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A potential role of TNFR gene polymorphisms in autoimmune thyroid diseases in the Tunisian population

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Abstract

Autoimmune thyroid diseases (AITDs) including Graves disease (GD) and autoimmune hypothyroidism (AH) are associated with TNF genes polymorphisms. TNF molecules bind to TNFRI and TNFRII. No genetic association was reported between TNFR and AITDs. In this study, we have analysed two polymorphisms in TNFRI gene (TNFRI+36A/G SNP and a microsatellite $(GT)_{17}$ $(GA)_n$) and one polymorphism in TNFRII gene (TNFRII +676 T/G). All these polymorphisms were studied in a large Tunisian family with high prevalence of AITDs, and on a case-control sample of 91 GD patients and 165 controls. The present study was undertaken to investigate the genetic association of these polymorphisms with AITDs development. We reported the implication of TNFRIA3 allele in AITDs pathogenesis in familial and case control studies, respectively ($\chi^2 = 4.13$, $p = 0.042$; $\chi^2 = 9.26$, $p_c = 0.005$). In addition, Case-control study has revealed for the first time that TNFRII+676G allele was associated with GD ($\chi^2 = 11.53$; $p = 0.0007$). Two TNFRI haplotypes were found to be associated with GD: TNFRI+36G-A8, TNFRI+36A-A3 ($\chi^2 = 88.07$; $p = 6.32 \times 10^{-21}$, $\chi^2 = 16.78$; $p = 4.2 \times 10^{-5}$, respectively). Our data showed that TNFRI polymorphisms have an important role in AITDs pathogenesis in both familial and case-control samples and that TNFRII was rather implicated in GD development in the Tunisian population.

Keywords: TNFRI; TNFRII; AITDs; Polymorphism; Association

1. Introduction

Tumor necrosis factor (TNF) is a pleiotropic cytokine with a potent immunomodulatory activity. TNF acts via binding to cell surface receptors: TNFRI (CD120a or p55) (OMIM 191190) and TNFRII (CD120b or p75) (OMIM 191191). TNFRI and TNFRII genes map to 12p13 and 1p36, respectively [1,2]. TNFRI is expressed in all nucleated cells, particularly those susceptible to the cytotoxic action of TNF, and TNFRII is expressed predominantly in cells of myeloid origine, particularly stimulated T and B lymphocytes. It has been suggested that TNFα plays an effector role in autoimmune thyroid diseases (AITDs) development [3] including Graves disease (GD) and autoimmune hypothyroidism (AH): primary idiopathic myxedema (PIM) and Hashimoto thyroiditis (HT). However, there was no association study reported between TNFR gene polymorphisms and AITDs. The genetic contribution of TNF to AITDs development was previously described in the Tunisian population [4]. Consequently, we were interested in checking whether there was any possible implication of TNFR genes in AITDs pathogenesis in our population. Several TNFRI gene polymorphisms were described in the promoter region, in exon 1 (silent substitution), intron1 (microsatellite marker) and in introns 2, 4, 6, 7 and 8. In TNFRII gene many polymorphisms were described in the 3′UTR region and exon 6. In our study, we focused on an SNP (+36A/G)

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and a microsatellite marker ($(GT)_{17}$ $(GA)_n$) in TNFRI gene and in an SNP in exon 6 (+676 T/G) in TNFRII gene. All these polymorphisms were investigated in both familial and case-control samples in the Tunisian population. The current study aims at a direct testing of the association between TNFRI and TNFRII polymorphisms with AITDs in both case-control and familial studies.

Our results showed the incrimination of TNFRIA3 in AITDs and particularly GD development, respectively, in familial and case control studies. TNFRIIG allele was an effector allele for GD development. Two TNFRI haplotypes: TNFRI+36A-A3 and TNFRI+36G-A8 were found to be associated with GD.

2. Materials and methods

2.1. Patients and controls

Patients were recruited from a large family in south Tunisia with a high prevalence of AITDs named Akr family [5]. The members of this family are distributed on 10 generations of 400 members including 176 healthy individuals and 65 patients. These latters were subdivided into 34 patients affected with GD, and 31 patients with AH (9 HT, 22 PIM). The case control study was performed on DNAs from 106 unrelated patients with GD and 199 controls with no history of AITDs recruited from blood donors. The diagnosis of GD was based on the presence of biochemical hyperthyroidism as indicated by a decrease of TSH (TSH<0.2 μUI/ml), an increase of T4 levels (FT4>21 pmol/l) and positive TSH receptor antibody, in association with diffuse goiter or the presence of exophtalmos. The diagnosis of HT was based on the presence of thyroid hormone replaced primary hypothyroidism, defined as a TSH level above the upper limits associated with positive titers of thyroid antibodies (anti-thyroid peroxidase, or anti-thyroglobulin) and a palpable goiter. PIM was diagnosed by the presence of hypothyroidism requiring T3 or T4 replacement. Patients with PIM have an atrophic gland.

2.2. Polymorphisms genotyping

DNA was extracted from peripheral blood as previously described [6]. TNFRI $(GT)_{17}$ $(GA)_n$ microsatellite polymorphism was performed as previously described [7]. Amplified products were resolved on 6% sequencing gels and detected by autoradiography. TNFRI and TNFRII SNPs were genotyped using PCR-RFLP. In the TNFRI gene, we have examined an SNP (CCA/CCG) at codon 12 at position 36 in exon 1 (rs767455). This polymorphism is a silent mutation Pro12Pro. Genotyping of this polymorphism was carried out as previously described [8]. The primer sequences and PCR conditions used for amplification of a 242 bp fragment of the TNFRII exon 6 T/G (rs1061622) were previously described [9]. The studied polymorphism is a T/G transversion at codon 196 (+676) (ATG to AGG) which creates a Met 196 Arg substitution.

2.3. Statistical analysis

In the familial study, we used the FBAT program (Family based Association Test) [10,11] to test for association in the presence of linkage [12] using three diagnostic models (i) AITDs model: all AITDs patients were considered as affected, (ii) GD model: only GD patients were considered as affected and AH patients were considered as unaffected, and (iii) AH model: only AH patients were considered as affected. We used the Version 1.5 of FBAT which provides a haplotypic test of association [10]. The distribution of alleles in patients with GD versus controls was compared using the chi-square test or Fisher's exact test for a 2x2 contingency table. A corrected p (p_c) value of <0.05 was considered significant. This corrected p-value was calculated according to the Bonferroni correction. Odds ratios (OR) were calculated by Woolf's formula [13] with 95% confidence intervals (95% CI). TNFRI Haplotypes were estimated from population genotype using PHASE software version 2.02 [14,15]. The power of the association study was evaluated using functions from the Genetics R-package (available on http://cran.r-project.org) based on the method of Long and Langley (1997) [16].

3. Results

We were interested in three polymorphisms: two polymorphisms in TNFRI gene: ($(GT)_{17}$ $(GA)_n$ microsatellite and +36 A/G SNP) and a +676 T/G SNP in TNFRII gene. In the familial study, the analysis of TNFRI microsatellite marker has revealed 8 alleles (A3, A4, A5, A6, A8, A9, A10, and A11). The FBAT analysis showed an association of A3 allele with AITDs ($\chi^2 = 4.13$ and $p = 0.042$, biallelic mode, additive model) but not with either GD or AH. The investigation of the diallelic polymorphism of TNFRI gene revealed similar allele frequencies (56.25% and 43.75% for G and T alleles, respectively). In order to search for any associated haplotype with AITDs, the application of hbat package on TNFRI gene polymorphisms ($(GT)_{17}$ $(GA)_n$ microsatellite and +36 A/G SNP) showed 14 different haplotypes in Akr family. No haplotype was found to be associated either with AITDs, GD or AH. No association was also detected with TNFRII exon 6 T/G for any of the three inheritance models tested by FBAT ($p > 0.05$).

Allele frequencies of each investigated polymorphism in both controls and GD patients were mentioned in Table 1. Departure from Hardy Weinberg Equilibrium (HWE) was tested in controls for each investigated polymorphism using a Chi-square test. Only TNFRI+36 A/G SNP was found to be in HWE (Table 1). In the case control study, the analysis of TNFRI microsatellite polymorphism revealed 11 alleles. The A3 allele was also found to be a predispositional allele for GD pathogenesis ($p_c = 0.005$) as in the familial study. However, the A6 allele was found to be protector ($p_c = 5.5\ 10^{-6}$). The analysis of TNFRI+36 A/G polymorphism showed no significant difference between GD patients and controls ($\chi^2 = 3.27$; $p = 0.07$). The application