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Transporter associated with antigen processing (TAP) 1 gene polymorphisms in patients with hypersensitivity pneumonitis

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Abstract

Hypersensitivity pneumonitis (HP) is a lung inflammatory disease caused by the inhalation of a variety of antigens. Previous studies support the role of the major histocompatibility complex (MHC) class II genes in the susceptibility to develop HP. However, the putative role of other MHC loci has not been elucidated. Transporters associated with antigen processing (TAP) genes are located within the MHC class II region and play an important role transporting peptides across the endoplasmic reticulum membrane for MHC class I molecules assembly. The distribution of single nucleotide polymorphisms (SNPs) in TAP1 genes was analyzed in 73 hypersensitivity pneumonitis (HP) patients and 58 normal subjects. We found a significant association of the allele Gly-637 (GGC) ($p=0.00004$, OR=27.30, CI=3.87–548.04) and the genotypes Asp-637/Gly-637 ($p=0.01$, OR=16.0, CI=2.19–631.21), Pro-661/Pro-661 ($p=0.006$, OR=11.30, CI=2.28–75.77) with HP. A significant decrease in the frequency of the allele Pro-661 (CCA) ($p=0.008$, OR=0.06, CI=0–0.45), the genotype Asp-637/Asp-637 ($p=0.01$, OR=0.17, 95% CI=0.05–0.58) and the haplotype [Val-333 (GTC), Val-458 (GTG), Gly-637 (GGC), Pro-661 (CCA)] was detected in HP patients compared with controls ($p=0.002$, OR=0.07, CI=0.0–0.57). These findings suggest that TAP1 gene polymorphisms are related to HP risk, and highlight the importance of the MHC in the development of this disease.

Keywords: Genetic susceptibility; Hypersensitivity pneumonitis; Major histocompatibility complex (MHC); Transporter associated with antigen processing genes; TAP1

Introduction

Hypersensitivity pneumonitis (HP) is an immunologically-mediated lung disease caused by repeated inhalation of dispersed antigens in susceptible individuals (Lacasse et al., 2003). Pigeon breeder's disease is one of the most common clinical forms of HP, caused by exposure to various avian-derived antigens. The clinical presentation is heterogeneous and the individuals exposed to low levels of avian antigens, can develop a sub-acute or chronic disease and some of them may evolve to pulmonary fibrosis (Selman et, 2004; Ramírez-Venegas et al., 1998).

Importantly, only a small proportion of individuals exposed to potential HP causing antigens develop the disease. In this context, HP is considered a multifactorial disease involving complex interactions between antigen exposure and perhaps other environmental factors and alleles of many genes (Selman, 2004; Ramírez-Venegas et al., 1998; Fink et al., 2005). MHC genes have been involved with the susceptibility to HP in

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different ethnic groups. In previous studies, we have described a significant association of the MHC class II haplotype: HLA-DRB1*1305-DQB1*0301 with the susceptibility to HP in Mexicans. Furthermore, our results suggested the existence of additional susceptibility loci within the MHC which might contribute to the disease susceptibility (Camarena et al., 2001). The transporters associated with antigen processing (TAP) genes are mapped within the MHC class II region and are critical for antigen processing and presentation by MHC class I molecules (Walsh et al., 2003; Powis et al., 1993). TAP translocates antigenic peptides from the cytosol into the endoplasmic reticulum (ER) lumen, where peptides are loaded onto MHC class I molecules (Koch et al., 2004). The functional TAP complex is a heterodimer composed by TAP1 and TAP2 and their function is dependent on adenosine triphosphate (ATP) hydrolysis (Koch et al., 2004; McCluskey et al., 2004). Because of their role in endogenous antigen processing, their location within MHC, and their polymorphisms, the TAP genes have been reported to be important candidates for disease association (Lajoie et al., 2003).

The aim of this study was to investigate the distribution of four single nucleotide polymorphisms (SNPs) located in the coding region of TAP1 gene in Mexican Mestizos and analyze their potential role in the susceptibility to HP.

Materials and methods

Subjects

Seventy three Mexican patients with diagnosis of HP were included in this study. Patients were recruited from the Interstitial Lung Diseases Clinic of the National Institute of Respiratory Diseases. Diagnosis of HP was established according to the following criteria: a) bird exposure preceding the disease and positive serum antibodies against avian antigens as determined by ELISA; b) shortness of breath with partial improvement after avoidance of avian antigen exposure; c) clinical and functional features of an interstitial lung disease; d) high-resolution computed tomography scan of the chest showing diffuse centrilobular poorly defined micronodules, ground glass attenuation, focal air trapping and mild/moderate fibrotic changes and e) >40% lymphocytes in bronchoalveolar lavage fluid (Ramírez-Venegas et al., 1998; Selman et al., 2006). Forty-five percent of the patients were biopsied and in all of them lung histology was compatible with the diagnosis of HP. Fifty eight healthy Mexican individuals, without history of connective tissue disorders or pulmonary disease, were included in the study as controls. For this study we enrolled only those individuals whose last two generations were born in Mexico, and thus considered to be Mexican Mestizo. Our control group has been genetically characterized and admixture estimation studies have revealed a proportion of 56% Indian genes, 40% Caucasian genes and 4% black genes (Lisker et al., 1998; Lisker et al., 1990).

The protocol was approved by the Institutional Review Board and only those patients who signed informed consent letter were included in the study.

DNA extraction

Genomic DNA from whole blood containing EDTA was isolated by a salting-out procedure.

TAP polymorphism typing

Variations in the TAP1 gene were identified by polymerase chain reaction and hybridization with sequence-specific oligonucleotide probes (PCR-SSOP) as previously described (Perry et al., 1998). PCR was performed in a 25 µl final volume containing 100 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1× Taq DNA polymerase buffer, 0.5 µM of each oligonucleotide primer and 2.5 U of recombinant *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). PCR conditions were initial denaturation at 94 °C during 5 min followed by 35 cycles of 94 °C during 30 s, 54 °C or 68 °C during 30 s, and 72 °C during 5 min. Cycling was carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City CA). PCR products were heat-denatured during 10 min at 94 °C and 5 µl of the sample was spotted onto positively charged nylon membranes (Amersham Biosciences, Bucks UK) previously soaked in SSC 10× (10 min) and then they were UV-cross linked. Blots were pre-hybridized with DIG Easy Hyb solution (Roche Diagnostics GmbH, Mannheim Germany) during 30 min at the calculated melting temperature for each oligonucleotide and then hybridized with mix Dig Easy Hyb/digoxigenin-labeled SSO probes (25 ng/ml) overnight at the calculated melting temperature for each SSO probe. Blots were washed twice with 2× SSC, 0.1% SDS, twice with 0.5× SSC, 0.1% SDS and washing buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5, 0.3% [v/v] Tween 20) at room temperature. Membranes were incubated with 10% blocking solution during 30 min (Roche Diagnostics GmbH, Mannheim Germany) and treated with anti-digoxigenin-Ab-alkaline phosphatase conjugate (Roche Diagnostics GmbH, Mannheim Germany) in blocking solution during 30 min at room temperature and then washed two times during 15 min in washing buffer. Membranes were incubated with detection buffer during 5 min, and washed with sterile double distilled water. Results were documented by Electrophoresis Documentation and Analysis System 290 (Kodak, Rochester NY).

Statistical analysis

Comparison of TAP1 allele, genotypic and haplotypic frequencies of TAP1 gene SNPs (corresponding to amino acid positions 333, 458, 637 and 661) were evaluated by the Mantel–Haenszel, Chi-square test that combined the 2×2 contingency tables in HP patients and control group using the EPIINFO statistical program (Version 6.04b). *P* values were corrected by the Bonferroni method multiplying the *p* value for the number of comparisons. Relative risks (RR) with 95% confidence interval (95% CI) were estimated as the odds ratios. Hardy–Weinberg equilibrium was also tested for all genotypic combinations of each TAP variant.

Results

The gene and genotype frequencies of the TAP1 polymorphisms in the positions 333, 458, 637 and 661 are presented in Table 1. The most common alleles for these positions were: Ile-333 (ATC) for position 333 (g.f.=0.808 in HP patients and 0.853 in healthy controls); Val-458 (GTG) for position 458 (g.f.=0.993 in HP patients and 0.965 in controls); Asp-637 (GAC) for position 637 (g.f.=0.801 in HP patients and 0.931 in controls) and Pro-661 (CCG) for position 661 (g.f.=0.979 in HP patients compared with 0.948 in controls). The most frequent genotypes were homozygous Ile-333/Ile-333 (ATC/ATC) for position 333; homozygous Val-458/Val-458 (GTG/GTG) for position 458; homozygous Asp-637/Asp-637 (GAC/GAC) for position 637 and homozygous Pro-661/Pro-661 (CCG/CCG) for position 661 (Table 1). Significant deviations from the Hardy–Weinberg equilibrium in the distribution of TAP1 SNP genotypes in HP patients and healthy controls were not detected.

We found a significant increase in the frequency of the allele Gly-637 (GGC) in HP patients compared with controls ($p=0.00004$, OR=27.30, 95% CI=3.87–548.04), Table 1. We also observed a significant decrease in the frequency of the allele Pro-661 (CCA) in HP patients ($p=0.008$, OR=0.06, 95% CI=0–0.45).

In regard to the genotype analysis we found a significant increase in the frequency of Asp-637/Gly-637 ($p=0.01$,