Paraoxonase-1 192/55 Polymorphisms and the Risk of Lung Cancer in a Turkish Population

PINAR AKSOY-SAGIRLI[1], BEDIA CAKMAKOGLU[2], TURGAY ISBIR[3], ESRA KAYTAN-SAGLAM[4], AHMET KIZIR[4], ERKAN TOPUZ[4] and HAKAN BERKKAN[5]

[1]*Department of Biochemistry, Pharmacy Faculty, and* [4]*Institute of Oncology,*
[2]*Molecular Medicine, Institute of Experimental Medicine Research, Istanbul University, Istanbul, Turkey;*
[3]*Medical Biology, Medical Faculty, Yeditepe University, Istanbul, Turkey;*
[5]*Department of Biochemistry, Medical Faculty, Istanbul Bilim University, Istanbul, Turkey*

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 polymorphsim 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).

Correspondence to: Pinar Aksoy-Sagirli, Ph.D., Department of Biochemistry, Pharmacy Faculty, Istanbul University, 34116, Istanbul, Turkey. Tel: +90 2124400000/13475, Fax: +90 2124400252, e-mail: aksoyp@istanbul.edu.tr, pinaraksoy22@yahoo.com

Key Words: Paraoxonase 1, *PON1*, polymorphism, lung cancer risk, SNP.

Human serum paraoxonase (PON1) is an esterase enzyme that has lipophilic antioxidant characteristics. Serum PON1 binds to high-density lipoprotein (HDL) and contributes to the elimination of organophosphorus compounds, such as paraoxon, and carcinogenic lipid-soluble radicals from lipid peroxidation (5-7).

The *PON1* gene has two common coding region polymorphisms: rs662 A>G in exon 6 that results in a glutamine-to-arginine exchange (Gln192Arg, Q192R, A192B), and rs854560 T>A in exon 3 that results in a leucine-to-methionine substitution (Leu55Met, L55M) (8-9). Studies showed that polymorphisms of *PON1* gene may change PON1 activity. In a study by Eckerson *et al*., the PON1 activity of *PON1* 192 Q allele carriers was reported to be lower than that of the R carriers (10). Reduced PON1 activities have been reported in different groups of patients, including those with diabetes mellitus, hypercholesterolemia and cardiovascular disease who are under increased oxidative stress (11-12). In last decade, studies have focused on the association of *PON1* polymorphisms with cancer (9, 13-16). But only few have studied the association between *PON1* and LC (17-18).

We assume that PON1, which has antioxidant and anti-inflammatory effects, may play an important role in lung cancer caused by oxidative stress and inflammation. Therefore, the aim of this study was to identify serum PON1 activities and genotypes and establish the correlation between genotype and phenotype in LC patients and controls. Moreover, this study will also help to determine the relationship between *PON1* polymorphisms and subgroups of LC patients in the Turkish population.

Materials and Methods

The study population. A total of 234 unrelated healthy volunteers and 223 patients with LC, diagnosed at Istanbul University Oncology Institute, were included in this case-control study. Blood

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'-CAC GCT AAA CCC AAA TAC ATC TC-3', which encompass the 192 polymorphic region of the human *PON1* gene, were used. For the *PON1* 55 polymorphism, sense primer 5'-GAA GAG TGA TGT ATA GCC CCA G-3' and antisense primer 5' TTT AAT CCA GAG CTA ATG AAA GCC-3' were used.

The PCR reaction mixture contained 100 ng DNA template, 0.5 μM of each primer, 1.5 mM $MgCl_2$, 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 95˚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*l 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 Bspl restriction site which results in 66- and 33-bp products and the Q genotype (glutamine) cannot be cut, allowing the 192 genotype to be determined (21). For the *PON1* 55 polymorphism, the PCR reaction and the cycling conditions were the same as above. The PCR product (170 bp) was digested with *Hsp*92 (Promega, Madison, WI, USA) in the presence of bovine serum albumin (BSA) (0.1 μg/μl final concentration, 37˚C, overnight) and the digested products were separated and identified as above. Allele L (leucine) did not contain the *Hsp*92I site, whereas M (methionine) contained the *Hsp*92II site giving rise to 126- and 44-bp products (20).

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 $CaCl_2$ and 2.63 M NaCl. Addition of 200 μl of 6 mM freshly prepared paraoxon (*O,O*-diethyl-*O*-*p*-nitrophenylphosphate; 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 18.05×10^3 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 Haploview program (http://www.broad.mit.edu/mpg/haploview/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

Table I. *Demographic characters and parameters of patient and control groups*.

	Controls (%)	Patients (%)	*p*-Value
Total	234	223	
Age mean±SD, years	56.7±10.4	57.9±11.6	0.315
PON activity mean±SD†	413.6±186.2	12.6±16.6	0.0001
Gender			
Male	149 (63.7)	197 (88.3)	0.001
Female	85 (36.3)	26 (11.7)	
Smoking status			
Never	49 (26.4)	12 (6.8)	
Former	53 (28.8)	68 (38.2)	0.049
Ever	82 (44.9)	98 (55.1)	0.0001
Unknown	50	45	
Family history of LC			
Yes	17 (9.1)	31 (16.6)	0.032
No	169 (90.9)	156 (84.3)	
Unknown	48	36	
Subgroup of LC			
Small cell carcinoma	-	28 (16.8)	
Squamous cell carcinoma	-	99 (59.3)	
Adenocarcinoma	-	40 (23.9)	
Unknown	-	56	

†mmol Paraoxon $min^{-1}l^{-1}$. *Classified as former + never and ever and then *p*-value calculated; **Classified as never and former + ever and then *p*-value calculated.

Table II. *Distributions of PON1 192/55 genotypes and alleles in controls and patients*.

		Controls (%) n=234	Patients (%) n=223	*p*-Value
PON1 192	Genotype			
	QQ	121 (51.7)	93 (41.7)	0.081
	QR	93 (39.7)	111 (49.8)	
	RR	20 (8.6)	19 (8.5)	
	RR+QR	113 (48.7)	130 (58.3)	0.032
	QQ	121 (51.3)	93 (41.7)	
	Allele			
	Q	335 (71.6)	297 (66.6)	0.100
	R	133 (28.4)	149 (33.4)	
PON1 55	Genotype			
	LL	118 (50.4)	119 (53.4)	0.693
	LM	102 (43.6)	94 (42.2)	
	MM	14 (6.0)	10 (4.5)	
	MM+LM	116 (49.6)	104 (46.6)	0.530
	LL	118 (50.4)	119 (53.4)	
	Allele			
	L	338 (72.2)	332 (74.4)	0.440
	M	130 (27.8)	114 (25.6)	

Table III. *Paraoxonase activity (mmol PON $min^{-1}l^{-1}$) according to PON1 192/55 genotype in patients and controls*.

	PON activity±SE		
	Controls	Patients	*p*-Value[a]
Overall	413.6±37.2	12.6±2.7	0.0001
PON1 192 Genotype			
QQ	300.8±42.0	10.8±4.0	<0.0001
QR	328.8±30.3	14.7±4.5	<0.0001
RR	566.1±76.0	10.8±2.4	0.002
PON1 55 Genotype			
LL	404.2±38.9	13.2±3.5	<0.0001
LM	305.1±41.0	12.6±4.6	<0.0001
MM	183.8±71.7	1.2±0.5	0.003

[a]ANOVA.

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.

Acknowledgements

This research was supported in part by the following grants: Istanbul University Scientific Research Projects, Project no: T-561/21102004 and UDP-4443/15102009.

References

1 Hoffman PC, Mauer AM and Vokes EE: Lung cancer. Lancet *355(9202)*: 479-485, 2000.

2 Minna JD, Roth JA and Gazdar AF: Focus on lung cancer. Cancer Cell *1(1)*: 49-52, 2002.

3 Gackowski D, Speina E, Zielinska M, Kowalewski J, Rozalski R, Siomek A, Paciorek T, Tudek B and Olinski R: Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. Cancer Res *63(16)*: 4899-4902, 2003.

4 Paz-Elizur T, Krupsky M, Blumenstein S, Elinger D, Schechtman E and Livneh Z: DNA repair activity for oxidative damage and risk of lung cancer. J Natl Cancer Inst *95(17)*: 1312-1309, 2003.

5 Li HL, Liu DP and Liang CC: Paraoxonase gene polymorphisms, oxidative stress, and diseases. J Mol Med *81(12)*: 766-779, 2003.

6 Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL and La Du BN: Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest *101(8)*: 1581-1590, 1998.

7 Mackness MI, Arrol S and Durrington PN: Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett *286(1-2)*: 152-154, 1991.

8 Aynacioglu AS, Cascorbi I, Mrozikiewicz PM, Nacak M, Tapanyigit EE and Roots I: Paraoxonase 1 mutations in a Turkish population. Toxicol Appl Pharmacol *157(3)*: 174-177, 1999.

9 Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY and Goodman MT: Genetic polymorphisms in the paraoxonase 1 gene and risk of ovarian epithelial carcinoma. Cancer Epidemiol Biomarkers Prev *17(8)*: 2070-2077, 2008.

10 Eckerson HW, Romson J, Wyte C and La Du BN: The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet *35(2)*: 214-227, 1983.

11 Ayub A, Mackness MI, Arrol S, Mackness B, Patel J and Durrington PN: Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol *19(2)*: 330-335, 1999.

12 Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M and Durrington PN: Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis *86(2-3)*: 193-199, 1991.

13 Hussein YM, Gharib AF, Etewa RL and Elsawy WH: Association of L55M and Q192R polymorphisms in paraoxonase 1 (*PON1*) gene with breast cancer risk and their clinical significance. Mol Cell Biochem *351(1-2)*: 117-123, 2011.

14 Karaman E, Uzun H, Papila I, Balci H, Ozdilek A, Genc H, Yanardag H and Papila C: Serum paraoxonase activity and oxidative DNA damage in patients with laryngeal squamous cell carcinoma. J Craniofac Surg *21(6)*: 1745-1749, 2010.

15 Ozturk O, Kagnici OF, Ozturk T, Durak H, Tuzuner BM, Kisakesen HI, Cakalir C and Isbir T: 192R allele of paraoxanase 1 (*PON1*) gene as a new marker for susceptibility to bladder cancer. Anticancer Res *29(10)*: 4041-4046, 2009.

16 Arpaci A, Gormus U, Dalan B, Berkman S and Isbir T: Investigation of *PON1* 192 and *PON1* 55 polymorphisms in ovarian cancer patients in Turkish population. In Vivo *23(3)*: 421-424, 2009.

17 Elkiran ET, Mar N, Aygen B, Gursu F, Karaoglu A and Koca S: Serum paraoxonase and arylesterase activities in patients with lung cancer in a Turkish population. BMC Cancer 7(48), 2007.

18 Lee CH, Lee KY, Choe KH, Hong YC, Kim YD, Kang JW and Kim H: Effects of oxidative DNA damage induced by polycyclic aromatic hydrocarbons and genetic polymorphism of the paraoxonase-1 (*PON1*) gene on lung cancer. J Prev Med Public Health *38(3)*: 345-350, 2005.

19 Miller SA, Dykes DD and Polesky HF: A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res *16(3)*: 1215, 1988.

20 Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ and Furlong CE: The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet *3(1)*: 73-76, 1993.

21 Adkins S, Gan KN, Mody M and La Du BN: Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. Am J Hum Genet *52(3)*: 598-608, 1993.

22 Furlong CE, Richter RJ, Seidel SL, Costa LG and Motulsky AG: Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. Anal Biochem *180(2)*: 242-247, 1989.
23 Kerridge I, Lincz L, Scorgie F, Hickey D, Granter N and Spencer A: Association between xenobiotic gene polymorphisms and non-Hodgkin's lymphoma risk. Br J Haematol *118(2)*: 477-481, 2002.
24 Lincz LF, Kerridge I, Scorgie FE, Bailey M, Enno A and Spencer A: Xenobiotic gene polymorphisms and susceptibility to multiple myeloma. Haematologica *89(5)*: 628-629, 2004.
25 Gallicchio L, McSorley MA, Newschaffer CJ, Huang HY, Thuita LW, Hoffman SC and Helzlsouer KJ: Body mass, polymorphisms in obesity-related genes, and the risk of developing breast cancer among women with benign breast disease. Cancer Detect Prev *31(2)*: 95-101, 2007.
26 Antognelli C, Mearini L, Talesa VN, Giannantoni A and Mearini E: Association of *CYP17, GSTP1*, and *PON1* polymorphisms with the risk of prostate cancer. Prostate *63(3)*: 240-251, 2005.
27 Ergen A, Kilicoglu O, Ozger H, Agachan B and Isbir T: Paraoxonase 1 192 and 55 polymorphisms in osteosarcoma. Mol Biol Rep, 2010.
28 Stevens VL, Rodriguez C, Pavluck AL, Thun MJ and Calle EE: Association of polymorphisms in the paraoxonase 1 gene with breast cancer incidence in the CPS-II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev *15(6)*: 1226-1228, 2006.
29 Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD and Furlong CE: Paraoxonase (*PON1*) phenotype is a better predictor of vascular disease than is *PON1(192)* or *PON1(55)* genotype. Arterioscler Thromb Vasc Biol *20(11)*: 2441-2447, 2000.
30 Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, Miller JE, Boulton AJ and Durrington PN: Serum paraoxonase (*PON1*) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin-dependent diabetes mellitus. Atherosclerosis *139(2)*: 341-349, 1998.
31 Abbott CA, Mackness MI, Kumar S, Boulton AJ and Durrington PN: Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. Arterioscler Thromb Vasc Biol *15(11)*: 1812-1818, 1995.
32 Mohamed RH, Karam RA and Abd El-Aziz TA: The relationship between paraoxonase1-192 polymorphism and activity with coronary artery disease. Clin Biochem *43(6)*: 553-558, 2010.
33 Akcay MN, Yilmaz I, Polat MF and Akcay G: Serum paraoxonase levels in gastric cancer. Hepatogastroenterology *50(Suppl 2)*: cclxxiii-cclxxv, 2003.
34 Akcay MN, Polat MF, Yilmaz I and Akcay G: Serum paraoxonase levels in pancreatic cancer. Hepatogastroenterology *50(Suppl 2)*: ccxxv-ccxxvii, 2003.
35 Ito H, Matsuo K, Hamajima N, Mitsudomi T, Sugiura T, Saito T, Yasue T, Lee KM, Kang D, Yoo KY, Sato S, Ueda R and Tajima K: Gene environment interactions between the smoking habit and polymorphisms in the DNA repair genes, *APE1* Asp148Glu and *XRCC1* Arg399Gln, in Japanese lung cancer risk. Carcinogenesis *25(8)*: 1395-1401, 2004.
36 Nakachi K, Imai K, Hayashi S, Watanabe J and Kawajiri K: Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. Cancer Res *51(19)*: 5177-5180, 1991.
37 Freedman ND, Leitzmann MF, Hollenbeck AR, Schatzkin A and Abnet CC: Cigarette smoking and subsequent risk of lung cancer in men and women: analysis of a prospective cohort study. Lancet Oncol *9(7)*: 649-656, 2008.
38 Bain C, Feskanich D, Speizer FE, Thun M, Hertzmark E, Rosner BA and Colditz GA: Lung cancer rates in men and women with comparable histories of smoking. J Natl Cancer Inst *96(11)*: 826-834, 2004.

Received March 4, 2011
Revised May 4, 2011
Accepted May 6, 2011