

L-myc Polymorphism in Head and Neck Nonmelanoma Skin and Lower Lip Cancers

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Objective: To evaluate the presence of L-myc gene variations as a genetic predisposition to head and neck non-melanoma skin cancer (HNNMSC) and lower lip cancer (LLC).

Design: A case-control study.

Setting: An academic institute laboratory.

Participants: Twenty-four patients with HNNMSC and 27 with LLC were compared with 51 age- and sex-matched control subjects.

Main Outcome Measures: Polymerase chain reaction restriction fragment length polymorphism and aga-

rose gel electrophoresis were used to determine the L-myc oncogene genotypes.

Results: The presence of the LS genotype was found to be significantly increased in the study group, whereas the LL genotype was not detected. The S allele was also more frequent in the study group. The SS genotype was found to correlate with aggressive tumor behavior in patients with HNNMSC and a family history of cancer. Patients with LLC displayed significantly less of the SS genotype.

Conclusions: The L-myc gene polymorphism may help detect and prevent HNNMSC and LLC in susceptible individuals. It may also contribute to estimation of tumor behavior in patients with HNNMSC.

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CANCER OF THE SKIN IS THE most common form of malignant disease, and the skin of the head and neck is the site most frequently involved. The number of basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) is believed to be rising at a rate of 5% per year.¹ Basal cell carcinoma is the most common type of skin cancer. Lip cancer is a form of oral cancer at the junction between the oral cavity and the skin. The lips are the most common site of cancer in the oral cavity, and the lower lip is more frequently affected than the upper lip. Cancers arising from the vermilion of the lip can be considered to be a unique group of tumors because they derive from a modified and external mucosal tissue that is exposed to different environmental factors than other sites of the oral cavity.² Squamous cell carcinoma is the most common malignant neoplasm of the lower lip.

The risk of BCC and SCC is associated mainly with long-term exposure to UV radiation but also with geographic location, race, immune status, and other as-yet-undetermined genetic factors. Early detection of high-risk populations for prevention of disease and estimation of biological tumor behavior for planning treatment favor the need for molecular studies of the genetic changes in head and neck nonmela-

noma skin cancer (HNNMSC) and lower lip cancer (LLC). Proto-oncogenes are normal cellular genes involved in the regulation of cellular proliferation that lead to neoplastic cell proliferation when they have mutations or are overexpressed. They mostly display a very broad tumor spectrum, whereas some tend to be activated primarily in certain cancer types. The *myc* family of oncogenes, which includes *c-myc*, *N-myc*, and *L-myc*, has been proved to be amplified late in the progression of many human tumors involving head and neck cancers and nonmelanoma skin cancers and is generally associated with an aggressively malignant phenotype.³⁻⁹

Since cloning of the L-myc gene (Genbank M19720) in 1985,³ many studies have investigated the possible role of the L-myc gene in various cancers. The L-myc protein is involved in the tissue-specific regulation of cell growth, and alterations in the expression of L-myc may participate in malignant transformation.^{3,4} The L-myc *EcoRI* polymorphism is a noncoding variation in the second intron of the L-myc gene, resulting in short (S) and long (L) alleles. It is the first genetic variation found to be associated with prognosis in cancer.⁵ Individuals carrying the S allele tend to have a poor prognosis and increased risk of several tumor types, although controversial results have been re-

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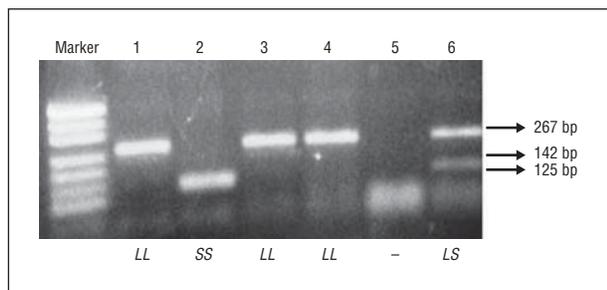


Figure. Direct visualization of polymerase chain reaction products by means of ethidium bromide staining. A 267–base pair (bp) *L-myc* fragment was amplified, cleaved with *EcoRI*, and electrophoresed on 2% agarose gel. Results of 5 representative control subjects are indicated. Lanes 1, 3, and 4 show the *LL* homozygote; lane 2, the *SS* homozygote; lane 5, no polymerase chain reaction product; and lane 6, the *LS* heterozygote. The 3 genotypes were the *LL* homozygote, appearing as a 267-bp fragment; the *LS* heterozygote, with 267-, 142-, and 125-bp fragments; and the *SS* homozygote, with 142- and 125-bp fragments.

Table 1. Distribution of *L-myc* Genotypes

	Participants, No. (%)			P Value
	<i>LL</i>	<i>LS</i>	<i>SS</i>	
Control group (n=51)	20 (39)	20 (39)	11 (22)	.001
Study group (n=51)	0	41 (80)	10 (20)	

ported.¹⁰ The literature includes no studies, to our knowledge, regarding the relation of the *L-myc* polymorphism with either HNNMSC or LLC. We aimed to evaluate them together because of their anatomical and etiologic proximity.

METHODS

Thirty-six men and 15 women aged 26 to 85 years (mean, 60.92 years) were included in the study. They were selected from among previously untreated patients with biopsy-confirmed head and neck skin cancer (n=24) and patients with LLC (n=27) who underwent surgery in the Plastic and Reconstructive Surgery Department at Vakif Gureba Research and Education Hospital between January 2, 2005, and April 30, 2006. The skin cancer subgroup included 10 patients with BCC, and all the other patients were diagnosed as having SCC. Fifty-one age- and sex-matched control subjects without any disease history were included in the study. Two standardized questionnaires were administered for each subgroup to record the clinical characteristics of patients. The institutional review board of Vakif Gureba Research and Education Hospital approved the study, and informed consent was obtained from all the participants.

Skin tumors were classified as clinically aggressive if they exceeded 2 cm in 1 surface dimension; invaded muscle, bone, or cartilage; or were metastatic to lymph nodes. Lower lip tumors were classified in accordance with the TNM staging system.¹¹ All the frozen or formalin-fixed tumor tissue samples were analyzed by the same pathologist.

Venous blood samples were collected in tubes containing EDTA. DNA was isolated from blood leukocytes in 10 mL of EDTA using the method of Miller et al.¹² Template DNA (0.5–1.0 µg) was used in a polymerase chain reaction under sterile conditions. One hundred nanograms 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-

Table 2. Distribution of Allelic Frequencies

	Participants, No. (%)		P Value	Relative Risk (95% Confidence Interval)
	<i>L</i>	<i>S</i>		
Control group (n=102)	60 (59)	42 (41)	.008	2.12 (1.21-3.71)
Study group (n=102)	41 (40)	61 (60)		

TCA-GGA-AGC-TTG-AG-3' in a volume of 50 µL containing 3mM magnesium chloride, 50mM potassium chloride, 10mM Tris hydrochloride (pH, 8.4), 0.5mM of each deoxynucleotide triphosphate (MBI Fermentas, Burlington, Ontario, Canada), and 1 U of *Taq* polymerase (MBI Fermentas). Amplification was performed using a DNA thermal cycler (MBI Fermentas) for 30 cycles, with denaturation steps at 94°C for 30 seconds, annealing at 50°C for 1 minute, and extension at 74°C for 1 minute. The polymerase chain reaction product exhibited a 267–base pair (bp) fragment. The amplification fragment was digested with 5 U of *EcoRI* (MBI Fermentas) at 37°C for 1 hour. The digested DNA fragments were separated by means of gel electrophoresis on 2% agarose gel in 1X Tris borate EDTA buffer and DNA visualized by means of ethidium bromide staining. The responsible *L-myc* restriction fragment length polymorphism alleles were identified in each sample.

Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, Illinois). Descriptive statistical methods (mean [SD]) and quantitative variables were compared using the 2-tailed *t* test. The χ^2 and Fischer exact tests were used to compare qualitative variables, and *P* < .05 was considered statistically significant.

RESULTS

The polymorphic *L-myc* locus was analyzed by means of polymerase chain reaction–restriction fragment length polymorphism for 51 patients and 51 control subjects. The 3 genotypes revealed were the *LL* homozygote, appearing as a 267-bp fragment; the *LS* heterozygote, with 267-, 142-, and 125-bp fragments; and the *SS* homozygote, with 142- and 125-bp fragments (**Figure**).

The presence of the *LS* genotype in the study group (41 patients [80%]) was found to be higher than that in the control group (20 patients [39%]). The *LL* genotype was present in 20 patients in the control group [39%], whereas it was not detected in the study group (**Table 1**). The difference in genotypic distribution between the groups was significant (*P* = .001). Allelic frequencies also displayed significant differences between them: the *S* allele was more frequent in the study group (**Table 2**). When tumor (LLC and HNNMSC) and histopathologic (SCC and BCC) subgroups were compared with the control group separately, the *LS* genotype dominance was significant in each subgroup (*P* < .01 for the LLC, HNNMSC, and SCC subgroups and *P* = .05 for the BCC subgroup). The frequency of the *SS* genotype in the LLC subgroup was significantly lower (*P* < .01) (**Table 3**), and *S* allele frequency in the HNNMSC, BCC, and SCC subgroups was significantly higher than the frequencies in the control group (HNNMSC: odds ratio [OR], 2.85; 95% confidence interval [CI], 1.3–5.8; *P* < .01; BCC: OR, 3.33; 95% CI, 1.1–9.3; *P* < .05; and SCC: OR, 1.91; 95% CI, 1.0–3.4; *P* < .05).

Table 3. Tumor-Specific Distribution of L-myc Genotypes

	Participants, No. (%)			P Value
	LL	LS	SS	
Control group (n=51)	20 (39)	20 (39)	11 (22)	.001
LLC subgroup (n=27)	0	25 (93)	2 (7)	

Abbreviation: LLC, lower lip cancer.

Of the clinical variables in the study group, family history of cancer (any cancer in first- and second-degree relatives), tumor subgroup, and tumor behavior were significant: the percentage of the SS genotype in patients with a positive family history (6 patients [50%]) was higher than the percentage in those without it (4 [10%]) (OR, 8.75; 95% CI, 1.8-40.5; $P=.002$) (**Table 4**). Patients with HNNMSC displayed a higher SS genotype presence than patients with LLC: 8 patients (33%) vs 2 (7%) (OR, 6.25; 95% CI, 1.1-33.2; $P=.02$). The frequencies of LS and SS genotypes in patients with HNNMSC and aggressive tumor behavior were found to be 53% (9 patients) and 47% (8 patients), respectively, whereas those with nonaggressive behavior displayed only the LS genotype ($P=.05$).

Although no significant correlation between the other clinical variables and the genotypic distribution was detected, SS genotype presence was considerably higher in smokers (9 patients [26%]) than in nonsmokers (1 [6%]) and in patients with BCC (4 patients [40%]) compared with patients with SCC (6 [15%]).

COMMENT

Since the first article regarding Japanese patients with lung cancer in 1988,⁵ the association of the L-myc polymorphism with cancer susceptibility and prognosis has produced conflicting results that may have been due to ethnic differences and methodological variations. The SS and LS genotypes were found to be associated with increased susceptibility to breast, gastric, and esophageal cancers and soft-tissue sarcoma, whereas no association was detected in lung, renal, oral, hepatic, and bladder cancers; neuroblastoma; and non-Hodgkin lymphoma.¹⁰

The heterozygous LS genotype abundance in accordance with increased S allele frequency in the study group confirmed the relation of the L-myc polymorphism with both cancers. This finding reveals that the L-myc polymorphism may serve as a reliable genetic marker to identify high-risk individuals for both cancers, at least in the present population. This has profound implications for the prevention and early diagnosis of cancer. It may also be promising for investigating its role in organ transplant recipients with a higher skin cancer incidence.

Despite values as low as 8 of 61 patients with gastric cancer (13%) for the LL genotype¹³ and 1 of 21 patients with hepatocellular cancer (5%) for the SS genotype,¹⁴ their absence was not reported previously. This unexpected finding in the present study group regarding the LL genotype may be due to the small sample size and needs to be clarified with further studies.

Table 4. Distribution of L-myc Genotypes in the Study Group According to Clinical Variables

	Patients, No. (%)		P Value	Relative Risk (95% CI) ^a
	LS	SS		
Sex				
Female	11 (73)	4 (27)	.41	...
Male	30 (83)	6 (17)		
Smoking				
Ever	25 (74)	9 (26)	.08	...
Never	16 (94)	1 (6)		
Sun exposure				
Yes ^b	27 (77)	8 (23)	.38	...
No	14 (88)	2 (12)		
Family history of cancer ^c				
Positive	6 (50)	6 (50)	.002	8.75 (1.88-40.53)
Negative	35 (90)	4 (10)		
Histopathologic group				
BCC	6 (60)	4 (40)	.07	...
SCC	35 (85)	6 (15)		
Tumor subgroup				
HNNMSC	16 (68)	8 (33)	.02	6.25 (1.17-33.25)
LLC	25 (93)	2 (7)		
Tumor behavior for HNNMSC subgroup				
Aggressive ^d	9 (53)	8 (47)	.05	...
Nonaggressive	7 (100)	0		
Stage for LLC subgroup				
Early ^f	14 (100)	0	.22	...
Advanced ^g	11 (85)	2 (15)		

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; HNNMSC, head and neck nonmelanoma skin cancer; LLC, lower lip cancer; SCC, squamous cell carcinoma; ellipses, not calculated.

^aRelative risk and 95% CI were calculated for statistically significant comparisons unless otherwise stated.

^bOccupational exposure or less sun-protective behavior.

^cAny cancer in first- and second-degree relatives.

^dTumors exceeded 2 cm, invaded deep structures, or were metastatic to lymph nodes.

^eThe relative risk and 95% CI could not be calculated because the SS genotype was found to be nil in the nonaggressive subgroup.

^fTNM stages I and II.

^gTNM stages III and IV.

The SS genotype displayed a significant correlation with aggressive behavior of HNNMSC in this study. In the meta-analysis of previous studies of the L-myc EcoRI polymorphism,¹⁰ the SS genotype was significantly associated with prognosis (ie, lymph node metastasis, distant metastasis, and stage) in lung cancer, whereas the heterozygous LS genotype showed intermediate risk. Combined analysis of these genotypes for other cancers (ie, bladder, oral, and renal cancers and glioma) revealed a significant association with tumor recurrence only. When all types of cancer were examined together, the SS genotype was associated with lymph node metastasis, distant metastasis, clinical stage, and cancer risk. The ability to estimate aggressive tumor behavior would enable physicians to design more precise treatment algorithms in patients with HNNMSC.

The significantly lower presentation of the SS genotype in patients with LLC compared with the control group and the HNNMSC subgroup was distinctive. Considering similar results available in hepatocellular can-

cer studies,^{14,15} a protective effect of the SS genotype against certain cancers can be proposed.

A higher predisposition to breast cancer was found in patients with a positive family history of any cancer in first-, second-, or third-degree relatives and S allele presence,¹⁶ but a significant association between family history of cancer and SS genotype was not detected previously. In a study¹⁷ including tissue samples from 65 patients with head and neck skin cancer about the role of p53 mutations in tumor behavior, 45 members of the cohort (69%) were reported to have a first-degree relative with cancer, whereas 25 of them (39%) were reported to have at least 1 relative with skin cancer (non-melanoma or melanoma). These data support the role of inheritance in HNNMSC and LLC development.

Skin SCC development is viewed as a multistep process, whereas BCCs are believed to develop de novo. Although an aberrant sonic hedgehog pathway was identified as the major cause of BCC development, the genetic mechanisms causing skin and lower lip SCCs are still poorly understood, despite the increased knowledge about the role of a variety of oncogenes, tumor suppressor genes, and signal transducing pathways.¹⁸⁻²³ In many studies investigating the association of gene polymorphisms with nonmelanoma skin cancers in the past decade, one of the most studied DNA repair genes, XRCC3, was found to be associated with a significantly decreased risk,²⁴ whereas patients with an XPD gene polymorphism were found to be susceptible to a second primary cancer development.²⁵ Despite lacking a relevant functional polymorphism,²⁶ the L-myc genomic region may be another candidate for further studies to enlighten the mechanisms of both cancers.

In conclusion, HNNMSC and LLC are common types of human cancer, and their incidences are increasing gradually, mainly owing to long-term sun exposure. Despite the relatively low mortality rates, their morbidity related to cosmetic and functional deformities is tremendous. We confirmed and extended the possible role of the L-myc polymorphism in the susceptibility and prognosis of another series of patients with cancer, which may help detect and prevent cancer in high-risk populations and estimate tumor behavior.

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