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The combination of ınterleukin-10 −1082 and tumor necrosis factor α −308 or ınterleukin-6 −174 genes polymorphisms suggests an association with susceptibility to Hashimoto's thyroiditis

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ABSTRACT

Background: The etiopathogenesis of Hashimoto's thyroiditis (HT) has not been clearly elucidated although the role of chronical inflammation and endothelial dysfunction has been established. The imbalance between pro- and anti-inflammatory cytokines may play a role in the etiology. The aim of the present study was to investigate whether cytokine gene polymorphisms are associated with HT, and to evaluate the relationship between genotypes and clinical/laboratory manifestation of HT.
Methods: Tumor necrosis factor α (TNFα) G-308A (rs 1800629), interleukin-6 (IL-6) G-174C (rs 1800795) and IL-10 G-1082A (rs 1800896) single nucleotide polymorphisms (SNPs) in DNA from peripheral blood leukocytes of 190 patients with HT and 231 healthy controls were investigated by real-time PCR combined with melting curve analysis using fluorescence-labeled hybridization probes.
Results: There was no notable risk for HT afflicted by TNFα −308, IL-6 −174 and IL-10 −1082 polymorphisms alone. However, carriers of variant alleles of both IL-10 −1082 and TNFα −308 polymorphisms had four-fold times higher risk for HT in comparison with non-carriers. Additionally, concomitant presence of both mutant IL-10 −1082 A and IL-6 −174 C alleles raised three-fold the HT risk.
Conclusion: Our results suggest that the combined effects of TNFα −308, IL-6 −174 and IL-10 −1082 variant alleles may be more decisive to induce functional differences and modify the risk for HT.

1. Introduction

Hashimoto's thyroiditis (HT) is the most common organ-specific autoimmune disorder affecting approximately 18% of overall population [1]. It is characterized by diffuse lymphocytic infiltration of the thyroid gland, elevated levels of serum anti-thyroid antibodies, evidence of goitrous or atrophic gland, and frequent thyroid dysfunction in varying degrees [1]. Although the exact pathophysiologic mechanisms of HT remain elusive, there is growing evidence that the disease is a consequence of interaction between genetic and environmental factors [2]. Low-grade chronic inflammation and imbalance between pro- and anti-inflammatory cytokines have been proposed to play a role in the pathogenesis [3,4]. Cytokines participate in the induction and effector phases of the immune and inflammatory responses and are therefore considered as markers involved in the development of autoimmune thyroid disease. A variety of cytokines including tumor necrosis factor α (TNFα), interleukin (IL)-1α, IL-6, IL-8, IL-10, IL-12 and interferon-γ (IFNγ) has been shown to be produced in thyroid follicular cells and intrathyroid inflammatory cells [5]. TNFα and IL-6 are main cytokines that play a central role in initiation and regulation of the cytokine cascade during an inflammatory response. On the other hand, IL-10 limits inflammation by reducing the synthesis of pro-inflammatory cytokines such as TNFα, IL-1, and IL-6, by suppressing cytokine receptor expression and by inhibiting receptor activation [6]. Elevated TNFα and IL-6 levels in serum and diminished IL-10 synthesis in peripheral blood mononuclear cells (PBMC) have been reported in patients with autoimmune thyroid disease [7–10].

Several single nucleotide polymorphisms (SNPs) in the regulatory regions of TNFα, IL-6 and IL-10 have been implicated to modulate the risk of autoimmune diseases, possibly by influencing the expression of the protein [11–20] and could be critical to the etiology of HT.

Abbreviations: Anti-Tg, anti-thyroglobulin antibody; Anti-TPO, anti-thyroid peroxidase antibody; BMI, body mass index; BP, blood pressure; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; HDL-C, high density lipoproteine-cholesterol; HT, Hashimoto's thyroiditis; IFNγ, interferon-γ; IL-10, interleukin-10; IL-1α, interleukin-1α; IL-6, interleukin-6; IL-8, interleukin-8; LDL-C, low density lipoproteine-cholesterol; OR, odds ratio; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; SNPs, single nucleotide polymorphisms; TNFα, tumor necrosis factor α; TSH, thyroid-stimulating hormone; VLDL-C, very low density lipoproteine-cholesterol.

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Therefore, in the present study, we aimed to investigate whether TNFα G-308A, IL-6 G-174C and IL-10 G-1082A polymorphisms could predispose to HT, and to evaluate the possible relationship between genotypes and clinical/biochemical characteristics of HT.

2. Materials and methods

One hundred and ninety patients with the diagnosis of HT were included in the study. Patients with symptoms or findings of hypothyroiditis, moderate rigidity of enlarged thyroidal gland, increased or normal TSH value, decreased or normal FT3 or FT4 values, and elevated levels of autoantibodies (anti-thyroid peroxidase antibody = anti-TPO, anti-thyroglobulin antibody = anti-Tg) in screening tests were diagnosed as HT. Loss of ecogenity in parenchyma and fibrotic separations and pseudonodular image in Doppler ultrasonography of thyroid gland were also detected. Fine needle aspiration was only performed to evaluate the patients having a thyroid nodule ≥1 cm in diameter ($n=17$). All HT patients were subjected to thyroxine therapy until euthyroid state has been achieved. The thyroxine dose was regulated according to TSH concentrations checked regularly in three month intervals. The control group consisted of 231 individuals matched for age and sex. None of the controls had personal or family history of thyroid disease and goiter on examination; they had normal thyroid functions and were negative for thyroid autoantibodies. Exclusion criteria were the existence of any comorbid cardiac, autoimmune, infectious, musculoskeletal or malignant disease and a recent history of operation or trauma. Height (m) and weigh (kg) were measured after fasting, without shoes and wearing light clothes. All measurements were conducted with the patient in a standing position. Body mass index (BMI) was calculated by dividing the weight by the height squared. Blood pressure (BP) was measured after 15 min of rest in a seated position with the right arm by using a sphygmomanometer. The study was approved by the Institutional Review Board at Şişli Etfal Research and Training Hospital. Informed consent was obtained from each subject.

Blood samples were taken in the morning subsequent to an overnight (12 h) fast. Peripheral venous blood samples were collected in plain tubes for routine biochemical analysis, and in EDTA-K_3 for genotype analysis. Serum triglyceride, cholestrerol, HDL-, LDL- and VLDL-cholestrol measurements were performed on ISE 1800 DPP Roche autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum TSH, freeT_3, free T_4, anti-Tg and anti-TPO were measured on Modular EEE Electrod Elecsys Roche autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Genomic DNA was isolated from peripheral blood leukocytes by using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). For detection of polymorphisms Light SNIP assays were used. Light SNIP assays are based on simple probe melting curve analysis. They consist of pre-mixed primers and probes. They were developed and optimized according to NCBI "rs" numbers of studied SNP's by Tib MolBiol (Berlin, Germany). The detection of polymorphisms was performed in a LightCycler (Roche Diagnostics, Mannheim, Germany).

Differences in genotype distributions and allele frequencies in the cases and the controls were compared for statistical significance using the chi-square (χ^2) test. The statistical significance for deviations from Hardy–Weinberg equilibrium (HWE) was determined using the Pearson χ^2-test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (CIs). The wild-type genotype/allele served as a reference category. We examined also the association between combined polymorphisms and HT in carriers and non-carriers of mutant allele of above mentioned genes, and computed interaction terms between TNFα and IL-10, TNFα and IL-6, IL-6 and IL-10 by using χ^2-test. Logistic regression (univariate) analysis was used to construct the model to explain the variatons in lipid parametrs. In this model BMI, age, gender, free T3 and free T4 were included as covariates, and genotype score was included as fixed factor. Mann–Whitney *U*, Kruskal–Wallis and Spearman correlation tests were used for the evaluation of clinical and biochemical parameters. The differences were considered significant if the value of probability (p) did not exceed 0.05. All statistical analyses were performed with SPSS 15.0 for Windows (Chicago, IL, USA). In addition the NCSS 2000 statistical package (Kaysville, Utah, USA) was used to evaluate the power analysis. We had a 97% power to detect an effect size (W) of 0.20 using a 2 degrees of freedom ($\alpha=0.05$).

3. Results

TNFα −308, IL-6 −174 and IL-10 −1082 promoter polymorphisms were analyzed in 190 patients with HT (169 women and 21 men) and 231 unrelated healthy controls (201 women and 30 men). The clinical characteristics of controls and patients with HT were given in Table 1. The genotypic and allelic distributions of TNFα −308, IL-6 −174 and IL-10 −1082 polymorphisms for cases and controls are shown in Table 2. All genotype distributions were in accordance with the HWE among the controls and cases. No notable differences were observed in allele or genotype frequencies for TNFα −308, IL-6 −174, and IL-10 −1082 genes alone (Table 2). We also investigated whether any combinations of TNFα −308, IL-6 −174 and IL-10 −1082 variant alleles affected the risk for HT. The concomitant presence of IL-10 A and TNFα A, as well as IL-10 A and IL-6C alleles significantly raised the risk for HT (OR 3.88 with 95% CI = 0.99–15.07 and OR 2.74 with 95% CI = 1.04–7.24, respectively; $p<0.05$) (Table 3). In addition, the possible relation between studied polymorphisms and certain clinical phenotypes, including age of onset, gender, TSH, free T_3, free T_4, anti-TPO, anti-Tg and lipid profile parameters was evaluated in patients with HT and we could not find any difference (data not shown).

4. Discussion

We have carried out a genetic association study of TNFα G-308A, IL-6 G-174C and IL-10 G-1082A polymorphisms in HT—the most

Table 1
Characteristics of controls and patients with Hashimoto thyroiditis.

	Control ($n=231$)	HT ($n=190$)
Age (years)	36.0 ± 9.9	40.6 ± 10.6
Mean ± SD	18–38	16–78
Range		
HT onset	–	99 (52.1)
<40 years *n* (%)		
>40 years, *n* (%)	–	91 (47.9)
Gender	30 (13.0)	21 (11.1)
Male, *n* (%)	201 (87.0)	169 (88.9)
Female, *n* (%)	–	99 (52.1)
Familial history, *n* (%)	104 (45.02)	99 (52.1)
Smoking, *n* (%)	–	624.4 ± 378.4
Anti-TPO (IU/mL) (mean ± SD)	–	562.4 ± 389.5
Anti-Tg (IU/mL) (mean ± SD)		
BMI (kg/m^2) (mean ± SD)	25.7 ± 4.45	27.81 ± 5.61
Systolic BP (mmHg)	111.2 ± 11.5	114.3 ± 13.3
Diastolic BP (mmHg) (mean ± SD)	75.7 ± 7.5	72.7 ± 9.2
TSH (mIU/L) (mean ± SD)	1.78 ± 0.9	4.0 ± 3.3*
FreeT_3 (pmol/L) (mean ± SD)	3.5 ± 0.35	3.1 ± 0.4
FreeT_4 (pmol/L) (mean ± SD)	14.8 ± 2.7	14.5 ± 3.7
Cholesterol (mg/dL) (mean ± SD)	170.27 ± 39.90	194.64 ± 36.00
Triglyceride (mg/dL) (mean ± SD)	100.78 ± 54.80	111.38 ± 55.33
HDL-C (mg/dL) (mean ± SD)	58.25 ± 12.76	59.00 ± 12.30
LDL-C (mg/dL) (mean ± SD)	100.80 ± 35.09	113.85 ± 31.76
VLDL-C (mg/dL) (mean ± SD)	20.57 ± 11.18	22.67 ± 12.24

Man–Whitney *U* test, *$p<0.05$.
Abbreviations: BMI (body mass index), BP (blood pressure), HT (Hashimoto thyroiditis), HDL-C (high density lipoproteine-cholesterol), LDL-C (low density lipoproteine-cholesterol), TSH (thyroid-stimulating hormone), VLDL-C (very low density lipoproteine-cholesterol).