
CC chemokine receptor polymorphism CCR5 Δ 32 in Portuguese Behçet's disease patients

A. Bettencourt¹, B. Leal¹, C. Carvalho¹, R. Oliveira², A. Martins Silva^{1,3}, J. Vaz Patto⁴, M. Bastos⁵, L. Costa², P.P. Costa^{1,2}, C. Vasconcelos^{1,3}, J. Correia³, B.M. Silva¹

¹UMIB - Instituto de Ciências Biomédicas
Abel Salazar (ICBAS-UP), Porto,
Portugal;

²Instituto Nacional de Saúde (INSA)
Dr Ricardo Jorge, Lisboa, Portugal;

³Unidade de Imunologia Clínica (UIC),
Hospital de Santo António, Porto, Portugal;

⁴Instituto Português de Reumatologia,
Lisboa, Portugal ;

⁵Hospital São Teotónio, Viseu, Portugal;

Andreia Bettencourt, MSc

Bárbara Leal, MSc

Cláudia Carvalho, MSc

Rita Oliveira, MSc

Ana Martins Silva, MD

José Vaz Patto, MD

Marina Bastos, MD

Luciana Costa, PhD

Paulo P. Costa, PhD

Carlos Vasconcelos, PhD

João Correia, MD

Berta Martins Silva, PhD

Please address correspondence to:

Andreia Bettencourt,

UMIB – Instituto de Ciências

Biomédicas Abel Salazar (ICBAS-UP),

Rua de Jorge Viterbo

Ferreira n.º 228,

4050-313 Porto, Portugal.

E-mail: ambettencourt@icbas.up.pt

Received on January 13, 2014; accepted in
revised form on April 14, 2014.

Clin Exp Rheumatol 2014; 32 (Suppl. 84):
S72-S74.

© Copyright CLINICAL AND
EXPERIMENTAL RHEUMATOLOGY 2014.

Key words: Behçet's disease,
chemokines, immunogenetics,
Portugal, CCR5, chemokines

ABSTRACT

Objective. To investigate whether CCR5 deletion is associated with susceptibility to Behçet's disease (BD) in a Portuguese population.

Methods. A total of 122 BD patients and 227 ethnically-matched controls were studied. Genotyping of the CCR5 Δ 32 polymorphisms was performed using polymerase chain reaction product sizing.

Results. No significant differences were observed in the allelic frequencies of CCR532 between patients and controls (OR=0.820; $p=0.512$). Stratification for gender and for the presence of HLA-B*51 did not reveal any significant differences.

Conclusions. These results indicate that CCR5 Δ 32 is unlikely to contribute to susceptibility to BD in Portuguese patients. This may be explained by the known functional redundancy of this signalling system.

Introduction

Behçet's disease (BD) is a chronic, multisystem, inflammatory disease characterised by oral and genital mucous ulcers, uveitis and skin lesions; it can also affect vascular, gastrointestinal and neurological systems and arthritis is common (1). The etiology of BD is still unknown, but is presumed to be multifactorial, implicating genetic predisposition, dysregulation of the immune system, infectious agents and external environmental factors (2). The strongest evidence of genetic susceptibility of BD is its association with HLA-B*51, a human leucocyte antigen (HLA) class I gene (3, 4).

Serum levels and single nucleotide polymorphisms of various cytokines and chemokines, which play an important role in the immune response and inflammation, have been studied extensively in BD (5-9). The CC chemokine

receptor 5 (CCR5) is one of the known chemokine receptors expressed by peripheral blood leucocytes and is involved in the recruitment of inflammatory cells. Very recently, new susceptibility loci for BD at CCR1-CCR3, STAT4, KLRK1-KLRC4 and ERAP1 were reported (10).

A common 32-basepair deletion (CCR5 Δ 32) in the coding region of CCR5 gene that originates a truncated non-functional receptor with reduced expression on the cell surface has been described, that protects against several autoimmune diseases (11). Elevated expression of this receptor in the tissue of oral and genital ulcers in BD has been reported (12, 13). Three previous studies addressed the role of CCR5 Δ 32 in BD susceptibility, the first one, by Yang *et al.*, found no association of this polymorphisms and the disease in three populations from different geographical areas (14), a second study, by Mojtahedi *et al.*, suggested that CCR532 may be a genetic risk factor for BD in Iranian women (15). More recently, Atzeni described an association between the polymorphism and susceptibility to BD in Italian patients (16). The aim of this study was to investigate whether the CCR5 deletion polymorphism is associated with susceptibility to BD in the Portuguese population.

Patients and methods

Patients

The study population included 122 unrelated Portuguese patients with BD (84 females and 38 males), aged between 15 and 72 years (average: 42.6 years), 95 from the Internal Medicine Department of Hospital de Santo António-Centro Hospitalar do Porto, 21 from the Portuguese Institute of Rheumatology and 6 from the Internal Medicine Department of Hospital São Teotónio. All of them fulfilled the International

Competing interests: none declared.

Study Group diagnostic criteria for BD (17) evaluated following a controlled protocol. The control group consisted of 227 healthy individuals unrelated to each other or to the patients. The study was approved by the local ethics committee and all participants gave written informed consent.

All relevant clinical manifestations developed since the onset of BD were recorded on a standard form. Patients were then divided into two severity groups: mild – patients with only mucocutaneous findings or acute attacks of arthritis; severe – patients with at least one of the following characteristics:

- i. one or more attacks of uveitis in a year
- ii. deep vein thrombosis of the lower extremities
- iii. neurological involvement, including sinus thromboses
- iv. thrombosis of the superior and/or inferior vena cavae, including the hepatic veins
- v. arterial aneurysms and occlusions,
- vi. intestinal involvement.

CCR532 genotyping

Genomic DNA was extracted from EDTA anticoagulated peripheral venous blood. Genotyping of CCR5Δ32 polymorphism was assayed by PCR fragment sizing using agarose gel electrophoresis. Comprised a one-step PCR method with forward primer 5'-CATCATCCTCCTGACAATCG-3' and reverse primer 5'-CCAGCCCCAAGATGACTATC-3' flanking of the region containing the 32-bp deletion. All assays included positive 225-bp and 193-bp ladder controls and a 100-bp commercial ladder. PCR products of 225 and 193 bp were analysed by electrophoresis in 3% agarose gels and visual-

ised using UV fluorescence after staining with ethidium bromide. A negative control containing no added DNA was included in each amplification reaction, and the absence of a PCR product under these conditions was confirmed. HLA Class I genotyping was carried out as previously described (3).

Statistical analysis

HLA frequencies in patients and controls were compared using the Pearson chi-squared test or the Fisher's exact test as appropriate. All analyses were undertaken using PASW Statistics 18 software (IBM Corporation, Somers, NY, USA). Hardy-Weinberg equilibrium was tested using the Gene [VA] website Tool.

Results

The CCR5 deletion polymorphism distribution was in Hardy-Weinberg equilibrium in both populations. No significant differences were observed in the allelic and genotypic frequencies of CCR5Δ32 between patients and controls (Table I). Gender effects were not observed (Table I). Also, when the data were stratified for the presence of HLA-B*51 no differences between patients and controls were found. Concerning severity, no differences were observed between mild and severe forms (data not shown). Table II summarises the results of previously performed studies regarding CCR5Δ32 in different populations of BD patients, including this study.

Discussion

Our results indicate that CCR5Δ32 polymorphism is unlikely to contribute to BD susceptibility in Portuguese

patients, even after stratification for the presence of HLA-B*51, a known genetic factor for this disease (3). This is in agreement with Yang *et al.* previous work (14) and may be explained by the known functional redundancy of this signaling system. The putative association between this deletion and female gender in BD, reported by Mojtahedi in Iran, was not replicated in Portuguese BD women. It should be noted that the association found in that study is marginal ($p=0.047$). Nevertheless and even though we could not demonstrate an association between BD and CCR5Δ32, the results from Mojtahedi in Iran (15) may warrant further investigation in particular subgroups of patients, taking in account different environmental factors. In 2012, an Italian study (16) found an association of CCR5Δ32 and susceptibility to BD but they stress that although this association may be a genuine finding, it can be limited to one or some ethnic groups. Interestingly, the CCR5Δ32 polymorphism is found mainly in Europe and western Asia, with higher frequencies generally in the north (18), where the prevalence of BD is low, in opposition to HLA-B*51 that has a higher frequency in areas in which BD is more prevalent (19). Population association studies may be complicated by genetic differences between ethnic groups. As distribution of CCR5Δ32 polymorphism varies throughout the world, we must stress that epidemiologic studies should be carried out at different latitudes, keeping in mind that the role of this polymorphism in BD may not be the same for all populations.

It should be acknowledged that this study is underpowered for identifying

Table I. Genotypic and allelic frequencies of the CCR5Δ32 in patients and controls.

Genotypes	Total		Female		Male	
	BD (n=122) n (%)	Controls (n=230) n (%)	BD (n=84) n (%)	Controls (n=131) n (%)	BD (n=38) n (%)	Controls (n=99) n (%)
CCR5, CCR5	106 (86.9)	194 (84.3)	72 (85.7)	110 (84.0)	34 (89.5)	84 (84.8)
CCR5, Δ32	15 (12.3)	34 (14.8)	11 (13.1)	19 (14.5)	4 (10.5)	15 (15.2)
Δ32, Δ32	1 (0.8)	2 (0.9)	1 (1.2)	2 (1.5)	0 (0.0)	0 (0.0)
<i>Allele</i>						
CCR5	227 (93.0)	422 (91.7)	155 (92.3)	239 (91.2)	72 (94.7)	183 (92.4)
Δ32	17 (7.0)	38 (8.3)	13 (7.7)	23 (8.8)	4 (5.3)	15 (7.6)

Table II. Results of previously performed studies regarding CCR5Δ32 in different populations of BD patients.

Genotypes	n (%)											
	Yang X <i>et al.</i> (14)						Atzeni F <i>et al.</i> (16)		Mojtahedi Z <i>et al.</i> (15)		Present study	
	UK Caucasoid		Turkish		Palestinian		Italian		Iranian		Portuguese	
	BD (n=131)	Controls (n=325)	BD (n=109)	Controls (n=96)	BD (n=110)	Controls (n=98)	BD (n=196)	Controls (n=180)	BD (n=100)	Controls (n=380)	BD (n=122)	Controls (n=230)
CCR5, CCR5	98 (74.8)	241 (74.1)	96 (88.1)	88 (91.7)	105 (95.5)	97 (99.0)	172 (87.8)	170 (94.4)	91 (91)	362 (95.3)	106 (86.9)	194 (84.3)
CCR5, Δ32	31 (23.7)	77 (23.6)	12 (11.0)	8 (8.3)	5 (4.6)	1 (1.0)	24 (12.2)	10 (5.6)	9 (9)	18 (4.7)	15 (12.3)	34 (14.8)
Δ32, Δ32	2 (1.5)	7 (2.1)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	2 (0.9)
<i>Allele</i>												
CCR5	227 (87.0)	559 (86.0)	204 (94.0)	184 (96.0)	215 (98.0)	195 (99.5)	368 (93.9)	350 (97.2)	191 (95.5)	742 (97.6)	227 (93.0)	422 (91.7)
Δ32	35 (13.0)	91 (14.0)	14 (6.0)	8 (4.0)	5 (2.0)	1 (0.5)	24 (6.1)*	10 (2.8)*	9 (4.5)	18 (2.4)	17 (7.0)	38 (8.3)

* $p=0.024$.

small genetic effects. For this reason, these results only exclude a moderate to high genetic effect ($OR > 1.5$) of this polymorphism in BD. In conclusion we suggest that CCR5Δ32 polymorphism is not associated with BD in the Portuguese population. Large-scale studies, from different populations, should be carried out to verify our findings.

References

- EVEREKLIOGLU C: Current concepts in the etiology and treatment of Behçet disease. *Surv Ophthalmol* 2005; 50: 297-350.
- MENDOZA-PINTO C, GARCIA-CARRASCO M, JIMENEZ-HERNANDEZ M *et al.*: Etiopathogenesis of Behçet's disease. *Autoimmun Rev* 2010; 9: 241-5.
- BETTENCOURT A, PEREIRA C, CARVALHO L *et al.*: New insights of HLA class I association to Behçet's disease in Portuguese patients. *Tissue Antigens* 2008; 72: 379-82.
- HUGHES T, COIT P, ADLER A *et al.*: Identification of multiple independent susceptibility loci in the HLA region in Behçet's disease. *Nat Genet* 2013; 45: 319-24.
- KANG EH, CHOI JY, LEE YJ, LEE EY, LEE EB, SONG YW: Single nucleotide polymorphisms in IL-10-mediated signalling pathways in Korean patients with Behçet's disease. *Clin Exp Rheumatol* 2014; 32 (Suppl. 84): S27-S32.
- YILMAZ S, CINAR M, PEKEL A *et al.*: The expression of transmembrane and soluble CXCL16 and the relation with interferon- α secretion in patients with Behçet's disease. *Clin Exp Rheumatol* 2013; 31: 84-7.
- NA SY, PARK MJ, PARK S, LEE ES: Up-regulation of Th17 and related cytokines in Behçet's disease corresponding to disease activity. *Clin Exp Rheumatol* 2013; 31: 32-40.
- WU Z, CHEN H, SUN F *et al.*: PTPN22 rs1893217 single-nucleotide polymorphism is associated with risk of Behçet's disease in a Chinese Han population. *Clin Exp Rheumatol* 2014; 32 (Suppl. 84): S20-S26.
- TURUNC G, COSKUN D, ALIBAZ-ONER F *et al.*: TIR-domain-containing adaptor protein gene TIRAP S180L polymorphism is not increased in Behçet's disease patients in two ethnic cohorts. *Clin Exp Rheumatol* 2013; 31: 54-6.
- KIRINO Y, BERTSIAS G, ISHIGATSUBO Y *et al.*: Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between HLA-B*51 and ERAP1. *Nat Genet* 2013; 45: 202-7.
- MARTENS HA, KALLENBERG CG, BIJL M: Role of CCR5 Delta32 bp deletion in RA and SLE. *Autoimmunity* 2009; 42: 260-2.
- ONDER M, KOKTURK N, POYRAZ A, OZTAS MO: Expression of CCR5 in Behçet's disease. *Adv Exp Med Biol* 2003; 528: 245-7.
- DALGHOUS AM, FREYSDOTTIR J, FORTUNE F: Expression of cytokines, chemokines, and chemokine receptors in oral ulcers of patients with Behçet's disease (BD) and recurrent aphthous stomatitis is Th1-associated, although Th2-association is also observed in patients with BD. *Scand J Rheumatol* 2006; 35: 472-5.
- YANG X, AHMAD T, GOGUS F *et al.*: Analysis of the CC chemokine receptor 5 (CCR5) Delta32 polymorphism in Behçet's disease. *Eur J Immunogenet* 2004; 31: 11-4.
- MOJTAHEDI Z, AHMADI SB, RAZMKHAH M, AZAD TK, RAJAEI A, GHADERI A: Association of chemokine receptor 5 (CCR5) delta32 mutation with Behçet's disease is dependent on gender in Iranian patients. *Clin Exp Rheumatol* 2006; 24: S91-94.
- ATZENI F, BOIARDI L, CASALI B *et al.*: CC chemokine receptor 5 polymorphism in Italian patients with Behçet's disease. *Rheumatology (Oxford)* 2012; 51: 2141-5.
- CRITERIA FOR DIAGNOSIS OF BEHÇET'S DISEASE: International Study Group for Behçet's Disease. *Lancet* 1990; 335: 1078-80.
- NOVEMBRE J, GALVANI AP, SLATKIN M: The geographic spread of the CCR5 Delta32 HIV-resistance allele. *PLoS Biol* 2005; 3: e339.
- VERITY DH, MARR JE, OHNO S, WALLACE GR, STANFORD MR: Behçet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. *Tissue Antigens* 1999; 54: 213-20.