



POLG mutations in Alpers syndrome

Abstract—Described are six patients with Alpers syndrome from four unrelated families. Affected individuals harbored the following combinations of *POLG* mutations: 1) A467T/W1020X, 2) W748S-E1143G/G848S, 3) A467T/A467T, and 4) A467T/G848S. Homozygosity for the A467T allele in one patient was associated with a later age at onset. Mitochondrial respiratory chain studies in skeletal muscle were normal in each case. Nine combinations of mutant *POLG* alleles that cause Alpers syndrome are summarized.

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Alpers syndrome (OMIM 203700; sometime 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 1931¹ and was recently found to be associated with mutations in the gene for mitochondrial DNA polymerase γ (*POLG*).² Alpers syndrome affects children and young adults and is characterized by the clinical triad of 1) refractory seizures that often have a focal component, 2) psychomotor regression that is often episodic, and 3) characteristic liver disease.³ Children and teens with Alpers syndrome are typically asymptomatic at birth and may 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.

In 2004, two mutations in *POLG* were identified as a cause of Alpers syndrome.² One of these was an

E873X mutation, converting a glutamate residue at position 873 (GAG) to a stop codon (TAG) located at the beginning of the polymerase domain of the protein. The other was a compound heterozygous alanine-to-threonine (A467T) substitution. We describe four combinations of *POLG* mutations that caused Alpers syndrome: A467T/W1020X, W748S-E1143G/G848S, A467T/A467T, and A467T/G848S.

Methods. *Patients.* We studied six patients with a diagnosis of Alpers syndrome from four families. The minimum diagnostic triad for patients in this 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. 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 CSF protein (>100 mg/dL); 2) brain proton MR spectroscopy showing reduced *N*-acetyl aspartate, normal total creatine, and elevated lactate; 3) cerebral volume loss (central $>$ cortical) on repeat brain MRI or CT studies; 4) EEG showing a multifocal paroxysmal activity with high-amplitude slow waves (200 to 1,000 μ V, 0.75 to 3 Hz), and asymmetric low-amplitude polyspikes (10 to 100 μ V, 12 to 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 *POLG* 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 three of the following eight histologic 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.

PCR and DNA analysis. See Appendix E-1 in the supplementary material on the *Neurology* Web site (go to www.neurology.org) for additional methods.

Results. *Clinical findings.* Clinical findings in our series are summarized in table 1. All patients 1) had refractory seizures that often initiated explosively with an episode of status epilepticus following shortly after an infectious illness and were mixed in type, with a strong focal component, sometimes with myoclonus and epilepsy partialis continua; 2) had psychomotor regression that was not monotonically degenerative, but episodic and some-

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Table 1 Clinical characteristics of patients with Alpers syndrome

	Patient no./sex					
	A3.II.3/F	A3.II.4/F	A4.II.2/M	A5.II.1/M	A6.II.1/M	A6.II.3/F
Characteristic						
Age at onset	10 mo	9 mo	13 mo	8.5 y	12 mo	11 mo
Age at death	18 mo	12.5 mo	20 mo	9 y	15 mo	21 mo
Presenting symptom	SE	SE	SE	EPC	LF	EPC
Presentation associated with intercurrent infection	+	+	+	-	+	+
Seizures	R, G, P	R, G, P	R, G, P, M, A, EPC	R, P, EPC	R, P, EPC	R, G, P, M, EPC
EEG	ND	ND	PLED, MF, HA	ND	ND	HA, LA
Psychomotor regression	+	+	+	+	+	+
Episodic deterioration	+	+	+	+	+	+
Cortical blindness or OA	-	-	-	-	-	-
Transient hypoglycemia	+	+	+	-	ND	+
Transient lactic acidemia	+	-	+	+	ND	+
Elevated CSF protein	ND	ND	ND	ND	ND	+
Weeks to valproate toxicity	7	ND	5	24	ND	ND
Other findings	HC	H	CH			
Early MRI abnormality	-	-	-	-	ND	-
Late MRI abnormality	CL, HE, PL	ND	CL, FC, PL, SD	CL	ND	ND
DWI abnormality	ND	ND	+	ND	ND	ND
MRS	ND	ND	AP	ND	ND	ND
liver failure	+	+	+	+	+	+
Liver histology	MS, F, BD	ND	MS, F, BD, RN, C	MS, PD, BD, O, C	ND	ND
Received liver transplant	+	-	+	-	+	-
Muscle ETC	N	ND	N	N	ND	N
Liver ETC	ND	ND	N	COX	ND	ND
<i>POLG</i> mutations	A467T/W1020X	A467T/W1020X	W748S-E1143G/G848S	A467T/A467T	A467T/G848S	A467T/G848S

SE = status epilepticus; R = refractory; EPC = epilepsia partialis continua; G = grand mal; P = focal/partial seizures; A = atonic; M = myoclonus; LF = liver failure; OA = optic atrophy; MS = microvesicular steatosis; RN = regenerative nodules; F = fibrosis; C = micronodular cirrhosis; BD = bile ductular proliferation; PD = liver parenchymal disarray; O = oncocytic change with mitochondrial proliferation and strongly eosinophilic cytoplasm; PLED = periodic lateralized epileptiform discharges; MF = migrating foci in serial studies; HA = high-amplitude slow waves; LA = low-amplitude polyspikes; CL = cerebral volume loss (central > cortical); HE = hydrocephalus ex vacuo; PL = periventricular leukomalacia; SD = subcortical demyelination; FC = focal cortical swelling; DWI = diffusion-weighted MRI; MRS = proton MR spectroscopy; ETC = mitochondrial electron transport chain enzymology; AP = Alpers pattern (reduced *N*-acetyl aspartate, elevated lactate, normal total creatine, and normal glutamine+glutamate); HC = hypertrophic cardiomyopathy; H = hypercoagulable state; CH = cerebellar microhemorrhages; COX = cytochrome *c* oxidase deficiency; N = normal; ND = no data.

times associated with intercurrent infection; and 3) had liver disease that was indolent and subclinical early in the disease (with aspartate transaminase and alanine transaminase typically only two- to threefold elevated for months to years). Lactic acidosis was documented transiently in four of the six patients but was short-lived and rapidly returned to normal throughout most of the course of the disease. None of the skeletal muscle biopsies (3/3; Subjects A3.II.3, A4.II.2, and A5.II.1) showed respiratory chain abnormalities. Liver respiratory chain studies were also normal in Subject A4.II.2. Liver biopsy in Subject A5.II.1 showed normal complex I, II, and III activities but reduced complex IV (cytochrome *c* oxidase). Quantitative mitochondrial DNA depletion studies or direct measurement of *POLG* enzymatic activity was not performed.

Molecular findings. All six patients were homozygous or compound heterozygous for mutations in the *POLG* gene (table 2). Pedigree analysis showed recessive inheritance of each of the mutant alleles (see figure E-1 on the *Neurology* Web site). All parents were asymptomatic and heterozygous carriers. Figure E-2 on the *Neurology* Web site illustrates the molecular architecture of the *POLG* locus and protein. The *POLG* protein is divided in three domains; exonuclease, linker, and polymerase. We determined the domain boundaries by computer comparison of the mitochondrial polymerases with other members of the Family A type DNA polymerases. We found that the strong homology between *POLG* and related polymerases within the exonuclease domain ended with exon 6 and did not start again until exon 14, at the beginning of the polymer-

Table 2 *POLG* allelic combinations associated with Alpers syndrome

Mutations	Published Alpers probands*				Genotype found in other phenotypes	Ref.	cDNA mutations†		
	A	B	C	D					
p.A467T/p.A467T		1		1	+	6	c.1681G→A in exon 7	/	c.1681G→A in exon 7
p.A467T/p.G848S		1		1	-	4	c.1681G→A in exon 7	/	c.2824G→A in exon 16
p.A467T/p.E873X	1				-	2	c.1681G→A in exon 7	/	c.2899G→T in exon 17
p.A467T/p.A957P		1			-	4	c.1681G→A in exon 7	/	c.3151G→C in exon 18
p.A467T/p.W1020X				1	-	New	c.1681G→A in exon 7	/	c.3339G→A in exon 19
p.A467T/c.3764 + 2 (T→C) splice		1			-	4	c.1681G→A in exon 7	/	c.3764 + 2 (T→C) in intron 21
p.W748S-p.E1143G/p.L244P		1			-	4	c.2525G→C in exon 13	+	c.3710A→G in exon 21 / c.731T→C in exon 3
p.W748S-p.E1143G/p.G848S			4	1	-	5	c.2525G→C in exon 13	+	c.3710A→G in exon 21 / c.2824G→A in exon 16
p.W748S-p.E1143G/p.Y1210fs1216X		1			-	4	c.2525G→C in exon 13	+	c.3710A→G in exon 21 / c.3912insC in exon 22
Proband totals (/15)	1	6	4	4					

* Proband references: A = ref. 2; B = ref. 4; C = ref. 5; D = current study.

† cDNA map positions are numbered according to *POLG* NCBI accession NM_002693. To convert to nucleotide numbers beginning with the "A" of the initiating methionine ATG, subtract 282 bp. In practice, genomic DNA (not cDNA) is amplified by PCR and used for mutation analysis.

ase domain (see figure E-2). The intervening sequence is called the linker region. It comprises exons 7 to 13 (amino acids 418 to 755). The *POLG* linker is conserved among the mitochondrial polymerases but shares no homology with the nonmitochondrial Family A type polymerases. All patients had at least one mutation in the linker region.

Discussion. We report four different combinations of *POLG* mutations that caused Alpers syndrome in four unrelated families (see table 2; also figure E-1). In three of four kindreds, the A467T allele was involved. The role of the A467T allele in Alpers syndrome has also been noted in other studies^{2,4,5} and in patients with other phenotypes, including ataxia and autosomal recessive progressive external ophthalmoplegia.⁶ In two of our kindreds, it was present in single copy and inherited in compound heterozygous condition with a second mutation (see figure E-1A and D). In the third kindred, it was inherited in homozygous condition (see figure E-1C). Clinically, the phenotypes differed mainly according to their age at onset, with the homozygous A467T genotype being associated with a later age at onset (8.5 years in Patient A5.II.1; see table 1). A delayed onset of Alpers syndrome at age 7 was recently reported in another patient who was homozygous for the A467T allele.⁴ The mean age at onset for the non-A467T homozygous patients was 11 months (n = 5; range = 9 to 13 months; see table 1).

Can other genes cause Alpers syndrome? This is certainly possible in some patients who are *POLG* mutation negative. The liver histology of some patients with *DGUOK* mutations may be similar to that in Alpers, but a similar neurologic phenotype has not yet been reported.⁷ Until the discovery of

causative *POLG* mutations, the only definitive tool for the diagnosis of Alpers syndrome was postmortem examination. Today, the study of choice for confirming the clinical diagnosis of Alpers syndrome is DNA testing. The Alpers phenotype appears to require the inheritance of two strongly pathogenic alleles of *POLG*. Inheritance of one or two weaker *POLG* alleles gives rise to non-Alpers phenotypes that are usually more delayed in their age at onsets and more diverse in their clinical manifestations. Currently, nine combinations of nine mutant *POLG* alleles are known to cause Alpers (see table 2), but more will undoubtedly be discovered as research progresses.

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Supplemental Data for www.neurology.org

Appendix (E)A-1

MATERIALS AND METHODS

PCR and DNA Sequencing

DNA was purified from blood (Puregene Blood Kit, Gentra Systems, Minneapolis, MN), or tissue samples by standard methods and amplified by polymerase chain reaction (PCR) using the following primers:

1. Exons 7/8-F 5'- GTC TTG CCT CCT GTG GTC ATT TAT
2. Exons7/8-R 5'- CAC CCA TGC TCC CCA CCT TTC CT
3. Exons19/21-F 5'- GAA GCA CTC CCG TGG AAT G
4. Exons19/21-R 5'-CAA GGA ACG CTC ACC CAA AG
5. Exons 11/18-F 5'-CCC GGG AAA GTG CTA TCT G
6. Exons 11/18-R 5'-AGG GGC TAG GTG AGA GTT CAA

Exons 7-8 and 19-21 were amplified by PCR under the following conditions: [95°Cx5 min]; [94°Cx30 sec, 65°Cx2 min, 72°Cx1 min] x 35 cycles; 72°Cx5 min; 4°Cx up to 16 hours, gel purified, and sequenced using Big Dye chemistry and an ABI 3700 sequencer. The use of primers 1 and 2 amplified a 503 bp fragment and permitted the detection of the A467T mutation. The use of primers 3 and 4 amplified a 919 bp fragment, and permitted the detection of the W1020X (TGA) mutation. Primers 3 and 4 also permitted the detection of the E1143G substitution. Primers 5 and 6 amplified exons 11 to 18 in a 3722 bp fragment with the Expand Long Template PCR kit (Roche). The following conditions were used for primers 5 and 6: [94°Cx2 min]; [94°Cx10 sec, 65°Cx30 sec, 68°Cx17 min] x 10 cycles; [94°Cx15 sec, 65°Cx30

sec, 68°Cx17 min + 20 sec per cycle] x 20 cycles; 68°Cx7 min; 4°C up to 16 hours. For sequencing this fragment, the following primers were used: exon 13-F 5'-TGT CAT TTC CCA GCT GAT GA, exon 13-R 5'-GAC AGT ATG TGC CTG AAA TC, which permitted the detection of the W748S mutation in exon 13; and exon 15-16-F 5'-CCT TGA GTC CAG TTA GTG A and exon 16-R 5'-TCA TGA TCC TCA CTA AAT AC, which detected the G848S mutation in exon 16.

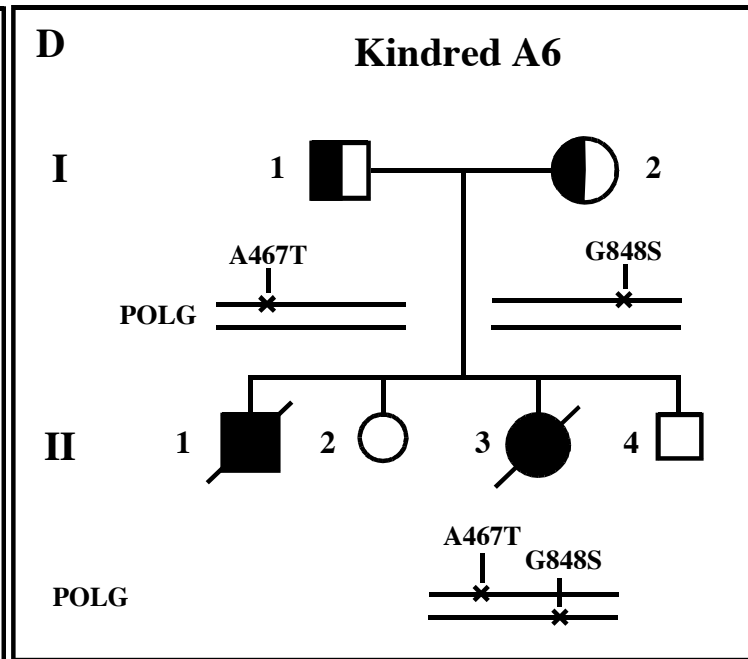
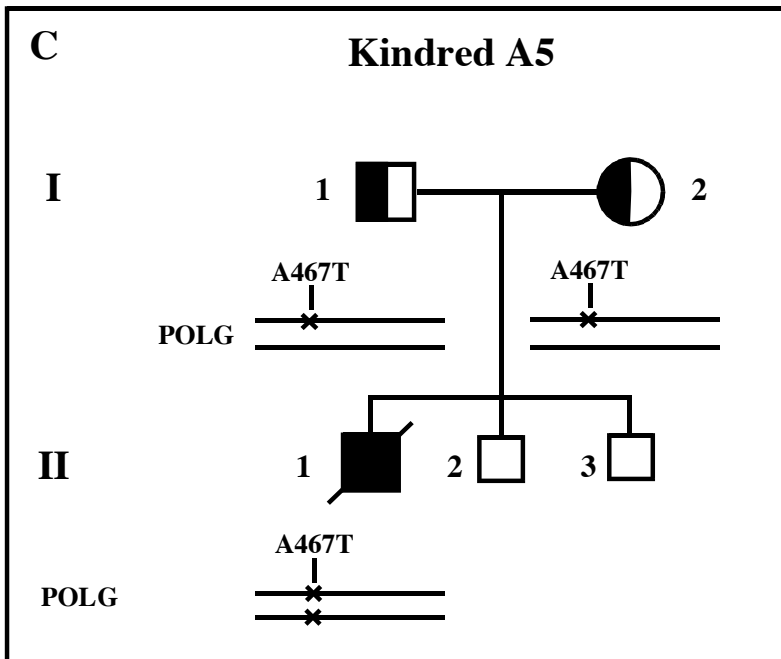
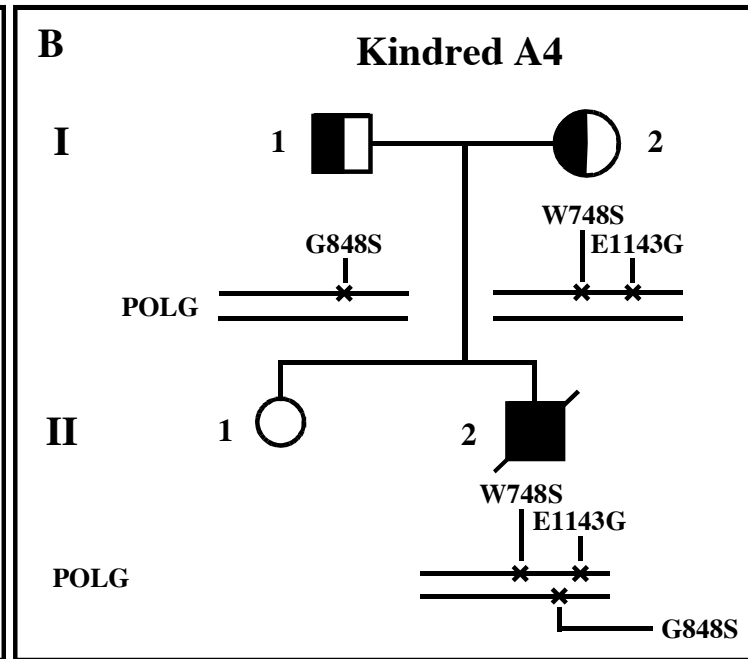
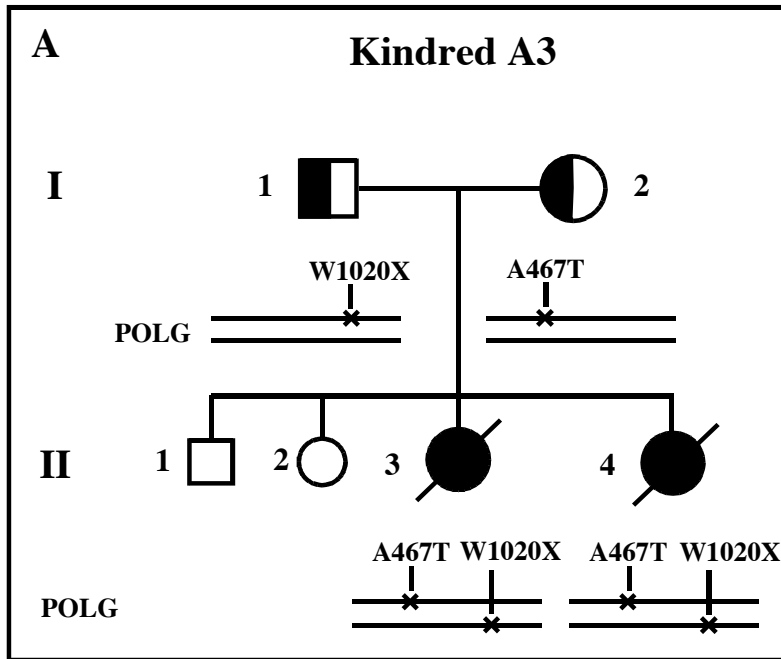
DNA Sequence Analysis

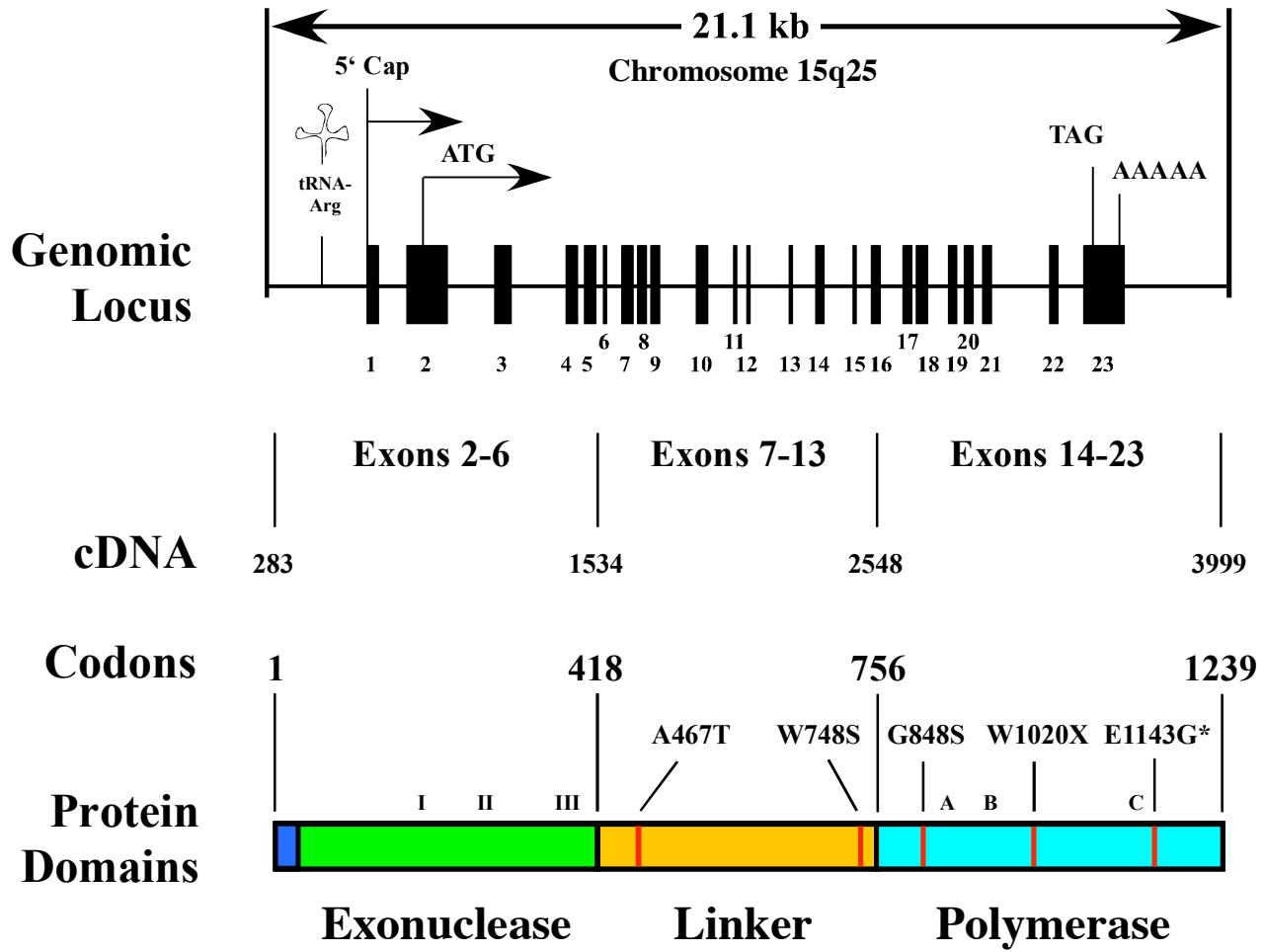
Computer analysis of the DNA sequence data was performed using DNASTAR's Lasergene v. 5.53 (Madison, WI, USA) for Macintosh OS X. Multiple alignments of POLG sequences were performed and the phylogenetic distance matrix was calculated using the clustal W method in MegAlign. The 21 kb human *POLG* sequence, containing the 23 exons and promoter region of the gene, was derived from NCBI AF497906 and annotated with GeneQuest. To establish the exon boundaries of the domains shared by the Family A type DNA polymerases and the mitochondrial polymerases, POLG DNA and the deduced amino acid sequences were aligned with the Family A DNA polymerases of: T7 bacteriophage (NCBI V01146), *E. coli* DNA polymerase I (NCBI V00317), *Thermus aquaticus* (NCBI U62584), bacteriophage T5 (NCBI M24354), mycobacteriophage L5 (NCBI Z18946), and *Bacillus subtilis* bacteriophage SPO2 (NCBI X01458). Chicken (*Gallus gallus*) *POLG* is unique among the vertebrate mitochondrial polymerases in lacking an exonuclease domain, and the first half of the linker, and could not be used in the alignment of sequences in those domains.

FIGURE LEGENDS

(E)F-1 Pedigree Analysis of *POLG* Genotypes in Alpers syndrome. A. Kindred A3, A467T/W1020X. B. Kindred A4, W748S-E1143G/G848S. C. Kindred A5, A467T/A467T. D. Kindred A6, A467T/G848S

(E)F-2 Molecular Architecture of the Mitochondrial DNA Polymerase γ (POLG). The A467T and W748S substitutions are located in the linker region. The G848S and W1020X mutations are located in the polymerase domain. The E1143G substitution is also located in the polymerase domain, but it was linked in *cis* with the W748S substitution and its independent role in pathogenesis has not yet been established.





■ **Statin use and cognitive decline in the elderly**

Studying an elderly community cohort, Bernick et al. found that statin drug use was associated with a slight reduction in cognitive decline vs nonusers over a 7-year period. The only MRI variables that differentiated statin users were a lower number of accumulated silent infarcts.

see page 1388

■ **Surrogate consent for high risk research?**

Research consent for incompetent subjects lacks explicit policy guidance in most jurisdictions. Kim et al. surveyed persons at elevated risk for Alzheimer disease and found that the majority support surrogate consent for research even when the risk and burdens to subjects are substantial.

see page 1395

■ **Patent foramen ovale in migraine with aura**

In a cross-sectional case control study, Schwerzmann et al. studied the characteristics of cardiac right-to-left cardiac shunts in patients with migraine with aura using transesophageal echocardiography. Nearly half of the patients with migraine with aura had a patent foramen ovale, and shunt size larger than in controls without migraine with aura.

see page 1415

■ **Botulinum toxin treatment for cervical dystonia**

A controlled study by Comella et al. directly compared two serotypes of botulinum toxin for the treatment of cervical dystonia. They found similar efficacy for serotypes A and B, with a modest prolongation of benefit using serotype A.

see page 1423

■ **Daytime sleepiness and PD**

In the Honolulu-Asia Aging Study Abbott et al. examined the relationship between excessive daytime sleepiness (EDS) and the development of Parkinson disease (PD). EDS was assessed in 3,078 elderly men from 1991 to 1993. Men were followed for incident PD to 2001. Findings suggest that EDS can predate PD.

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■ **Ictal monoparesis with primary somatosensory lesion**

Matsumoto et al. report three patients with ictal monoparesis of the arm. A well-circumscribed lesion in the primary arm somatosensory area was associated with epileptic activity.

see page 1476

■ **POLG mutations in Alpers syndrome**

Nguyen et al. identified mutations in the mitochondrial DNA polymerase (*POLG*) as the cause of Alpers syndrome. Here they summarize nine causative mutations in *POLG*. Genetic testing may now be possible for this neurodegenerative disease of children and young adults.

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■ **Long-term 24-hour duodenal levodopa infusion**

Nyholm et al. treated five patients with PD with continuous 24-hour duodenal levodopa infusion for 13 to 37 months. Motor responses were stable and without increased side effects. Daily dosage increased slightly (14%) over the study period.

see page 1506

■ **Levodopa addiction in PD**

Levodopa may be addictive. Borek et al. describe two parkinsonian patients, one of whom was quadriplegic, for whom psychological benefit rather than motor response was seen with levodopa.

see page 1508

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