Abstract. Aim: The purpose of the present study was to investigate the possible association of paraoxonase-1 (PON1) 192/55 polymorphisms with lung cancer (LC) risk in a Turkish population. 

Materials and Methods: A population-based, case-control study was carried out, including 223 patients with LC and 234 controls. The frequencies of PON1 192/55 genotypes were compared in patient and control groups using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. 

Results: Distribution of PON1 192 R (+) genotype was found to be significantly higher in patients with LC compared to the controls (odds ratio: 1.497, 95% confidence interval: 1.034-2.166). This difference was especially noteworthy in patients with small cell carcinoma and squamous cell carcinoma.

Conclusion: This is the first case-control study on the association between PON1 polymorphisms and LC susceptibility in a Turkish population. Our results suggest that PON1 192 polymorphism is associated with an increased risk of LC in the Turkish population and may be a useful genetic marker for small cell and squamous cell carcinoma.

Lung cancer (LC) is among the most common malignancies in the world and is the leading cause of cancer deaths in adults (1-2). It is well known that increased oxidative stress is a main cause of development of lung cancer (3, 4). Any change in the antioxidant system of organisms causes increased oxidative stress. Therefore, antioxidant enzymes may play a key role on carcinogenesis (5).

Key Words: Paraoxonase 1, PON1, polymorphism, lung cancer risk, SNP.
samples were collected between May 2002 and June 2003. Patients were newly diagnosed with histopathologically confirmed primary LC. Cases with secondary or recurrent tumours were excluded. Information such as metastasis, type of LC, stage and size of tumour were assembled from the archive of the Istanbul University Oncology Institute. As a control group, data from healthy ethnically matched individuals were obtained during the same period to examine the association between PON1 genotypes and susceptibility to LC. Healthy individuals had no evidence of cancer. All information, including cigarette smoking, history of chronic disease and tuberculosis, regarding controls and patients were collected by interviewing each individually and excluding those who had chronic bronchitis and tuberculosis. Smoking status of participants was classified as ‘never’, those who had never smoked or smoked less than/up to 100 cigarettes in their life; ‘former’, those who had quit smoking one year and more previously; ‘ever’, those who were currently smoking or had quit smoking within the previous year. All participants gave their consent for this study. To be able to determine gene frequencies correctly, age distributions of patients and controls were balanced. The study protocol was approved by the local Ethical Committee.

Genotype analysis for PON1 55/192 polymorphism. Blood samples were taken from patients prior to any form of treatment and collected in tubes containing ethylenediaminetetra-acetate (EDTA), and DNA was isolated from leukocyte pellets by sodium dodecyl sulfate (SDS) lysis, ammonium acetate extraction, and ethanol precipitation (19). PON1 genotypes were determined following polymerase chain reaction (PCR) according to previously published protocols (20-21). For the PON1 192 polymorphism, sense primer 5’-TAT TGT TGC TGT GGG ACC TGA G-3’ and antisense primer 5’- TGT ATA GCC CCA G-3’ were used. The PCR reaction mixture contained 100 ng DNA template, 0.5 μM of each primer, 1.5 mM MgCl2, 200 μM dNTPs and 1 U Taq DNA polymerase (MBI Fermentas, Lithuania). After denaturing the DNA for 5 min at 94°C, the reaction mixture was subjected to 35 cycles of denaturation for 1 min at 94°C, 1 min annealing at 60°C, and 1 min extension at 72°C for the 192 genotype. The 99-bp PCR product was digested with 8 U Bsp restriction endonuclease (MBI Fermentas) and the digested products separated by electrophoresis on 2% agarose gel (FMC BioProducts, Rockland, ME, USA) and visualized using ethidium bromide. The R genotype (arginine) contains a unique Bsp1 restriction site which results in 66- and 33-bp products.

Serum paraoxonase activity assay. Paraoxonase activities were measured according to Furlong et al. (22). The assay buffer contained 0.132 M Tris-HCl (pH 8.5), 1.32 mM CaCl2 and 2.63 M NaCl. Addition of 200 μl of 6 mM freshly prepared paraoxon (O,O-diethyl-O-p-nitrophenylphosphor; Sigma, Poole, UK) and 40 μl of serum initiated the assay. The rate of generation of p-nitrophenol was determined at 37°C with the use of a continuously recording spectrophotometer at 405 nm. A molar extinction coefficient of 18.05x103 was used for calculation of p-nitrophenol using paraoxon as substrate. Paraoxonase activity is expressed as a mmol paraoxon hydrolysed per minute per litre.

Statistical analysis. Statistical analyses were performed using the SPSS software package, version 13.0 (SPSS Inc, Chicago, IL, USA). Student’s t-test was used for mean age comparison. For comparison of PON activities, ANOVA test was used. Differences in the distribution of PON1 genotypes between patients with LC and controls were tested using either chi-square or Fisher’s exact tests. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the risk for LC. The degree of linkage disequilibrium (LD) between polymorphisms was assessed using the Haplovew program (http://www.broad.mit.edu/mpg/haplovew/documentation.php). All results were considered significant when the p-value was less than 0.05.

Results

The demographic characteristics of patients with LC and controls are presented in the Table I. Individuals with a family history of LC in first-degree relatives had a significantly increased LC risk. As expected, history of tobacco smoking was also associated with LC risk (Table I).

PON1 polymorphisms and LC. Genotype and allelic frequencies of the PON1 192/55 polymorphisms in patients with LC and control groups are shown in Table II. The frequency of the combined variant genotypes PON1 192 RR and QR (PON1 192 R (+)) was significantly higher in patients with LC compared to the controls (OR=1.497, 95% CI=1.034-2.166) (Table II). However, frequencies of the other genotypes and alleles were similar in both patient and control groups.

In addition to single nucleotide polymorphism analyses, haplotypes were evaluated for association with LC. Haplotype analysis confirmed the association of PON1 variants with LC and revealed that the PON1 192 R:55L haplotype was significantly more frequent in patients with LC as compared with the controls (p=0.027).

PON1 polymorphism and subgroup of LC. To evaluate the correlation between PON1 polymorphism and subgroups of LC, the distribution of the genotypes in patients with small cell, squamous cell and adenocarcinoma was compared with that of the controls. The frequency of PON1 192 R (+) genotype was found to be higher in patients with small cell (p=0.021; OR=2.677, 95% CI=1.134-6.320) and squamous cell carcinoma (p=0.040; OR=1.647, 95% CI=1.022-2.656) compared to the controls. Although the frequency of the PON1 55 LL genotype was higher in patients with
adenocarcinoma (62.5%) compared to the controls (50.4%), this result was not statistically significant ($p=0.158$; OR=0.610, 95% CI=0.306-1.216).

Serum PON1 activity. Serum PON1 activity was significantly lower in the patients with LC compared to the control group as shown in Table I and such a decrease was apparent for all genotype groups as shown in Table III. Although PON1 activity was higher in $PON1$ RR and $PON1$ 55 LL genotype carriers in controls compared to that of the other genotypes (QQ, QR and MM, LM, respectively) ($p<0.05$), we did not find this association in the patients with LC ($p>0.05$).

### Discussion

Our present study reports for the first time that $PON1$ polymorphisms are associated with increased risk of LC in this Turkish population. We first observed that carrying the $PON1$ 192 R (+) genotypes is a significant risk factor for LC, especially for small and squamous cell carcinoma. Similar to our results, some studies indicated that the R allele is associated with an increased risk of ovarian cancer, non-Hodgkin’s lymphoma and multiple myeloma (9, 23-24). However, only one paper has so far been published about the relationship between $PON1$ polymorphisms and lung cancer (18). According to this paper, the $PON1$ QQ genotype was found to be associated with an increased risk of lung cancer in a Korean population (18). Similarly, some studies also showed association between $PON1$ 192 QQ genotype and an increased risk of breast and prostate cancer, and osteosarcoma (25-27). Our findings are inconsistent with these results (18, 25-27). In addition, several studies reported that $PON1$ 55 polymorphism is associated with higher breast, prostate and ovarian cancer risk (9, 26, 28); however, we did not find any difference for $PON1$ 55 polymorphism between both controls and patients in our study.

We also found PON1 activity increased in the order of QQ<QR<RR for $PON1$ 192 and LL<LM<MM for $PON1$ 55...
polymorphisms in our study. Although this association in patients was not shown to be statistically significant, serum PON1 activity in patients was significantly lower compared with that in controls. These results are consistent with the findings of other studies (17, 29-34) but some studies have failed to find such a relationship (31).

Similar to the previous studies (35-38), we observed that smoking and gender were associated with LC risk.

Polymorphisms in genes coding for antioxidant enzymes, such as PON1, may cause defects in antioxidant/oxidant balance (14, 29-32). This can trigger oxidative stress and the formation of reactive oxygen species. The present results, which indicate an association of the PON1 192 R (+) genotypes with LC, are consistent with the hypothesis that PON1 activity altered by possible genotypic effect may be involved in the mechanism of developing LC due to a reduction in antioxidant and anti-inflammatory activity. Although we found that there was no significant genotypic effect on the phenotypic expression of PON in LC patients, we believe that increased frequency of R and L (+) genotypes in LC patients may be the body’s response in order to improve its defective antioxidant defence system.

A limitation of this study is the sample size of patients and controls and subgroups of patients according to the histological type of tumor. In this study, we cannot exclude there being a false-negative result due to the sample size. Nevertheless, the study is sufficiently powered to rule out a major association of PON1 polymorphism with LC risk.

Taken together, our data suggest that PON1 192 R (+) genotype may affect the LC risk, especially for small cell and squamous cell carcinoma in our population. The importance of PON1 as a predictive risk factor for LC should be assessed.

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References


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