Brief communication

A TAP2 genotype associated with Alzheimer’s disease in APOE4 carriers

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Abstract

Sporadic Alzheimer’s disease (AD) appears to be the consequence of the interaction between combinations of genes and environmental factors. Binding with the transporter associated with antigen processing (TAP) is thought to be the main way in which herpes simplex virus type 1 (HSV-1) evades immune surveillance. Several TAP gene polymorphisms were examined and a TAP2 SNP (rs241448) associated with AD found in two independent case–control samples, especially in carriers of the APOE4 allele. These findings are consistent with the hypothesis that human genetic variants facilitating the access of HSV-1 to the brain might result in susceptibility to AD.

Keywords: Alzheimer’s disease; Genetic risk factor; TAP; APOE; Interaction

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by deteriorating cognitive ability in later life. Its worldwide prevalence is about 1% in 60–64-year olds, rising to approximately 50% by age 95 [11]. The vast majority of AD cases have a complex etiology, with multiple genetic and environmental factors influencing pathogenesis. The e4 allele of the apolipoprotein E gene (APOE4) is the main genetic risk factor for sporadic AD [4,8], although the mechanisms responsible for this association are not well understood. It has been reported that the possession of APOE4 plus the presence of the herpes simplex virus type 1 (HSV-1) genome in the brain confers an increased risk of developing AD compared to the possession of APOE4 alone [12]. We have reported that some APOE promoter polymorphisms lead to variations in transcriptional activity that result in increased susceptibility to AD [5]. In addition we have recently reported the involvement of apoE in the hematogenous route of HSV-1’s access to the central nervous system, the APOE dose being directly linked to the degree of neuroinvasiveness [6], and apoE4 being more efficient than apoE3 in allowing the virus access to the brain [7].

Given this background, the present work used genetic association to test the hypothesis that human genes important in HSV-1 biology participate in AD pathogenesis. The transporters associated with antigen processing (TAP) genes

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are interesting candidates for such a study since binding to TAP is thought to be the main way in which HSV-1 evades the immune response [2]; the viral protein ICP47 blocks the peptide-binding site of TAP, thus preventing antigen presentation to the immune system. TAP1 and TAP2 belong to the ATP-binding cassette (ABC) superfamily of transporter molecules and play a pivotal role in the adaptive immune response, forming heterodimeric complexes that delivers cytosolic peptides to the lumen of the endoplasmic reticulum where they associate with MHC class I molecules [15].

TAP genes map within the class II MHC region of chromosome 6 (6 p21.3, 30 cM), relatively close (about 30 cM away) to one of the AD loci consistently identified by genetic linkage and association studies [3] (see up-to-date reports at the Alzforum web page: http://www.alzforum.org). There are seven haplotypes of TAP1 and four of TAP2 with significant frequencies in Caucasian populations (see sequences and official nomenclature for TAP haplotypes at http://www.anthonynolan.com/HIG/nomen/nomen_index.html). These haplotypes are defined by combinations of single nucleotide polymorphisms (SNPs), from which those tagging major haplotypes were selected for a genetic association study in a case–control sample of AD. An independent case–control sample, with pathological confirmation, was then analyzed to validate the findings.

2. Subjects and methods

2.1. Patients and controls

The test sample consisted of 571 individuals from central Spain. These included 253 patients with sporadic AD (mean age at onset 71.2 ± 10.8 years; mean ± S.D.; 63% females) recruited at the neurology departments of the participating hospitals. The controls were 318 healthy individuals (mean age at examination 73.3 ± 12.8 years; 60% females). All subjects gave their informed consent to be included in the study. All ‘case’ subjects had a diagnosis of probable AD according to the NINCDS-ADRA [16] or DSMIV [17] criteria for Alzheimer’s dementia. The controls were subjected to a Mini Mental test. This sample had previously been genotyped for APOE and other polymorphic genes [5].

The Canadian sample was composed of 100 patients with AD (mean age at onset 70.2 ± 10.6 years; mean ± S.D.; 63% females) and 178 controls (mean age at examination 76.3 ± 12.8 years; 48% females). All these patients underwent an autopsy at the London Health Sciences Center, University of Western Ontario. The criteria for AD included a diagnosis of dementia by a neurologist and a NIA/Reagan score showing a high probability that dementia was due to AD. The criteria for controls was a documented normal mental status (not necessarily recorded by a neurologist) on clinical exam prior to death, and a NIA/Reagan score showing a low probability of AD [1].

2.2. Genotyping

APOE, TAP1333 (dbSNP rs4148880), TAP1637 (rs1800453) and TAP2379 (dbSNP rs4148873) genotyping was performed by RFLP with the appropriate restriction enzymes following standard methods (detailed protocols available upon request). TAP2687 (rs241448), that discriminates TAP2*0201 from the other major TAP2 haplotypes (TAP2*0101, *0102 and *0103), was analyzed with MGB-Taqman probes (AB assay Id C_2961793_10) following the manufacturer’s instructions.

2.3. Statistics

Genotypes and allele distributions were compared using the \( \chi^2 \) test. Logistic regression models adjusted by age at AD onset, gender and sample origin were used to calculate the strength of association between the genotypes and AD (expressed as the odds ratio [OR] and 95% confidence intervals [CI]). All calculations were performed with SPSS12.0 software.

3. Results

Table 1 shows there were no differences in the distribution of the genotypes between cases and controls for any of the SNPs in TAP1. The same was observed for TAP2379. However, for TAP2687, the C allele and the CC genotype were significantly more common in AD patients than in controls (odds ratio 1.51 (95% CI 1.16–1.97) and 2.14 (95% CI 1.16–3.93), respectively). Since TAP2 alleles are in linkage disequilibrium with HLA-DRB1 haplotypes, the possibility that this might be the reason for this association was explored. The HLA-DRB1 genotypes of a subsample of 100 individuals were determined and, as reported for other Caucasian populations [10], significant linkage was found between TAP2687C (687 Stop) and HLA DR3 and DR4 \((p = 0.0001 [\chi^2 \text{ test}]; \text{data not shown})\). The TAP2687 genotypes in the DR3/DR4 carriers showed (as in the whole sample) an increased frequency of the TAP2687C allele in the cases (18% compared to 7% in controls), although this difference did not reach significance \((p = 0.12 [\chi^2 \text{ test}])\). This suggests that the association of TAP2687C with AD is not attributable to a linkage disequilibrium with the HLA DRB haplotypes, although we cannot completely exclude this possibility.

In the context of the working hypothesis, APOE and TAP are important for HSV-1 infectivity, and both showed genetic association with AD in the test sample. If there were any biological interaction between these two genes with respect to AD pathogenesis, this would be reflected in the risk of developing the disease. A search was therefore made for possible interactions between the APOE4 and TAP2687C alleles.

We first studied the effect of TAP2687 in the sample stratified for APOE genotype. As shown in Table 2A, the relative risk for TAP2687CC genotype was higher in carriers of the
APOE4 allele compared to non-carriers, suggesting a possible interaction of the two genotypes with respect to AD risk.

The validity of this observation was then tested in the Canadian, autopsy-based, case–control sample. The results, shown in Table 2A, showed the same tendency as in the test sample, i.e., a significant increase in the homozygous frequency of TAP2687C among cases who were carriers of the APOE4 allele. Since the two samples showed the same tendency, the results were pooled to increase the statistical power of a logistic regression analysis (although both samples showed the same results when studied separately; not shown). This was used to assess the individual effect of each gene, adjusting by age at onset, gender and the origin of the sample. APOE and TAP2687 genotypes were coded as dichotomic variables (APOE—one or two 4 alleles or no 4 allele at all; TAP2687—any T allele or CC genotype). Both APOE and TAP2687 contributed significantly to the risk of developing AD, although as expected from the raw data the risk of AD attributable to APOE (OR 4.1; 95% CI 3.1–5.3) was higher than that corresponding to TAP2687 (OR 1.7; 95% CI 1.1–2.6).

Finally, the risk associated with the genotype combinations of the two genes was evaluated in a logistic regression model adjusted for age at AD onset, gender and sample origin. Table 2B clearly indicates that carrying the APOE4 and two TAP2687C alleles is strongly associated with AD.

In summary, the different analyses performed suggest a combined effect of the APOE4 allele and the TAP2687CC genotype in the two samples studied, with individuals carrying both factors showing a high probability of belonging to the AD patients.

4. Discussion

APOE4 is the only universally accepted genetic risk factor for sporadic AD [4]; higher apoE levels also seem associated with an increased risk [5,14]. We recently reported that the entry of HSV-1 into the brain via the hematogenous route is better facilitated by APOE4 compared to APOE3 [7] and by a higher dose of apoE [6]. Together with the previously reported potentiation of the risk conferred by APOE4 in the presence of HSV-1 in the brain [12], the above evidence suggests that HSV-1, somehow interacting with APOE, is involved in AD pathogenesis. In addition, Lambert et al. [13] have reported an association between LBP-1c/CP2/LSF and the risk of AD; this transcription factor is involved in the activation of human immunodeficiency and herpes simplex viruses.

The present work explores the possibility that TAP, via which HSV-1 evades the immune system [2], might participate in the pathogenic process of AD and in susceptibility to
The disease. Of the SNPs analyzed, the TAP2687C allele (a tag SNP of TAP2*0201 haplotype) appears to be clearly associated with AD susceptibility. In particular, homozygosity for TAP2687C in carriers of the APOE4 leads to a risk comparable to that reported for APOE4 homozygosity [4]. This observation was made in two case–control samples (one clinically and one autopsy-based) obtained independently from two countries.

The results indicate that carrying the APOE4 and two TAP2687C alleles confers a high risk of developing AD. Thus, although the two patient populations studied were clearly different, as reflected by the different frequency of APOE4 in the patients (very low in the Canadian sample compared to the Spanish test sample), the combined effect of APOE4 and TAP2687C was observed in both. This suggests that this genotype combination is a strong risk factor observable over the noise caused by different population backgrounds, sampling designs, etc., although the possibility of a false positive association due to multiple testing cannot be completely excluded.

Functional anomalies in TAP due to somatic mutations or to rare polymorphisms have been reported in a variety of cancers. Although the most common TAP polymorphisms do not alter peptide binding or translocation to the ER [9], the possibility that they affect the interaction of TAP with ICP47, thus influencing HSV-1’s evasion of the immune system, has not been analyzed and cannot be ruled out. HSV-1 has been associated with sporadic AD [12], and both apoE [6,7] and TAP2 [2] are involved in HSV-1 neuroinvasiveness, although it cannot be discarded that TAP2 involvement in AD is unrelated to HSV-1.

The present results suggest that the combined APOE/TAP2 genotype is a good genetic marker of the possibility of developing AD, whether TAP2 is the primary risk factor or a marker of another locus. They also leave open the interesting possibility of a link between the central nervous system’s genetic susceptibility to viral infections and the onset of neurodegenerative diseases.

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**References**


