, and complete *POLG1* sequencing is required for DNA diagnosis. Furthermore, distinguishing a mutation from a polymorphic variant continues to be a challenge in DNA diagnosis, because amino acid variants are common and functional tests are not available. The 3-dimensional structure of the protein may become an important tool to evaluate the structural consequences and potential pathogenic role of such variants.

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DISCLOSURE

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