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Vascular endothelial growth factor polymorphisms increase the risk of developing Graves' disease

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ABSTRACT

Background: Graves' disease (GD) is a consequence of genetic and environmental factors. Vascular endothelial growth factor (VEGF) is a strong angiogenic and mitogenic factor, which plays a key role in lymphocyte infiltration, and hypervascularization in the thyroid gland of patients with GD.
Aim: The aim of this study is to investigate the relationship between GD and A-2578C, T-460C and G+405C single nucleotide polymorphisms (SNPs) of VEGF gene, as well as to evaluate whether there are any relationships between genotypes and some clinical/laboratory parameters of GD.
Methods: We analyzed the genotype and allele distributions of the above mentioned SNPs in 167 patients with established GD diagnosis and 203 healthy controls by real-time PCR combined with melting curve analysis using fluorescence-labeled hybridization probes.
Results: The distribution of VEGF A-2578C and T-460C genotypes and allele frequencies in control and GD groups were not significantly different. With regard to the +405 polymorphism, the frequency of C allele was 1.8-fold increased in GD patients compared to controls, and the CC genotype was associated with a 4.6-fold increased disease risk. There was no relationship between some clinical/laboratory parameters with G+405C polymorphism. However, in −2578C allele carrying GD patients the anti-thyroid antibody levels were increased according to wild homozygous. Additionally, −2578C and −460T alleles were related with early (at age before 40) disease onset.
Conclusion: VEGF +405 polymorphism may be a risk factor for GD, while the −2578 SNP is related with increased autoantibody production.

1. Introduction

Graves' disease (GD) is an autoimmune disorder characterized with increase of anti-thyroid antibodies, diffuse goiter and hyperthyroidism. Although the etiopathogenesis has not been clearly elucidated yet, it has been suggested that this common endocrinological disease is a consequence of genetic and environmental factors. The hypervascularization, and lymphocyte infiltration seen in thyroid gland of patients with GD have been linked with increased expression of vascular endothelial growth factor (VEGF) — a strong angiogenic and mitogenic factor [1–3]. It is well known that this growth factor initiates the migration and proliferation of endothelial cells, suppresses the apoptosis of epithelial cells, increases vascular permeability, and initiates monocyte/macrophage chemotaxis [4]. The human VEGF gene is on chromosome 6p21.3 and consists of eight exons [5–11]. A number of single nucleotide polymorphisms (SNPs) have been described in this gene, including A-2578C and T-460C in the promoter region, and G+405C in the 5′-untranslated region, reported to be related with protein production [5–11]. Increased expression of VEGF in subjects carrying −460T [7,8] and −2578C [6,8] alleles has been detected. With regard to +405 polymorphism, some authors reported that high VEGF production was associated with C allele [9–11], whereas others reported that it is associated with G allele [5,7]. The relationship between the above mentioned polymorphisms and some autoimmune diseases such as psoriasis [12,13], type 1 diabetes mellitus [14] and Behçet's disease [15] has been found. To our knowledge, there is no study in the literature regarding the relationship of these polymorphisms and GD. Therefore, in the present study we aimed to investigate whether A-2578C, T-460C and G+405C SNPs of the VEGF gene might predispose to GD, and to evaluate the possible relationships between genotypes and clinical/laboratory characteristics of GD.

Abbreviations: Anti-TG, anti-thyroglobulin; Anti-TPO, anti-thyroid peroxidase; AT, anti-thyroid; BMI, body mass index; CI, confidence interval; GD, Graves' disease; HWE, Hardy–Weinberg equilibrium; MMI, methimazole; OR, Odds ratio; PTU, propylthiouracil; SNPs, single nucleotide polymorphisms; TRAb, TSH receptor antibody; TSH, thyroid-stimulating hormone; VEGF, vascular endothelial growth factor.

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2. Materials and methods

A total of 167 previously treated GD patients were included in this study. GD was diagnosed on the basis of clinical and laboratory evidence of thyrotoxicosis, palpable diffuse goiter and diffuse thyroidal uptake on radioactive scan. In addition, patients had at least one of the following findings: ophthalmopathy (class 2–6 according to the NOSPECS classification), positive TSH receptor antibody (TRAb), anti-thyroid peroxidase (anti-TPO), and/or anti-thyroglobulin (anti-TG) antibodies. Characteristics of the GD patients are shown in Table 1. Patients with GD were treated with initial dosages of propranolol (40–60 mg/day), propylthiouracil (PTU; 300–400 mg/day) or methimazole (MMI; 10–30 mg/day), which were reduced gradually to maintain euthyroidism as serum thyroid hormone concentrations declined. GD patients were followed in our clinic for at least 1 year after cessation of the treatment. Relapse was confirmed by clinical presentation and laboratory data. Recurrence was diagnosed when serum free T4 and/or free T3 levels exceeded the upper limit of the normal range of our laboratory. The control group consisted of 203 individuals matched for age and sex. None of the controls had personal or family history of thyroid disease and goiter on examination (goiter size was classified according to WHO); they had normal thyroid functions and were negative for thyroid autoantibodies. The study was approved by the Institutional Review Board at Şişli Etfal Training and Research Hospital. Informed consent was obtained from each subject.

Blood samples were taken in the morning subsequent to an overnight (12 h) fasting. Peripheral venous blood samples were collected in plain tubes for routine biochemical analysis, and in EDTA-K_3 for genotype analysis. Serum TSH, free T_3, free T_4, anti-TPO and anti-TG levels were measured on a Modular EEE Electrode Elecsys Roche autoanalyzer (Roche Diagnostics, Mannheim, Germany). TRAb levels were assayed using a commercially available kit (Immunotech, Marseille Cedex, France).

Genomic DNA was isolated from peripheral blood leukocytes by using High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). Detection of the mentioned polymorphisms was done by rapid capillary PCR with melting curve analysis using fluorescence-labeled hybridization probes in a LightCycler (Roche Diagnostics, Mannheim, Germany) as has been previously described [16].

Differences in genotype distributions and allele frequencies in study and control groups were compared for statistical analysis 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. Differences between individual clinical variables and genotypes were assessed in two ways. Categorical data were analyzed using χ^2-test. For numerical data, Kruskal–Wallis, Mann–Whitney *U* and Spearman correlation tests were used. Statistical analyses were performed with SPSS 15.0 for Windows (Chicago, IL, USA). Linkage disequilibrium (LD) and haplotype frequencies were estimated using the Haploview software and compared between cases and controls using a contingency χ^2-test [17]. In addition, the NCSS 2000 statistical package (Kaysville, Utah, USA) was used to evaluate the power analysis. We had a 94% power to detect an effect size (W) of 0.20 using 2 degrees of freedom ($\alpha = 0.05$).

3. Results

In this study, VEGF −2578, −460 and +405 polymorphisms were analyzed in 167 patients with GD and 203 healthy controls. The mean age of GD patients was 40.9. Patient and control groups were matched for age (40.6). Table 1 summarizes the clinical and laboratory characteristics of GD patients. The genotype and allele distributions of VEGF −2578, −460 and +405 polymorphisms for cases and controls are shown in Table 2. All genotype distributions were in accordance with the HWE among the controls ($p = 0.864$, $p = 0.237$, and $p = 0.869$, respectively). However, the HWE for VEGF +405 was not respected in GD patients ($p = 0.03$). The allelic frequencies of A (0.58) and C (0.42) for VEGF −2578; T (0.59) and C (0.41) for VEGF −460; G (0.86) and C (0.14) for VEGF +405 found in our control population were similar with reported previous studies [16,18]. No notable differences were observed in allele or genotype frequencies for −2578 and −460 SNPs between GD and controls (Table 2). Individuals carrying CC genotype at the +405 locus had a 4.6-fold risk for developing GD compared to wild type homozygotes ($p = 0.004$, 95% CI 1.47–14.68). Moreover, the frequency of C allele was significantly increased in patients compared to controls ($p = 0.002$; OR = 1.81, 95% CI 1.23–2.66).

The relations between the studied SNPs and the age, gender, goiter size, presence of ophthalmopathy, type of anti-thyroid treatment, family history and number of patients relapsed after 1 year of follow-up, and the results of thyroid ultrasonography were evaluated in patients with GD, and no differences were detected (data not shown). However,

Table 1
Characteristics of the patients with Graves' disease (GD).

Variables	GD patients
Age (years)	
Mean ± SD	40.90 ± 12.56
Range	16–78
Gender	
Male, *n* (%)	44 (26.3)
Female, *n* (%)	123 (73.7)
GD onset	
<40 years, *n* (%)	93 (55.7)
>40 years, *n* (%)	74 (44.3)
Family history, *n* (%)	36 (21.6)
Smoking, *n* (%)	89 (53.3)
Ophthalmopathy, *n* (%)	55 (32.9)
PTU treatment, *n* (%)	141 (84.4)
MMI treatment, *n* (%)	26 (15.6)
Recurrence, *n* (%)	48 (28.7)
Goiter size 1, *n* (%)	97 (58.1)
Goiter size 2, *n* (%)	51 (30.5)
Goiter size 3, *n* (%)	19 (11.4)
Duration of AT treatment, months (mean ± SD)	22.7 ± 17.5
Anti-TPO (IU/mL) (mean ± SD)	630.4 ± 365.4
Anti-TG (IU/mL) (mean ± SD)	578.2 ± 345.5
TRAb (IU/L) (mean ± SD)	13.5 ± 12.9

Abbreviations: PTU (propylthiouracil), MMI (methimazole), AT (anti-thyroid), Anti-TPO (anti-thyroid peroxidase), Anti-TG (anti-thyroglobulin), TRAb (TSH receptor antibody).

Table 2
Distribution of genotypes and allele frequencies for patients with Graves' disease (GD) and control group.

	Controls *n* (%)	GD *n* (%)	OR (95% CI)	p
VEGF A-2578C				
AA	68 (33.5)	60 (35.9)	1.0[a]	–
AC	100 (49.3)	86 (51.5)	0.97 (0.62–1.53)	0.91
CC	35 (17.2)	21 (12.6)	0.68 (0.35–1.29)	0.23
A allele frequency	0.58	0.62	1.0[a]	
C allele frequency	0.42	0.38	0.86 (0.64–1.16)	0.32
VEGF T-460C				
TT	75 (36.9)	61 (36.5)	1.0[a]	–
CT	90 (44.3)	82 (49.1)	1.12 (0.71–1.75)	0.62
CC	38 (18.8)	24 (14.4)	0.77 (0.42–1.43)	0.41
T allele frequency	0.59	0.61	1.0[a]	
C allele frequency	0.41	0.39	0.92 (0.68–1.23)	0.58
VEGF G+405C				
GG	152 (74.9)	106 (63.5)	1.0[a]	–
CG	47 (23.2)	48 (28.7)	1.46 (0.93–2.34)	0.11
CC	4 (1.9)	13 (7.8)	4.66 (1.47–14.68)	0.004
G allele frequency	0.86	0.78	1.0[a]	
C allele frequency	0.14	0.22	1.81 (1.23–2.66)	0.002

Each p-value was based on chi-square (χ^2) analysis.
[a] Reference values for OR.