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 gene2 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

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chromosome 12. 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. Furthermore, a polymorphism in the translated region of exon 24 of the A2M gene (Val1000->Ile1000) has been associated with an increased risk for AD. The α2 macroglobulin protein (A2M) is a high molecular weight plasma protease inhibitor (720 kD) with a large variety of postulated functions. In the brain, several lines of evidence suggest that A2M plays an important role in neuronal repair following injury. In AD, A2M has been localized immunohistochemically by several laboratories to neuritic plaques. 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. 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 non-demented 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 women, 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 [NINCDS-ADRDA] criteria for probable AD or International Classification of Diseases [ICD]-10 criteria 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. The patients met National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD or International Classification of Diseases (ICD)-10 criteria 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) scores ≥ 26.

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). 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 neuropsychological 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). 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. 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. 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 χ² test in SU patients and by McNemar's χ² 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. 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-e4/e4 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. The interaction between APOE and of A2M was assessed by logistic regression in the SU cohort and for the sample overall, and by conditional logistic regression in IU and MU samples.

**Results.** The distributions of A2M and APOE-e4 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-e4 and AD. 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 = 0.038). 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).

Next, we examined the joint distribution of A2M and APOE-e4 in the entire data set. Our data are consistent with previous observations in which increased allele frequencies of the APOE-e4 allele is associated with AD. Logistic regression analyses adjusted for the effect of APOE-e4 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. 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 to be similar to the risk for those with the APOE-e4 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.
Table 3 Odds ratios and a test for APOE-ε4−α2 macroglobulin (A2M) interaction on the AD risk for the three centers

<table>
<thead>
<tr>
<th>Location</th>
<th>APOE ε4−</th>
<th>APOE ε4+</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU</td>
<td>3.04 (0.92–10.07)</td>
<td>0.43 (0.12–1.5)</td>
<td>0.032</td>
</tr>
<tr>
<td>MU</td>
<td>1.21 (0.43–3.39)</td>
<td>1.74 (0.67–4.48)</td>
<td>0.461</td>
</tr>
<tr>
<td>SU</td>
<td>0.78 (0.12–1.50)</td>
<td>1.38 (0.63–3.04)</td>
<td>0.393</td>
</tr>
</tbody>
</table>

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.,4 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 Blacker et al.4 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.4 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.

References
NeuroImages

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 intramural 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 non-traumatic 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.