

Contribution of the *STAT4* rs7574865 gene polymorphism to the susceptibility to autoimmune thyroiditis in healthy Turk population and psoriatic subgroups

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Abstract

Introduction: *STAT4* is an important transcription factor that activates gene transcription as a response to cytokines. Recently, the influence of *STAT4* gene on autoimmune disease has been widely studied in many different immune-related diseases. Autoimmune, metabolic and cardiovascular disorders are more common in psoriatic patients. *STAT4* may be a unique gene that switches on in autoimmune-related thyroid disease in psoriatic patients.

The aim of the study: To explore the association of a *STAT4* rs7574865 polymorphism to autoimmune thyroid diseases in the general Turkish population and psoriatic subgroups.

Material and methods: A total of 132 psoriatic patients and 118 non-psoriatic volunteers were genotyped for *STAT4* rs7574865 using real time PCR. Twenty-four of the psoriatic patients and 15 of the non-psoriatic volunteers have autoimmune-related thyroid diseases.

Results: The prevalence of the T allele [OR = 4.37; 95% CI: 1.05-19; $p = 0.03$] of the *STAT4* rs7574865 was higher in individuals with autoimmune-related thyroid diseases among the all non-psoriatic volunteers. The volunteers with autoimmune-related thyroid diseases has an increased allele positivity and carriers having at least one of the risk allele was significantly higher than in counterparts with a GG wild genotype [ORGTTT vs. GG: 1.73; 95% CI: 0.09-32; $p = 0.03$]. Yet, there was no evidence of an association between rs7574865 and autoimmune-related thyroid disease in psoriatic patients.

Conclusions: The *STAT4* rs7574865 polymorphism increases autoimmune-related thyroid disease susceptibility among the general population but not in psoriatic patients.

Key words: psoriasis, polymorphism, Signal transducer and activator of transcription 4 (*STAT4*), autoimmune disease, JAK / STAT, autoimmune thyroiditis, rs7574865.

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Introduction

STAT4 is a transcription factor that specifically mediates signals that comes from interferon-induced tyrosine phosphorylation of target cytokine receptors [1, 2]. *STAT4* has a DNA-binding domain and specifically binds several DNA sequences among the promoter regions of cytokine genes as well as receptors and signal factors [3]. *STAT4* activated gene expression has a critical role in an autoimmune-mediated response by effecting functional regulation and differentiation of natural killer cells, mast cells, dendritic cells and T-helper cells [4]. As an example, *STAT4* activation causes a specific Th1 dif-

ferentiation resulting in a Th1/Th2 ratio shift to the Th1 side, which triggers psoriasis [5-7]. Thus many therapeutic methods aim to inactivate *STAT4* [8]. Recent studies show that many autoimmune diseases share similar immune pathogenesis and psoriasis associated other immune system disorders [6]. *STAT4* may be a major transcription factor that switches on several genes to trigger many unrelated disease at the same time. Based on this point of view, autoimmune-related thyroiditis and psoriasis may share the same immune pathogenesis through *STAT4* activation. In this study, we aimed to examine the risk of autoimmune thyroiditis and psoriasis due to *STAT4* rs7574865 polymorphism.

Material and methods

Ethics statement

This study was approved by the local ethics committees of the Çanakkale Onsekiz Mart University School of Medicine, Çanakkale, Turkey, and written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Design and setting

A case-control study was performed to define genotype and allelic profiles of polymorphic STAT4 region in autoimmune-related thyroid diseases among a general population and psoriatic sub-groups.

Patient profiles

The study population consisted of 132 psoriatic patients [24 autoimmune thyroiditis (18.18%)] and 118 non-psoriatic volunteers [15 autoimmune thyroiditis (12.71%)]. The inclusion criteria for the psoriatic sub-groups were at least a one-year follow-up examination by the same dermatologist. The exclusion criteria for control group were that there was no prior psoriasis history over the last three generations. A questionnaire on personal and family medical histories, current medication was completed during a face-to-face interview. Autoimmune thyroiditis diagnosis was based on clinical, laboratory and ultrasound tests.

Genotyping

Genotyping blood samples were collected at EDTA tubes from all individuals in Canakkale Onsekiz Mart University Training Research Hospital by the collaboration with Medical Genetic Department. Genotype analyses were performed by real-time PCR reaction after genomic DNA isolation from leukocytes from peripheral blood by using commercial DNA isolation kit (Genomic DNA Purification Kit, Thermo) according to the manufacturer's protocol. STAT4 rs7574865 polymorphism in the 3rd intron of the gene was genotyped in cases and controls. Real time PCR reaction was performed in a total volume of 20 µl PCR reaction that consist of 5 µl genomic DNA, 7.4 µl PCR-grade fluid, 1.6 µl Mg⁺² solution, 4 µl of primer and probe mixture, and 2 µl Master mix real-time PCR. The melting curve analyses was performed to determine genotypes.

Statistical analysis

Allele and genotype frequencies were determined by gene-counting method and were compared within the each subgroups controls by the chi-square test and the Pearson chi-square and Fisher exact test. All statistical analysis was performed by SPSS statistical software package and probability values less than 0.05 were assumed statistically sig-

nificant. Genotype associations and relative risk of were assessed via Odds Ratio by performing the Armitage trend test.

Results

The study included 132 psoriatic patients (72 women/60 men; average age: 44.61 ±15.95/47.85 ±17.08) and 118 controls (67 women/51 men average age: 43.9 ±17.4/50.04 ±21.69) without a prior history of psoriasis. The control group's age was 46.65 ±19.49 (median ± SD) and psoriasis' group age was 46.11 ±16.49. The correlation of STAT4 rs7574865 polymorphism to autoimmune thyroiditis was examined by comparing them with non-psoriatic healthy volunteers (Table 1). The autoimmune thyroiditis was detected in 13 out of the 72 cases with wild (GG) genotype and two out of 41 with heterozygote genotype (GT), and none of the participants had a mutant genotype (TT). The psoriatic subgroup showed and increased risk (1.53 times) for autoimmune thyroiditis compared to the general population; yet, the results were not statistically significant [OR = 1.53; 95% CI: 0.76-3.07; *p* = 0.24]. The distribution of autoimmune thyroiditis was in 17 out of 110 cases with a wild (GG) genotype and seven out of 45 with a heterozygote genotype (GT). In psoriatic sub-groups, one patient was TT genotyped and was not diagnosed with autoimmune thyroiditis.

In our study, rs7574865 T alleles were seen more frequently in patients with autoimmune thyroiditis in comparison to non-psoriatic healthy subjects [OR = 4.39 95% CI: 1.0-19; *p* = 0.03]. This result means that T allele carriers have a 4.4 times higher risk for development of autoimmune thyroiditis. Similarly, allele positivity also increased the risk of autoimmune thyroiditis 4.75 times more in the general population [OR: 4.75; 95% CI: 0.04-0.96; *p* = 0.029].

Moreover, in our study we aimed to examine the relationship of STAT4 rs7574865 T alleles to the development of autoimmune thyroiditis among the psoriatic patients. Based on this point of view, both autoimmune thyroiditis and psoriasis may be share the similar immune pathogenesis that contains common genetic marker such as *STAT4*. For that purpose, the association of STAT4 rs7574865 to autoimmune thyroiditis was analysed within the psoriatic subgroups (Table 2). The results showed that a lack of allelic (T as risk allele) or genotypic (TT or GT) association for rs7574865 polymorphism in psoriatic subgroups. The present study indicates that rs7574865 polymorphism of *STAT4* gene might be a risk factor for the development of autoimmune thyroiditis in the general population; yet, it did not have an additional risk in psoriatic subgroups.

Discussion

STAT4 is an important player in the immunogenetics of several diseases and increases the autoimmune-related

Table 1. The correlation of STAT4 rs7574865 polymorphism with autoimmune thyroiditis in non-psoriatic Turkish population

SNP	Tests for deviation from Hardy-Weinberg equilibrium		Tests for association (95% CI)				
	Turk population without autoimmune thyroid diseases	Turk population with autoimmune thyroid diseases	Allele frequency difference	Heterozygous	Homozygous	Allele positivity	Armitage's trend test
STAT4 rs7574865	nGG = 59 (59.83)	nGG = 13 (13.07)	Risk allele T				
	nGT = 39 (37.34)	nGT = 2 (1.87)	[G]<->[T]	[GG]<->[GT]	[GG+]<->[TT]	[GG]<->[GT+TT]	Common odds ratio
	nTT = 5 (5.83)	nTT = 0 (0.07)	OR = 0.229	OR = 0.233	OR = 0.401	OR = 0.206	OR = 0.222
	f _{a1} = 0.76 ± 0.029	f _{a1} = 0.93 ± 0.044	CI: [0.053-0.995]	CI: [0.050-1.089]	CI: [0.021-7.692]	CI: [0.044-0.961]	χ ² = 4.65
	F = -0.04433	F = -0.07143	χ ² = 4.53	χ ² = 3.94	χ ² = 1.09	χ ² = 4.75	p = 0.03107
	p = 0.652811 (Pearson)	p = 0.782055 (Pearson)	p = 0.03329 (P)	p = 0.04714	p = 0.29732	p = 0.02925	
	p = 0.648842 (Llr)	p = 0.705337 (Llr)	Risk allele G				
	p = 0.789704 (Exact)	p = 1.000000 (Exact)	[T]<->[G]	[TT]<->[GT]	[TT]<->[GG]	[GG+GT]<->[TT]	common odds ratio
			OR = 4.369	OR = 0.696	OR = 2.496	OR = 1.731	OR = 7.738
			CI: [1.005-19.003]	CI: [0.029-16.487]	CI: [0.130-47.911]	CI: [0.091-32.880]	χ ² = 4.65
			χ ² = 4.53	χ ² = 0.25	χ ² = 1.09	χ ² = 0.76	p = 0.03107
			p = 0.03329 (P)	p = 0.61358	p = 0.29732	p = 0.38321	

OR – odds ratio

Table 2. The correlation of STAT4 rs7574865 polymorphism with autoimmune thyroiditis in psoriatic sub groups of Turkish population

SNP	Tests for deviation from Hardy-Weinberg equilibrium		Tests for association (CI: 95% confidence interval)				
	Psoriasis patients without autoimmune thyroid diseases	Psoriasis patients with autoimmune thyroid diseases	Allele frequency difference	Heterozygous	Homozygous	Allele positivity	Armitage's trend test
STAT4 rs7574865	nGG = 76 (77.52)	nGG = 17 (17.51)	Risk allele T				
	nGT = 31 (27.96)	nGT = 7 (5.98)	[G]<->[T]	[GG]<->[GT]	[GG+]<->[TT]	[GG]<->[GT+TT]	Common Odds Ratio
http://ihg.gsf.de/cgi-bin/hw/finetti2.pl?nco_11=59&nco_12=39&nco_22=5&nca_11=13&nca_12=2&nca_22=0&snp=SNP1	nTT = 1 (2.52)	nTT = 0 (0.51)	OR = 0.947	OR = 1.009	OR = 1.457	OR = 0.978	OR = 0.966
	f _{a1} = 0.85 ± 0.023	f _{a1} = 0.85 ± 0.046	CI: [0.391-2.290]	CI: [0.381-2.674]	CI: [0.057-37.298]	CI: [0.370-2.586]	χ ² = 0.02
	F = -0.10879	F = -0.17073	χ ² = 0.01	χ ² = 0.00	χ ² = 0.22	χ ² = 0.00	p = 0.89707
	p = 0.258220 (Pearson)	p = 0.402924 (Pearson)	p = 0.90339 (P)	p = 0.98484	p = 0.63665	p = 0.96414	
	p = 0.214314 (Llr)	p = 0.273123 (Llr)	Risk allele G				
	p = 0.457099 (Exact)	p = 1.000000 (Exact)	[T]<->[G]	[TT]<->[GT]	[TT]<->[GG]	[GG+GT]<->[TT]	Common Odds Ratio
			OR = 1.056	OR = 0.714	OR = 0.686	OR = 0.684	OR = 1.213
			CI: [0.437-2.554]	CI: [0.026-19.331]	CI: [0.027-17.566]	CI: [0.027-17.294]	χ ² = 0.02
			χ ² = 0.01	χ ² = 0.22	χ ² = 0.22	χ ² = 0.22	p = 0.89707
			p = 0.90339 (P)	p = 0.63563	p = 0.6366	p = 0.63607	

OR – odds ratio

disease risks such as SLE, RA, T1D, SSc, pSS [9-11]. The scientists hope to treat autoimmune diseases with the suppression of the STAT4 gene to immunosuppress the disease in the future. The logic behind this treatment is because of the major role that the STAT4 gene plays in both inflammation and autoimmunity. On a molecular basis, the STAT4 protein has a linker domain that binds to a specific DNA target. In animals, a STAT4 knock out and knock in studies prove that STAT4 has an active role in autoimmune diseases. Recent studies showed that STAT4 deficient mice were protected from developing autoimmune diabetes when compared to wild types [12, 13]. In addition, when STAT4 deficient mice were compared to wild type mice, the initial period and severity of diabetes were delayed in the STAT4 deficient groups [14, 15].

Furthermore, any genetic alteration in the *STAT4* gene, such as single nucleotide polymorphism (SNP) that affects the DNA binding affinity of the STAT4 protein, triggers an autoimmune disease [5, 10, 11, 15-17].

Liang *et al.* reported that STAT4 rs7574865 polymorphism is associated with immune-related diseases and T-allele carriers increase their risk for the multiple autoimmune diseases [18].

Land *et al.* previously reported that a STAT4 deficient mice developed hyperthyroidism more often than the wild type group as well as showed higher severity of hyperthyroidism [19]. Yan *et al.* performed a study to evaluate STAT4 rs7574865 polymorphism with Graves' disease (GD) and Hashimoto's thyroiditis (HT). According to their data, genotype and the T allele distribution of STAT4 rs7574865 was significantly higher in GD patients; in contrast, the same association was not found in HT patients [5]. Park *et al.* reported that STAT4 rs7574865 polymorphism is significantly associated with autoimmune thyroid disease (1.5 times); Graves' disease (1.43 times) and Hashimoto's thyroiditis (1.58 times) more than in the general population [20]. In contrast, STAT4 rs7574865 polymorphism had no association to autoimmune thyroid diseases in the Tunisian population, but that polymorphism was found to be a risk factor for rheumatoid arthritis [21].

In our study, we examined the genetic and immune basis of thyroiditis in Turkish patients in general population and in psoriatic subgroups. Our results revealed that, T allele frequency difference is significantly higher in autoimmune thyroid disease group compared to the general population. Allele positivity increases the risk of thyroiditis is higher in the Turkish population and conversion of the G allele to T increases autoimmune thyroiditis risk 4.36 times more and general risk increases 7.7 times more in the Turkish population. When various ethnic groups were evaluated, STAT4 rs7574865 polymorphism may be a risk factor for autoimmune thyroiditis and our results are similar with previous studies that were performed by Yan *et al.* and also Park *et al.* [5, 20]. The major finding of the

present study is that STAT4 rs7574865 polymorphism has a role in the development of autoimmune thyroid disorders in the general population. However, no significant association with the risk of autoimmune thyroid was found in psoriatic subgroups.

Conclusions

This is the first report that researched the association of STAT4 rs7574865 to autoimmune thyroiditis in psoriatic subgroups. Also, this is the first case-control study on the association between STAT4 polymorphisms and autoimmune thyroiditis susceptibility in a Turkish population. Our results suggest that STAT4 polymorphism is associated with an increased risk of autoimmune thyroiditis in the Turkish population but not in a psoriatic sub-population. Thus we could not conclude that both autoimmune thyroiditis and psoriasis share the common genetic marker for the STAT4 allele.

The authors declare no conflict of interest.

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