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No association between the alpha-2 macroglobulin I1000V polymorphism and Alzheimer's disease

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Abstract

Recent reports have suggested that variability in the α2-macroglobulin gene is a genetic risk factor for Alzheimer's disease. Here we have both tested a common polymorphism in the gene (I1000V) for association with the disease in a four-site case control study design, and tested the locus for linkage in a large series of sibpairs afflicted with late onset disease. Our results fail to show an association between this polymorphism and disease.

Keywords: Alzheimer's disease; Genetics; α2-macroglobulin; Apolipoprotein E

Apolipoprotein E (ApoE) is a major genetic risk factor locus for late-onset Alzheimer's disease [4]. However, it does not explain all the risk for the development of this form of the disease. As part of a strategy to find other genetic risk factors, several groups have reported the use of non-parametric linkage searches in families with late onset AD. Each of these studies has reported some evidence for a locus on the short arm of chromosome 12 [9,12,15]. However, the precise position of the linkage peak in each case has been different, probably because of the inherently poor resolution of the genetic analysis.

There are two attractive candidate genes for Alzheimer loci on this chromosome: the LRP receptor gene and the α2 macroglobulin (α2M) gene. LRP is the predominant receptor for ApoE in the brain [14] and α2M is another ligand for this receptor as well as being a serum pan-protease inhibitor [3,13]. Recently, an association between an intronic deletion polymorphism in the α2M gene [10,11] and AD has been reported [2]. We have been unable to confirm this association between this intronic polymorphism in a large case control series (unpublished data). However, there is a coding variant of the α2M gene: an I1000V variant, which has an allele distribution of ~70%/30% in Caucasian populations [10,11]. Therefore we determined to examine whether this polymorphism showed an association with disease in the same case control series. In particular, we considered it possible that this I1000V mutation was the biologically relevant polymorphism and that the intronic

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polymorphism was in linkage disequilibrium with this in some, but not all populations. The case control series we used for our analysis comprised four independent samples of AD patients with onset over 50 years and relevant controls (these controls were a mixture of population controls and the spouses of affected individuals) (see Table 1). AD patients were diagnosed according to NINCDS-ADRDA [8] criteria with either probable or definite AD. We also examined whether this polymorphism showed genetic linkage to late-onset AD in a large series of sibpairs. These sibpairs comprised a series of 266 sibpairs whose collection was funded by the National Institute of Mental Health [1] and a similar series of sibpairs collected through the auspices of the National Institute on Aging at the Indiana Alzheimer cell repository (131 sibpairs). The former series overlaps with that reported by Blacker et al. [1]. In addition, within the sibpairs, we tested whether the allele frequencies were similar in the affected probands and their unaffected sibs.

The I1000V polymorphism was detected following standard PCR and RFLP methods (see Table 1). The genotype distributions of the polymorphism in relation to APOE status are shown in Table 1. Pearson's chi-square was used to test for association. The frequencies were not different between the AD cases and the controls in the sporadic samples, or in the familial samples (sibpair series). We failed to observe an association between AD and possession of at least one copy of the polymorphism (all $P > 0.1$). As Blacker et al. [1] observed a stronger effect in individuals without an ApoE4 allele, we stratified our sample according to ApoE4 status. We found no evidence of either a genotypic or allelic association in those with no ApoE4 alleles (all $P > 0.2$).

We then did a linkage study on the whole set of sibpairs using the program SPLINK to compute single-point maximum lod scores under 'possible triangle' restrictions [5,6]. This linkage data is shown in Table 2. The lod scores were marginally, though not significantly, positive and consistent with the data we have previously published for this chromosomal region [15].

These data, taken together with our data concerning the intronic deletion polymorphism suggest that α2M locus is not a risk factor locus for AD. The two simplest explanations are that the original report of an involvement of α2M constituted a type 1 statistical error (false positive) or that our failure to find an association in this series constitutes a type 2 error (false negative). However, both studies are statistically powerful and so other possibilities should also be considered. Chief among these is the fact that the ascertainment in the linkage studies is different from ascertainment in case control studies. In the linkage studies reported by Blacker et al., the genetic linkage strategy used looks for genetic differences in multiply affected families between affected and unaffected sibs. While this study was under revision, in a follow-up paper, Liao et al. [7] reported an association between the V1000 polymorphism and disease in a case control study of similar size to that we report here.

Table 1

Genotype distributions of the α2 macroglobulin gene. Genotype distributions of the I1000V polymorphism in the four-case control series and in the sibpairs used in the linkage series of experiments. The latter were not pooled with the former. The cases were diagnosed using NINCDS/ADRDA criteria. In the case/control series, the controls were either spouse controls or elderly unaffected persons from the same population. The linkage series consisted of data derived from a proband (AD case) and a random unaffected sib ('Control'). The primers used to generate the data had the sequences 5′GAGACATATTAGGCTCTGCC and 5′CAGTGTTGAGATAGCCAATG. Use of these primers generates a fragment of 180 bp. Digestion with *Sau3A1* or *DpnII* cuts allele 1 (I1000) to yield 140 bp/40 bp fragments: allele 2 (V1000) is not cut. The genotype frequencies (whole dataset) were: controls, 1/1, 42%; 1/2, 46%; 2/2, 12%; AD cases, 1/1, 44%; 1/2, 46%; 2/2, 10%. Allele frequencies are controls: 1, 65%; 2, 35% and AD cases: 1, 67%; 2, 33%.

APOE	Total			E4 +			E4 –		
A2M	1/1	1/2	2/2	1/1	1/2	2/2	1/1	1/2	2/2
Cardiff									
AD	39	64	16	25	37	9	14	27	7
Controls	41	56	17	13	12	5	28	44	12
Jacksonville									
AD	143	141	45	80	81	24	62	60	21
Controls	200	226	61	52	43	16	148	183	45
St. Louis									
AD	56	66	14	21	39	6	35	27	8
Controls	59	72	14	12	23	5	47	49	9
Lille									
AD	290	275	51	171	154	31	119	121	20
Controls	289	283	76	65	55	12	224	228	64
Total									
AD	528	546	126	297	311	70	230	235	56
Controls	589	637	168	142	133	38	447	504	130
Sibpairs									
AD	72	50	15	57	38	13	15	12	2
Controls	60	60	17	31	35	4	29	26	13

Table 2

Linkage data in sibpair series between I1000V polymorphism and Alzheimer's disease in 397 sibpairs. Linkage data in 397 sibpairs computed using the SPLINK program [5,6].

	Whole	E4+	E4–
Log-likelihood Ratio-chi-squared	1.67	1.78	0.01
	($P = 0.1$)	($P = 0.12$)	($P = 0.5$)
Equivalent to a lod score	0.4	0.4	0

They report a modest, but statistically significant increase in cases homozygote for the V1000 allele and a modest, but not statistically significant increase in the frequency of this allele in AD cases. In contrast, we find a modest, but not statistically significant decrease in both the proportion of V1000 homozygotes and in the frequency of the V1000 allele in cases. We regard our failure to find an association between the V1000 polymorphism and AD as evidence that α2M is not a locus of major effect in AD, though it remains possible that there are protective alleles of α2M which are found in the unaffected sibs of families multiply affected by AD. Exhaustive sequencing of the locus will be required to determine whether this is the case.

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