



ORIGINAL RESEARCH

THE ASSOCIATION OF P53 CODON 72 POLYMORPHISM WITH THYROID CANCER IN TURKISH PATIENTS

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ABSTRACT

Objective: Thyroid cancer is the most common endocrine malignancy. Molecular mechanisms underlying this disorder are heterogeneous and need to be clarified. Different polymorphic variants of the *p53* gene have been demonstrated to be in association with several human malignancies. Of those, codon 72 polymorphism is the most extensively studied variant; however, so far only two studies have investigated the association of this polymorphism with thyroid cancer. Thus, we aimed to investigate the association of *p53* codon 72 polymorphism with thyroid cancer in a group of Turkish patients.

Material and Methods: 58 thyroid cancer patients and 115 healthy individuals were genotyped by PCR-based RFLP analysis.

Results: Polymorphic proline allele frequency was higher in patients than in the controls. Pro/pro genotype was found to be a risk factor for thyroid cancer development. Arginine/proline genotype frequencies in patients and controls did not show any significant differences. There was no statistical difference between follicular adenoma and papillary carcinoma patients or TNM stages of papillary carcinoma patients for codon 72 genotypes.

Discussion: Further studies involving codon 72 polymorphism of the *p53* gene along with other susceptibility factors in different populations may be useful to clarify the molecular mechanisms underlying thyroid cancer development.

Key words: Thyroid cancer, *p53* codon 72, polymorphism

TİROİD KANSERLİ TÜRK HASTALARDA P53 KODON 72 POLİMORFİZMİ

ÖZET

Amaç: Tiroid kanseri en sık görülen endokrin malignitedir. Hastalığa yol açan moleküler mekanizmalar çeşitlilik göstermektedir. *p53* geninin polimorfik varyantları ile çeşitli insan kanserleri arasındaki ilişki gösterilmiştir. Bunlar arasında kodon 72 en sık çalışılan varyanttır ancak tiroid kanseri ile ilgili sadece 2 çalışma mevcuttur. Bu çalışmada Türk hastalarda *p53* kodon 72 polimorfizmi ile tiroid kanseri arasındaki ilişkinin araştırılması amaçlanmıştır.

Materyal ve Metot: 58 tiroid kanser hastasının ve 115 sağlıklı bireyin genotip analizi PCR-RFLP yöntemi ile yapılmıştır.

Sonuçlar: Polimorfik prolin allel sıklığı hastalarda kontrollerden daha yüksektir. Pro/Pro genotipi tiroid kanser gelişimi için bir risk faktörü olarak bulunmuştur. Arg/pro genotip sıklığı açısından hasta ve kontrol grupları arasında farklılık bulunmamıştır. Foliküler adenom ve papiller karsinom hastaları veya papiller karsinom hastalarının TNM evrelendirmeleri açısından kodon 72 genotipleri açısından bir fark bulunmamıştır.

Tartışma: Tiroid kanser gelişimine neden olan moleküler mekanizmaların aydınlatılabilmesi için farklı populasyonlarda kodon 72 polimorfizmi ile birlikte diğer genetik faktörlerin de çalışılmasına gereksinim vardır.

Anahtar kelimeler: Tiroid kanseri, *p53* kodon 72, polimorfizm

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INTRODUCTION

Tumors of the thyroid gland represent a variety of lesions from well-differentiated benign tumors to anaplastic malignant cancer. Approximately less than 5-10% of hyperfunctioning thyroid nodules develop thyroid cancer and the prevalence of these nodules is estimated to be 5 to more than 20% in humans¹. In the United States, it is reported that approximately 1% of all malignancies are thyroid tumors. Several genetic factors have been associated with thyroid cancer such as p53, RET, BRAF, p21, Rb². Of note, the association of mutations and polymorphisms of p53 have been reported in a variety of human tumors.

Although several polymorphisms have been identified in both coding and non-coding regions, only two of them were shown to alter the amino acid sequence of p53. One of these variants is a proline (pro) to serine change at codon 47 and the other is an arginine (arg) to proline change at codon 72. The latter is located in the proline-rich region of the protein and may affect the structure of the putative SH3-binding domain³. p53 codon 72 polymorphism have been studied in several types of cancers; however, the results are controversial. Although codon 72 pro/pro genotype is associated with an elevated risk of lung cancer^{4,5} other studies have not confirmed such an association in the same malignancy^{6,7}. A similar controversy is observed in breast cancer and several different tumor types^{8,9}, most likely due to ethnic differences of the populations studied.

To date, only two studies have investigated the association of codon 72 polymorphism in thyroid tumors. Botze et al.¹⁰ have examined Caucasian thyroid carcinoma patients and concluded that the presence of the proline variant was a potential risk factor for the induction of thyroid carcinoma and was also associated with a relatively more poor prognosis. Consistent with this report, Granja et al.¹¹ showed an increased risk for pro/pro genotype for thyroid cancer in the Brazilian population.

The aim of this study was to investigate the association of p53 codon 72 polymorphism

among a group of Turkish patients with thyroid cancer by performing genotyping in 58 patients and 115 healthy controls by a PCR-based RFLP analysis.

PATIENTS AND METHODS

Tissue specimens and DNA extraction

Paraffin-embedded tissue specimens were selected from 58 thyroid cancer patients. Briefly, 6 µm thick sections were cut from blocks that had been selected for maximal tumor content. Total DNA extraction was performed by using a DNA isolation kit according to the manufacturer's instructions (MagneSil genomic, fixed tissue system, Promega, USA). Isolated DNA was aliquoted and stored at -20°C. DNA from the peripheral leucocytes of 115 healthy controls was also extracted by proteinase K digestion followed by phenol/chloroform extraction as previously described by John et al.¹². The study protocol was approved by the Research Council and Ethics Committee of Gulhane Military Medical School.

Genotype analysis

DNA samples were analyzed for p53 codon 72 polymorphism using PCR-based RFLP analysis. First, a 296 bp fragment was amplified using forward (5'-ATCTACAGTCCCCCTTGCCG-3) and reverse (5'-GCAACTGACCGTGCAAGTCA-3) primers. The 50 µl PCR mixture contained 0.1 µg genomic DNA, 0.6 U Taq DNA polymerase, 10 pmol of each primer, 200 µM of each dNTPs, and 1.5 mM MgCl₂. PCR products were checked by 2% agarose gel electrophoresis and then subjected to restriction enzyme digestion. Briefly, 10 µl of PCR product was mixed with 10 U of Bsh1236I restriction enzyme (Fermentas, Lithuania) in 1x buffer containing 10 mM tris, 10 mM MgCl₂, 100 mM KCl, 0.1 mg/ml BSA pH 8.5. The samples were incubated in 37 °C for 19 hours to ensure complete digestion. Digestion products were visualized under UV transilluminator after they were separated in 4% agarose gel electrophoresis containing ethidium bromide. The presence of wild-type arginine allele was indicated by bands of 169 and 127 bp, whereas no digestion was



observed in the case of the polymorphic proline allele.

Data comparison was made by chi-square test. A p value <0.05 was considered as statistically significant.

RESULTS

A total of 58 thyroid cancer patients consisting of 36 males and 22 females with the mean age of 40.01 years were retrospectively enrolled into the study. The patient group represented a mixed population of different malignant and benign thyroid tumors including 26 papillary carcinomas, 23 follicular adenomas, 4 follicular carcinomas, 2 medullar carcinomas, 2 papillary carcinomas with Hashimoto's thyroiditis and one anaplastic carcinoma. Clinical data such as tumor size, metastasis and lymph nodes were available for only 16 follicular adenomas and 18 papillary cancers. One hundred and fifteen healthy individuals consisting of 31 males and 84 females were also investigated for codon 72 polymorphism and were studied as the control group (mean age= 55.6 years).

The allele and genotype frequencies of patients and controls are shown in Table I. Of note, proline allele frequency is significantly higher in patients than in the controls and appears to be a risk factor for thyroid cancer ($p<0.05$) (Odds ratio=0.523, 95% CI:0.331-0.828). Comparison of genotype frequencies of patients and controls indicates that pro/pro genotype is significantly higher in patients ($p<0.05$) suggesting a risk factor for thyroid cancer (Odds ratio= 0.330, 95% CI:0.135-0.807). On the other hand, arg/pro genotype frequencies in both groups did not show any significant differences, implicating that the presence of the arginine variant may have a protective effect against carcinogenesis.

Sixteen follicular adenoma and 18 papillary carcinoma patients with clinical data were further evaluated. The genotype frequencies of 16 follicular adenoma patients were 31.25 % (5/16) Arg/Arg, 62.5 % (10/16) Arg/Pro and 6.25 % (1/16) Pro/Pro. The genotype frequencies of 18 papillary carcinoma patients were 20.8 % (5/18) Arg/Arg, 50 % (9/18) Arg/Pro and 22.2 % (4/18) Pro/Pro. Although homozygosity for proline in patients with papillary carcinoma seemed to be higher than in the adenoma group, there was no significant difference between follicular adenoma and papillary carcinoma patients for p53 codon 72 variants ($p>0.05$). The TNM stages of the papillary carcinoma patients were determined according to the American Joint Committee on Cancer (AJCC)¹³. If the tumor size was 2 cm or less in greatest dimension limited to the thyroid, it was considered as T1 and if the tumor size was more than 2 cm but not more than 4 cm, it was considered as T2. Six out of 18 papillary carcinomas were T2 stage while the rest were T1 stage. Fourteen out of 18 papillary carcinoma patients had no lymph node metastasis (N0), while 4 patients had lymph node metastases with different degrees (2 N1, 1 N1a and 1 N1b). None of the papillary carcinoma patients had distant metastasis (M0). The percentages of Arg/Arg, Arg/Pro, Pro/Pro genotypes for T1 and T2 stage patients were 16.7% (2/12), 58.3% (7/12), 25% (3/12) and 50% (3/6), 33.3 % (2/6), 16.7 %, respectively. The genotype frequencies of patients with no lymph node metastasis were 21.4% (3/14) Arg/Arg, 64.3% (9/14) Arg/Pro and 14.3% (2/14) Pro/Pro. No significant difference was found between The TNM of the papillary carcinoma patients ($p>0.05$).

Table I: p53 codon 72 genotype and allele frequencies

Alleles Groups	Genotype frequency			Allele frequency	
	Arg/Arg	Arg/Pro	Pro/Pro	Arg	Pro
Patients (n=58)	0.293	0.483	0.224*	0.534	0.466**
Controls (n=115)	0.461	0.452	0.087*	0.687	0.313**



DISCUSSION

Variation at codon 72 that leads to an adenine to proline substitution is one of the most frequent single nucleotide polymorphisms (SNP) of p53, and has been reported to alter the primary structure of the protein¹⁴. The arginine variant was reported to be more efficient than the proline variant at inducing apoptosis due to the varied tendency of protein localization in the mitochondria¹⁵. On the other hand, Marin et al.¹⁶ reported that the proline variant is more effective for inducing G1 arrest than the other variant, probably due to altered binding affinity to p73.

p53 codon 72 polymorphism has been investigated in a variety of human tumors including breast, colorectal, esophageal, and lung^{4,8,9,17,18}. Of note, only two previous studies have investigated the association of this polymorphism with thyroid cancer^{10,11}. Both studies have reported an increased risk of thyroid cancer development in the presence of proline allele.

In the present study, we found that allele and genotype frequencies of the control group for codon 72 of p53 are very similar to NCBI SNP database records for The European population (RefSNP ID: rs1042522), and also consistent with reports of Boltze et al.¹⁰ and Granja et al.¹¹. In conjunction with these two previous studies, we found that proline allele is a risk factor for thyroid cancer. Moreover, in consistence with Granja et al.¹¹ only pro/pro genotype was a risk factor for both benign and malignant thyroid cancers. On the other hand, no significant differences were found between the stage of the tumor and p53 codon 72 status. In contrast with the report of Boltze et al.¹⁰, we found that the prevalence of the homozygote proline allele in either carcinoma or adenoma groups did not differ significantly. These differences among different studies may be due to geographical-ethnic variations in the studied populations.

In conclusion, screening for p53 codon 72 polymorphisms along with other susceptibility factors may be useful for determining the tendency of thyroid cancer development. Besides, further studies

involving other common polymorphisms of p53 together with codon 72, and a haplotype analysis may be more informative for delineating the association of p53 polymorphisms with thyroid cancer.

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