

TIR-domain-containing adaptor protein gene *TIRAP* S180L polymorphism is not increased in Behçet's disease patients in two ethnic cohorts

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ABSTRACT

Objective. The single nucleotide polymorphism (SNP) of *TIRAP* (Serine 180 leucine, S180L) that is shown to be associated with Behçet's disease (BD) in a European-derived cohort, but not in Middle Eastern patients is investigated in two other populations.

Methods. Two cohorts of BD patients and controls from Turkey (n=797) and Italy (n=633) were genotyped by sequence specific primer-polymerase chain reaction (SSP-PCR) or TaqMan q-PCR assays.

Results. Genotype and allele frequencies in *TIRAP* S180L (rs8177374) were not different between BD patients and controls in either ethnicity. Furthermore, a meta-analysis between the Turkish and the Italian BD cohorts did not reveal an association between this non-synonymous SNP in *TIRAP* and BD (meta-analysis OR=0.94, meta-analysis p=0.61, Q statistic heterogeneity p=0.11).

Conclusion. *TIRAP* S180L gene polymorphism, which was previously shown to be associated with BD in a Caucasian population, has been replicated in either Turkish or Italian population in our study.

Introduction

TIRAP, a MyD88-adaptor-like molecule (toll-interleukin-1 receptor domain containing adaptor protein, MAL) has a regulatory role in Toll-like receptor-2 (TLR2) and TLR4 signalling (1). A single nucleotide polymorphism (SNP) of *TIRAP* causing an amino acid change (Serine 180 leucine, S180L) is shown to have a protective effect in various infections, first in tuberculosis, invasive pneumococcal disease, bacteremia and malaria and later in HIV-1 and Chagas cardiomyopathy (2, 4).

Behçet's disease (BD) is a chronic, multisystemic, inflammatory disease characterised by recurrent attacks of mucocutaneous, ocular, musculoskeletal, vascular, central-nervous system and gastrointestinal manifestations. Both innate and adaptive immune responses have been demonstrated in BD (5). TLR2 and TLR4 have been identified as signalling receptors activated by bacterial cell wall components in BD and possibly have a major role in innate-activation (6). Previously, an increased TLR expression in buccal mucosa of BD patients and an association of *TIRAP* S180L polymorphisms is observed in a European BD population, but not in Middle Eastern patients (7).

Differences in both clinical presentations such as the frequency and severity of different manifestations and genetic susceptibility such as HLA-B*51 presence are observed among different ethnicities in BD. With this background, we aimed to investigate *TIRAP* further in two cohorts of BD patients, from Turkey and Italy to replicate the previous results.

Materials and methods

The Turkish BD cohort (245 M/195 F, mean age: 42 years) is composed of four subgroups followed in different institutions: Marmara University, School of Medicine, Department of Rheumatology (n=220), Akdeniz University, Faculty of Medicine, Department of Dermatology (n=56), Ankara University, Faculty of Medicine, Department of Rheumatology (n=126) and Department of Internal Medicine II, University Hospital Tübingen, Germany (n=38). The Italian BD cohort (61 M/57 F, mean age: 30 years) is similarly followed in the Department of Internal Medicine, Reggio Emilia, Northern Italy. All patients fulfilled the

Table I. Study groups with the genotype and minor allele frequencies, and P values using a Pearson's chi-squared test.

GROUP	SS	SL	LL	L allele frequency	χ^2 and <i>p</i> -value (Pearson) S vs. L	OR	0.95 CI
<i>Turkish</i>							
BD (n=440)	345 (0.78)	84 (0.19)	11 (0.03)	0.12	0.34; 0.56	1.07	0.79–1.46
Control (n=357)	279 (0.78)	75 (0.21)	3 (0.01)	0.11	0.34; 0.56		
<i>Italian</i>							
BD (n=118)	87 (0.74)	29 (0.25)	2 (0.02)	0.14	2.53; 0.11	0.72	0.48–1.08
Control (n=515)	347 (0.67)	147 (0.29)	21 (0.04)	0.18	2.53; 0.11		

1990 International Study Group classification criteria for Behçet's disease (8). The study protocols were approved by the ethics committees and Institutional Review Boards of all institutions. All study participants signed an informed written consent.

The single nucleotide polymorphism (rs8177374) was detected by SSP-PCR method in 227 BD patient and 213 healthy controls (HC) as described elsewhere (9). Briefly, two different sequence-specific forward primers for the polymorphic C/T alleles and a common reverse primer were used in the PCR (F-C-CACCATCCCCCTGCTGTC, F-T-CACCATCCCCCTGCTGTT and R-GATACAAACCCCGACAGCC). Human growth hormone (hGH) was used as PCR amplification control for each DNA simultaneously. The amplified DNA's were analysed by agarose gel electrophoresis.

For the second set of 331 BD patients and 659 HC q-PCR method was used. While using ViiA 7 System (Applied Biosystems) the standard protocol for TaqMan SNP Genotyping Assays (hold 10 min at 95°C for enzyme activation and 40 cycles of each including both denaturation at 92°C for 15 sec and annealing/extending 60°C for 1 min) was applied with 15 ng of DNA samples. Allele and genotype frequencies were calculated by direct counting. Pearson's chi-square test was used to compare distribution of genotypes and alleles between patients and controls, odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated.

Results

Table I shows the genotype and minor allele frequencies, and *p*-values using a Pearson's chi-squared test. Genotype

and allele frequencies in rs8177374 were not different between BD patients and controls in either ethnicity (Turkish cohort: BD vs. controls: CC: 78.4 vs. 78.2%, CT: 19.1 vs. 21% and TT: 2.5 vs. 0.8%, Italian cohort: CC: 73.7 vs. 67.3%, CT: 24.5 vs. 28.5% and TT: 1.6 vs. 4.0%, respectively). The T allele frequency in Turkish BD patients and controls were 12.0 vs. 11.3%, respectively ($2 \times 2 \chi^2=0.34$; $p=0.56$; OR, 1.07 [95% CI, 0.79-1.46]). In the Italian BD group the T allele frequency was 14.0 vs. 18.3% ($2 \times 2 \chi^2=2.53$; $p=0.11$; OR, 0.72 [95% CI, 0.48-1.08]), without a significant difference. Furthermore, a meta-analysis between the Turkish and the Italian BD cohorts did not reveal an association between this non-synonymous SNP in *TIRAP* and BD (meta-analysis OR=0.94, meta-analysis $p=0.61$, Q statistic heterogeneity $p=0.11$).

Discussion

TIRAP (rs8177374) gene polymorphism, which was previously shown to be associated with BD in a European cohort from the UK, has not been replicated in either Turkish or Italian patient populations in our study. Considering the relationship between geographic location and genetic resemblance, some similarity in the results of the Middle East and Turkish population was expected, however, results from a UK European cohort were also not replicated in the Italian population. Among the possible reasons for this discrepancy, one may be the low number of BD patient DNA samples in both populations studied (n=118 in an Italian population, n=146 in a UK population). Clinical differences among the study groups, especially in major organ manifestations, might also have influenced the results.

The lack of S180L *TIRAP* variant homozygosity among Middle Eastern populations is also seen in African and Asian cohorts and Durrani *et al.* suggests that this is due to the increased pathogen burden in these areas, where increased TLR signalling would be more dangerous (7).

TIRAP as a candidate gene associated with inflammation, has been previously studied in other rheumatological diseases with conflicting results. A protective effect of S180L *TIRAP* variant was observed in SLE, but not in rheumatoid arthritis (10, 11).

Although attractive candidate genes, genetic susceptibility of innate dysregulation is rarely shown in BD. We also could not demonstrate an association of *NLRP3* (*NALP3*) Q705K gene polymorphism, linked to cryopyrin-associated periodic syndrome, with BD (unpublished observation). Currently, among innate immunity-associated mechanisms, only *MEFV*, which encodes pyrin protein linked to the inflammasome and familial Mediterranean fever, is associated with both ankylosing spondylitis and BD, especially as a *MEFV* heterozygote carrier state (12, 13). However, as expected, this association is mainly observed in Middle Eastern populations with a high frequency of heterozygote carriers in the healthy population.

In conclusion, we could not confirm the association of *TIRAP* (rs8177374) polymorphism with BD in both Turkish and Italian cohorts in our study. However, as common genetic susceptibility loci between BD and spondyloarthropathies are increasing being demonstrated, such as *IL23R* and *IL10*, further studies of other innate immunity associated gene polymorphisms are warranted.

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