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Effect of PON1 gene polymorphisms in Turkish patients with hepatocellular carcinoma ☆

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ABSTRACT

Background: Reactive oxygen species (ROS) can oxidize biological molecules that mediate carcinogenesis by causing metabolic malfunction and damage to DNA. Human serum paraoxonases (PON1, PON2 and PON3) play a role in antioxidant defense and protect the cell against ROS. *PON1* polymorphisms Q192R and L55M have been shown to be associated with several human cancers, but their association with hepatocellular carcinoma (HCC) has yet to be investigated. *Methods*: We performed genotyping analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in a hospital-based case–control study of 217 confirmed HCC patients and 217 age-, gender-, smoking- and alcohol consumption-matched cancer-free controls in Turkish population. *Results*: Q192R and L55M polymorphisms were in significant linkage disequilibrium (LD) ($D' = 0.77$). However, allele, genotype and haplotype analysis showed no significant differences between the risks of HCC and PON1 polymorphisms. Moreover, no significant differences were found between clinical findings, clinico-pathological features and sex in comparison with the *PON1* genotypes in HCC group. *Conclusion*: Our results suggest for the first time that neither the Q192R polymorphism nor the L55M polymorphism has relationship with the risk of developing HCC. Further independent studies are required to clarify the possible role of *PON1* gene Q192R and L55M

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polymorphisms on the risk of developing HCC in a larger series and also in patients of different ethnic origins.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer death. The incidence and mortality rates of HCC are approximately equal because it has high fatality rates (Parkin et al., 2005). It is now well established that multiple risk factors contribute to hepatocarcinogenesis, including chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, cirrhosis, carcinogen exposure (such as aflatoxin B1), oxidative stress, excessive alcohol drinking, and a number of genetic and epigenetic alterations (El-Serag and Rudolph, 2007; Farazi and DePinho, 2006). Although HBV and HCV infections are the major cause of HCC, only a fraction of infected patients develop HCC during their lifetime; therefore, the identification of other risk characteristics (such as genetic polymorphisms) to stratify those individuals into high-risk populations is needed.

Reactive oxygen species (ROS) can oxidize biological molecules that mediate carcinogenesis by causing metabolic malfunction and damage to DNA (Cejas et al., 2004). Human serum paraoxonases (PON1, PON2 and PON3) are a family of calcium-dependent hydrolases that are involved in antioxidant defense and the metabolism of various organophosphorus compounds, including paraoxon, neurotoxins and insecticides (Cowan et al., 2001; Mackness et al., 1998). Several studies have indicated that PON1 enzymatic activity is decreased in chronic hepatitis, steatohepatitis, and liver cirrhosis (LC) patients when compared with that in healthy people (Baskol et al., 2005; Ferré et al., 2002). Additionally, the authors reported that determination of low PON1 activity may serve as a useful additional test in assessing liver diseases including acute viral hepatitis B, chronic alcoholic hepatitis, cirrhosis with portal hypertension, HCV-related cirrhosis, hereditary hemochromatosis, and non-alcoholic fatty liver disease (NAFLD) (Keskin et al., 2009; Matineli et al., 2013; Mogarekar and Talekar, 2013).

Genetic polymorphisms in the coding region of the PON1 gene, such as Q192R, L55M, -909 (C/G), -832 (A/G), -162 (A/G), -126 (C/G) and -108 (C/T) may alter the expression and activity of the PON1 enzyme. Especially PON1 Q192R, L55M and -108 C/T polymorphisms are responsible for an up to 13-fold interpersonal variation in PON1 enzyme activity and concentration (Kang et al., 2013; Macharia et al., 2012). *PON1* polymorphisms are characterized by amino acid substitution of leucine to methionine at the 55th position (L55M) and by a change from glutamine to arginine at position 192 (Q192R) (Leviev et al., 2001). Epidemiologic and molecular studies revealed that the PON1 activity of the *PON1* 192 Q and the *PON1* 55 L allele carriers was reported to be lower than that of the other allele carriers (Davies et al., 1996; Li et al., 2000; Mackness et al., 1997). Several case–control studies have investigated the association between polymorphisms of *PON1* gene and cancer risk, such as brain, breast, bladder, colorectal and ovarian (Yuzhalin and Kutikhin, 2012). However, the results have not been consistent.

According to our recent knowledge, no research has been conducted to evaluate *PON1* Q192R and L55M polymorphisms and risk of HCC. To test the hypothesis that the polymorphisms of *PON1* gene Q192R and L55M are associated with the risk of developing HCC, we performed genotyping analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in a hospital-based case–control study of 217 confirmed HCC patients and 217 age-, gender-, smoking- and alcohol consumption-matched cancer-free controls in Turkish population.

2. Patients and methods

The study was approved by the Committee for Ethics of Medical Experiment on Human Subjects, Faculty of Medicine, Çukurova University, Adana. Submission of the individuals to the study was conditioned by an obtained written informed consent form regarding the use of their blood samples for research studies. The study proceeded in agreement with the Helsinki declaration approved on the World Medical Association meeting in Edinburgh. Two hundred and seventeen consecutively diagnosed patients with HCC were enrolled as the case group. During the same time, 217 unrelated community

residents who entered the hospital for health check-ups were enrolled as the control group. Each healthy control was pair-matched by sex, age (±2 years), smoking and alcohol consumption to a patient with HCC.

The HCC diagnostic criteria were based on the guideline proposed by the European Association for the Study of the Liver (EASL) (Bruix et al., 2001). We gave a diagnosis of HCC when a patient had one or more risk factors (i.e. hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, or cirrhosis) and one of the following: >400 ng/mL α-fetoprotein (AFP) and at least one positive finding following examination using spiral computed tomography (CT), contrast-enhanced dynamic Magnetic Resonance Imaging (MRI), or hepatic angiography; or <400 ng/mL AFP and at least two findings following CT, MR, or hepatic angiography. A positive HCC finding using dynamic CT or MRI is indicative of arterial enhancement followed by venous washout in the delayed portal/venous phase. In addition, we performed histopathological diagnosis for cases that did not fulfill all of the clinical noninvasive diagnostic criteria of HCC. Cirrhosis was diagnosed with liver biopsy, abdominal sonography and biochemical evidence of parenchymal damage plus endoscopic esophageal or gastric varices (Tsai et al., 1994). Patients with cirrhosis were classified into three Child–Pugh grades based on their clinical status (Pugh et al., 1973). Serum HBsAg and anti-HCV were assessed using an immunoassay (Abbott Laboratories, Abbott Park, IL, USA). Serum AFP concentration was measured by microparticle enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA). Heavy alcohol intake was defined as a daily minimum consumption of 160 g alcohol for at least 8 years. Technicians who performed the blood tests were blinded to the identity and disease status of the participants. Peripheral blood samples were taken from patients and controls in the Department of Gastroenterology between September 2005 and December 2012. Blood specimens were stored at +4 °C and serum specimens were frozen at −20 °C until analysis.

3. *PON1* gene Q192R and L55M genotype detection

Genotyping for the *PON1* gene Q192R and L55M polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as described previously (Rajković et al., 2011). Genomic DNA was extracted from peripheral whole blood using High Pure PCR Template Preparation Kit (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's protocol. For detection of the Q192R and L55M polymorphisms, a 238-base pair (bp) and a 172-bp fragment were amplified by using primers 5′-TAT TGT TGC TGT GGG ACC TGA G-3′ (forward), 5′-CCT GAG AAT CTG AGT AAA TCC ACT-3′ (reverse) and 5′-TGA AAG ACT TAA ACT GCC AGT C-3′ (forward), and 5′-CCT GCA ATA ATA TGA AAC AAC CTG-3′ (reverse), respectively. Amplification was performed in GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Singapore). The 20 μL PCR mixture contained approximately 250 ng DNA, with 0.25 μM of both primers, 0.1 mM of each dNTP, 1× PCR buffer, 1.5 mM $MgCl_2$ and 1 U Taq polymerase (Promega, Madison, WI, USA). The following cycling conditions were used: 95 °C for 5 min, followed by 35 cycles of 94 °C for 60 s, 58 °C for 60 s (57 °C for L55M) and 72 °C for 60 s, with a final extension at 72 °C for 10 min. After confirmation of successful PCR amplification by 1.5% agarose gel electrophoresis, each PCR product was digested overnight with 5 U AlwI (for Q192R) and 5 U NlaIII (for L55M) restriction endonuclease enzymes at 37 °C (New England Biolabs Inc., Beverly, MA) and electrophoresed on 3% agarose gel containing 0.5 μg/mL ethidium bromide and visualized under UV illumination. Samples yielding the 175- and 63-bp fragments were scored as RR, 238, 175, and 63 bp fragments as QR, and 238 bp as QQ. For L55M, samples yielding the 107- and 65-bp fragments were scored as MM, 172, 103, and 65 bp fragments as LM, and 172 bp as LL.

4. Statistical analysis

Effective sample sizes for case–control study, and for obtaining 80% power was calculated by Quanto 1.1 ver. software (http://hydra.usc.edu/gxe) using minor allele frequency data from HapMap (http://hapmap.ncbi.nlm.nih.gov/). Data analysis was performed using the computer software Statistical Package for Social Sciences (SPSS; SPSS, Inc., Chicago, IL, USA) for Windows (version 10.0). Continues variables are presented as the mean (standard deviation, SD) or median (min — max) for abnormal distributions and categorical variables are presented as frequencies (%). Comparisons in the distributions of demographical characteristics between the patients with hepatocellular carcinoma and control subjects were evaluated

using Student's *t*-test or Mann–Whitney *U* test for continuous variables (each when adequate) depending on their Gaussian distribution and chi-square test for categorical variables. The observed genotype frequencies were compared with expected values calculated from Hardy–Weinberg equilibrium theory. Significant variables after univariate regression analysis were entered into a stepwise logistic regression analysis to identify factor of HCC risk. Statistical analysis of genotypes, haplotype estimation and linkage disequilibrium (LD) were analyzed using the website for SNP statistics: http://bioinfo.iconcologia.net/snpstats/start.htm. Logistic regression analysis was used to analyze the association of genotypes in inheritance models (codominant, dominant, recessive, overdominant and log-additive) in the case and control groups. Results are expressed as odds ratios with 95% confidence interval (CI). All tests were two-sided and *P* value < 0.05 was considered significant.

5. Results

The clinical and demographic characteristics of the 434 Turkish subjects are shown in Table 1. The characteristics of patients with HCC and those of control subjects did not differ with regard to age, sex, smoking status and alcohol consumption.

5.1. The allele and genotype frequencies of PON1 gene Q192R and L55M polymorphisms

The overall allelic frequencies of *PON1* gene Q192R polymorphism were 72.7% and 27.3% for Q and R, respectively. On the other hand, the overall allelic frequencies for L55M polymorphism were 67.1% and 32.9% for L and M, respectively. There was no association between the control group and the HCC group for L55M and Q192R allele frequencies. In addition, the genotype frequencies of Q192R and L55M polymorphisms were not significantly different between the two groups ($P = 0.79$, $P = 0.69$, respectively). The patient and

Table 1
Distribution of selected characteristics in patients with hepatocellular carcinoma and controls.

Characteristic	Patients (%) (n = 217)	Controls (%) (n = 217)	*P*-value*
Age (years) mean (±SD) (range)	61.63 ± 10.95 (20–87)	60.23 ± 10.97 (20–90)	NS
Male sex	168 (77.4)	168 (77.4)	NS
Female sex	49 (22.6)	49 (22.6)	NS
Smoking status			NS
Ever	121 (55.9)	121 (55.9)	
Never	96 (44.1)	96 (44.1)	
Alcohol status			NS
Drinker	67 (31.1)	67 (31.1)	
Nondrinker	150 (68.9)	150 (68.9)	
Viral infection			
HBsAg positive	125 (57.7)	–	
Anti-HCVAb positive	51 (23.5)	–	
Both positive	4 (2)	–	
Both negative	37 (16.8)	–	
Liver cirrhosis			
Present	198 (91.2)	–	
Absent	19 (8.8)	–	
Tumor size (cm)			
≤5	111 (51.2)	–	
>5	106 (48.8)	–	
Child–Pugh classification			
A	54 (28.7)	–	
B	80 (37.1)	–	
C	64 (34.2)	–	
α-fetoprotein (ng/mL)			
<100	116 (53.5)	–	
100–400	29 (13.5)	–	
>400	72 (33)	–	

NS, not significant, n = total number of case patients or control subjects. *P-values were derived from Pearson's chi-square test except age; Student's *t*-test was used for age. All *P*-values are two-sided.

control frequencies were also in Hardy–Weinberg equilibrium and no selection bias (for Q192R frequency of the patients and controls; n: 217, df: 1, x^2: 1; n: 217, df1, x^2: 0.97, respectively. For L55M frequencies of the patients and controls; n: 217, df: 1, x^2: 0.94; both of them were the same). The allele and genotype frequencies are shown in Table 2.

5.2. The association between PON1 (Q192R, L55M) genotypes and HCC risk

To identify whether there was a statistically significant increased risk of HCC in terms of the *PON1* Q192R and L55M genotypes, we performed logistic regression analysis (Table 2). For the Q192R polymorphism, logistic regression analysis showed that subjects heterozygous for the QR genotype had a 1.16-fold increased risk of developing HCC compared those with RR genotype but not statistically significant associated with the risk of HCC (OR = 1.16; 95% 0.52–2.61; $P = 0.72$). Similarly, the homozygous QQ genotype had also increased risk but not statistically significant associated with the risk of HCC (OR = 1.02; 95% 0.46–2.27; $P = 0.96$). For the L55M polymorphism, logistic regression analysis showed that subjects homozygous for the MM genotype had a 1.1-fold increased risk of developing HCC compared those with LL genotype but not statistically significant

Table 2
Allele frequency and model inheritance for PON1 Q192R and L55M polymorphisms among cases and controls as well as the association with hepatocellular cancer risk.

	Cases (%), n = 217	Control (%), n = 217	OR (95% CI)	*P*-value[a]	AIC[b]	BIC[c]
Q192R						
Allele frequency						
R	121 (27.9)	116 (26.7)	1.00 (Reference)		–	–
Q	313 (72.1)	318 (73.3)	1.02 (0.94–1.10)	0.70	–	–
Codominant					607.2	619.4
RR	13(6)	14 (6.5)	1.00 (Reference)			
QR	95 (43.8)	88 (40.5)	1.16 (0.52–2.61)	0.72		
QQ	109 (50.2)	115 (53)	1.02 (0.46–2.27)	0.96		
Dominant					605.6	613.8
RR	13 (6)	14 (6.5)	1.00 (Reference)			
QR + QQ	204 (94)	203 (93.5)	1.08 (0.50–2.36)	0.84		
Recessive					605.3	613.5
RR + QR	108 (49.8)	102 (47)	1.00 (Reference)			
QQ	109 (50.2)	115 (53)	0.90 (0.61–1.30)	0.57		
Overdominant					605.2	613.3
RR + QQ	122 (56.2)	129 (59.5)	1.00 (Reference)			
QR	95 (43.8)	88 (40.5)	1.14 (0.78–1.67)	0.5		
Log-additive	–	–	0.94 (0.69–1.28)	0.69	605.5	613.6
L55M						
Allele frequency						
L	291 (67.1)	291 (67.1)	1.00 (Reference)			
M	143 (32.9)	143 (32.9)	1.00 (0.83–1.21)	1.00		
Codominant					606.9	619.1
LL	105 (48.4)	101 (46.5)	1.00 (Reference)			
LM	81 (37.3)	89 (41)	0.86 (0.58–1.31)	0.52		
MM	31 (14.3)	27 (12.5)	1.10 (0.62–1.98)	0.74		
Dominant					605.5	613.6
LL	105 (48.4)	101 (46.5)	1.00 (Reference)			
LM + MM	112 (51.6)	116 (53.5)	0.93 (0.64–1.35)	0.70		
Recessive					605.3	613.5
LL + LM	186 (85.7)	190 (87.6)	1.00 (Reference)			
MM	31 (14.3)	27 (12.4)	1.17 (0.67–2.04)	0.57		
Overdominant					605	613.2
LL + MM	136 (62.7)	128 (59)	1.00 (Reference)			
LM	81 (37.3)	89 (41)	0.86 (0.58–1.26)	0.43		
Log-additive	–	–	1.00 (0.76–1.31)	1.00	605.7	613.8

[a] Data were calculated by logistic regression analysis.
[b] AIC: Akaike's information criterion.
[c] BIC: Bayesian information criterion.

Table 3
Association between clinicopathological features, alcohol consumption, and sex according to the PON1 genotypes in HCC group.

Q192R genotypes, n (%)					L55M genotypes, n (%)			
Valuables	RR	QR	QQ	*P* value	LL	LM	MM	*P* value
Sex				0.81[b]				0.83[b]
Male	11 (6.5)	73 (43.5)	84 (50)		83 (49.4)	61 (36.3)	24 (14.3)	
Female	2 (4.1)	22 (44.9)	25 (51)		22 (44.9)	20 (40.8)	7 (14.3)	
Alcohol status				0.72[b]				0.92[b]
Drinker	4 (5.9)	32 (47.8)	31 (46.3)		31 (46.3)	26 (38.8)	10 (14.9)	
Non-drinker	9 (6.0)	63 (42)	78 (52)		74 (49.3)	55 (36.7)	21 (14)	
Smoking status				0.17[b]				0.99[b]
Ever	4 (3.3)	54 (44.6)	63 (52.1)		59 (48.8)	45 (37.2)	17 (14)	
Never	9 (9.4)	41 (42.7)	46 (47.9)		46 (47.9)	36 (37.5)	14 (14.6)	
Tumor size (cm)				0.13[b]				0.13[b]
≤5	5 (4.5)	43 (38.7)	63 (56.8)		61 (55)	37 (33.3)	13 (11.7)	
>5	8 (7.5)	52 (49.1)	46 (43.4)		44 (41.5)	44 (41.5)	18 (17)	
Child–Pugh class. (n = 198)				0.17[b]				0.21[b]
A	6 (11.1)	19 (35.2)	29 (53.7)		27 (50)	17 (31.5)	10 (18.5)	
B	6 (7.5)	25 (31.2)	49 (61.3)		34 (42.5)	37 (46.2)	9 (11.3)	
C	7 (10.9)	31 (48.4)	26 (40.6)		36 (56.3)	19 (29.6)	9 (14.1)	
α-fetoprotein (ng/mL)				0.77[b]				0.15[b]
≤400	9 (6.2)	61 (42.1)	75 (51.7)		73 (50.3)	56 (38.7)	16 (11)	
>400	4 (5.6)	34 (47.2)	34 (47.2)		32 (44.4)	25 (34.8)	15 (20.8)	
ALT (U/L)[†]	46 (18–346)	46 (11–421)	46 (7–324)	0.90[a]	46.5 (7–421)	53 (11–173)	30 (13–181)	0.37[a]
AST (U/L)[†]	78 (31–375)	82 (16–439)	70 (14–721)	0.49[a]	76.5 (14–439)	77.5 (16–721)	61 (20–401)	0.85[a]
Albumin (g/L)[†]	3 (1.8–4.4)	2.8 (2–4.7)	3.1 (1.9–5.1)	0.67[a]	2.95 (1.9–5.1)	2.95 (1.8–4.7)	3.2 (2.0–4.1)	0.51[a]
Ca (mg/dL)[†]	8.8 (7.6–10.3)	8.7 (7–11.6)	8.7 (7.4–10.9)	0.97[a]	8.7 (7.0–11.3)	8.6 (7.4–11.6)	9.1 (7.9–10.2)	0.11[a]
PT (s)[†]	13.6 (11.6–20)	15.9 (11.9–26.2)	15.1 (11.5–39)	0.16[a]	15.6 (11.6–39)	15.9 (11.5–29.6)	14.4 (11.5–19.9)	0.054[a]
INR[†]	1.2 (0.9–1.9)	1.3 (0.9–2.4)	1.3 (0.9–2.9)	0.67[a]	1.3 (0.9–2.9)	1.3 (0.9–2.3)	1.2 (1.0–1.8)	0.47[a]

Ca: Calcium. PT: Prothrombin time. INR: International normalized ratio. ALT: Alanine transaminase. AST: Aspartate aminotransferase.
[a] *P* value was calculated by Kruskal–Wallis test.
[b] *P* value was calculated by *chi-square test*.
[†] : Median(min — max).

associated with the risk of HCC (OR = 1.1; 95% 0.62–1.98; $P = 0.74$). Moreover, no significant differences were found between clinical findings, clinicopathological features and sex in comparison with the *PON1* genotypes in HCC group (Table 3).

5.3. Linkage disequilibrium and haplotype analysis of PON1 Q192R and L55M polymorphisms in case and control groups

On linkage disequilibrium (LD) analysis, PON1 Q192R polymorphism was found to be in strong LD with PON1 L55M polymorphism ($D' = 0.77$). However, as shown in Table 4, no significant association was found between neither global test nor individual haplotypes and the susceptibility to hepatocellular carcinoma.

6. Discussion

Single nucleotide polymorphisms in many functionally critical genes have been suggested as a risk factor for a variety of cancers, including HCC (Kato et al., 2005; Kim and Lee, 2005). For multifactorial diseases such as HCC, the identification of regulator genes should make it possible to identify hepatocarcinogenic risk factors. The prevention of HCC may be ensured in HBV carriers and in HCV infected persons through determination of genetic biomarkers (Ludwig and Weinstein, 2005). The present investigation was conducted to explore the association between the *PON1* (Q192R, L55M) polymorphisms and hepatocellular carcinoma in a Turkish population. Since the serum paraoxonase enzyme hydrolyzes activated organophosphates in the bloodstream, *PON1* (Q192R, L55M) polymorphisms were selected as the candidate polymorphism. Oxidative stress occurring through activated organophosphates has been associated with an increased risk of various types of cancers (Ames, 1983; Klaunig and Kamendulis, 2004). Therefore, the paraoxonase enzyme has a critical role in protection of cells from organophosphate intoxication (Cowan et al., 2001). Additionally, the relationship between *PON1* polymorphisms and the risk of HCC has not been investigated so far.

Sun et al. reported that the protein expression level of PON1 in both LC and early HCC decreased to about half compared with those in healthy controls (Sun et al., 2012). Crystal structure analysis indicated that Q192R influences the active histidine dyad of PON1. Consequently, the catalytic consequences are different. It is suggested that the R allele may lead to the production of PON1 enzyme with higher detoxification activity against reactive oxygen species and lipid peroxidation. On the other hand, the M variant decreases the stability of the PON1 enzyme, thus lowering the concentration of PON1 which subsequently affects the activity of the enzyme. PON1 activity has therefore been suggested as a marker for liver impairment (Riedmaier et al., 2011; Sun et al., 2012).

According to the previous results, the R and M alleles of *PON1* gene between the populations are distributed as follows; 0.59 and 0.09 in Japanese, 0.57 and 0.04 in Chinese, 0.31 and 0.21 in Indian, 0.26 and 0.36 in British, and 0.24 and 0.38 in French, respectively (Aynacioglu et al., 1999). Our findings were 0.27 and 0.33 for R and M, respectively. In this study, the distribution of *PON1* gene (Q192R, L55M) polymorphism genotypes and alleles was not different between HCC cases and controls. Statistical analysis revealed that there were not significant relationship between the risk of HCC and *PON1* (Q192R, L55M) polymorphisms. Thus, *PON1* polymorphisms seem to have no direct effect on the risk of HCC. Our results are in agreement with previous findings showing that there was no relationship between *PON1* (Q192R and L55M) polymorphisms and risk of various cancers including brain cancer (Martínez et al., 2010),

Table 4
Frequency distribution of haplotypes of PON1 Q192R and L55M polymorphisms in HCC and healthy controls.

Haplotypes PON1 (Q192R and L55M)	Frequency		OR (95% CI)	*P*-value
	Cases	Controls		
QL	0.4171	0.4238	1.00 (Reference)	–
QM	0.3042	0.3119	0.99 (0.72–1.37)	0.95
RL	0.2562	0.2464	1.06 (0.74–1.52)	0.74
RM	0.0225	0.0179	1.31 (0.36–4.83)	0.69

Global haplotype association *P*-value: 0.95.

colorectal cancer (Van Der Logt et al., 2005), multiple myeloma (Gold et al., 2009), breast cancer (for Q192R) (Hussein et al., 2011; Stevens et al., 2006) and lung cancer (for L55M) (Aksoy-Sagirli et al., 2011; Wang et al., 2012). On the contrary, a relationship with some cancers including breast cancer (for L55M) (Hussein et al., 2011; Stevens et al., 2006), lung cancer (for Q192R) (Aksoy-Sagirli et al., 2011; Wang et al., 2012), non-Hodgkin's lymphoma cancer (De Roos et al., 2006; Kerridge et al., 2002), prostate cancer (Stevens et al., 2008) and ovarian cancer (Lurie et al., 2008) has already been shown. Furthermore, Fang et al. reported a meta analysis that the M allele of L55M polymorphism is a major marker for risk of cancer susceptibility, but there was no relationship between the Q192R and cancer risk (Fang et al., 2012; Yuzhalin and Kutikhin, 2012). It is possible that the significant difference in the results of studies may be due to differences in the studied population, as well as on several environmental and other factors that influence that population.

There are a number of limitations to this study. Firstly, patients were selected at a single institution (Çukurova University Balcalı Hospital in Adana from South Turkey) and thus may have been unrepresentative of HCC patients in the general population. In addition, it should be noted that the control subjects were recruited at the same hospital. Furthermore, the patient and control frequencies were also in Hardy–Weinberg equilibrium and there was no selection bias according to Hardy–Weinberg model. Secondly, we limited our study to a Turkish population owing to variation in allele frequencies between different ethnic groups which have been observed for the Q192R and L55M polymorphisms. Thirdly, this study had only focused on two loci on a single gene without taking into consideration gene–environment interactions, gene–gene interactions, *PON1* gene expression and interactions between different loci on the *PON1* gene, which may affect the risk of HCC. Fourth, although the desired power of our study is set at 80%, this study is limited by the relatively small number of the subgroup analysis.

In conclusion, our results demonstrate for the first time that *PON1* gene Q192R and L55M polymorphisms have no major effect on the risk of HCC. Further independent studies are required to clarify the possible role of *PON1* gene Q192R and L55M polymorphisms in a larger series, as well as in patients of different ethnic origins and to better understand the mechanisms between *PON1* gene Q192R and L55M polymorphisms and the developing risk of HCC. Additionally, further independent studies should include measurement of the enzyme activity and its genetic polymorphisms.

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Conflict of interest statement

All of the authors declare no conflicts of interest.

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References

Aksoy-Sagirli, P., et al., 2011. Paraoxonase-1 192/55 polymorphisms and the risk of lung cancer in a Turkish population. Anticancer Res. 31, 2225–2229.

Ames, B.N., 1983. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science 221, 1256–1264.

Aynacioglu, A.S., Cascorbi, I., Mrozikiewicz, P.M., Nacak, M., Tapanyigit, E.E., Roots, I., 1999. Paraoxonase 1 mutations in a Turkish population. Toxicol. Appl. Pharmacol. 157, 174–177.

Baskol, M., Baskol, G., Deniz, K., Ozbakir, O., Yücesoy, M., 2005. A new marker for lipid peroxidation: serum paraoxonase activity in non-alcoholic steatohepatitis. Turk. J. Gastroenterol. 16, 119–123.

Bruix, J., et al., 2001. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J. Hepatol. 35, 421–430.

Cejas, P., et al., 2004. Implications of oxidative stress and cell membrane lipid peroxidation in human cancer (Spain). Cancer Causes Control 15, 707–719.

Cowan, J., Sinton, C.M., Varley, A.W., Wians, F.H., Haley, R.W., Munford, R.S., 2001. Gene therapy to prevent organophosphate intoxication. Toxicol. Appl. Pharmacol. 173, 1–6.

Davies, H.G., Richter, R.J., Keifer, M., Broomfield, C.A., Sowalla, J., Furlong, C.E., 1996. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. Nat. Genet. 14, 334–336.
De Roos, A.J., et al., 2006. Metabolic gene variants and risk of non-Hodgkin's lymphoma. Cancer Epidemiol. Biomark. Prev. 15, 1647–1653.
El-Serag, H.B., Rudolph, K.L., 2007. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132, 2557–2576.
Fang, D.H., Fan, C.H., Ji, Q., Qi, B.X., Li, J., Wang, L., 2012. Differential effects of paraoxonase 1 (PON1) polymorphisms on cancer risk: evidence from 25 published studies. Mol. Biol. Rep. 39, 6801–6809.
Farazi, P.A., DePinho, R.A., 2006. Hepatocellular carcinoma pathogenesis: from genes to environment. Nat. Rev. Cancer 6, 674–687.
Ferré, N., et al., 2002. Serum paraoxonase activity: a new additional test for the improved evaluation of chronic liver damage. Clin. Chem. 48, 261–268.
Gold, L.S., et al., 2009. Associations of common variants in genes involved in metabolism and response to exogenous chemicals with risk of multiple myeloma. Cancer Epidemiol. 33, 276–280.
Hussein, Y.M., Gharib, A.F., Etewa, R.L., ElSawy, W.H., 2011. Association of L55M and Q192R polymorphisms in paraoxonase 1 (PON1) gene with breast cancer risk and their clinical significance. Mol. Cell. Biochem. 351, 117–123.
Kang, Y.H., et al., 2013. Association of PON1 genotype and haplotype with susceptibility to coronary artery disease and clinical outcomes in dual antiplatelet-treated Han Chinese patients. Eur. J. Clin. Pharmacol. 69, 1511–1519.
Kato, N., et al., 2005. Large-scale search of single nucleotide polymorphisms for hepatocellular carcinoma susceptibility genes in patients with hepatitis C. Hepatology 42, 846–853.
Kerridge, I., Lincz, L., Scorgie, F., Hickey, D., Granter, N., Spencer, A., 2002. Association between xenobiotic gene polymorphisms and non-Hodgkin's lymphoma risk. Br. J. Haematol. 118, 477–481.
Keskin, M., et al., 2009. Baseline and salt-stimulated paraoxonase and arylesterase activities in patients with chronic liver disease: relation to disease severity. Intern. Med. J. 39, 243–248.
Kim, Y.J., Lee, H.S., 2005. Single nucleotide polymorphisms associated with hepatocellular carcinoma in patients with chronic hepatitis B virus infection. Intervirology 48, 10–15.
Klaunig, J.E., Kamendulis, L.M., 2004. The role of oxidative stress in carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 44, 239–267.
Leviev, I., Deakin, S., James, R.W., 2001. Decreased stability of the M54 isoform of paraoxonase as a contributory factor to variations in human serum paraoxonase concentrations. J. Lipid Res. 42, 528–535.
Li, W.F., et al., 2000. Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying organophosphorus compounds. Pharmacogenetics 10, 767–779.
Ludwig, J.A., Weinstein, J.N., 2005. Biomarkers in cancer staging, prognosis and treatment selection. Nat. Rev. Cancer 5, 845–856.
Lurie, G., et al., 2008. Genetic polymorphisms in the paraoxonase 1 gene and risk of ovarian epithelial carcinoma. Cancer Epidemiol. Biomarkers Prev. 17, 2070–2077.
Macharia, M., Hassan, M.S., Blackhurst, D., Erasmus, R.T., Matsha, T.E., 2012. The growing importance of PON1 in cardiovascular health: a review. J. Cardiovasc. Med. (Hagerstown) 13, 443–453.
Mackness, B., Mackness, M.I., Arrol, S., Turkie, W., Durrington, P.N., 1997. Effect of the molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon. Br. J. Pharmacol. 122, 265–268.
Mackness, B., Durrington, P.N., Mackness, M.I., 1998. Human serum paraoxonase. Gen. Pharmacol. 31, 329–336.
Martínez, C., Molina, J.A., Alonso-Navarro, H., Jiménez-Jiménez, F.J., Agúndez, J.A., García-Martín, E., 2010. Two common nonsynonymous paraoxonase 1 (PON1) gene polymorphisms and brain astrocytoma and meningioma. BMC Neurol. 19 (10), 71.
Matineli, N., et al., 2013. Paraoxonase-1 status in patients with hereditary hemochromatosis. J. Lipid Res. 54, 1484–1492.
Mogarekar, M.R., Talekar, S.J., 2013. Serum lactonase and arylesterase activities in alcoholic hepatitis and hepatitis B. Indian J. Gastroenterol. 32, 307–310.
Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., 2005. Global cancer statistics, 2002. CA Cancer J. Clin. 55, 74–108.
Pugh, R.N., Murray-Lyon, I.M., Dawson, J.L., Pietroni, M.C., Williams, R., 1973. Transection of the esophagus for bleeding esophageal varices. Br. J. Surg. 60, 646–649.
Rajković, M.G., Barišić, K., Juretić, D., Grubišić, T.Ž., Flegar-Meštrić, Z., Rumora, L., 2011. Polymorphisms of pon1 and pon2 genes in hemodialyzed patients. Clin. Biochem. 44, 964–968.
Riedmaier, S., Klein, K., Winter, S., 2011. Paraoxonase (PON1 and PON3) polymorphisms: impact on liver expression and atorvastatin-lactone hydrolysis. Front. Pharmacol. 2, 41.
Stevens, V.L., Rodriguez, C., Pavluck, A.L., Thun, M.J., Calle, E.E., 2006. 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.
Stevens, V.L., et al., 2008. Paraoxonase 1 (PON1) polymorphisms and prostate cancer in the CPS-II Nutrition Cohort. Prostate 68, 1336–1340.
Sun, C., Chen, P., Chen, Q., 2012. Serum paraoxonase 1 heteroplasmon, a fucosylated, and sialylated glycoprotein in distinguishing early hepatocellular carcinoma from liver cirrhosis patients. Acta Biochim. Biophys. Sin. (Shanghai) 44, 765–773.
Tsai, J.F., Chang, W.Y., Jeng, J.E., Ho, M.S., Lin, Z.Y., Tsai, J.H., 1994. Hepatitis B and C virus infection as risk factors for liver cirrhosis and cirrhotic hepatocellular carcinoma: a case–control study. Liver 14, 98–102.
Van Der Logt, E.M., et al., 2005. No association between genetic polymorphisms in NAD(P)H oxidase p22phox and paraoxonase 1 and colorectal cancer risk. Anticancer Res. 25, 1465–1470.
Wang, H., Li, L., Ding, L., Zhang, Z., Pu, C., 2012. Association of genetic polymorphisms in the paraoxonase 1 gene with the risk and prognosis of non-small cell lung cancer in Chinese Han population. J. Investig. Med. 60, 592–597.
Yuzhalin, A.E., Kutikhin, A.G., 2012. Common genetic variants in the myeloperoxidase and paraoxonase genes and the related cancer risk: a review. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 30, 287–322.