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Short communication

# Common variants of the neuropeptide expressing tachykinin genes and susceptibility to asthma: A case–control study

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ABSTRACT

Since tachykinins appear to be involved in the pathogenesis of allergic asthma, we investigated a possible association between 28 single nucleotide polymorphisms of the tachykinin genes *TAC1*, *TAC3* and *TAC4*, and asthma susceptibility. A case–control study was conducted on 102 patients and 100 healthy subjects from the Canary Islands (Spain). A significant association with asthma was observed for two SNPs: rs2291855 in the *TAC3* gene conferring asthma protection (Odds ratio [OR]: 0.46; 95% Confidence Interval [CI]: 0.22–0.97; $P = 0.038$), and rs4794068 in the *TAC4* gene associated with an increased risk for asthma (OR: 1.94; 95% CI: 1.06–3.54; $P = 0.03$). The present study represents a preliminary step in elucidating the association between tachykinin gene polymorphisms and asthma susceptibility.



## 1. Introduction

Tachykinins are a family of related peptides implicated in many physiological and pathological processes (Patacchini and Maggi, 2001; Almeida et al., 2004; Greco et al., 2004). They derive from three distinct genes: *TAC1* encodes for substance P (SP) and neurokinin A (NKA), *TAC3* encodes for neurokinin B (NKB), and *TAC4* encodes for hemokinin-1 (HK-1) (Pennefather et al., 2004). Tachykinins exert most of their actions by interaction with three specific membrane receptors and although they can act as full agonists on the three receptors, SP and HK-1 show preference for NK1, NKA for NK2, and NKB for NK3 (Almeida et al., 2004; Pennefather et al., 2004). Several evidences support a role for the tachykinin peptides in bronchial asthma (Joos et al., 2000; Groneberg et al., 2006). In lung, tachykinin receptors are widely distributed among different cell types (Joos et al., 2000; Pinto et al., 2004), and coupling to them produce bronchoconstriction, vasodilatation, plasma protein extravasation and mucus secretion. Consequently, these peptides have been implicated in many aspects of asthma pathogenesis, including airway narrowing, inflammation, hyperresponsiveness, mucus hypersecretion and leukocyte recruitment (Joos et al., 2000; Daoui et al., 2000; Kraneveld and Nijkamp, 2001; Nénan et al., 2001; Grant et al., 2002; Feistritzer et al., 2003; Groneberg et al., 2006). Sensory nerves are traditionally considered the main source of tachykinins in human airways, which are released from nerve terminals by mechanical and chemical stimuli (Belvisi, 2003). Several studies found increased levels of SP and NKA in bronchoalveolar lavage (BAL) of asthmatic patients after antigen challenge (Nieber et al., 1992; Heaney et al., 1998). In addition, plasma SP concentration was elevated in acute asthma exacerbations (Cardell et al., 1994). However, the high level of tachykinins in asthmatic patients does not correlate with an increased level of tachykinin-containing nerve fibers in the airway (Howarth et al., 1995; Chanez et al., 1998). In recent years, it has become clear that immune and inflammatory cells may form an additional source of tachykinins (Springer et al., 2005). Among human inflammatory cells, *TAC1* mRNA and SP protein have been detected in lymphocytes (Lai et al., 1998) and monocytes/macrophages (Ho et al., 1997). In addition, immunoreactivity for SP has been observed in eosinophils and neutrophils (Aliakbari et al., 1987). We have recently demonstrated NKB immunoreactivity in lymphocytes, monocytes, eosinophils and neutrophils as well as *TAC4* mRNA expression in all these cell types, except monocytes (Klassert et al., 2008). Thus, increased numbers of cells attracted to the inflamed airways may be responsible for the increased tachykinin levels observed in asthma patients. In fact, it has been shown that airway tachykinin input due to immune cells is essential for the progression of the inflammation (Chavolla-Calderón et al., 2003).

Despite the evidence that tachykinins play an important role in inflammatory disease, association studies of tachykinin genes with asthma susceptibility have not been reported so far. The aim of this

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study was to test a possible association of common variants in the tachykinin genes *TAC1*, *TAC3* and *TAC4*, with asthma susceptibility.

## 2. Material and methods

### 2.1. Study participants

A case–control study was performed in the Canary Islands population (Spain), where asthma has a high prevalence (Torres-Galván et al., 2000). Our study population was composed of 102 adult atopic asthma patients diagnosed according to the criteria of the Global Initiative for Asthma (GINA). The control group consisted of 100 healthy ethnically matched adults which passed the European Community Respiratory Health Survey II (ECRHS II) screening questionnaire. All subjects gave written consent and the study was approved by the Institutional Review Boards.

### 2.2. Polymorphism selection and genotyping

We selected 28 single nucleotide polymorphisms (SNPs) from the tachykinin genes: 11 from *TAC1*, 9 from *TAC3* and 8 from *TAC4*. The goal of the SNP selection was to cover each gene region with at least one SNP per 1–2 kb. To ensure collection of known tag-SNPs, the selection was guided by the HapMap phase II data using the tSNP_pairwise_Tagger with an $r^2$ threshold of 0.8 and minor allele frequency (MAF) >5%, based on the data from CEU (CEPH residents of Utah with ancestry from northern and western Europe). Fig. 1 shows selected SNPs and their location in the tachykinin genes.

Genomic DNA was isolated from EDTA preserved whole blood by standard proteinase K digestion and phenol–chloroform methods (Sambrook et al., 1989). All SNPs were genotyped using the SNaPshot (Applied Biosystems) multiple simultaneous SNP genotyping system involving multiplex PCR and subsequent multiple single base extension (SBE) reactions. Two assays were carried out in order to cover the 28 SNPs. Primers used in PCR reactions are listed on the Appendix. A detailed protocol for multiplex SNP genotyping system is available elsewhere (Sánchez et al., 2006). The purified SBE products were electrophoresed on an ABI PRISM 3130x1 Genetic Analyzer and analyzed using GeneScan software (Applied Biosystems).

### 2.3. Statistical analysis

SNPs with Minor Allele Frequencies (MAF) <5% in this population were discarded from the analysis. Hardy–Weinberg equilibrium (HWE) for each SNP was tested by the Pearson chi-square procedure. Linkage disequilibrium (LD) among genotyped SNPs was obtained using the Haploview 2.05 program (Barrett et al., 2005).

Based on the logistic regression method, association of single locus genotypes with the outcome of asthma was tested under five inheritance models (codominant, dominant, recessive, overdominant and log-additive) using SNPStats software (Solé et al. 2006; http://www.bioinfo.iconcologia.net/SNPstats). Odds ratios (OR) and 95% confidence intervals (CI) were calculated, and Akaike Information Criterion (AIC) was used to choose the genetic model that best fits the data.

Haplotype frequencies were estimated and tested for association using the expectation–maximization (EM) algorithm as implemented in the haplo.stats package (http://www.mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm). The most common haplotypes were automatically selected as the reference category and rare haplotypes (frequency <0.01) were pooled together. A log-additive inheritance model was assumed by default. The significance level of all these tests was set to 0.05.

## 3. Results

In total, 102 patients with asthma and 100 controls were evaluated. Of the 28 SNPs genotyped in all three tachykinin genes, 12 SNPs were monomorphic in our population. To determine whether the

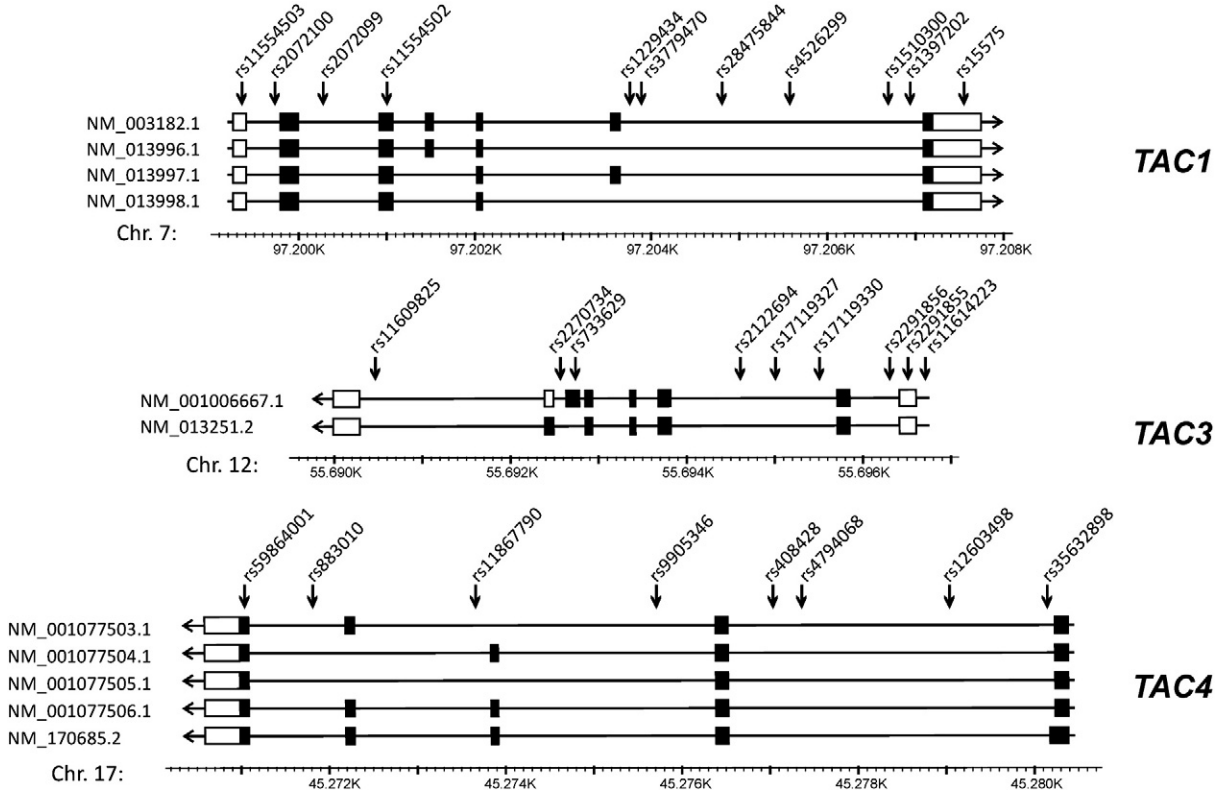


**Fig. 1.** Organization of the human tachykinin genes (*TAC1*, *TAC3* and *TAC4*) and location of analyzed SNPs. Different transcripts for each gene are shown. Boxes indicate exons, and lines indicate introns. Coding exons are marked by shaded blocks. Polymorphisms were expressed as their dbSNP ID.