Cholinergic dysfunction in diseases with Lewy bodies

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Article abstract—*Objective:* To evaluate cholinergic activity in diseases with Lewy bodies (LB; LB variant of AD [LBV], diffuse LB disease [DLBD], and Parkinson's disease [PD]) to determine if 1) AD changes are requisite to cholinergic dysfunction, 2) cholinergic activity declines to the same extent in neocortical and archicortical areas, and 3) cholinergic loss is influenced by *APOE* genotype. *Background:* Like AD, diseases with LB are associated with decreased choline acetyltransferase (ChAT) activity. Increased *APOE* ϵ4 allele frequency has been reported in LBV. Whether *APOE* genotype affects cholinergic function in LBV remains unclear. *Methods:* An autopsy series of 182 AD (National Institute on Aging and Consortium to Establish a Registry for Alzheimer's Disease criteria), 49 LBV, 11 PD, 6 DLBD, and 16 normal control (NC) subjects. *APOE* genotype and ChAT activity (nmol/h/100 mg) in the midfrontal and hippocampal cortices were determined. *Results:* Mean midfrontal ChAT activity was markedly reduced in diseases with LB (LBV: 53.3 ± 39.0; PD: 54.8 ± 35.7; DLBD: 41.3 ± 24.8) compared to NC (255.4 ± 134.6; $p < 0.001$) and AD (122.6 ± 78.9; $p < 0.05$). Among diseases with LB, midfrontal ChAT activity was decreased to a similar extent in patients *with* (LBV) and *without* (DLBD and PD) AD pathology. Although mean ChAT activity for LBV was less than half that for AD in the midfrontal cortex, it was similar to that for AD in the hippocampus (LBV: 243.5 ± 189.7; AD: 322.8 ± 265.6; $p > 0.05$). However, hippocampal ChAT activity for both AD and LBV was lower than that for NC (666.5 ± 360.3; $p < 0.001$). The ϵ4 allele dosage did not influence midfrontal ChAT activity in LBV. *Conclusions:* Marked losses in midfrontal ChAT activity occur in diseases with LB, independent of coexistent AD changes. A greater midfrontal, as opposed to hippocampal, cholinergic deficit may differentiate LBV from AD. The lack of a relationship between ϵ4 allele dosage and midfrontal ChAT activity suggests that other factors may play a role in its decline in LBV. **Key words:** Cholinergic dysfunction—Lewy body disease—*APOE*—AD.

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Decrements in choline acetyltransferase (ChAT) activity have been found in AD,[1] AD with Lewy bodies (LB; LB variant of AD [LBV]),[2,3] and, irrespective of presence of dementia, Parkinson's disease (PD).[2,4] Marked reductions are also likely to occur in pure diffuse LB disease (DLBD), but the extent to which ChAT activity declines in this disorder has been less clearly defined, primarily owing to heterogeneity of patient samples.[5,6] The primary anatomic explanation for this reduced cholinergic activity has been reported to be loss of neurons in the basal forebrain, especially in the nucleus basalis of Meynert (nbM).[7,8]

Although correlations between ChAT activity and global measures of cognitive impairment have been found in both AD[9,10] and LBV,[2,6] more extensive neocortical cholinergic deficits may differentiate LBV from AD[2-4,11] and possibly contribute to its characteristic clinical profile.[11] In particular, it has been hypothesized that the higher prevalence of psychotic symptoms (visual hallucinations and delusions) and severe visuospatial dysfunction reported for LBV[12,13] may be related to greater reductions in ChAT activity in the temporal and parietal cortices, respectively.[11] Due to this remarkable cholinergic hypoactivity, it has also been suggested that LBV may respond more positively than AD to cholinergic therapy.[2,12]

Both AD and LBV are associated with an increased frequency of the *APOE* ϵ4 allele.[14,15] Although it has been proposed that the number of ϵ4 allele copies may influence cholinergic dysfunction in AD,[16] whether this occurs in LBV has not been carefully examined. In addition to a possible relationship between midfrontal (MF) cholinergic losses and *APOE* genotype, we also investigated whether hippocampal (Hip), in addition to MF, cholinergic deficit differentiated LBV from AD. Our principal objective, however, was to evaluate ChAT activity not only in LBV, but also in other diseases with LB (DLBD, PD) to determine whether concomitant AD changes (present in LBV alone) were requisite to MF cholinergic dysfunction.

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Methods. *Subjects.* Most of the patients in the current study were followed clinically at the University of California, San Diego (UCSD), Alzheimer's Disease Research Center (ADRC), Senior's Only Care, or in the private practices of its senior clinicians. They represent all patients who have come to autopsy between 1985 and the present with a neuropathologic diagnosis of AD, LBV, DLBD, or PD, or normal controls (NC) for whom apoE genotype or ChAT activity were available. There were 182 AD, 49 LBV, 11 PD, 6 DLBD, and 16 NC patients. All AD and LBV patients met both National Institute on Aging (NIA)[17] criteria for a pathologic diagnosis of AD and Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[18] criteria for definite or probable AD. They also met either Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised (DSM-III-R)[19] criteria for a clinical diagnosis of dementia or National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association[20] criteria for probable or possible AD. In addition, the LBV group had concomitant LB in the brainstem, archicortex, and neocortex. Dementia in these patients preceded the occurrence of extrapyramidal signs. Most of these cases would be labeled by other investigators as combined AD and PD,[21] AD with PD-related changes,[22] or senile dementia of the LB type (SDLT).[2]

The PD group consisted of patients with clinically diagnosed idiopathic PD whose brains showed neuronal loss and gliosis in subcortical nuclei—i.e., substantia nigra, locus coeruleus, and nucleus basalis of Meynert—accompanied by single or multiple LB in surviving neurons. LB were also present in the archicortex and, although not invariably, in the neocortex. Neuropathologic features of AD were absent. In particular, plaques were not sufficient to meet NIA or CERAD criteria for a diagnosis of AD. Despite the lack of an obvious pathologic basis, all PD subjects also fulfilled DSM-III-R criteria for a clinical diagnosis of dementia prior to death. Parkinsonism, however, was the presenting complaint.

The DLBD group consisted of patients with a clinical diagnosis of dementia according to DSM-III-R criteria and parkinsonism whose brains showed typical PD pathology accompanied by sparse or plentiful LB in the brainstem, archicortex, and neocortex, in the absence of significant AD changes. These subjects presented with a progressive dementing syndrome.

LBV, PD, and DLBD patients met the Consortium on Dementia with Lewy Bodies (DLB) criteria[12] for a pathologic diagnosis of DLB. No attempt was made to define subtypes on the basis of the relative distribution of LB in the brainstem, allocortical, and neocortical regions.

The NC group consisted of patients whose brains failed to meet NIA and CERAD criteria for AD and were considered neuropathologically normal. Most of the NC had been evaluated on an annual basis through the UCSD ADRC and had been given a clinical diagnosis of NC.

Neuropathologic examination. Pathologic assessment was made by one observer (L.A.H.). Autopsy was performed within 8 hours of death using a protocol described by Terry et al.[23] The left hemibrain was fixed by immersion in 10% formalin for 5 to 7 days, at which time blocks were taken for paraffin embedding from MF, rostral superior temporal, and inferior parietal areas of neocortex, anterior cingulate gyrus, posterior cingulate gyrus, anterior hippocampus, basal ganglia/substantia innominata, mesencephalon, and pons. The cortical areas correspond to Brodmann areas 46, 38, and 39. The paraffin blocks of neocortex were cut at 7-mm thickness for hematoxylin and eosin (H-E) staining for oversight purposes. Ten-μm–thick sections were made for thioflavin S stains. Total plaque, neuritic plaque, and neurofibrillary tangle counts were determined by the same examiner with the same criteria used consistently. Plaques were assessed with thioflavin S fluorescent microscopy. Each brain was staged for degree of neurofibrillary pathology according to a modification[24] of Braak and Braak's criteria.[25] As recommended by the Consortium on DLB,[12] subcortical LB were identified with H-E; cortical LB were detected by H-E or antiubiquitin immunostaining.

APOE *genotyping.* The *APOE* genotype was extracted and determined in either peripheral blood samples or in postmortem brain tissue (by homogenizing 500 mg of frozen brain tissue over ice, adding lysis buffer and proteinase K, and rocking overnight at 37 °C, followed by phenol/chloroform extraction). Genomic DNA was amplified by polymerase chain reaction (PCR) using the primers prescribed by Wenham et al.[26] After amplification, DNA was digested with the Hha restriction enzyme, electrophoresed on 6% nondenaturing polyacrylamide gels, and visualized by ethidium bromide staining.

ChAT activity. Samples were taken from MF and Hip areas of frozen unfixed right hemibrain neocortex and homogenized in 1 mM ethylenediamine tetraacetic acid, pH 7.0, containing 0.1% Triton X-100. Analysis of ChAT activity (nmol/h/100 mg) was performed in triplicate by the modified Fonnum technique.[27,28] The coefficient of variation is 3% with an intra-assay variability of 7.9%.

Statistical analysis. Mean values among AD, LBV, PD, DLBD, and NC subjects and the three LBV genotypes (two ε4, one ε4, and no ε4) were compared using Kruskal-Wallis ANOVA. A nonparametric test was chosen because of small samples or significant differences among standard deviations. When a significant global result ($p < 0.05$) was obtained, Kruskal-Wallis ANOVA was followed by Dunn's multiple comparison test to compare each pair of means.

Results. As shown in table 1, mean MF ChAT activity was markedly reduced in diseases with LB (LBV: 53.3 ± 39.0; PD: 54.8 ± 35.7; DLBD: 41.3 ± 24.8) compared to NC (255.4 ± 134.6; $p < 0.001$) and AD subjects (122.6 ± 78.9; $p < 0.05$), despite similar ages at death. MF ChAT activity was actually lowest in DLBD; however, there were no statistically significant differences between this group and either PD or LBV.

Mean Hip ChAT activity (not available on PD and DLBD subjects) was reduced in LBV (243.5 ± 189.7) and AD (322.8 ± 265.5) compared to NC (666.5 ± 360.3; $p = 0.004$) subjects. In Hip, as opposed to MF cortex, there were no significant differences in ChAT activity between LBV and AD subjects.

APOE genotyping was available on 45 of the 49 LBV subjects. In this cohort, the distribution of genotypes included: 2/2, n = 0; 2/3, n = 0; 2/4, n = 0; 3/3, n = 13; 3/4, n = 27; 4/4, n = 5. Overall, the ε4 allele frequency was 41% in LBV, 38% in AD, 14% in PD, 8% in DLBD, and 11% in NC.

As shown in table 2, there was no relationship between mean MF ChAT activity and number of ε4 allele copies in

Table 1 *Ages at death and choline acetyltransferase (ChAT) activities in our patient cohorts*

Characteristic	DLBD	PD	LBV	AD	NC	*p* Value*
MF ChAT (nmol/h/100 mg)	41.3† (24.8), 6	54.8† (35.7), 11	53.3‡ (39.0), 49	122.6 (78.9), 182	255.4 (134.6), 16	0.0001
Hip ChAT (nmol/h/100 mg)	NA	NA	243.5§ (189.7), 38	322.8 (265.6), 111	666.5 (360.3), 12	0.004
Age at death, y	80.2 (6.9), 6	76.5 (4.5), 11	78.7 (7.7), 49	79.2 (8.0), 182	75.5 (12.1), 16	0.22

Values are mean (SD), n.

* Kruskal-Wallis ANOVA.
† $p < 0.05$ Compared to AD; $p < 0.001$ compared to NC (Dunn's multiple comparisons test).
‡ $p < 0.001$ Compared to AD and NC (Dunn's multiple comparisons test).
§ $p < 0.001$ Compared to NC (Dunn's multiple comparisons test).

DLBD = diffuse Lewy body (LB) disease; PD = idiopathic PD; LBV = LB variant of AD; NC = normal controls; MF = midfrontal; Hip = hippocampal; NA = not applicable.

LBV patients. Although ChAT activity was slightly lower in patients with two ε4 alleles (ε4/ε4: 38.6 ± 32.2), there were no statistically significant differences compared to those with one (ε−/ε4: 53.1 ± 37.9) or no ε4 alleles (ε−/ε−: 46.9 ± 26.2). MF ChAT activity of ε4 carriers (50.9 ± 37.8, n = 32) was nearly identical to that of noncarriers (42.6 ± 26.4, n = 13, $p = 0.7$). Mean age at death for LBV subjects with two ε4 (72.8 ± 12.3 years), one ε4 (79.0 ± 7.8 years), and no ε4 alleles (79.1 ± 5.1 years) did not differ significantly. Neither did the duration of illness (ε−/ε−: 7.3 ± 2.3; ε−/ε4: 8.3 ± 3.6; ε4/ε4: 6.5 ± 3.0) or age at onset (ε−/ε−: 72.8 ± 5.7; ε−/ε4: 72.3 ± 7.5: ε4/ε4: 66.0 ± 10.4).

Discussion. The current findings suggest that, irrespective of clinical diagnosis, the occurrence of LB in dementia is associated with a MF cholinergic deficit more extensive than that observed in AD. Among diseases with LB, MF ChAT activity in patients *without* significant AD changes (DLBD and PD) was similar to that found in those *with* significant AD changes (LBV). Thus, LB pathology alone, independent of coexistent AD, may be sufficient to cause severe reductions in MF cholinergic levels.

Heterogeneity of patient samples is likely the main reason for conflicting results previously reported for DLBD, ranging from the observation of lower ChAT activity in DLBD compared to AD[6] to that of similar levels in both conditions.[5] Like Samuel et al.,[6] we reserved the designation of DLBD for brains with subcortical and cortical LB lacking concomitant neocortical AD. In contrast, Dickson et al.[5] have not made such a distinction, as most of the "DLBD" cases in their report had variable degrees of AD pathology, with 4 of 6 patients displaying enough plaques to meet NIA neuropathologic criteria for AD and one a substantial number of neocortical tangles. In our series, designation of DLBD was reserved for LB pathology alone; however, no distinctions were made between cases with sparse or plentiful LB, and some neocortical LB were also found, albeit not invariably, in PD. Therefore, DLBD and PD patients, although different from each other in initial clinical presentation (dementia in the former; parkinsonism in the latter), were likely to be neuropathologically indistinguishable.

All the LBV, PD, and DLBD cases in the current study met the Consortium criteria for a pathologic diagnosis of DLB.[12] We emphasize that our brain sampling procedures were consistent with those recommended by the Consortium on DLB[12]; that the current study stressed a qualitative (presence versus absence of LB pathology) rather than a quantitative distinction (frequency of LB), as the main objective was to investigate if pure LB pathology was sufficient to decrease cholinergic function.

Reduction in frontal ChAT activity was distinctly greater in LBV than AD, a difference previously observed in other studies,[3,6] but not in those by Gibb et al.[29] and Perry et al.,[2,4,11] in which frontal ChAT losses in AD patients with or without LB were found to be comparable. It is likely that the failure to detect differences in these studies were due in part to small sample sizes. Additionally, in the study by Gibb et al.,[29] the two cohorts were not matched for age, which has been reported to influence cholinergic function in AD markedly.[30] The greater frontal ChAT reduction found in our LBV cohort appears to be

Table 2 *Clinical features and midfrontal (MF) choline acetyltransferase (ChAT) activity across* APOE *ε4 genotypes in Lewy body variant of AD*

Characteristic	No ε4 alleles	One ε4 allele	Two ε4 alleles	*p* Value*
MF ChAT (nmol/h/100 mg), mean (SD), n	46.9 (26.2), 13	53.1 (37.9), 27	38.6 (32.3), 5	0.72
Age at death, y, mean (SD), n	79.1 (5.1), 13	79.0 (7.8), 27	72.8 (12.3), 5	0.49
Age at onset, y, mean (SD), n	72.8 (5.7), 13	72.3 (7.5), 23	66.0 (10.4), 5	0.34
Duration, y, mean (SD), n	7.3 (2.3), 13	8.3 (3.6), 23	6.5 (3.0), 5	0.74

* Kruskal-Wallis ANOVA.

unrelated to the severity of AD pathology, as both neuritic plaques[31] and neurofibrillary tangles in the neocortex are reduced in LBV compared to AD.[31-33]

In our series, although mean ChAT activity for LBV was less than half that for AD in the frontal cortex, it was similar in the hippocampus. In the series by Perry et al.,[2,4,11] in contrast, Hip ChAT activity was significantly higher in LBV (designated as SDLT) than AD. In addition, although parietal and temporal ChAT activities in their studies were greatly reduced in LBV as compared to AD patients, frontal ChAT activity, as noted above, was similar in the two cohorts. Despite such discrepancies, our results remain compatible with the hypothesis of Perry et al. that neocortical, as opposed to archicortical, cholinergic deficit differentiates LBV from AD and may contribute to its characteristic clinical profile. In this respect, our finding of a more extensive frontal cholinergic deficit in LBV than AD may be anatomically consistent with the more "frontosubcortical" pattern of cognitive impairment (prominent deficit in executive function, attention, and verbal fluency) reported for LBV.[13,34]

Although the condition of the basal forebrain was not assessed, our observations support the idea that LB, more profoundly than AD, pathology may affect neocortical pathways projecting from the nbM, which supplies the neocortex with most of its cholinergic input. However, the extent of cholinergic deficit is not necessarily predicted by degree of nbM degeneration. In fact, correlations between neuronal counts in this nucleus and ChAT activities have been reported for PD, but not consistently for AD.[8] In addition, a greater decline in neocortical ChAT has been found in LBV compared to AD patients,[2,4] despite similar numbers of neurons in nbM. It appears therefore that the presence of LB pathology, in addition to nbM cell loss, contributes to functional alterations in the cholinergic system in LBV, whereas other factors, including the presence of neocortical plaques and tangles, may play a more important role in AD.

What also emerges from our findings is that, in diseases with LB, coexistent AD pathology may not be requisite either to neocortical cholinergic deficit or to dementia. Although it has been previously suggested that concomitant AD pathology is necessary for dementia in PD,[35] neuropathologic correlates of cognitive decline in this disorder can be more variable and complex. In the report of Hughes et al.,[36] for example, although a third of PD patients with dementia had concomitant AD at autopsy, a fifth had widespread neocortical LB, and most, like our cases, displayed only the typical pathology of PD.

Consistent with other studies,[14,15] the *APOE* ϵ4 allele was overrepresented in patients *with* (LBV and AD) as opposed to those *without* (DLBD and PD) AD pathology, in whom its frequency was comparable to that observed in NC. This increased frequency in LBV (41%) was similar to that measured for our AD cohort (38%) and slightly higher than that previously found by Galasko et al.[14] (29%) in a smaller cohort of LBV patients from our institution. Because the frequency of the ϵ4 allele in diseases with LB was increased only in the presence of concomitant AD, its prevalence is likely associated with AD rather than LB pathology.

Although the possible relationship between *APOE* genotype and cholinergic losses in AD has been investigated in several studies,[16,37,38] only Morris et al.[38] have previously examined whether *APOE* genotype influenced cholinergic function in DLB. Utilizing parietal cortex from a small sample of patients (n = 9) comparable to our LBV, these investigators did not find a relationship between the presence of the ϵ4 allele and ChAT activity; however, owing to the unavailability of homozygotes, the possible effect of ϵ4 allele dosage on cholinergic function could not be evaluated. In the MF cortex of our large cohort of LBV patients, cholinergic activity was lowest in those with two ϵ4 alleles, highest in those with one ϵ4 allele, and intermediate between the two in patients with no ϵ4 alleles; nevertheless, there were no statistically significant differences across genotypes. In addition, MF ChAT activity of ϵ4 carriers was nearly identical to that of noncarriers. This pattern was similar to that found for the AD cohort (data not presented), suggesting that factors other than *APOE* genotype are operative in MF cholinergic dysfunction in AD *with* or *without* LB.

Finally, our findings may have therapeutic implications. It has been postulated that cholinergic therapy can play a substantial role in LBV, as three patients with combined AD/LB pathology and very low neocortical ChAT activity were reported to be positive responders in a tacrine treatment trial.[39] There is also some evidence that cholinesterase inhibitors may have neuropsychiatric benefits,[40] including reduction of agitation, delusions, and hallucinations, which are seen in great frequency in LBV.[12] Moreover, increased muscarinic receptor binding, possibly reflecting sparing of intracortical neuronal systems, has been reported in LBV and PD, but not AD.[4] These observations raise the possibility that cholinergic replacement therapy may be particularly effective in DLB patients, despite possible worsening of extrapyramidal function. The risk/benefit of cholinesterase inhibitors in DLB has not been carefully studied. Ideally, this should be assessed in well-designed pharmacologic trials; nevertheless, the difficulty in clinically differentiating these disorders may raise major obstacles to their feasibility. In clinical contexts, however, PD patients with dementia, irrespective of further distinctions, may be the best candidates for replacement cholinergic therapy, in light of their remarkable cholinergic losses, despite an absence of AD pathology.

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APOE ε4 does not predict mortality, cognitive decline, or dementia in the oldest old

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Article abstract—*Objective:* To examine the effect of the ε4 allele on cognitive decline in the oldest old. *Methods:* We studied all 601 citizens of the city of Vantaa age 85 years and older in 1991. A total of 553 subjects (92%) took part in the study, which used the Mini-Mental State Examination (MMSE) and assessment of dementia according to the Diagnostic and Statistical Manual of Mental Disorders, third ed., revised (DSM-III-R) criteria. The survivors were re-examined 3 years later. *APOE* genotype was determined in 510 subjects, representing 83.2% of the original population. *Results:* Approximately one-half of the subjects (n = 250) died before the follow-up, and 253 subjects (97.3% of the survivors) were re-examined. The occurrence of the *APOE* ε4 allele did not have any significant effect on survival. Of the 187 previously nondemented subjects, 58 (31%) had developed dementia. The OR for the ε4 carriers to develop dementia was not significant: OR = 1.78; 95% CI = 0.88 to 3.60. In individuals with a follow-up MMSE score (n = 222), the mean decline in the score was 3.1 points. *APOE* ε4 carrier status did not have a significant effect on the mean MMSE change except in the previously demented subjects, among whom the drop was larger in the *APOE* ε4 carriers. *Conclusions:* The lack of association between *APOE* ε4 carrier status and mortality, or development of dementia, or cognitive decline in these very elderly people, whether analyzed in the whole population or among the nondemented subjects only, suggests that the *APOE* ε4 effect in younger subjects is age-dependent, and that it is no longer present in very old age. **Key words:** *APOE*—Dementia—Mortality.

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The *APOE* ε4 allele has been associated with increased risk of AD.[1-6] It also has been reported to impair the cognitive capacity in people without dementia,[7-10] and to increase the risk for cognitive decline on follow-up.[11-13] Some studies have suggested that the effect associated with the *APOE* ε4 allele is strongest in people in their 60s and 70s and diminishes with increasing age.[6,14]

We examined the effect of the *APOE* ε4 allele on the risk of developing dementia and cognitive decline in a population of people age 85 years and older, taking advantage of a population-based sample and a prospective follow-up setting.

Subjects and methods. The basic population of the Vantaa 85+ study consisted of all the people living in the city of Vantaa (a city of 170,000 inhabitants just beside the city of Helsinki) born before April 1, 1906 (n = 610). Of these persons, 36 (6%) died before the examination, 11 (2%) refused to participate, and 1 could not be contacted. Therefore, it was possible to examine 553 persons, representing 92% of these very old people.

The basic study included an interview and examination by a trained public health nurse and a neurologist. The Mini-Mental State Examination (MMSE)[15] and an assessment of dementia according to the Diagnostic and Statistical Manual of Mental Disorders, third ed., revised (DSM-III-R)[16] were carried out. A clinical diagnosis of AD also was assessed but not used in analyses because completion of some autopsies on the study subjects during the follow-up indicated that the accuracy of the clinical diagnosis of AD, compared with neuropathologic findings, was not reliable enough to justify its systematic use. The interviews and examinations were conducted in the spring and fall of 1991.

The 260 subjects who survived were re-examined in the spring of 1994. The same interviews, tests, and examinations were carried out by the persons who performed them 3 years earlier. The cognitive change was measured by the difference in MMSE score between the basic and follow-up examinations. *APOE* genotyping was carried out by the minisequencing technique.[17]

Statistics were carried out by SPSS software using *t*-test, χ^2, Fisher's exact test, nonparametric Kruskal-Wallis test, and Wilcoxon survival function when appropriate. Odds ratios (OR) were calculated against the reference population with *APOE* ε3/ε3 genotype. The study was ap-

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Table 1 *Mortality of* APOE *ϵ4 carriers and noncarriers at the 3 years' follow-up investigation in the whole population and among the initially nondemented and initially demented subjects*

Group/ follow-up	ϵ4 Carriers	ϵ4 Noncarriers	All	*p* Value*
All				
Alive	71 (27.3)	189 (72.7)	260 (100)	
Dead	78 (31.2)	172 (68.8)	250 (100)	0.34
Nondemented				
Alive	45 (23.2)	149 (76.8)	194 (100)	
Dead	23 (19.3)	96 (80.7)	119 (100)	0.42
Demented				
Alive	26 (39.4)	40 (60.6)	66 (100)	
Dead	55 (42.0)	76 (58.0)	131 (100)	0.72

Values are n (%).

* Pearson chi-square.

proved by the Ethical Review Committee of the Health Center of the city of Vantaa.

Results. The *APOE* genotype could be determined in 510 (92.2%) of the 553 persons originally taking part in the study. There was no difference in mean age or sex distribution between those who were analyzed for their *APOE* genotypes and those who were not.

Before the follow-up examination, 250 persons (49%) had died, and 7 persons (1.4%) refused to take part in the reexamination or had moved into another district. The remaining 253 persons (97.3% of the survivors) were reexamined. At the time of the basic study 3 years earlier, 187 of these persons had not been demented.

When the deceased and those re-examined were compared, no gender difference was found, but the deceased subjects had been somewhat older at the time of the baseline study (88.2 years versus 87.7 years, respectively; Kruskal-Wallis, $p = 0.03$). Dementia was significantly associated with mortality. Before the re-examination, 66.5% of the demented and 38% of the nondemented persons had died (χ^2, $p = 0.000$).

The proportion of *APOE* ϵ4 carriers was 29.2% at the baseline study (n = 510). It was 27.7% among the re-examined and 31.2% among those deceased. This difference is not statistically significant (χ^2, $p = 0.34$). The difference remained insignificant in the subgroups of demented and nondemented subjects (table 1), and in all these groups divided by gender (data not shown; *p* values for χ^2 test ranged from 0.24 to 0.99). We also made a life table analysis (Wilcoxon statistic) on survival of *APOE* ϵ4 carriers and noncarriers. No differences in survival were found between these groups, neither in the whole population ($p = 0.30$) nor among women ($p = 0.29$) or men ($p = 0.73$), nor among the demented ($p = 0.53$) or nondemented subjects ($p = 0.23$).

At the 3-year follow-up examination, 124 persons were assessed as demented, corresponding to 49% of those reexamined. Among these, there were 58 new cases of dementia, with 18 persons carrying at least one ϵ4 allele. For the initially nondemented *APOE* ϵ4 carriers, the OR for developing dementia during the follow-up was 1.78 (95% CI = 0.88 to 3.60), and thus statistically insignificant.

Table 2 *Mean Mini-Mental State Examination (MMSE) scores at follow-up in the whole population and in the initially nondemented and demented subjects by* APOE *ϵ4 carrier status*

Group	*APOE* ϵ4 −	*APOE* ϵ4 +*	All	*p* Value†
All	167 (20.1)	55 (17.9)	222 (19.6)	0.08
Initially nondemented	141 (21.6)	42 (20.9)	183 (21.4)	0.40
Initially demented	26 (11.3)	13 (8.4)	39 (10.3)	0.10

Values are n (mean MMSE).

* At least one *APOE* ϵ4 allele.
† Kruskal-Wallis test.

At the baseline examination, MMSE score was assessed in 500 subjects with a known *APOE* genotype. There were no *APOE* genotype-related differences in the mean MMSE score in the separate groups of demented subjects (8 points for *APOE* ϵ4 carriers and 9.2 for the noncarriers; *t*-test, $p = 0.24$) and nondemented subjects (23.7 points for carriers and 23.3 noncarriers; *t*-test, $p = 0.57$) at the baseline study.

The follow-up MMSE score was known in 222 subjects (87.7% of those re-examined). The mean follow-up MMSE score for the re-examined population was 19.6 ± 7.2 points: 21.4 ± 5.9 for those not demented at the time of the baseline study (n = 183) and 10.3 ± 5.4 for those demented then (n = 39). There were no differences in mean MMSE scores between those with and those without the ϵ4 allele in the whole re-examined population, nor among the initially demented or nondemented subjects (table 2). Analysis of all these groups divided by gender did not alter this, except that previously demented women with the *APOE* ϵ4 allele had a lower MMSE score (7.1) than those without this allele (11; $p = 0.05$). Other differences were insignificant (χ^2 *p* values ranged from 0.11 to 0.65).

Cognitive decline was measured by change in MMSE score. The mean decline in the MMSE scores in the whole population was 3.1 points: 3.2 for women and 2.5 for men (Kruskal-Wallis, $p = 0.26$).

Among the initially nondemented subjects, the drop in the mean MMSE score was 2.9 points. No differences in the mean MMSE change between the ϵ4 carriers and noncarriers were found, whether analyzed in the whole population or among the initially nondemented subjects, or in men and women separately. In contrast, there was an ϵ4-associated difference in the MMSE change among the initially demented subjects: the decline in the mean MMSE score was 5.7 points in ϵ4 carriers and 2.7 points in noncarriers (Kruskal-Wallis, $p = 0.03$; table 3).

Discussion. The Vantaa 85+ study is a population-based study in which 510 (83.2%) of the 601 citizens in the city of Vantaa age 85 years or older were examined and had their *APOE* genotypes determined. During the 3 years of follow-up, 49% of the population had died, whereas 97.3% of the surviving persons (n = 253) were re-examined. This population can be regarded as representative for people of this very old age.

The *APOE* ϵ4 allele was not associated with mor-

Table 3 *Mean Mini-Mental State Examination (MMSE) change (difference in individual MMSE scores at the baseline study and at the follow-up) in the whole population and in the initially nondemented and demented subjects by* APOE *ϵ4 carrier status*

Group	*APOE* ϵ4 −	*APOE* ϵ4 +*	All	*p* Value†
All	167 (−2.8)	55 (−3.9)	222 (−3.1)	0.12
Initially nondemented	141 (−2.8)	42 (−3.3)	183 (−2.9)	0.67
Initially demented	26 (−2.7)	13 (−5.7)	39 (−3.7)	0.03

Values are n (MMSE change).

* At least one *APOE* ϵ4 allele.
† Kruskal-Wallis test.

tality of these very elderly people. At first glance, this lack of association may appear unexpected because this allele has been strongly associated with both coronary heart disease[18,19] and AD,[1-4] and also with crude mortality.[20] The explanation for the lacking association may be inherent for the extremely old age of this population. Therefore, the negative influence of the ϵ4 allele on longevity is likely to become apparent earlier in life, and may have worn out by very old age. This hypothesis accords with earlier findings showing that the effect of *APOE* ϵ4 allele on the risk of AD is strongest in people in their 60s and 70s.[6,14]

In contrast, in an elderly Swedish population, the *APOE* ϵ4 allele increased mortality of cognitively unimpaired people older than 85 years, whereas it had no effect on the mortality of people ages 75 to 79 years, or on that of cognitively impaired persons of any age.[21] The impact of *APOE* genotypes on the risk of coronary heart disease has not been studied in very old populations.

Among the nondemented persons in the current study, the *APOE* ϵ4 allele did not increase the risk of developing dementia during the follow-up, nor did it increase the risk for cognitive decline. This constitutes an additional support for the hypothesis that the risk-increasing effect of the ϵ4 allele on the risk of AD and dementia has mainly disappeared by this age, whatever its underlying mechanism.

A correlation between the *APOE* ϵ4 allele and the prevalence of AD has been found in this same population older than 85 years.[22] However, there was no ϵ4 allele–related association in the mean MMSE score at the follow-up examination or in the change in MMSE score during the follow-up among the initially nondemented persons nor among the whole reexamined population. Among the demented subjects the cognitive decline was greater in *APOE* ϵ4 carriers.

As reported in several previous studies, the presence of the *APOE* ϵ4 allele was associated with impaired cognitive capacity of people without dementia[7-10] and increased risk of cognitive decline among unaffected people during follow-up.[11-13] Only in one study was an *APOE*-related difference in the MMSE score noticed.[9] In other studies, the differences were revealed only by neuropsychological tests. A study of cognitive decline assessed by MMSE found no differences between *APOE* ϵ4 carriers and noncarriers.[23] The lack of association between the ϵ4 allele and MMSE score as well as MMSE change in the nondemented population therefore could result from robustness of the MMSE test.

The lack of evidence for significant association between the *APOE* ϵ4 allele and cognitive decline seems to support the hypothesis that the harmful effect of this allele on cognitive functions is age-dependent and has worn out before this very old age.

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Early-life risk factors and the development of Alzheimer's disease

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Article abstract—*Objective:* To investigate the association of early-life factors with AD. *Background:* The early-life environment and its effect on growth and maturation of children and adolescents are linked to many adult chronic diseases (heart disease, stroke, hypertension, and diabetes mellitus), and these effects are also linked to maternal reproduction. AD may have an early-life link. The areas of the brain that show the earliest signs of AD are the same areas of the brain that take the longest to mature during childhood and adolescence. A poor-quality childhood or adolescent environment could prevent the brain from reaching complete levels of maturation. Lower levels of brain maturation may put people at higher risk for AD. *Methods:* In a community-based case-control study (393 cases, 377 controls), we investigated the association of early-life factors and AD. Early-life variables include mother's age at patient's birth, birth order, number of siblings, and area of residence before age 18 years. Patient education level and apolipoprotein E (*APOE*) genotypes were also included in the analysis. *Results:* Area of residence before age 18 years and number of siblings are associated with subsequent development of AD. For each additional child in the family the risk of AD increases by 8% (OR = 1.08, 95% CI = 1.01 to 1.15). More controls compared with cases grew up in the suburbs (OR = 0.45, 95% CI = 0.25 to 0.82). *APOE* ϵ4 and the patient's education level did not confound or modify the associations. *Conclusions:* The early-life childhood and adolescent environment is associated with the risk of AD. **Key words:** Early life—Childhood—Adolescence—AD—Chronic disease.

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The early-life environment and its effect on growth and maturation in children and adolescents are linked to many adult chronic diseases (heart disease, stroke, hypertension, diabetes mellitus, and chronic obstructive lung disease)[1] and to female reproductive outcomes.[2] AD may also have an early-life link.[3-5] Understanding growth, maturation, and aging of the brain may be the key to this link. The brain grows most in size during the prenatal period and in childhood[6-8] but continues to complete its maturation during adolescence.[6-9] Brain maturation refers to the development of connectivity patterns, synapses, branching of dendrites, and myelination.[7-9] The areas of the brain that take longest to mature during childhood and adolescence (e.g., hippocampal formation, intracortical association areas, reticular formation)[7,9] are the same areas of the brain that show the earliest signs of AD.[10-13] An association between early-life growth and development and later-life cognitive decline was first suggested by Conel[14] in 1939.

Environmental factors can affect brain maturation. Studies on rats[15-19] show that brain maturation can be retarded with only mild malnutrition and that catch-up growth is not always attainable. Mild malnutrition has a slowing effect on development and myelination patterns and interferes with normal development by decreasing dendritic growth. Increasing nutrition later in the rats improves brain maturation (as measured by amount of myelin) but not to the level of those who were never malnourished. This finding parallels studies on human children[20,21] showing that children who were marginally malnourished are shorter, lighter, and score lower on cognitive ability than their larger, heavier, and better nourished peers. Improved nutrition and environment later in childhood modified, but did not

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eliminate, the difference between marginally undernourished and well-nourished children, although both groups of children scored within the normal intelligence range. Therefore, poor growth early in life could increase the risk of AD. The effects of impaired development could produce a brain that is normal but functions less efficiently because of less myelin, less branching of dendrites, and less developed connectivity patterns. This impaired development affects speed and specificity of nerve transmissions and requires increased energy to function properly.[22,23] The negative effects of this less efficient brain would likely be marginal until aggravated by the aging process.

We investigated the association of AD and early-life factors: mother's age at subject's birth, birth order, sibship size, and area of residence before the age of 18 years. Babies born to mothers who are younger than 20 or older than 35 years of age tend to have lower birth weights.[24] Although there is an increase in birth weight with each successive birth, in cross-sectional studies babies born to mothers over 35 years of age also tend to be smaller because mothers who continue having babies later in life usually are in lower socioeconomic levels.[25] The number of children in a family is related to socioeconomic level.[26-29] During the early 1900s when the subjects in this study were children, the optimal/preferred family size was three or four children.[27,29] Families with five or more children were more likely to be from the lower socioeconomic levels[27,28] and therefore were more likely to have poor growth rates.[25,30-32] If deficient maturation is associated with a less developed brain, then these measures that influence early growth could be associated with AD. We also investigated whether the potential association of these early-life factors and AD changed after adjusting for education level and apolipoprotein E genotype (*APOE*), or whether the potential associations are modified by *APOE* (i.e., whether the strength of the association differs between those with and those without one or more *APOE* ϵ4 alleles).

Methods. *Study population and design.* Patients for this case-control study were drawn from the Group Health Cooperative (GHC), a large health maintenance organization in Seattle, WA. GHC was established in 1949; the Seattle area membership of people aged 60 years or over included about 23,000 people. The attrition rate, excluding deaths, is about 1% per year. Most of the GHC population are longtime members who originally enrolled through their employers and remain after retirement. GHC members are representative of the surrounding community with respect to age distribution, gender, and ethnicity, but have a slightly higher education level in this age group.

The AD cases for this study were obtained from patients enrolled in the University of Washington/GHC AD Patient Registry (ADPR) (U01 AG 06781) from 1987 to 1996. Specifically, they were probable AD cases also enrolled in the Genetic Differences Case-Control Study (R01 AG 07584). Cases were patients in whom dementia had been diagnosed according to the Diagnostic and statistical manual of mental disorders, 3rd ed., revised,[33] and who had a diagnosis of probable AD as defined by the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association[34] working group criteria, or definite AD if they died after ADPR enrollment and had a neuropathologic diagnosis of AD. Controls were patients selected at random from GHC enrollment lists during approximately the same period as the cases (case selection started 6 months earlier than control selection) and frequency matched on gender and age within 2 years. Potential controls were excluded if they had dementia or other neurologic disease causing dementia. To verify that the potential control subjects did not have dementia, they were required to achieve a score of 28 of 30 on the Mini-Mental State Examination[35] (27 if over age 80 years) and to have no other indications of dementia based on other test results, medical record review, or observations of the research nurse-interviewer. For more details on the ADPR case surveillance, enrollment, and diagnostic protocol, see Larson et al.[36]; for description of the Genetic Differences Case-Control Study see Kukull et al.[37]

Collection of early-life variables. Early-life information and other types of epidemiologic information were obtained by in-person interviews between research nurses and proxy informants for both case and control subjects. The variables used for this study include mother's, patient's, and siblings' birth dates, patient's education level, and patient's area of residence before age 18 years. If a patient lived in more than one type of area prior to age 18, he or she was asked to choose the area in which he or she lived in the longest. The primary source of birth date information was a family history questionnaire. This questionnaire was completed by the proxy with consultation with other family members.

A greater proportion of case, compared with control, proxies answered fewer of the family history questions or did not complete the family history questionnaire. Missing information is unlikely to have biased reporting of mother's age and other early-life variables because the reasons for not completing the questionnaire were not related to mother's age or the early-life environment. If the exact birth date of the mother was unknown or missing from the family history questionnaire, approximate information from the in-person epidemiologic interview was used. At the epidemiologic data interview, the proxy was asked whether the mother's age at patient's birth was less than 20, 20 to 24, 25 to 29, 30 to 34, or greater than 35 years, rather than the date of birth as on the family history questionnaire.

APOE genotypes had been determined as part of the Genetic Differences Case-Control Study by the restriction enzyme digestion method of Hixson and Vernier,[38] using DNA prepared from blood and brain tissue samples. Laboratory personnel were blinded to case/control status. *APOE* genotypes were unavailable for 74 of the case subjects and for 11 of the control subjects because either the family (n = 36) or the patient (n = 23) refused to give a blood sample, they discontinued participation (n = 11), the study ended before a blood sample was taken (n = 7), or samples were degraded or failed the PCR (n = 9).

Statistical analysis. The strength of association between early-life factors and AD is described by ORs and 95% CIs.[39,40] For categorical variables, ORs and 95% CIs

were calculated in the conventional manner from frequency tables, and Mantel-Haenzsel adjusted ORs were calculated for stratified data.[39,40] For continuous variables, unconditional logistic regression was used to estimate the crude and adjusted ORs and 95% CIs.[39,40] Multiple logistic regression (unconditional) was also used to obtain adjusted effect estimates.[39,40] For crude and adjusted ORs the variables are coded in the following ways: number of siblings was analyzed as a continuous variable, as a single dummy variable (<5 versus >5), and as three dummy variables (5 to 6 siblings, 7 to 9 siblings, and >10 siblings), using less than five siblings as the reference category. This value was chosen because families with five or more children were more likely to be from the lower socioeconomic levels[26-29] and therefore were more likely to have poor growth rates.[25,30-32] Education was coded as high school graduate or less versus more than high school. *APOE* genotype was coded as one or more ε4 alleles versus none.

Results. Characteristics of the study population are shown in table 1. The mean birth order is similar in case and control subjects. The mean number of siblings is higher in case than in control subjects (3.8 versus 3.3). The area of residence the patient lived in prior to age 18 years is similar for farm, rural, and urban residence; however, more control compared with case subjects reported growing up in the suburbs. Cases had a higher frequency of *APOE* ε4–containing genotypes and an overall lower education level. The difference in level of education between case and control subjects is primarily due to response bias among controls. A substudy conducted as part of the Genetic Differences Case-Control Study showed that persons selected as potential controls who refused to participate in the study were more similar to the enrolled AD cases in their level of education, whereas control subjects who agreed to enter the study were more highly educated. Therefore, the appearance of an association between lower education and AD in this data is thought to be spurious.

Increased number of siblings is associated with an increased risk of AD (table 2). The risk of AD increases by 8% for each additional sibling in the family (OR = 1.08, 95% CI = 1.02 to 1.15). Growing up in a family with five or more siblings increases the risk of developing AD by 39% (OR = 1.39, 95% CI = 0.99 to 1.95). There is a linear trend of increasing risk with increasing sibship size. Compared with families with less than five siblings, having seven to nine siblings is associated with an almost twofold risk (OR = 1.72, 95% CI = 1.01 to 2.43), and in extremely large families with 10 or more siblings the risk is greater than twofold (OR = 2.66, 95% CI = 0.92 to 7.99). The area of residence prior to age 18 years is associated with AD. Specifically, more control compared with case subjects grew up in the suburbs (OR = 0.46, 95% CI = 0.25 to 0.83). The presence of at least one *APOE* ε4 allele is associated with a 3.6-fold increased risk of AD. We found no association between mother's age at patient's birth and subsequent onset of AD.

Having more than a high school education is inversely correlated with number of siblings (Spearman's $r = -0.17$, $p = 0.0014$). The presence/absence of *APOE* ε4 allele shows no correlation to growing up in the suburbs, level of education, or number of siblings. Table 3 shows the results of six different multiple logistic regression models used to obtain adjusted ORs and 95% CIs for the risk of AD. The ORs of the variables of interest are quite similar for each model and to the crude ORs shown in table 2. Model 1 shows the association of growing up in the suburbs while adjusting for patient's education. Only a few patients are missing area of residence information and are not in this model. Model 2 shows the association of more than five siblings while controlling for presence of the *APOE* ε4 allele. The

Table 1 *Characteristics of case and control subjects in a study of early-life factors and the development of AD*

Characteristic	Cases, n = 393	Controls, n = 377
Male sex	142 (36)	140 (37)
Patient's age at intake, y	78 ± 6.7	78 ± 6.8
Age distribution, y		
<60	3 (1)	3 (1)
60–69	30 (8)	36 (10)
70–79	163 (42)	163 (43)
80–89	183 (47)	165 (44)
90+	14 (4)	10 (3)
White race	346 (88)	362 (96)
Mother's age, y		
<20	22 (6)	23 (6)
20–34	265 (67)	282 (75)
35+	48 (12)	63 (17)
Missing	58 (15)	9 (2)
Birth order	2.8 ± 2.2	2.7 ± 1.9
Missing	145 (37)	57 (15)
Number of siblings	3.8 ± 2.9	3.3 ± 2.5
Missing	91 (23)	40 (11)
Distribution of siblings		
0–2	131 (34)	152 (40)
3–4	67 (17)	92 (24)
5–6	49 (12)	56 (15)
7–9	42 (11)	30 (8)
10+	13 (3)	6 (2)
Missing	91 (23)	40 (11)
Residence before age 18 y		
Farm	76 (20)	67 (18)
Rural	125 (32)	132 (35)
Suburb	17 (4)	34 (9)
Urban	171 (44)	141 (38)
Missing	4 (<1)	3 (<1)
Education level		
< High school	98 (25)	61 (16)
High school graduate	141 (36)	104 (28)
> High school	154 (39)	212 (56)
Any ε4 allele		
No	141 (36)	271 (72)
Yes	178 (45)	95 (25)
Missing	74 (19)	11 (3)

Values are n (%) or mean ± SD.

Table 2 *Crude associations of early-life factors and AD*

Early-life factor	OR (95% CI)
Per additional sibling	1.08 (1.02–1.15)
Sibship size	
<5	1.0
5–6	1.07 (0.68–1.68)
7–9	1.72 (1.01–2.43)
10+	2.66 (0.92–7.99*)
Sibship size	
5+	1.39 (0.99–1.95)
<5	1.0
Suburb	0.46 (0.25–0.83)
Other place	1.0
Area of residence before age 18 y	
Farm	0.94 (0.62–1.42)
Rural	0.78 (0.55–1.10)
Suburb	0.41 (0.21–0.80)
Urban	1.0
APOE status	
Any ε4	3.60 (2.56–4.85)
No ε	1.0
Mother's age, y	
<20	1.02 (0.53–1.95)
20–34	1.0
35+	0.89 (0.71–1.12)
Birth order	1.04 (0.96–1.12)

Chi-square for linear trend: 6.94; p value: 0.008.

large amount of missing information is from sibling size and *APOE*; even so, the adjusted associations of models 1 and 2 shown in table 3 are very similar to the crude associations shown in table 2. Model 3 shows the associations of both area of residence and number of siblings adjusting for education, and model 4 shows the associations of area of residence and more than five siblings adjusting for both potential confounders (*APOE* and education). These four models individually and together show that the associations are stable for area of residence and sibship size when investigating individual associations or combined associations while adjusting for one or both of the potential confounders and across variations in the sample size. As can be seen in the variation in number of case and control subjects across the six models, there is notable but not complete overlap in the amount of missing information on each patient. Models 5 and 6 show the linear trend of sibship size in the presence of one or both confounders. Again the amount of variation between the associations of sibship size is minimal and the increasing risk with increasing sibship size is stable. Thus, the large amount of missing data on level of education or *APOE* ϵ4 allele does not appear to confound the association between AD, sibship size, or growing up in the suburbs.

Stratifying by the presence/absence of *APOE* ϵ4 allele (table 4) revealed no significant variation in the strength of association between the variables of interest and AD. Increasing sibship size was not associated with AD in the presence of *APOE* ϵ4; however, the large percent of missing information for the number of siblings in the case subjects combined with the lower frequency of *APOE* ϵ4 in the control subjects may have compromised the ability to evaluate this association. The Breslow-Day test for homogeneity of ORs across strata was not significant ($p > 0.1$), indicating no statistical evidence for effect modification by *APOE* genotype.

Discussion. The relationship of the process of growth and development of the brain and the pathology of AD describes a biologic connection. We as well as others[3-5] have concluded that the early-life environment may be associated with the development of AD. Each of these studies used different measures of early life (head circumference,[3] adult height,[5] and early-adult linguistic ability[4]) to investigate an early-life association with AD. We used information collected by interview to retrospectively collect factors in the early-life environment that influence growth

Table 3 *ORs (95% CIs) relating various combinations of risk factors to AD*

	Models					
Variable	1	2	3	4	5	6
Cases/controls, n	389/374	248/328	298/334	246/326	248/328	248/328
Other variables						
Area of residence suburb	0.46 (0.3–0.8)	–	0.36 (0.2–0.7)	0.37 (0.2–0.8)	–	–
Per additional sibling	–	–	1.06 (1.0–1.12)	–	–	–
5+ Sibling	–	1.39 (0.9–2.0)	–	1.25 (0.9–1.9)	–	–
5–6 Siblings	–	–	–	–	1.04 (0.6–1.7)	1.0 (0.6–1.6)
7–9 Siblings	–	–	–	–	1.64 (0.9–2.9)	1.43 (0.8–2.5)
10+ Siblings	–	–	–	–	2.43 (0.8–7.1)	2.10 (0.7–6.3)
APOE ε4 allele	–	3.50 (2.5–5.0)	–	3.96 (2.7–5.7)	3.47 (2.4–4.9)	3.80 (2.6–5.5)
> High school education	0.49 (0.4–0.7)	–	0.49 (0.4–0.7)	0.42 (0.3–0.6)	–	0.44 (0.3–0.6)

The dash (–) indicates the variable is not included in the model. Each column represents a separate unconditional logistic regression model. The number of subjects is specified followed by the adjusted odds ratio and 95% CI for each independent variable.

Table 4 *Results of stratified analysis of early-life factors and AD by presence of any* APOE *ε4 allele*

Early-life factor	Any ε4	OR (95% CI)
Area of residence before age 18y/suburb	No	0.33 (0.12–0.88)
	Yes	0.67 (0.24–0.85)
Per additional sibling	No	1.11 (1.02–1.21)
	Yes	1.01 (0.58–2.64)
Number of siblings 5+	No	1.62 (1.00–2.62)
	Yes	1.01 (0.56–1.83)

to further explore the association between early life and AD.

Area of residence and number of siblings are related to socioeconomic level and therefore to quality of the living environment. The association between living in the suburbs and AD could reflect the benefits of higher socioeconomic status and less exposure to infectious disease. During the early 1900s infectious diseases were known to be less frequent in less densely populated rural areas compared with urban areas.[26] The suburbs were less densely populated and, especially during this era, were an area of at least middle to upper socioeconomic levels. Therefore, children growing up in the suburbs may have been more likely to have better nutrition and less exposure to infectious disease, leaving more energy for normal growth and development. Following this logic, we expected to see a protective association with living on a farm. We did not. However, many farming families during this era who experienced economic hardship migrated into other urban-based occupations. Without being able to differentiate between patients who grew up on economically successful farms versus those whose families lost their farms, we are unable to fully test this association.

The number of siblings in the family also reflects the economic level.[26-29] Simply, more people living in one family stretches the resources more thinly. Also, it was more common for persons of lower socioeconomic status to marry younger and begin childbearing at an earlier age, continuing reproduction into their middle and late 30s.[26,27] Persons of higher socioeconomic status tended to marry later in life after furthering their education and as a result tended to have fewer children.[27]

The association of education level needs to be interpreted with caution. In this study sample 39% of the case subjects and 56% of the control subjects have more than a high school education. Prior to 1940 when people of this age group were on average 28 years old, less than 25% of the population over 24 years of age had more than a high school education.[41] GHC, the Seattle area health maintenance organization from which this study population is selected, has approximately 20% more members with more then a high school education[42] compared with the surrounding population.[43] Another factor complicating the analysis of education level is the effect of selection bias. There is likely selection bias for education in this study because potential control subjects who declined to participate were similar in education level to the enrolled case subjects. More highly educated control subjects participated; therefore, the larger proportion of control subjects with more than high school education is likely to be at least partially the result of selection factors, and thus the association with AD cannot be correctly estimated. Case subjects display less selection effect because they were motivated to participate to obtain expert diagnosis by ADPR clinicians of a very serious problem.

We see in our data that number of siblings, a socioeconomic indicator, and greater than high school education are correlated, albeit modestly (Spearman's $r = -0.17$). The complexities of understanding the meaning of the association of education level with disease are not unique to this study. Level of education can be a risk factor, a confounder, and a reason for selection bias. Therefore, we suggest the association be viewed with caution. Adjusting for educational level in this study did not significantly change the association between AD and the variables of interest to the study's hypothesis.

This study has other limitations. The early-life variables are obtained from proxy interviews; we interviewed proxies for *both* case and control subjects to avoid obvious asymmetry in data collection that would occur if we interviewed proxies of case subjects and then interviewed control subjects directly.[44] We expect that proxy interviews, generally speaking, may lead to nondifferential misclassification of information obtained and potentially bias effect measures toward the null.[44] Data on mother's age at patient's birth, birth order, and number of siblings are unavailable for many patients. Although missing data were more prevalent in case than in control subjects, we have no evidence that these missing data would bias our finding away from the null. However, better, more objective means of collecting these data are necessary to understand the association of these variables. We are currently developing another project to follow up on these preliminary findings and to further explore the association of the early-life environment and the development of AD.

The strengths of this study are that the results indicate associations consistent with a biologically plausible connection seen between growth and development and the pathology of AD. Although the associations of sibling size and area of residence before age 18 years are not particularly large, they are stable when controlling for potentially confounding effects, and in sibship size there is a consistent and significant linear trend.

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Lu 25-109, a muscarinic agonist, fails to improve cognition in Alzheimer's disease

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Article abstract—*Objective:* To evaluate the therapeutic effect of the selective muscarinic receptor m1 partial agonist, m2 antagonist, Lu25-109—a compound that directly stimulates muscarinic cholinergic receptors—in patients with probable AD. *Methods:* A 6-month, randomized, double-blind, placebo-controlled, parallel group trial comparing three doses of Lu25-109 with placebo was carried out. A total of 496 patients with probable AD with a Mini-Mental State Examination score between 10 and 26 were enrolled at 29 centers and randomized to placebo or Lu25-109 25, 50, or 100 mg tid. The primary efficacy measures were the AD Assessment Scale–Cognitive subscale and the AD Cooperative Study Clinical Global Impression of Change. Secondary efficacy variables included the AD Cooperative Study Inventory of Activities of Daily Living and the Behavioral Symptoms in AD Scale. *Results:* In both an intent-to-treat and a completer's analysis there were no significant differences for either the two primary or the secondary variables. There was a trend for patients on the highest drug dose to worsen in the completer's analysis. Adverse events included dizziness, nausea, diarrhea, fatigue, increased sweating, and anorexia, all of which increased with increasing drug dose. *Conclusion:* Lu25-109, a selective partial m1 agonist and an m2/m3 antagonist, failed to improve cognition in patients with mild to moderate AD.
Key words: AD—Cognition—Cholinergic—Muscarinic agonist.

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In AD, a chronic neurodegenerative disorder,[1] markers of cholinergic functioning including neocortical choline acetyltransferase (ChAT)[2] are decreased. Basal forebrain cholinergic neurons that project to the cortex and the hippocampus are diminished.[3] Treatment with cholinesterase inhibitors, which augments cholinergic functioning by inhibiting acetylcholine (ACh) catabolism, improves cognition in patients with AD.[4-8]

Less attention has been directed toward agonist replacement therapy in AD. Five muscarinic cholinergic receptor subtypes have been identified.[9] The m1 receptor subtype is found predominantly in the cerebral cortex and hippocampus, is postsynaptic, and is maintained or increased in AD despite the loss of presynaptic input from the forebrain. The m2 receptor subtype is located presynaptically and its stimulation increases ACh release. Previous trials of nonselective muscarinic agonists have been limited primarily by toxicity. More recent selective muscarinic agonists, which are highly active at the m1 site, have been tested.[10] In theory, a drug with an agonist effect on the m1 receptor and an antagonist effect on the m2 receptor should be optimal for therapy as it would directly stimulate postsynaptic receptors as well as enhance the release of ACh by blocking presynaptic inhibition of ACh release.[11] Lu 25-109 acts as a partial m1 agonist and an m2/m3 antagonist, thus stimulating postsynaptic muscarinic receptors and inhibiting presynaptic receptors. Because of its optimal receptor profile for facilitating cholinergic transmission and its favorable preclinical profile that demonstrated increased cortical ACh release in hippocampus and improvement of spatial memory in aged rats,[12] a dose-finding efficacy study was carried out in patients with AD.

Methods. *Objective.* The primary objective of this trial was to evaluate the effects of Lu25-109 on cognition and global functioning across a wide range of doses. Secondary objectives were to evaluate its effects on activities of daily living and behavior, and to determine its safety profile.

Patients. Male and female outpatients with a reliable caregiver to ensure compliance with the protocol were selected according to the following criteria: 45 years of age or older, diagnosis of probable AD according to National Institute of Neurologic and Communicative Disorders–Alzheimer's Disease and Related Disorders Association criteria,[13] a Mini-Mental State Examination (MMSE) score[14] between 10 and 26, and a Modified Hachinski Ischemic Score[15] of 4 or less. Patients were excluded if they had a medical condition that required concurrent medication known to affect the CNS, a neurologic disorder other than AD, or evidence of major depression on the Cornell Scale.[16] Informed consent was obtained from each patient and caregiver or legal guardian. Either local or national insti-

*Contributors to the Lu25-109 Study Group are listed in the Appendix on page 425.

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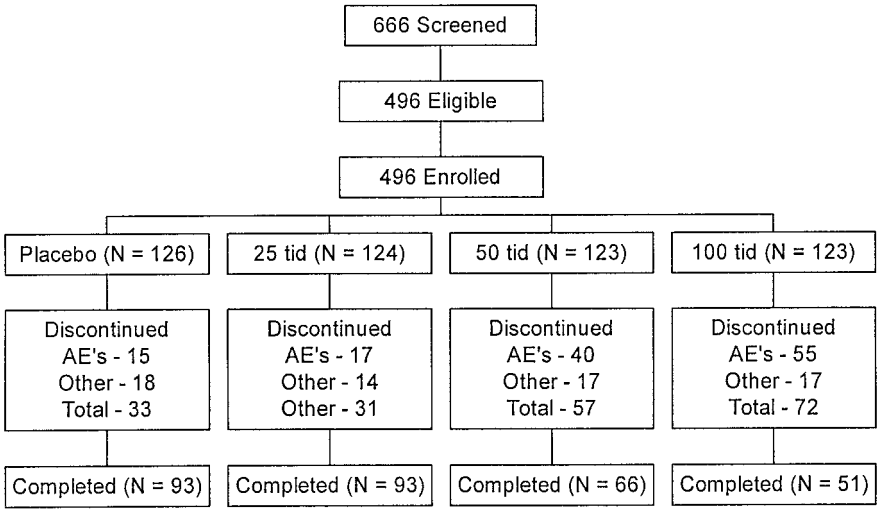

Figure. Patient distribution. AE = adverse event.

tutional review boards approved the study for each of 29 centers that contributed patients for this clinical trial.

Study design. The design of the study consisted of a 3-week screening phase, a 2-week dose-titration phase, followed by a 24-week fixed dose treatment with placebo or 25, 50, or 100 mg tid of Lu25-109 daily. A follow-up evaluation occurred 5 weeks later. During the study, patients were evaluated nine times, with cognitive measures collected at baseline and months 1, 3, and 6. The highest selected dose (300 mg daily) was two-thirds of the established maximum tolerated dose and was considered to be well-tolerated. A tid dose regimen was selected based on a half-life of approximately 2 hours. Following completion of the trial, all patients were allowed to enter an open-label treatment protocol.

Outcome measures. Efficacy was assessed using two primary and two secondary outcome measures, each of which were assessed at baseline and months 1, 3, and 6.

Primary measures. The cognitive component of the Alzheimer's Disease Assessment Scale (ADAS-Cog),[17] a composite neuropsychologic measure consisting of 11 items and scored from 0 to 70, was performed at baseline and months 1, 3, and 6. The Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC)[18] was the second primary outcome measure. In this seven-point scale, the study clinician rates each patient along a continuum from very much worse (−3) to very much improved (+3).

Secondary measures. Two secondary outcome measures included the ADCS Inventory of Activities of Daily Living (ADCS-IADL)[19] and a Behavioral Symptoms in AD Scale (BEHAVE-AD)[20] in which information about behavior was obtained from the caregiver and the patient.

At baseline, apolipoprotein E status was determined as it has been reported to predict response to acetylcholinesterase inhibitors.[21] CYP2D6 genotyping was performed to determine if fast or slow metabolizers of drug by cytochrome P450 would result in excessively high drug levels in some patients.[22] Biochemical, hematologic, and urine analyses, as well as EKGs, were performed at intervals during the study. Vital signs were monitored at each visit.

Randomization, blinding, and sample size. Patients were randomly allocated in equal numbers to the four treatment groups. The tablets were indistinguishable; drug and placebo were identical in taste and smell. The number of tablets to be given at each dose was the same throughout the study. Subjects receiving the highest dose (300 mg per day) underwent blinded dose titration during the first 2 weeks. Individual code breaker envelopes were available to break the code on an individual patient if needed. The blind was not broken in any cases.

The sample size calculation was based on the detection of a three-point difference on the ADAS-Cog between placebo and any of the tested doses of drugs assuming a SD of 7, an alpha of 0.05, and a power at 80%. Using this calculation, a sample size of 120 subjects per group was considered appropriate. Owing to the short duration of the trial, no interim analysis was planned.

Statistical analysis. Statistical analyses were performed using an intent-to-treat (ITT) approach for all patients using the last observation carried forward (LOCF) principle. A completer's analysis was also carried out.

For the change in ADAS-Cog, a general linear analysis of covariance (ANCOVA) with baseline ADAS-Cog, site, and treatment groups as covariates was applied. Analysis of ADCS-CGIC, site, and treatment groups as covariates was performed with an ANCOVA analysis. Analysis of the ADCS-IADL and BEHAVE-AD were performed as described above for the ADAS-Cog. Demographic differences and treating emergent adverse events (AE) were compared by Fisher's exact test.

Results. *Patient flow.* A total of 666 subjects were screened for this trial and 496 were eligible and enrolled. Discontinuation rates were 26% for the placebo group, 25% for 25 mg, 46% for 50 mg, and 58% for 100 mg tid of Lu25-109. The increase in discontinuation rate reflected an increasing prevalence of AE in the middle and highest dose (figure). The discontinuation rate due to AE at the lowest dose was similar to that on placebo (14% versus 12%).

Patient demographics and baseline characteristics. Other than a slight imbalance in race, patient demographics with respect to age, sex, weight, and education did not differ among the groups (table 1). The Hachinski Ischemic Score

Table 1 *Patient characteristics at baseline*

Characteristic	Placebo, n = 126	Lu25–109			*p* Value
		25 mg tid, n = 124	50 mg tid, n = 123	100 mg tid, n = 123	
Age, y, mean (range)	76 (47–91)	74 (53–91)	76 (54–92)	76 (50–95)	0.28
Sex, n, F/M	70/56	74/50	73/50	72/51	0.91
Race, white/other	108/18	116/8	115/8	117/6	0.03
Weight, kg, mean (range)	69.2 (34–127)	69.3 (42–119)	66.9 (41–98)	69.2 (37–127)	0.51
Education, high school or more, %	96	94	92	95	0.10
Hachinski Ischemic Scale, mean ± SD	0.6 ± 0.8	0.6 ± 0.8	0.7 ± 0.8	0.6 ± 0.7	0.63
Cornell Scale, mean ± SD	3.0 ± 2.4	3.6 ± 2.8	3.1 ± 2.5	3.4 ± 2.6	0.29
MMSE, mean ± SD	20.1 ± 4.8	20.5 ± 4.5	20.1 ± 4.8	19.7 ± 5.0	0.70
APOE, % ϵ4	53	59	50	55	0.62
CYP2D6, % B	36	33	39	3.8	0.95

MMSE = Mini-Mental State Examination.

was low in all cases. There were no differences in depression on the Cornell Scale. The mean MMSE was 20 and did not differ by group assignment. Similarly, there were no differences in the efficacy variables at baseline (table 2). Fifty-four percent of the sample had one or two ϵ4 alleles; 37% carried the CYP2D6 B allele (see table 1).

Efficacy parameters—ITT analysis. There were no significant differences for either of the two primary outcome measures, the ADAS-Cog or the ADCS-CGIC, across the treatment groups (table 3). An analysis by baseline MMSE severity showed that patients with more severe disease declined more during the study but that the treatment–

Table 2 *Efficacy variables at baseline*

Measure	Placebo, n = 126	Lu25–109			*p* Value
		25 mg tid, n = 124	50 mg tid, n = 123	10 mg tid, n = 123	
ADAS-Cog	22.7 ± 12.9 (3–66)	23.0 ± 12.5 (3–60)	23.1 ± 12.2 (4–60)	24.1 ± 13.3 (3–66)	0.81
ADCS-ADL	59.5 ± 15.1 (19–78)	60.7 ± 15.0 (6–78)	59.6 ± 14.4 (6–78)	59.6 ± 15.9 (14–78)	0.91
BEHAVE-AD	5.6 ± 5.7 (0–29)	5.7 ± 5.2 (0–23)	5.7 ± 5.3 (0–31)	5.6 ± 5.6 (0–25)	0.99

Values are mean ± SD (range).

ADAS-Cog = Alzheimer's Disease Assessment Scale–cognitive component; ADCS-ADL = Alzheimer's Disease Cooperative Study Inventory of Activities of Daily Living; BEHAVE-AD = Behavioral Symptoms in AD Scale.

Table 3 *Adjusted mean differences at 24 weeks for Lu25–109 versus placebo—intention-to-treat analysis*

Outcome measures	Placebo, n = 126	Lu25–109			*p* Value
		25 mg tid, n = 124	50 mg tid, n = 123	100 mg tid, n = 123	
Primary					
ADAS-Cog	1.16	1.04	0.90	1.90	0.51
ADCS-CGIC	0.25	0.34	0.22	0.33	0.63
Secondary (n = 117–126)					
ADCS-ADL	−2.62	−2.79	−2.40	−3.13	0.91
BEHAVE-AD	0.07	−0.72	−0.15	−0.26	0.35

Higher scores indicate worsening for the Alzheimer's Disease Assessment Scale–cognitive component (ADAS-Cog), Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC), and Behavioral Symptoms in AD Scale (BEHAVE-AD); lower scores indicate worsening for the ADCS Inventory of Activities of Daily Living (ADCS-ADL).

Table 4 *Adjusted mean differences at 24 weeks for Lu25–109 versus placebo—completers*

Outcome measures	Placebo, n = 92–97	Lu25–109			*p* Value
		25 mg tid, n = 85–92	50 mg tid, n = 62–68	100 mg tid, n = 45–51	
Primary					
ADAS-Cog	0.95	0.96	0.86	3.40	0.08
ADCS-CGIC	0.27	0.37	0.30	0.37	0.88
Secondary					
ADCS-ADL	−3.04	−2.85	−2.38	−4.65	0.61
BEHAVE-AD	0.10	−0.48	−0.24	−1.16	0.30

Higher scores indicate worsening for the Alzheimer's Disease Assessment Scale–cognitive component (ADAS-Cog), Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC), and Behavioral Symptoms in AD Scale (BEHAVE-AD); lower scores indicate worsening for the ADCS Inventory of Activities of Daily Living (ADCS-ADL).

placebo differences did vary by baseline severity. Similarly, there were no significant differences on the secondary outcome measures, the ADCS-IADL and the BEHAVE-AD, by treatment assignment (see table 3).

Completer's population. The completer's population showed a similar pattern to the ITT population. There were no significant differences on any of the primary or secondary outcome measures. However, there was a trend toward worsening on the ADAS-Cog in those subjects receiving the highest dose of Lu25-109 (table 4).

ApoE and cytochrome P-450 (CYP) genotyping. Statistical analysis was performed using apoE and CYP genotype as covariates interacting with treatment within the ANCOVA modeling. Nothing significant was found. In addition, analysis of treatment effect by genotype was nonsignificant.

Safety analysis. Treatment-emergent AE present in ≥5% in any group are listed in table 5. As would be expected of a cholinergic agent, the treatment-emergent AE related primarily to stimulation of peripheral cholinergic receptors. Side effects occurring with increased frequency in the drug-treated groups included dizziness, nausea, diarrhea, fatigue, sweating, anorexia, increased salivation, vomiting, loss of weight, and asthenia. In general, the treatment-emergent side effects were dose dependent and occurred most frequently on the two highest doses. AE

Table 5 *Percent treatment-emergent adverse events ≥5%*

Adverse event	Placebo, n = 126	Lu25–109			*p* Value
		25 mg tid, n = 124	50 mg tid, n = 123	100 mg tid, n = 123	
Dizziness	10	24	42	38	0.00
Nausea	11	15	24	28	0.00
Diarrhea	4	13	7	20	0.00
Fatigue	5	10	12	15	0.04
Sweating ↑	3	6	12	18	0.00
Anorexia	2	5	8	24	0.00
Depression	12	10	9	7	0.67
Headache	6	14	10	5	0.07
Somnolence	4	7	11	12	0.08
Confusion	6	8	7	11	0.52
UTI	6	11	10	6	0.25
Saliva ↑	2	<1	8	19	0.00
Abdominal pain	5	6	11	7	0.35
Vomiting	4	4	7	12	0.04
Agitation	8	6	7	6	0.92
Weight ↓	2	5	6	13	0.00
Asthenia	2	3	7	11	0.02

Figures are percentages.

UTI = urinary tract infection.

were responsible for the high drop-out rate for patients assigned to the highest drug doses. The percent of patients experiencing treatment-emergent AE at the lowest dose (85%) was no different from the percent of patients on placebo (87%). AE were responsible for the high drop-out rate for patients assigned to the highest drug dose.

There were no significant changes in vital signs, EKG, or chemistries.

Discussion. Treatment with Lu25-109 failed to improve cognition in patients with mild to moderate AD. No effect was detected at 25 and 50 mg tid; at the highest dose of 300 mg daily, subjects clearly worsened. This is most striking in the completer's analysis for the high-dose group where subjects on drug were about 2.5 points worse on the ADAS-Cog compared with those on placebo. Similarly, there were no improvements on the global measure of behavior or activities of daily living.

There were no safety findings of particular clinical concern. AE increased with dose and 58% of patients on 300 mg per day discontinued participation from the study, principally because of AE. AEs were typical of cholinergic stimulation and included gastrointestinal disturbance, increased sweating, dizziness, anorexia, and weight loss.

Cholinergic agonists acting directly on the postsynaptic receptor have been tried in previous AD trials. Their use has generally been limited by peripheral side effects. With arecoline, slight improvement was noted on a picture recognition task[23] and on a verbal learning task[24]; a third study demonstrated no improvement.[25] Trials with oxotremorine,[26] RS86,[27] and pilocarpine[28] were all negative. A single-blind trial of intraventricular bethanechol was encouraging,[29] but a larger double-blind study failed to demonstrate improvement in cognition.[30] A large multicenter trial of xanomeline, a selective m1 and m4 agonist, demonstrated a small cognitive improvement on a completer's analysis but not on an ITT LOCF analysis on the highest dose tested.[10] A similar response was seen on the Clinician's Interview Based Impression of Change Plus (CIBIC+), with improvement noted on highest dose on the completer's analysis but not on the ITT analysis. A post-hoc analysis also appeared to show some improvement in behavioral symptoms with reductions in verbal outbursts, suspiciousness, delusions, agitation, and hallucinations. As in the current study, side effects secondary to cholinergic stimulation including sweating, nausea, vomiting, and dyspepsia were common and occurred in 59% of the high-dose xanomeline-treated patients.

Use of SB 202026, a partial muscarinic m1 agonist, resulted in improvement on the ADAS-Cog and a trend toward improvement on a CIBIC+.[31] Side effects included increased sweating, diarrhea, vomiting, nausea, and insomnia.

Unlike cholinesterase inhibitors, which elevate brain ACh levels, direct stimulation of postsynaptic muscarinic receptors does not appear to improve cognition in AD. There are several potential reasons for this. First, continuous stimulation of postsynaptic muscarinic receptors may lead to rapid receptor desensitization. Both in vitro and in vivo experiments have shown that the more potent and full a muscarinic agonist is, the more rapidly the agonist induces downregulation with receptor internalization.[32] This rapid receptor downregulation might prevent the appearance of clinical improvement even after only a minimum time of exposure to drug. Second, tonic stimulation of receptors may be suboptimal for improvement of learning and memory. Impulse-mediated release of presynaptic neurotransmitters may be necessary for optimal and appropriate encoding of new material. Finally, the emergence of dose-limiting peripheral side effects may limit the ability to stimulate CNS postsynaptic muscarinic receptors pharmacologically.

Appendix

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Gender differences in the treatment of behavior problems in Alzheimer's disease

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Article abstract—*Objective:* To define gender differences in noncognitive behavioral problems of patients with AD and differences in the associated treatment of those problems. *Design/Methods:* We performed an observational study using the Systematic Assessment and Geriatric drug use via Epidemiology (SAGE) database, which contains data collected with the Minimum Data Set on a cross-section of nursing home residents in five US states. Behavior problems were documented at the first assessment of 28,367 residents with AD. We evaluated the role of gender differences in behavior as predictors of differences in nonpharmacologic versus specific pharmacologic therapies with psychoactive medications using logistic regression. *Results:* Men were more likely than women to exhibit behavior problems such as wandering, abusiveness, and social impropriety (59% versus 50% for any behavior problem). Hallucinations and delusions as well as depression were equally prevalent in men and women. Nevertheless, men were more likely to receive psychoactive medications. Among the specific drug categories examined, and controlling for age and degree of cognitive impairment, men were more likely to receive antipsychotic drugs and less likely to be receiving antidepressants. *Conclusion:* Gender appears to play an important role in determining the frequency of behavioral problems in nursing home residents with AD, which may influence choice of treatments as well as the decision whether to treat. The use of more potent tranquilizers in men with problem behaviors has potential implications for morbidity, deserving further investigation. **Key words:** Gender—Behavior—Treatment—AD—Dementia.

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Alzheimer's disease is associated with a wide variety of so-called noncognitive psychiatric and behavioral disturbances, including apathy, depression, psychosis with delusions and hallucinations, sleep disruption, wandering, inappropriate sexual behavior, and aggression. Despite the many reports indicating a relationship of behavior disturbances with dementia severity,[1-5] there are limited and somewhat contrasting data suggesting that men and women with AD differ in the frequency of specific types of behavior disturbances.

In a study of outpatients with AD, Ott and coworkers[5] reported that aggression, apathy, and vegetative disturbances such as overeating and oversleeping were seen more commonly in men. Reclusiveness, resistiveness, hoarding, and emotional lability were more characteristic of women. An increased prevalence of agitation in male outpatients with AD has been reported using the Neuropsychiatric Inventory.[6] Other studies of AD have reported an association between physical,[4,7,8] verbal,[4,9] and sexual aggression[10] and male gender. Data have shown a higher incidence of physical and verbal aggression in men that did not reach statistical significance, possibly because of the low prevalence of aggressive behaviors in this outpatient sample.[11] Sexual indifference was reportedly common among community dwelling residents with AD.[12] There was no gender difference in this factor; however, men were more likely to show changes in actual sexual behavior and exhibited more embarrassing sexual behaviors.

Whereas apathy is more common among men with AD,[5,9] other psychiatric symptoms appear to predominate in women. Depression is more prevalent in women.[13-16] Psychotic symptoms such as delusions and hallucinations occurred at an earlier stage of disease in women.[17] Three other separate studies found that delusions occurred more commonly in women.[1,2,13]

Considerably less is known about how such differences affect the choices of nonpharmacologic and specific pharmacologic approaches to treatment of behavior problems in men and women with AD. In the current study, we examined a large sample of nursing home residents with AD to extend previous observations about behavior differences between the sexes that have been reported in studies of outpatients. Because most behavior problems are more prominent in the advanced stages of dementia, it was predicted that aggressive behaviors would be clearly more prevalent among men in this sample.

*Members of the Systematic Assessment of Geriatric drug use via Epidemiology (SAGE) Study Group are listed in the Appendix on page 431.

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The pharmacologic and nonpharmacologic therapies for behavior problems were then examined to define the gender differences in treatment associated with these problems.

Methods. We used the Systematic Assessment of Geriatric drug use via Epidemiology (SAGE) database, a multisource, cross-linked, long-term care database.[18,19] The SAGE database combines data collected during the Health Care Financing Administration's Multi-State, Case-Mix, and Quality Demonstration Project. Data were collected on the federally mandated Resident Assessment Instrument that includes a 300-item Minimum Data Set (MDS).[20] All Medicare/Medicaid certified nursing homes (n = 1,492) in five US states (Kansas, Maine, Mississippi, New York, and South Dakota) collected MDS data on over 450,000 residents living in long-term care from 1992 through 1996. Detailed information regarding the specific characteristics of the database is found elsewhere.[19,21] For this study we used only data obtained from the initial assessment performed in the nursing home. We provide a brief summary of the SAGE database below.

SAGE database. Information such as sociodemographics, numerous clinical items including all active clinical diagnoses, and variables describing the degree of physical dependence and cognitive functioning is included in the MDS.[18,20] An extensive list of signs, symptoms, syndromes, treatments, and indicators describing each resident's behavior and mood is also included in the MDS.[18,20] Trained clinical professionals (e.g., nurses, social workers, therapists) are called on to assess resident performance over all shifts during the prior 7-day period. Each item has its own explicit definition. Cues are provided for how to ask questions, what to observe, and who to contact for information. Each assessor is to interact directly with the resident, review the record, and gather information on resident performance from direct care and licensed professional staff.[22]

Summary scales to describe the performance on activities of daily living (ADL), cognition, mood status, behavioral problems, social engagement, communication, mobility, and urinary continence are embedded in the MDS.[22,23] The ADL score, a 5-item, 6-level score, measures performance in physical function. This scale is based on the resident's dependency in the areas of dressing, eating, toileting, bathing, locomotion, transferring, and incontinence.[23] We classified residents as mildly impaired (ADL score 0 to 1), moderately impaired (ADL score 2 to 3), or severely impaired (ADL score 4 to 5).

To measure level of cognitive impairment, we used a seven-level cognitive performance scale (CPS) whose reliability and validity have been demonstrated in comparison with other research instruments, including the Mini-Mental State Examination and clinical criteria.[22,24,25] Seven separate direct measures of cognitive performance were included in the MDS: short- and long-term memory, orientation items (recall of season, of location of room, of identity of staff, and that the resident is in a nursing home), and a single item assessing the ability of the resident to make decisions about activities that are a daily part of life. The MDS also includes a number of indirect measures of cognition: an item indicating comatose status, two measures of communication skills, and eight measures of ADL. Based on these items, we categorized residents as having minimal impairment (CPS score 0 to 1), moderate cognitive impairment (CPS score 2 to 4), or severe cognitive impairment (CPS score 5 to 6).

We considered the following behaviors associated with dementia: wandering, verbal abusiveness, physical abusiveness, and socially inappropriate/disruptive behavior. The MDS defines wandering as movement with no rational purpose, seemingly oblivious to needs or safety. Nurses documented verbal abusiveness when residents threatened, screamed, or cursed at other residents. If residents hit, shoved, scratched, or sexually abused others, nurses documented the behavior as physical abusiveness. Socially inappropriate or disruptive behavior included sexual behavior or disrobing in public, smearing/throwing food or feces, self-abusive acts, hoarding, rummaging through others' belongings, making disruptive sounds, or screaming. The presence of depressive symptoms and hallucinations/delusions were also assessed. Except for the presence of depressive symptoms, presence of behaviors referred to the 7 days preceding the assessment.

To create an indicator variable of presence of depressive symptoms, we used the following MDS items:

1) expresses sadness/anger/empty feelings over lost roles/status;
2) verbal expressions of distress;
3) tearfulness, emotional groaning, sighing;
4) sad or anxious mood;
5) motor agitation;
6) failure to eat or take medications;
7) pervasive health concerns;
8) recurrent thoughts of death.

These items have been documented to have adequate reliability[26] that does not differ by level of cognitive impairment.[27]

Nursing home staff also recorded as many as 18 different drugs received by each resident in the 7 days preceding the assessment. Information on the resident's drug therapy included brand/generic name, dosage, route and frequency of administration, and whether the order was standing or as needed.[19] We translated the drugs initially coded using national drug codes into therapeutic classes and subclasses using the Master Drug Data Base (MediSpan Inc., Indianapolis, IN).[19,21] We considered antidepressants, antipsychotics, hypnotics (antihistamines, benzodiazepines, barbiturates), anxiolytics (benzodiazepines and nonbenzodiazepines), and antidementia agents (cholinomimetics and nootropics) as possible pharmacologic treatments of the behavior problems of interest.

In addition to considering the pharmacologic management of behavior problems, we evaluated the use of physical restraints and behavior management programs. Physical restraints included bed rails, trunk restraints, limb restraints, and chairs used to prevent rising. Behavior management was defined as a plan of management other than medications or restraints based on an understanding of causal factors for a behavior problem identified by staff members. Written documentation of the structure of the program as well evidence of continuity of care was required.

Study population. From an initial population of 424,503 residents who were 65 years of age or older, we identified 42,216 people with an active clinical diagnosis of AD. We defined AD based on section K of the MDS, which is the listing of active clinical diagnoses, at their initial MDS assessment. The AD diagnosis is based on the physician's judgment using information obtained from the medical record, including the physical examination of the patient, medication and other treatment orders, and hospital discharge documentation.[23,28] The MDS diagnoses have excellent reliability and have been used in previous studies employing SAGE data.[21,26]

We excluded people with no information on gender (n = 161), people with delirium (n = 5,678), and people with other primary psychiatric illness other than dementia on admission (n = 8,010). The remaining 28,367 people constituted our sample, which included 7,080 men and 21,287 women.

Analysis. Using a cross-sectional study design, we compared distributions of sociodemographics, functional characteristics, and manifestations of behaviors associated with AD of men and women. Because our sample size was large, statistical significance was meaningless in this study. Therefore, we considered absolute differences of 5% to 10% as a clinically meaningful difference, depending on the variable of interest.[29] To evaluate whether the gender differences in manifestation of behaviors were confounded by level of cognitive impairment, we also performed these analyses stratified by CPS score.

We determined whether gender differences existed in the pharmacologic management of behavior problems in two ways. First, for the subgroups of people exhibiting each type of behavior, we compared the gender differences in the prevalence of use of each type of pharmacologic agent (i.e., antipsychotics or antidepressants). We also developed a multiple logistic regression model to quantify the effect of gender on receipt of psychoactive agents while simultaneously controlling for confounding factors including age, level of cognitive impairment, and behavior problems. All analyses were performed using SAS (version 6.12, Cary, NC).

Table 1 *Sociodemographic characteristics of patients with AD by gender (%)*

Characteristic	Men (n = 7,080)	Women (n = 21,287)
Age, y		
65–74	16	12
75–84	50	44
85+	34	45
Admitted from		
Hospital	53	59
Home	28	32
Race/ethnicity		
African American	8	7
Hispanic	2	2
American Indian	1	1
Asian/Pacific Islander	0.4	0.4
White	89	90
Cognitive function		
Mildly impaired	6	6
Moderately impaired	52	49
Severely impaired	42	45
Physical function		
Mildly dependent	8	10
Moderately dependent	36	33
Severely dependent	55	57

Results. Sociodemographic characteristics for the study sample are detailed in table 1. Women were older than men, but were otherwise comparable in race and ethnicity, severity of cognitive impairment, and physical functional impairment.

In this sample of predominantly moderate to severely impaired AD patients, overall behavior problems were common, particularly among men (59% versus 50%). Wandering, verbal and physical abusiveness, and inappropriate behavior were more common among men than women. This trend was seen across different levels of cognitive impairment, but was most evident among those with more severe cognitive impairment (table 2).

Among patients with severe cognitive impairment, the use of physical restraints for disturbed behavior was comparable by gender, with daily bed rails being used in 67% of men versus 68% of women, daily trunk restraints being used in 23% of men versus 20% of women, and daily chair restraints being used in 17% of men versus 15% of women. Similar usage between men and women but with lower prevalence was seen for those with less severe cognitive impairment.

Among patients with severe dementia, behavior management programs were used more frequently in men (58%) than women (50%). Comparable differences were present in those with moderate dementia (53% in men versus 45% in women) and in those with mild dementia (33% in men versus 26% in women).

The male gender predominance in behavior disturbances was not associated with a gender difference in psychiatric symptoms such as hallucinations, delusions, and depression. Nevertheless, there were gender differences in the use of psychoactive medications (table 3). Among the specific drug categories examined, and controlling for age and degree of cognitive impairment, men were more likely to receive antipsychotic drugs (OR = 1.35; 95% CI = 1.27 to 1.45) and less likely to be receiving antidepressants (OR = 0.88; 95% CI = 0.80 to 0.97) than women.

There was an apparent relationship of dementia severity to medication usage between the sexes. For the sample as a whole, men were more likely to receive psychoactive medications. This was seen particularly with the use of antipsychotics in patients with moderate to severe dementia. A reverse prescription pattern was seen in patients with mild dementia, with the exception of those with hallucinations or delusions, where men still had a higher frequency of antipsychotic prescriptions. The frequency of antipsychotic use in patients with any behavior problem rose from 17% to 40% in men and 24 to 30% in women

Table 2 *Presence of behavior problems stratified by gender and level of cognitive impairment (%)*

Impairment	Men (n = 7,080)	Women (n = 21,287)
Mild		
Wandering	7	4
Verbally abusive	7	7
Physically abusive	4	2
Inappropriate behavior	5	5
Hallucinations/delusions	0.5	1
Depression	21	22
Any behavior	33	30
Moderate		
Wandering	34	26
Verbally abusive	19	14
Physically abusive	18	12
Inappropriate behavior	20	15
Hallucinations/delusions	3	3
Depression	29	32
Any behavior	61	56
Severe		
Wandering	30	19
Verbally abusive	17	11
Physically abusive	25	14
Inappropriate behavior	24	20
Hallucinations/delusions	3	3
Depression	25	25
Any behavior	60	47
Total sample		
Any behavior	59	50

There were 128 people with no information on level of cognitive impairment.

comparing mild with severe dementia cases. The frequency of antidepressant use dropped from 15% to 10% in men and 17% to 10% in women comparing mild with severe dementia cases.

The greatest gender discrepancy in antipsychotic use within the different behavior categories was seen for hallucinations and delusions, ranging from 17% in severe to 67% in mild dementia cases.

Discussion. The findings of our study extend the observations made in outpatient studies of behavior problems in AD by demonstrating clearly that men are more likely than women to exhibit physically abusive and socially inappropriate behavior, particularly in the more advanced stages of dementia. Furthermore, we found that verbal abusiveness and wandering behavior were also more common in men. Some of the previous studies in outpatients did not find a significant gender difference for verbally aggressive behavior,[11,30] perhaps because of their smaller sample size or because the dementia severity of their subjects was milder.

Gender differences in behavior have not been examined in most of the studies performed in nursing homes. Wagner et al.[31] reported that men in special care units were more likely to sit inappropriately, have an unusual gait, and talk little. The types of gender-related behavioral disturbances reported in different series likely reflect not only differences in the instruments used to record the behaviors, but also differences in the severity of dementia of the subjects and differences in the environment.

Unlike outpatient studies, we found no gender difference in the frequency of psychiatric symptoms of hallucinations, delusions, or depression. Such psychiatric symptoms as hallucinations and delusions have been proposed to underlie physical[11,30] and verbal[30] aggression. Lyketsos et al.[7] found a relationship between depression and physical aggression. Consequently, it is possible that the substrate of aggressive behavior in the nursing home setting may consist of other factors besides psychiatric symptoms, such as social and environmental triggers.[32] Also, frontal lobe degeneration may disinhibit premorbid aggressive characteristics of male behavior in reaction to misperceived threat.

Another important finding in this study is the increased use of behavior management programs and major tranquilizers in the treatment of men with AD. The aggressive nature of abnormal behaviors in men may be perceived as being of greater threat to residents and staff, leading to more frequent use of behavior management programs and major tranquilizers. Arguing somewhat against this explanation is the observation that the use of physical restraints was similar between the sexes.

In a related study of tacrine use in the nursing home employing the SAGE database, men were more likely to receive this antidementia drug than were women, suggesting that physicians may be using the drug for reasons unrelated to its known efficacy, such as wandering and physical abusiveness.[33] We acknowledge that there are certain limitations to the interpretation of this data. Although it seems likely that antipsychotic agents may be used in men for their nonspecific tranquilizing effects on agitated behavior, information was not specifically collected regarding the indications for the medications. Also, the design of this study was cross-sectional. Therefore, one cannot draw conclusions about whether there could be a gender effect on the response to treatment that affects the prevalence of target symptoms. This should be an important area of future research.

The use of large databases such as SAGE have proved to be of great interest and relevance to neurologic health services research.[34] The large sample size of SAGE data gained from a wide geographic area lends itself to accurate description of actual clinical practices in the nursing home; however, the results of this study should be interpreted with caution. This was a cross-sectional study, in that we did

Table 3 *Treatment for behavior problems by gender (% males/% females) stratified by dementia severity*

Level of cognitive impairment and drug type	Wandering	Verbal abuse	Physical abuse	Inappropriate behavior	Hallucinations	Depression	Any behavior	No behavior
Mild								
Antipsychotics	38/32	20/26	27/34	15/36	100/33	12/22	17/24	14/11
Antidepressants	8/10	17/15	20/28	5/15	0/7	17/20	15/17	11/11
Moderate								
Antipsychotics	41/33	47/39	48/40	41/37	51/41	38/30	37/30	18/15
Antidepressants	11/13	10/14	10/14	10/12	10/17	14/16	11/13	7/9
Severe								
Antipsychotics	45/35	49/40	45/35	43/36	54/37	40/30	40/30	16/10
Antidepressants	11/10	9/10	10/10	10/10	18/13	10/12	10/10	4/4

not examine specific outcomes of these differences, and their clinical significance will require further research.

There are both pharmacoeconomic and health care implications of this particular study. Because of the increased time demands on nursing home staff to carry out behavior management programs and the increased use of psychoactive medications in men with AD, the cost of care for men is likely to be higher than for women. This increased cost may be balanced by the shortened life span of men with AD residing in nursing homes.[35,36] Another question that arises is whether care of men with advanced dementia carries a greater risk for physical harm to staff.

The increased use of antipsychotic drugs may also have an effect on morbidity of men with AD, because of potential side effects such as rigidity, immobility, falls, sedation, and hypotension. Longitudinal studies of behavior treatment outcomes should address this issue. It should be noted, however, that since the 1992–1996 time period of this study there has been more widespread use of selective serotonin reuptake inhibitors and atypical antipsychotics as well as donepezil. The advent of such agents with safer side effect profiles may limit the relevance of our observations to present-day drug prescription practices in the nursing home. Because the process of changing prescription habits in nursing homes is often slow and difficult, however, we believe this information is still applicable.

The social, developmental, hormonal, and pathologic interactions that produce differences in the clinical manifestations of AD between men and women are very complex.[37] Our understanding of these interactions is evolving. A great deal of work has been done in the study of gender differences in normal development and aging; however, studies of gender differences in AD are just beginning. A deeper understanding of these issues and how they influence drug treatment and response may ultimately lead to improvements in the health care of nursing home residents.

Appendix

The Systematic Assessment of Geriatric drug use via Epidemiology (SAGE) Study Group is composed of the following: *Steering Committee:* R. Bernabei, C. Gatsonis, L. Lipsitz, V. Mor; *Coordination:* G. Gambassi, K. Lapane; *Data Management:* J. Hiris, C. Brostrup-Jensen, B. Jesdale, S. Gonzalez; *Bio-statistics:* C. Gatsonis, J. Hogan, O. Intrator; *Participants:* M. Barbour, K. Berg, D. Gifford, J. Friedman, A. Hume, F. Landi, B. Ott, A. Sgadari, K. Steel, and A. Van Haaren.

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Lack of association of the α2-macroglobulin locus on chromosome 12 in AD

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Article abstract—*Objective:* Analysis of AD has revealed that the apolipoprotein E locus (*APOE*) cannot account for all of the genetic risk associated with AD. Whole genome scanning in AD families suggests that a chromosome 12 locus may contribute significantly to disease development. The α2-macroglobulin gene (*A2M*) has been suggested as a candidate locus for AD based on analysis of familial AD. *Method:* We determined, in 195 neuropathologically verified AD cases and 107 age-matched control subjects, the association of two common polymorphisms in *A2M* (a pentanucleotide deletion 5′ to the bait domain exon, and a valine-1000-isoleucine polymorphism in the thiolester site of the protein). *Results:* Evidence was observed for linkage disequilibrium between the deletion and Ile1000 polymorphisms. No evidence was observed for an association between the thiolester polymorphism and AD alone or when accounting for the *APOE*-ε4 allele. No alteration in the frequency of the bait domain deletion was observed, although a small excess (4%) of deletion homozygotes was found in the AD group, which were absent in the control population. *Conclusions:* The *A2M* deletion polymorphism at most accounts for a small fraction of the genetic contribution toward AD, and this is small compared with *APOE*. Furthermore, reverse transcriptase PCR of *A2M* RNA from the brains of patients homozygous for the deletion polymorphism showed that the bait domain exon still is present in the RNA. This suggests that the *A2M* deletion polymorphism may be nonfunctional and that the chromosome 12 AD locus is situated elsewhere. **Key words:** AD—Polymorphism—Apolipoprotein E—α2-Macroglobulin—Chromosome 12.

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The finding that the ε4 allele of the *APOE* gene is a major risk factor for late-onset sporadic and familial AD emphasizes the pivotal role played by genetics in AD development and progression.[1,2] The molecular basis of the action of apoE in the biology of AD is not clear, although several mechanisms of action have been investigated. ApoE is known to interact with β-amyloid and to promote fibrillary aggregation, with apoE ε4 showing a greater propensity for this property than apoE ε3.[3] This may underscore the suggestion of a dose-dependent increase in senile plaque deposition with the ε4 allele in AD.[4] ApoE ε3 also is known to interact with tau protein and can bind to dephosphorylated tau and prevent its phosphorylation, although apoE ε4 has reduced affinity.[5]

Because of the biologic effects of apoE, one focus of attention in its neurobiologic mechanism has been the interaction with other proteins, or proteins that may have similar effects. In particular, proteins that may show genetic polymorphisms have been studied. One of the major ligands for β-amyloid within the CNS has been identified as α2-macroglobulin (A2M), which also acts as a scavenger for the amyloid precursor protein (APP) and for β-amyloid.[6] Potentially, A2M has the capacity to behave toward β-amyloid in a similar manner to apoE-ε3, binding to β-amyloid and targeting it for degradation through the low-density lipoprotein receptor–like protein (LRP). In contrast to apoE-ε4, native A2M may have beneficial effects in preventing aggregation, possibly by interaction with the bait domain of the protein.[7] Functional polymorphisms in this protein may, therefore, produce differential effects on β-amyloid metabolism.

Two commonly occurring polymorphisms in A2M are known. One is a valine-to-isoleucine change at codon 1000 (Val1000Ile) in the thiolester site of the protein, which has been suggested to functionally alter the enzyme.[8] The second polymorphism within *A2M* causes a five base pair (bp) deletion adjacent to a consensus splice site in intron 17 (5′ to exon 18) of

See also pages 438 and 443

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the gene, which may cause exon skipping.[9] The *A2M* gene is located on chromosome 12, an area of the genome that has received attention in late-onset AD families after a whole genome scan.[10] This pericentromeric region on chromosome 12 was identified using a novel statistical approach in a family-based study. Using this method applied to the *A2M* deletion polymorphism, a highly significant association was observed between *A2M* and AD.[11] Additionally, this group also reports in a case–control study that the Val1000Ile polymorphism associates with AD.[12] We therefore analyzed a large series of neuropathologically confirmed cases with AD and control subjects for both polymorphisms in *A2M* to determine their effect in this population.

Methods. All case and control subjects were from the north of England and were part of a large, ongoing study. A total of 195 cases with AD (age range 57 to 98 years; mean 79.8 ± 0.6 SEM years; male–female ratio 1:1.9) fulfilled National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria, and all subjects had a neuropathologic verification of AD. A total of 107 nondemented control subjects (age range 60 to 100 years; mean age at death 79.0 ± 0.9 SEM years; male–female ratio 1:1.4) were studied; they had no history of neuropsychiatric disease and, on neuropathologic examination, showed age-related neuropathologic features.

Genomic DNA was isolated from frozen brain tissue of case and control subjects using standard proteinase K digestion followed by phenol/chloroform extraction. The *A2M* deletion polymorphism[9] was detected using a 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein–labeled forward primer. The reactions were performed in a final volume of 20 μL standard buffer containing 1.5 pmol of each primer, 0.2 U Taq polymerase (Pharmacia, UK), 200 μmol/L each deoxynucleotide, and 200 ng of DNA. Reaction conditions were an initial denaturation at 94 °C for 5 minutes followed by 35 cycles of annealing at 55 °C for 20 seconds, extension at 72 °C for 30 seconds, and denaturation at 94 °C for 30 seconds, and reactions were run on an ABI377 automated sequencer with 6-carboxy-X-rhodamine–labeled internal size standards (Perkin-Elmer, Warrington, UK). The *A2M* Val1000Ile polymorphism at the thiolester site[8] was detected using the following primers: forward 5′CTCTGCCATGCAAAACACAC3′, reverse 5′TGGAAA TCCAGTTGAATAACATT3′ (designed using primer 3 at the Whitehead Institute: http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi/).The reactions were performed in a final volume of 20 μL standard buffer containing 15 pmol of each primer, 0.5 U Taq polymerase (Pharmacia, Amersham, UK), 200 μmol/L each deoxynucleotide, and 200 ng of DNA. Reaction conditions were an initial denaturation at 94 °C for 5 minutes followed by 35 cycles of annealing at 58 °C for 20 seconds, extension at 72 °C for 30 seconds, and denaturation at 94 °C for 30 seconds. The resultant amplification products were then digested overnight at 37 °C with 2.5 U of *Nde*II enzyme (Boehringer, Lewes, UK) in the buffer supplied. After digestion, the PCR products were run on a 4% composite agarose (3% NuSieve: 1% standard agarose, Flowgen, Lichfield, UK) gel, and the bands visualized using ethidium bromide fluorescence. Bands of 129 and 88 denoted the presence of the Ile1000 variant, and a band of 217 (uncut PCR product), the Val1000 variant. Amplification of the *APOE* gene containing the allelic sites was performed on all case and control subjects by previously described methods.[13,14]

Linkage disequilibrium between the exon 18 deletion polymorphism and the exon 24 Val1000Ile polymorphism was assessed in an extended series of case subjects and non–age-matched controls homozygous for the deletion or insertion polymorphism only, and compared against expected frequencies using the χ^2 test.

Additionally, we designed primers located in exons 17 and 19 to amplify across the putatively deleted bait domain exon (exon 18) for use in reverse transcriptase (RT)-PCR to determine if the deletion polymorphism leads to exon skipping. RNA was extracted from frozen frontal cortex from AD patients homozygous for the deletion polymorphism and AD patients with the normal or heterozygous genotype using standard methods. Complementary DNA (cDNA) was primed using antisense primer 5′TTCCACTCGGTGATGGTGT3′ and synthesized using Moloney murine leukaemia virus (MMLV) RT (Promega, Southampton, UK), and one tenth of the reaction volume used as a template for PCR using 20 pmol antisense and sense primer (5′GGCTTAAAGGCATTCACCAA3′) with conditions of 40 cycles of annealing at 63 °C for 30 seconds, extension at 72 °C for 1 minute, and denaturation at 94 °C for 15 seconds. Products were visualized on 2% agarose gels. The band was excised from the gel and purified using Qiagen spin columns according to the manufacturer's instructions (Qiagen UK, Crawley, UK). PCR products were sequenced using Big Dye Terminator ready reaction (Perkin-Elmer) and reactions run on an ABI 377 automated sequencer. The genomic sequences of these cases in and around the insertion/deletion polymorphism also were sequenced and examined using unlabeled primers identical to those used for the gene scan analysis.[9]

Quantitative neuropathologic data were available on a subset of the AD patients. Tissue blocks from the formalin-fixed right hemisphere were taken from the frontal, temporal, occipital, and parietal cortex; hippocampus; and the cerebellum, and embedded in paraffin wax. Sections were cut at 10-μm thickness on a microtome. Routine histologic study was carried out on the four cortical regions, and senile plaques (SP) and neurofibrillary tangles (NFT) were stained with von Braunmühl's method and Palmgren's silver method, respectively, for AD diagnosis and quantification of SP and NFT.[15] Mean neocortical SP and NFT densities were determined by using a graticule and counting five fields as a strip running from the pial surface to the gray–white interface in each section, from each of the four cortical lobes. The resultant count per square millimeter was averaged for each lobe.

Statistical analysis of allele frequencies was by the χ^2 test with Yates' correction for small sample sizes when appropriate. Logistic regression analysis was performed using apoE, sex, and age as conditional variables when analyzing for an effect of *A2M* genotypes. Genotype data were analyzed for an effect on age at onset of AD and duration of illness where accurate data were available. Clinical and neuropathologic data were analyzed by a General Linear Model or analysis of variance with post hoc *t*-tests where appropriate.

Table 1 *Allele frequency of the α2-macroglobulin (A2M) deletion (D)/insertion (I) polymorphism in AD*

Subjects	Age, y, mean ± SEM	*A2M* frequencies			
		A2M-D	*D/D*	*D/I*	*I/I*
Control (103)	78.6 ± 0.61	0.14	0.00 (0)	0.27 (28)	0.73 (75)
AD (195)	79.3 ± 0.61	0.18	0.04 (8)*	0.28 (54)	0.68 (133)

Numbers in parentheses indicate the no. of cases studied.
* $p < 0.05$ (chi-square test).

Results. The *A2M* deletion polymorphism (table 1) was not found to be altered between the AD and control cases, with a slight excess of the deletion allele being found in the total AD group ($\chi^2 = 1.86$, $p = 0.17$). Analysis of the data showed that this effect was caused by an excess of deletion homozygotes in the AD population, with eight being present in the AD group and none in the control group ($\chi^2 = 4.34$, $p = 0.037$). The frequency of insertion homozygotes ($\chi^2 = 0.68$, $p = 0.41$) and deletion heterozygotes ($\chi^2 = 0.009$, $p = 0.92$) was not altered between groups. The finding of an increased frequency of the deletion heterozygotes was not enhanced by the presence of the *APOE*-ϵ4 allele overall ($\chi^2 = 3.23$, $p = 0.072$). The frequency of deletion homozygotes was not enhanced in *APOE*-ϵ4 homozygotes ($\chi^2 = 3.74$, $p = 0.053$) or in the absence of the ϵ4 allele ($\chi^2 = 1.83$, $p = 0.19$) (table 2). Logistic regression analysis did not demonstrate an effect of the deletion polymorphism or gender on the development of AD (del Wald = 0.6 df_1, $p = 0.45$; gender Wald = 0.8 df_1, $p = 0.36$), although *APOE* genotype (Wald = 21.5 df_1, $p < 0.0001$) was associated with an effect. Stratification of the groups into early onset AD (onset when younger than 65 years) and late-onset AD (onset when older than 65 years) did not reveal any further significant effects (data not shown).

Results of *A2M* Val1000Ile polymorphism analysis are shown in table 3. The frequency of the Val allele was found to be unaltered between control and AD populations (AD versus control: $\chi^2 = 0.092$, $p = 0.762$), and no changes were found in the frequency of Val homozygotes, Val heterozygotes, and Ile homozygotes between the case and control subjects (data not shown). No effect was found for the Val1000Ile polymorphism when stratifying for the presence of the *APOE*-ϵ4 allele (data not shown) or when applied to a logistic regression model (Wald = 0.003, $p = 0.96$).

The *A2M* polymorphisms were assessed for their influence on the age at onset and duration of dementia alone and in the presence and absence of the ϵ4 allele. No effect was found for either the deletion (table 4) or Val1000Ile (data not shown) polymorphisms on age at onset or duration of disease, although a minor trend toward a later age at onset of disease of the deletion homozygotes was observed ($p = 0.08$, two-way *t*-test; see table 4). A trend toward increased senile plaque densities was observed when comparing the deletion homozygotes to the deletion heterozygotes and insertion homozygotes (GLM F = 4.37, $p = 0.013$) (figure 1), although post hoc testing failed to identify this (data not shown). No effect was observed for either of the *A2M* polymorphisms on neurofibrillary tangle densities (figure 2) (GLM F = 0.42, $p = 0.654$).

We calculated expected frequencies of *A2M* haplotypes in homozygotes and related this to the findings in the control and AD groups combined (data not shown). The two *A2M* polymorphisms were found to be in linkage disequilibrium with each other ($\chi^2 = 19.25$, $df = 5$, $p = 0.002$), with the deletion allele being strongly associated with the Ile1000 polymorphism ($\chi^2 = 11.56$, $df = 2$, $p = 0.003$), although the Val1000 polymorphism was not as strongly linked to the insertion allele ($\chi^2 = 7.69$, $df = 2$, $p = 0.02$). All eight of the deletion homozygous AD patients were homozygous for the Ile1000 allele.

RT-PCR of the *A2M* mRNA flanking the bait domain in patients homozygous for the deletion polymorphism failed to identify any band shift in the products compared with mRNA extracted from the brains of insert homozygotes with a 278-bp band being produced in all patients (data not shown). Sequencing of the RT-PCR products from the deletion or insert homozygotes revealed no alteration in sequence from that expected, despite the DNA sequence showing loss of 5 bp adjacent to the exon in deletion ho-

Table 2 *Stratification of α2-macroglobulin (*A2M*) deletion allele frequencies in AD according to presence of the* APOE-ϵ4 *allele*

Subjects	*A2M* deletion allele frequency		
	APOE-ϵ4-ve	1 *APOE*-ϵ4	2 *APOE*-ϵ4
Control	0.14 (21)	0.13 (7)	0.0 (0)
AD	0.15 (24)	0.17 (26)	0.27 (17)*

Numbers in parentheses indicate the no. of cases carrying the deletion allele studied (insert allele homozygotes not included).
* $p < 0.1 > 0.05$ (χ^2 test, compared with 1 *APOE*-ϵ4 control group).

Table 3 *Allele frequency of the α2-macroglobulin (*A2M*) valine 1000 isoleucine polymorphism alleles in AD*

Subjects	Age, y, mean ± SEM	*A2M* Val1000Ile frequencies			
		A2M-Val	*Val/Val*	*Val/Ile*	*Ile/Ile*
Control (107)	78.5 ± 0.77	0.39	0.14 (15)	0.50 (53)	0.36 (39)
AD (191)	79.0 ± 0.61	0.40	0.17 (32)	0.47 (89)	0.37 (70)

Numbers in parentheses indicate the no. of cases studied.

Table 4 *Effect of the α2-macroglobulin (*A2M*) Ins/Del allele on age at onset and duration of dementia, and age at death in AD*

Allele	Onset	Duration	Death
Del/Del (4)	77.5 ± 3.0*	5.6 ± 0.2	80.1 ± 3.6 (8)
Del/Ins (20)	71.6 ± 2.4	5.2 ± 0.8	79.4 ± 1.1 (54)
Ins/Ins (44)	69.9 ± 1.6	6.0 ± 1.6	79.2 ± 0.8 (133)

Values are age in y ± SEM. Numbers in parentheses indicate no. of cases analyzed where accurate data were available.

* $p < 0.1 > 0.05$, two-sample *t*-test.

mozygotes (data not shown). A difference in the published cDNA sequence was, however, identified at nucleotide 2293 of the published sequence (Database accession no. M11313), causing a G-to-T change, although without any change at the predicted protein level (GG**G**→GG**T**, glycine→glycine), this being present in all cases sequenced.

Discussion. Previously, an association with the *A2M* locus on chromosome 12 in AD has been described.[11] These findings were based on a family-based approach using affected individuals and siblings older than the affected case as control subjects, and applying the sibship-disequilibrium test approach.[16] In this study, we used a case–control analysis with age-matched individuals who were assessed as being free of disease at time of death compared with a neuropathologically verified AD population. In this population, there is no association with the *A2M* gene and AD other than a small excess of deletion homozygotes. The ages of the case and control subjects in the previous study were not given, and neuropathologic verification of the cases was not stated. One possibility is that there may be a bias in disease classification because non-AD patients may have been included in the previous studies.[11] An age effect also may be present, and again, this may lead to sample bias if there is differential survival associated with *A2M* genotypes, the previous study having used controls in the sibships who were older than the probands.[11] Our findings suggest only a weak effect, and several differences are apparent. Unlike the original findings,[11] which identified a dose-independent effect of the *A2M* deletion polymorphism, in the current study, the effect appears to be, at most, associated with the deletion homozygotes. This effect is, however, small, as it can be attributable to less than 5% of the AD population. This effect may result from the deletion homozygotes having a family history of AD, although we have not been able to ascertain any such individuals from study of the case notes. The possibility exists, therefore, that the *A2M* deletion polymorphism is a risk factor for only familial forms of AD. Whereas the small excess of deletion homozygotes has been noted in one report using a Canadian sample (AD 3.4% versus control 0.6%), the same authors failed to find any change in a US sample (AD 2.7% versus control 3.2%), and in neither sample was there any association between *A2M* and AD.[17] Two other reports[18,19] also have not shown an excess of deletion homozygotes, and the current results may indicate a type 1 error. Whereas previous studies identified an effect independent of the *APOE*-ϵ4 allele,[11] in this population, there appears to be some association of the deletion homozygotes with *APOE*-ϵ4 homozygotes.[1,2] The finding of an increased

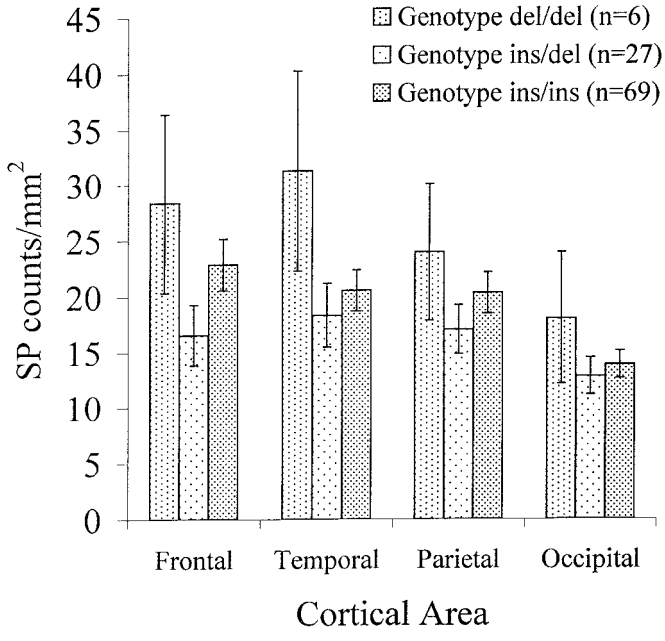

Figure 1. Senile plaque (SP) densities in AD stratified according to the A2M *deletion/insertion polymorphism. SP density was determined on von Braunmuhl–stained sections from the areas indicated on a random subset of the patients used in the main study. An increased level of SPs was found overall in deletion homozygotes (GLM,* $p < 0.05$*), although this was not evident on post hoc testing.*

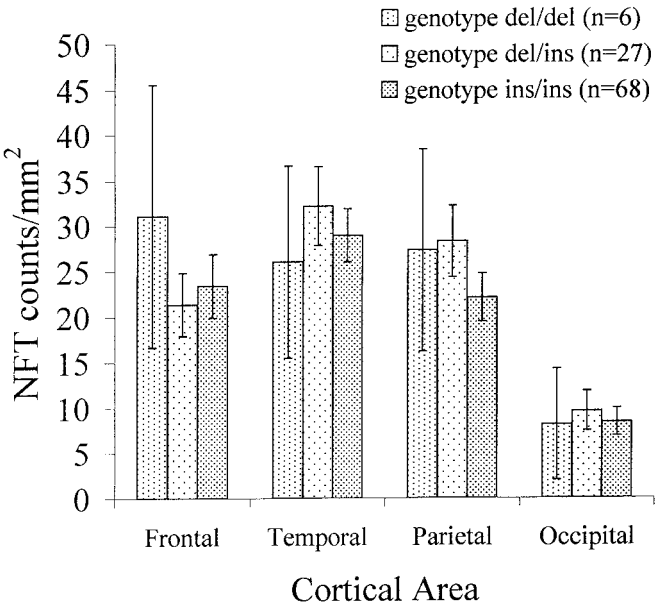

Figure 2. Neurofibrillary tangle (NFT) densities in AD stratified according to the A2M *deletion/insertion polymorphism. Tangle density was determined on Palmgren-stained sections on a random subset of the patients used in the main study from the areas indicated.*

senile plaque burden in deletion homozygotes may be an indication of effect, as the *APOE*-ϵ4 allele has been suggested to influence senile plaque deposition in a dose-dependent manner.[4] However, the deletion homozygotes showed a later age at onset of disease, suggesting a slowing of the disease process, although the numbers where accurate data were available was small, and it is not possible to state this with any certainty (see table 4). This contrasts with the *APOE*-ϵ4 allele, where homozygotes generally have a lower age at onset of disease.[1,2] Overall, there appears to be no convincing evidence that deletion homozygotes are more susceptible to AD on the basis of this and other[17-19] data.

We have attempted to determine if the *A2M* deletion polymorphism has a functional effect by determining if there is exon skipping of the *A2M* mRNA in deletion homozygotes caused by the pentanucleotide deletion being in close proximity to the splice acceptor junction of the bait domain exon (exon 18).[9] No size or sequence differences were found between mRNA from deletion homozygotes compared with wild-type mRNA, suggesting that the deletion polymorphism is not functional at the RNA/protein level. It was not possible to analyze the A2M protein itself because the predicted size change was below the resolving power of our system (wild-type tetramer molecular weight $\approx$ 750,000, predicted deletion $\approx$ 730,000). This finding suggests that even if there is an association of the *A2M* deletion polymorphism with AD, it is not because of the polymorphism itself but possibly because of a functional polymorphism within or near to *A2M*.

The suggestion has been made that there are additional genetic loci that account for the genetic susceptibility to AD[20] and that loci may be present on chromosomes 4, 6, 12, and 20.[10] We have ruled out the possibility that the common thiolester polymorphism may be the locus responsible, as no association was found in this study in contrast to a previous study.[12] This polymorphism in *A2M*, however, appears to be in linkage disequilibrium with the deletion polymorphism. Our study also contrasts in that the frequency of the valine allele is higher in our control population (0.39 versus 0.32). One possibility is that there is ethnic variation in *A2M* allele frequencies, with our north-of-England population being of a different racial background from the mixed ethnic background found in the previous study.[11] Another possibility is that there is a bias in our control population because of differential survival being associated with certain *A2M* genotypes, although we are unaware of any data regarding this. The cohorts used previously[11] may be of a different age than our patients (but this information was not given), although we have not observed any differences in allele frequencies in a younger cohort of controls (unpublished observation).

The *A2M* gene is orientated on chromosome 12 such that the 5′ end is distal to the centromere.[21] A report suggests that the chromosome 12 locus may be situated between the markers D12S1057 and D12S309 located at the telomeric end of the short arm of chromosome 12, at the 5′ end of *A2M*.[10] Our results suggest that a locus other than the deletion polymorphism or the thiolester polymorphism is responsible for AD susceptibility, and is elsewhere on chromosome 12 and possibly not with the *A2M* gene. The suggestion has been made that the *LRP* gene on the long arm of chromosome 12 may be the AD locus[22,23]; however, linkage analysis does not indicate the long arm of chromosome 12 to be the locus associated with AD.[10]

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α2 Macroglobulin and the risk of Alzheimer's disease

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Article abstract—*Background:* α2 Macroglobulin is a panproteinase inhibitor that is found immunohistochemically in neuritic plaques, a requisite neuropathologic feature of AD. Recently, a pentanucleotide deletion near the 5′ end of the "bait region" of the α2 macroglobulin (*A2M*) gene was reported to be associated with AD in a large cohort of sibpairs, in which the mutation conferred a similar odds ratio with AD as the *APOE*-ϵ4 allele for carriers of at least one copy of the *A2M* gene (Mantel–Haenszel odds ratio, 3.56). *Methods:* We studied three independent association samples of AD patients (n = 309) with an age range of 50 to 94 years and representative controls (n = 281) to characterize the allele frequency of the pentanucleotide deletion in this cohort. We detected the mutation near the 5′ splice site of exon 18 using standard PCR and restriction fragment length polymorphism methods. The results were adjusted for age, gender, education, and *APOE* polymorphism. *Results:* We found that the *A2M* gene polymorphism conferred an increased risk for AD, with an estimated Mantel–Haenszel ratio of 1.5 (95% CI 1.1 to 2.2; $p = 0.025$). There was no age- or gender-dependent increase in *A2M* gene allele frequencies in AD patients compared with controls. The combined sample showed the expected association between AD and *APOE*-ϵ4. In one of our three samples there was an interaction between the *A2M* and *APOE*-ϵ4 genes, but the other two samples showed no interaction between the two risk factors. *Conclusions:* Our data support an association between the *A2M* gene and AD. This association is less pronounced, however, in our cohort than in the previously reported sample of sibpairs. **Key words:** AD—Polymorphism—Apolipoprotein E—α2-Macroglobulin.

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Genetic studies on AD have identified several genes that cause or predispose carriers to AD. Mutations in the genes encoding the β-amyloid precursor proteins presenilin-1 and presenilin-2 cause relatively rare early-onset forms of AD.[1] Susceptibility polymorphisms associated with the risk of the more common late-onset variants of AD have been established for the *APOE* gene[2] and suggested for α1-antichymotrypsin (ACT),[3] the LDL receptor–related protein (LRP),[4] an intron of presenilin-1,[5] and the *APOE*-promoter region.[6] Although the *APOE*-ϵ4 allele is a significant risk factor for AD, it is neither necessary nor sufficient to cause disease, suggesting the presence of additional genetic and perhaps nongenetic susceptibility factors, which either alone or in concert with *APOE*-ϵ4 alter the risk as well as the age at onset of AD. In addition, linkage studies have presented statistical evidence for several putative new loci including one on

See also pages 433 and 443

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chromosome 12.[7] The *LRP* gene as well as the α2 macroglobulin (*A2M*) gene are located within the putative 30 cM region on chromosome 12.

Recently, an intronic pentanucleotide deletion polymorphism near the 5′ end of exon 18 of the *A2M* gene was reported to confer a higher risk of AD in a sample of affected sibpairs.[8] Furthermore, a polymorphism in the translated region of exon 24 of the A2M gene ($Val^{1000} \rightarrow Ile^{1000}$) has been associated with an increased risk for AD.[9] The α2 macroglobulin protein (A2M) is a high molecular weight plasma panprotease inhibitor (720 kd) with a large variety of postulated functions.[10] In the brain, several lines of evidence suggest that *A2M* plays an important role in neuronal repair following injury.[11,12] In AD, A2M has been localized immunohistochemically by several laboratories to neuritic plaques.[13] We, and others, have recently identified A2M as a high-affinity binding protein for the β-amyloid (Aβ) peptides and proposed that the binding of A2M to Aβ may represent a clearance or sequestration mechanism for Aβ, affecting the amount of amyloid deposition.[14-18] Moreover, in vitro, A2M attenuates the propensity of Aβ peptides to form fibrils and to be neurotoxic. Polymorphisms in *A2M* may therefore be less efficient in the clearance of Aβ, thereby promoting aggregation, fibril formation, and deposition of Aβ and increasing the number of plaques, which may be responsible for the pathogenesis of AD.

We investigated the association of *A2M* in a case-control cohort of patients with AD and nondemented elderly. DNA from AD patients recruited from three different centers was genotyped for *A2M* and *APOE* alleles and compared with an age- and ethnicity-matched control group.

Methods. *Study population.* In our initial analysis we enrolled 316 individuals, who were consecutively recruited in outpatient clinics for cognitive disorders from the Indiana AD Center, Indiana University Medical School, Indianapolis, Indiana (IU), and the Department of Psychiatry, Technische Universität, München, Germany (MU).

The IU group consisted of 83 white American patients with AD (mean age, 72.7 ± 9.5 years; range, 50 to 94 years; 44 women, 39 men) and 83 unrelated age-matched control individuals (mean age, 72.9 ± 9.4 years; range, 51 to 94 years; 43 woman, 40 men). All IU AD patients had complete medical, psychiatric, neurologic, and neuropsychological evaluations (e.g., Consortium to Establish a Registry for Alzheimer's Disease[19] neuropsychological test battery), as well as laboratory studies to exclude reversible causes of dementia. The patients met National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[20] criteria for probable AD or International Classification of Diseases (ICD)–10 criteria[21] at entry into the study. Control individuals were either unrelated healthy spouses of AD patients or healthy volunteers recruited by advertisement, who had no family history of dementia. All control individuals had Folstein Mini-Mental State Examination (MMSE)[22] scores ≥ 26.[23]

The MU group consisted of 75 white German AD patients (mean age, 71.8 ± 10.5 years; range, 50 to 95 years) and 75 unrelated age-matched control individuals (mean age, 72.7 ± 10.1 years; range, 50 to 95 years; 45 female, 30 male). The MU control individuals were either unrelated healthy spouses of AD patients or volunteers who had no family history or evidence of neurologic disease with potential to affect cognition and who attained a score of 28 or higher on the MMSE (German version).[24,25] Each AD patient had a clinical examination, including neuropsychological testing, to document deficits in cognition and activities of daily living, as well as laboratory studies and a neurologic examination to exclude reversible causes of dementia. All AD patients met the diagnostic research criteria for dementia of ICD-10.

We also included a third group from the Department of Psychiatry and Behavioral Sciences, Stanford University Medical Center, Stanford, California (SU).[26] The SU group consisted of 151 unrelated white American AD patients (mean age, 74.8 ± 7.6 years; range, 51 to 90 years; 72 female, 79 male) and 123 unrelated white control individuals (mean age, 68.2 ± 8.1 years; range, 50 to 87 years; 83 women, 40 men). This SU control group differed from the other two groups in that the control individuals were not age-matched to AD patients. SU AD patients were recruited from the Stanford/VA Palo Alto Alzheimer's Center, which provides diagnostic and treatment services to the San Francisco peninsula and South Bay regions. Study patients included those individuals who consented to donate blood for genetic analysis. The SU control individuals were either unrelated healthy spouses of AD patients at the center or community-dwelling volunteers who were recruited by newspaper advertisements and by word of mouth. Thus, control individuals were drawn from the same geographic region as AD patients. All SU AD individuals had complete medical, psychiatric, neurologic, and neuropsychological evaluations and met NINCDS-ADRDA criteria for probable AD at entry into the study cohort. In making the diagnosis of probable AD at SU, the following cognitive and functional tests were performed: MMSE, the AD Assessment Scale cognitive subscale, the Boston Naming Test, Category Fluency test, Trail Making A and B tests, Wechsler Adult Intelligence Scale (WAIS)–R Block Design, Wechsler Memory Scale (WMS)–R Logical Memory I and II, the Blessed–Roth Dementia Rating Scale, and the Global Deterioration Scale. SU control individuals who were spouses of AD patients were tested with the MMSE to rule out cognitive impairment. All spousal controls had MMSE scores above the 25th percentile for age, based on the criteria of Bleecker et al.[23] All SU community-dwelling volunteer control individuals had MMSE scores greater than or equal to 25. None of the SU control individuals reported a history of neurologic disease.

Only patients and control individuals who were older than 50 years were included. For some AD patients, informants reported a family history of cognitive impairment, whereas others had no reported family history. All individuals (or, for significantly cognitively impaired individuals, their legal guardians or caregivers with power of attorney) had given written informed consent.

Genotyping of A2M and *APOE*. Genomic DNA was extracted from blood or buccal epithelial swab samples according to standard protocols. Genotyping of *APOE* and of *A2M* was performed by a PCR-based assay as described

previously.[27,28] Restriction fragments were resolved on 4% (*APOE*) or 2% (*A2M*) agarose gels with 1 mg/mL ethidium bromide for 20 minutes at 200 V and directly detected under ultraviolet light.

Statistical analysis. Age and gender. The difference in age between AD and control individuals was assessed by a two-sample *t*-test in the SU sample and by a paired *t*-test in IU and MU samples. The difference in gender distribution was examined by a χ^2 test in SU patients and by McNemar's χ^2 test in the IU and MU samples. Note that test statistics were employed that matched the study design in each center. Differences in age (gender distribution) among the three *A2M* genotype groups were assessed by Mantel–Haenszel analysis of variance test.

Odds ratios. Patients and controls were subdivided into age younger than 55 years, between 55 and 64 years, between 65 and 74 years, between 75 and 84 years, and 85 years or older to control for the effect of age. To combine patients from the three centers together, we first assessed the effect of the individual matching employed in the IU and MU centers. Matching has two effects: first, groups are balanced with respect to the matching variable; and second, if the matching is efficient, then the outcomes from matched pairs are correlated.[29] If only the first effect occurs, then an unmatched analysis is efficient. For the IU and MU samples individually and for the two centers combined, age group–stratified Mantel–Haenszel odds ratios (ORs) and McNemar's ORs were estimated and compared. Differences in magnitude of the ORs were less than 0.1 for the *A2M* outcome and 1.2 for the *APOE*-ϵ4/ϵ4 outcome. In addition, kappa statistics were estimated for each outcome to assess the concordance of outcomes in matched pairs. The maximum kappa statistic estimated was 0.07, and in all cases the asymptotic 95% CI for kappa contained 0. We concluded that there was no effect of the individual matching beyond that of balancing the AD and control groups with respect to age. Thus, all further analysis of ORs was conducted by center– and age group–stratified Mantel–Haenszel ORs, CIs, and hypothesis tests. To assess the appropriateness of combining all three centers, the homogeneity of the ORs across centers was assessed by the Breslow–Day homogeneity of OR test.[30] The interaction between *APOE* and of *A2M* was assessed by logistic regression[30] in the SU cohort and for the sample overall, and by conditional logistic regression in IU and MU samples.[30]

Results. The distributions of *A2M* and *APOE*-ϵ4 polymorphisms in AD and control individuals are presented in table 1. The genotype distributions for both genes were in Hardy–Weinberg equilibrium in both AD and control individuals. Our data confirm previous observations on the association of *APOE*-ϵ4 and AD.[2,31] The estimated ORs for being affected as a function of carrying at least one *A2M* allele were 1.6 for the IU group, 1.8 for the MU group, and 1.3 for the SU group, compared with having no *A2M* allele (table 2). The *p* values in each group if tested individually did not achieve statistical significance, because of the limited number of individuals in each stratum; however, if all groups were combined, the estimated OR was statistically significant for all ages (OR 1.5; 95% CI 1.1 to 2.8; $p = 0.026$), and there was no evidence of a lack of homogeneity of ORs across centers ($p = 0.692$). In older patients (>60 years) the results were similar (OR 1.5; 95% CI 1.0 to 2.3; $p = 0.035$). Because age is a major risk factor, we investigated whether there was a change in allele distribution depending on the individual's age. AD and control groups were stratified into subgroups of older than 65 years, older than 75 years, and older than 85 years. In AD patients, we found no association between *A2M* allele frequency and age ($p = 0.129$). Likewise, stratification by gender did not influence the association of *A2M* and AD ($p = 0.776$).

Table 1 *Number and frequency of α2 macroglobulin gene (*A2M*) alleles (%) in the combined cohort of AD patients and in the combined cohort of controls*

	Controls			AD		
Gene	1/1	1/2	2/2	1/1	1/2	2/2
A2M						
n	210	62	9	201	101	7
Genotype frequency, %	74.7	22.1	3.2	65.0	32.7	2.3
APOE						
ϵ2	7.5	4.7	5.6	3.3	6.8	8.3
ϵ3	81.2	80	77.8	64.7	52.9	58.3
ϵ4	11.3	15.3	16.6	32.0	40.3	33.3

Next, we examined the joint distribution of *A2M* and *APOE*-ϵ4 in the entire data set. Our data are consistent with previous observations in which increased allele frequencies of the *APOE*-ϵ4 allele is associated with AD. Logistic regression analyses adjusted for the effect of *APOE*-ϵ4 on risk for AD for the three different centers are shown in table 3. There was no statistically significant change in the magnitude of risk in the MU and SU group if both genes were included; in the IU group, however, we observed an interaction at $p = 0.032$ (table 3).

Discussion. Our data support a report suggesting that a deletion in the 5′ end of exon 18 of the *A2M* gene increases the risk of AD.[8] In a large group of AD patients and controls that included 590 individuals, we found a statistically significant increase in the risk of AD as a function of carrying at least one *A2M* allele. The observed OR, however, was lower than that described using an affected sibpair sample. Although the OR in patients carrying the *A2M* allele was previously estimated[8] to be similar to the risk for those with the *APOE*-ϵ4 allele, we found an OR of less than one-half this value. This OR is similar to those calculated for other polymorphisms investigated as risk factors for AD, such as ACT and LRP.[3,4]

Another group has recently reported a weak but statistically significant association of *A2M* and AD in

Table 2 *Mantel-Haenszel odds ratio (OR) estimates for the effect of α2 macroglobulin gene (*A2M*),* APOE-ϵ4/ϵ4*, and* APOE-ϵ4 *on risk for AD for the entire sample*

A2M carrier			APOE-ϵ4/ϵ4			APOE-ϵ4		
OR	(95% CI)	*p* Value	OR	(95% CI)	*p* Value	OR	(95% CI)	*p* Value
1.5	(1.1–2.2)	0.025	9.0	(3.8–21.6)	0.001	4.1	(2.9–5.8)	0.001

Table 3 *Odds ratios and a test for* APOE-ε4$^+$–α2 *macroglobulin (*A2M*) interaction on the AD risk for the three centers*

Location	*A2M* carrier		*p* Value
	APOE ε4$^+$	APOE ε4$^-$	
IU	3.04 (0.92–10.07)	0.43 (0.12–1.5)	0.032
MU	1.21 (0.43–3.39)	1.74 (0.67–4.48)	0.461
SU	0.78 (0.12–1.50)	1.38 (0.63–3.04)	0.393

Values are OR (95% CI). Conditional logistic regression was done in Indiana University (IU) and Munich University (MU) and logistic regression in Stanford University (SU).

a subgroup of their cohort,[32] but two other recent studies did not detect an association of *A2M* and AD.[33,34] There are several possible explanations for the apparent difference in observed ORs in our study compared with that of Blacker et al.,[8] including population stratification, differing sampling methods, differences in diagnostic criteria, and other factors.[35] Additionally, because a higher OR for *A2M* was observed in the sibpairs examined by Blaker et al.[8] than in our sample, which included sporadic AD, our results imply that this variant may be a stronger risk factor in cases in which a familial clustering of AD exists. In AD patients, there is accumulating evidence suggesting that multiple genes contribute to disease risk or susceptibility. We found no evidence for an interaction between *APOE*-ε4, an established susceptibility marker, and *A2M* in the MU and SU groups. However, in the IU group, the risk resulting from *A2M* was stronger among *APOE*-ε4 carriers than among noncarriers. These results are at variance with those of Blacker et al.,[8] who reported a stronger *A2M* effect among *APOE*-ε4 noncarriers.

It is interesting that many of the described genetic polymorphisms that have been associated with AD are related to LRP or LRP ligands. We and others have postulated that apoE and A2M may serve to bind Aβ peptides and either enhance or retard clearance via an LRP-dependent mechanism. It is tempting to speculate that A2M polymorphisms associated with AD may be defective in this clearance function. It is not clear, however, how this polymorphism affects the primary structure and function of A2M, because the deletion is located in a portion of the untranslated sequence. Further characterization of the A2M protein will clarify this issue, and studies are underway.

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Neuro*Images*

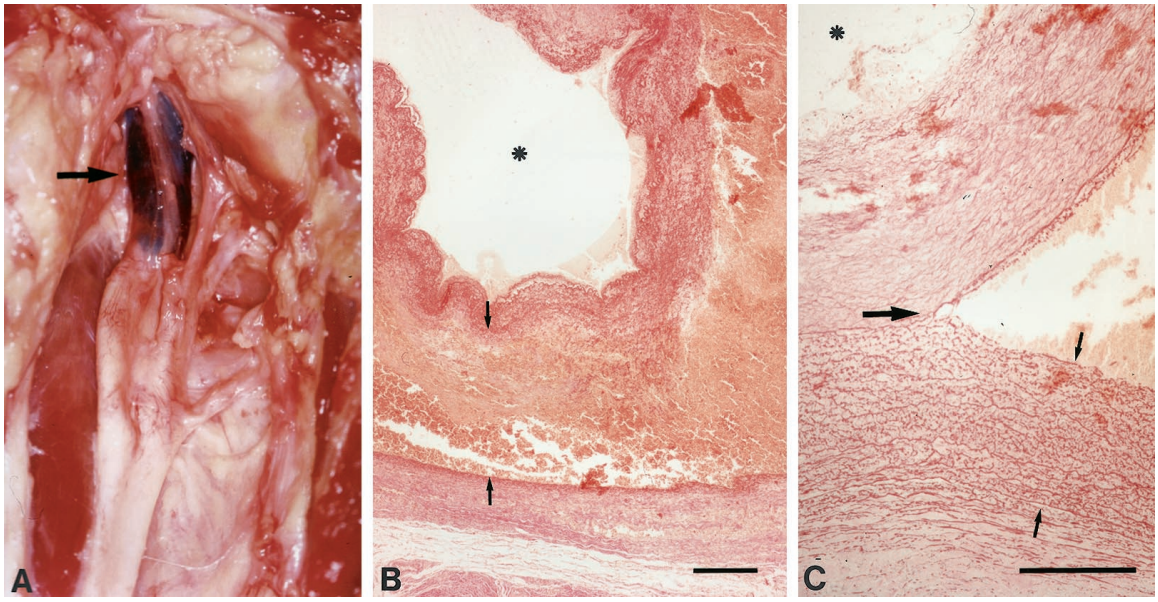

Figure. (A) Postmortem exposition of intramural hematoma of the right internal carotid artery above the bifurcation (arrow). (B) Cross-section of the dissected artery shows subintimal and intramedial hematoma (small arrows). (C) Longitudinal section with zipper-like separation within the arterial wall (large arrow) corresponding to the false lumen and myxoid degeneration of the media (small arrows). Asterisk = true lumen; Elastica van Gieson, scale bar = 0.25 mm.

Internal carotid artery dissection

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A 61-year-old patient was admitted with spontaneous acute holocephalic headache and left hemiplegia. Results of initial cranial CT scan were normal, but CT angiography revealed an occlusion of the right internal carotid artery (ICA) about 1.5 cm above the carotid bifurcation with typical string sign indicative for arterial dissection. The next day, transcranial Doppler detected a distal occlusion of the M1 segment of the right middle cerebral artery (MCA). Three days later, cranial CT showed complete infarction of the right anterior cerebral artery and MCA territory with severe midline shift. Despite immediate decompressive hemicraniectomy, the patient died 3 days later. Postmortem examination confirmed ICA dissection about 1.5 cm above the carotid bifurcation and showed fibromuscular dysplasia (FMD) in the entire extension of both ICAs as the underlying disease.

In community-based studies, the incidence rate of nontraumatic cervical artery dissections (CAD) is about 2.9 per 100,000.[1] CAD accounts for about 2% of patients with stroke and 10% of stroke patients under 50. In most cases the pathogenesis of nontraumatic CAD is unknown. FMD is one of the known pre-existing diseases and is found in up to 20% of patients with CAD.[2] FMD is an idiopathic, systemic, multifocal vascular disease of unknown origin that affects cephalic arteries in 25% of reported cases.

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α2-Macroglobulin polymorphism is not associated with AD or AD-type neuropathology in the Japanese

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Article abstract—*Background:* α2-Macroglobulin (A2M) forms the complex with amyloid β-protein (Aβ) and is associated with degradation of Aβ. It has been reported that the A2M gene (*A2M*) exon 18 splice acceptor deletion polymorphism influences the development of AD, regardless of apolipoprotein E-ε4 (*APOE*-ε4) status. *Objective:* To determine the effect of *A2M* polymorphism on the development of AD and AD-type neuropathologic changes. *Methods:* The authors examined the *A2M* and *APOE* genotypes, the densities of the senile plaques (SPs), SPs with dystrophic neurites (NPs), and neurofibrillary tangles (NFTs) in the brains of 62 postmortem-confirmed sporadic AD and 90 nondemented patients from an autopsy series of elderly Japanese subjects. *Results:* There was no association of the *A2M* polymorphism with AD, age at onset, or duration of illness in AD. The *A2M* polymorphism was not associated with the SPs, NPs, or NFTs in AD or nondemented patients. The results remained insignificant, even when the *A2M* genotype groups were divided into subgroups by *APOE*-ε4 status. *Conclusion:* The *A2M* polymorphism does not affect the development of sporadic AD or formation of AD-type neuropathologic changes. **Key words:** AD—Apolipoprotein E—Neuropathologic change—Risk factor—Japanese—α2-Macroglobulin—Polymorphism—Senile plaque—Neurofibrillary tangle.

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Sporadic AD is a polygenic disease characterized by progressive dementia. The major genetic risk factor for sporadic AD is apolipoprotein E-ε4 (*APOE*-ε4) allele, which influences the development of AD in many ethnic populations. Thirty to forty percent of patients with sporadic AD do not carry the *APOE*-ε4 allele.[1] In the Japanese population, the frequency of the *APOE*-ε4 allele is lower, and *APOE*-ε4 has less of an effect on the development of AD compared with other ethnic populations.[2,3] This suggests the presence of other genes increasing the risk of AD, especially in the Japanese population. Studies suggest that the presenilin 1 gene,[4] α1-antichymotrypsin gene,[5] butyrylcholinesterase gene,[6] and low-density lipoprotein receptor-related protein (LRP) gene[7] are genetically associated with the development of sporadic AD, but these relationships are unclear.

A new gene that may be associated with the development of sporadic AD is the α2-macroglobulin (A2M) gene (*A2M*). A2M is a major protease inhibitor that binds to amyloid-β protein (Aβ) to form the A2M–Aβ complex.[8] Endocytosis of the A2M–Aβ complex through the cell surface receptor, LRP, is mediated by A2M and is degraded within the cell.[8] A2M also inhibits the secretion of amyloid protein precursor from neuronal cells.[9] A2M is located in the senile plaques (SPs) and prevents amyloid fibril formation of Aβ.[10-12] A2M concentration increases in the brain of AD patients,[13] and the immunoreactivities of A2M in the brains of AD patients are stronger than in normal elderly individuals.[14] Interleukin-6 induces the synthesis of A2M, indicating that A2M is an acute-phase protein in the brain.[9,14] A2M might be involved in the pathogenesis of AD.

It has been reported that the *A2M* exon 18 splice acceptor deletion polymorphism was genetically associated with sporadic AD in AD patients and their unaffected siblings in an American population.[15] This association was independent of the *APOE*-ε4 status. The risk of the *A2M* polymorphism for the development of AD was comparable with that of *APOE*-ε4.[15] Another common polymorphism in the *A2M*, Val-1000-Ile, was reported to be genetically associated with the development of AD, and the GG genotype of this polymorphism was overrepresented in AD.[16] A significant increase of Aβ deposition also was found

See also pages 433 and 438

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Table 1 *Genotypic distributions of the* A2M *polymorphism in AD and nondemented patients*

Subjects	*A2M* genotype, n (%)		*A2M* allele, n (%)		*p* Value
	A2M-1/A2M-1	*A2M-1/A2M-2*	*A2M-1*	*A2M-2*	
AD, n = 62	59 (95.2)	3 (4.8)	121 (97.6)	3 (2.4)	0.53
Nondemented, n = 90	82 (91.1)	8 (8.9)	172 (95.6)	8 (4.4)	

A2M = α2-macroglobulin gene; *A2M-1* = normal allele; *A2M-2* = deletion mutant allele.

in the brains of the G allele carriers. Further, *A2M* is located on chromosome 12, which has been reported to be genetically linked with the development of AD.[17,18] A previous report shows a genetic association between AD and the LRP gene, the receptor of A2M.[7] This suggests that the *A2M* polymorphism might be involved in the pathogenesis of AD.

To verify the role of the *A2M* polymorphism on AD, a similar study using a population with another ethnic background should be done. The reason that A2M is related to AD may result from the prevention of amyloid fibril formation or deposition of Aβ.[11,12] Therefore, in addition to genotypic distribution analysis of the *A2M* polymorphism in AD and nondemented patients, it is important to examine the relationship between the *A2M* polymorphism and severity of AD-type neuropathologic changes. We examined the *A2M* and *APOE* genotypes and severity of AD-type neuropathologic changes in postmortem-confirmed sporadic AD and nondemented patients.

Methods. *Patients.* The patients were 62 postmortem-confirmed sporadic AD patients (age at death, 62 to 104 years; mean ± SD, 85.8 ± 7.8 years) and 90 nondemented individuals (age at death, 65 to 101 years; mean ± SD, 85.2 ± 7.8 years) from an autopsy series at Yokufukai Geriatric Hospital, Tokyo. All patients were Japanese. The difference of the ages at death between the two groups was not significant. Nondemented patients did not have any neurodegenerative disorders. The neuropathologic diagnosis of AD was based on the neuropathologic criteria of the Consortium to Establish a Registry for Alzheimer's Disease and clinical diagnosis of AD at onset was based on criteria of the National Institute for Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association and the Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised.[19-21]

Neuropathologic examination. The analysis of AD-type neuropathologic changes was performed as previously described.[2] We quantified the densities of the SPs, SPs with dystrophic neurites (NPs), and neurofibrillary tangles (NFTs) in the hippocampus (HP) and superior temporal gyrus (STG) from the brains of all patients by using sections stained with methenamine-Bodian. Ten ×100 microscopic fields (field size 2.56 mm^2) were randomly chosen and examined for SPs and NPs, and 10 ×200 microscopic fields (field size 0.64 mm^2) for NFTs. As for diffuse plaques, those smaller than 25 μm in diameter were excluded from the count. We confirmed that the methenamine-Bodian stain could detect all types of SPs with almost equal sensitivity to the Aβ immunohistochemical method.[22]

Identification of polymorphism. Genomic DNA was extracted from the frozen brain by phenol/chloroform extraction and amplified by PCR with oligonucleotide primers.[23] The PCR product was digested with *Hph*I (New England BioLabs, Beverly, MA), and the restriction fragments were resolved on 2.0% agarose gel. The *A2M* deletion polymorphism consists of two common alleles, the normal allele (*A2M-1*) and mutant allele with the deletion (*A2M-2*).[23] *A2M-1* has the sequence that is recognized by restriction enzyme *Hph*I, and *A2M-2* is not cut by *Hph*I. *A2M-1* is characterized by two fragments, and *A2M-2* by a single fragment of 326 base pairs (bp). The *APOE* genotype examination was performed as previously described.[24]

Statistical analysis. Fisher's exact test was used to examine genotypic distribution of the *A2M* polymorphism in all cases and in the subgroups divided by *APOE*-ε4 status. The densities of the SPs, NPs, and NFTs in HP and STG in the brains of AD and nondemented patients, age at onset, and duration of illness in AD were compared with the *A2M* genotypes using the Mann-Whitney test. Similar statistical analyses were done among the subgroups divided by *APOE*-ε4 status. Statistical significance was defined as two-tailed probabilities of <0.05. All analyses were performed using the computer software StatView J-4.5 (Abacus Concepts, Berkeley, CA).

Results. The *A2M* and *APOE* genotypes were in Hardy-Weinberg equilibrium for both AD and nondemented patients. The allelic frequency of *A2M-2* in nondemented patients in our study was 4.4%. There were no significant differences in the frequency of *A2M* alleles or genotypes between AD and nondemented patients in the total number of patients (table 1), *APOE*-ε4 carriers, or non–*APOE*-ε4 carriers (data not shown). The allelic or genotypic distribution of the *A2M* polymorphism was not associated with *APOE*-ε4 in the total number of cases, AD patients, or nondemented patients (data not shown).

The *A2M* genotypes and densities of the SPs, NPs, and NFTs in the HP and STG in AD and nondemented patients, age at onset, and duration of illness in AD patients are shown in table 2. There was no association of the *A2M* genotypes with the densities of SPs, NPs, or NFTs in the HP or STG in either AD or nondemented patients. The *A2M* genotypes did not influence the age at onset or duration of illness in AD patients. The results remained insignificant when we analyzed the association between the *A2M* polymorphism and AD-type neuropathologic changes in AD or nondemented patients, age at onset, or duration of illness in the subgroups divided by *APOE*-ε4 status (data not shown).

The *APOE*-ε4 allele was associated with AD in total patients ($p = 0.0008$), as we previously reported.[2,3]

Table 2 A2M *genotypes and the densities of senile plaques (SPs), SPs with dystrophic neurites (NPs), and neurofibrillary tangles (NFTs) in the hippocampus and superior temporal gyrus in AD and nondemented patients, and age at onset and duration of illness in AD*

	AD (n = 62)			Nondemented (n = 90)		
A2M genotype	*A2M-1/A2M-1* (n = 59)	*A2M-1/A2M-2* (n = 3)	*p* Value	*A2M-1/A2M-1* (n = 82)	*A2M-1/A2M-2* (n = 8)	*p* Value
Hippocampus						
SPs	19.6 ± 11.1	12.6 ± 3.6	0.23	2.2 ± 5.6	1.7 ± 4.8	0.47
NPs	18.0 ± 11.2	12.6 ± 3.6	0.42	1.6 ± 4.4	1.7 ± 4.8	0.52
NFTs	47.5 ± 29.1	45.8 ± 19.0	0.82	4.0 ± 8.0	2.0 ± 3.0	0.38
Superior temporal gyrus						
SPs	74.0 ± 31.4	76.8 ± 56.4	0.77	9.3 ± 24.5	2.6 ± 7.3	0.33
NPs	20.9 ± 20.4	12.7 ± 8.5	0.61	1.8 ± 3.8	1.0 ± 2.9	0.26
NFTs	5.8 ± 8.0	4.2 ± 5.9	0.69	0.0 ± 0.1	0.0 ± 0.0	0.53
Age at onset, y	79.3 ± 8.1	81.3 ± 7.8	0.42			
Duration of illness, y	6.3 ± 3.9	7.0 ± 4.6	0.74			

Values are medians ± SD. The density represents the average counts in 2.56 mm^2 for SPs and NPs, and in 0.64 mm^2 for NFTs.

A2M = α2-macroglobulin gene; *A2M-1* = normal allele; *A2M-2* = deletion mutant allele.

Discussion. We did not find an association between the *A2M* polymorphism and the development of AD in the total patients or subgroups divided by *APOE*-ϵ4 status. However, *A2M-2* has been shown to increase the risk of AD.[15] There are four possible reasons why our study did not support this previous positive correlation. First, there was a difference in the sample population. The allelic frequency of the *A2M polymorphism in our study with the single Japanese population (A2M-2* = 4.4%) was significantly different from that in the previous study in European and Mediterranean populations (*A2M-2* = 18%).[23] The strong association of the *APOE*-ϵ4 allele with the development of AD in our study indicates that our samples have the same risk factors as other populations, indicating that it was not biased. Although the low frequency of *A2M-2* could reduce statistical power, we did not find any positive trend between the *A2M* polymorphism and AD, suggesting that this is not a major reason for the negative results in our study. The influence of the *A2M* polymorphism on AD might be different among the sample populations and weak in Japanese patients. Second, all patients were postmortem-confirmed subjects in our study. The difference might reflect incorrectness of the diagnosis of AD in the studies using clinically diagnosed patients. Third, our patients are older than those in other studies. This factor was inevitable in a study using autopsy-confirmed subjects. Last, there is difference in the experimental design. Our study is a population-based case–control study, whereas Blacker et al. used a family-based association test method.[15] Although there might be a difference of the sensitivity to detect the effect of *A2M-2* on AD between the two designs, even a positive trend could not be found between the *A2M* polymorphism and AD in our study. Recent reports of family-based as well as population-based studies found no association of *A2M-2* with AD,[25-27] although one family-based analysis indicates a weak association.[26]

Our results reveal a lack of association of the *A2M* polymorphism with AD-type neuropathologic changes in AD or nondemented patients, even when we divided the total number of patients into subgroups by *APOE*-ϵ4 status. This observation does not support the hypothesis that the *A2M* polymorphism affects the development of AD by accelerating the formation of AD-type neuropathologic changes.

It has been reported that Val-1000-Ile polymorphism in *A2M* was associated with AD.[16] A significant increase of Aβ deposition in the brains from G allele carriers also was reported. However, the following study did not confirm this positive association.[28] The statistical associations between *A2M* and AD in some populations imply that the other region relevant to AD in *A2M* or another adjacent gene could be in linkage disequilibrium with the *A2M-2* or Val-1000-Ile polymorphism. Further studies are needed to determine the correlation of intronic deletion or Val-1000-Ile polymorphism in *A2M* with AD in various ethnic backgrounds. This may clarify the effect of A2M on the development of AD.

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Prevalence and outcomes of vascular cognitive impairment

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Article abstract—*Objective:* To assess the importance of vascular cognitive impairment and its three subgroups (cognitive impairment, no dementia; vascular dementia; and AD with a vascular component) to the prevalence and burden of cognitive impairment in elderly people. *Background:* Vascular lesions may produce a spectrum of cognitive changes. Omitting elderly patients whose cognitive impairment falls short of dementia (vascular cognitive impairment, no dementia) may give a falsely low indication of the prevalence and burden of disease. To test this proposition, we compared the rates of adverse outcomes for patients with no cognitive impairment, vascular cognitive impairment (and its subgroups), and probable AD. *Methods:* The Canadian Study of Health and Aging is a prospective cohort study of 10,253 randomly selected community-dwelling and institution-dwelling respondents aged 65 years or older. In the community, all participants (n = 9,008) were screened for cognitive impairment; those who screened positive and a sample of those who screened negative received a clinical assessment (n = 1,659). All patients living in institutions received a clinical assessment (n = 1,255). Participants were reassessed 5 years after the original survey. *Results:* Vascular cognitive impairment without dementia was the most prevalent form of vascular cognitive impairment among those aged 65 to 84 years. Rates of institutionalization and mortality for those with vascular cognitive impairment were significantly higher than those of people who had no cognitive impairment, and the mortality rate for patients with vascular cognitive impairment was similar to that of patients with AD. *Conclusions:* Failure to consider vascular cognitive impairment without dementia underestimates the prevalence of impairment and the risk for adverse outcomes associated with vascular cognitive impairment. **Key words:** Vascular cognitive impairment—Dementia—Diagnosis.

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The foundations for the diagnosis of vascular dementia (VaD) are under scrutiny.[1-14] Dementia criteria are typically modeled on AD, whereby the predilection for involvement of the mesial temporal lobes results in prominent memory impairment. By contrast, patients with vascular lesions have no such predilection. As such, it may be too restrictive to require patients with cognitive impairment arising from vascular causes to meet currently accepted criteria for dementia before considering them as significant cases.[1] The emphasis on dementia may underestimate the burden of disease and distract from a focus on prevention.[15] Cognitive impairment with no dementia (vascular CIND) resulting from vascular causes has been identified as a component of vascular cognitive impairment (VCI).[16] VCI forms a spectrum that includes VaD (of the multi-infarct and white-matter rarefaction/infarction types), mixed AD with a vascular component, and VCI that does not meet dementia criteria (i.e., vascular CIND).

Data from the Canadian Study of Health and Aging (CSHA) bear on this controversy.[17-19] Including subjects with VCI who do not meet the dementia criteria increases the prevalence of VCI, although the exact effect has not been estimated. More importantly, we can now test the hypothesis that discounting VCI patients who do not meet the traditional dementia criteria underestimates the societal burden associated with vascular cognitive disease. The 5-year follow-up data for the CSHA cohort allow us to compare the rates of institutionalization and mortality of patients with VCI with those of patients with dementia and age-matched individuals without cognitive impairment. We report prevalence estimates for the spectrum of VCI and examine the risk for institutionalization and death with regard to VCI, comparative cognitive diagnoses, and VCI subtypes.

*See the Appendix on page 451 for a listing of the Vascular Cognitive Impairment Investigators of the Canadian Study of Health and Aging.

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Methods. The CSHA is described elsewhere.[17,20] In a prospective cohort study, randomly selected community-dwelling and institution-dwelling respondents were surveyed in 1991 and 1992, recontacted after 18 months, and re-examined in 1996 and 1997. At baseline, the response rate was 63.9% for the community-dwelling sample and 79.1% for the institution-dwelling sample.[17] A total of 9,008 community-dwelling individuals were screened for cognitive impairment using the Modified Mini-Mental State (3MS) Examination.[21] A relatively high cutoff score of 77/78 was used to increase the sensitivity of screening with regard to dementia.[22] Among the community-dwelling participants, 1,106 screened positive (i.e., scores < 78) and received a clinical examination; 494 randomly selected individuals who screened negative and 59 who could not be screened because of hearing or other difficulties were also selected for clinical examination (n = 1,659). All participants living in institutions during the original survey received a clinical assessment (n = 1,255).[17] The choice of cutoff score as a diagnostic filter demonstrated efficiency in that 398 (80.6%) of the community-dwelling individuals who screened negative were diagnosed as having no cognitive impairment on clinical examination. Diagnostic methods during clinical assessment included administration of the 3MS, section H of the Cambridge Mental Disorders of the Elderly Examination,[23] and a battery of neuropsychologic tests.[17] Neuroimaging studies were not included in the protocol because of budget considerations.

Throughout the study, case conferences to arrive at consensus diagnoses between physicians and neuropsychologists were held. To assess diagnostic consistency, 210 randomly selected clinical assessment forms were subsequently sent, in batches of 14 assessments, to each of 15 study clinicians for blind reassessment. The kappa index of agreement for the classification of no cognitive impairment, CIND, AD, and all other types of dementia was 0.79 (95% CI 0.62 to 0.78).[17]

At baseline, cognitive function was classified as no cognitive impairment, cognitive impairment that did not meet the criteria for dementia (i.e., CIND),[18,24] dementia from AD, VaD, or other types of dementia. In keeping with one proposal,[25] we subsequently classified patients as having VCI to include three subtypes: vascular CIND, AD with a vascular component (mixed AD), and VaD.

The dementia syndrome was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised (DSM-III-R) criteria.[27] Etiologic diagnoses followed the International Classification of Diseases of World Health Organization (ICD)–10 criteria[28] for VaD and National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria[29] for AD. Both criteria were used to define mixed AD. The guidelines specified by ICD-10 and NINCDS-ADRDA presuppose dementia. The ICD-10 criteria state that the cognitive deficits in patients with VaD are typically asymmetrically distributed and require antecedent evidence of vascular features (e.g., sudden onset or stepwise progression) on examination (focal findings) or neuroimaging (i.e., infarction or white matter changes). Mixed AD (i.e., AD with vascular components) was diagnosed in patients with typical AD presentation who had clinically significant vascular features on assessment. The diagnostic category CIND is described in detail elsewhere.[24] Vascular CIND was diagnosed as mentioned earlier, except that although cognitive impairment had to be present, it did not meet the criteria for dementia.

Follow-up information regarding the adverse outcomes of institutionalization or death was obtained for 98% of participants approximately 5 years after the baseline assessment. Exact dates of institutionalization or death were obtained. The time-to-event data for individuals without these outcomes were defined as the date of first contact at the time of follow-up. Nonresponse proportions were similar among community-dwelling (3%) and institution-dwelling (1%) individuals.

In this analysis, comparisons are made between the classifications of no cognitive impairment, probable AD, VCI, and within the three categories of VCI. The category of no cognitive impairment was subdivided into those who had vascular risk factors (defined as a history of hypertension, stroke, or diabetes) and those who had no such risk factors. The category of CIND was subdivided into cases caused by vascular causes and those resulting from other causes. Age-specific and gender-specific estimates of prevalence were calculated using sample weights that adjusted the sample design, which was stratified by age, region and residence in community or institution, and the likelihood of having a clinical assessment based on the screening test. Weighted data are reported for all prevalence estimates.

The assumption of proportional hazards was tested and verified, and the Cox regression model was used in the subsequent analyses. Relative risk (RR), adjusted for age, sex, and severity of cognitive impairment as measured by the 3MS score, was calculated to estimate the likelihood of institutionalization and death for individuals diagnosed with VCI compared with those with no cognitive impairment. Also, population-attributable risk percentages (PAR%) were computed to evaluate the risk for adverse outcomes for those diagnosed with VCI compared with those with no cognitive impairment.[26] Cox regression was also used to compare time to institutional admission and time to death for those with no cognitive impairment, probable AD, and VCI (and its subgroups). Also, survival analysis was performed to compare length of time before adverse outcomes for no cognitive impairment (with and without vascular risk factors), nonvascular CIND, and vascular CIND.

Results. The table shows the prevalence of categories of VCI, at baseline, among Canadians aged 65 years or older, by age group and by sex. The prevalence of AD is included in the table for the purposes of comparison and is described in detail elsewhere.[17] All types of VCI combined increase with age. Vascular CIND was the most prevalent VCI subgroup to age 84 years, after which mixed AD and VaD predominated.

Residential environment strongly reflected cognitive status. Only 0.8% of men and 1.5% of women diagnosed with no cognitive impairment resided in institutions at baseline. Among individuals diagnosed with VCI, 30.8% of men and 40.9% of women lived in institutional settings. Of those diagnosed with probable AD, 33.3% of men and 52.1% of women were institutionalized. Approximately 5 years after the baseline survey, 445/1,606 (27.7%) of those originally residing in the community who received a clinical assessment had been institutionalized before follow-up

Table *Age- and gender-specific prevalence estimates per 1,000 for each of the three subgroups of vascular cognitive impairment (VCI) and for AD among Canadians age 65 years or older (1991–1992)*

Demographic	Vascular CIND (n = 149)	Mixed AD (n = 136)	VaD (n = 208)	All VCI (n = 493)	AD (n = 747)
Age, y					
65–74	14	0	6	20	10
75–84	45	14	24	83	69
85+	38	51	48	137	260
Total (65+)	26	9	15	50	51
Sex					
Men	28	7	18	53	38
Women	24	11	13	49	58

Prevalence estimates for AD are taken from reference 17.

CIND = cognitive impairment, no dementia; mixed AD = AD with a vascular component; VaD = vascular dementia.

(data unavailable for 53 subjects). Participants diagnosed with VCI during the initial CSHA survey demonstrated an increased risk for institutionalization 5 years later (RR 3.1; range 2.1 to 4.6) compared with those with no cognitive impairment. A marked overall effect was found for VCI as a diagnosis with regard to institutionalization among the elderly (PAR% = 17.4). The likelihood of institutionalization was somewhat lower for individuals diagnosed with all types of VCI compared with those with probable AD (RR 0.7; range 0.5 to 1.0). Time to institutionalization was compared for those with no cognitive impairment, VCI, and probable AD (figure 1A). Individuals having no cognitive impairment remained in the community significantly longer before institutionalization than did individuals with VCI and those with probable AD ($p < 0.001$). Also, people diagnosed with VCI lived in the community longer than did those with AD ($p = 0.03$). Within the diagnosis of VCI (figure 2A), individuals with vascular CIND remained in the community significantly longer than did those with mixed AD ($p = 0.01$). No significant difference was found in the amount of time patients remained in the community among patients with vascular CIND or VaD, nor among patients with VaD or mixed AD. Time before institutionalization was also compared among patients with no cognitive impairment and CIND (figure 3A). Cognitively normal people, with and without vascular risk factors, remained in the community significantly longer than did those with nonvascular or vascular CIND ($p = 0.001$). Significant differences in time to institutionalization were not found among subjects within the classifications of no cognitive impairment or within subjects within the categories of CIND.

The mortality rate, between baseline and follow-up, was 869/1,245 (69.8%) for the institutionalized sample (data unavailable for 10 subjects) and 652/1,614 (40.4%) for community-dwelling participants (data unavailable for 45 subjects). People diagnosed with VCI at baseline demonstrated an increased risk for death 5 years later (RR 1.8; range 1.5 to 2.3) compared with cognitively normal people. Also, the impact of VCI on the mortality rate among the elderly was substantial (PAR% = 18.7). The risk for death was similar among individuals diagnosed with VCI and those with AD (RR 1.0; range 0.9 to 1.2). Time to death was compared for those with no cognitive impairment,

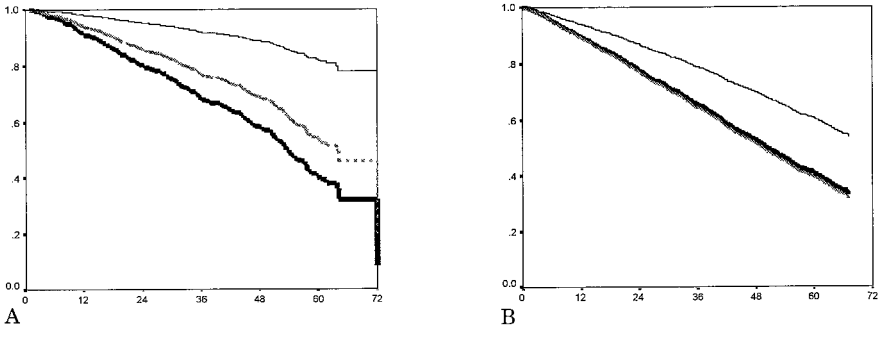

Figure 1. Time to (A) institutionalization and (B) death for no cognitive impairment (thin line), vascular cognitive impairment (gray line), and AD (black line) groups, adjusted for age, sex, and Modified Mini-Mental State (3MS) Examination score.

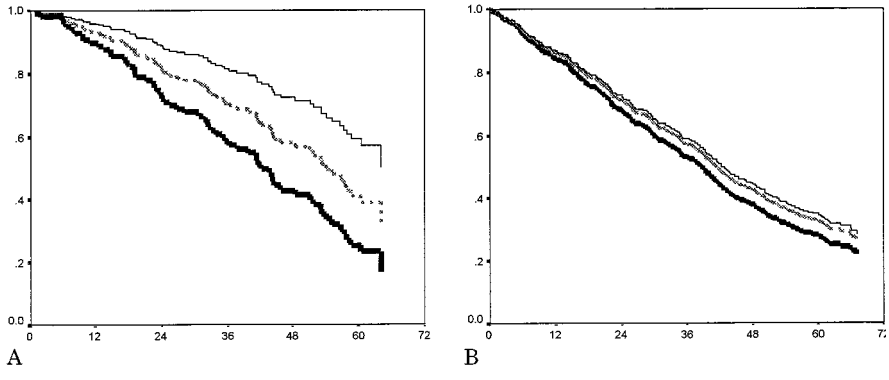

Figure 2. Time to (A) institutionalization and (B) death for vascular cognitive impairment with no dementia (thin line), mixed AD (gray line), and vascular dementia (black line) groups, adjusted for age, sex, and Modified Mini-Mental State (3MS) Examination score.

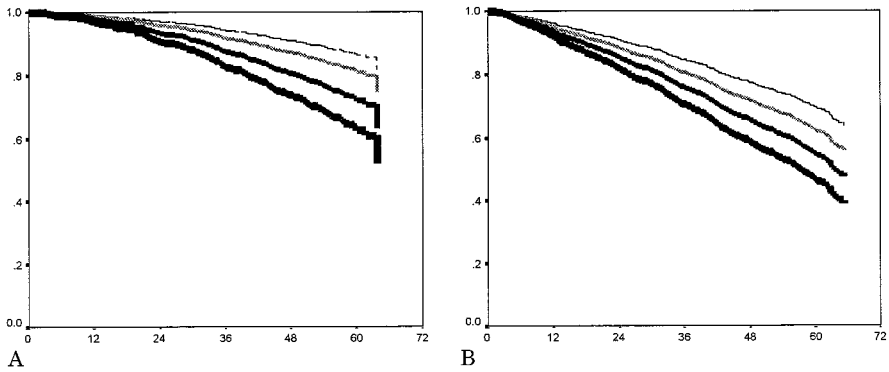

Figure 3. Time to (A) institutionalization and (B) death for no cognitive impairment (NCI) without risk factors (thin line), NCI with risk factors (gray line), nonvascular cognitive impairment with no dementia (CIND) (medium black line), and vascular CIND (heavy black line) groups, adjusted for age, sex, and Modified Mini-Mental State (3MS) Examination score.

VCI, and probable AD (figure 1B). Cognitively normal people lived longer than did individuals diagnosed with VCI and with probable AD ($p < 0.001$), but no significant difference in survival time was found among patients with VCI and those with probable AD. Within the diagnosis of VCI (figure 2B), survival curves for vascular CIND, mixed AD, and VaD were compared for the outcome of death. The survival times for patients with vascular CIND, mixed AD, and VaD, although decreasing in the expected direction, were not significantly different. Survival times were also compared among patients with no cognitive impairment and those with CIND (figure 3B). Cognitively normal people without vascular risk factors lived longer than did those with vascular risk factors ($p = 0.04$), who in turn lived longer than did individuals with nonvascular CIND ($p = 0.001$) and vascular CIND ($p = 0.001$). The survival time did not differ significantly among patients with the different classifications of CIND.

Discussion. In keeping with an earlier CSHA report,[19] patients with VCI seem to form a distinctive group, with outcomes clearly different from those with no cognitive impairment and somewhat different from those with AD. These data provide additional evidence of the importance of all levels of VCI as contributors to the burden of cognitive impairment in the elderly. These results support the view that VCI criteria that require the diagnosis of dementia underestimate the prevalence and burden of vascular cognitive disease.[1,3] Vascular CIND is the most common form of VCI among Canadians aged 65 to 84 years. Vascular CIND is associated with an increased risk for adverse outcomes compared with no cognitive impairment (with and without vascular risk factors). The rate of institutionalization among individuals with vascular CIND is similar to that of those with VaD, and the mortality rate of patients with vascular CIND is similar to that of patients with VaD or mixed AD. Moreover, all three subgroups of VCI demonstrate much higher rates of adverse outcomes than do those associated with no cognitive impairment; the mortality rate of VCI is comparable with that found for probable AD.

Although this is a large, representative, prospective cohort study, these data must be interpreted with some caution for the following reasons. First, the survival curves were based on a prevalence sample of people who were originally identified at varying stages of their disease at baseline in 1991 and 1992. Although the exact extent of such bias is unknown, it is likely to be conservative because vascular CIND likely progressed to VaD over the period of observation in some cases. Second, during the original survey, neuroimaging data were not available to support the diagnosis of VCI. Nevertheless, these data represent the first comprehensive estimate of the prevalence and outcomes of VCI from a representative population sample.

These data do not include neuropathologic studies, so we cannot address whether "pure" VaD has been underestimated or overestimated, but neuropathologic information provides evidence of one form of criterion validity of this diagnosis. We found an alternate form of criterion testing—namely, predictive validity—with regard to the risk for adverse outcomes.

Our estimate of the mortality rate associated with VCI is similar to that for probable AD. This finding is contrary to other reports,[30,31] in which patients with VaD had worse outcomes than did patients with AD.

VCI remains a clinical syndrome and not a disease. All subgroups of VCI (including VCI coincident with AD) encompass vascular insults that can arise from many causes (e.g., thromboembolic or other types of stroke or chronic hemodynamic abnormalities) and can be expressed in different ways on neuroimaging studies (e.g., white matter changes and single or multiple cortical or subcortical strokes). Additional multicenter clinic data are needed to provide numbers sufficiently large to decipher patterns classified by, for example, cause or neuroradiologic abnormality.

These data show that disregard for patients with VCI who fail to meet criteria for dementia excludes most elderly Canadians with VCI despite their high institutionalization and mortality rates. We suggest that the label of VCI should be used to describe the spectrum from vascular CIND to VaD.[32] Perhaps more attention to this redefined disease entity will help to foster increased preventive measures for these patients. Treatment with aspirin and management of hypertension may reduce the incidence of dementia and slow the progression of cognitive impairment in patients who are already impaired.[33,34]

Appendix

The Vascular Cognitive Impairment Investigators in the Canadian Study of Health and Aging: Erika Ebly, David B. Hogan, Univer-

sity of Calgary, Alberta; Howard Feldman, University of British Columbia, Vancouver, British Columbia; John Fisk, Stephen Phillips, Kenneth Rockwood, Robert Vandorpe, Dalhousie University, Halifax, Nova Scotia; Serge Gauthier, Christina Wolfson, McGill University, Montreal, Quebec; Vladimir Hachinski, Truls Østbye, Runa Steenhuis, University of Western Ontario, London, Ontario; Ian McDowell, University of Ottawa, Ontario; René Verreault, Université Laval, Quebec City, Quebec.

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