PTPN22 is not associated with Behçet's disease. Study spanning the complete gene region in the Spanish population and meta-analysis of the functional variant R620W

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ABSTRACT

Objective. *The functional variant R620W of the protein tyrosine phosphatase non receptor-22 (PTPN22) gene plays an important role in susceptibility to several immuno-mediated pathologies. Behçet's disease (BD) is a complex disease related to the immune system with a demonstrated genetic base. The HLA class I genes are the most important genetic factors in BD although other genes are also involved in the susceptibility to this disease. The PTPN22 has been proposed as a candidate gene in BD but this association has not been clearly demonstrated yet. The aim of this study was to assess the association of PTPN22 with BD.*

Methods. *A cohort composed of 404 Spanish BD patients and 1517 unrelated healthy individuals ethnically matched was genotyped in rs2476601 (R620W). Five tag SNPs: rs1217412, rs2476599, rs3789607, rs3765598 and rs1217419 (spanning a 57 Kb region between 3'UTR and 5'UTR) and rs2488457 (located at the promoter region) were also studied in order to perform a screening of the complete gene. Genotyping was performed using TaqMan® assays. The rs2476601 data were included in a meta- analysis together with those published till the date. The rest of the SNPs were used in a case-control study.*

Results. *No evidence of the association of rs2476601 with BD in the meta-analysis (p=0.504 in the model of alleles) was found. In the case-control study, no statistically significant differences were observed when comparing the distribution of variants in patients and controls.*

Conclusion. *Our results do not support a major role of the PTPN22 gene in BD.*

Introduction

Behçet's disease (BD) is an immune-mediated and complex disease in which certain environmental factors such as infectious agents are the triggers of the disease in genetically predisposed individuals (1). This rare condition characterised by recurrent oral, genital ulcerations and other manifestations such as ocular affectation, mainly uveitis, is most common along the old route named "Silk Road", stretching from China to the Mediterranean area (2, 3). This particular geographical distribution in conjunction with the familial aggregation and association with HLA class I molecules (specifically with HLA-B51) are evidences supporting a genetic base into the pathogenesis of the disease (4, 5). The contribution of the HLA region to the genetic component has been estimated in approximately 20% (6). Some genes such as IL23R, IL10 and others have been related with BD in different populations (7, 8), whereas in other cases the association seems to be limited to one ethnic group or specific population (9-13).

The protein tyrosine phosphatase non-receptor-22 (*PTPN22*) gene, which is located on 1p13.2, encodes a lymphoid protein tyrosine phosphatase known as Lyp. *PTPN22* is a good candidate to be involved in susceptibility to autoimmune diseases, in fact, a functional variant, C1858T (R620W), has been related with multiple immuno-mediated pathologies such as rheumatoid arthritis (14), systemic lupus erythematosus (15) and systemic sclerosis (16). This variant causes disruption of the binding between Lyp and Csk which suppresses T cell activation and, consequently, individuals with the variant have, at

least theoretically, hyperresponsive T Two previous studies have investigated the association between this variant of *PTPN22* gene and BD but without conclusive results (17, 18). The main problem is the low frequency of R620W variant in different populations and its virtual absence in others, such as Asian populations (http://hapmap.ncbi.nlm.nih.gov/). This circumstance makes more difficult to evaluate the role of this gene in a rare condition such as BD. To our knowledge, no study covering the entire region of the gene has been performed in order to evaluate the relationship of the complete *PTPN22* gene with BD. The aim of the present study was to contribute to clarify the potential role of *PTPN22* in BD by investigating a) the contribution of the R620W variant to the susceptibility to the disease by performing a meta-analysis including data from our cohort together with those reported till the date and b) whether variants located in other positions of this gene contribute to the susceptibility to BD by performing a case-control study with tag SNPs.

Material and methods

Study subjects

This study included a total of 404 BD patients (43.7% males) who fulfilled the 1990 International Study Group classification criteria for Behçet's disease (19). Moreover, 1517 healthy individuals (43% males) were included as control group. All the subjects were Spanish from European origin and they were recruited from different Spanish hospitals. The study was approved for the local ethics committees of the corresponding hospitals and all the study participants gave written informed consent according to the declaration of Helsinki. Clinical features of the patient group were: 100% had oral ulcers, 59.5% genital ulcers, 54% uveitis, 42% arthritis, and 21% vascular, 18.2% neurological and 15.5% gastrointestinal involvement**.** The distribution of the frequencies of the different markers in the cohorts from different hospitals was not significantly different.

DNA extraction

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

Genotyping of rs2476601 and meta-analysis

Genotyping of the rs2476601 was performed using TaqMan® SNP Genotyping Assays (Applied Biosystems, Barcelona, Spain) in a 7900HT Fast Real Time PCR System (Applied Biosystems). A meta-analysis, which included the results of genotyping in our cohort together with those previously published in studies that investigated the relationship of this variant of the *PTPN22* gene and BD was conducted. References included in the analysis were selected from the databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed/.) and Scopus (http://www.scopus.com) using the searching criteria: "PTPN22" or "protein tyrosine phosphatase non-receptor 22" and "polymorphism" and "Behçet's disease". The date of the last revision was July 2015 and the references included in the retrieved papers were also checked. One study was considered as eligible when they met the following criteria: association studies written in English, in which commonly accepted classification criteria for Behçet's disease were used, containing information of the population under study and where the genotype distribution in the patient and control groups were displayed in detail. Exclusion criteria were deviation of Hardy-Weinberg equilibrium (HWE), insufficient information and redundant or overlapping results. One of the eligible studies did not include genotype data of R620W therefore, it was excluded (20).

Tag SNPs selection and genotyping

A *PTPN22* region (Chr1:114158106..114215631) LD plot was generated with the "Utah residents with Northern and Western European ancestry from the CEPH collection" (CEU) data obtained from HapMap (data release 28, Phase II+III, NCBI build 36 assembly, dbSNP b126) (http://hapmap.ncbi.nlm.nih.gov/). The confidence intervals approach was used to define haplotype blocks in the CEU population using the Haploview program (Haploview, v. 4.1. Broad Institute, Cambridge, MA, USA). With these conditions, five tag SNPs (minor allele frequency MAF>0.1, r^2 threshold>0.8) were selected to genotype: rs1217412, rs2476599, rs3789607, rs3765598 and rs1217419 in addition to rs2476601. These six tag SNPs span a 57 Kb region between 3'UTR and 5'UTR in the *PTPN22* gene capturing the 27 SNPs in the region (100% coverage). In addition, rs2488457, which is located in the promoter region, was also included in the study because it is associated with different pathologies (21. (Genotyping was performed using TaqMan® SNP Genotyping Assays (Applied Biosystems) in a 7900HT Fast Real Time PCR System (Applied Biosystems).

Statistical analysis

The software CaTS Power Calculator (http://www.sph.umich.edu/csg/abecasis/CaTS/) was used for calculating the statistical powers (22). The χ^2 test was used to test HWE. The different association models: genotypic, allelic, dominant and recessive were tested using the χ^2 test and *p*-values ≤0.05 were considered statistically significant. The ORs and 95% confidence intervals (95% CI) were calculated according to Woolf's method. For the meta-analysis, the combined data were summarised in two by two tables. The Brewslow-Day method, the Cochran c^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 DerSimonian-Laird method for random effects because heterogeneity was de-

tected and *p*-values (P_{DL}) lower than 0.05 were considered significant. All the associations were tested under the allelic model. In addition, to examine the extent to which an individual study affects the overall estimate, sensitivity analysis were performed to study how the elimination of one study each time affects to the pooled data The Linux software PLINK v. 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) and StatsDirect v. 2.6.6 (StatsDirect, Altrincham,UK) software were used to carry out statistical analyses.

Results

The rs2476601 genotyping success rate was higher than 95% and no divergence from HWE was observed nor in controls neither in cases ($p>0.05$). The allelic frequencies in the control group (MAF=0.064) were similar to those reported in the 1000 Genomes Project for the Iberian population in Spain (IBS, 0.08) and Tuscan in Italy (TSI, 0.066) (http://browser.1000genomes.org). The meta-analysis, conducted with the data pooled of this study together with those included in two papers previously published, incorporates a total of 794 patients and 1876 controls from four different populations: United Kingdom (UK), Middle East (ME), Turkey and Spain. A summary of the data of all these studies are displayed in Table I. To note that, two separate studies (17, 18) detected significant differences in two different populations (UK and Turkey), whereas in other two independent studies, the present and other (17), performed also in two different populations (ME and Spain), significant differences were not observed. Heterogeneity across the different studies was detected in the analysis of this polymorphism ($P_{BD}=0.002$, $P_Q=0.021$ and $I^2=69.1\%$), therefore, the meta-analysis was performed using the random effect method. No evidence of association of the rs2476601 (R620W) with BD was observed in the meta-analysis ($P_{DL}=0.504$; OR= 0.723; 95%CI 0.28 to 1.87 in the allelic model) (Fig. 1).

Next, Table II displays the allele and genotype frequencies in BD patients and healthy controls for the six tag SNPs (including the rs2476601) which were studied in Chr1:114158106.114215631 in order to check the whole gene region from 3'UTR until 5'UTR. This Table includes also data of the rs2488457, which is located at the promoter region, and also the statistical power values for all the SNPs included considering three different ORs: 1.3, 1.5 and 2.0. The allelic frequencies in the control group were similar to those reported in the 1000 Genomes Project for IBS and TSI populations (http://browser.1000genomes.org). No statistically significant differences were observed when distributions in patients and controls were compared.

Discussion

The *PTPN22* gene, specifically its variant C1858T (R620W), is a very interesting candidate in the susceptibility to immune mediated diseases; in fact, it has been described in association with many of these pathologies (14-16). Nevertheless, results of the present study do not support a major role of this gene in BD, although, the association with an specific population can not be completely ruled out.

Although two independent studies performed in populations from UK and Turkey suggested association of the MAF of this variant of the *PTPN22* as a protective factor in BD, the *p*-values were only marginally significant (17-18). Replication studies are essential to avoid type I errors, the present study includes the largest cohort of patients and controls genotyped for the variant R620W in BD and it is well powered to detect association with similar OR as the original study, nevertheless, no significant association was detected. The origin of the discrepancies may be, among other reasons, the use of different typing methods because these have evolved from less reproducible methods, to others more reliable. Limitations caused by genotyping methods are more important in small cohorts, in which the Type I errors are more likely.

BD is a rare condition, this fact conditioned the sample size and it makes more difficult to achieve an adequate statistical power in individual studies (Table I). This disadvantage is compounded because this variant, which has a low worldwide frequency, is extremely rare or absent in populations with a relatively high prevalence of the disease such as Turkish, Japanese and Chinese. For this reason a meta-analysis by pooling all the available data for this variant in BD was performed. The meta-analysis with data pooled of the four populations has an adequate statistical power (≥86%) to detect associations with OR≥1.4 and it supports non-association of the BD with this variant of the *PTPN22* gene.

Table I. Characteristics of the studies included in the meta-analysis of the rs2476601.

First author	Year	Population	Genotyping methods	Sample Size		MAF (T)		Statistical power[1]		*p*-value	OR
				Cases	Controls	Cases	Controls	OR=1.5	OR=2.0		
Baranathan V	2007	UK[2]	PCR-SSP	116	169	4.5%	9.7%	30%	60%	0.04	0.46[3]
		ME		140	65	1.8%	0%	<10%	<10%	0.180	5.2
Sahin N	2007	Turkish	PCR + RFLP	134	177	0%	2.5%	15%	30%	0.012	0.65
Ortiz-Fernández L	2014	Spanish	Real Time PCR (TaqMan)	404	1465	6.7%	6.4%	76%	100%	0.723	1.06
Overall				794	1876					0.504	

[1]Statistical powers were calculated for OR values of 1.5 and 2.0 taking into account the allelic frequency and the sample size of each population. [2]UK [-AC/ME]; UK cohort without patients from Afro-Caribbean or Middle Eastern (ME) origin. [3]The OR (95% IC) shown in the paper (2.1) corresponds with the most common allele (C). MAF: minor allele frequency.

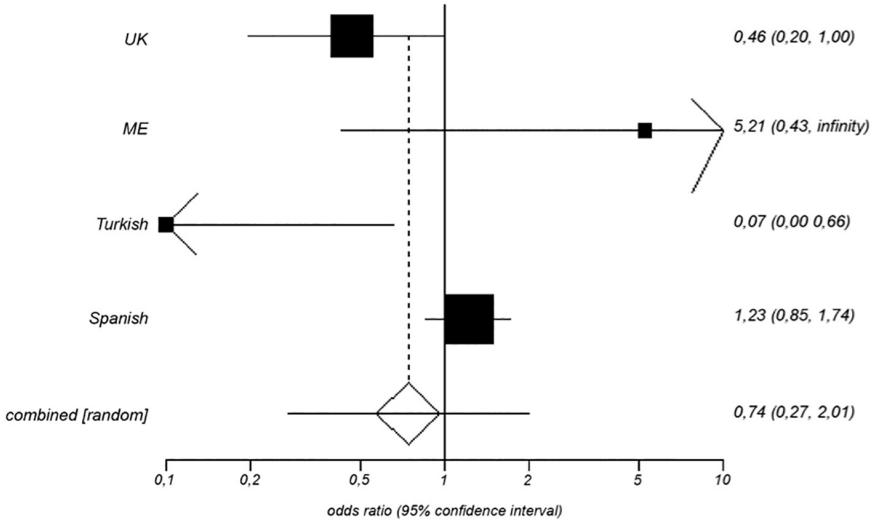

Fig. 1. Forest plots of *PTPN22* rs2476601. The Forest plots of the rs2476601 in the *PTPN22* gene pooling the four populations included in the meta-analysis was performed with the DerSimonian-Laird method for random effects because heterogeneity was detected. The inheritance model considered was the allelic inheritance model. The odds ratios and 95% confidence intervals are displayed.

Other SNPs of this gene have been associated with immune mediated diseases in populations in which the C1858T (R620W) is absent. Thereby, rs2488457, rs1310182 and rs3789604 have been associated with type 1 diabetes and rheumatoid arthritis in Asian populations (23-24), nevertheless, association with BD has been discarded in the Chinese Han population in a large cohort of patients with ocular involvement (20). Because of the interest of this gene in the immune mediated diseases and in order to investigate whether other variants are associated with BD, a study of tagSNPs was performed. This study, that spanning the whole *PTPN22* gene region with 100% coverage of all the 27 SNPs located within this zone and including in addition the rs2488457 (located at the promoter region), discards association of this gene with BD, at least for ORs ≥1.3, for which the study was adequately powered. In agreement with our results, none of the three GWAS performed in BD have reported association with *PTPN22* (7, 8, 25) Although complete information derived from GWAS is not always available, in those cases where it has been reported, no association of this gene with BD was found (7).

In conclusion, our results of case-control study and meta-analysis do not suggest that *PTPN22* plays a major role in Behçet's disease.

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Table II. Distribution of genotypes and alleles of the SNPs included in this study.

SNP	1/2	Subject	Genotype n (%)			MAF	Allele test		Statistical power		
			1/1	1/2	2/2		*p*-value*	OR (CI 95%)	OR 1.3	OR1.5	OR 2.0
rs1217412	A/G	BD	162 (58.3)	105 (37.8)	9 (3.2)	0.223	0.863	0.98 (0.79-1.22)	81%	99%	100%
		Controls	903 (59.5)	517 (34.1)	81 (5.3)	0.226					
rs2476599	A/G	BD	16 (5.8)	115 (41.4)	145 (52.2)	0.266	0.571	1.06 (0.86-0.30)	84%	100%	100%
		Controls	99 (6.5)	565 (37.2)	833 (54.9)	0.255					
rs3789607	A/G	BD	123 (44.2)	122 (43.9)	33 (11.9)	0.338	0.763	1.03 (0.85-1.25)	88%	100%	100%
		Controls	676 (44.6)	676 (44.6)	165 (10.9)	0.332					
rs2476601	C/T	BD	346 (86.5)	54 (13.5)	0 (0)	0.067	0.723	1.06 (0.77-1.44)	38%	76%	100%
		Controls	1323 (87.2)	190 (12.5)	2 (0.1)	0.064					
rs3765598	A/G	BD	6 (2.2)	81 (29.1)	190 (68.3)	0.168	0.270	0.87 (0.69-1.11)	76%	99%	100%
		Controls	56 (3.7)	457 (30.1)	1003 (66.1)	0.188					
rs1217419	A/C	BD	37 (13.3)	145 (52.2)	96 (34.5)	0.394	0.406	0.92 (0.77-1.11)	100%	100%	100%
		Controls	266 (17.5)	717 (47.3)	530 (34.9)	0.413					
rs2488457	C/G	BD	185 (66.5)	88 (31.7)	5 (1.8)	0.176	0.447	0.91 (0.72-1.16)	76%	99%	100%
		Controls	998 (65.8)	460 (30.3)	58 (3.8)	0.190					

*All the *p*-values were calculated in the allelic model. MAF: minor allele frequency.

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References

1. MENDES D, CORREIA M, BARBEDO M *et al*.: Behçet's disease: a contemporary review. *J Autoimmun* 2009; 32: 178-88.
2. SAKANE T, TAKENO M, SUZUKI N, INABA G: Behçet's disease. *N Engl J Med* 1999, 341: 1284-91.
3. SAZZINI M, GARAGNANI P, SARNO S *et al*.: Tracing Behçet's disease origins along the Silk Road: an anthropological evolutionary genetics perspective. *Clin Exp Rheumatol* 2015; 33 (Suppl. 94): S60-66.
4. 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.
5. 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.
6. YAZICI H, FRESKO I, YURDAKUL S: Behçet's syndrome: disease manifestations, management, and advances in treatment. *Nat Clin Pract Rheumatol* 2007; 3: 148-55.
7. 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.
8. 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.
9. LEE YJ, HORIE Y, WALLACE GR *et al*.: Genome-wide association study identifies GIMAP as a novel susceptibility locus for Behçet's disease. *Ann Rheum Dis* 2013; 72: 1510-6.
10. ORTIZ-FERNÁNDEZ L, GARCÍA-LOZANO JR, MONTES-CANO MA *et al*.: Association of CCR5Δ32 and Behçet's disease: new data from a case-control study in the Spanish population and meta-analysis. *Clin Exp Rheumatol* 2015; 33 (Suppl. 94): S96-100.
11. HATEMI G, SEYAHI E, FRESKO I, TALARICO R, HAMURYUDAN V: Behçet's syndrome: a critical digest of the 2014-2015 literature. *Clin Exp Rheumatol* 2015; 33 (Suppl. 94): S3-14.
12. ORTIZ-FERNANDEZ L, GARCÍA-LOZANO JR, MONTES-CANO MA *et al*.: Lack of association of TNFAIP3 and JAK1 with Behçet's disease in the European population. *Clin Exp Rheumatol* 2015; 33 (Suppl. 94): S36-9.
13. ORTIZ-FERNÁNDEZ L, CONDE-JALDÓN M, GARCÍA-LOZANO JR *et al*.: GIMAP and Behçet disease: no association in the European population. *Ann Rheum Dis* 2014; 73: 1433-4.
14. TOTARO MC, TOLUSSO B, NAPOLIONI V *et al*.: *PTPN22* 1858 C>T Polymorphism distribution in Europe and association with rheumatoid arthritis: Case-Control Study and meta-Analysis. *PLoS ONE* 2011; 6: e24292.
15. KYOGOKU C, LANGEFELD CD, ORTMANN WA *et al*.: Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 2004; 75: 504-7.
16. DIEUDÉ P, GUEDJ M, WIPFF J *et al*.: The PTPN22 620W allele confers susceptibility to systemic sclerosis: findings of a large case-control study of European Caucasians and a meta-analysis. *Arthritis Rheum* 2008; 58: 2183-88.
17. BARANATHAN V, STANFORD MR, VAUGHAN RW *et al*.: The association of PTPN22 620W polymorphism with Behçet's disease. *Ann Rheum Dis* 2007; 66: 1531-33.
18. SAHIN N, BICAKCIGIL M, ATAGUNDUZ P, DIRESKENELI H, SARUHAN-DIRESKENELI G: PTPN22 gene polymorphism in Behçet's disease. *Tissue Antigens* 2007; 70: 432-34.
19. International Study group for Behçet's disease. Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
20. ZHANG Q, HOU S, JIANG Z *et al*.: No Association of PTPN22 Polymorphisms with Susceptibility to Ocular Behçet's Disease in Two Chinese Han populations. *PLoS ONE* 2012; 7: e31230.
21. CHEN Z, ZHANG H, XIA B *et al*.: Association of PTPN22 gene (rs2488457) polymorphism with ulcerative colitis and high levels of PTPN22 mRNA in ulcerative colitis. *Int J Colorectal Dis* 2013; 28: 1351-8.
22. SKOL AD, SCOTT LJ, ABECASIS GR, BOEHNKE M: Joint analysis is more efficient than replication based analysis for two-stage genome-wide association studies. *Nat Genet* 2006; 38: 209-13.
23. KAWASAKI E, AWATA T, IKEGAMI H *et al*.: Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): association between a promoter polymorphism and type 1 diabetes in Asian populations. *Am J Med Genet A* 2006; 140: 586-93.
24. FENG X, LI YZ, ZHANG Y *et al*.: Association of the PTPN22 gene (-1123G > C) polymorphism with rheumatoid arthritis in Chinese patients. *Tissue Antigens* 2010; 76: 297-300.
25. 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: 1836-1842.