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## LETTER TO JMG

Interaction between the  $\alpha$ -T catenin gene (VR22) and APOE in Alzheimer's disease

E R Martin, P G Bronson, Y-J Li, N Wall, R-H Chung, D E Schmechel, G Small, P-T Xu, J Bartlett, N Schnetz-Boutaud, J L Haines, J R Gilbert, M A Pericak-Vance

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**Background:** APOE is the only gene that has been consistently replicated as a risk factor for late onset Alzheimer's disease. Several recent studies have identified linkage to chromosome 10 for both risk and age of onset, suggesting that this region harbours genes that influence the development of the disease. A recent study reported association between single nucleotide polymorphisms (SNPs) in the VR22 gene (CTNNA3) on chromosome 10 and plasma levels of A $\beta$ 42, an endophenotype related to Alzheimer's disease.

**Objective:** To assess whether polymorphisms in the VR22 gene are associated with Alzheimer's disease in a large sample of Alzheimer's disease families and an independent set of unrelated cases and controls.

**Results:** Several SNPs showed association in either the family based or case-control analyses ( $p < 0.05$ ). The most consistent findings were with SNP6, for which there was significant evidence of association in both the families and the unrelated cases and controls. Furthermore, there was evidence of significant interaction between APOE-4 and two of the VR22 SNPs, with the strongest evidence of association being concentrated in individuals carrying APOE-4.

**Conclusions:** This study suggests that VR22 or a nearby gene influences susceptibility to Alzheimer's disease, and the effect is dependent on APOE status.

Alzheimer's disease is the most common form of dementia in the elderly, and currently affects more than four million people in the USA. Four major genes (amyloid precursor protein (APP), presenilin 1 and 2 (PS1 and PS2), and apolipoprotein E (APOE)) have been identified, but these genes collectively account for only 45–55% of the genetic aetiology in this disorder. Though mutations in APP, PS1, and PS2 cause early onset Alzheimer's disease, APOE is the only gene that has been consistently replicated as a risk factor for late onset disease. Other genes contributing to the common late onset type of disease remain to be determined.

Several studies have identified linkage to chromosome 10 for both risk of Alzheimer's disease and age of onset of the disease, often in overlapping datasets.<sup>1–4</sup> Further, association has been reported with several candidate genes in the region in different studies, including insulin degrading enzyme (IDE), plasminogen activator urinary (PLAU), and glutathione S transferase-omega (GSTO1).<sup>5–7</sup> A recent study<sup>8</sup> reported association between single nucleotide polymorphisms (SNPs) in the VR22 gene (chromosome 10q21.3), lying in the region of linkage, and plasma amyloid  $\beta$  protein (specifically A $\beta$ 42) levels in a small set of families with Alzheimer's disease. Amyloid  $\beta$  aggregates in amyloid fibrils in senile plaques found in the brains of Alzheimer patients.<sup>9</sup>

Plasma A $\beta$ 42 levels have been shown to be increased in individuals with Alzheimer's disease, and these levels have been used as an endophenotype in attempts to map genes contributing to the disease.<sup>10–12</sup> The finding of association between VR22 SNPs and A $\beta$ 42 levels suggests that this gene may be involved in Alzheimer's disease susceptibility. However, the previous study did not specifically test the polymorphism for association with Alzheimer's disease; therefore, a direct link between VR22 and disease risk has not been established.

The VR22 gene (also called CTNNA3) codes for the  $\alpha$ -catenin-like protein ( $\alpha$ -T catenin), which is believed to be involved in cell adhesion. To examine whether polymorphisms in the VR22 gene are associated with Alzheimer's disease, we tested for association with several SNPs identified by Ertekin-Taner *et al*<sup>8</sup> as being the most significantly associated with A $\beta$ 42 levels and additional surrounding SNPs. We tested a large sample of Alzheimer families and an independent set of unrelated cases and controls for association with Alzheimer's disease.

## METHODS

## Study samples

Our analysis set includes both a family sample and an independent case-control sample. The family sample, described in table 1, consists of 738 families collected by the following: the Collaborative Alzheimer Project (CAP: The Joseph and Kathleen Bryan ADRC and the Center for Human Genetics at Duke University, The Center for Human Genetics research at Vanderbilt University Medical Center, and the University of California at Los Angeles Neuro-psychiatric Institute); National Institutes of Mental Health (NIMH); and the National Cell Repository for Alzheimer's Disease at Indiana University Medical Center (IU).

The dataset contains 371 families with at least one sampled affected family member and at least one sampled unaffected family member (discordant sibling pairs) informative for association analysis. The multiplex dataset consists of 580 families informative for linkage analysis.

All affected individuals met the NINDS/ADRDA criteria for probable or definite Alzheimer's disease.<sup>13</sup> Unaffected relatives from the CAP and NIMH sites were examined and showed no signs of dementia. Unaffected individuals from IU were classified based on self report. The mean (SD) age at onset (AAO) in affected individuals was 72.31 (9.09) years, and the mean (SD) age at examination (AAE) was 74.82

**Abbreviations:** AAE, age at examination; AAO, age at onset; APL, association in the presence of linkage; CAP, Collaborative Alzheimer Project; HWE, Hardy-Weinberg equilibrium; IU, Indiana University Medical Center; LD, linkage disequilibrium; NIMH, National Institutes of Mental Health; PDT, pedigree disequilibrium test; SNP, single nucleotide polymorphism

**Table 1** Description of family dataset

Family type	Total families	CAP families	NIMH families	IU families	Discordant sibling pairs	Affected relative pairs
Multiplex (n)	580	87	349	124	1111	1153
Singleton (n)	158	78	3	29	161	0

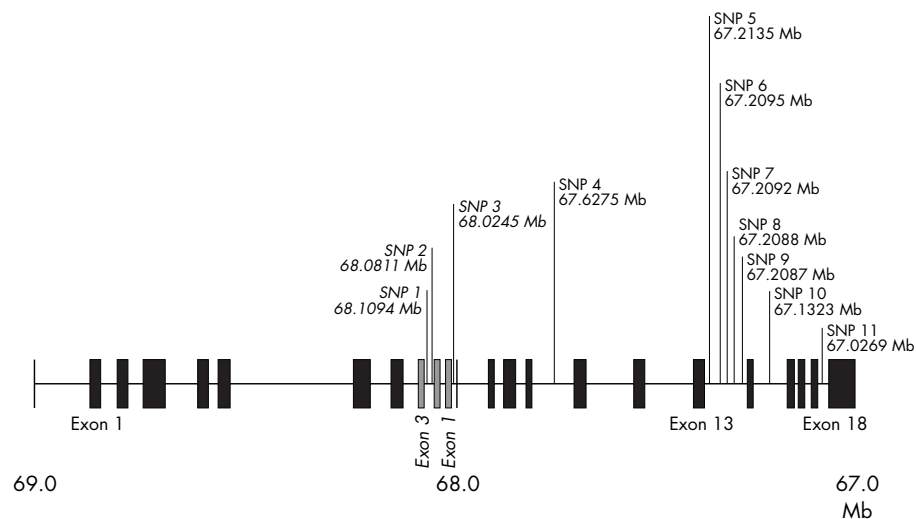
CAP, Collaborative Alzheimer Project; IU, National Cell Repository for Alzheimer's Disease at Indiana University Medical Center; NIMH, National Institutes of Mental Health.

(11.75) years. A positive family history of Alzheimer's disease was reported for 82% of the families.

The case-control sample consisted of 584 unrelated Alzheimer cases ascertained through the CAP and IU and 858 unrelated controls collected through the CAP. Cases had an average AAO of 71.14 (6.66) years and controls were age matched at the time of examination, with an average AAE of 71.94 (6.29) years. The cases were predominantly sporadic, with less than 4% reporting a positive family history of Alzheimer's disease in first degree relatives. All cases met the NINDS/ADRDA criteria for probable or definite Alzheimer's disease.<sup>13</sup> Controls had no obvious signs of cognitive or neurological impairment when enrolled in the study, as determined by personal interview by clinical personnel at the ascertainment site. All individuals included in the family and case-control analyses were white. Written consent was obtained from all participants, in agreement with protocols approved by the institutional review board at each contributing centre.

### SNPs and genotyping

We studied 11 SNPs for association with Alzheimer's disease (fig 1). These SNPs included six (SNP5-SNP9 and SNP11) that were studied previously by Ertekin-Taner *et al*<sup>8</sup> and two (SNP4 and SNP10) selected from public databases. Intron 7 of the VR22 gene contains another gene, the leucine-rich repeat transmembrane neuronal 3 (LRRTM3) gene, which encodes a brain specific protein expressed in the hippocampus. Three of the SNPs lie in the LRRTM3 gene (SNP1-SNP3, fig 1). We extracted DNA for individuals ascertained by CAP with the Puregene system (Gentra Systems, Minneapolis, Minnesota, USA). SNPs were genotyped using the ABI 7900 Taqman<sup>®</sup> system.<sup>14</sup> APOE alleles (corresponding to allele combinations at SNP +3937/rs429358 and SNP +4075/rs7412) were genotyped as previously reported.<sup>15</sup> Genotyping efficiency was greater than 90%, and quality control was achieved by including two sets of 12 control samples and four sets of two standard samples on each 384 well plate. The



Marker	SNP ID	Alternative IDs	Allele frequencies (nucleotides)
SNP1	rs1925617	cv11735825	0.43/0.57 (G/T)
SNP2	rs942780	cv8934576	0.20/0.80 (C/T)
SNP3	rs1925583	cv11735884	0.45/0.55 (T/G)
SNP4	rs997225	cv8040922	0.21/0.79 (A/G)
SNP5	rs6480140	cv11295092	0.39/0.61 (C/A)
SNP6	rs7074454	cv3096478	0.37/0.63 (T/C)
SNP7	rs7070570	4360*	0.28/0.72 (G/A)
SNP8	rs12357560	4783*	0.23/0.77 (G/A)
SNP9	rs7911820	4825*	0.37/0.63 (A/C)
SNP10	rs2126750	cv3096510	0.35/0.65 (T/A)
SNP11	rs1786927	cv1380042	0.41/0.59 (A/G)

\* From Ertekin-Taner *et al* 2003

**Figure 1** SNPs analysed in VR22 and LRRTM3 genes on chromosome 10. Locations are from NCBI Build 34. VR22 exons are black and SNPs non-italic, LRRTM3 exons are in grey, and SNPs in italics.

laboratory was blinded to family relations, affection status, and quality control samples.

### Statistical analysis

Tests for deviations from Hardy–Weinberg equilibrium (HWE) were conducted in unrelated cases, unrelated controls, and families (selecting one affected individual and one unaffected individual per family) using the exact test from the Genetic Data Analysis software.<sup>16</sup> Measures of linkage disequilibrium (LD) were computed with GOLD.<sup>17</sup> We report the squared correlation coefficient ( $r^2$ ) and the normalised disequilibrium coefficient ( $D'$ ) between all pairs of SNPs.

Family based association analysis for Alzheimer's disease risk was conducted with the pedigree disequilibrium test (PDT) and genotype-PDT.<sup>18–20</sup> Haplotype association analysis was conducted for pairs of markers in families using the test for association in the presence of linkage (APL).<sup>21</sup> Haplotypes with frequencies  $<0.05$  were not used in the analysis. Single point parametric linkage analysis was conducted using Fastlink<sup>22</sup> under affected-only dominant and recessive models. Disease allele frequencies were 0.001 and 0.20 for dominant and recessive models, respectively. Marker allele frequencies were estimated in the family dataset. We used the HOMOG program to test for linkage in the presence of heterogeneity.<sup>23</sup> All tests were conducted in the overall sample and conditional on APOE genotype. For analysis in APOE-4 carriers, all affected individuals not carrying APOE-4 were reclassified as having an unknown phenotype. For analysis of individuals without APOE-4, all affected individuals who have an APOE-4 allele were reclassified as unknown.

Case–control analysis was conducted in the independent unrelated case–control sample using SAS<sup>24</sup> (SAS Institute Inc, Cary, North Carolina, USA). We carried out a logistic regression including terms for AAE, sex, and SNP genotype. Two models were considered to dichotomise SNP genotypes: one testing carriers versus non-carriers of the major allele, denoted as 1 (11+12  $\nu$  22); and the other testing carriers versus non-carriers of the minor allele, denoted as 2 (22+12  $\nu$  11). Case–control tests were conducted in the overall case–control sample and in the sample stratified by APOE genotype. Case–control analyses were conducted in two stages; thus sample sizes used in analyses vary for different SNPs. Initially five SNPs (SNP4–SNP7 and SNP10) were genotyped in a sample of 381 cases and 326 controls. The remaining six SNPs were genotyped later in the enlarged overall case–control dataset described above.

Tests for interaction were conducted in the unrelated case–control sample. We tested SNP genotypes for interaction with APOE genotypes for any SNP that was found significant in the case–control analyses described above. Logistic regression was used, adjusting for AAE and sex. We used genotype

coding for the SNPs that led to significant main effects and considered carriers of APOE-4  $\nu$  non-carriers to code APOE genotype. We included terms for AAE, sex, SNP genotype code, APOE genotype code, and (SNP $\times$ APOE).

## RESULTS

### Hardy–Weinberg and linkage disequilibrium analyses

We found no evidence for Hardy–Weinberg disequilibrium in the samples of cases, controls, or families. Pairwise LD measures are shown in table 2 for unaffected siblings (one per family). LD measures were similar in affected and unaffected individuals from families and from the unrelated case–control dataset. We found a strong correlation ( $r^2 \geq 0.77$ ) between alleles at SNP6 and SNP9, and between SNP7 and SNP8. All other marker pairs showed  $r^2$  values smaller than 0.3. Values of  $D'$  indicated that SNP6–SNP10 form a block with  $D' \geq 0.87$ , suggesting there has been little ancestral recombination in the region bounded by these markers. Other markers showed little evidence of LD, in particular SNP1–SNP3 in LRRTM3 showed very little LD with VR22 markers by both  $r^2$  and  $D'$  measures.

### Family based analyses

Results from family based tests of association are given in table 3. The PDT gave significant results for SNP6 ( $p = 0.011$ , allele C positively associated) and SNP9 ( $p = 0.028$ , allele C positively associated) in the overall sample, showing a significant association with Alzheimer's disease. The genotype-PDT (GenoPDT) showed evidence of genotype association with Alzheimer's disease for SNP1 in LRRTM3 ( $p = 0.002$ ). Examination of individual genotypes at SNP1 shows that the TT genotype is significantly more frequent in affected siblings than in unaffected siblings ( $p = 0.007$ ).

Considering the known APOE-4 effect on Alzheimer's disease risk, we conducted a conditional PDT. In the first analysis we considered only individuals without an APOE-4 allele (107 informative families). In the second analysis, we considered only individuals who are carriers of APOE-4 (185 informative families). The results are given in table 3. We found that significant association was restricted to the stratum of APOE-4 carriers, with SNP6 and SNP9 showing significant association consistent with results in the overall dataset.

Haplotype analysis at pairs of SNPs gave significant global test results in the overall dataset for SNP10–SNP11 ( $p = 0.037$ ) and SNP7–SNP4 ( $p = 0.032$ ). The result for SNP10–SNP11 increased in significance ( $p = 0.010$ ) in individuals carrying APOE-4, and pairs containing SNP9–SNP11 ( $p = 0.044$ ) and SNP7–SNP11 ( $p = 0.043$ ) also became significant in that subset. No global haplotype tests were significant in individuals not carrying APOE-4. Examination of individual haplotype results showed that the most significantly associated haplotype for pairs was for the A-T

**Table 2** Pairwise linkage disequilibrium measures,  $r^2$  (above diagonal) and  $D'$  (below diagonal), in unaffected individuals

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11
SNP1		0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SNP2	0.10		0.07	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01
SNP3	0.05	0.55		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
SNP4	0.12	0.02	0.11		0.00	0.02	0.01	0.01	0.02	0.01	0.01
SNP5	0.01	0.10	0.02	0.06		0.05	0.10	0.17	0.06	0.01	0.00
SNP6	0.01	0.12	0.01	0.22	0.24		0.21	0.16	0.97	0.30	0.05
SNP7	0.09	0.20	0.03	0.10	0.67	0.98		0.77	0.20	0.17	0.02
SNP8	0.01	0.20	0.01	0.07	0.97	0.97	0.98		0.16	0.14	0.02
SNP9	0.00	0.09	0.04	0.19	0.25	0.99	0.97	0.98		0.29	0.05
SNP10	0.07	0.04	0.07	0.29	0.10	0.97	0.87	0.89	0.97		0.01
SNP11	0.02	0.15	0.11	0.17	0.03	0.24	0.25	0.29	0.26	0.13	

**Table 3** Probability (p) values for the pedigree disequilibrium test (PDT) and genotype-PDT (GenoPDT) in the overall dataset and the PDT conditional on APOE genotype in the family dataset

Marker	Overall dataset		PDT stratified by APOE genotype	
	PDT	GenoPDT	No APOE-4 (107 families, 253 DSPs)	Carriers of APOE-4 (185 families, 477 DSPs)
SNP1	0.492	<b>0.002</b>	0.125	0.653
SNP2	0.099	0.294	0.205	0.291
SNP3	0.531	0.835	0.228	0.612
SNP4	0.662	0.567	0.560	0.502
SNP5	0.558	0.133	0.679	0.523
SNP6	<b>0.011</b>	0.077	0.827	<b>0.014</b>
SNP7	0.114	0.107	0.655	0.347
SNP8	0.099	0.065	0.945	0.614
SNP9	<b>0.028</b>	0.123	0.473	<b>0.013</b>
SNP10	0.160	0.329	0.366	0.109
SNP11	0.925	0.962	0.562	0.305

DSP, discordant sibling pair.

haplotype at SNP10 and SNP11 ( $p = 0.004$  in APOE-4 carriers and  $p = 0.037$  in the overall family dataset), respectively. This haplotype (with frequency of approximately 0.14) was observed more often in affected individuals than expected by chance, indicating a positive association with disease.

Though our primary focus was on detecting association, we also looked for evidence of linkage at the VR22 SNPs in our sample. Testing for linkage in the presence of heterogeneity, we obtained a maximum two point LOD (log of odds) score of 1.29 under a dominant model for SNP7 in the overall sample. Consistent with our conditional PDT analysis above, the linkage evidence increased in individuals carrying APOE-4. Specifically, for SNP7 the LOD score for the dominant model increased from 1.29 in the overall sample to 2.78 in APOE-4 carriers, for SNP6 it increased from 0.74 in the overall sample to 1.40 in APOE-4 carriers, and for SNP4 it increased from 0.35 in the overall sample to 1.42 in APOE-4 carriers.

### Case-control analyses

Using the independent set of unrelated cases and controls, we did not find significant evidence of association in the overall sample. However, when cases and controls were stratified based on APOE genotype, significant ( $p < 0.05$ ) evidence of association was found for four SNPs (table 4). SNP8 was the only marker significant in the stratum with no APOE-4 alleles (odds ratio (OR) = 4.67 for carriers of allele A (95% confidence interval (CI), 1.08 to 20.19),  $p = 0.039$ ). In the stratum with carriers of APOE-4, significant association was found for SNP3 (OR = 1.57 (1.05 to 2.35) for carriers of allele T;  $p = 0.030$ ), SNP4 (OR = 2.21 (1.14 to 4.32) for carriers of allele A;  $p = 0.020$ ), and SNP6 (OR = 2.12 (1.01 to

4.45) for carriers of allele C;  $p = 0.047$ ). The results at SNP6 are consistent with the family based analyses, showing positive association of carriers of the C allele with Alzheimer's disease.

### Tests for genetic interactions

Because of the differences observed when we stratified by APOE genotype, we conducted tests for interaction between SNP genotypes and APOE-4 carrier status in unrelated cases and controls. We conducted tests for interaction with APOE for the four SNPs with significant results in the stratified case-control analyses (SNP3, SNP4, SNP6, and SNP8). SNP4 and SNP8 both showed significant evidence of interaction with APOE-4 carrier status, with logistic regression adjusted for AAE and sex (table 5).

For a better understanding of the nature of the interactions, we examined the SNP genotype frequencies in the case-control sample stratified by APOE genotype. Table 5 shows the unadjusted SNP genotype frequencies from the stratified analyses reported in table 4. For SNP4, genotypes with the A allele were significantly more frequent in cases than in controls in carriers of APOE-4, but the trend was reversed in individuals without the APOE-4 allele. For SNP8, carriers of the A allele occurred significantly more often in cases than in controls in individuals without an APOE-4 allele, but again the trend was reversed in carriers of the APOE-4 allele.

The test for interaction with SNP6 was close to significant but did not reach our nominal level of  $p < 0.05$ . However, this result is still notable because SNP6 was the only marker with significant results across both family and case-control

**Table 4** Probability (p) values for case-control tests in the independent case-control dataset stratified by APOE genotype

Marker	No APOE-4 (189 cases, 619 controls)		Carriers of APOE-4 (395 cases, 239 controls)	
	11+12 v 22	22+12 v 11	11+12 v 22	22+12 v 11
SNP1	0.070	0.409	0.505	0.697
SNP2	0.395	0.988	0.103	0.580
SNP3	0.693	1.000	0.812	<b>0.030</b>
SNP4	0.563	0.754	0.880	<b>0.020</b>
SNP5	0.414	0.221	0.348	0.901
SNP6	0.515	0.348	<b>0.047</b>	0.707
SNP7	0.728	0.472	0.820	0.545
SNP8	<b>0.039</b>	0.488	0.322	0.545
SNP9	0.827	0.182	0.883	0.703
SNP10	0.363	0.478	0.234	0.505
SNP11	0.265	0.589	0.937	0.910

Tests were conducted for genotypes 11+12 v 22 and for 22+12 v 11, where 1 indicates the major allele.



**Table 5** Tests for interaction with APOE-4 and stratum specific genotype frequencies in unrelated cases and controls

Marker	Test of interaction (p value)	SNP genotype	Genotype frequencies No APOE-4		Genotype frequencies, carriers of APOE-4	
			Cases (n=189)	Controls (n=619)	Cases (n=395)	Controls (n=239)
SNP3	0.150	GT+TT	0.68	0.69	0.72	0.64*
SNP4	<b>0.048</b>	AA+AG	0.39	0.43	0.45	0.32*
SNP6	0.057	CC+CT	0.85	0.87	0.87	0.80*
SNP8	<b>0.027</b>	AA+AG	0.99	0.95*	0.92	0.95

\*Significant difference in genotype frequencies between cases and controls within APOE-4 stratum ( $p < 0.05$ ).

analyses. Comparison of SNP genotype frequencies (table 5) shows that genotypes carrying the C allele were more frequent in cases than in controls among carriers of APOE-4, but less frequent among non-carriers. Though the trends were reversed in APOE-4 carriers and non-carriers, the test for interaction was not statistically significant. However, the trends are consistent with the frequencies observed in the family data. Among carriers of APOE-4, genotypes with the C allele were more frequent in affected siblings than in unaffected siblings (90% in affected  $v$  85% in unaffected). Among siblings without APOE-4, carriers of C were less frequent in affected siblings than in unaffected siblings (83% in affected  $v$  87% in unaffected).

## DISCUSSION

This study provides the first evidence of a direct effect of the VR22 gene on risk for Alzheimer's disease. We have examined several SNPs for association with Alzheimer's disease in a large family sample and an independent sample of unrelated Alzheimer cases and controls. Though several of the SNPs showed association in either the family based or case-control analyses, the most consistent findings were with SNP6, which showed significant evidence of association in the families and in the unrelated case-control sample.

Taken individually, our results are not overwhelmingly significant and certainly would not hold up to a correction for multiple comparisons; however, the compilation of evidence from multiple independent sources lends strength to the possible role of VR22 in Alzheimer's disease. VR22 is both a strong locational and functional candidate. Our previous genomic screen in 466 Alzheimer families identified peak linkage on chromosome 10 at a marker D10S1211 (MLOD = 1.41) lying only 1.1 Mb from the VR22 gene<sup>25</sup>; and others have identified linkage nearby,<sup>2-26</sup> making VR22 a good locational candidate. Additionally, the role of VR22 in cell adhesion and possible interaction with presenilin 1<sup>27</sup> make it a strong functional candidate for involvement in Alzheimer's disease.

Further support is given by the consistency of our results with the previous study of the relation of VR22 to A $\beta$ 42 levels.<sup>8</sup> That study identified SNP6 as being associated with plasma A $\beta$ 42 levels in two sets of Alzheimer pedigrees. We have confirmed that the same marker is associated with Alzheimer status in a large sample of families, and in addition that it shows association in an independent sample of Alzheimer cases and unrelated controls. We were, however, unable to find association with other SNPs tested by Ertekin-Taner *et al.*<sup>8</sup> Specifically, their most significant SNP (SNP7) showed no evidence of association with Alzheimer's disease in any of our analyses, while SNP8, which also showing significant association with A $\beta$ 42 levels, was significant only in our case-control analysis. These discrepancies could be explained by the differences in the phenotypes studied, or by the large sampling variability that would be expected considering that the previous study involved samples of 22 pedigrees or fewer.

Interestingly, our significant findings were concentrated in individuals carrying APOE-4 alleles, and in fact two of the SNPs showed significant evidence of interaction with the APOE genotype. This suggests that the effect of the VR22 SNPs is dependent on APOE status. Inspection of SNP genotype frequencies among carriers and non-carriers of APOE-4 in table 5 shows that for SNP4 and SNP6, SNP genotype frequencies were similar in cases and controls without APOE-4 and in cases with APOE-4; however, the frequency in controls carrying APOE-4 differs. This suggests a possible protective effect of the SNPs, or a polymorphism in linkage disequilibrium with them, among individuals with APOE-4. The trends for SNP8 (table 5) do not offer the same simple interpretation, but the low frequency of the GG genotype makes the results at this marker difficult to judge.

The dependence on APOE-4 is also reflected in our linkage analysis of the VR22 SNPs, in which the maximum LOD score at the SNPs was strongest when only carriers of APOE-4 were used in the analysis. These results are consistent with other linkage screens, which found the evidence of linkage in this region to be stronger in affected siblings who shared APOE-4 than in those who did not in overlapping family samples.<sup>2-26</sup> However, despite the higher LOD score in the APOE-4 positive group, Myers *et al.*<sup>2</sup> found that the ibd estimates in the two groups were similar, showing increased sharing. They suggested this meant that their linkage did not depend on sharing at APOE, but they provided no conclusive evidence of linkage in the APOE-4 negative subset. Still it is possible that the VR22 polymorphisms act only in individuals carrying APOE-4, while other polymorphism in VR22 or other genes act in an APOE independent fashion. Only 15% of our multiplex family sample overlap with Myers *et al.*,<sup>2</sup> so genetic heterogeneity could lead to discrepancies in the results.

Other studies have implicated regions of linkage further telomeric.<sup>3-4</sup> There is evidence that this region may harbour genes contributing to risk or age of onset of Alzheimer's disease. Our own study found evidence that the GSTO1 gene (10q25.1) is associated with age of onset.<sup>5</sup> This gene is more than 38 Mb away from VR22 and shows no evidence of linkage disequilibrium with VR22 SNPs; thus it is likely that these represent distinct regions, each involved in Alzheimer's disease aetiology.

The polymorphisms that we studied are all intronic with no known function. Thus it is unlikely that they directly affect Alzheimer's disease risk. Instead it is more likely that an as yet undiscovered variant or variants lead to the observed association. The analysis of linkage disequilibrium by Ertekin-Taner *et al.*<sup>8</sup> suggests that the association does not extend beyond the boundaries of the VR22 gene. However, we cannot rule out the involvement of the nested LRRTM3 gene contained in intron 7 of the VR22 gene. The LRRTM3 gene encodes a leucine-rich repeat that tends to form the amyloid fibrils that compose Alzheimer's disease amyloid plaques. The LRRTM3 protein is a brain specific protein expressed in the hippocampus. We did find some evidence of association at SNPs within LRRTM3, though these were not our most significant findings. While it is possible that these

results simply reflect association with VR22 polymorphisms, we found little linkage disequilibrium between LLRTM3 SNPs and SNPs in VR22 in our samples.

## Conclusions

Our results, combined with previous findings, support the involvement of the VR22 gene or a nearby gene in Alzheimer's disease. As we do not have data on plasma A $\beta$ 42 levels in our samples, we cannot rule out the possibility that the association merely reflects association with A $\beta$ 42 levels. Further biological studies are required to distinguish the effect. Our results do strongly point to the importance of taking into account APOE genotype as we try to understand the role of this gene in Alzheimer disease.

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