Ubiquilin 1 Polymorphisms Are Not Associated with Late-Onset Alzheimer's Disease

Scott Smemo, AB,[1] Petra Nowotny, PhD[1] Anthony L. Hinrichs, PhD,[1] John S. K. Kauwe, MSc,[1] Sara Cherny, AB,[1] Katherine Erickson,[1] Amanda J. Myers, PhD,[2] Mona Kaleem, BSc,[2] Lauren Marlowe, BSc,[2] Alison M. Gibson, BSc,[3] Paul Hollingworth, PhD,[4] Michael C. O'Donovan, PhD,[4] Chris M. Morris, PhD,[4] Peter Holmans, PhD,[5] Simon Lovestone, PhD,[6] John C. Morris, MD,[7] Leon Thal, MD,[8] Yonghong Li, PhD,[9] Andrew Grupe, PhD,[9] John Hardy, PhD,[2] Michael J. Owen, PhD,[4] Julie Williams, PhD,[4] and Alison Goate, DPhil[1,7,10]

Several studies have reported evidence for linkage of late-onset Alzheimer's disease (LOAD) to chromosome 9. Recently, an intronic polymorphism affecting alternative splicing of exon 8 of ubiquilin 1 (UBQLN1) was reported to be associated with LOAD. We attempted to replicate this observation by genotyping this polymorphism, rs12344615 (also known as UBQ-8i), in a large sample of 1,544 LOAD cases and 1,642 nondemented controls. We did not find any evidence that this single nucleotide polymorphism, or any of six others tested in *UBQLN1*, increases risk for LOAD.

Ann Neurol 2006;59:21–26

Late-onset Alzheimer's disease (LOAD) is a complex neurological disorder, undoubtedly influenced by several genetic and environmental factors. The only widely recognized genetic risk factor currently identified is a polymorphism in the apolipoprotein E gene (*APOE*), which is associated with increased disease risk and decreased age of onset.[1] However, this susceptibility locus accounts for less than half of the genetic variance in Alzheimer's disease, and Warwick Daw and colleagues[2] estimated that at least four other genes with an effect of similar magnitude on age of onset remain to be identified.

The ubiquilin1 (*UBQLN1*) gene on chromosome 9q22 lies in a region that has been implicated in several linkage studies of LOAD.[3,4] UBQLN1 has been shown to interact with the presenilin (PS) proteins and to increase their accumulation.[5] Massey and colleagues[6] have shown that overexpression of ubiquilin 1 decreases ubiquitination and degradation of presenilin proteins, which is notable because mutations in the presenilins are associated with early-onset AD.[7,8] *UBQLN1* therefore is a good positional and functional candidate as a novel LOAD susceptibility gene. A recent family-based association study reported an association between a single nucleotide polymorphism (SNP) in *UBQLN1*, rs12344615, and LOAD.[9] We have attempted to replicate this finding in a large sample of unrelated cases and controls from the United States and the United Kingdom.

Materials and Methods

Samples

Five case–control series studying white subjects composed of unrelated individuals were used: three series were clinically assessed (WashU, UCSD, MRC-LOAD) and two were neuropathologically assessed (Newcastle-Brain, US-Brain). Cases in the clinically assessed series had a clinical diagnosis of dementia of the Alzheimer's type (probable/definite) according to National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[10] criteria with a minimum age at which the first symptoms of AD were observed

From the [1]Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; [2]National Institute for Aging, Bethesda, MD; [3]Institute for Ageing and Health, Newcastle General Hospital, University of Newcastle upon Tyne, Newcastle upon Tyne; [4]Department of Psychological Medicine, School of Medicine and [5]Biostatistics and Bioinformatics Unit, Cardiff University, Cardiff; [6]Institute of Psychiatry, Kings College London, London, United Kingdom; [7]Department of Neurology, Washington University School of Medicine, St. Louis, MO; [8]Department of Neuroscience, University of California, San Diego, CA; [9]Celera Diagnostics, Alameda, CA; [10]Department of Genetics, Washington University, School of Medicine, St. Louis, MO.

Received May 20, 2005, and in revised form Aug 24. Accepted for publication Aug 25, 2005.

Published online Nov 8, 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20673

Address correspondence to Dr Goate, Samuel and Mae S. Ludwig Professor of Genetics in Psychiatry, Professor of Genetics and Neurology, Department of Psychiatry, Campus Box 8134, Washington University School of Medicine, 660 S. Euclid Street, St. Louis, MO 63110. E-mail: goate@icarus.wustl.edu

Table 1. Demographic Data

Demographic	Newcastle-Brain Cases	Newcastle-Brain Controls	US-Brain Cases	US-Brain Controls	Combined Cases	Combined Controls	WashU Cases	WashU Controls	MRC-LOAD Cases	MRC-LOAD Controls	UCSD Cases	UCSD Controls
No. of subjects	160	112	196	116	1544	1642	376	353	633	731	179	330
Female (%)	66	50.5	57.2	45.6	66.9	62.4	64.6	62.0	76.5	66.6	49.2	63.3
AAO (SD)	n/a	—	n/a	—	77.4 (6.4)	—	76.2 (6.8)	—	77.0 (6.1)	—	73.4 (5.4)	—
AAE/AAD (SD)	81.4 (6.8)	77.7 (8.0)	81.7 (6.8)	80.0 (9.0)	—	77.7 (6.8)	—	77.5 (7.4)	—	76.6 (5.9)	—	79.3 (7.2)
APOE4+ (%)	60	23.2	68.6	23.6	61.9	23.3	58.0	24.1	61.9	23.5	64.2	21.8

The MRC-LOAD series is an expansion of what was previously referred to as the UK1 series in Nowotny and colleagues.[12] The Newcastle-Brain series was referred to as the UK2 series in the same study. The controls of both neuropathologically assessed series have also been described elsewhere.[13] n/a = not available; AAO = age of onset; AAE/AAD = age at examination/age at death; SD = standard deviation.

("age of onset") of 60 years. Nondemented controls in these three series were also at least 60 years old when screened for dementia using neuropsychological tests and/or clinical interviews. Controls were age- and sex-matched with cases. Both the Newcastle-Brain and US-Brain series were composed of samples that were pathologically confirmed AD cases at autopsy, with 30% of cases having Braak and Braak staging[11] of 5 or 6. Controls had no histopathological abnormalities with 40% of controls having Braak and Braak staging less than 2. The US-Brains were identified through the National Institute on Aging National Alzheimer's Coordinating Center database and were collected by the Neuropathology Cores of Alzheimer's Disease Research Centers. The total sample size is 1,544 cases/1,642 nondemented controls and breaks down as follows: WashU 376/353, MRC-LOAD 633/731, UCSD 179/330, Newcastle-Brain 160/112, US-Brain 196/116. Detailed demographics are provided in Table 1. Informed consent was obtained from all subjects or appropriate proxy, and the respective institutional review boards approved all experimental protocols.

Polymorphisms Investigated

Rs12344615 (UBQ-8i[9]), which identifies the H3 haplotype and was most significantly and consistently associated with LOAD as reported by Bertram and colleagues,[9] was genotyped in all five sample sets. Rs2780995, which, ignoring one rare haplotype, identifies the H1 haplotype reported by Bertram and colleagues[9] was genotyped in the WashU series, as were five additional SNPs. These five were chosen because they tag more than 93% of the haplotypic diversity observed in the region. This region (haplotype block) extends from 12.5kb upstream of the *UBQLN1* coding region, through the approximately 43kb of *UBQLN1* and terminates approximately 10kb downstream of the terminal exon.

Genotyping and Quality Control

The WashU, MRC-LOAD, and UCSD samples were genotyped using allele-specific real-time polymerase chain reaction.[14] The Newcastle-Brain, US-Brain, and a subset of the WashU series were genotyped using Pyrosequencing technology (http://www.pyrosequencing.com). Sequences for the polymerase chain reaction and SNP primers are available from the authors by request. Blinded, duplicate genotyping of rs12344615 at two centers, using the two methods above, yielded 0 discrepant and 515 concordant genotypes.

Statistical Analyses

χ^2 tests were used to check genotypic frequencies for deviation from Hardy–Weinberg equilibrium in case and control samples. A Fisher's exact test was used to test for allelic and genotypic association for each SNP, as well as intersite and intrasite heterogeneity. A nominal p value of less than or equal to 0.05 was used as the threshold for significance in all tests. Because no single test was significant at this level, no corrections were made for the multiple tests performed. A combined analysis including all sites, and using sex and *APOE* ε4 status (positive or negative) as covariates, was performed using a logistic regression. An analysis of variance (ANOVA), also using sex and *APOE* ε4 status as covariates, was used to test whether rs12344615 modified age of onset. Both the regression and the ANOVA were performed using SAS (SAS System for Solaris, Release 8.0. 1999; SAS Institute Inc., Cary, NC).

Linkage disequilibrium (LD) was analyzed using the program COCAPHASE from the UNPHASED software suite.[15] Haplotype-tagging SNPs were identified from publicly available data from the International HapMap Project[16] using the confidence interval block-delimiting algorithm in Haploview.[17] Tests for population stratification within and among the WashU, MRC-LOAD, and UCSD series have been described in Li and colleagues.[18]

Results

In an attempt to replicate the main findings of Bertram and colleagues,[9] we genotyped UBQ-8i (rs12344615) in five case–control series totaling 1,544 cases and 1,642 unrelated, nondemented controls. The SNP did not significantly deviate from Hardy–Weinberg equilibrium in either the cases or the controls of each series, or in the whole data set. We did not observe allelic or genotypic association at the p value less than or equal to 0.05 level between LOAD and rs12344615 in any of the individual series, or in any *APOE* ε4 substratum (Table 2). The Newcastle-Brain series (allelic model; unstratified $p = 0.074$; *APOE* ε4 negative $p = 0.099$) and the US-Brain series (genotypic model; *APOE* ε4 positive $p = 0.098$) showed a trend toward significance. However, the trends were in the opposite direction: in the Newcastle-Brain series, the risk allele

Table 2. Genotyping and Fisher's Exact Test Results for rs12344615

		Case						Control						p	
Series	Stratum	CC	CT	TT	Sum	mAF	HWE	CC	CT	TT	Sum	mAF	HWE	Allelic	Genotypic
WashU	All	10	109	257	376	0.172	0.93	15	99	239	353	0.183	0.52	0.5835	0.5120
WashU	ε4−	5	39	115	159	0.154	0.76	11	82	178	271	0.192	0.92	0.1673	0.3858
WashU	ε4+	5	70	142	217	0.184	0.56	4	16	61	81	0.148	0.15	0.3332	0.0528
UCSD	All	9	59	111	179	0.215	0.95	9	96	225	330	0.173	0.95	0.1101	0.2188
UCSD	ε4−	5	17	41	63	0.214	0.29	8	77	173	258	0.180	0.99	0.3746	0.2350
UCSD	ε4+	4	41	70	115	0.213	0.79	1	19	52	72	0.146	0.88	0.1336	0.3049
MRC-LOAD	All	28	205	400	633	0.206	0.97	31	225	475	731	0.196	0.80	0.5336	0.7910
MRC-LOAD	ε4−	11	82	146	239	0.218	0.99	22	173	360	555	0.196	0.98	0.3403	0.5802
MRC-LOAD	ε4+	17	122	253	392	0.199	0.90	9	50	113	172	0.198	0.55	1.0000	0.8017
Newcastle-Brain	All	4	51	105	160	0.184	0.75	2	24	86	112	0.125	0.98	0.0744	0.1525
Newcastle-Brain	ε4−	3	18	43	64	0.188	0.83	1	18	67	86	0.116	0.99	0.0994	0.2174
Newcastle-Brain	ε4+	1	33	62	96	0.182	0.32	1	6	19	26	0.154	0.85	0.8375	0.2573
US-Brain	All	6	58	132	196	0.179	0.99	6	39	71	116	0.220	0.98	0.2106	0.4341
US-Brain	ε4−	3	18	37	58	0.207	0.92	3	26	55	84	0.190	1.00	0.7628	0.8681
US-Brain	ε4+	2	39	89	130	0.165	0.61	2	10	14	26	0.269	0.99	0.1137	0.0976
All	All	57	482	1005	1544	0.193	1.00	63	483	1096	1642	0.185	0.57	n/a	n/a
All	ε4−	27	174	382	583	0.196	0.46	45	376	833	1254	0.186	0.95	n/a	n/a
All	ε4+	29	305	616	950	0.191	0.49	17	101	259	377	0.179	0.23	n/a	n/a

n/a = not available; mAF = minor allele frequency; HWE = Hardy–Weinberg equilibrium.

(C) proposed by Bertram and colleagues[9] was more prevalent in the cases whereas for the US-Brain series the C allele was more prevalent in the controls.

A Fisher's exact test for heterogeneity between cases and between controls in each series showed a significant difference in genotype and allele frequencies for rs12344615 observed in the Newcastle-Brain controls compared with the other series (Table 3). A logistic regression that included sample series ("site") as a variable therefore was used to combine all the data and test for allelic or genotypic association in the entire sample. No significant associations between LOAD and genotype or allele were observed (see Table 4). Because the Bertram and colleagues study[9] reported a dose-dependent increase in risk, we analyzed the effect of *APOE* status, sex, and rs12344615 allele/genotype on age of disease onset using ANOVA. There was no significant association for the allelic ($p = 0.856$) or genotypic ($p = 0.953$) tests (data not shown).

Bertram and colleagues[9] also reported moderate association between LOAD and rs2780995. We therefore genotyped this SNP in the WashU series, our standard exploratory sample set. Because we did not observe association with LOAD in this sample (Table 5), we did not genotype it in the other series.

To further assess the role of variation in the ubiquilin 1 gene as a risk factor for LOAD, we genotyped, in the WashU series only, five additional SNPs that were identified as haplotype-tagging SNPs. None of the SNPs deviated significantly from Hardy–Weinberg equilibrium, and none were significantly associated with LOAD, irrespective of *APOE* genotype (see Table 5). LD, as measured by D′, was greater than 0.77 for all pairs of markers within this region, but the six

Table 3. Tests of Heterogeneity for rs12344615

		Genotypic p		Allelic p	
Series	Series	Controls	Cases	Controls	Cases
WashU	UCSD	0.5595	0.1835	0.6711	0.0834
WashU	MRC-LOAD	0.6607	0.1567	0.4850	0.0620
WashU	Newcastle-Brain	0.1621	0.7802	0.0514	0.6609
WashU	US-Brain	0.4079	0.9219	0.2130	0.8053
UCSD	MRC-LOAD	0.3933	0.8792	0.2088	0.7128
UCSD	Newcastle-Brain	0.2374	0.4443	0.1138	0.3377
UCSD	US-Brain	0.2424	0.4388	0.1165	0.2314
MRC-LOAD	US-Brain	0.6560	0.5404	0.4261	0.2478
MRC-LOAD	Newcastle-Brain	0.0469	0.5882	0.0099	0.4356
US-Brain	Newcastle-Brain	0.0307	0.8696	0.0091	0.8456

Fisher's exact tests for allelic and genotypic heterogeneity between all pairs of sample sets. Shaded areas indicate significant differences.

Table 4.
Combined Analysis

Test	OR (95% CI)	*p*
Genotypic: TT vs CC	0.939 (0.624–1.414)	0.4677
Genotypic: TC vs CC	1.047 (0.687–1.597)	0.5403
Allelic: T vs C	0.924 (0.804–1.062)	0.2641

Logistic regression analyzing all sites together (1,544 cases and 1,642 nondemented controls), corrected for *APOE* and sex. OR = odds ratio; CI = confidence interval.

SNPs showed relatively low r^2 values, except for rs12344615 and rs944947, and rs2780995 and rs2811925 (r^2 = 1 for each pair; Table 6). We performed pairwise haplotype analysis with rs12344615 and the six other markers, using the expectation maximization algorithm to estimate haplotype frequencies (data not shown). No haplotype was significantly associated with LOAD at the *p* value equal to 0.05 level after removing those with a frequency of less than 1%. These htSNPs were not genotyped in the larger data set, and no additional analyses (ANOVA) were performed, because the results were uniformly negative in the WashU series.

Discussion

The primary focus of this study was to attempt to replicate the reported association between AD and rs12344615[9] in the ubiquilin 1 gene. The earlier study used a family-based test of association in two series of LOAD families, whereas we have used a case–control design to test the role of rs12344615 in risk for LOAD. Our sample is large, consisting of five case–control series of white subjects totaling 1,544 cases and 1,642 controls. No evidence of association was observed between rs12344615 and risk for disease or age of onset in any individual series or in the combined data set. We therefore were unable to detect any evidence in support of rs12344615 (UBQ-8i) as a common risk factor for LOAD in our samples.

Rs2780995 and rs12344615 tag the LOAD-associated haplotypes, H1 and H3, respectively, reported by Bertram and colleagues. [9] Neither of these haplotypes was associated with risk for LOAD in our study. To determine whether other alleles within the *UBQLN1* gene might influence risk for LOAD, we genotyped five additional SNPs in the WashU series. Although these SNPs were not identical to those used in the original study, a similar haplotype block structure, at this gross scale,[19] was observed across the gene, and no evidence for association between any tested haplotype and LOAD was observed.

A common shortcoming of many case–control studies has been low power to replicate because of small sample size. With respect to rs12344615, Bertram and colleagues[9] reported an odds ratio of 1.5 for a dominant model and an odds ratio of 2.1 for a recessive model. Assuming a similar effect size, in our study of more than 1,500 cases and 1,500 controls, we have greater than 99% power to detect an association with an α of 5% and a risk allele frequency of 25% (or the 20% observed in our own data). However, at the lower

Table 5.
Genotypes and Analyses for Five htSNPs and rs2780995 in the WashU Series

SNP ID and Alleles	Series	Stratum	Case 11	Case 12	Case 22	Case Sum	Case mAF	Case HWeq	Control 11	Control 12	Control 22	Control Sum	Control mAF	Control HWeq	*p* Allelic	*p* Genotypic
rs1333862	WashU	All	15	102	143	260	0.254	0.85	20	99	143	262	0.265	0.88	0.7242	0.7007
1=C	WashU	ε4−	9	41	67	117	0.252	0.75	16	76	112	204	0.265	0.83	0.7794	0.9508
2=T	WashU	ε4+	6	60	76	142	0.254	0.38	4	23	30	57	0.272	0.99	0.7057	0.6963
rs2811925	WashU	All	34	119	108	261	0.358	0.99	27	115	120	262	0.323	1.00	0.2402	0.4779
1=T	WashU	ε4−	18	49	50	117	0.363	0.59	24	87	93	204	0.331	0.87	0.4370	0.6317
2=C	WashU	ε4+	16	69	58	143	0.353	0.80	2	28	27	57	0.281	0.26	0.1960	0.2160
rs944947	WashU	All	6	68	187	261	0.153	1.00	11	76	174	261	0.188	0.77	0.1617	0.3099
1=G	WashU	ε4−	4	26	87	117	0.145	0.52	7	60	136	203	0.182	0.99	0.2731	0.3587
2=A	WashU	ε4+	2	42	99	143	0.161	0.57	4	16	37	57	0.211	0.50	0.2459	0.1214
rs3814507	WashU	All	11	77	154	242	0.205	0.94	6	74	176	256	0.168	0.86	0.1430	0.2805
1=A	WashU	ε4−	6	32	70	108	0.204	0.67	5	59	136	200	0.173	0.89	0.3829	0.3845
2=G	WashU	ε4+	5	44	84	133	0.203	0.97	1	15	39	55	0.155	0.95	0.3124	0.6387
rs4877797	WashU	All	17	107	136	260	0.271	0.80	19	117	121	257	0.302	0.43	0.3022	0.4917
1=T	WashU	ε4−	10	45	63	118	0.275	0.89	15	89	97	201	0.296	0.68	0.5886	0.5612
2=C	WashU	ε4+	7	61	73	141	0.266	0.44	4	28	23	55	0.327	0.51	0.2615	0.4313
rs2780995	WashU	All	46	175	156	377	0.354	0.96	38	158	154	350	0.334	0.96	0.4397	0.7349
1=C	WashU	ε4−	22	73	64	159	0.368	0.99	29	123	117	269	0.336	0.92	0.3732	0.5879
2=A	WashU	ε4+	24	102	92	218	0.344	0.86	9	35	36	80	0.331	0.99	0.8454	0.8965

Genotyping and Fisher's exact test results for five haplotype tagging SNPs and rs2780995, all of which were genotyped in the WashU series. mAF = minor allele frequency; HWeq = Hardy–Weinberg equilibrium.

Table 6.
Linkage Disequilibrium among Five Haplotype Tagging Single Nucleotide Polymorphisms and rs12344615

Intermarker Distance (bp)		rs2811925	rs12344615	rs2780995	rs944947	rs4877797	rs3814507	rs1333862
–	rs2811925		1.000	1.000	1.000	0.936	1.000	1.000
9,492	rs12344615	0.109		1.000	1.000	0.765	1.000	1.000
9,973	rs2780995	1.000	0.117		1.000	1.000	1.000	1.000
8,896	rs944947	0.109	1.000	0.116		0.796	1.000	1.000
6,149	rs4877797	0.184	0.058	0.210	0.060		1.000	0.989
17,096	rs3814507	0.100	0.046	0.103	0.046	0.466		1.000
941	rs1333862	0.176	0.083	0.175	0.082	0.830	0.564	

Linkage disequilibrium calculated in the WashU controls. The shaded values represents D′; the unshaded are r2. rs12344615 and rs2780995 were not present in the HapMap data. Hence, it was unknown that their genotyping obviated that of rs944947 and rs2811925, with whom they were in complete linkage disequilibrium, respectively.

limit of the reported 95% confidence interval (1.1 for both inheritance modes), our power to detect association is only 22%. To achieve 80% power with a risk allele frequency of 25% and odds ratio of 1.1, we would need approximately 16,000 samples[20] (data not shown).

Although case–control studies generally are considered to provide a more powerful means of detecting association than similarly sized family-based studies, they are subject to artifacts due to undetected population admixture differences between the cases and controls. The inherent shared genetic component present in family-based studies makes stratification less of an issue. By conditioning on heterozygous parents, many family-based association tests are robust to stratification. However, by testing for population stratification in case–control samples, one can help reduce the risk of spurious results. In three of our five series, comprising 901 cases and 1,068 controls, we have previously[18] tested for population stratification within and between these data sets and found no evidence thereof. Using only these samples in a combined test yields no evidence for association (allelic $p = 0.49$, genotypic $p = 0.74$; power to detect = 0.999) (data not shown). Therefore, we think it is unlikely that our failure to replicate the original finding is caused by differences within our samples that obfuscate a true association.

Our failure to replicate the main finding of Bertram and colleagues could be caused by many factors. The original finding may, in fact, represent a type 1 error (false-positive); no mention is made in the original published article about multiple test correction. However, the fact that the observation was replicated in their second sample series does somewhat mitigate the omission. As we have addressed above, we do not believe population stratification is a complicating factor in our study. Another possibility is genetic heterogeneity between our sample sets and those of the original study. This would not be surprising, given the fundamental difference in ascertainment strategies (case–control or family based) and could be reflected in the significant difference in minor allele frequency for rs12344615 between the two studies. Our ascertainment may also have caused reduced genetic loading in our case samples because they were not selected for having affected first-degree relatives. For more detailed discussions on the above issues, see Cardon and Bell[21] or Thomas and Witte.[22]

With respect to the five SNPs genotyped only in the WashU series, our power to detect an effect of similar magnitude to that reported by Bertram and colleagues[9] is greater than 87% but decreases rapidly with smaller effect size[20] (data not shown). Our analyses of these five additional SNPs and haplotypes suggest that it is unlikely that a common variant in the ubiquilin 1 gene explains a significant portion of risk for LOAD, although it remains possible that it confers a minor effect on risk that we did not have the power to detect.

This work was supported by the NIH (Alzheimer's Disease Research Center Grants P50 AG05681, J.C.M.; P01 AG03991, J.C.M.; AG16208, A.G.; P50 AG05131, L.T.) and the Medical Research Council, UK (J.W., M.J.O., S.L., M.C.O., A.M.G., C.M.M.), the Alzheimer's Research Trust (J.W., M.J.O., M.C.O., S.L., C.M.M.). J.H. was supported by National Institute on Aging (NIA) intramural program and the Verum Foundation; A.J.M. is a resident research associate of the National Academy of Sciences (USA). Many data and biomaterials for the US-Brain series were collected from several NIA National Alzheimer's Coordinating Center–funded sites.

We thank Drs M. Morrison-Bogorad, T. Phelps, and W. Kukull for helping to coordinate this collection. The directors, pathologist, and technicians involved include Drs R. Seemann, J. C. Troncoso, O. Pletnikova, H. Vinters, J. Pomakian, C. Hulette, D. Horoupian, A. Salehi, J. P. Vonsattel, E. Tessa Hedley-Whyte, K. Fitch, R. Albin, L. Bain, E. Gombosi, W. Markesbery, S. Anderson, D. W. Dickson, N. Thomas, C. A. Miller, J. Tang, D. Diaz, D. McKeel, J. C. Morris, E. Johnson, V. Buckles, D. Carter, T. Montine, and A. Schantz. We are also thankful to the families for their participation in this study. M. Coats and E. Grant coordinated the Washington Univer-

sity case material; M. Sundsmo coordinated the University of California at San Diego case material.

References

1. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977–1981.
2. Warwick Daw E, Payami H, Nemens EJ, et al. The number of trait loci in late-onset Alzheimer disease. Am J Hum Genet 2000;66:196–204.
3. Blacker D, Bertram L, Saunders AJ, et al. Results of a high-resolution genome screen of 437 Alzheimer's disease families. Hum Mol Genet 2003;12:23–32.
4. Myers A, Wavrant-De Vrieze F, Holmans P, et al. Full genome Screen for Alzheimer disease: stage II analysis. Am J Med Genet 2002;114:235–244.
5. Mah AL, Perry G, Smith MA, et al. Identification of ubiquilin, a novel presenilin interactor that increases presenilin protein accumulation. J Cell Biol 2000;151:847–862.
6. Massey LK, Mah AL, Ford DL, et al. Overexpression of ubiquilin decreases ubiquitination and degradation of presenilin proteins. J Alzheimers Dis 2004;6:79–92.
7. Sherrington R, Rogaev EI, Liang Y, et al. Cloning a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 1995;375:754–760.
8. Levy-Lahad E, Wasco W, Poorkaj P, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 1995;269:973–977.
9. Bertram L, Hiltunen M, Parkinson M, et al. Family-based association between Alzheimer's disease and variants in UBQLN1. N Engl J Med 2005;352:884–894.
10. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
11. Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol Aging 1997;18: 351–357.
12. Nowotny P, Hinrichs Al, Smemo S, et al. Association studies between the risk for late-onset Alzheimer's disease and variants in insulin degrading enzyme. Am J Med Genet 2005;136:62–68.
13. Pittman AM, Myers AJ, Abou-Sleiman P, et al. Linkage disequilibrium fine-mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration. J Med Genet 2005; Online ahead of print. DOI: 10.1136/jmg.2005.031377.
14. Germer S, Holland MJ, Higuchi R. High-throughput SNP allele-frequency determination in pooled DNA samples by kinetic PCR. Genome Res 2000;10:258–266.
15. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 2003;25:115–121.
16. The International HapMap Consortium. The International HapMap Project. Nature 2003;426:789–796.
17. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005: 21:263–265.
18. Li Y, Nowotny P, Holmans P, et al. Association of late-onset Alzheimer's disease with genetic variation in multiple members of the GAPD gene family. Proc Natl Acad Sci USA 2004;101: 15688–15693.
19. Ke, Xiayi, Hunt S, Tapper W, et al. The impact of SNP density on fine-scale patterns of linkage disequilibrium. Hum Mol Genet 2004:13:577–588.
20. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–150.
21. Cardon LR, Bell JI. Association study designs for complex diseased. Nat Rev Genet 2001:2:91–99.
22. Thomas DC, Witte JS. Point: Population stratification: a problem for case-control studies of candidate-gene associations. Caner Epidemiol Biomarkers Prev 2002:11:505–512.