Investigation of the Relationship Between Genome Wide Association Studies-derived Polymorphisms and Differentiated Thyroid Cancer Risk in a Turkish Population

Bir Türk Popülasyonunda Genom Çapında İlişkilendirme Çalışmalarından Kaynaklanan Polimorfizmler ile Farklılaşmış Tiroid Kanseri Riski Arasındaki İlişkinin İncelenmesi

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ABSTRACT

Background: Thyroid cancer is the most common malignancy of endocrine system. Genome Wide Association Studies (GWAS) revealed a number of common variants associated with thyroid cancer risk. In this study, we aimed to investigate the association of these known variants with thyroid cancer risk in a Turkish population living in Trakya region.
Methods: The study included 97 cases of differentiated thyroid cancer and 379 healthy controls. Real-Time Polymerase Chain Reaction (RT-PCR) method was used for the genotyping of rs965513, rs944289, rs966423 rs2439302 polymorphisms.
Results: There was no statistically significant difference between patients and controls in terms of SNP genotype and allele frequencies. The distribution of cumulative genetic risk scores between patients and controls was also not significantly different. In the multiple logistic regression analysis (MLR), it was observed that the relationship of rs2439302 polymorphism GG genotype with thyroid cancer risk has a trend to be significant ((p = 0.067, 95%CI: 2.947 (0.928-9.357)).
Conclusion: We suggest that the confirmation of the association of common variants with thyroid cancer in different populations will contribute to make a consensus on global risk alleles. The marginal significance of the association of rs2439302 with thyroid carcinoma risk shown in our study supports the need for functional studies on the role of this polymorphism in thyroid carcinoma.

Keywords: Thyroid carcinoma, GWAS, genetic risk

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ÖZET

Giriş: Tiroid kanseri, endokrin sistemin en sık görülen malignitesidir. Genom Boyu İlişki Çalışmaları (GBİÇ), tiroid kanseri riskiyle ilişkili bir dizi yaygın varyantı ortaya çıkarmıştır. Bu çalışmada, bilinen bu varyasyonların, Trakya bölgesinde yaşayan bir Türk popülasyonunda, tiroid kanseri riski ile ilişkisini araştırmayı amaçladık.
Materyal ve Yöntem: Çalışmaya 97 diferansiye tiroid kanseri vakası ve 379 sağlıklı kontrol dahil edildi. rs965513, rs944289, rs966423 rs2439302 polimorfizmlerinin genotiplendirilmesi için Gerçek Zamanlı Polimeraz Zincir Reaksiyonu (RT-PCR) yöntemi kullanıldı.
Bulgular: SNP genotipi ve allel frekansları açısından hastalar ve kontroller arasında istatistiksel olarak anlamlı bir fark yoktu. Kümülatif genetik risk skorlarının hastalar ve kontroller arasındaki dağılımı da önemli ölçüde farklı değildi. Çok değişkenli lojistik regresyon analizinde rs2439302 polimorfizmi GG genotipinin tiroid kanseri riski ile sınırda anlamlı bir ilişkisinin olduğu görüldü ((p = 0.067,% 95 CI: 2.947 (0.928-9.357)).
Sonuç: Farklı popülasyonlarda tiroid kanseri ile ortak varyantların ilişkisinin doğrulanmasının global risk alelleri üzerinde bir fikir birliğine varılmasına katkıda bulunacağını düşünüyoruz. Çalışmamızda gösterilen rs2439302 ile tiroid karsinom riski arasındaki ilişkinin sınırdaki önemi, bu polimorfizmin tiroid karsinomundaki rolü üzerine fonksiyonel çalışmalara olan ihtiyacı desteklemektedir.

Anahtar Kelimeler: Tiroid karsinomu, GBİÇ, genetik risk

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INTRODUCTION

Thyroid cancer (TC) constitutes approximately 90% of all malignancies of the endocrine system as the most common endocrine malignancy (1). Over 500000 new thyroid cancer patients have been reported In the 2018 report of the World Health Organization (2). The incidence of thyroid cancer increased both in males and females in Turkey, according to the statistics of the Turkish Cancer Research Association in 2008 (3). About 90% of all thyroid cancers are differentiated thyroid cancers (DTC) arising from follicular cells (FNMTC) (4). Papillary thyroid carcinoma (PTC) is the most common subtype of differentiated thyroid carcinoma. Several different factors including radiation exposure, smoking, iodine deficiency, hormonal factors, and genetic factors have been shown to contribute to the development of DTC (5).

The genome-wide association study (GWAS) approach became a preferred method to identify genetic factors involved in complex diseases via analyzing genetic variants across the genomes of many individuals (6). GWASs studies on thyroid cancer revealed some risk-associated single-nucleotide polymorphisms (SNPs) in different populations (Table 1) (4, 7-15). Germline candidate risk factors for thyroid carcinoma were mainly located close to the genes including *FOXE1, DIRC3, NKX2-1, NRG1,* and *PTSC2* (Table 1).

Results of the GWAS studies can differ between populations due to the divergence of frequencies of previously unpublished SNPs and their associations with diseases may not be the same with the reference population of other studies. So, confirmation of the associated variants across multi-ethnic, founder, admixed, and highly consanguineous populations is important (6). Here, we aimed to evaluate the role of GWAS-derived TC variants in a population living in the Trakya region of Turkey.

MATERIALS and METHODS

Study design and samples

This was a case-control study. We selected the thyroid cancer-related polymorphisms from the previous study performed by Wang et al (12) and a priori power analysis was done by using the G-power 3.1.2 version (16) . The sample size was calculated as 412 samples based on an effect size of 0.153 at the rs944289 genetic variant (12), with an alpha level of 5%, and a power of 80%. However, we included 478 samples considering possible missing data (16). After providing written informed consent, 97 nonmedullary TC cases (13 males and 84 females) and 381 cancer-free control individuals (282 females, 99 males) were included in the study. All patients have histologically confirmed thyroid cancer patients. For controls, we prospectively scanned apparently healthy volunteers with thyroid ultrasonography, and individuals who do not have a thyroid nodule were recruited for the genetic analysis. They were cancer-free nonconsanguineous individuals who did not have any thyroid-related disorder and who did not have a first-degree relative with thyroid cancer. Ninety-four out of 97 thyroid cancer patients had papillary thyroid carcinoma whereas only 3 had follicular thyroid carcinoma. fT3, fT4, and TSH levels of patients and controls were biochemically analyzed.

The study was approved by the Trakya University Faculty of Medicine Scientific Research Ethics Committee (TÜTF-BAEK, 2015/174) and financially supported by Trakya University Scientific Research Projects Unit (TÜBAP 2016/132).

SNPs genotyping

We isolated genomic DNA from peripheral blood samples using the EasyOne Automated DNA Isolation System (Qiagen, Hilden, Germany). Quality control and purity of the isolated genomic DNA samples was determined using a NanoDrop Spectrophotometer (NanoDrop 2000C; ThermoFisherScientificInc.,Wilmington, MA, USA). Samples with a A260/280 values of between1.8–2.0 were included in while low-quality samples were re-isolated from stored blood samples. We genotyped rs2439302, rs944289, rs965513, and rs966423 SNPs using Applied Biosystems Step One Plus Real-Time PCR (Thermo Fisher Scientific, USA) with TaqMan® SNP Genotyping Assay kit (ThermoFisherScientificInc., Wilmington, MA, USA) according to the manufacturer's instructions.

Statistical analysis

All the statistical analyses were performed using SPSS 20.0 (Statistical Package for the Social Sciences 20.0; IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Hardy–Weinberg equilibrium was tested by chi-square tests for the four SNPs. We compared the patients and controls in terms of sex (male, female), and family history of cancer (present, absent), metastasis (absent, present) using the Chi-square test. The Student t-test was used in the comparison of age between patients and controls. Unweighted Cumulative genetic risk scores (UCGRS) were calculated as the total count of disease alleles from four SNPs studied (possible score range of 0–8) (17), samples with at least one or more missing genotypes were excluded in the genetic risk score calculations to avoid misinterpretation. The effect of SNPs on DTC was tested by using multiple logistic regression analysis (MLR).

RESULTS

Age, sex, clinical and biochemical parameters in DTC patients and controls are shown in Table 2. We studied 97 DTC patients (mean age: 50.2 ± 12.2 years). and 381 healthy control subjects (mean age: 37.1 ± 8.7 years). Of the 97 patients, 94 were diagnosed with papillary thyroid carcinoma and 3 with follicular thyroid carcinoma. The mean age was higher between the DTC patients compared to the control groups ($p<0.001$). the number of female patients was higher in the DTC group ($p < 0.01$) (Table2).

Even the risk allele frequencies were higher between the patients compared to controls for rs965513, rs944289, and rs2439302 polymorphisms, the differences were not statistically significant (Table 4). It was interesting that the frequency of the risk allele of rs966423 polymorphism (*T*) was higher among DTC patients than the controls. Genotype distribution was not significantly different among patients in terms of lymph node metastasis or family history (Table 4). Mean Unweighted cumulative genetic risk was not statistically significant between DTC patients and controls (Table 5). Analysis of demographic, biochemical, and genetic factors for thyroid carcinoma risk with logistic regression analysis revealed that the *GG* genotype of rs2439302 polymorphism has a trend to be significant (P = 0.067) between the patients with DTC and control subjects (OR=2.947 95% *CI*: 0.928-9.357)(Table 6).

Table 1. A summary of SNPs (Single Nucleotide Polymorphisms) previously associated with thyroid cancer in GWAS studies. The table is adapted from GWAS Catalog (lit).

rsID (Risk Allele)	Mapped Gene	Risk Allele Frequency	Association with	Reference
rs965513 (A)	*PTCSC2*	NR	thyroid carcinoma	Köhler et al, 2013
		NR	thyroid carcinoma	Takahashi et al, 2010
		0.34	thyroid carcinoma	Gudmundsson et al, 2009
		0.34	thyroid carcinoma	Mancikova et al, 2015
rs2439302 (G)	*NRG1*	0.351	thyroid carcinoma	Gudmundsson et al, 2012
rs966423 (C)	*DIRC3*	0.442	thyroid carcinoma	Gudmundsson et al, 2012
rs944289 (T)	*PTCSC3 - AL162511.1*	0.57	thyroid carcinoma	Gudmundsson et al, 2009
rs10069690 (T)	*TERT*	0.275	thyroid carcinoma	Gudmundsson et al, 2017
rs10122541 (G)	*FOXE1 - TRMO*	0.33	thyroid carcinoma	Mancikova et al, 2015
rs11175834 (T)	*LINC02454, AC025419.1*	0.15	differentiated thyroid carcinoma	Son et al, 2017
rs114692817 (T)	*THEGL*	0.014383	thyroid carcinoma	Rashkin et al, 2020
rs116909374 (T)	*AL162511.1 - MBIP*	0.034	thyroid carcinoma	Gudmundsson et al, 2017
		0.017	thyroid carcinoma	Gudmundsson et al, 2012
rs11693806 (C)	*DIRC3*	0.319	thyroid carcinoma	Gudmundsson et al, 2017
rs117401978 (A)	*AC007603.3 - CNEP1R1*	0.010953	thyroid carcinoma	Rashkin et al, 2020
rs11947482 (T)	*AC093323.1 - LINC02481*	NR	thyroid carcinoma	Rashkin et al, 2020
rs12002967 (C)	*FOXE1 - TRMO*	NR	thyroid carcinoma	Rashkin et al, 2020
rs12129938 (A)	*PCNX2*	0.795	thyroid carcinoma	Gudmundsson et al, 2017
rs12990503 (G)	*DIRC3*	0.63	differentiated thyroid carcinoma	Son et al, 2017
rs1588635 (A)	*PTCSC2*	0.396	thyroid carcinoma	Gudmundsson et al, 2017
rs1874564 (G)	*SOWAHB - SEPTIN11*	0.69	papillary thyroid carcinoma	Son et al, 2017
rs2289261 (C)	*SMAD3*	0.683	thyroid carcinoma	Gudmundsson et al, 2017
rs2466074 (C)	*NRG1*	NR	thyroid carcinoma	Rashkin et al, 2020
rs2466076 (G)	*NRG1*	0.484	thyroid carcinoma	Gudmundsson et al, 2017
rs34081947 (T)	*LINC00609*	0.41	differentiated thyroid carcinoma	Son et al, 2017
rs368187 (G)	*AL133304.3, AL133304.2*	0.581	thyroid carcinoma	Gudmundsson et al, 2017
rs4649295 (T)	*PCNX2*	0.82	differentiated thyroid carcinoma	Son et al, 2017
rs4915076 (T)	*VAV3*	0.7	differentiated thyroid carcinoma	Son et al, 2017
rs555678255 (C)	*FTH1P21 - AC096736.3*	0.898888	thyroid carcinoma	Rashkin et al, 2020
rs56062135 (T)	*SMAD3*	0.253	thyroid carcinoma	Gudmundsson et al, 2017
rs6697759 (T)	*TGFB2 - LINC02869*	0.439535	thyroid carcinoma	Rashkin et al, 2020
rs6759952 (T)	*DIRC3*	0.43	thyroid carcinoma	Köhler et al, 2013
rs6793295 (T)	*LRRC34*	0.759	thyroid carcinoma	Gudmundsson et al, 2017
rs6996585 (G)	*NRG1*	0.23	differentiated thyroid carcinoma	Son et al, 2017
rs7030280 (C)	*PTCSC2*	NR	thyroid carcinoma	Rashkin et al, 2020
rs7037324 (A)	*FOXE1 - TRMO*	0.34	thyroid carcinoma	Mancikova et al, 2015
rs72753537 (C)	*FOXE1 - TRMO*	0.07	differentiated thyroid carcinoma	Son et al, 2017
rs72806259 (T)	*CHST3 - AC022392.1*	0.093896	thyroid carcinoma	Rashkin et al, 2020
rs73227498 (A)	*EPB41L4A*	0.872	thyroid carcinoma	Gudmundsson et al, 2017
rs74518511 (T)	*DBX1 - HTATIP2*	0.017275	thyroid carcinoma	Rashkin et al, 2020
rs76032629 (A)	*LARP7, MIR302CHG*	0.040918	thyroid carcinoma	Rashkin et al, 2020
rs77166399 (T)	*LINC00923*	0.148487	thyroid carcinoma	Rashkin et al, 2020
rs772695095 (T)	*DIRC3*	NR	thyroid carcinoma	Rashkin et al, 2020
rs7902587 (T)	*STN1 - SLK*	0.109	thyroid carcinoma	Gudmundsson et al, 2017
rs925489 (T)	*PTCSC2*	0.332	thyroid carcinoma	Zhou et al, 2018
rs9858271 (G)	*AC126121.3*	0.43	differentiated thyroid carcinoma	Son et al, 2017

NR: Not reported

Table 2. Biochemical and demographic properties of the studied population.

		N	Mean	Std. Deviation	*p*
	Patient	94	50.2	12.2	*<0.001*
Age	**Control**	381	37.1	8.7	
	Patient	94	2.9	1.3	*<0.001*
fT3 level	**Control**	372	3.4	0.6	
	Patient	94	1.1	0.5	*<0.001*
fT4 level	**Control**	371	0.9	0.1	
	Patient	94	11.9	23.5	*0.01*
TSH level	**Control**	371	1.7	1.2	
Gender					
		Male (%)	**Female (%)**		*p*
	Patient	13 (13.4)	84 (86.6)		*0.01*
	Control	99 (74)	282 (26)		

P values were calculated with the Chi-Square test. $P < 0.05$ indicates statistical significance.

Table 3. Genotype and allele distribution comparison among DTC patients and controls.

rs no	*Gene/ Consequence*	*Genotype/ Alleles*	*No of cases (Frequency)*	*No. Of controls (Frequency)*	*OR (95% CI)*	*p*
		GG	40 (0.412)	181 (0.475)		
		AG	47 (0.485)	161 (0.423)	1.32 (0.82 - 2.11)	0.247
rs965513 (9q22.33)	*PTCSC2, intron variant*	AA	10 (0.103)	39 (0.102)	0.28 (0.13 - 0.58)	0.706
		A*	67 (0.345)	239 (0.313)	1.15 (0.82 - 1.61)	0.397
		CC	18 (0.186)	72 (0.196)		
		TC	51 (0.526)	193 (0.524)	1.05 (0.57 - 1.92)	0.856
rs944289 (14q13.3)	*PTCSC3 - AL162511.1*	TT	28 (0.289)	103 (0.28)	0.77 (0.39 - 1.52)	0.451
		T*	107 (0.551)	399 (0.542)	1.03 (0.75 - 1.42)	0.814
		CC	32 (0.33)	137 (0.361)		
		GC	47 (0.485)	192 (0.507)	1.04 (0.63 - 1.72)	0.854
rs2439302 *(8p12)*	*NRG1, intron variant*	GG	18 (0.186)	50 (0.132)	1.54 (0.79 - 2.98)	0.198
		G*	83 (0.427)	292 (0.385)	1.19 (0.86 - 1.64)	0.278
		CC	22 (0.227)	67 (0.176)		
		TC	44 (0.454)	192 (0.504)	0.69 (0.39 - 1.25)	0.224
rs966423 (2q35)	*DIRC3, intron variant*	TT	31 (0.32)	122 (0.32)	0.77 (0.41 - 1.44)	0.418
		T*	106 (0.546)	436 (0.572)	0.90 (0.65 - 1.23)	0.517

P values were calculated with the Chi-Square test.
$P < 0.05$ indicates statistical significance.
Risk alleles were designated with * symbol. OR: Odds Ratio, CI: Confidence Interval

Table 4. Comparison of the genotypes of patients according to metastasis and family history.

SNP	Genotypes	Lymph Node Metastasis (-)		P	Family History of Thyroid carcinoma		P
		Absent	Present		Absent	Present	
rs965513	*GG*	34	3		23	12	
	GA	35	8	*0.35*	29	13	*0.97*
	AA	8	2		7	3	
rs966423	*TT*	22	5		14	11	
	TC	39	4	*0.41*	31	12	*0.29*
	CC	16	4		15	5	
rs944289	*TT*	22	5		21	5	
	TC	42	4	*0.25*	27	18	*0.18*
	CC	13	4		12	3	
rs2439302	*GG*	15	2		11	5	
	GC	39	5	*0.51*	29	14	*0.98*
	CC	23	6		20	9	

P values were calculated with the Chi-Square test. P < 0.05 indicates statistical significance.

Table 5. Comparison of unweighted cumulative genetic risk scores (CGRS) between the patients and controls.

	Mean CGRS	Std. Deviation	Minimum CGRS	Maximum CGRS	p
Patients *(n=97)*	3.74	1.35	1	7	
					0.48
Controls *(n=367)*	3.61	1.38	0	7	

P values were calculated with the Chi-Square test. P < 0.05 indicates statistical significance.

Table 6. Analysis of demographic, biochemical, and genetic factors for thyroid carcinoma risk

	OR (95% CI)	*p*
Gender		
Male	***1 (Reference)***	***0.008***
Female	6.988 (1.666-29.310)	
Age	1.075 (1.035-1.116)	***<0.001***
fT3 (IUI/ml)	0.260 (0.138-0.490)	***<0.001***
fT4 (IUI/ml)	19361 (1275-293942)	***<0.001***
TSH (IUI/ml)	1.383 (1.173-1630)	***<0.001***
rs965513		
GG	***1 (Reference)***	
AG	0.655 (0.186-2.308)	*0.510*
AA	0.319 (0.087-1.166)	*0.084*
rs944289		
CC	***1 (Reference)***	
TC	0.419 (0.122-1.441)	*0.167*
TT	0.926 (0.317-2.703)	*0.888*
rs2439302		
CC	***1 (Reference)***	
GC	0.815 (0.321-2.072)	*0.667*
GG	**2.947 (0.928-9.357)**	***0.067***
rs966423		
CC	***1 (Reference)***	
TC	0.595 (0.195-1.809)	*0.360*
TT	0.449 (0.134-1.505)	*0.194*

P values were calculated with MLR analysis. P < 0.05 indicates statistical significance. OR: Odds Ratio, CI: Confidence Interval

DISCUSSION

The role of genetic risk factors in the pathogenesis of thyroid carcinoma is widely accepted and (18; 19). The pieces of evidence of those studies are needed to be confirmed in discrete populations. Although some studies are focusing on the genetic risk factors for thyroid carcinoma in the Turkish population (20,21,22), no studies are investigating the association of previously defined GWAS-derived TC risk SNPs. In this study, we showed that the risk allele frequencies of rs96513, rs944289, and rs2439302 were higher between the thyroid cancer patients compared to healthy controls but the differences were not statistically significant for rs965513 and rs944289. However, we found a trend to be significant for the rs2439302 ($p = 0.067$) between the DTC patients and control subjects. The risk allele frequency of rs966243, on the other hand, was higher between the controls compared to DTC patients in our study, suggesting that this polymorphism is unlikely a risk factor for thyroid carcinoma risk in the Turkish population.

The first GWAS study for thyroid carcinoma, performed in a European population including 192 thyroid cancer patients and 37.196 controls revealed that rs965513 on 9q22.33 and rs944289 on 14q13.3 were related to both papillary and follicular thyroid cancer risk (8). *FOXE1* gene, the nearest gene to the 9q22.33 locus was further associated with thyroid cancer in a study performed in Spanish and Italian populations. But rs1867277, rather than rs965513 have been reported to be associated with the TC risk in this study (23) In a subsequent study, rs965513 on 9q22.33 and rs944289 on 14q13.3 alleles were reported to be associated with low concentrations of thyroid-stimulating hormone (TSH), and the 9q22.33 allele is associated with a low concentration of thyroxin (T(4)) and a high concentration of triiodothyronine. This was a preliminary study suggesting an association between rs2439302 on 8p12 and the expression of *NRG1* gene, encoding for a signaling protein in the blood (11) and confirmed by a functional study (24).

FOXE1 locus was also associated with Papillary thyroid cancer (PTC) in a study focusing on patients exposed to radioactive iodine in their childhood or adolescence but the rs944289 at reported not to associated with radiation-related PTC in the same study (9). rs944289 has been shown to predisposes to PTC through a long intergenic noncoding RNA gene (*PTCSC3*) (25). Association of rs944289, rs965513, rs966423 rs2439302, and PTC risk have been confirmed in a Chinese population, too (12). However, the association of rs944289 with thyroid carcinoma could not have been shown in a study performed in a population of Italian familial nonmedullary thyroid cancer patients and controls (18). rs944289 was not associated with DTC in a study performed in a German population (26) as in our study.

Cumulative effects of the risk alleles of common variants have been shown to correlate with thyroid carcinoma in some studies (17,27). In our study, cumulative genetic risk scores did not show a significant association with DTC risk. But it was an interesting finding that the number of the patients with ≥7 risk allele count were higher compared to controls, however, the difference was not statistically significant. This finding supports the need for a larger sample size to be studied for defining the possible effects of CGRS on thyroid carcinoma risk.

The strongest candidate for thyroid carcinoma risk was rs2439302 polymorphism in our study with a trend to be associated (p=0.067) with increased risk of DTC (OR 2.947 95% CI: 0.928-9.357). The risk allele (G) of rs2439302 was previously shown to be correlated with the expression of multiple *NRG1* isoforms in unaffected thyroid tissue in a functional study (24). We suggest that the possible association of this polymorphism with DTC can be confirmed with a study including larger samples of DTC patients and controls.

There are some limitations of our study. First of all, benign thyroid nodules are very common in the normal population and this is restricting the inclusion of larger control groups. Second, as a disease with a higher frequency with advanced age, our control samples were younger than the patient group which may reflect the results. Finally, dietary and environmental differences which may affect thyroid cancer were not considered inpatient and control groups.

In conclusion, we suggest that the confirmation of the association of common variants with thyroid cancer performed in larger sample groups will contribute to making a consensus on global risk alleles. Especially, the marginal significance of the association of rs2439302 with thyroid carcinoma risk shown in our study urges the need for functional studies on the role of this polymorphism in thyroid carcinoma. However, our results also support that caution must be taken when extrapolating GWAS results from one population to directly predict disease risks in other populations (28,29).

Conflict of interest

No conflict of interest was declared by the authors.

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