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L-MYC GENE POLYMORPHISM AND RISK OF THYROID CANCER

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***L-myc* gene polymorphism is a representative genetic trait responsible for an individual's susceptibility to several cancers. However, there have been no reports concerning the association between thyroid cancer and *L-myc* gene polymorphism. *Aim:* To analyze the distribution of *L-myc* gene polymorphism in Turkish patients with thyroid disorders and thyroid cancers. *Methods:* We used a molecular genotyping method, polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP). We studied 138 patients of whom 47 had multinodular goiter, 13 had follicular cancer and 69 had papillar cancer, in comparison with control group of 109 healthy individuals. *Results:* No significant difference in the distribution of genotypes was observed between thyroid patients and controls. Carrying SS or LS genotype revealed a 1.96-fold (95% CI 0.573–6.706) risk for the occurrence of follicular cancer when compared with controls, and 3.11-fold (95% CI 0.952–10.216), when compared with multinodular goiter patients ($p = 0.04$). *Conclusion:* We suggest that L-myc genotype profiling together with other susceptibility factors, may be useful in the screening for thyroid nodular malignancy.**

Key Words: **allele, cancer, *L-myc*, polymorphism, thyroid gland.**

L-myc gene belonging to the myc family is an oncogene, localized to chromosome 1p34 and thought to be activated during late tumorigenesis [1, 2]. Since the cloning of *L-myc* gene in 1985, enormous amount of research has been conducted to help elucidate the role of this gene in human malignancy [3]. A possible role of the myc oncogene in the neoplastic transformation of the human thyroid gland has been investigated. Some researchers suggest that myc-oncogene alterations might be involved in malign transformation of the human thyroids [4, 5]. The non-coding variation in the second intron of the *L-myc* gene, generating an EcoRI polymorphism, is associated with increased risk of several tumor types, although controversial results have been reported [6, 7]. Some of the results suggested that *L-myc* gene polymorphism had some influence on the susceptibility to cancer. However, no study on the relevance of *L-myc* gene polymorphism to thyroid carcinogenesis has yet been reported. Identification of susceptibility factors that predispose individuals to thyroid cancer could possibly give further insight into the etiology of this malignancy. In this study, we have tried to establish a relationship between differentiated thyroid cancers and *L-myc* gene polymorphism. With this aim, we analyzed the *L-myc* gene status of differentiated thyroid cancer patients, multinodular goiter patients and healthy people for a comparison.

MATERIALS AND METHODS

Material. Blood samples from patients operated for nodular (multinodular) euthyroid goiter in Istanbul Medical Faculty, Haseki Educational Hospital and Cerrahpaşa Medical Faculty in 2003–2005 were collected and used. The study population consisted of 47 patients with benign thyroid nodules including only goiter patients and 82 patients with malignant nodules, including 69 cases of papillary carcinoma (PC) and 13 cases of follicular carcinoma (FC). The diagnosis of differentiated thyroid carcinoma (papillary and follicular carcinomas) and multinodular goiter patients was made by the pathological analysis of thyroid specimens after surgery. All patients underwent total or subtotal (for a few benign goiter patients) thyroidectomy. None of the patients had a history of accidental or medical radiation exposure. Control group included patients of the general surgery and orthopedic clinics of the same hospital, which were being treated for non-neoplastic diseases such as inguinal hernia or trauma. All data, including pathological diagnoses, age, gender and surgical findings were recorded. Subjects reporting cigarette smoking still in the year prior to examination were determined. Demographic data of patients and controls were shown in Table 1. Blood samples were collected from all patients preoperatively and *L-myc* gene polymorphism was performed after obtaining the result of pathological analysis.

Table 1. Demographic data of our study groups

Study groups	Males n (%)	Females n (%)	Total	Mean age (years)	Smoking history (+) n (%)
Controls	31 (32.6)	64 (67.4)	95	41.2	30 (31.6)
Follicular carcinomas	3 (23.1)	10 (76.9)	13	42.2	2 (15.4)
Papillary carcinomas	19 (27.5)	50 (72.5)	69	34.8	2 (2.9)
Multinodular goiter	9 (19.1)	38 (80.9)	47	40.8	1 (2.1)

Note. n = number of cases.

Isolation of DNA. Blood specimens from all subjects were collected into tubes containing EDTA. DNA was isolated from the blood leukocytes in 10 ml EDTA by the method of Miller *et al*. based on sodium

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Abbreviations used: FC – follicular carcinoma; PC – papillary carcinoma; PCR-RFLP – polymerase chain reaction-based restriction fragment length polymorphism

dodecyl sulphate lysis, ammonium acetate extraction and ethanol precipitation [8].

Polymerase Chain Reaction (PCR) for L-myc Oncogene. Template DNA (0.5–1.0 μg) was used in a PCR under sterile conditions. 100 ng of primer was used for the reaction — the forward primer was 5'-AGT-TCA-CTC-ACA-GGC-CAC-AT-3' and the reverse primer was 5'-TGC-ATA-TCA-GGA-AGC-TTG-AG-3' in a volume of 50 μl containing 3 mM $MgCl_2$, 50 mM KCl 10, mM Tris HCl (pH 8.4) 0.5 mM of each dNTP (MBI Fermentas) and 1 unit of Taq DNA Polymerase (MBI Fermentas). Amplification was performed in a DNA thermal cycler (MBI Fermentas)for 30 cycles with denaturation steps at 94 °C for 30 s, annealing at 50 °C for 1 min and extension at 74 °C for 1 min. The PCR product exhibited a 267 base pair fragment. Amplification fragment was digested with 5 units EcoRI (MBI Fermentas) at 37 °C for 1 h [9]. The digested DNA fragments were separated by gel electrophoresis on a 2% agarose gel in 1 x Tris Borate EDTA buffer and DNA visualized by ethidium bromide staining. The responsible L-myc RFLP alleles were identified in each sample .The three genotypes were the LL homozygote appearing as 267-base pair (bp) fragment, the LS heterozygote with 267, 142, and 125-bp fragments and the SS homozygote with 142 and 125-base pair fragments (Figure).

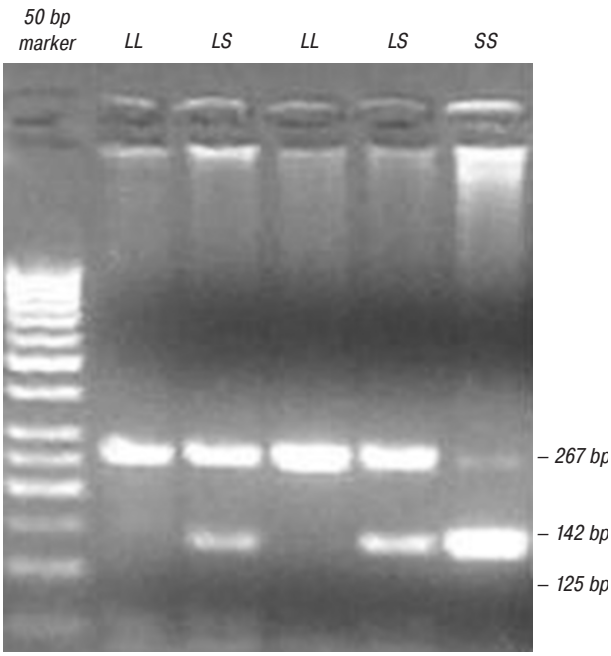

Figure. Direct visualization of PCR products by ethidium bromide staining. A 267-base pair L-myc fragment was amplified, cleaved with Eco RI and electrophoresed on a 2% agarose gel. Results for five representative tyroid cancer patients are shown. Molecular weight markers are at the left and the uncut 267 base pair L-allele, and 142 and 125 base pair S allele are designated at the right

Statistical Analysis. Statistical analyses were performed using the SPSS version 7.5 including the Chi Square (χ^2) test and Yate's correction for genotype and allele frequencies composition. Odds ratios and 95% confidence intervals were calculated for all three genotypes.

RESULTS

The number of females was higher in our thyroid patients than in control groups. However, we were not able to find any possible correlation between genotype and possible risk factors like age, gender and smoking habit. Histologic classification of thyroid tumors was as follows: 53.5% papillary tumors (69 patients), 10.1% follicular tumors (13 patients), 36.4% multinodular goiter (47 patients). Table 2 summarizes the data of the overall proportion of the L-myc genotypes in control population and in patients with benign and malignant thyroid diseases.

Table 2. Comparison between the different L-myc genotypes in the control population and in the benign and malignant thyroid disease patients

Genotypes	Controls, n (%)	Multinodular goiter, n (%)	Papillar cancer, n (%)	Follicular cancer, n (%)
LL	37 (38.9%)	24 (51.1%)	28 (40.6%)	3 (23.1%)
LS	47 (49.5%)	15 (31.9%)	30 (43.5%)	10 (76.9%)
SS	11 (11.6%)	8 (17.01%)	11 (15.9%)	0 (0%)
Total of cases	95	47	69	13
LL	37 (38.9%)	26 (55.3%)	28 (40.6%)	3 (23.1%)
LS + SS	58 (61.1%)	21 (44.7%)	41 (59.4%)	10 (76.9%)

Note. n = number of cases.

There was no statistically significant difference found between various types of thyroid disorders in regard to *L-myc* gene polymorphism expect in allele frequencies among patients with benign and malign nodules. The L-myc S allele tended to be more frequent among the follicular thyroid cancer patients (frequency 76.9%) than among controls (61.1%) but without reaching a significant level. In our follicular thyroid cancer patients, the frequency of L-myc S allele was higher than it was in multinodular goiter patients and the difference is statistically significant ($\chi^2 = 4.23$, $p = 0.04$). Patients with S allele (SS or LS genotypes) showed a 1.96-fold (95% CI 0.573–6.706) risk for follicular thyroid cancer when compared with controls, and 3.11-fold (95% CI 0.952–10.216; $p = 0.04$) when compared with multinodular goiter patients.

DISCUSSION

L-myc is one of the three major members of the myc proto-oncogene family. These genes encode proteins that play distinct but overlapping roles in a wide range of normal and aberrant cellular processes including cell proliferation, differentiation, apoptosis and tumorigenesis [10, 11]. L-myc has been much less intensively studied than the other two members of the myc gene family, c-myc or N-myc. No previous studies have reported genetic polymorphism of *L-myc* gene associated with thyroid carcinomas. It is known that mutations of genes involved in the control of cellular growth and/or differentiation, such as c-myc, affect the development of thyroid neoplasms [4, 5]. The frequency of females was significantly higher in the study population, as well as in the groups of papillary and follicular cancer, which is in agreement with the gender distribution usually observed in this type of cancers [12]. Burman *et al.* showed that c-myc oncogene expression is comparable in normal thyrocytes and in thyroid nodules or thyroid cancer samples [13]. Those findings support a role for c-myc in both normal and neoplastic thyrocyte growth. On the other

hand, a study by Bieche *et al.* reported that none of the neoplastic thyroid samples overexpressed myc oncogene [14].

The L-myc EcoRI polymorphism is a non-coding variation in the second intron of the *L-myc* gene resulting in S and L alleles. Individuals carrying the S allele tend to have poor prognosis and increased risk of several tumor types, although controversial results have been reported [6]. Since the first study of the L-myc RFLP in Japanese lung cancer patients reported in 1988, many researchers have studied the L-myc RFLP types in different cases of lung cancer and other malignant tumors. Some of these studies have shown a positive relationship between L-myc genotypes and susceptibility to some types of cancer [15–17], but other studies found no relationship between L-myc genotypes and susceptibility to the same and other types of cancer [7, 18]. These results suggest that further studies are required to clarify the relationship between L-myc genotypes and susceptibility to cancer [19]. The present study indicated that there was no significant difference in the distribution of L-myc RFLP between patients with thyroid cancer and control group. The only positive finding observed was for follicular thyroid cancer patients classified into two genotype groups "LS type plus SS genotype" and "LL genotype", where the "LS plus SS genotype" group, which showed a higher value of relative risk, was thought to be a higher risk group compared with the "LL genotype" group. We showed that having S allele (SS or LS genotype) caused a 1.96-fold risk of follicular thyroid cancer when compared with controls, which was 3.11-fold risk on comparison with multinodular goiter patients.

Although clinical and epidemiological features are helpful, there are still no accepted serological markers that can help identify patients at risk for malignancy among individuals with thyroid nodules [20, 21]. The association of *L-myc* gene polymorphism with cancer susceptibility has produced conflicting results. This may have been due to racial/ethnic differences and methodological variations in the studies, such as control selection and case stratification [22]. In our female patients, papillary and follicular carcinomas were two to four times more frequent than men, as supported by the previous literature [12], particularly in reproductive years. This has lead to the hypothesis that female hormones or X chromosomal genes might be involved in the etiology or pathogenesis of the disease [23]. This hypothesis is supported by data of molecular genetic analysis of thyroid cancers, which also suggest the existence of different molecular mechanisms leading to papillary and follicular thyroid cancers [24, 25]. Papillary and follicular tumors should be examined separately for the identification of putative risk genotypes for thyroid cancer. Conversely, when considered alone, polymorphic *L-myc* gene is not strongly associated with thyroid cancer, as has been observed for other types of cancer. We suggest that L-myc genotype profiling together with other susceptibility factors, may be useful in the screening for thyroid nodule malignancy.

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ПОЛИМОРФИЗМ ГЕНА *L-MYC* И РИСК РАЗВИТИЯ РАКА ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Для ряда опухолей человека показана корреляция между риском развития опухоли и определенным вариантом гена *L-MYC*. Данные о наличии такой связи при раке щитовидной железы к настоящему времени отсутствуют. *Цель:* проанализировать распределение полиморфных типов гена *L-MYC* в популяции больных с доброкачественными и злокачественными поражениями щитовидной железы, включая рак щитовидной железы, в Турции. *Методы:* для анализа полиморфизма гена *L-MYC* использован метод молекулярного генотипирования, в частности, метод определения полиморфизма длины рестрикционных фрагментов, основанный на полимеразной цепной реакции (PCR-RFLP). Определение проводили в лейкоцитах 138 больных, в том числе 48 больных с узловым зобом, 13 больных фолликулярным раком щитовидной железы и 69 больных папиллярным раком. Контрольную группу составляли 109 здоровых лиц. *Результаты:* статистически достоверных различий в распределении исследуемых генотипов у больных с патологией щитовидной железы и здоровых лиц не выявили. Показано, что относительный риск фолликулярного рака щитовидной железы у больных-носителей генотипа SS или LS составляет 1,96 по сравнению со здоровыми лицами (при 95% доверительном интервале от 0,573 до 6,706) и 3,11 по сравнению с больными с узловым зобом (при 95% доверительном интервале от 0,952 до 10,216) (p = 0,04). *Выводы:* по нашему предположению, определение профиля полиморфизма гена *L-MYC* с учетом других факторов, определяющих предрасположенность к развитию опухолей, может быть полезным при скрининге озлокачествления узелковых образований щитовидной железы.

***Ключевые слова*: аллель, рак, *L-myc*, полиморфизм, щитовидная железа.**

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