

Brief communication

A *TAP2* genotype associated with Alzheimer's disease in *APOE4* carriers

María J. Bullido^a, Ana Martínez-García^{a,1}, María J. Artiga^{a,1}, Jesús Aldudo^a,
Isabel Sastre^a, Pedro Gil^b, Francisco Coria^c, David G. Muñoz^d,
Vladimir Hachinski^e, Ana Frank^f, Fernando Valdivieso^{a,*}

^a Departamento de Biología Molecular and Centro de Biología Molecular Severo Ochoa (C.S.I.C.-U.A.M.),
Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

^b Servicio de Geriatria, Hospital Clínico de San Carlos, 28003 Madrid, Spain

^c 07014 Palma de Mallorca, Spain

^d University of Toronto & St. Michael's Hospital, Toronto, Canada

^e University of Western Ontario, London, Canada

^f Servicio de Neurología, Hospital Universitario La Paz (UAM), 28034 Madrid, Spain

Received 2 December 2005; received in revised form 16 February 2006; accepted 18 February 2006

Available online 3 April 2006

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.

© 2006 Elsevier Inc. All rights reserved.

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 $\epsilon 4$ 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

* Corresponding author. Tel.: +34 91 497 84 71; fax: +34 91 497 48 70.

E-mail address: fvaldivieso@cbm.uam.es (F. Valdivieso).

¹ These authors contributed equally to this work.

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.athornynolan.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 \pm 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 \pm 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 χ^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 ($p = 0.0001$ [χ^2 test]; 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 ($p = 0.12$ [χ^2 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

Table 1
TAP1, *TAP2* and *APOE* genotype distribution in a case–control AD sample

	<i>n</i> ^a	<i>TAP1333</i> (rs4148880) ^b			OR ^c (χ^2)
		AA	AG	GG	
Cases	215	0.71	0.28	0.02	0.92 (n.s.)
Controls	315	0.68	0.30	0.02	
	<i>n</i> ^a	<i>TAP1637</i> (rs1800453) ^b			OR ^c (χ^2)
		AA	AG	GG	
Cases	215	0.70	0.29	0.01	1.00 (n.s.)
Controls	315	0.70	0.29	0.01	
	<i>n</i> ^a	<i>TAP2379</i> (rs4148873) ^b			OR ^c (χ^2)
		AA	AG	GG	
Cases	207	0.80	0.20	0.01	0.90 (n.s.)
Controls	318	0.77	0.22	0.01	
	<i>n</i> ^a	<i>TAP2687</i> (rs241448) ^b			OR ^c (χ^2)
		TT	TC	CC	
Cases	231	0.45	0.43	0.12	1.51 (0.002)
Controls	318	0.56	0.38	0.06	
	<i>n</i> ^a	<i>APOE</i> (number of <i>APOE4</i> alleles) ^b			OR ^c (χ^2)
		0	1	2	
Cases	240	0.51	0.38	0.11	3.17 (10 ⁻¹⁵)
Controls	318	0.86	0.13	0.06	

^a Number of individuals. The differences among groups are due to “missed” genotypes.

^b Figures represent the frequencies of each genotype. The SNPs are denoted by their dbSNPs reference codes.

^c OR for the minority allele of each polymorphism, plus the *p*-value obtained in the χ^2 test.

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

Table 2
Association of *TAP2687* and *APOE* with AD

<i>APOE</i> genotype	<i>TAP2687</i> genotype ^a			OR ^b (χ^2 ; <i>p</i>)
	TT	TC	CC	
Effect of <i>TAP2687</i> in the sample stratified for <i>APOE</i> ^c				
Spain				
No 4				
Cases	53 (0.46)	49 (0.42)	14 (0.12)	1.95 (n.s.)
Controls	146 (0.57)	95 (0.37)	17 (0.07)	
Any 4				
Cases	47 (0.45)	45 (0.43)	13 (0.12)	5.93 (0.05)
Controls	25 (0.58)	17 (0.40)	1 (0.02)	
Canada				
No 4				
Cases	51 (0.68)	20 (0.27)	4 (0.05)	1.30 (n.s.)
Controls	93 (0.65)	45 (0.31)	6 (0.04)	
Any 4				
Cases	12 (0.49)	5 (0.20)	8 (0.31)	7.50 (0.01)
Controls	18 (0.53)	14 (0.41)	2 (0.06)	
<i>APOE</i> genotype	<i>TAP2687</i> genotype	Relative risk (OR)	95% CI	
Combined effect of <i>APOE</i> and <i>TAP2687</i> ^d				
No 4	Any T	Ref		
	CC	1.3	0.8–2.2	
Any 4	Any T	3.8	2.9–4.9	
	CC	14.7	5.1–42.5	

^a Figures are numbers of individuals. Frequencies are shown in parentheses.

^b OR for *TAP2687*CC vs. TT + TC (any T allele), plus *p* value (χ^2 test).

^c *TAP2* genotype distribution in the AD case–control test sample (Spain) and contrast sample (Canada) stratified by apoE genotype.

^d Effect of *APOE/TAP2* genotype combinations. Figures are the estimated OR and 95% CI. The logistic regression model was adjusted for age at onset, gender and sample origin.

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 *TAP* [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.

Acknowledgements

This work was supported by the Obra Social Caja Madrid, Comunidad Autónoma de Madrid (Programa de Grupos Estratégicos and GR/SAL/0783/2004), the Ministerio de Educación y Ciencia (GEN2003-20235-C05-05), and the Ministerio de Sanidad y Consumo (Instituto de Salud Carlos III; RTIC C03/07 and RTIC C03/06). The institutional grant awarded by the Fundación Ramón Areces to the Centro de Biología Molecular Severo Ochoa is gratefully acknowledged. We thank Dr. E. Díez-Tejedor for advice on clinical work, and to the Asociación de Familiares de Alzheimer de Madrid (AFAL) for continuous encouragement and help. This work was made possible by the generous participation of the patients, the controls and their families.

References

- [1] Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging* 1997;18(4 Suppl):S1–2.
- [2] Bauer D, Tampe R. Herpes viral proteins blocking the transporter associated with antigen processing TAP—from genes to function and structure. *Curr Top Microbiol Immunol* 2002;269:87–99.
- [3] Bertram L, Tanzi RE. Alzheimer's disease: one disorder, too many genes? *Hum Mol Genet* 2004;13 Spec(1):R135–41.
- [4] Bertram L, Tanzi RE. The genetic epidemiology of neurodegenerative disease. *J Clin Invest* 2005;115(6):1449–57.
- [5] Bullido MJ, Artiga MJ, Recuero M, Sastre I, Garcia MA, Aldudo J, et al. A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. *Nat Genet* 1998;18(1):69–71.
- [6] Burgos JS, Ramirez C, Sastre I, Bullido MJ, Valdivieso F. Involvement of apolipoprotein E in the hematogenous route of herpes simplex virus type 1 to the central nervous system. *J Virol* 2002;76(23):12394–8.
- [7] Burgos JS, Ramirez C, Sastre I, Bullido MJ, Valdivieso F. apoE4 is more efficient than E3 in brain access by herpes simplex virus type 1. *Neuroreport* 2003;14(14):1825–7.
- [8] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele

- and the risk of Alzheimer's disease in late onset families. *Science* 1993;261(5123):921–3.
- [9] Daniel S, Caillat-Zucman S, Hammer J, Bach JF, van Endert PM. Absence of functional relevance of human transporter associated with antigen processing polymorphism for peptide selection. *J Immunol* 1997;159(5):2350–7.
- [10] Djilali-Saiah I, Benini V, Daniel S, Assan R, Bach JF, Caillat-Zucman S. Linkage disequilibrium between HLA class II (DR, DQ, DP) and antigen processing (LMP, TAP, DM) genes of the major histocompatibility complex. *Tissue Antigens* 1996;48(2):87–92.
- [11] Fratiglioni L, De Ronchi D, Aguero-Torres H. Worldwide prevalence and incidence of dementia. *Drugs Aging* 1999;15(5):365–75.
- [12] Itzhaki RF, Dobson CB, Shipley SJ, Wozniak MA. The role of viruses and of APOE in dementia. *Ann NY Acad Sci* 2004;1019:15–8.
- [13] Lambert JC, Goumidi L, Vrieze FW, Frigard B, Harris JM, Cummings A, et al. The transcriptional factor LBP-1c/CP2/LSF gene on chromosome 12 is a genetic determinant of Alzheimer's disease. *Hum Mol Genet* 2000;9(15):2275–80.
- [14] Laws SM, Hone E, Gandy S, Martins RN. Expanding the association between the APOE gene and the risk of Alzheimer's disease: possible roles for APOE promoter polymorphisms and alterations in APOE transcription. *J Neurochem* 2003;84(6):1215–36.
- [15] McCluskey J, Rossjohn J, Purcell AW. TAP genes and immunity. *Curr Opin Immunol* 2004;16(5):651–9.
- [16] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34(7):939–44.
- [17] Wilson HS, Skodol A. Special report: DSM-IV: overview and examination of major changes. *Arch Psychiatr Nurs* 1994;8(6):340–7.