Association of TNF-α and PTPN22 SNPs with the risk and clinical outcome of type 1 diabetes

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Abstract: Type 1 Diabetes mellitus (T1DM) begins with aberrant inflammatory process followed by auto-destruction in genetically susceptible individuals. Therefore, we hypothesized that gain-of-function allelic variants TNF-α-238A, -308A and PTPN22 1858T could be associated not only with T1DM development but also with the clinical outcome in patients of Bosnia and Herzegovina. A total of 402 subjects were enrolled in the association study. SNPs were determined by PCR-RFLP. Data was analyzed by GraphPad Prism and Sigma Stat 3.5 software. Genotypes frequencies at TNF-α-238 and -308 loci were not statistically different between patients and controls. In contrast, distribution of genotypes at the 1858 position of PTPN22 was significantly different, due to higher frequency of gain-of-function gene variants in patients than controls. Moreover, long term glucose regulation (based on HbA1c level) was significantly worse in patients with the risk TNF-α-308A allele than in patients with non-risk (G) allele. However, patients with the risk allele of both genes (TNF-α-308A and PTPN22 1858T) had the worst glycemic control, suggesting that those two work synergistically. In conclusion, in a cohort from Bosnia and Herzegovina TNF-α-308A allele is significantly associated with the worse long-term glucose control, but PTPN22 1858T allele is significantly associated with diabetes development.

Keywords: Polymorphism • Autoimmunity • Cytokine

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1. Introduction

Type 1 Diabetes mellitus (T1DM) is an autoimmune disease whose complex etiology is not completely understood. It is recognized to be caused by both genetic and environmental components. During T1DM pathogenesis macrophages and lymphocytes infiltrate the islets. Thereby, the intensity of the β-cell destructive process is modulated by the interaction of a large number of susceptibility genes and both known (nutritional factors and viral infection) and unknown environmental factors [1].

Activated macrophages produce proinflammatory cytokines that can destroy pancreatic β-cells. Cytokines were therefore proposed as mediators of β-cell death in vivo [2], that is confirmed in NOD mice for TNF-α [3]. It was known that G-238A and G-308A SNPs in TNF-α promoter have a direct effect on transcriptional activity, where the A allele for both loci interprets high cytokine-producing variant. Genetically determined inter-individual variation in the production of this cytokine could predispose certain individuals with the risk genotype to develop T1DM [4].

The critical events leading to loss of T cell tolerance to pancreatic β-cells are still largely unknown. It seems that inefficient thymic negative selection increases the frequency of specific CD4+ and CD8+ T cells in the periphery, while a defective peripheral tolerance contributes to the expansion and differentiation of autoreactive T cells [5]. Effector functions of T cells are regulated by the activation of intracellular signaling pathways in response to triggering of the T-cell receptor. Lymphoid-specific phosphatase (Lyp), encoded by the PTPN22 gene, has direct role in setting thresholds for signaling through T-cell receptor. Thereby, the 1858T gene variant (not 1858C) is a gain-of-function form of the enzyme with significantly higher phosphatase activity.
activity and a dominant suppressing effect on receptor signaling [6,7].

Taking these facts in consideration we have several reasons to expect that these SNPs in the TNF-α and PTPN22 genes might have a vital effect on the appearance of T1DM. Conventionally, association studies are applied to specific candidate genes of presumed importance in the disease. To our knowledge, no other study has so far examined the genetic variability of TNF-α or PTPN22 in Bosnia and Herzegovina subjects. Therefore, we undertook case-control study design to clarify whether TNF-α G-75A (rs2476601), G-308A (rs1800629) and PTPN22 C1858T (rs2476601) polymorphisms correlate with increased risk of T1DM and also the clinical outcome of disease among diabetic patients of Bosnia and Herzegovina.

2. Experimental Procedures

2.1 Subjects
A total of 402 subjects were enrolled in this association study. The patient group consisted of randomly selected 241 subjects (male/female 124/117, median age 55 years (25th-75th percentiles 50.75-66.0 years), fasting plasma glucose 8.84±2.1 mmol/dm³) with T1DM recruited from the Medical center Vitez, central part of Bosnia and Herzegovina, where the diabetes is diagnosed according to revised recommendation of World Health Organization and American Diabetes Association criteria (http://www.who.int/diabetes/publications/Definition; http://www.aacc.org/members/nacb/lmpg/onlinetguide/draftguidelines).

All patients need insulin for glycemic control (the average dose/kg/day 0.74±0.23 units). Glycated haemoglobin (HbA1c) was measured in a central laboratory of the Medical center. All patients need insulin for glycemic control (the average dose/kg/day 0.74±0.23 units). Glycated haemoglobin (HbA1c) was measured in a central laboratory of the Medical center. The relevant research ethics committee gave ethical approval (No: 11-1-08/05) for this study. All patients and control subjects were informed of the study’s purpose and gave their informed consent before anonymously participating in the study.

2.2 DNA extraction and genotyping
DNA was isolated from 3 ml of peripheral blood by salting-out procedure [8], whose concentration was spectrophotometrically measured (Biospec, Shimadzu).

The appropriate fragments of TNF-α or PTPN22 genes were amplified by polymerase chain reaction (PCR) using two sets of primers for each gene [9,10]:

TNF-α-238 5’-ATCTGGAGGAAGCGGTAGTG-3’, 5’-AAGCTTCTGAGGCGCAG-3’;  
TNF-α-308 5’-GGGACACACAAAGCATCAAAG-3’, 5’-AAATGGTTTTGAGGGCCATG-3’;  
PTPN22 1858 5’-ACTGATAATGTTGCTTCAACGG-3’, 5’-TCACCCAGCTCTTCAACAGC-3’.

Primers for both polymorphic sites in TNF-α contain a single base-pair mismatch adjacent to the polymorphic sites. These changes introduce a restriction site into the wild-type nucleotide sequences after amplification [9].

The PCR mixture contained 100 ng DNA, 10 pmol of each primer, 200 µM of dNTP, 1x PCR buffer with MgCl₂, 1 U Taq DNA polymerase (Eppendorf, Hamburg, Germany) and QH₂O up to 25 µl. PCR was started with initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C (TNF-α) or 62°C (PTPN22) for 30 s and extension at 72°C for 30 s. The reaction was completed by final extension at 72°C for 10 min.

PCR products were checked by electrophoresis on 2.5% agarose gel containing ethidium bromide and visualized under UVC light (Alliance 4.7 UVTech, Cambridge).

The resulting PCR products (3 µL) were further digested with 4 units of appropriate restriction enzyme: MspI (Fermentas, Germany) for detection G/A SNP at TNF-α-238 locus (rs 361525, 139 bp +16 bp G allele, 152 bp A allele), Ncol (New England, BioLabs, SAD) for detection G/A SNP at TNF-α-308 locus (rs 1800629, 126 bp +16 bp G allele, 142 bp A allele) and Rsal (Fermentas, Germany) for detection C/T SNP at 1858 locus of PTPN22 (rs2476601, 172 bp + 46 bp C allele, 218 bp T allele). The restriction mixture (15 µl) was incubated at 37°C overnight. Genotype of each sample was determined by vertical electrophoresis on 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light (Alliance 4.7 UVTech, Cambridge). 15% randomly selected samples were re-tested for these SNPs and obtained results fully corroborated earlier findings.