Q192R and L55M Polymorphisms of Paraoxonase 1 Gene in Chronic Myelogenous Leukemia and Chronic Lymphocytic Leukemia

AYŞE GAYE TOMATIR[1], SACIDE PEHLIVAN[2], HANDAN HAYDAROĞLU SAHIN[3],
SIBEL OGUZKAN BALCI[4], SELIN BUDEYRI[3] and MUSTAFA PEHLIVAN[3]

[1]Department of Medical Biology, Faculty of Medicine, Pamukkale University, Denizli, Turkey;
[2]Department of Medical Biology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey;
Departments of [3]Hematology, and [4]Medical Biology and Genetics,
Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Abstract. *Aim: The aim of the present study was to investigate the association between Paraoxonase 1 (PON1) gene polymorphisms Q192R, and L55M in patients with Chronic Myelogenous Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL) patients. Materials and Methods: We analyzed samples from 60 patients with CML, 60 with CLL and 84 healthy controls. Polymerase Chain Reaction (PCR) - Restriction Fragment Length Polymorphism (RLFP) was performed and samples were run in agarose gel. Results: We found statistically significant results showing an increase in both the RR genotype (p=0.044) and the R allele (p=0.011) for PON1 Q192R, and an increase in the MM genotype (p=0.007) and a decrease in the LL genotype (p=0.004) and R allele (p=0.001) in PON1 L55M in patients with CLL. Conclusion: We concluded that both the Q192R gene polymorphism with an increase in the genotype R allele, and the M/L55 with an increase in the MM genotype play a role in CLL susceptibility, and a decrease in the LL genotype can act against disease in the Turkish population.*

Reactive oxygen species (ROS) can oxidize biological molecules that mediate carcinogenesis by causing both metabolic malfunction, and damage to DNA. Human serum paraoxonases (PONs) play a role in anti-oxidant defense and protect cells against ROS (1). PON1 is a serum esterase synthesized in the liver. PON1 is a member of a family of proteins that include PON2 and PON3, the genes for which are clustered on the long arm of the human chromosome 7(q21.22) (2). *PON1* has two common polymorphisms that influence both PON1 concentration and activity. Epidemiological and molecular studies have shown that there are two important genetic polymorphisms in positions 55 and 192 of the *PON1* gene. These two polymorphisms arise from the substitution of amino acids in positions 55 and 192. The substitution of glutamine (Q genotype) in position 192 of the PON1 gene by the arginine (R genotype) leads to the first polymorphism rs662 (Q192R). Similarly, the substitution of leucine (L genotype) in position 55 by methionine (M genotype) leads to the second polymorphism rs854560 (L55M). The most common genotype is homozygous QQ, the second is heterozygous QR, and the least common is homozygous RR. The hydrolyzing activity of the protein encoded by R is 8-fold more than that encoded by the Q allele. This polymorphism also affects the serum protein concentration. Homozygous RR individuals have higher enzyme concentrations than the homozygous QQ individuals (3, 4). In addition to the polymorphisms at these positions, five polymorphisms were reported for the *PON1* promoter region. Due to these genetic polymorphisms, PON1 activity may differ by up to 10- to 40-fold among different individuals and populations (5, 6).

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm related to the presence of the Breakpoint Cluster Region (BCR) – Abelson (Abl)1 fusion gene, which is linked to t(9;22)(q34;q11). It originates from an abnormal hematopoietic stem cell that is characterized from its normal counterpart by long-term self-renewal and multi-lineage differentiation. Both leukemia and quiescent normal hematopoietic stem cells preferentially reside in the osteoblastic niche. Mesenchymal stromal cells are located near them, playing a critical role in their regulation (7).

Correspondence to: Associate Professor Ayşe Gaye Tomatir, Ph.D., Faculty of Medicine, Department of Medical Biology, Pamukkale University, Kınıklı/Denizli, Turkey. Tel: +90 2582962578, Fax: +90 2582961765, e-mail: tomatir@pau.edu.tr; aysegaye@hotmail.com

B-Cell chronic lymphocytic leukemia (CLL) is the most common type of human leukemia (8). This disease is characterized by the monoclonal expansion of the B-lymphocytes in the peripheral blood, bone marrow, and lymphoid organs, and by an indolent course, which ultimately becomes aggressive and invariably lethal. CLL cells have a low proliferative rate and a prolonged life-span, suggesting that their primary alteration may be a defect in apoptosis (9). The cellular origin of CLL is unknown, and has been the subject of recent controversy. CLL cells are characterized by the expression of the cell surface markers CD5, CD23, CD19, and low levels of IgM/IgD, a pattern not shared by any known B-cell subpopulations (9-12). CLL is not associated with reciprocal balanced chromosomal translocations, but rather with specific deletions (13), suggesting the loss of known tumor-suppressor genes. The 13q14 deletions are the most common chromosomal alterations in CLL (8). Palamarchuk *et al*. reported that miR-15/16 is a target of 13q14 deletions, and plays a tumor-suppressor role by targeting B-cell lymphoma 2 (BCL2). In addition, they found that deleted in lymphocytic leukemia, 7 (DLEU7) expression in A549 lung cancer cells resulted in a decrease in the proportion of S-phase cells and increased apoptosis (8).

Several studies have focused on PON1 activity and genotype in various diseases. For example, PON1 has been identified as an independent risk factor for atherosclerosis. Serum paraoxanase 1 activity was significantly lower in patients with atherosclerosis (14). In addition, Serum PON1 activity was found to be significantly lower in patients with pancreatic (15) gastric (16) and lung (17) cancer when compared with healthy controls. A relationship between *PON1* genotype and the risk of breast (18, 19) prostate (20) and lung (21) cancer, non-Hodgkin's lymphoma (22) multiple myeloma (23), and osteosarcoma (24) has also been shown.

PON1 gene polymorphisms Q192R and L55M have been shown to be associated with several human cancer types, but their association with CML and CLL has yet to be investigated. In this study, we examined the *PON1* 192 and *PON1* 55 genotypes in relation to the risk of Turkish patients developing CML and CLL.

Materials and Methods

Patients. The study began by obtaining the approval of the Gaziantep University Ethics Committee (07-2007/40). The patient group consisted of 60 patients (33 males and 27 females; mean age of 48 years, range=18-78 years) with the diagnosis of or under treatment for CML and 60 patients (32 males, 28 females; mean age of 62 years, range=30-76 years) with the diagnosis of or under treatment for CLL at the Hematology Clinic, Gaziantep University Hospital. A total of 84 people (43 males, 41 females; mean age of 52 years, range=18-78 years) were included in the study as a control

group, who were apparently healthy volunteers. No one in the control group had a smoking history or a chronic use of any drugs. Written-informed consent was obtained from the patients and controls after the explanation of the study details. After a 20-min rest, 2 ml blood samples were collected from the antecubital vein of each participant into tubes prepared with EDTA. All the blood samples were stored at −20°C until DNA purification.

Determination of the PON1 192 gene polymorphism. Genomic DNA was extracted from mononuclear cells obtained from EDTA-treated peripheral venous blood using the salting-out method (25). The polymerase chain reaction (PCR) (25 μl) for the *PON1* 192 gene polymorphism was performed in 10 mM Tris-HCl, pH8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 20 pmol of each primer (Biobasic, Ontario, Canada), and 1 U Taqpolymerase (MBI Fermentas,Vilnius, Lithuania) in a thermocycler under the following conditions: 94°C for 5 min (initial denaturation) followed by 35 cycles at 95°C for 1 min (denaturation), 60°C for 1 min (annealing), 72°C for 1 min (extension), and a final extension at 72°C for 10 min. A 99-bp fragment was amplified from 10 ng genomic DNA. The PCR products were restricted using restriction endonuclease *Alw*I (MBI Fermentas) and separated by 2% agarose gel electrophoresis. The PCR products of 99 bp were digested with 2U AlwI at 55°C for 20 h, and resulted in 99-, 69- and 30-bp fragments for the QR genotype, 69- and 30-bp fragments for the RR genotype, and a non-digested 99-bp fragment for the QQ genotype. The digested products were resolved by gel electrophoresis (2% agarose gel) and visualized by ethidium bromide staining.

Determination of the PON1 55 gene polymorphism. For the genomic DNA samples of the individuals, the alleles of the PON1 55 locus were amplified by PCR. The PCR (25 μl) was performed in 10 mMTris-HCl, pH8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 20 pmol of each primer (Biobasic), and 1 U *Taq* polymerase (MBI Fermentas) mixed in a thermocycler under the following conditions: 94°C for 2 min (initial denaturation) followed by 35 cycles at 94°C for 1 min (denaturation), 60°C for 1 min (annealing), 72°C for 1 min (extension), and a final extension at 72°C for 10 min. A 170-bp fragment was amplified from 10 ng genomic DNA. After the PCR amplification, a fragment belonging to the *PON1* 55 locus was digested by using Hsp92II (Promega, Madison, USA) a restriction endonuclease and separated in 2% agarose gel. The PCR products (170 bp) were digested with 2U *Hsp*92II at 37°C for 20 h, and resulted in 170-, 126- and 44-bp fragments for the ML genotype, 126- and 44-bp fragments for the MM genotype, and a non-digested 170-bp fragment for the LL genotype. The digested products were resolved by gel electrophoresis (2% agarose gel) and visualized by ethidium bromide staining.

Statistical analysis. The analysis of the data was performed by using the computer software SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). The statistical significance of the differences between the patient and the control groups were estimated by logistic regression analysis. The adjusted odds ratios (ORs) were calculated with a logistic regression model that checked for gender and age, and reported with 95% confidence intervals (CI). Differences in *PON1* allelic frequencies between the control group and patients were compared with the chi-square test, with the Fisher's exact test being used when required. The Hardy–Weinberg equation was used to calculate the estimated and observed genotypic

Table I. *Comparison of Q/R192 polymorphism of the paraoxonase 1 (PON1) gene between patients with chronic lymphocytic leukemia (CML), with chronic myelogenous leukemia (CLL), and the healthy controls.*

PON1 (192)	CML	CLL	Healthy controls	HWE	OR	95% CI	p-Value
Genotypes	N[a] (%)	N[b] (%)	n[c] (%)				
QQ	36 (60)	33 (55)	58 (69)	0.596[a]	0.669[a]	0.329-1.364[a]	0.269[a]
				0.343[b]	0.674[b]	0.329-1.382[b]	0.282[b]
QR	20 (33.3)	21 (35)	24 (28.6)	0.793[c]	1.216[a*]	0.590-2.505[a*]	0.595[a*]
					1.469[b*]	0.695-3.106[b*]	0.314[b*]
RR	4 (6.7)	6 (10)	2 (2.4)		2.457[a*]	0.420-14.387[a*]	0.319[a*]
					6.046[b*]	1.053-34.914[b*]	0.044[b*]
Allele							
Q	92 (76.7)	87 (72.5)	140 (83.3)		1.639[a&]	0.904-1.084[a&]	0.070[a&]
R	28 (23.3)	33 (27.5)	28 (16.7)		2.042[b&]	1.144-3.646[b&]	0.011[b&]

HWE: Hardy–Weinberg Equation; OR: Odds Ratio; CI: Confidence Intervals. [a]n=60, [b]n=60, [c]n=84. *Adjusted by age and gender, [&]Fisher's exact test, [a]comparison of genotypic frequencies between CLL and healthy controls; [b]comparison of genotypic frequencies between CML and healthy controls.

Table II. *Comparison of M/L55 polymorphism of the paraoxonase 1 (PON1) gene between patients with chronic lymphocytic leukemia (CML), with chronic myelogenous leukemia (CLL), and the healthy controls.*

PON1 (55)	CML	CLL	Healthy controls	HWE	OR	95% CI	*p*-Value
Genotype	n[a] (%)	n[b] (%)	n[c] (%)	0.657[a]	0.753[a]	0.341-1.662[a]	0.482[a]
MM	13 (21.7)	28 (46.7)	22 (26.2)	0.438[b]	2.819[b]	1.328-5.984[b]	0.007[b]
ML	28 (46.7)	24 (40)	28 (33.3)	0.084[c]	1.714[a*]	0.862-3.405[a*]	0.124[a*]
					1.495[b*]	0.724-3.087[b*]	0.277[b*]
LL	19 (31.6)	8 (13.3)	34 (40.5)		0.618[a*]	0.303-1.263[a*]	0.187[a*]
					0.275[b*]	0.113-0.670[b*]	0.004[b*]
Allele							
M	54 (45)	80 (66.7)	72 (42.9)		1.007[a&]	0.628-1.615[a&]	0.536[a&]
R	66 (55)	40 (33.3)	96 (57.1)		0.412[b&]	0.253-0.671[b&]	0.001[b&]

HWE: Hardy–Weinberg Equation; OR: Odds Ratio; CI: Confidence Intervals. [a]n=60, [b]n=60,Cn=84. *Adjusted by age and gender, [&]Fisher's exact test, [a]comparison of genotypic frequencies between CLL and healthy controls; [b]comparison of genotypic frequencies between CML and healthy controls.

frequencies (26). A *p*-value of less than 0.05 was considered as statistically significant.

Results

The comparison of the Q/R192 polymorphism of the *PON1* gene between the patients with CML, CLL, and the healthy controls is shown in Table I. While the comparison of the patients with CML with the control group did not show any statistically significant relationship between the RR genotype or R allelic frequency and CML, both the RR genotype (*p*=0.04) and the R allele were statistically significantly more frequent in the CLL group (*p*=0.011).

The comparison of the M/L55 polymorphism of the *PON1* gene between the patients with CML, CLL, and the healthy controls is shown in Table II. While the comparison of patients with CML with the control group did not show any

statistically significant relationship for the genotypic and allelic frequencies, in CLL, the increase in the frequency of both MM (*p*=0.007) and a decrease in LL genotype (*p*=0.004) and in the R allele (*p*=0.001) were found to be statistically significant.

We did not observe any deviation from the Hardy–Weinberg equation in any of the groups (data not shown).

Discussion

Oxidative stress and free radicals have been associated with an increased risk in various types of cancers (27, 28). The PON gene family consists of three members, *PON1, PON2*, and *PON3*. PON1 is found exclusively extracellular, and is associated solely with the high-density lipoprotein (HDL) particles in the circulation, and, in part, confers the anti-oxidant and anti-inflammatory properties associated with

HDL (29). Our study shows that *PON1* polymorphisms are risk factors in the development of CML and CLL exclusively.

In the present study, we did not find any significant relationship for the genotype nor the allelic frequency in patients with CML regarding the *PON1* 192 and *PON1* 55 polymorphisms. In a similar result, Rajaraman *et al.* did not find any relationship between brain tumors and the *PON1* genotype (30). Akkız *et al.* suggested that neither the Q192R polymorphism nor the L55M polymorphism have any relationship to the risk of developing hepatocellular carcinoma (1). Uyar *et al.* found that there were no significant differences in the L55M polymorphism and in L and M allelic frequencies in patients with renal cell carcinoma and those without. They concluded that the R allele may protect against renal cell carcinoma (31). Martinez *et al.* found that common non-synonymous *PON1* polymorphisms are not related to the risk of developing astrocytoma and meningioma (32). Van Der Logt *et al.* found that *PON1* does not contribute to the susceptibility to colorectal cancer (33).

In contrast to our findings, Stevens *et al.* suggested that *PON1* L55 M allele may be associated with an increased risk of breast cancer in postmenopausal women (18). Deroo *et al.* found that the PON1 rs757158 polymorphism was associated with breast cancer (34). Conesa-Zamora *et al.* showed that *PON1* GG genotype in rs662 polymorphism could be a risk factor for B-cell lymphoma (35). Vecka *et al.* suggested that PON1 plays an important role in pancreatic cancer, especially in patients with cancer-associated malnutrition (36). Marchesani *et al.* concluded that *PON1* appears to be associated with an increased risk of prostate cancer (37). In another study, women carrying the *PON1* 55 L allele, compared with women with the AA genotype, had an increased risk of ovarian epithelial carcinoma (38).

In the present study, we found a statistically significant increase in frequency of both the RR genotype and the R allele for *PON1* 192, and an increase in the MM genotype, and a decrease in the LL genotype and R allele for *PON1* 55 in patients with CLL. Antognelli *et al.* showed that *PON1* 192 polymorphism, and the R allele were associated with a decreased risk of developing breast cancer, while polymorphisms in *PON1* 55 were associated with an increased risk of this neoplasia (39). Antognelli *et al.* found that PON1 192/QQ was associated with a significant increased risk of prostate cancer compared to the *PON1* 192/RR genotype (20). Ahmed *et al.* thought that the R allele may protect against colorectal cancer (40). In another study, Lee *et al.* showed that carriers of the *PON1* 192 QQ genotype have an increased risk of lung cancer (21). It was suggested in previous studies that the Q to R substitution leads to the production of the enzyme against carcinogenic products of oxidative stress (20, 41). This theory is confirmed by studies showing that individuals either had

higher PON1 activity or had a higher frequency of R and L alleles (41, 42). Ozturk *et al.* concluded that QQ type PON1 enzyme activity may be protective against bladder carcinogenesis (43). Their findings support the notion that genotypical differences of *PON1* might contribute to prognosis and pathogenesis of bladder cancer. Lee *et al.* found that *PON1* polymorphism was associated with an increased risk of lung cancer. Individuals carrying the Q/Q genotype of the *PON1* gene were found to be at higher risk of developing lung cancer (21). Eom *et al.* suggested that the protective effect of *PON1* rs662 SNP against lung carcinogenesis, and the induction of oxidative stress, might be modulated by the interaction between *PON1* genetic polymorphisms and tobacco smoking (44). Fang *et al.* suggested that the *PON1* 55M allele is a risk factor in the development of cancer, in particular of breast cancer (45). Arpacı *et al.* concluded that the *PON1* 192 AA genotype may play an important role as a risk factor for ovarian cancer in the Turkish population, and that the A and L alleles may be associated with the early onset of disease (46).

We did not find any significant relationship in the genotypic and allelic frequency in the CML group regarding the *PON1* Q192R and *PON1* M/L55 polymorphisms. We conclude that the R allele of *PON1* Q192R gene polymorphism, and the MM genotype of *PON1* M/L55 gene polymorphism play a role in CLL susceptibility, while the LL genotype may protect against CLL in the Turkish population. However, this is the first study on *PON1* in CML and CLL, and study of a larger number of individuals is necessary.

Acknowledgements

References

1 Akkız H, Kuran S, Akgöllü E, Üsküdar O, Bekar A, Bayram S, Yıldırım S, Ülger Y, Kaya BY, Şansal M and Çınar E: Effect of *PON1* gene polymorphisms in Turkish patients with hepatocellular carcinoma. Meta Gene *1*: 93-101, 2013.

2 Connolly JL, Schnitt SJ, Wang HH, Longtine JA, Dvorak A and Dvorak HF: Principles of Cancer Pathology. *In*: Cancer Medicine. Kufe DW, Pollock RE and Weichselbaum RR (eds.). London, Hamilton-BC Decker, pp. 487-502, 2003.

3 Mackness B, Mackness MI, Arrol S,Turkie W and Durrington PN: Effect of the molecular polymorphisms of human paraoxonase (*PON1*) on the rate of hydrolysis of paraoxon. Br J Pharmacol *122*: 265-268, 1997.

4 Azarsiz E and Sonmez EY: Paraoxonase and clinical importance. Turk J Biochem *25*: 109-119, 2000.

5 Playfer JR, Eze LC, Bullen MF and Evans DA: Genetic polymorphism and interethnic variability of plasma paroxonase activity. J Med Genet *13*: 337-342, 1976.

6 Durrington PN, Mackness B and Mackness MI: Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol *21*: 473-480, 2001.

7 Aristizábal JA, Chandia M, Del Cañizo MC and Sánchez-Guijo F: Bone marrow microenvironment in chronic myeloid leukemia: implications for disease physiopathology and response to treatment. Rev Med Chil *142*: 599-605, 2014.

8 Palamarchuk A, Efanov A, Nazaryan N, Santanam U, Alder H, Rassenti L, Kipps T, Croce CM and Pekarsky Y: 13q14 deletions in CLL involve cooperating tumor suppressors. Blood *115*: 3916-3922, 2010.

9 Caligaris-Cappio F and Hamblin TJ: BCell chronic lymphocytic leukemia: a bird of a different feather. J Clin Oncol *17*: 399-408, 1999.

10 Kipps TJ: Chronic lymphocytic leukemia. Curr Opin Hematol *5*: 244–253, 1998.

11 Chiorazzi N, Rai KR and Ferrarini M: Chronic lymphocytic leukemia. N Engl J Med *352*: 804-815, 2005.

12 Demir V, Kahraman S, Katgı A, Pişkin Ö, Özsan GH, Demirkan F, Ündar B and Özcan MA: General clinical evaluation of the chronic lymphocytic leukemia patients. DEÜ TIP FAKÜLTESİ DERGİSİ *26*: 9-9, 2012.

13 Döhner H, S, Stilgenbauer S, Döhner K, Bentz M and Lichter P: Chromosome aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogeneticanalysis. J Mol Med *77*: 266-281, 1999.

14 Ruiz J, Blanche H, James RW, Garin MC, Vaisse C, Charpentier G, Cohen N, Morabia A, Passa P and Froguel P: Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet *346*: 869-872, 1995.

15 Akcay MN, Polat MF, Yilmaz I and Akcay G: Serum paraoxonase levels in pancreatic cancer. Hepatogastroenterology 50: ccxxv-ccxxvii, 2003.

16 Akcay MN, Yilmaz I, Polat MF and Akcay G: Serum paraoxonase levels in gastric cancer. Hepatogastroenterology 50: cclxxiii-cclxxv, 2003 .

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 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*: 1226-1228, 2006.

19 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*: 95-101, 2007.

20 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*: 240-251, 2005.

21 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 Pub Health *38*: 345-350, 2005.

22 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*: 477-481, 2002.

23 Lincz LF, Kerridge I, Scorgie FE, Bailey M, Enno A and Spencer A: Xenobiotic gene polymorphisms and susceptibility to multiple myeloma. Haematologica *89*: 628-629, 2004.

24 Ergen A, Kılıcoglu O, Ozger H, Agachan B and Isbir T: Paraoxonase 1 192 and 55 polymorphisms in osteosarcoma Mol Biol Rep *38*: 4181-4184, 2011.

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

26 https://ihg.gsf.de/cgi-bin/hw/hwa2.pl

27 Cejas P, Casado E, Belda-Iniesta C, De Castro J, Espinosa E, Redondo A, Sereno M, García-Cabezas MA, Vara JA, Domínguez-Cáceres A, Perona R and González-Barón M: Implications of oxidative stress and cell membrane lipid peroxidation in human cancer. Cancer Causes Control *15*: 707-719, 2004.

28 Behrend L, Henderson G and Zwacka RM: Reactive oxygen species in oncogenic transformation. Biochem Soc Trans *31*: 1441-1444, 2003.

29 Devarajan A, Shih D and Reddy ST: Inflammation, infection, cancer and all that…the role of paraoxonases. Adv Exp Med Biol *824*: 33-41, 2014.

30 Rajaraman P, Hutchinson A, Rothman N, Black PM, Fine HA, Loeffler JS, Selker RG, Shapiro WR, Linet MS and Inskip PD: Oxidative response gene polymorphisms and risk of adult braintumors. Neuro Oncol *10*: 709-715, 2008.

31 Uyar OA, Kara M, Erol D, Ardicoglu A and Yuce H: Investigating paraoxonase-1 gene Q192R and L55M polymorphism in patients with renal cell cancer. Genet Mol Res *10*: 133-139, 2011.

32 Martínez C, Molina JA, Alonso-Navarro H, Jiménez-Jiménez FJ, Agúndez JA and García-Martín E: Two common nonsynonymous paraoxonase 1 (*PON1*) gene polymorphisms and brain astrocytoma and meningioma. BMC Neurol *10*: 71, 2010.

33 Van Der Logt EM, Janssen CH, Van Hooijdonk Z, Roelofs HM, Wobbes T, Nagengast FM and Peters WH: No association between genetic polymorphisms in NAD(P)H oxidase p22[PHOX] and paraoxonase 1 and colorectal cancer risk. Anticancer Res *25*: 1465-1470, 2005.

34 Deroo LA, Bolick SC, Xu Z, Umbach DM, Shore D, Weinberg CR, Sandler DP and Taylor JA: Global DNA methylation and one-carbon metabolism gene polymorphisms and the risk of breast cancer in the Sister Study. Carcinogenesis *35*: 333-338, 2014.

35 Conesa-Zamora P, Ruiz-Cosano J, Torres-Moreno D, Español I, Gutiérrez-Meca MD, Trujillo-Santos J, Pérez-Ceballos E, González-Conejero R, Corral J, Vicente V and Pérez-Guillermo M: Polymorphisms in xenobiotic metabolizing genes (*EPHX1, NQO1* and *PON1*) in lymphoma susceptibility: a case–control study. BMC Cancer *13*: 228, 2013.

36 Vecka M, Jáchymová M, Vávrová L, Kodydková J, Macášek J, Urbánek M, Krechler T, Slabý A, Dušková J, Muravská A and Zák A: Paraoxonase-1 (*PON1*) status in pancreatic cancer: relation to clinical parameters. Folia Biol *58*: 231-237, 2012.

37 Marchesani M, Hakkarainen A, Tuomainen TP, Kaikkonen J, Pukkala E, Uimari P, Seppälä E, Matikainen M, Kallioniemi OP, Schleutker J, Lehtimäki T and Salonen JT: New paraoxonase 1 polymorphism I102V and the risk of prostate cancer in Finnish men. J Natl Cancer Inst *95*: 812-818, 2003.

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

39 Antognelli C, Del Buono C, Ludovini V, Gori S, Talesa VN, Crinò L, Barberini F and Rulli A: *CYP17, GSTP1, PON1* and *GLO1* gene polymorphisms as risk factors for breast cancer: an Italian case–control study. BMC Cancer *9*: 115, 2009.

40 Ahmed NS, Shafik NM, Elraheem OA and Abou-Elnoeman SE: Association of paraoxonase-1 (Q192R and L55M) gene polymorphisms and activity with colorectal cancer and effect of surgical intervention. Asian Pac J Cancer Prev *16*: 803-809, 2015.

41 Ferre' N, Camps J, Fernandez-Ballart J, Arija V, Murphy MH, Cerveco S, Biarnes E, Vilella E, Tous M and Joven J: Regulationof serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population. Clin Chem *49*: 1491–1497, 2003.

42 Mackness B, Durrington PN and Mackness MI: Human serum paraoxonase. Gen Pharmacol *31*: 329-336, 1998.

43 Oztürk O, Kağnici OF, Oztürk T, Durak H, Tüzüner 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*: 4041-4046, 2009.

44 Eom SY, Yim DH, Lee CH, Choe KH, An JY, Lee KY, Kim YD and Kim H: Interactions between Paraoxonase 1 Genetic polymorphisms and smoking and their effects on oxidative stress and lung cancer risk in a Korean population. PLoS One *10*: e0119100, 2015.

45 Fang DH, Fan CH, Ji Q, Qi BX, Li J and Wang L: Differential effects of paraoxonase 1 (*PON1*) polymorphisms on cancer risk: evidence from 25 published studies. Mol Biol Rep *39*: 6801-6809, 2012.

46 Arpaci A, Görmüs 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*: 421-424, 2009.