Our third sample was composed of individuals from an epidemiologically based cohort from Cambridge aged 84 and older in which cognitive function was assessed using the MMSE 3. Of 229 individuals analysed, 47 had MMSE scores less than 22 and 159 had MMSE scores greater than 23 (used as controls in sample 1, above). The odds ratio reported previously for A2M (3.56; ref. 2) is detectable with 37 cases and 74 controls ($80%$ power, $5\%$ significance; ref. 5), thus our combined sample of 230 cases and 218 controls should detect this effect. Our autopsy-confirmed sample alone should detect an odds ratio of 2 ($80\%$ power, $5\%$ significance).

We designed new primers to facilitate A2M genotyping (Amar$'$, 5'-TTCTCTCTAGAAGCTTTATCTGATG-3'; Amra, 5'-AAGTACCCCAGAAGGTTTGAC-3'). These gave products of 138 bp (wild-type) and 133 bp (deletion), which corresponded with alleles determined using the previous method.

We compared frequencies of carriers for at least one $A2M*2$ allele with wild-type homozygotes and allele frequencies in cases and controls from our three groups and in the pooled cases with controls (Table 1). We saw no significant influence of A2M*2 on AD or dementia risk in any of these comparisons (P>0.05, $\chi^2$ tests) or on MMSE score (Cambridge cohort; P>0.05), even after using a multiple regression approach to account for the effect of age on MMSE score. A2M*2 is not strongly associated with AD risk in our UK samples. Our 95% confidence intervals for the odds ratio of AD associated with carriers of A2M*2 did not overlap with that in the Blacker study (1.80–7.03) when we compared autopsy-confirmed cases with all non-demented controls, Cambridge cases with Cambridge controls and all cases with all controls (Table 1). The proportion of A2M*2 carriers was 32.6% in our elderly, non-demented controls versus 29.5% in the Blacker et al. cases, and the A2M*2 allele frequencies were 17.9% and 16.4% in our controls and the Blacker et al. cases, respectively. Our data are unlikely to be confounded by population stratification, because they include two epidemiologically based cohort studies from confined regions including almost exclusively English individuals of European descent.

The discrepancies between our data and those reported previously may be due to regional population differences in AD susceptibility associated with this polymorphism, or the gene may be associated with AD confined to particular ages and the UK and USA samples may have different age distributions. There may be another functional variant closely linked to A2M*2 conferring AD risk, which is in linkage disequilibrium with the deletion allele in the USA sample but not in the UK samples. Family based association studies may give different odds ratios from population-based case-control studies, either because these families have a high density of disease, or because affected and unaffected individuals in family based studies are more likely to share other familial factors than population-based cases and controls. We have looked at autopsy-confirmed cases, however, avoiding the diagnostic uncertainties associated with clinical assessments and at epidemiologically based samples, which reflect the impact of this gene in the general population, as opposed to highly selected families.

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### α-2 macroglobulin gene and Alzheimer disease

Blacker et al. reported an association between a deletion in exon 18 of the α-2 macroglobulin (A2M) gene and Alzheimer disease (AD) in a sample of affected and unaffected siblings from families segregating AD. They observed that the degree of conferred risk for AD in A2M allele 2 (A2M*2) carriers was similar in magnitude to that for carriers of the apolipoprotein (APOE) ε4 allele (APOE*E4). We set out to test for a similar association in a powerful, case-control sample composed of 2,616 individuals taken from populations from Europe and the United States. In addition, we extended our analysis of the National Institute of Mental Health (NIMH) family samples studied by Blacker et al. using linkage and association approaches. We pooled this NIMH series (270 sibpairs) with a similar series of 125 sibpairs collected through the auspices of the National Institute of Aging (NIA) at the Indiana Alzheimer cell repository to increase the power of our analysis.

We studied four independent association samples of unrelated AD patients with onset over 50 years and relevant controls (Table 1) and diagnosed AD patients according to NINCDS-ADRDA criteria with either probable or definite AD. We calculated that this sample had over 99% power to detect an effect of equivalent size to that reported by Blacker et al. in their familial sample (odds ratio (OR)=3.56), assuming a stringent α level of 0.001. Furthermore, we calculated that our sample was sufficiently powerful (80%) to detect an extremely small effect (OR=1.2) assuming an α level of 0.05.

We detected the exon 18 mutation using standard PCR and RFLP methods (available on request). We used the Woolf method to test for association in the combined data set and MAPMAKER/SIBS (ref. 4) to compute single-point maximum lod scores.

The genotype distributions of A2M*2 in relation to APOE status are shown...
(Table 2), as well as the summary statistics (Table 1). We did not observe an association between AD and possession of at least one copy of the deletion (P=0.7) in the sample overall. We also failed to detect an allelic association (P=0.4), although the combined sample showed the expected association between AD and APOE*E4 (P<10\(^{-10}\), OR=4.6, 95% confidence interval (CI)=3.9–5.5). As Blacker et al. observed a stronger effect in individuals without an APOE*E4 allele, we stratified our sample according to APOE*E4 status. We found no evidence of either a genotypic (A2M*2/*2 and A2M*2/*1 versus A2M*1/*1) or allelic association in individuals with no APOE*E4 alleles (P=0.5 and P=0.4). All samples of cases and controls were in Hardy-Weinberg equilibrium.

We speculated that the difference between affected and unaffected siblings observed by Blacker et al. may reflect the action of a confound, such as age-related differences in exon 18 frequencies between groups. We tested this in the NIMH sample used by Blacker and colleagues and found some evidence of age differences in affected (mean age=75.7±10.9) versus unaffected siblings (mean age=82.5±7.2). We then tested for age differences (50–79 versus 80+ years) in proportions of individuals with the exon 18 deletion in our control samples. We found no association between the A2M*1/*1 genotype and age (P=0.2). Indeed, we found that the trend was slightly in the opposite direction (data not shown), suggesting that age did not act as a confound.

Our collaborative group recently obtained evidence consistent with linkage (MLS=1.91) to polymorphic markers on chromosome 12 in a region encompassing the A2M locus in the same NIMH sample studied by Blacker et al. This did not meet Lander and Kruglyak’s criterion for suggestive linkage but was the second highest score obtained in our genome scan and was observed in 68 APOE*E4-negative families. We therefore tested for linkage between AD and the exon 18 polymorphism analysed in the NIMH/NIA sample using the exon 18 polymorphism and obtained a lod score of 0.7 in APOE*E4-negative affected sibling pairs and a lod score of 0.17 for the sample overall. We thus observed a similar trend towards excess allele sharing in APOE*E4-negative siblings, but with a reduced level of significance that might reflect a lack of informativity of the polymorphism.

Finally, we were concerned that the tests used by Blacker et al., which included all members of sibships, may have overestimated the size of the association in their sample. We tested for association using one pair of affected/unaffected siblings chosen at random from each family. We found significant but weaker genotypic (A2M*2/*2 versus A2M*1/*1; P=0.04) and allelic (A2M*2; P=0.01) associations in the NIMH sample than those observed by Blacker.

### Table 1 • Association between A2M and AD

<table>
<thead>
<tr>
<th>Research centre</th>
<th>Sample descriptions</th>
<th>Whole samples *2/*2 and *1/*2 versus *1/*1</th>
<th>APOE*E4–4 *2/*2 and *1/*2 versus *1/*1</th>
<th>Whole sample *2/*1 and *2/*2</th>
<th>APOE*E4–4 *2/*1 and *1/*2 versus *1/*1</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK: Cardiff</td>
<td>152 AD patients: 63% F, AO 71.9±8.13</td>
<td>1.5 (0.8–2.8)</td>
<td>1.3 (0.6–2.8)</td>
<td>1.4 (0.8–2.4)</td>
<td>1.2 (0.6–2.5)</td>
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<tr>
<td></td>
<td>99 controls: 54% F, age 72.6±6.55</td>
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<tr>
<td>France: Lille</td>
<td>616 AD patients: 63% F, AO 69.4±8.5</td>
<td>1.1 (0.9–1.5)</td>
<td>1.1 (0.8–1.6)</td>
<td>1.1 (0.9–1.4)</td>
<td>1.1 (0.8–1.4)</td>
</tr>
<tr>
<td></td>
<td>648 controls: 63% F, age 73.1±8.5</td>
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<tr>
<td>US: Wash U</td>
<td>143 AD patients: 62.4% F, AO 73.7±9.51</td>
<td>1 (0.6–1.6)</td>
<td>1.7 (0.9–3.3)</td>
<td>1 (0.6–1.5)</td>
<td>1.6 (0.9–2.7)</td>
</tr>
<tr>
<td></td>
<td>144 controls: 65.2% F, age 77±9.2</td>
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<tr>
<td>US: Mayo</td>
<td>327 AD patients: 65.5% F, AO 75.5±7.7</td>
<td>0.8 (0.6–1.1)</td>
<td>0.8 (0.5–1.2)</td>
<td>0.8 (0.6–1.1)</td>
<td>0.9 (0.6–1.2)</td>
</tr>
<tr>
<td></td>
<td>487 controls: 67.1% F, age 82.7±7.5</td>
<td></td>
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<tr>
<td>Combined Sample</td>
<td>1,238 AD patients</td>
<td>1 (0.9–1.2)</td>
<td>1.1 (0.9–1.4)</td>
<td>1 (0.9–1.2)</td>
<td>1.1 (0.9–1.3)</td>
</tr>
<tr>
<td></td>
<td>1,378 controls</td>
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</table>

AO, age at onset.
et al., showing ORs of 2.13 and 2.17, respectively. Similar though non-significant trends were also observed in the NIA sample (P = 0.1; P = 0.1). Combined analyses of the NIMH and NIA samples showed significant genotypic (P = 0.007, OR = 2.13, CI = 1.22–3.7) and allelic (P = 0.003, OR = 2.02, CI = 1.3–3.2) associations with AD. We did not find associations between AD and A2M*2, or genotypes containing A2M*2, in a powerful, case-control sample. We did, however, detect significant but weaker associations in the NIMH sample than those quoted by Blacker et al. using the same sample, together with similar but non-significant trends in an independent family sample (NIA). We believe the ORs we observed provide more accurate estimates of the effect sizes than those quoted originally. We conclude that if these data are not due to chance, A2M, or a gene in linkage disequilibrium with it, may be weakly associated with AD in some samples and may reflect either an interaction with other susceptibility gene/s segregating in these families or a stronger genetic effect in some of these families. Either case would limit the power of conventional case-control samples to detect association.

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An α-2-macroglobulin insertion-deletion polymorphism in Alzheimer disease

Blacker et al. reported an association between Alzheimer disease (AD) and the deletion allele (A2M*2) of an intronic polymorphism in the α-2-macroglobulin gene (A2M; ref. 2), which confers a risk for AD (OR = 3.55, 95% CI = 1.90–9.47) and is strongly associated with AD in some samples. We tested several different antibodies, even when the “stringent unaffecteds” of AD (OR = 3.55, 95% CI = 1.90–9.47) and allelic (OR = 2.02, 95% CI = 1.3–3.2) associations with AD. We did not find associations between AD and A2M*2, or genotypes containing A2M*2, in a powerful, case-control sample. We did, however, detect significant but weaker associations in the NIMH sample than those quoted by Blacker et al. using the same sample, together with similar but non-significant trends in an independent family sample (NIA). We believe the ORs we observed provide more accurate estimates of the effect sizes than those quoted originally. We conclude that if these data are not due to chance, A2M, or a gene in linkage disequilibrium with it, may be weakly associated with AD in some samples and may reflect either an interaction with other susceptibility gene/s segregating in these families or a stronger genetic effect in some of these families. Either case would limit the power of conventional case-control samples to detect association.

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