ELSEVIER

Journal of Neuroimmunology 183 (2007) 208 – 213

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

The neuropeptide genes *TAC1*, *TAC3*, *TAC4*, *VIP* and *PACAP* (*ADCYAP1*), and susceptibility to multiple sclerosis

Stephen Cunningham [a], Catherine O'Doherty [a], Chris Patterson [b], Gavin McDonnell [c], Stanley Hawkins [c], Marria G. Marrosu [d], Koen Vandenbroeck [a,*]

[a] *Applied Genomics Research Group, School of Pharmacy, Center for Cancer Research and Cell Biology (CCRCB), Queen's University of Belfast, UK*
[b] *Epidemiology Research Group, School of Medicine and Dentistry, Queen's University of Belfast, UK*
[c] *Neurology Department, Royal Victoria Hospital, Belfast, UK*
[d] *Department of Neuroscience, University of Cagliari, Italy*

Received 26 June 2006; received in revised form 31 October 2006; accepted 6 November 2006

Abstract

The related immunomodulatory neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase activating peptide (PACAP; gene symbol *ADCYAP1*) have recently been proposed as novel therapeutics for the treatment of multiple sclerosis (MS). These neuropeptides, as well as those belonging to the tachykinin family exert pleiotropic effects, many of which are of relevance to central nervous system inflammation. In the present study, we have analysed 14 single nucleotide polymorphisms (SNPs) and 4 microsatellite markers in the *VIP*, *ADCYAP1*, *TAC3* and *TAC4* genes for susceptibility to MS in a case-control collection from Northern Ireland. Following correction for multiple comparisons, we did not find any significant associations between single polymorphic markers or multiple-marker haplotypes and susceptibility to MS. Furthermore, we analysed 2 SNPs in the *TAC1* gene in a set of Sardinian trio MS families, based on our previous observation of association of these SNPs with MS in the Northern Irish (Genes Immun. 2005, 6, 265–270). Analysis of these SNPs in the Sardinians was not significant though a similar trend to that originally observed in the Northern Irish was present. Meta-analysis of the Sardinian and Northern Irish *TAC1* SNP genotype data revealed a Mantel–Haenszel Common OR Estimate for the *TAC1* intron 1 SNP *rs2072100* of 0.76 (95% CI 0.63–0.92; $P=0.005$; A allele) and for the *TAC1* promoter SNP *rs7793277* of 0.76 (95% CI 0.615–0.95; $P=0.014$; C allele). Our data advocate a need for further exploration of the *TAC1* gene region in MS.

Keywords: Multiple sclerosis; Neuropeptide; Tachykinin; Vasoactive intestinal peptide; Single nucleotide polymorphism

1. Introduction

A number of neuropeptides with immunomodulatory effects relevant to inflammation in the central nervous system have been identified. Of these, the related neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase activating peptide (PACAP) have recently been proposed as novel therapeutics for the treatment of multiple sclerosis (Abad et al., 2006). VIP was reported to reduce incidence and severity of experimental allergic encephalomyelitis (EAE), to suppress EAE neuropathology and to prevent recurrence of the disease (Gonzalez-Rey et al., 2006). Similarly, PACAP was reported to exert beneficial effects on clinical and pathological manifestations of EAE, probably through suppression of antigen presenting cell functions (Kato et al., 2004). VIP and PACAP exert a number of effects in the central nervous system (CNS) that could generally be regarded as being anti-inflammatory and neuroprotective (Gressens et al., 1997; Gozes et al., 1997; Gomariz et al., 2001). Upon axotomy, expression of PACAP is rapidly

* Corresponding author. Tel.: +44 2890 272214; fax: +44 2890 247794.
E-mail address: k.vandenbroeck@qub.ac.uk (K. Vandenbroeck).

0165-5728/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2006.11.002

induced (Mulder et al., 1999). VIP and PACAP, released by injured neurons act as "neuron survival factors" by down-regulating synthesis of pro-inflammatory factors such as iNOS, IL-1β, TNF-α and chemokines by activated microglia (Delgado et al., 2002a,b, 2003). Activated microglia have been implicated in early stages of myelin destruction in MS (Trapp et al., 1999a). Axon trans-section in MS occurs at sites of inflammation, and correlates with neurological disability (Trapp et al., 1999b; Anthony et al., 2000). Thus, dysregulation of the VIP/PACAP neuroimmune circuitry might be of relevance to processes leading to inflammatory demyelination and axon loss in MS. Notwithstanding this rather convincing biological data, a putative role for the VIP and PACAP (*ADCYAP1*) genes in genetic susceptibility to MS has not yet been explored.

We have recently reported association between a two-marker haplotype spanning the promoter-intron-1 region of the neuropeptide tachykinin-1 (*TAC1*) gene on chromosome 7q21-22 and multiple sclerosis in Northern Ireland (Vandenbroeck et al., 2002; Cunningham et al., 2005). *TAC1* generates the neuropeptide substance P, known to display a range of immunomodulatory activities some of which are of relevance to CNS inflammation (McCluskey and Lampson, 2001; Annunziata et al., 2002). Two further tachykinins with overlapping and distinct functions have been recognized. The *TAC3* precursor gives rise to a mature peptide of 10 amino acids that is identical to neurokinin B of other mammalian species. *TAC4*, originally called hemokinin-1, was discovered in 2000 as a survival factor for B cell precursors (Zhang et al., 2000).

The chromosomal locations of the *VIP* gene (6q26-27), *ADCYAP1* (18p11), *TAC3* (12q13-21) and *TAC4* (17q21.33) have all been highlighted as potential regions of interest in some individual genome-wide linkage and/or association studies (Kuokkanen et al., 1997; Akesson et al., 2002; Ban et al., 2002). In a meta-analysis of raw genotype data from three full genome scans, of these, only the *VIP* and *TAC4* chromosome regions emerged more strongly with non-parametric linkage (NPL) scores of 2.01 (6qtel) and 2.30 (17q22), respectively (The Transatlantic Multiple Sclerosis Genetics Cooperative, 2001). In the recent high-density screen for linkage in MS, of these regions, only 17q23 showed suggestive evidence for linkage (International Multiple Sclerosis Genetics Consortium, 2005).

The present study was designed in two parts. First, in order to shed light on a potential role of the neuropeptide genes *VIP*, *TAC3*, *TAC4* and *ADCYAP1* in susceptibility to MS, we have genotyped a selection of 4 microsatellite and 14 single nucleotide polymorphisms (SNPs) located in these genes in a collection of multiple sclerosis patients and healthy control subjects from Northern Ireland. We primarily analysed SNPs in *VIP*, *TAC3*, *TAC4* and *PACAP* for association with susceptibility to MS as single markers, and used haplotype analysis only as subsidiary to the single-marker analysis. Second, starting from our previous study in which we demonstrated significant association of the *TAC1* SNPs *rs7793277* and *rs2072100* with MS in the Northern Irish collection (Cunningham et al., 2005), we have scrutinized these SNPs in an independent trio family-based collection from Sardinia.

2. Materials, subjects and methods

2.1. Patients and controls

Northern Irish collection: A total of 451 MS patients and 206 healthy controls all of Northern Irish origin were employed in this study. The control group is composed of a combination of both (i) non-affected friend clinical attendee and (ii) blood bank donors. Both of these groups are assumed to neither have MS or any other neurological condition, and are residents of Northern Ireland. The ratio of males to females in the cases and controls were 1:2.08 and 1:1.13, respectively. *Sardinian family collection*: A total of 199 trio families (maternal, paternal and affected MS patient) from a Sardinian population were implemented for the *TAC1* part of the study. All patients were diagnosed as having MS according to the Poser criteria (Poser et al., 1983). Clinical and demographic data have been published before (Cunningham et al., 2005; Goris et al., 2002).

2.2. Genotyping

Polymorphic sites within the four candidate genes *TAC3*, *TAC4*, *VIP* and *ADCYAP1* were selected from the NCBI database. An initial sequencing strategy was employed for the *TAC3* gene to screen for polymorphisms in the exon sequences and immediately surrounding regions in 24 individuals (12 MS/12 Controls). Post-PCR cycle sequencing reactions were carried out using Big Dye v3.1 (Applied Biosystems, Foster City, USA) performed on an ABI Prism 3100 (Applied Biosystems, USA) and the data generated were examined by means of Sequencer v4.05 (Gene Codes Corp; MI, USA).

SNP genotyping was performed using either (i) Taqman SNP Genotyping Assays (Applied Biosystems, USA), in conjunction with a DNA Opticon 2 (MJ Research; Waltham) or (ii) a Fluorescence Polarisation Single Base Extension (FP-SBE) method (Kwok, 2002), adopted for a number of SNPs using AcycloPrime assays (PerkinElmer, USA) and performed on an Analyst AD (Molecular Devices; Sunnyvale, CA). Taqman assays were performed using the Quantitect Probe PCR master mix (Qiagen, USA) in 10 μl reaction volumes. Amplification of templates for FP-SBE genotyping was performed using standard amplification conditions using HotStarTaq polymerase (Qiagen, USA) prior to enzymatic cleanup. FP-SBE analysis was performed as per manufacturer's protocol. The genotyping of *rs8192597* and *rs2856966* was performed by a direct sequencing approach as these SNPs are in close proximity. A list of all genotyping assays, primers and probes are given in Table 1. Primer and probes used for *TAC1* are those previously described (Cunningham et al., 2005).