Association of CCR5Δ32 and Behçet's disease: new data from a case-control study in the Spanish population and meta-analysis

L. Ortiz-Fernández[1], J.-R. García-Lozano[1], M.-A. Montes-Cano[1], M. Conde-Jaldón[1], N. Ortego-Centeno[2], M.-J. Castillo-Palma[3], G. Espinosa[4], G. Graña-Gil[5], J. Sánchez-Bursón[6], M.R. Juliá[7], R. Blanco[8], A.-C. Barnosi-Marín[9], R. Solans[10], P. Fanlo[11], M. Rodríguez Carballeira[12], T. Camps[13], S. Castañeda[14], J. Martín[15], M.-F. González-Escribano[1]

*Lourdes Ortiz-Fernández**
José-Raúl García-Lozano
Marco-Antonio Montes-Cano
Marta Conde-Jaldón
Norberto Ortego-Centeno
María-Jesús Castillo-Palma
Gerard Espinosa, Genaro Graña-Gil
Juan Sánchez-Bursón, María Rosa Juliá
Ricardo Blanco, Ana-Celia Barnosi-Marín
Roser Solans, Patricia Fanlo
Mónica Rodríguez Carballeira
Teresa Camps, Santos Castañeda
Javier Martín
María-Francisca González-Escribano

**Authors' affiliations on page S-99.*

This work should be attributed to: Servicio de Inmunología, IBiS, Hospital Universitario Virgen del Rocío/CSIC/ Universidad de Sevilla, Sevilla. Spain.

Please address correspondence to: María Francisca González-Escribano, Servicio de Inmunología. HU Virgen del Rocío, 41013 Sevilla, Spain. E-mail: mariaf.gonzalez.sspa@juntadeandalucia.es

Received on February 3, 2015; accepted in revised form on June 4, 2015.

Clin Exp Rheumatol 2015; 33 (Suppl. 94): S96-S100.

Key words: Behçet's disease, CCR5 gene, CCR5 delta 32 deletion, genetic contribution, meta-analysis

Funding: this work was supported by Fondo de Investigaciones Sanitarias (10/1701 and 13/01118), Fondos FEDER, Plan Andaluz de Investigación (CTS-0197), Red Enfermedades Inflamatorias y Reumáticas (RD08/0075/0013) and Consejería de Salud de la Junta de Andalucía (PI411/10).
L. Ortiz-Fernández is the recipient of a fellowship (FI11/00547).

Competing interests: none declared.

ABSTRACT

Objective. *Behçet's disease (BD) is an immune-mediated and complex disease associated with HLA class I and other genes. The aim of this study was to contribute to a better understanding of the relationship of the 32-bp deletion in the* CCR5 *gene (*CCR5Δ32*) and this disease by conducting a case-control study in the Spanish population and also a meta-analysis including all the studies available to date.*

Methods. *A cohort composed of 348 BD Spanish patients and 477 unrelated healthy and ethnically matched individuals were genotyped in* CCR5Δ32 *using polymerase chain reaction (PCR) and capillary electrophoresis with fluorescent detection. In the meta-analysis, data from a total of seven populations extracted from four previous studies along with data of the present study were included.*

Results. *Regarding the case-control study, no statistically significant differences were observed when the patient and control groups were compared (allelic model: 0.07 in patients versus 0.06 in controls, p=0.303). In the meta-analysis, no evidence of association of the* CCR5Δ32 *polymorphism with BD was observed (p_{MH}= 0.091; OR= 1.22; 95%CI 0.98 to 1.52 in the allelic model).*

Conclusion. *The results of this meta-analysis discard a major role of the* CCR5Δ32 *polymorphism in BD.*

Introduction

Behçet's disease (BD) is a multisystemic inflammatory disorder characterised by recurrent oral and genital ulcerations; ocular affectation, mainly uveitis, and skin lesions, additional manifestations which involve other organs such as joints and central nervous system are relatively common. The aetiology of BD remains elusive, although it has been suggested that the disease is the result of complex interactions between environmental factors (*e.g.* certain infectious agents) and genetic predisposition (1).

The evidences of genetic contribution to the pathogenesis of the disease are based on familial aggregation, predominance in patients with Mediterranean or Asian ancestry and the association with human histocompatibility complex (HLA) in several ethnic groups (2-4). The contribution of the HLA region has been estimated to represent approximately 20% of the genetic component of this disease therefore other genes should be involved in the predisposition to this pathology (5). As a result of different studies designed to establish the contribution of genes located outside the HLA class I region with the disease, a relationship of BD with IL23R, IL10 and other genes has been established in different populations (6-12).

One of the non-HLA genes proposed as a candidate in BD is the chemokine receptor type 5 (CCR5), a G protein-coupled receptor which is expressed on Th1 cells, monocytes and dendritic cells. Binding of CCR5 to its ligands mediates the migration of mononuclear cells to the inflammation site and, in addition, the CCR5 molecule is also a co-receptor of the HIV. The most extensively studied polymorphism in this gene is a deletion of 32 bp (CCR5Δ32) which results in the introduction of a premature stop codon by a reading frameshift producing, as a consequence, a truncated protein unable to

bind to the natural ligands of CCR5 (13). The *CCR5Δ32* allele is related to the resistance to HIV infection (14) and it has also been associated with immune-based diseases such as rheumatoid arthritis and multiple sclerosis (15, 16). The role of the CCR5Δ32 variant in immuno-mediated diseases is not clear because in some conditions such as rheumatoid arthritis it appears as a protective factor (17) because it is associated with a reduced risk whereas in others such as multiple sclerosis it increases the risk to develop the disease (18). CCR5 mediates mononuclear cell recruitment to sites of inflammation by interacting with its ligands CCL3, CCL4 and CCL5. The lack of functional CCR5 causes up-regulation of its ligands which can exert their biological effects by engaging other available receptors (19). Thus, lymphocytes from CCR5Δ32 homozygous subjects secrete CCL5 at levels that are 5–10 times higher than the CCR5 non-mutated control subjects (20). The elevated levels of this chemokine can, by engaging other available receptors such as CCR3 and CCR1 result in increased recruitment of inflammatory cells and production of pro-inflammatory cytokines.

So far, four studies investigating the association between CCR5 gene and BD have been published (21-24). Three of these studies found no association of the CCR5Δ32 variant with susceptibility to BD. Nevertheless, they include cohorts with a relatively small sample size and this fact, along with the low frequency of the variant, determines that the statistical power of the individual studies is inadequate. The aim of this study was to contribute to improve the current knowledge about the relationship of this functional variant of the CCR5 gene and BD by investigating whether the *CCR5Δ32* variant is associated with BD in the Spanish population and also by conducting a meta-analysis including all the available data.

Material and methods

Case-control study

This part of the study includes 348 BD patients (153 males and 195 females) with a mean age at onset (years) ± SD of 48.22±12.19 who fulfilled the 1990 International Study Group classification criteria for Behçet's disease (25) and 477 unrelated healthy individuals recruited in the same geographic regions and matched by age and gender with BD patients. All the subjects were Spanish from European origin, patient group was partially included in the Registry of the Spanish network of Behçet's disease (26) and they were recruited from different Spanish hospitals. The study was approved by the local ethics committees of all the participant hospitals., A Coruña (CHU A Coruña), Almería (H. Torredecárdenas), Barcelona (H. Clinic, Vall d'Hebron and Mútua Terrassa), Granada (H. Clínico San Cecilio), Madrid (H. de la Princesa), Málaga (H. Carlos Haya), Palma de Mallorca (H, Universitari Son Espases), Pamplona (H. Virgen del Camino), Santander (H. Marques de Valdecilla) and Sevilla (H. Virgen del Rocío y H. Virgen de Valme) and a written informed consent was obtained from all participants. Clinical features of the patient group were: 100% had oral ulcers, 66% genital ulcers, 59% uveitis, 51.6% arthritis, and 22% vascular, 23% neurological and 20% gastrointestinal involvement.

Peripheral blood or saliva were used as the starting material. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Quiagen, Barcelona, Spain) according to the manufacturer's recommendations and stored at -20ºC until use. The purity of DNA was determined using NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Only those DNA samples having a 260/280 ratio between 1.7 and 2.0 and a final concentration of 10-20 ng/µl were genotyped.

Genotyping was performed by polymerase chain reaction (PCR) using primers spanning the region of the 32 bp deletion in the *CCR5* gene. The sequence of the primers were those previously described 5'CTTCATCATCCTCCTGACAATCG3' (sense) and 5'GACCAGCCCAAGTTGACTATC3' (antisense) (27), labelling the sense primer with a fluorochrome (Fam) at the 5' end. The PCR products were separated by capillary electrophoresis using a 3130/3130xl Genetic Analyzer (Applied Biosystems, Barcelona, Spain) with fluorescent detection. The expected size of the amplified DNA fragments for the wild-type and deletion were 262 bp and 230 bp respectively. About 5% of the samples were studied in duplicate to verify genotyping consistency which was identical in all the cases.

The statistical powers were calculated with the Statistical Power test of the Research Tool Kit of the DSS Researh Software (https://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx) to detect associations with odds ratios (OR) greater than 1.5 in the allelic model and taking into account the allelic frequency of the variant in each population. All the statistical analyses were performed with PLINK v.1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). The χ^2 test was used to test Hardy Weinberg equilibrium (HWE). To check association of the variant CCR5Δ32 with disease, the distribution of alleles and genotypes in patient and control groups were compared using χ^2 (or Fisher exact test when appropriated) from 2x2 contingency tables. Odds ratios (ORs) and 95% confidence intervals (CI 95%) were calculated according to the Woolf method.

Meta-analysis

We conducted a meta-analysis which includes the data of the present study together with those previously published regarding the relationship of the CCR5Δ32 variant and BD. Literature included in the analysis was selected using the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/.) and Scopus (http://www.scopus.com) searching for "CCR5 or chemokine receptor type 5" and "polymorphism" and "Behçet's disease". References from the retrieved papers were also checked. Papers published up to January 2015 were considered to be included in the meta-analysis. The eligible studies were included when they met the following criteria: association studies written in English, in which commonly accepted classification criteria for Behçet's disease were used, reporting data of the population under study and displaying genotype distribution for the CCR5Δ32 polymorphism both in the patient and control groups. Exclusion criteria were HWE

deviation, insufficient information and redundant or overlapping results.

The combined data were summarised in two by two tables. The Brewslow-Day method, the Cochran χ^2-based Q test and the inconsistency index (I^2) were used to assess heterogeneity in the different populations. Brewslow-Day and Q test with *p*-values (P_{BD} and P_Q) lower than 0.05 were considered statistically significant. Those I^2 values from 0 to 25% were considered non heterogeneity, 25–50% moderate, 50–75% large and 75–100% extreme heterogeneity. The ORs were pooled using the Mantel-Haenszel, Robins-Breslow-Greenland methods for fixed effects because non significant heterogeneity was detected and *p*-values (P_{MH}) lower tan 0.05 were considered significant. All associations were tested under the allelic and the dominant model and the meta-analysis was conducted using StatsDirect v. 2.6.6 (StatsDirect, Altrincham,UK) software. In addition, to examine the degree to which an individual study affects the overall estimate, sensitivity analyses were conducted by removing one study at a time and analysing the change of the pooled effect.

Results

Case-control study

In the cohort included in our study, the successful rate of genotyping was 95%, the study population was found to be in the Hardy-Weinberg equilibrium ($p>0.05$) and distribution of the *CCR5Δ*32 variant in the cohorts from different hospitals was not significantly different. Table I displays the data of genotyping of *CCR5Δ*32 in our cohort of BD patients and healthy controls. Frequency of the *CCR5Δ*32 variant in our control population was 0.06 similar to that described in other South European populations and lower than that found in the North of Europe where the highest frequency of the *CCR5Δ*32 variant has been described. No statistically significant differences were observed when the patient and control groups were compared under the allelic model (0.07 in patients *vs*. 0.06 in controls, $p=0.303$). Regarding the genotypes distribution, no significant differences were found comparing the distribution in patients and controls under the dominant model ($p=0.390$). Statistical analyses of the other inheritance models were not performed because of the low number of individuals CCR5Δ32/CCR5Δ32. Regarding gender and HLA-B*51 subgroups, frequencies of the variant in our cohort were 0.07 in males *vs*. 0.06 in females ($p=0.688$) and 0.06 in the subjects B*51 positive *vs*. 0.07 in those negative ($p=0.551$). Therefore, no significant differences attributable to gender neither to the presence of B*51 were found. The possible association between the CCR5Δ32 variant and the main clinical characteristics of BD were analysed but non significant differences were found (data not shown).

Table I. Frequency of the CCR5Δ32 genotypes in Spanish BD patients and healthy controls. The allelic and dominant models of inheritance are also displayed.

	Patients (n=348)		Controls (n=477)		*p*-value
Genotype	n	%	n	%	
Δ32/Δ32	1	0.3	0	0	0.325
Δ32/wt	46	13.2	54	11.3	
wt/wt	301	86.4	423	88.7	
Allelic Model					
Δ32	48	6.9	54	5.7	0.303
wt	648	93.1	900	94.3	
Dominant model					
Δ32/Δ32 + Δ32/wt	47	13.5	54	11.3	0.390
wt/wt	301	86.5	423	88.7	

wt: Wild type; Δ32: CCR5Δ32 variant.

Meta-analysis

To avoid the low statistical power of the individual studies published to date regarding the relationship of the *CCR5Δ32* polymorphism and BD (see Table II), a meta-analysis was conducted. This meta-analysis includes the data pooled of the four papers previously published (all of them fulfilled the above-mentioned criteria) together with data of the present study. A summary of the data of all these studies are displayed in Table II. No heterogeneity was detected in the analysis of this polymorphism in the homogeneity analysis ($P_{BD}=0.1881$, $P_Q= 0.2089$ and $I^2=28.7\%$). Therefore, the subsequent meta-analysis was performed using the fixed effect method with the pooled data from a total of 1114 patients and 1786 healthy controls from seven different populations. The statistical power of the pooled data to detect an association with OR≥1.5 is 93%. As a result of this meta-analysis, no evidence of association of the *CCR5Δ32* polymorphism with BD was observed ($P_{MH}= 0.091$; OR= 1.22; 95%CI 0.98 to 1.52 in the allelic model) (Fig. 1). The results obtained considering a dominant model were very similar to those obtained with the allelic (data not shown).

Discussion

The results of the present study suggest no association of the *CCR5Δ32* with the disease in the Spanish population. Most of the four individual studies published until the present regarding the association of this polymorphism have reported the same conclusion. In fact, only one study performed in an Italian cohort reported *CCR5Δ*32 as a risk factor for BD (23). Although most of the studies discarded influence of this variant in the susceptibility to the disease, the statistical power of the individual studies, including the present, is low to reach a definitive conclusion. In fact, in all the populations except in the UK and the Portuguese populations (21-24) the frequency of the variant is higher in patients and therefore, differences could not reach significance as a consequence of limitation in size. Nevertheless, according to data of the meta-analysis presented in this study, which is well powered, a major role of the *CCR5Δ32* polymorphism in BD is discarded.

In some of the previous studies, authors suggested association of this variant with a specific subgroup. Thereby, in a study in an Iranian population, the authors suggested association be-

Table II. Characteristics of the studies included in the meta-analysis.

Author	Year	Population	Genotyping methods	Sample Size		Allelic frequency of the Δ32 variant		% SP*	*p*-value	OR (95%CI)
				Cases	Controls	Cases	Controls			
Yang *et al*.	2003	UK	PCR and agarose gel electrophoresis	131	325	0.13	0.14	67.3	>0.05	
		Turkish		109	96	0.06	0.04	21.7	>0.05	
		Palestinian		110	98	0.02	0.005	9.0	>0.05	
Mojtahedi *et al*.	2006	Iranian	PCR and agarose gel electrophoresis	100	380	0.09	0.05	37.2	>0.05	
Atzeni *et al*.	2012	Italian	PCR and agarose gel electrophoresis	196	180	0.06	0.03	25.0	0.02	2.28 (1.1-4.8)
Bettencourt *et al*.	2015	Portuguese	PCR and agarose gel electrophoresis	122	230	0.07	0.08	27	>0.05	
Present study	2014	Spanish	PCR and capillary electrophoresis	348	477	0.07	0.06	68.2	>0.05	

*SP-Statistical power was calculated taking into account the allelic frequency and the sample size of each population and an expected OR ≥1.5.

tween *CCR5Δ32* and BD but only in the case of female patients (22). Nevertheless, data in our population, consistently with the study in the Portuguese population (24), do not confirm an influence of the *CCR5Δ32* variant which would be dependent on gender because the distribution of the variant was not different in males and females. Additionally, data in the UK population, suggest that the frequency of the *CCR5Δ32* variant is increased among patients B*51 positive (21). This fact could reflect association only among individuals B*51 and/or a higher homogeneity of the patients with this HLA risk factor. On this sense, a previous meta-analysis studying the influence of this CCR5 variant in BD and other diseases, suggested association of the *CCR5Δ32* among B*51 carriers (28). Nevertheless, this association was not found in the Portuguese population (24) neither in the present study in the Spanish population. The global distribution of this CCR5 variant is well known mainly because of its interest in the outcome of the HIV infection. In general, this mutation is more common among people with European ancestry but in the southern Europe, including Mediterranean populations, the frequency is usually lower than in the northern Europe. Frequencies that are found in the Middle East populations are similar to those found in the Mediterranean area and its presence is sporadic in the Asian populations (29). For this reason, although BD has a relatively high incidence in the Japanese and Chinese populations the influence of this variant in susceptibility to this disease in Asian populations did not have investigated because this variant practically does not exist in these populations. The question about association of CCR5 remains open in Asian populations because the influence of other functional variants of this gene which are present in these populations has not been yet investigated.

In conclusion, according to data of the meta-analysis presented in this study, a major role of the *CCR5Δ32* polymorphism in BD can be discarded.

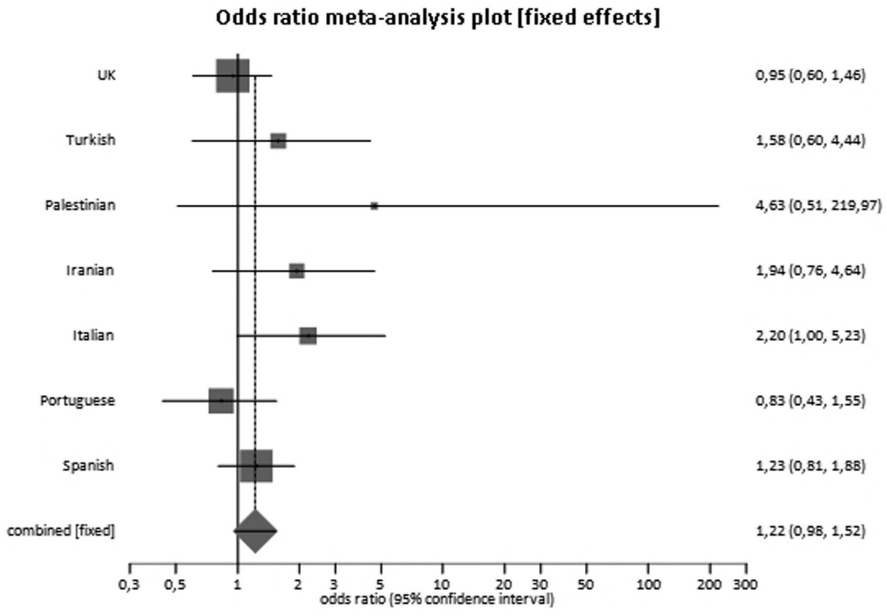

Fig. 1. Forest plots displaying the odds ratios and 95% confidence intervals of CCR5Δ32 polymorphism pooling the six populations included in the meta-analysis. The meta-analysis was conducted under fixed-effects and considering an allelic inheritance model.

Acknowledgments

The authors would like to thank the Asociación Andaluza de Enfermedades Autoinmunes (AADEA) and all patients and donors enrolled in the present study for their cooperation.

Authors' affiliations

[1]*Servicio de Inmunología, IBiS, Hospital Universitario Virgen del Rocío/ CSIC/Universidad de Sevilla, Sevilla;*
[2]*Servicio de Medicina Interna, Hospital Clínico San Cecilio, Granada;*
[3]*Servicio de Medicina Interna, Hospital Universitario Virgen del Rocío, Sevilla;*
[4]*Servicio de Enfermedades Autoinmunes, Hospital Clinic, Barcelona;*
[5]*Servicio de Reumatología, Complejo Hospitalario Universitario, A Coruña;*
[6]*Servicio de Reumatología, Hospital Universitario de Valme, Sevilla;*
[7]*Servicio de Inmunología,*

Hospital Universitari Son Espases, Palma de Mallorca;
[8]Servicio de Reumatología, Hospital Marqués de Valdecilla, Santander;
[9]Servicio de Medicina Interna, Hospital de Torrecárdenas, Almería;
[10]Servicio de Medicina Interna, Hospital Vall d'Hebron, Barcelona;
[11]Servicio de Medicina Interna, Hospital Virgen del Camino, Pamplona;
[12]Servicio de Medicina Interna, Hospital Universitari Mútua, Terrassa;
[13]Servicio de Medicina Interna, Hospital Universitario Carlos Haya, Málaga;
[14]Servicio de Reumatología, Hospital de la Princesa, Madrid;
[15]Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas, Granada, Spain.

References

1. MENDES D, CORREIA M, BARBEDO M *et al*.: Behçet's disease: a contemporary review. *J Autoimmun* 2009; 32: 178-88.
2. KONÉ-PAUT I, GEISLER I, WECHSLER B *et al*.: Familial aggregation in Behçet's disease: high frequency in siblings and parents of pediatric probands. *J Pediatr* 1999; 135: 89-93.
3. SAKANE T, TAKENO M, SUZUKI N, INABA G: Behçet's disease. *N Engl J Med* 1999; 341: 1284-91.
4. MENTHON DE M, LAVALLEY MP, MALDINI C, GUILLEVIN L, MAHR A: HLA-B51/B5 and the risk of Behçet's disease: a systematic review and meta-analysis of case-control genetic association studies. *Arthritis Rheum* 2009; 61: 1287-96.
5. YAZICI H, FRESKO I, YURDAKUL S: Behçet's syndrome: disease manifestations, management, and advances in treatment. *Nat Clin Pract Rheumatol* 2007; 3: 148-55.
6. 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.
7. MEGURO A, INOKO H, OTA M *et al*.: Genetics of Behçet disease inside and outside the MHC. *Ann Rheum Dis* 2010; 69: 747-54.
8. KARASNEH J, GÜL A, OLLIER WE, SILMAN AJ, WORTHINGTON J: Whole-genome screening for susceptibility genes in multicase families with Behçet's disease. *Arthritis Rheum* 2005; 52: 1836-42.
9. FEI Y, WEBB R, COBB BL, DIRESKENELI H, SARUHAN-DIRESKENELI, SAWALHA AH: Identification of novel genetic susceptibility loci for Behçet's disease using a genome-wide association study. *Arthritis Res Ther* 2009; 11: R66.
10. REMMERS EF, COSAN F, KIRINO Y *et al*.: Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. *Nat Genet* 2010; 42: 698-702.
11. MIZUKI N, MEGURO A, OTA M *et al*.: Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci. *Nat Genet* 2010; 42: 703-6.
12. HATEMI G, SEYAHI E, FRESKO I, TALARICO R, HAYAMURYUDAN V: Behçet's sindrome: a critical digest of the 2013-2013 literature. *Clin Exp Rheumatol* 2014; 32 (Suppl. 84): S112-122.
13. CHARO IF, RANSOHOFF RM: The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006; 354: 610-21.
14. LIU R, PAXTON WA, CHOE S *et al*.: Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996; 86: 367.
15. RODRÍGUEZ-RODRÍGUEZ L, GONZÁLEZ-JUANATEY C, GARCÍA-BERMÚDEZ M *et al*.: CCR5Δ32 variant and cardiovascular disease in patients with rheumatoid arthritis: a cohort study. *Arthritis Res Ther* 2011; 13: R133.
16. PULKKINEN K, LUOMALA M, KUUSISTO H *et al*.: Increase in CCR5 Delta32/Delta32 genotype in multiple sclerosis. *Acta Neurol Scand* 2004; 109: 342-7.
17. PRAHALAD S: Negative association between the chemokine receptor CCR5-Delta32 polymorphism and rheumatoid arthritis: a meta-analysis. *Genes Immun* 2006; 7: 264-8.
18. FAVOROVA OO, ANDREEWSKI TV, BOIKO AN *et al*.: The chemokine receptor CCR5 deletion mutation is associated with MS in HLA-DR4-positive Russians. *Neurology* 2002; 26: 1652-5.
19. CHARO IF, RANSOHOFF RM: The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006; 354: 610-21.
20. PAXTON WA, KANG S: Chemokine receptor allelic polymorphisms: relationships to HIV resistance and disease progression. *Semin Immunol* 1998; 10: 187-94.
21. YANG X, AHMAD T, GOGUS F *et al*.: Analysis of the CC chemokine receptor (CCR5) Delta32 polymorphism in Behçet's disease. *Eur J Immunogenet* 2004; 31: 11-4.
22. MOJTAHEDI Z, AHMADI SB, RAZMKHAH M, AZAD TK, RAJAEE A, GHADERI A: Association of chemokine receptor 5 (CCR5) Δ32 mutation with Behçet's disease is dependent on gender in Iranian patients. *Clin Exp Rheumatol* 2006; 24: S91-4.
23. ATZENI F, BOIARDI L, CASALI B *et al*.: CC chemokine receptor 5 polymorphism in Italian patients with Behçet's disease. *Rheumatology* 2012; 51: 2141-5.
24. BETTENCOURT A, LEAL B, CARVALHO C *et al*.: CC chemokine receptor polymorphism CCR5Δ32 in Portuguese Behçet's disease patients. *Clin Exp Rheumatol* 2014; 32: S72-74.
25. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
26. RODRÍGUEZ-CARBALLEIRA M, ALBA MA, SOLANS-LAQUÉ R *et al*.: Registry of the Spanish network of Behçet's disease: a descriptive analysis of 496 patients. *Clin Exp Rheumatol* 2014; 32 (Suppl. 84): S33-9.
27. KRISTIANSEN TB, KNUDSEN TB, OHLENDORFF S, EUGEN-OLSEN J: A new multiplex PCR strategy for the simultaneous determination of four genetic polymorphisms affecting HIV-1 disease progression. *J Immunol Methods* 2001; 252: 147-51.
28. SONG GG, KIM JH, LEE YH: The chemokine receptor 5 delta32 polymorphism and type 1 diabetes, Behçet's disease, and asthma: a meta-analysis. *Immunol Invest* 2014; 43: 123-36.
29. MARTINSON JJ, CHAPMAN NH, REES DC, LIU YT, CLEGG JB: Global distribution of the CCR5 gene 32-basepair deletion. *Nat Genet* 1997; 16: 100-3.