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Association between an α_2 Macroglobulin DNA Polymorphism and Late-Onset Alzheimer's Disease

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An association between a five-base-pair deletion/insertion DNA polymorphism at the α_2 macroglobulin gene (A2M) and late-onset Alzheimer's disease (LOAD) has been recently described. We developed a PCR assay to analyze this polymorphism in 190 LOAD patients (older than 65 years) and 400 controls from Spain. Controls were stratified into three groups: <65 years (n = 200), 65 to 80 years (n = 100), and 81 years or older (n = 100). We found a significantly higher frequency of carriers of the D allele in patients older than 81 years compared to controls older than 81 years (p = 0.0012). In addition, the frequency of the D allele was significantly lower in controls older than 81 years compared to controls younger than 65 (p = 0.048). Our work suggests that the D allele confers an age-dependent increased risk to develop late-onset Alzheimer's disease.

Polymorphisms in the genes that encode proteins involved in amyloid-β (Aβ) production and secretion have been associated with an increased risk for the late-onset form of Alzheimer's disease (LOAD). The best known of these is a polymorphism at the apolipoprotein E gene, with the ApoE-E4 allele being at a significantly higher frequency among LOAD patients (1–5). Recently, by employing a candidate gene approach Blacker *et al.* described an association between a 5 bp deletion/insertion (D/I) polymorphism at the α_2 macroglobulin gene (A2M) and LOAD (6). α_2 macroglobulin is a serum protease inhibitor expressed in brain, that binds Aβ mediating its clearance and degradation, thus preventing the accumulation and deposition of Aβ. In addition, the A2M-gene maps to chromosome 12p, where linkage evidence for a susceptibility locus for LOAD has been reported (7). The association between the A2M polymorphism and LOAD was confirmed by some authors (8), while others did not find such association (9, 10). To define the role of the A2M polymorphism in the risk for LOAD, we genotyped 400 healthy controls and 190 patients.

METHODS

A total of 190 consecutive LOAD patients were recruited at the Neurology Departments of the five major Hospitals from the region of Asturias (Northern Spain, population 1 million). All the patients fulfilled the NINCDS–ADRDA criteria for clinical probable Alzheimer's disease. All the patients were older than 65 years (average age 72 years ± 9.14), and were divided into two groups, according to the age at onset: 65 to 80 years (n = 122) and 81 years or older (n = 68). We also analyzed a total of 400 healthy controls from the same population. These controls were Hospital staff, blood bank donors, or eligible residents from Asturias, and gave their consent to participate in this study. Controls were recruited as part of an analysis of genetic factors involved in the risk of cardiovascular and Alzheimer's diseases (11). Controls were stratified into 3 groups, <65 years (n = 200), 65 to 80 (n = 100), and 81 or older (n = 100). We excluded the presence of clinical symptoms of Alzheimer's disease in controls older than 65 years through a Minimental test.

DNA was obtained from patients and controls. For the analysis of the 5-bp deletion/insertion polymorphism at the 5′ splice site of exon 18 in the A2M gene, a 170-bp sequence was amplified with primers CTTTCCTTGATGACCCAAGCGCC (forward) and CATGGCCTCTTCCCATTACATCTGACT (reverse; annealing at 68°C, 30 cycles). Reactions also contained 0.1 μCi of [^{32}P]dCTP, and were electrophoresed on a 6% polyacrylamide (sequencing) gel, followed by autoradiography. Figure 1 shows the three genotypes for this polymorphism. Patients and controls were also genotyped for the ApoE-polymorphism as previously described (12).

Genotypes and allele frequencies were compared through a χ^2 test. The relative risk of Alzheimer's disease was estimated as an odds ratio (OR), and the 95% confidence interval (95% CI) was also calculated. For the statistical analysis we used the computer program BMDP-New Systems (BMDP Statistical Software, Cork, Ireland).

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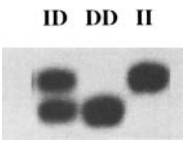

FIG. 1. Autoradiography showing the three genotypes for the A2M-I/D polymorphism.

RESULTS

Using our method for the analysis of the A2M-polymorphism we were able to unambiguously define the genotype for each patient and control (Fig. 1). Genotype and gene frequencies are summarized in Table 1. Among the healthy controls, the frequency of carriers of the D allele decreased from 0.33 in the youngest group (<65 years, $n = 200$) to 0.22 in the oldest group (81 years or older, $n = 100$) ($p = 0.048$).

Carriers of the D allele were at a significantly higher frequency in patients older than 81 years, compared to controls older than 81 years ($p = 0.0012$) (Table 1). In the group aged 65 to 80 years, the difference between patients and controls was not significant ($p = 0.23$). Taken together, our data suggest that the A2M-D allele is associated with an increased risk to develop late onset Alzheimer's disease. However, this association was age dependent, and DD/ID individuals would have an increased risk to develop LOAD as they become older.

The frequency of the A2M-D allele was higher in patients without the APOE4-allele compared to those who were E4-carriers (29% vs 24%, respectively). However, this was a non-significant difference (data not shown).

DISCUSSION

According to our results, the A2M-D allele is associated with an overall increased risk to develop Late Onset Alzheimer's Disease (OR = 1.46; 95% CI = 0.94, 2.27; patients and controls aged 65 years or older). However, this risk would be age-dependent. Gene and genotype frequencies did not differ between patients and healthy controls aged 65 to 80 years, but the difference between healthy controls and patients older than 81 years was highly significant; as was the difference between controls younger than 65 years and 81 years or older, or between patients aged 65–80 and older than 81 years. These data suggest than in our population, carriers of the A2M-D allele have an increased risk to develop Alzheimer's disease as they become older. It is possible that the A2M-polymorphism is associated with the development of the disease at older ages, compared with polymorphisms at other genes, that would confer a higher risk to develop the disease at younger ages. Similarly, some authors have suggested that the risk conferred by the APOE-E4 allele would be age-dependent (13).

At least four recent reports have analyzed the association between the A2M polymorphism and LOAD. Blacker *et al.* and Rudrassingham *et al.* reported an association between the A2M-D allele and LOAD in a sample of affected and unaffected siblings from families segregating the disease (6, 8). In contrast, Dow *et al.* and Rogaeva *et al.* failed to confirm such association by comparing cases and controls from several populations worldwide (9, 10). However, in these two case-control studies neither patients nor controls were stratified by age. In our population, the association between A2M-D and LOAD is only apparent when we compared patients and healthy controls older than 81 years. In agreement with Dow *et al.* and Rogaeva *et al.* we only find a marginal difference when total patients and controls (aged 65 years or older) were compared ($p = 0.079$; OR = 1.46).

In conclusion, our results indicate that the A2M D allele is associated with an increased risk for LOAD. Our data also suggest that the risk would be age dependent, with those individuals carrying the D allele having a higher risk of developing AD as they become older. This effect is confirmed by the fact that the frequency of this allele is lowest among the oldest healthy controls. Because α_2 macroglobulin is present in senile plaques, binds amyloid, and can attenuate amyloid toxicity, any polymorphism at the A2M gene is a strong candidate for conferring risk of LOAD. The A2M-D allele could be less effective in the clearance of amyloid, thus defining a higher risk of developing develop AD for carriers of this allele. In addition to the I/D polymorphism, other mutations at the A2M-gene could contribute to the risk of developing LOAD (14).

TABLE 1

Distribution of A2M and apoE Genotypes in Patients (Pats.) and Controls (Ctls.), Distributed by Age Groups

		Genotypes			
Age		II	ID	DD	ID + DD
<65	Ctls.	134 (68)	62 (31)	4 (2)	66 (33)*
65–80	Ctls.	65 (65)	33 (33)	2 (2)	35 (35)#
	Pats.	83 (68)	35 (29)	4 (2)	39 (32)
>81	Ctls.	78 (78)	20 (20)	2 (2)	22 (22)[&]
	Pats.	37 (55)	25 (37)	6 (8)	31 (40)

Note. Parentheses indicate percentages.
* $p = 0.048$, ctls. <65 vs ctls. >81.
# $p = 0.23$, ctls. vs pats. 65–80.
[&] $p = 0.0012$, ctls. vs pats. >81.

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