Genetic Linkage Evidence for a Familial Alzheimer's Disease Locus on Chromosome 14

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Linkage analysis was used to search the genome for chromosomal regions harboring familial Alzheimer's disease genes. Markers on chromosome 14 gave highly significant positive lod scores in early-onset non–Volga German kindreds; a Z_{max} of 9.15 ($\theta = 0.01$) was obtained with the marker D14S43 at 14q24.3. One early-onset family yielded a lod score of 4.89 ($\theta = 0.0$). When no assumptions were made about age-dependent penetrance, significant results were still obtained ($Z_{max} = 5.94$, $\theta = 0.0$), despite the loss of power to detect linkage under these conditions. Results for the Volga German families were either negative or nonsignificant for markers in this region. Thus, evidence indicates a familial Alzheimer's disease locus on chromosome 14.

Defective genes are responsible for at least some cases of Alzheimer's disease (AD). Numerous families have been described in which early-onset AD appears to segregate as an autosomal dominant trait (*1–7*). In a subset of early-onset familial AD (FAD) kindreds, mutations at codons 670, 671, and 717 of the amyloid precursor protein (APP) gene are responsible for the disease (*8, 9*). However, the majority of early-onset FAD families, including the Volga German (VG) kindreds, do not show linkage to (*7, 10–12*) and do not appear to have mutations in the APP gene (*12–14*). Defective genes may also be responsible for some or possibly all late-onset AD (*15*).

The observation that APP mutations account for AD in only a fraction of families indicates that FAD is genetically heterogeneous (*7, 10–14, 16*). Additional FAD loci may exist on chromosome 21 (*4, 16, 17*) and chromosome 19 (*18, 19*), although these sites do not account for all FAD (*7, 11, 12, 20*). We describe genetic linkage evidence for an FAD locus on chromosome 14.

Different chromosomal locations were tested for the presence of an FAD locus with the use of the logarithm of the likelihood ratio for linkage (lod) score method (*21*). The families studied (Table 1 and Fig. 1) had three or more affected subjects in two or more generations and at least one case of autopsy-documented AD. Three groups defined for separate analysis (*7*) were: VG (*5*), early-onset (onset mean ≤ 60 years) non-VG (ENVG), and VG and ENVG combined. Two models assuming autosomal dominant inheritance were used. In the first, cumulative normal age-dependent penetrance was assumed with a maximum penetrance of 100%. For the second model, penetrance was set at 1% such that no assumptions about the age-dependence of FAD onset were made. For both conditions, lod scores were computed at FAD gene frequencies of 0.001, 0.01, and 0.06. The VG and ENVG groups were negative for linkage to the APP gene and to more centromeric markers on chromosome 21 (*7, 11, 12*), and for chromosome 19 q12-13.3 loci (*22*). In addition, affected subjects from these families do not have mutations

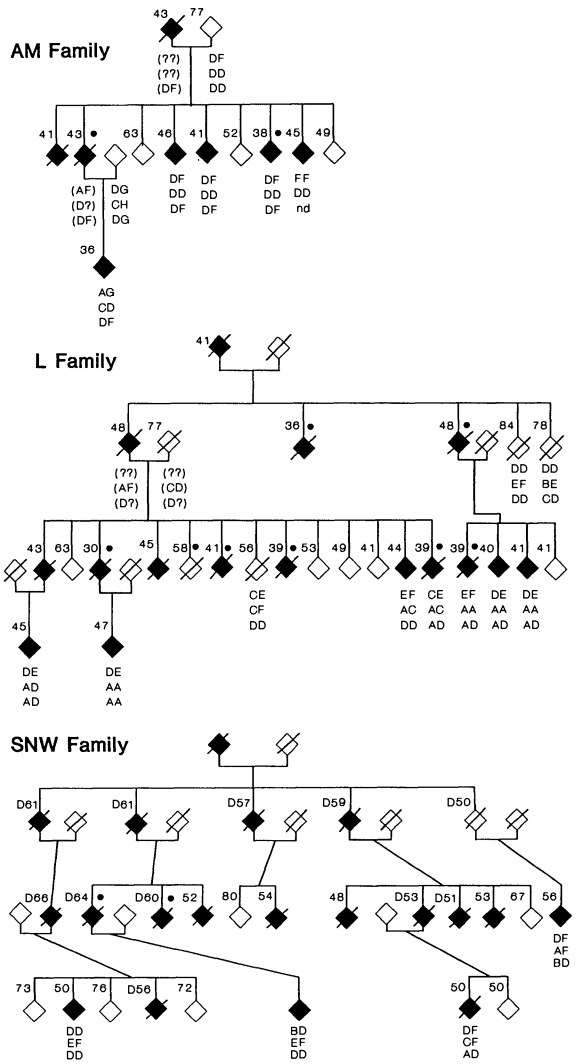

Fig. 1. Segregation of marker alleles in FAD families AM, L, and SNW. Alleles for D14S52, D14S43, and D14S53 are shown below subjects from top to bottom, respectively. Alleles for D14S52 are: A, 57 bp; B, 69 bp; C, 81 bp; D, 83 bp; E, 85 bp; F, 87 bp; G, 93 bp; and H, 95 bp. Alleles for D14S43 are: A, 159 bp; B, 179 bp; C, 181 bp; D, 183 bp; E, 185 bp; F, 187 bp; G, 175 bp; and H, 171 bp. Alleles for D14S53 are: A, 144 bp; B, 148 bp; C, 149 bp; D, 151 bp; E, 153 bp; F, 155 bp; and G, 157 bp. "nd" indicates genotype not determined. Genotypes in parentheses were deduced from spouse and offspring genotypes. Solid symbols represent affected subjects, and a slash indicates the subject is deceased. The pedigrees have been altered to protect confidentiality and not all subjects genotyped are shown. The number above and to the left of subjects is the age-of-onset for affected subjects, the current age for living unaffected subjects, or the age at death for unaffected subjects. For affected subjects, if the age-of-onset is not known, the age of death is shown preceded by "D." A dot above and to the right of a subject indicates an autopsy was performed.

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in exons 16 or 17 of the APP gene (*12, 13*). A total of 64 markers were analyzed; 14 were on chromosome 21 clustered at 21 q11.1-21.1, 17 were on chromosome 19 clustered at 19 q12-13.3, and the remaining 33 were scattered on other autosomes.

When chromosome 14 markers were tested, results with the initial markers D14S1 and the α1-antitrypsin (PI)–α1-antichymotrypsin (AACT) gene cluster gave small nonsignificant positive lod scores. D14S43 and T-cell receptor delta (TCRD) were then selected to test whether these positive lod scores were the result of a more centromeric FAD locus (Fig. 2). D14S43 gave strong positive lod scores for ENVG families at all recombination fractions for both the age-corrected penetrance and the affected only (1% penetrance) models (Tables 2 and 3). Significant results ($Z_{max} > 3.00$) were obtained for both models at all FAD gene frequencies tested. When an FAD gene frequency of 0.001 and a group-specific standard deviation for the onset means was used, the ENVG group yielded a maximum lod score of 9.15 ($\theta = 0.01$) with D14S43 for the age-corrected penetrance model and 5.94 ($\theta = 0.0$) for the 1% penetrance model (Table 3). Much of the positive evidence for linkage came from families L ($Z_{max} = 4.89$, $\theta = 0.00$) and SNW ($Z_{max} = 2.17$, $\theta = 0.0$).

The VG kindreds gave negative lod scores for D14S43 at all recombination fractions when the age-corrected penetrance function was used, but yielded small nonsignificant positive lod scores under the 1% penetrance model (Table 4). When a between-group heterogeneity test (*21*) was used to compare the ENVG to the VG group, highly significant evidence for heterogeneity ($\chi_1^2 = 9.99$, $P = 0.0015$) was obtained. When the age-dependent model was used, this test gave only marginally significant results ($\chi_1^2 = 3.91$, $P = 0.048$) for the 1% penetrance model. There was no evidence for heterogeneity within the ENVG or the VG group. However, the lack of statistical evidence for heterogeneity does not exclude the possibility that some of the ENVG families may not map to chromosome 14.

D14S43 and PI were placed on a recent-

Table 1. FAD kindreds used for linkage analysis. Volga German and early-onset non–Volga German kindreds are described. Families were evaluated as previously described (*4–7, 18*). Pedigrees for each family are published (*1–3, 5–7*). The SNW family has also been referred to as FAD3 (*4*) and SW (*1*), and the LH family referred to as family 603 (*7, 13, 18*). Abbreviations: *n*, number; SD, standard deviation.

Family	Affected (*n*)	Autopsied (*n*)	Subjects sampled (*n*)	Affecteds sampled (*n*)	Mean age of onset ± SD, (*n*), range	Ethnic origin
R	20	4	29	3	51 ± 7.1 (17) 40–67	Volga German
W	4	1	4	2	54 ± 3.9 (4) 48–58	Volga German
HD	23	1	11	6	59 ± 10.4 (18) 46–82	Volga German
HB	21	4*	24	4	60 ± 7.2 (20) 47–75	Volga German
KS	12	3	24	6	65 ± 5.1 (11) 55–71	Volga German
L	16	9*	23	7	42 ± 4.6 (16) 30–48	German
AM	8	2†	10	5	42 ± 3.2 (8) 36–46	Japanese
HR-XV	12	1†	7	4	42 ± 3.9 (6) 37–47	Hispanic
KG	5	3	11	1	44 ± 1.0 (5) 43–45	British
V	8	1	9	2	46 ± 3.8 (7) 41–50	British (?)
HR-I	13	6	25	3	47 ± 4.6 (12) 40–55	German
HR-XIII	4	4	11	0	47 ± 3.6 (4) 41–51	Scottish-Irish
LH(603)	23	6	27	5	48 ± 6.5 (18) 37–68	French-Canadian
SNW	19	5	17	4	52 ± 2.5 (7) 48–56	Russian-Jewish

*Includes an autopsy on a normal subject. †Includes one biopsy.

Table 2. Lod scores for linkage of FAD to chromosome 14 loci. Genotype analysis of STRP loci was performed by conventional methods (*19, 24, 29–31*). For D14S43, genotype determinations were repeated for all family subjects with a final error rate of less than 1%. Lod scores were calculated with the use of an FAD gene frequency of 0.001. For screening purposes (markers AACT, D14S1, D14S43, PI, AACT, and TCRD), a standard deviation of 11.99 years for the mean age of onset was used, which was derived from the early-onset non–Volga German (ENVG) and Volga German (VG) families in Table 1 as well as a group of late-onset families (*12*). For the remaining markers, a standard deviation of 9.52 years, derived from the VG and ENVG families, was used. The choice of standard deviations did not significantly affect the results (compare Tables 2 and 3). PIC, polymorphic information content; nd, not determined.

Marker	PIC	Group	Recombination fraction						
			0.001	0.05	0.10	0.15	0.20	0.30	0.40
TCRD	0.74	ENVG	−10.34	−4.52	−2.84	−1.86	−1.21	−0.44	−0.09
		VG	−8.44	−3.63	−2.28	−1.50	−0.99	−0.38	−0.09
D14S47	0.58	ENVG	−5.29	−0.87	−0.01	0.35	0.48	0.39	0.15
		VG	−6.25	−2.76	−1.80	−1.22	−0.82	−0.34	−0.10
D14S52	0.58	ENVG	2.02	4.59	4.56	4.19	3.64	2.25	0.08
		VG	−4.60	−1.26	−0.52	−0.17	0.01	0.08	0.01
D14S43	0.72	ENVG	8.91	8.40	7.67	6.79	5.79	3.55	1.33
		VG	−6.41	−1.51	−0.47	−0.02	0.16	0.16	0.03
D14S53	0.68	ENVG	4.24	7.12	6.88	6.19	5.28	3.10	0.99
		VG	−3.58	−2.49	−1.74	−1.21	−0.82	−0.30	−0.06
D14S55	0.51	ENVG	−1.32	0.43	0.66	0.70	0.64	0.43	0.19
		VG	−6.97	−3.12	−1.51	−0.69	−0.27	0.00	−0.05
D14S48	0.73	ENVG	−10.96	−2.56	−0.93	−0.15	0.25	0.41	0.13
		VG	nd	nd	nd	nd	nd	nd	nd
AACT		ENVG	−2.35	0.06	0.52	0.68	0.69	0.46	0.16
		VG	−3.58	−1.77	−1.22	−0.86	−0.60	−0.25	−0.06
PI	0.89	ENVG	−9.72	−2.52	−0.69	0.16	0.55	0.61	0.24
		VG	−9.08	−3.43	−2.01	−1.29	−0.87	−0.43	−0.18
D14S1	0.79	ENVG	−17.70	−5.10	−2.08	−0.59	0.20	0.69	0.47
		VG	−13.33	−6.38	−3.76	−2.27	−1.37	−0.44	−0.11

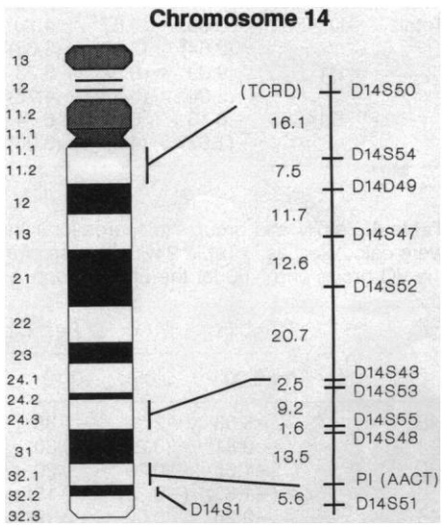

Fig. 2. Location of markers on chromosome 14. The genetic map was constructed with data from the CEPH families with the use of the computer program CRIMAP (*24*). Distances are given in centimorgans (sex-averaged) with the use of the Kosombi map function. The order for D14S43 and D14S53 was favored over the reverse order by a likelihood ratio of 7700:1. The order for loci D14S48, PI, and D14S51 was favored by 10^{12}:1 odds. Band locations were determined by others (*26*).

ly described short tandem repeat polymorphism (STRP) map of chromosome 14 (*24*) (Fig. 2). When the two markers flanking D14S43 (D14S52 and D14S53) were tested for linkage to FAD, both gave significant positive lod scores for the ENVG group and were negative for the VG families. The AM family, which was uninformative for D14S43, gave positive lod scores with D14S53 (Z_{max} = 1.49, θ = 0.0).

Table 3. Family and group lod scores for linkage of FAD to D14S52, D14S43, and D14S53 for the ENVG families. Lod scores were calculated as in Table 2 with the use of a group-specific standard deviation of 5.60 years for the mean age of onset. Lod scores in parentheses were calculated under the assumption of 1% penetrance.

Family	Locus	Recombination fraction							Z_{max} (θ)
		0.00	0.05	0.10	0.15	0.20	0.30	0.40	
AM	D14S52	−2.70	−0.70	−0.31	−0.12	−0.01	0.08	0.07	
	D14S43	0.00	0.01	0.02	0.02	0.02	0.01	0.00	
	D14S53	1.49	1.36	1.22	1.07	0.91	0.57	0.22	
HR-I	D14S52	2.17	2.09	1.92	1.71	1.46	0.91	0.32	
	D14S43	1.44	1.23	1.04	0.86	0.68	0.35	0.08	
	D14S53	−6.38	0.11	0.26	0.28	0.25	0.13	0.03	
HR-XIII	D14S52	0.12	0.10	0.08	0.06	0.05	0.02	0.00	
	D14S43	0.30	0.26	0.22	0.19	0.14	0.07	0.02	
	D14S53	−0.75	−0.46	−0.29	−0.18	−0.10	−0.02	0.00	
HR-XV	D14S52	1.12	1.01	0.89	0.77	0.64	0.37	0.12	
	D14S43	−0.91	−0.40	−0.22	−0.13	−0.07	−0.02	0.00	
	D14S53	−0.50	−0.03	0.11	0.16	0.17	0.12	0.04	
KG	D14S52	−6.80	−0.02	0.16	0.22	0.22	0.15	0.05	
	D14S43	0.88	0.84	0.77	0.69	0.59	0.36	0.13	
	D14S53	1.21	1.09	0.96	0.82	0.67	0.38	0.12	
L	D14S52	1.82	1.65	1.48	1.30	1.10	0.66	0.24	
	D14S43	4.89	4.47	4.03	3.57	3.09	2.03	0.89	
	D14S53	−4.57	2.44	2.36	2.13	1.83	1.07	0.28	
LH(603)	D14S52	0.00	−0.01	−0.03	−0.04	−0.04	−0.03	−0.01	
	D14S43	1.33	1.23	1.11	0.96	0.79	0.45	0.16	
	D14S53	2.32	2.06	1.78	1.50	1.22	0.68	0.24	
SNW	D14S52	0.87	0.77	0.67	0.57	0.47	0.28	0.10	
	D14S43	2.17	2.02	1.81	1.56	1.29	0.71	0.22	
	D14S53	0.66	0.65	0.59	0.52	0.44	0.28	0.12	
V	D14S52	−0.30	−0.22	−0.17	−0.13	−0.10	−0.06	−0.02	
	D14S43	−0.99	−0.75	−0.56	−0.40	−0.28	−0.11	−0.02	
	D14S53	−1.60	−0.48	−0.24	−0.13	−0.07	−0.03	−0.02	
Totals	D14S52	−3.69	4.67	4.70	4.34	3.79	2.37	0.86	4.76 (0.08)
		(2.64)	(3.89)	(3.60)	(3.13)	(2.58)	(1.44)	(0.48)	3.96 (0.06)
	D14S43	9.09	8.92	8.23	7.32	6.25	3.86	1.47	9.15 (0.01)
		(5.94)	(5.44)	(4.78)	(4.07)	(3.33)	(1.84)	(0.61)	5.94 (0.0)
	D14S53	−8.13	6.73	6.74	6.18	5.32	3.18	1.03	6.85 (0.07)
		(1.67)	(4.08)	(3.98)	(3.54)	(2.95)	(1.66)	(0.55)	4.11 (0.06)

Table 4. Family and group lod scores for linkage of FAD to D14S43 for VG kindreds. Lod scores were calculated as in Table 2 with the use of a group-specific standard deviation of 8.62 years for the VG group and 5.60 for the ENVG group.

Family	Recombination fraction							Z_{max} (θ)
	0.00	0.05	0.10	0.15	0.20	0.30	0.40	
HB	−5.58	−2.29	−1.38	−0.88	−0.57	−0.23	−0.08	
	(−0.81)	(0.21)	(0.30)	(0.29)	(0.23)	(0.10)	(0.00)	0.31 (0.11)
HD	−1.65	0.09	0.20	0.19	0.13	−0.03	−0.09	0.20 (0.10)
	(−1.63)	(−0.32)	(−0.15)	(−0.09)	(−0.06)	(−0.04)	(−0.03)	
KS	−2.26	0.00	0.19	0.26	0.26	0.20	0.10	0.26 (0.15)
	(−1.01)	(−0.10)	(0.05)	(0.10)	(0.11)	(0.09)	(0.05)	0.11 (0.20)
R	0.15	0.10	0.05	0.01	−0.02	−0.03	−0.01	0.15 (0.0)
	(0.17)	(0.11)	(0.06)	(0.03)	(0.01)	(0.0)	(0.0)	0.17 (0.0)
W	−0.98	−0.54	−0.35	−0.24	−0.16	−0.06	−0.01	
	(0.29)	(0.25)	(0.21)	(0.17)	(0.13)	(0.06)	(0.02)	0.29 (0.001)
Total (VG only)	−10.32	−2.65	−1.28	−0.65	−0.35	−0.15	−0.10	
	(−2.99)	(0.14)	(0.48)	(0.50)	(0.42)	(0.20)	(0.04)	0.50 (0.15)
Total (VG and ENVG)	−1.23	6.27	6.95	6.66	5.91	3.71	1.37	6.95 (0.10)
	(2.96)	(5.58)	(5.26)	(4.57)	(3.75)	(2.04)	(0.65)	

These data provide strong evidence for an early-onset FAD locus at 14q24.3. The lod scores substantially exceed 3.0, the threshold value for statistical significance for both penetrance models, despite the loss of power to detect linkage resulting from use of the 1% penetrance model. Therefore, the evidence for linkage is independent of assumptions about the age-dependent penetrance of FAD. The probability of linkage obtained from these analyses exceeds 99.99% (*23*) even after the original screening Z_{max} of 8.91 for D14S43 (Table 2) is corrected for the two penetrance models used and the 64 markers tested, reducing the Z_{max} to 6.8. However, it is probably unnecessary to adjust for the moderate number of independent markers used in this study (*23, 25*).

The location of the chromosome 14 FAD gene can be estimated from evidence for recombination events obtained from discordant genotypes among affected individuals. In pedigrees HRI and L there are recombinant events between the FAD locus and D14S53, but none for D14S43 or D14S52. In pedigree AM, there is a recombinant event between the FAD locus and D14S52 but not D14S53 (D14S43 was uninformative). Because D14S53 is much closer to D14S43, these three recombinants put the FAD locus between D14S43 and D14S52. A location between D14S43 and D14S52 is supported by the distance estimates obtained with the two-point analyses: the estimated recombination distance of the FAD locus from D14S53 is 7% and from D14S52 is 8% (Table 3). It may be possible to obtain a more precise estimate of the FAD gene location using multipoint analysis. However, because of the large amount of missing data in FAD pedigrees (deceased subjects who cannot be sampled) and because the markers used are highly polymorphic resulting in a large number of alleles segregating in each family, multipoint analysis requires prohibitive amounts of computer time. We estimate that the multipoint analysis of FAD, D14S52, D14S43, and D14S53 would require at least 3 months of computing time with a workstation-type computer. Given that several families are more informative for D14S53 than for D14S43, it is likely that the maximum multipoint lod score in the region will be over 11.0 for the ENVG families.

These results have several implications for both the genetics of FAD and the pathogenetic mechanisms responsible this disease. FAD is indeed genetically heterogeneous; early-onset FAD can be caused by

mutations either at the APP gene or a chromosome 14 locus. Additional FAD loci are not excluded. Unraveling the genetics of FAD will ultimately contribute to resolving the contribution of inheritance to apparently "sporadic" AD, the most common form of the disease.

The chromosome 14 locus may be involved in APP processing. Several chromosome 14 genes that might be candidates include the protease inhibitors encoded by the AACT and PI genes and the protease cathepsin G at band 14 q11. However, these loci do not appear to be located in the correct region and the AACT-PI cluster appears to be excluded by the linkage data (Table 2). Candidate loci that map to the 14 q24 region include c-FOS and HSPA2 (heat shock 70-kD protein) (*26*). c-FOS may be involved in transcriptional regulation of the APP gene (*27*). The HSPA2 gene product is a molecular chaperon potentially involved in protein assembly and degradation (*28*) and thus could act in one of the APP processing pathways. In addition, the gene products from both c-FOS and HSPA2 have been implicated in injury response mechanisms in the central nervous system (*27, 28*). The possibility also exists that the chromosome 14 locus defines a pathogenetic mechanism that is completely distinct from APP processing.

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21. Lod scores were calculated with the use of the computer program LIPED modified to handle up to 20 alleles and with the capacity to handle different alleles for each family. Possible heterogeneity between the two groups of families was examined with the M test [N. E. Morton, *Am. J. Hum. Genet.* **8**, 80 (1956)] and heterogeneity within groups was tested with the A test [C. A. B. Smith, *Proc. 2nd Int. Congr. Hum. Genet.* **1**, 212 (1961); S. E. Hodge, C. E. Anderson, K. Neiswanger, R. S. Sparkes, D. L. Rimoin, *Am. J. Hum. Genet.* **35**, 1139 (1983)].
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29. For the STRP loci (D14S43, D14S47, D14S48, D14S52, D14S53, D14S55, and TCRD), allele frequencies, primers, and amplification conditions were as described [(*26*) and V. Sharma, L. Smith, L. Allen, R. E. Magenis, M. Litt, *Nucleic Acids Res.* **19**, 1722 (1991); S. A. Jordan, P. McWilliam, D. S. O'Briain, P. Humphries, *ibid.*, p. 1959]. For all STRP markers, allele frequencies used were those published, combined with data from 100 additional unrelated unaffected Caucasians. For D14S43, D14S52, and D14S53, allele frequencies used for the AM family were from 90 unrelated unaffected Japanese subjects.
30. D14S1 and AACT genotypes were determined by Southern blotting techniques (*7*). For AACT, a partial cDNA clone was used to identify the following polymorphic sites: (1) a Taq I bi-allelic RFLP with restriction fragment sizes of 2.8 and 5.8 kb and allele frequencies of 0.3 and 0.7, respectively; (2) a Stu I bi-allelic RFLP with restriction fragment sizes of 17.5 and 7.0 kb and allele frequencies of 0.64 and 0.36, respectively; (3) a Stu I polymorphic site with restriction fragment sizes of 1.1 and 0.95 kb and allele frequencies of 0.12 and 0.88, respectively; (4) an Apa I bi-allelic RFLP with restriction fragment sizes of 2.1 and 1.65 kb and allele frequencies of 0.11 and 0.89, respectively.
31. The PI gene was genotyped by amplifying three portions of the gene followed by digestion with restriction enzymes. Primer sequences were taken from the published PI gene sequence [G. L. Long, T. Chandra, S. L. Woo, E. W. Davie, K. Kurachi, *Biochemistry* **23**, 4828 (1983), GenBank accession number K02212] and were numbered according to the position of the 5′ nucleotide. Primers PI4047L (AGTGAAGTCATTTACCCCAGG) and PI4047R (GGTTCATTATGCCTCCCAAGC) produce a 466-bp product, beginning at base 2096, which contains two bi-allelic restriction fragment length polymorphism (RFLP) sites detected by digestion with Msp I and Ava II [D. W. Cox, S. L. C. Woo, T. Mansfield, *Nature* **316**, 79 (1985)]. Primers PI7595L (GCTGACACTCACGATGAAATCC) and PI7595R (ACTTATCCACTAGCTTCAGGC) produce a 181-bp product, starting at base 5644, which contains a bi-allelic RFLP at codon 101 detected by digestion with Rsa I [T. Nukiwa, M. L. Brantly, F. Ogushp, G. A. Fells, R. G. Crystal, *Am. J. Hum. Genet.* **43**, 322 (1988)]. Primers PI9350L (TCTGCTACACTCTTCCAAACC) and PI9350R (GGTGAGTTCATTTACCAGGTGC) produce a 292-bp product, beginning at base 7399, which contains an RFLP site at the codon for Val^{213} that can be detected by digestion with Bst EII [T. Nukiwa *et al.*, *J. Biol. Chem.* **261**, 15989 (1986)].
32. Supported by grants from the National Institute on Aging for an Alzheimer's Disease Research Center at the University of Washington (AG08017), the National Institute of Mental Health (R01MH43240), the National Center for Human Genome Research (HG00248), Veterans Affairs Research Funds, the American Health Assistance Foundation, and the Washington State Friends of Alzheimer's Research Program. We thank G. Shiely and K. Kiech for gifts to the University of Minnesota; D. Nochlin, M. Sumi, M. Ball, C. Altshuler, and G. Glenner for neuropathologic characterization of many of the families, E. Loomis, S. Ito, and J. Lundgren for technical help, H. Lipe and T. Lampe for obtaining blood samples and medical records, S. Ahn for assistance with computations, and A. Roses and P. St George-Hyslop for some of the cell lines for families LH (603) and SNW, respectively. Informed consent was obtained from each subject or next-of-kin with approval of the University of Washington Human Subjects Review Committee.

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