

Molecular diagnosis of Alpers syndrome

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Background/Aims: Alpers syndrome is a developmental mitochondrial DNA depletion syndrome leading to fatal brain and liver disease in children and young adults. Mutations in the gene for the mitochondrial DNA polymerase (*POLG*) have recently been shown to cause this disorder.

Methods: The *POLG* locus was sequenced in 15 sequential probands diagnosed with Alpers syndrome. In addition, the *POLG* mutations found to cause Alpers syndrome in the 20 cases published to date were analyzed.

Results: *POLG* DNA testing accurately diagnosed 87% (13/15 = 87%; 95% confidence interval = 60–98%) of cases. Five new *POLG* amino acid substitutions (F749S, R852C, T914P, L966R, and L1173fsX) were found that were associated with Alpers syndrome in five unrelated kindreds, and 14 different allelic combinations of *POLG* mutations were found to cause Alpers syndrome in the 20 probands published to date. The most common Alpers-causing mutation was the A467T substitution, located in the linker region of the pol γ protein, which accounted for about 40% of the alleles and was present in 65% of the patients. All patients with *POLG* mutations had either the A467T or the W748S substitution in the linker region.

Conclusions: Screening for A467T and W748S substitutions in *POLG* now constitutes the most rapid and sensitive test available for confirming the clinical diagnosis of Alpers syndrome.

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1. Introduction

Alpers syndrome (MIM 203700; sometimes called Alpers–Huttenlocher syndrome, Alpers hepatopathic

poliodystrophy, or hepatocerebral degeneration of childhood) is one of the oldest recognized phenotypes associated with mitochondrial disease. It was first described by Bernard Alpers in 1931 [1], and recently found to be associated with mutations in the gene for the mitochondrial DNA polymerase (*POLG*) [2–4]. It is also the first reported disorder to result from a biochemical deficiency in any human DNA polymerase [5]. Alpers syndrome is inherited as a monogenic, autosomal recessive disorder. It affects children and young adults, and is characterized by the clinical triad of: (1) refractory seizures that include a focal component, (2)

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psychomotor regression that is often episodic, and (3) characteristic liver disease [6]. Children and teens with Alpers syndrome are asymptomatic at birth and develop normally over the first few weeks to years of life. Later, they develop the signs and symptoms of the disease in stepwise fashion, with few patients surviving beyond their teens [7].

The mitochondrial DNA polymerase (pol γ) is essential for mitochondrial DNA replication and repair [8,9]. Pol γ is comprised of a 140 kDa catalytic (α) subunit that contains DNA polymerase, 3'–5' exonuclease, and dRP lyase activities, and a 55 kDa accessory (β) subunit that functions as a processivity and DNA binding factor [10]. The holoenzyme appears to function as a $\alpha\beta_2$ heterotrimer [11]. The catalytic subunit is encoded by the *POLG* (sometimes called the *POLG1*) locus, which includes 23 exons, and is located on chromosome 15q25 [12]. The accessory subunit is encoded by the *POLG2* locus, which includes 8 exons, and is located on chromosome 17q23. *POLG* is arranged in three domains as illustrated in Fig. 2: exonuclease (codons 1–417), linker (codons 418–755), and polymerase domain (codons 756–1239).

Of 16 eukaryotic DNA polymerases discovered to date [13], only *POLG* and *POLH* (pol η) have been associated with inherited human disease [14]. Here, we show that all 20 of the Alpers syndrome patients with *POLG* mutations reported to date have had at least one copy of the A467T or W748S substitution. Screening for these 2 mutations in *POLG* now constitutes the most rapid and sensitive test available for confirming the clinical diagnosis of Alpers syndrome.

2. Materials and methods

2.1. Diagnostic criteria for Alpers syndrome

We analyzed DNA samples for *POLG* mutations from 15 sequential, unrelated patients clinically diagnosed with Alpers syndrome [6]. The minimum diagnostic triad for inclusion of patients in this prospective series was: (1) refractory, mixed-type seizures that often included a focal component, (2) psychomotor regression that was often episodic and triggered by intercurrent infection, and (3) hepatopathy with or without acute liver failure. The hepatopathy was triggered by Valproic acid in some patients, but persisted after stopping the drug, and did not require Valproic acid to occur. In addition, all patients had either a liver biopsy with characteristic features (see below), or at least 2 of the following 11 findings: (1) elevated cerebral spinal fluid (CSF) protein (> 60 mg/dl); (2) brain proton magnetic resonance spectroscopy showing reduced N-acetyl aspartate, normal total creatine (creatinine + creatine-phosphate), and elevated lactate in affected areas of the basal ganglia, thalamus, or cerebral cortex (sampled voxels were 9–13 ml, or about 25×25×20 mm); (3) cerebral volume loss (central > cortical, with ventriculomegaly in excess of cortical thinning) on repeat brain MRI or CT studies, (4) at least one EEG showing asymmetric or posterior high amplitude slow waves (200–1000 μ V, 0.75–3 Hz) intermixed with lower amplitude polyspike discharges (10–100 μ V, 12–25 Hz), (5) cortical blindness or optic atrophy, (6) abnormal visual evoked potentials and normal electroretinogram, (7) quantitative mitochondrial DNA depletion in skeletal muscle or liver ($\leq 35\%$), (8) deficiency in DNA polymerase γ (pol γ enzymatic activity $\leq 10\%$) in

skeletal muscle or liver, (9) elevated blood or CSF lactate (≥ 3.0 mM) on at least one occasion in the absence of acute liver failure, (10) isolated complex IV, or a combined I, III, and IV electron transport complex defects ($\leq 20\%$ of normal) upon liver respiratory chain testing, or (11) a sibling confirmed to have Alpers syndrome. The characteristic features on liver biopsy required exclusion of Wilson disease, and at least 3 of the following 8 histological findings: (1) microvesicular steatosis, (2) bile ductular proliferation, (3) hepatocyte dropout or focal necrosis with or without portal inflammation, (4) collapse of liver cell plates, (5) parenchymal disarray or disorganization of the normal lobular architecture, (6) bridging fibrosis or cirrhosis, (7) regenerative nodules, and (8) oncocytic change (mitochondrial proliferation associated with intensely eosinophilic cytoplasm) in scattered hepatocytes not affected by steatosis.

2.2. DNA purification and PCR primers

DNA was purified from blood (Puregene Blood Kit, Gentra Systems, Minneapolis, MN), buccal washes [15], or tissue samples by standard methods [16] and amplified by polymerase chain reaction (PCR) using primers and thermocycling conditions described below.

1. Exon 3F 39195 5'-CCT GAC CTG TAG CTG TTT GAG TTA G
2. Exon 3R 38659 5'-GGG GAC ATA CAA GAC CTA GGC ACA G
3. Exons 4/6F 38031 5'-AGC ATG AGG ATT CTG TAT TTG GTG T
4. Exons 4/6R 36997 5'-ACA CAA GCA CAA CTC CAT GGC CCT C
5. Exons 7/9F 36232 5'-TGC CGT CTG ACT TTT GAG CTG TGC C
6. Exons 7/9R 35210 5'-GCA AAT GAG AAT AGG ACT GGA AGA C
7. Exons 7/8F 5'-GTC TTG CCT CCT GTG GTC ATT TAT
8. Exons 7/8R 5'-CAC CCA TGC TCC CCA CCT TTC CT
9. Exon 10F 34535 5'-AGG GTG GGT GGG GAC ATT GTG AGA G
10. Exon 10R 33790 5'-CCC AGA GAG TGA GTG AGT GAG TGA G
11. Exons 11/14F 33146 5'-TGT CAA TCA ATC CCT GTC TAA AAC C
12. Exons 11/14R 31239 5'-CGG CTC CAG CAG TTA CAC CAA GAA G
13. Exon 13F 5'-CCT TGC TGA ATG CAG GTG C
14. Exon 13R 5'-GTG GGC CTT GAG CAG AAT G
15. Exons 15/18F 30982 5'-GGC CGG TTC CCA GAT GGT TTA TGC T
16. Exons 15/18R 29431 5'-TGG GCA GGA GAT AGA ACA GAT GGT A
17. Exon 16F 5'-CTA AGA CCC ATT TCC TTC CC
18. Exon 16R 5'-CAG ACC TGG GAG AGG AAG A
19. Exons 17/18F 5'-GAA TGG GGT AGG AAG AGT CT
20. Exons 17/18R 5'-CGG GTC CTG GGT GTT AAA G
21. Exons 19/21F 28375 5'-GAG TCT TTG TCC TTT ATT GGG CTA C
22. Exons 19/21R 27034 5'-ACA ATT CCC CTA ACC TCA CTG CTT C
23. Exons19/21F 5'-GAA GCA CTC CCG TGA AAT G
24. Exons19/21R 5'-CAA GGA ACG CTC ACC CAA AG
25. Exons 22/23F 26481 5'-TGG GAG GTT AGG CTG CTG GAT GGA A
26. Exons 22/23R 25401 5'-GGT CCT GCT ACT GAA AAA TGG CTG G
27. Exon 22/23F 5'-GAT GCA TCT GTT CAC AGG AG
28. Exon 22/23R 5'-GCT GAA AGC CTG AGT TTG GG

2.3. PCR primer validation

All primers were screened for the incidental inclusion of single nucleotide polymorphisms (SNPs) or short insertions and deletions (indels) in *POLG* by performing a BLASTN search against the NCBI SNP database (dbSNP build 124). The 'C' at nucleotide position 4 in primer no. 5 (7/8-F) above was found to be polymorphic (SNP ID = rs3176176). The frequency of C at this position is 99% and the frequency of 'T' at this position is 1%. No other primers contained reported SNPs.

2.4. PCR conditions and DNA sequencing

Genomic DNA was amplified by either of 2 PCR thermocycler programs: (95°C×5 min); [94°C×30 s, 65°C×2 min, 72°C×1 min]×35 cycles; 72°C×5 min; 4°C×up to 16 h; or by Touchdown PCR under the following conditions: (95°C×10 min); (94°C×30 s, 65–67°C (depending on the primer)×1 min decreasing 0.5°C after each cycle, 72°C×2 min)×20 cycles; (94°C×30 s, 65–67°C (depending on the

Table 1
POLG Allelic combinations associated with Alpers syndrome

	POLG mutations	Published Alpers probands					Genotype found in other phenotypes	Non-Alpers references	cDNA ^a mutations
		A	B	C	D	E			
1.	p.A467T/p.A467T	1			1	+	[20,21,30]	c.1681G > A in exon 7/c.1681G > A in exon 7	
2.	p.A467T/p.F749S				1	–		c.1681G > A in exon 7/c.2528T > C in exon 13	
3.	p.A467T/p.G848S		1		1	–		c.1681G > A in exon 7/c.2824G > A in exon 16	
4.	p.A467T/p.R852C				1	–		c.1681G > A in exon 7/c.2836C > T in exon 16	
5.	p.A467T/p.E873X	1				–		c.1681G > A in exon 7/c.2899G > T in exon 17	
6.	p.A467T/p.T914P				1	–		c.1681G > A in exon 7/c.3022A > C in exon 18	
7.	p.A467T/p.A957P		1			–		c.1681G > A in exon 7/c.3151G > C in exon 18	
8.	p.A467T/p.L966R				1	–		c.1681G > A in exon 7/c.3179T > G in exon 18	
9.	p.A467T/p.W1020X				1	–		c.1681G > A in exon 7/c.3339G > A in exon 19	
10.	p.A467T/c.3764 + 2 (T > C) splice		1			–		c.1681G > A in exon 7/c.3764 + 2 (T > C) in intron 21	
11.	p.A467T/p.L1173fsX				1	–		c.1681G > A in exon 7/c-3800insGACT in exon 22	
12.	p.W748S-p.E1143G/p.L24P		1			–		c.2525G > C in exon 13 - c.3710A > G* ^b in exon 21/c.371T > C in exon 3	
13.	p.W748S-p.E1143G/p.G848S			4	1	–		c.2525G > C in exon 13 - c.3710A > G in exon 21/c.2824G > A in exon 16	
14.	p.W748S-p.E1143G/p.Y1210fs1216X		1			–		c.2525G > C in exon 13 - c.3710A > G in exon 21/c.3912insC in exon 22	
Proband totals (out of 20):		1	6	4	4	5			

A, [2]; B, [4]; C, [3]; D, [19]; E, The present study.

^a cDNA map positions are numbered according to *POLG* NCBI Accession NM_002693. In practice, genomic DNA (not cDNA) is used for testing.

^b The independent role of the p.E1143G substitution in pathogenesis has not yet been established (see text).

primer) x1 min, 72 °C×2 min)×10 cycles; 20 °C×4 min; and hold at 4 °C×up to 16 h. Amplified DNA was gel purified, and sequenced using either Big Dye chemistry or dRhodamine dye terminator and an ABI 3700 sequencer or an ABI Prism 3100 Genetic Analyzer. Each new mutation was confirmed with top and bottom strand sequence from at least 2 independent PCR amplifications performed on different days to achieve a minimum of 3-fold redundancy.

2.5. Criteria for ‘mutation-negative’ cases

Patients were designated ‘mutation-negative’ if they did not carry any of the 14 *POLG* alleles known to cause Alpers syndrome (Table 1), and a new mutation was not found after sequencing 100% of the 3057 coding nucleotides from exons 3–23, and 36% of the 13.8 kb of the introns with at least 2-fold redundancy. All intronic splice donor and splice acceptor sites (at least 10 nucleotides on each side of the exon borders) were sequenced that flanked exons 3–23. Exon 1 in the 5’ untranslated region, and exon 2 were not successfully amplified from any of the DNA samples with PCR primers synthesized to date. A total of 379,827 nucleotides of *POLG* DNA were analyzed from the 15 sequential samples from probands with Alpers syndrome.

2.6. Statistical analysis

The probability that all 20 probands with Alpers syndrome carried mutations in the linker region of the pol γ protein was calculated using the binomial distribution. We started with the null hypothesis that *POLG* mutations were randomly distributed along the amino acid coding length of the cDNA. We next calculated the fraction of the total represented by each domain based on the *POLG* cDNA (GenBank Accession no. NM_002693). The exonuclease, linker, and polymerase domains constitute 33.6, 27.3, and 39.1% of the 3717 amino acid coding nucleotides, respectively. Since the linker region represents 27.3% of the total AA coding length of the gene, the chance of a person receiving 1 mutation is 0.273. The chance of not receiving a linker mutation is 1–0.273 = 0.727. The chance of receiving 2 linker mutations is 0.273×0.273 = 0.0745. The probability of receiving 1 mutation after receiving 2 *POLG* alleles is 2×(0.273×0.727) = 0.397. The probability of receiving 1 or 2 linker mutations after receiving 2 alleles is 0.0745 + 0.397 = P = 0.471. The chance of not receiving a linker mutation is 1–P = q = 0.529. The probability that 20 out of 20 patients received linker region mutations by chance = P²⁰ = 0.47147²⁰ = 2.94×10⁻⁷, or about 1 in 30 million.

3. Results

3.1. New *POLG* mutations

We found 5 new compound heterozygous *POLG* mutations that were associated with Alpers syndrome: (1) A467T/F749S, (2) A467T/R852C, (3) A467T/T914P, (4) A467T/L966R, and (5) A467T/L1173fsX (Table 1). The role of the A467T allele in Alpers syndrome has been previously reported [2,4]. The F749S substitution was caused by a c.2528T > C mutation, located in exon 13. The R852C substitution was caused by a c.2836C > T mutation, located in exon 16. The T914P substitution was caused by a c.3022A > C mutation, located at the beginning of exon 18. The L966R substitution was caused by a c.3179T > G mutation, located in exon 18. The L1173fsX was caused by the 4-nucleotide insertion (c.3800insGACT) in exon 22, immediately following the wild-type GACT, which encodes amino acid 1172 and the first nucleotide of codon 1173. This resulted in a 4-nucleotide tandem duplication, and a TGA stop codon at position 1173, just downstream of motif C within the highly conserved polymerase domain in pol γ (Figs. 1 and 2). None of these mutations was observed in over 300 chromosomes sequenced as part of the National Institute of Environmental Health Sciences GeneSNPs program (NIEHSb in the Web Resources section). Each substitution occurred in a highly conserved region of the pol γ protein (Fig. 1A–D).

The results of mitochondrial DNA depletion and mitochondrial respiratory chain studies were dependent on the timing of the test, and were neither sensitive nor specific for Alpers syndrome. Among the 5 probands we report here, quantitative mtDNA depletion studies

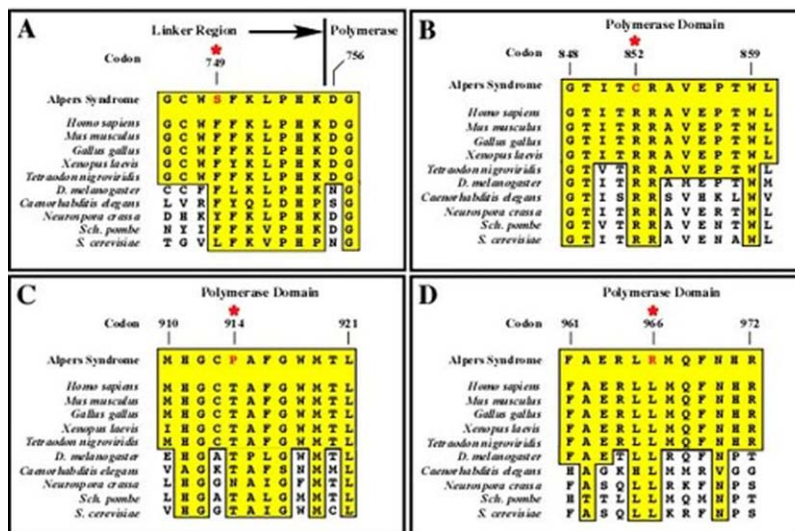


Fig. 1. Conservation of *POLG* mutations associated with Alpers syndrome. (A) F749S, (B) R852C, (C) T914P, (D) L966R.

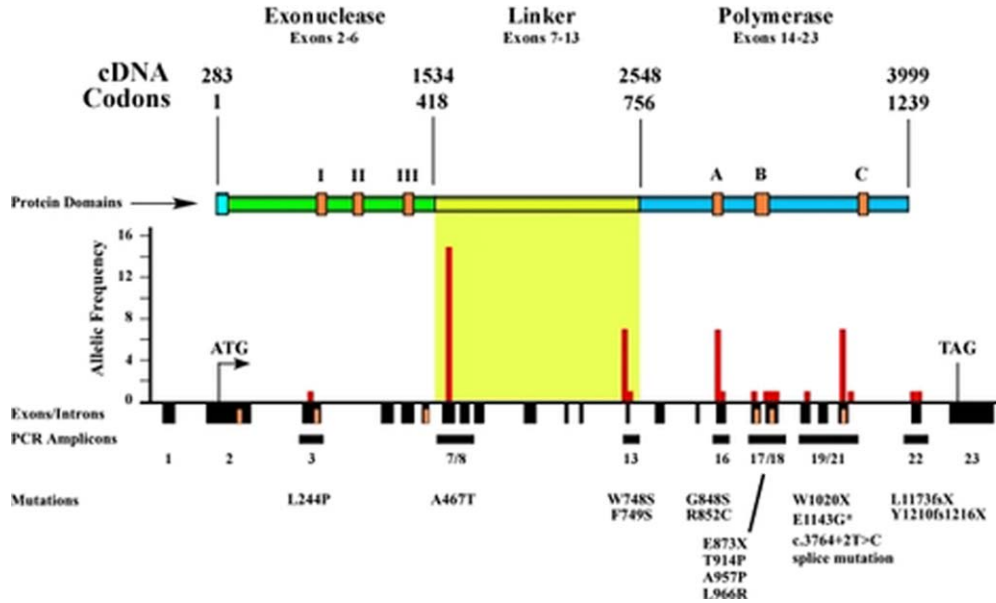


Fig. 2. Allelic frequencies and genomic location of POLG mutations associated with Alpers syndrome. The allelic frequencies of the 14 alleles and 15 mutations known to cause Alpers syndrome in 20 reported probands are indicated as red bars. The locations of motifs I–III, and A–C in the exonuclease, and polymerase domains, respectively, are indicated in orange. Each mutation was found on an independent allele, with the exception of W748S-E1143G mutations, which were always found linked in *cis*. *The independent role of the E1143G mutation in Alpers syndrome is not yet known. All patients reported to date had at least one mutation in the linker region of the protein, highlighted in yellow.

Table 2
POLG allelic combinations associated with recessive, non-Alpers syndrome phenotypes

	POLG mutations	Probands	POLG Domains	Phenotype	Refs.
1.	R232G/T251I + P587L	1	AA	Infantile hepatocerebral	[4]
2.	T251I/R227W	1	AA	PEO	[36]
3.	T251I/R309L	1	AA	PEO	[37]
4.	W312R/W312R	1	AA	PEO/neuropathy	[38]
5.	G268A/G268A	1	AA	PEO/retinopathy/amenorrhea	[38]
6.	A467T/R3P	1	AB	PEO	[25]
7.	A467T/G268A	1	AB	PEO	[38]
8.	A467T /L304R	1	AB	PEO	[25]
9.	A467T/A467T	1	BB	ANS/MEMS/VLT	[30]
		2	BB	ANS/VLT	[20]
		2	BB	ANS	[21]
10.	A467T/R627W	1	BB	ANS	[26]
11.	A467T/R627Q + Q1236H	1	BB	ANS/myopathy	[23]
12.	A467T/W748S + E1143G	1	BC	ANS	[20]
13.	A467T/S1104C	1	BC	PEO	[36]
14.	L424stop/G431V	1	BB	PEO	[36]
15.	N468D/A1105T	1	BC	PEO	[23]
16.	Q497H/W748S + E1143G	2	BC	ANS	[21]
17.	R579T/A889T	1	BC	PEO	[39]
18.	R227W/S1176L	1	AC	PEO	[40]
19.	T251I/G848S	1	AC	PEO	[37]
20.	T251I/c.2354Gins(G785insG-806X)	1	AC	PEO	[37]
21.	T251I + P587L/N864S	1	AC	PEO	[28]
22.	T251I + P587L/R807P	4	AC	PEO/neuropathy	[38]
23.	T251I + P587L/H932Y	1	AC	PEO	[38]
24.	T251I + P587L/R709X	1	AC	PEO	[38]
25.	H932Y/G1051R	1	CC	ANS-PEO	[41]
26.	W748S + E1143G/W748S + E1143G	1	CC	ANS	[20]
Probands		34			

Abbreviations: POLG domains: A, exonuclease; B, first 2/3 of the linker region; C, last 1/3 of the linker region and the polymerase. Allelic combinations involving A467T are highlighted in gray. PEO-progressive external ophthalmoplegia. ANS-ataxia-neuropathy spectrum. MEMS, myoclonus, epilepsy, myopathy spectrum. VLT, valproic acid-associated liver toxicity.

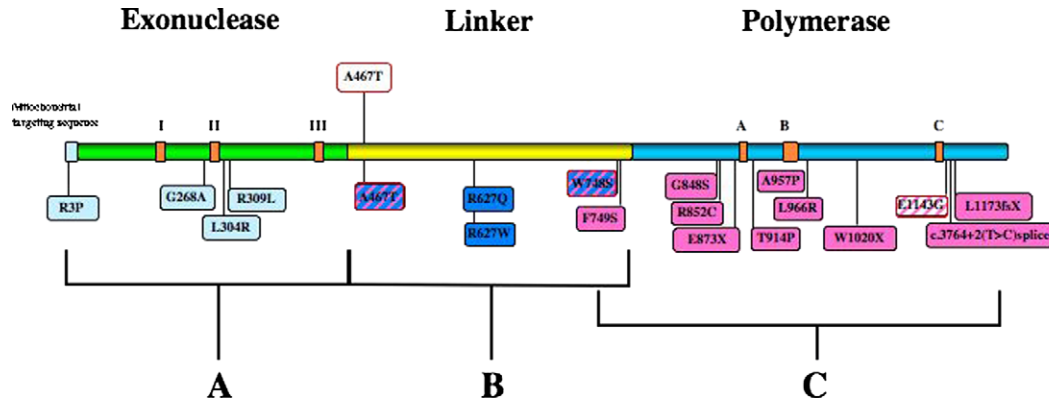


Fig. 3. Mutations found in trans with A467T in recessive *POLG* disorders. The allelic combinations represented are A467T/'X', where 'X' is one of the 20 mutations indicated. Second alleles bearing mutations in the exonuclease domain (A) are associated with autosomal recessive (ar) PEO, and cause mtDNA deletions and point mutations. Second alleles with mutations in the first 2/3 of the linker region (B) are associated with ANS, or Alpers syndromes, and causes mtDNA deletions in some, but not all cases. Second alleles with mutations in the last 1/3 of the linker region and the polymerase domain (C) are typically associated with Alpers syndrome, and cause mtDNA depletion. Mutations colored in light blue are associated with arPEO; dark blue, ANS; and pink, Alpers syndrome. The blue and pink striped mutations are found in both Alpers syndrome ANS disorders. The locations of motifs I–III, and A–C in the exonuclease, and polymerase domains, respectively, are indicated in orange.

were normal, ie not depleted, in the early stages of the disease in 2 of the 2 patients who were tested. Later in the course of disease, mtDNA depletion was documented in 4 of 4 patients tested. One of the late-positive patients had normal levels of mtDNA early in the disease course, confirming that: (1) Alpers syndrome is a progressive disease, and (2) the early phenotype can be very severe in the absence of mtDNA depletion. We concluded that the early phenotype in Alpers syndrome may result from a more subtle, non-replicative consequence of *POLG* mutation. An example of such a non-replicative function of pol γ is DNA repair [17,18].

3.2. Frequency of *POLG* mutation-negative patients in Alpers syndrome

We next set out to determine the frequency of patients with *POLG* mutations in a sequential series of 15 DNA samples from probands with Alpers syndrome. The *POLG* mutations we found were: (1) A467T/E873X, (2) A467T/L1173fsX, (3) No *POLG* mutation found, (4) A467T/T914P, (5) No *POLG* mutation found, (6) A467T/W1020X, (7) W748S-E1143G/G848S, (8) A467T/G848S, (9) A467T/G848S, (10) A467T/c.3764 + 2T > C, (11) A467T/A467T, (12) A467T/W748S-E1143G, (13) A467T/F749S, (14) A467T/R852C, and (15) A467T/L966R. We found that 13 of the 15 DNA samples had pathogenic mutations in *POLG*. Two samples (2/15 = 13.3%; 95% Confidence Interval = 1.6%–40%) were mutation-negative, despite 100% sequence coverage of exons 3–23 and at least 10 nucleotides of intron DNA on both sides of each of the sequenced exons containing the mRNA splice donor and acceptor sites.

3.3. Absence of phenotypic differences in the mutation-negative cases of Alpers syndrome

Retrospective analysis of the clinical, neurological, radiologic, and biochemical data for the 2 patients who were mutation-negative confirmed that these patients were indeed phenocopies, and could not be distinguished from the 13 other rigorously diagnosed patients with Alpers syndrome on the basis of the diagnostic criteria used in our study. Both patients had mtDNA depletion late in the course of disease. Pol γ enzymatic activity was not measured in either. These data suggest that about 13% of cases (about 1 in 7) with Alpers syndrome are phenocopies that result from mutations in other genes.

3.4. *POLG* mutation frequencies in Alpers syndrome

We next reviewed the *POLG* mutations found in the 20 probands with Alpers syndrome published to date [2–4,19]. Fourteen alleles and 15 *POLG* mutations were found (Table 1). The allelic frequencies and location of each of the mutations are shown in Fig. 2. The most common mutation was A467T, which accounted for 15 of 40 alleles, and was present in 13 (65%) of the 20 probands. The next most common mutations were W748S and G848S, which accounted for 7 alleles each. The E1143G substitution was found linked in *cis* with W748S in each case. The independent role of the E1143G allele in pathogenesis, is not yet known. The E1143G mutation is seen independently of the W748S mutation in about 4% of US population (NIEHSb in the Web Resources section) and has formerly been considered a neutral polymorphism. However, it is not neutral in standardized computer models of protein

structure (Yu 2005, in Web Resources below). Eleven of the 14 *POLG* mutations known to cause Alpers syndrome were unique to individual families (Table 1).

3.5. Linker region mutations

All of the 20 Alpers patients published to date had either the A467T or W748S allele in the linker region of the pol γ protein (Table 1, Fig. 2). The probability that this happened by a chance distribution and transmission of mutations in the linker region is 2.94×10^{-7} , or about 1 in 30 million (see Section 2). In comparison, linker region mutations were seen in just 50% (17 of 34) probands in the recessive, non-Alpers phenotypes of *POLG*-associated diseases (Table 2). We also found that when a non-Alpers phenotype involved the A467T allele, the other *POLG* allele typically contained a mutation in the exonuclease domain, or the first 2/3 of the linker region (Table 2, Fig. 3). When patients are homozygous for the A467T allele, they developed either a later-onset form of Alpers syndrome [2,4,19], or a more chronic spectrum of disorders characterized by progressive ataxia, sensory neuropathy, and epilepsy, valproate sensitivity, encephalopathy or dementia, [20,21]. For simplicity this spectrum is referred to as the ataxia-neuropathy spectrum (ANS) of *POLG* associated disorders (Table 2, Fig. 3).

Allelic mutations paired with A467T in Alpers syndrome were found to be in the last 1/3 of the linker, or the polymerase domain (Fig. 3). What are the biochemical consequences of this mutation? The A467T mutation forces the pol γ enzyme into an altered conformation that possesses only about 4% of the wild-type DNA polymerase activity with only modest effect on the exonuclease [22,23]. Additionally, the A467T pol γ protein fails to interact with the p55 accessory subunit that is normally required for highly processive DNA synthesis [22,23].

4. Discussion

Over 60 mutations in *POLG* have been described [24] (NIEHSa in the Web Resources section). Different allelic combinations are associated with 4 partially-overlapping groups of disease. These are: (1) Progressive External Ophthalmoplegia (PEO, \pm multisystem disorder) [25–28], (2) Ataxia, Neuropathy Spectrum (ANS) disorders (\pm seizures, dementia, liver dysfunction, or sensitivity to valproic acid; includes SANDO and MIRAS) [20,21,29], (3) Myoclonus, Epilepsy, Myopathy Spectrum (MEMS) disorders [30], and (4) Hepatocerebral Disorders (which include Alpers syndrome) [2–4]. Alpers syndrome, ANS, and MEMS appear to represent points on a spectrum of *POLG* disorders involving the brain and liver. In general, Alpers syndrome, which carries

with it the prognosis of neurodegeneration and fatal encephalopathy or liver failure within 2 months to 15 years of diagnosis, presents between 1 month and 25 years of age, and usually within the first 2 years of life. ANS presents later, with a median age of onset of 28 years and a range of 7–41 years [29]. Often the specific combination of *POLG* mutations is helpful in establishing the diagnosis and prognosis. For example, 13 of the 14 allelic combinations of *POLG* mutations summarized in Table 1 have been found to be diagnostic for Alpers syndrome, and are not seen in ANS or other phenotypes studied to date. Only 1 of the 14 allelic combinations is known to produce both Alpers syndrome, and a non-Alpers phenotype; homozygosity for the A467T allele can cause Alpers syndrome with a later onset and peripheral neuropathy, after age 7 [2,4,19], or may cause ANS [20,21].

In theory, all of *POLG* disorders might share mtDNA depletion or deletion as a common pathogenetic feature, since they all result from mutations in the mitochondrial DNA polymerase. However, in practice mtDNA depletion/deletions are not always present at the time when clinical symptoms first appear. When performed early in the course of disease, tests for mtDNA depletion and deletions were normal in 2 of 2 patients among the five new patients reported here. Both of these patients had classic Alpers syndrome, and one of the patients was tested again, later in the course of disease. The later test showed the development of mtDNA depletion. Therefore, normal mtDNA depletion or deletion test results cannot be used to exclude the diagnosis of *POLG* disease [20,21,26,29].

Alpers syndrome, and a related hepatocerebral disorder with neuropathy (patient no. 9 in Ferrari, et al. [4]), are the earliest onset forms of any of the described *POLG* disorders. The typical age of onset for Alpers syndrome is before 2 years. However, patients with 2 copies of the A467T allele have a later age of onset, typically after 7 years [2,4,19], or may have ANS [20,21], or MEMS [30]. Mono-allelic expression of a *POLG* genotype containing a single copy of the A467T mutation, with selective elimination of transcripts bearing a premature stop codon expressed from the other allele, results in a more typical age of onset of Alpers syndrome before age 2 [31]. Mutations in other genes, such as deoxyguanosine kinase (*DGUOK*) are known to cause brain and liver disease with nystagmus in children [32]. However, seizures and EEG abnormalities similar to those of Alpers syndrome have been absent in the *DGUOK* pedigrees reported to date [32–35].

We found that the sensitivity of *POLG* DNA testing for Alpers syndrome was 87% ($n = 13/15$ patients = 86.7; 95 CI = 60–98%). Future studies may reveal subtle differences that may prove helpful in identifying the *POLG* mutation-negative cases of Alpers syndrome, but in the present study, these 13% of cases were

clinically indistinguishable from the *POLG* mutation-positive forms. The ascertainment of patients in our prospective series was made on the basis of clinical criteria. No patient was either excluded or included solely on the basis of biochemical phenotype. Our results show that no specialized biochemical, or mtDNA depletion testing is required prior to confirmation by *POLG* DNA testing, once the diagnosis of Alpers syndrome has been made on clinical grounds.

Among the 20 probands with Alpers syndrome and *POLG* mutations published to date [2–4,19], we found that 100% of the cases could be ascertained by screening for just 2 mutations; the A467T and the W748S substitutions in the linker region (Table 1, Fig. 2). These results provide the first molecular epidemiologic evidence that the linker region of the pol γ protein may play an important role in the pathogenesis of Alpers syndrome in patients with *POLG* mutations (Figs. 2 and 3), and show that *POLG* DNA testing is a powerful and sensitive tool for confirming the clinical diagnosis of this classic neurogenetic disease.

5. Web resources

1. National Library of Medicine Online Mendelian Inheritance in Man (OMIM), www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM Entries no. 203700 and no. 174763. Date Accessed July 19, 2005.
2. National Institute of Environmental Health Sciences (NIEHSa). Human DNA Polymerase Gamma Mutation Database. URL: <http://dir-apps.niehs.nih.gov/polg/index.cfm> Date Accessed: July 19, 2005.
3. National Institute of Environmental Health Sciences (NIEHSb). GeneSNPs of *POLG*. URL: <http://www.genome.utah.edu/genesnps> Date Accessed: July 19, 2005.
4. Yu SNPs 3D: Molecular functional effects of non-synonymous SNPs based on structure and sequence analysis. URL: <http://www.snps3d.org> Date Accessed: July 19, 2005.

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