In search for genetic markers for cancer susceptibility as well as for diagnostic prognosis, polymorphism of known oncogenes, such as L-myc, are particularly consideration. In our studies we examined patients with lung, breast, gastric and thyroid cancer to determine whether genetic variations in L-myc gene were associated with higher susceptibility to cancer. Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP), Agarose Gel Electrophoresis Techniques were used to determine the L-myc genotypes in patient and control groups. The results were evaluated using SPSS 7.5 statistical software. Shown below are the conclusions from our research regarding the investigated genetic factor and their association as predisposition factors with the diseases of cancer. These studies in Turkish lung cancer patients demonstrate that the L-myc gene polymorphism is not associated with smoking status, cancer susceptibility or prognosis, and especially increased risk of metastasis either to the lymph nodes or to other organs. According to these results, we found no significant difference both in the distribution of the LL, LS and SS genotypes and in the allelic frequencies between the patient group and the control group. Our data concerning age, sex, size of tumours, histological type of tumours showed no significant association with L-myc genotype. However, a higher frequency of L-myc S-allele in the epidermoid carcinomas compared to other histological groups was found, although this difference was not statistically significant. In our group of gastric cancer patients, there was a significant difference in the distribution of both L-myc genotypes (p=0.004) and allele frequencies (p=0.005) between patients with gastric cancer and control groups. In our group of breast cancer patients, the frequency of S allele was significantly higher in breast cancer patients than in normal individuals (p<0.01). No correlation was observed between the presence of L-myc S allele and several parameters in the history of each patient. We also studied 138 patients of whom 47 had multi nodular goiter, 13 follicular cancer, 69 papiller cancer against a control group of 109 healthy individuals. We found no significant difference in the distribution of LL, LS, SS genotypes among these groups. However, the frequency of S allele was significantly higher in follicular thyroid cancer patients than in patients with multinodular goiter (p=0.04). Testing several genetic polymorphisms, simultaneously has the potential to identify individuals with an extremely high risk of developing cancer. This has profound implications for prevention since such high-risk individuals may be screened intensively as well as potentially treated with several preventive approaches. These studies constitute the basis for genetic susceptibility to lung, breast, gastric and thyroid cancer in Turkish patients.

**Key words:** Cancer, polymorphism, L-myc

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Accepted: August 08, 2005*
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Proto-oncogenes are normal cellular genes, the products of which have been shown to be important components of intracellular signalling pathways. The oncogenes, on the other hand, are not found in normal cells, but are generated by activation of their corresponding proto-oncogenes during tumour development. Many protooncogenes encode transcription factors that are normally induced in response to growth factor stimulation. These proteins regulate the expression of growth control genes by binding to specific DNA sequences. The c-myc proteins (c-myc, N-myc, L-myc) all contain basic, helix-loop-helix and leucine zipper domains and form heterodimers with the protein Max. MycError! Hyperlink reference not valid. family proteins form high-affinity heterodimers with Max that are capable of recognizing and binding to specific DNA E-box elements (CACGTG) to active transcription. Several genes have been identified whose expression is upregulated in response to c-myc expression, but it remains unclear whether increased expression is a direct result of myc-max transactivation or an indirect consequence of myc-induced cell cycle progression. In cell cycle regulation, myc is required for G1 to S phase progression of primary cells, and induction of c-myc expression stimulates quiescent non-transformed cells to transverse G1 phase and enter S phase. Myc-max heterodimers function as sequence specific transcriptional regulators and dimerization with Max is essential for DNA binding. Max also forms heterodimers with other leucine zipper proteins, including Mad, MxII. These heterodimers bind to the same DNA sequences as the Myc-Max heterodimers and will therefore repress transcription activation by myc-max complexes. The principal functions of the myc-gene products are the induction of cell proliferation and the inhibition of terminal differentiation in response to mitogenic stimuli. These genes are converted oncogenes by either amplification and/or overexpression, which occurs commonly in a wide range of human tumours’ C-myc is the normal cellular homology of v-myc, the viral-transforming oncogene from avian myelostomatosis virus MC29. The c-myc gene consists of three exons: a long,
untranslated first exon (exon 1) and two exons encompassing the protein coding sequences (exons II and III). Members of myc-gene family directly involved in the transformation process include the cellular N-myc and L-myc genes. Both N-myc and L-myc genes conserve the three exon structure of c-myc, with coding sequences contained exclusively in exons II and III. The N-myc and L-myc proteins share several regions of at least 80% amino acid sequence homology to c-myc, including the myc box I (MbI) myc box II (MbII) domains as well as a basic helix-loop helix and leucine zipper region.

L-myc gene was initially identified as a gene with structural similarity to c-myc and N-myc from a human small cell lung cancer line. L-myc is an oncogene localized to chromosome 1p34 and is thought to be activated during late tumorigenesis. The protein encoded by L-myc is a nuclear phosphoprotein that acts as a transcription factor and regulates cell proliferation and differentiation. The expression of L-myc gene is restricted to fetal brain, kidney, thymus, pancreas, skin, lung, as well as to adult lung tissue. In search for genetic markers for cancer susceptibility as well as for diagnostic prognosis, polymorphism of known oncogenes, such as L-myc, are particularly consideration.

Alleles of protooncogenes may also serve as markers of genetic susceptibility to cancer. The assays for protooncogene alleles can be done by using blood samples, making epidemiologic studies of protooncogene alleles relatively easy to carry out using prevalent cancer cases. The use of protooncogene alleles in cancer risk assessments may even be practical in prospective studies using existing banks of frozen peripheral lymphocytes.

A polymorphic EcoR1 restriction site is located in the second intron of the L-myc gene. Three different genotypes (LL, LS ve SS), consisting of two different alleles L (large) and S (small), can be seen. Although, the functional difference between L and S variants is unclear, there are data indicating a direct or direct involvement of L-myc polymorphism in cancer susceptibility and progression. Regarding the cancer susceptibility that is analyzed by examining the differences in genotype distribution between cancer patients and controls, the S allele was more common in patients with non-Hodgkin's lymphoma bone and soft-tissue sarcomas and esophageal cancer. However, the S allele was not increased in patients with lung cancer, glioma, bladder cancer or colorectal cancer and appears to protect against hepatocellular cancer. Individuals carrying the S allele tend to have poor prognosis and increased risk of several tumor types, although controversial results have been reported.

In terms of progression, Kawashima et al showed that the presence of the S allele was associated with metastases and/or an adverse prognosis of lung cancer among Japanese. However, this association was not found in population of Norwegian, North American, Australian and Turkish lung cancer patients. For the correlation of L-myc polymorphism with cancer progression in other tumor types, a positive association was found in esophageal, oral tumors neuroblastoma tumors but not bladder and renal tumors.

Several investigations have been published on the implications of restriction fragment length polymorphism (RFLP) of the L-myc gene as a marker for metastatic potential in patients with non-small cell lung cancer. Kawashima et al showed a close correlation between L-myc RFLP, the extent of metastasis, the development of multiple cancers and prognosis. These findings of the Japanese studies were not confirmed by some studies including other populations. Ge et al suggested that the L-L genotype being associated with poor prognosis and shorter survival after surgery in the Hong Kong Chinese lung cancer patients. This observation differs from that seen among Japanese lung cancer patients who were less likely to have lymph node involvement with L-L genotype. Recently, Shih et al suggested that the S allele of the L-myc polymorphism may be associated with lung cancer progression. According to their findings, the S allele of L-myc polymorphism was associated with an increased risk of developing advanced-stage lung cancer after adjusting for age and sex. They found that the S allele frequency was significantly increased in stages III+IV patients with lung cancer. The S allele frequency was significantly increased in stages III+IV patients (P=0.005).

On the other hand, the studies of Tefre et al failed to find an association between L-myc allelotype and prognosis, family history of cancer or metastasis in a Norwegian lung cancer population. Suzuki et al investigated association between the histological pattern of invasion and the prognosis in Japanese lung adenocarcinoma patients to evaluate the L-myc RFLP as a prognostic marker. According to their results, although the
histological pattern of invasion was correlated with the prognosis, no correlation was observed between the L-myc RFLP and the prognosis. There was no association between the L-myc RFLP and the histological pattern of invasion in their study. In Turkish lung cancer patients, there was also no correlation of the genotypes with age, sex, smoking history, tumor size, node classification or TNM stage. The three different genotypes are represented in two major histological groups except adenocarcinoma where no SS genotype was found. There was no difference between the genotypes of the two major non-small-cell lung cancer (NSCLC) subtypes; epidermoid carcinoma (SCC) and adenocarcinoma. Tefre et al. suggested that an increased risk of adenocarcinoma linked to the SS allele would be consistent. This observation was different from ours. Individuals carrying the S allele tend to have poor prognosis and increased risk of several tumor types, although controversial results have been reported.

Breast cancer is a genetic disease, with most breast cancer cases resulting from a dysregulation of genetically determined cellular pathways. The occurrence of cytogenetic abnormalities affecting chromosome 1 in a number of breast tumors led us to examine, at the molecular level, loci on chromosome 1 in primary breast tumor DNAs. Bieche et al. found that a significantly shorter period after relapse was observed for patients with loss of heterozygosity at L-myc in primary tumor DNAs compared with patients with tumor DNAs lacking this alteration. Champeme et al. reported that there was a statistical correlation between L-myc RFLP and lung metastasis in breast cancer patients who relapsed. In this study, a significant difference was found in the distribution of L-myc genotypes between breast cancer patients and healthy individuals. Our results supported the hypothesis that the L-myc locus is involved in a genetic predisposition to breast cancer. In contrast to our results, Champeme et al. reported that no differences in the patterns of L-myc RFLP were found between breast cancer patients and healthy individuals. The results of our study supported that L-myc gene is related to genetic susceptibility to breast cancer as Togo et al. suggested. They reported that the statistically significant effect observed in their study was due to the increased prevalence of the S allele in breast cancer group and they analyzed women separately. In our group of breast cancer patients, the frequency of S allele was significantly higher in breast cancer patients than in normal individuals (p<0.01). We studied women as a control group for L-myc RFLP analysis.

Multiple epidemiologic studies have documented that a reported history of breast cancer among relatives is a reproducible predictor of breast cancer risk. In general a "positive family history" of breast cancer confers a relative risk of 2.0 to 3.0 for breast cancer. The relationship between risk factors for breast cancer in women with or without a positive family history of this disease has been explored. Additional work is needed to further characterize the molecular reasons for the increased risk seen in individuals with positive family history. It would be interesting to study whether or not a specific distribution of the L-myc RFLP can also be observed in breast cancer and to test linkage in high-risk families.

There was a significant difference in the distribution of L-myc RFLP between patients with gastric cancer and healthy Turkish subjects in the present study. This positive findings persisted when the patients were classified in two genotypes, LS plus SS and LL types or when they were analyzed according to allele frequency. In our group of gastric cancer patients, there was a significant difference in the distribution of both L-myc genotypes (p=0.004) and allele frequencies (p=0.005) between patients with gastric cancer and control groups. These findings are incompatible with two previous studies of gastric cancer. Mironov et al. reported that the ratio of L-myc genotypes in patients with gastric cancer was very close to that found for a healthy white Caucasian population. Dlugosz A et al found that a significant correlation between S-allele presence and regional nodal metastasis was found. The results of our study supported that L-myc gene is located to genetic susceptibility to gastric cancer as Shibuta et al. suggested. The reason why polymorphism of the L-myc gene is related to genetic susceptibility to gastric cancer is unknown. It is possible that a gene or genes that are closely associated with L-myc on the same chromosome are related. Although the L-myc genotype has consistently been analysed using Southern blotting in previous studies, this study utilized a molecular genotyping method involving PCR-RFLP. As the epidermal risk factors to gastric cancer involve individual acquired conditions such as smoking, alcohol intake and gastritis. There was also no correlation between the genotypes and smoking history, alcohol intake and gastritis in Turkish gastric cancer patients.
Growth of thyroid cancer cells is also stimulated by various growth factors via signal transduction pathways. Myc oncogenes are activated in some thyroid neoplasms.  

Sheikh et al suggested that frequency of allelic loss in L-myc gene may provide a useful adjunctive prognostic test in medullary thyroid carcinoma. We designed a study about thyroid cancer. Our aim was to test whether there was an association between L-myc S allele in thyroid cancer and predisposition to the disease. For this purpose, we studied 138 patients of whom 47 had multi nodular goiter, 13 follicular cancer, 69 papiller cancer against a control group of 109 healthy individuals. In our study, we found no significant difference in the distribution of LL, LS, SS genotypes among these groups but the frequency of S allele was significantly higher in follicular thyroid cancer patients than in patients with multinodular goiter (p=0.04).

Our results suggested that L-myc gene polymorphism is not suitable as a prognostic marker of metastatic development in Turkish NSCLC patients. We also suggested that L-myc RFLP analysis may therefore be an important factor predicting a higher risk for breast cancer, at least in the Turkish population. However, further studies of well characterised large patient groups of defined ethnicity are needed to ascertain the role of S allele in breast cancer. In the same way, L-myc polymorphism may be significant in an individual's susceptibility to gastric cancer in Turkey and may be useful for identifying patients at high risk of developing gastric and follicular thyroid cancer.

There are several possible reasons for the discrepancy between those previous studies and ours. For the first, limited number of patients may be included in our study. Second, ethnic differences may have an important role and for the last, the sampling bias might have contributed to these different results. Further evaluation with more patients should be carried out to be able to define whether there is a correlation between L-myc S allele and prognostic factors of non-small cell lung cancer.

An alternative explanation is that the L-myc gene is not involved, but is in linkage disequilibrium with a gene or genes that are important in various type of cancer. Linkage disequilibriuim of L-myc with another disease-related polymorphic gene(s) appears to be a plausible explanation for the effects observed by various investigators.

They suggested that the differences in allele frequency and linkage disequilibrium patterns in different populations may explain the contrasting results in the Asian and Caucasian population on the role of L-myc EcoR1 polymorphism in lung tumor prognosis. PCR-RFLP analysis should be applicable to individuals at high risk in an attempt to select such individuals more objectively. Testing L-myc polymorphism, simultaneously may be useful to identify individuals with an extremely high risk of developing cancer. This has profound implications for prevention since such high-risk individuals may be screened intensively as well as potentially treated with several preventive approaches.

References


