Pathol. Oncol. Res. (2010) 16:533–540
DOI 10.1007/s12253-010-9267-5

Genetic Polymorphisms of Paraoxonase 1 (PON1) Gene: Association Between L55M or Q192R with Breast Cancer Risk and Clinico-Pathological Parameters

Rakesh Naidu · Yip Cheng Har · Nur Aishah Mohd Taib

Received: 13 January 2010 / Accepted: 29 March 2010 / Published online: 15 April 2010

Abstract The aim of the present study was to evaluate the association between the paraoxonase 1 (PON1) L55M and Q192R polymorphisms and breast cancer risk as well as clinico-pathological characteristics of the patients. Genotyping of these polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in a hospital-based Malaysian population. Peripheral blood samples were collected from 387 breast cancer patients and 252 normal and healthy women who had no history of any malignancy. The genotype (P=0.023) and allele (P=0.008) frequencies of L55M polymorphism were significantly different between the breast cancer cases and normal individuals. However, the distribution of genotype (P=0.333) and allele (P=0.163) frequencies of Q192R polymorphism showed lack of statistical significance. Women who were MM homozygotes (OR=2.229; 95% CI, 1.219–4.075) and carriers of M allele genotype (OR=1.429; 95% CI, 1.035–1.974) or M allele (OR=1.397; 95% CI, 1.093–1.785) were associated with increased risk of breast cancer. However, women who were heterozygous (OR=0.793; 95% CI, 0.567–1.110) or homozygous (OR=0.746; 95% CI, 0.407–1.370) for R allele or carriers of R allele (OR=0.838; 95%, 0.654–1.074) were not associated with breast cancer risk. The M allele genotype was significantly associated with estrogen receptor negativity (P=0.046) and nodal involvement (P=0.004) but R allele genotype was not associated with any of the clinico-pathological characteristics. In conclusion, our findings suggest that the polymorphic variant of L55M polymorphism could be a useful genetic marker for tumor prognosis and to identify women who might be at greater risk of developing breast cancer in a hospital-based Malaysian population.

Keywords Breast cancer · Paraoxonase 1 · Polymorphism

R. Naidu (✉)
School of Medicine and Health Sciences,
Monash University Sunway Campus,
Jalan Lagoon Selatan,
46150 Bandar Sunway, Selangor Darul Ehsan,
Malaysia
e-mail: kdrakeshna@hotmail.com

Y. C. Har · N. A. M. Taib
Department of Surgery, University of Malaya,
50603 Kuala Lumpur, Malaysia

Y. C. Har · N. A. M. Taib
University Malaya Medical Center, University of Malaya,
50603 Kuala Lumpur, Malaysia

Introduction

The PON1 gene encodes paraoxonase 1 protein which is predominantly synthesized in the liver, and found associated with high-density lipoproteins (HDLs) in the serum [1]. This has led to the suggestion that the enzyme might have a role in lipid metabolism. The protein is also part of the endogenous free-radical scavenging system that catalyses the hydrolysis of toxic organophosphate compounds, lactones, aromatic esters including estrogen monoesters and diesters, oxidized phospholipids, lipid hydroperoxides and H_2O_2, and carbamates, but the natural substrates and physiological functions of the gene have not been fully understood [2–4]. The PON1 metabolizes lipid-soluble radicals and also prevents oxidative modification of low-density lipoproteins (LDLs) through HDL-mediated protection. This suggests that the enzyme can act as an antioxidant and also plays an important role in the anti-inflammatory effect of HDLs [5].

The PON1 gene, located on the long arm of chromosome 7q21.3, is polymorphic and the expression levels vary widely in human populations [6]. The two common functional single nucleotide polymorphisms, L55M and Q192R, have been identified in the coding region of the gene [1, 6]. The L55M polymorphism (T→A) is characterized by a substitution of a leucine residue by methionine in exon 3 and has been noted to effect the enzyme concentration [7]. The Q192R polymorphism (A→G) led to glutamine to arginine exchange in exon 6 and the variants differ in their hydrolytic activities towards lipid peroxides [8]. Its ability to detoxify carcinogenic oxidative stress products has led investigators to hypothesize that PON1 polymorphisms might contribute to the increased risk of cancer. Serum PON1 activity was shown to be lower in gastric [9], pancreatic [10] and lung [11] cancers. Some studies have reported association between L55M and/or Q192R polymorphism(s) and increased risk of breast cancer [12–14], lung cancer [15], epithelial ovarian cancer [16], prostate cancer [17], non-Hodgkin's lymphoma [18] and multiple myeloma [19] while others failed to demonstrate any association with colorectal [20] and brain [21] cancers. Based on the limited number of studies published on cancer risk, these polymorphisms require further investigations particularly in relation to breast cancer risk.

In Malaysia, breast cancer is the commonest cancer in three ethnic groups namely the Malays, Chinese and Indians. There was a marked difference in the incidence rate among these groups [22]. The age pattern in 2003 showed a peak age specific incidence rate at the 50–59 age group in Malays, Chinese, and Indians. The life time risk of developing breast cancer for a woman in Malaysia is 1 in 20. Based on ethnic groups, it was noted that 1 in 16 Chinese women, 1 in 16 Indian women and 1 in 28 Malay women will develop the disease at some stage in their lives [22]. The present study was aimed to investigate whether L55M and Q192R polymorphisms of PON1 gene are associated with breast cancer risk in a hospital-based Malaysian population. The genotype and allele frequencies of these polymorphic variants will be determined in breast cancer patients and normal healthy individuals. We also examined association between these polymorphisms and clinico-pathological parameters such as age at diagnosis, estrogen receptor (ER) status, progesterone receptor (PgR) status, nodal status, histological grade, tumor staging and ethnicity.

Materials and Methods

Patients and Tissues

Peripheral blood samples were collected from 387 women who were histologically confirmed as having breast cancer. Written informed consent was obtained from these patients who were admitted to University Malaya Medical Center, University of Malaya, Malaysia before proceeding further for collection of blood. The approval has been obtained from the Medical Ethics Committee of University Malaya Medical Centre. A total of 252 women who were healthy and had no history or a family history of any malignancy were recruited as normal controls. The healthy individuals were matched for age with the breast cancer patients. The clinical characteristics of the patients and controls were summarized in Table 1. Histopathological evaluation of the tissues confirmed that 387 patients had invasive ductal carcinoma. All the invasive ductal carcinomas (IDCs) were graded according to the modified criteria as described by Elston and Ellis, [23] and Bloom and Richardson, [24]. The IDCs were staged according to the American Joint Committee on Cancer (AJCC) staging system [25, 26]. In Malaysia, the age pattern in 2003 showed that the age specific incidence rate peaks in the 50–59 years age group [22]. Since breast cancer incidence is closely related with the age, the patients and healthy individuals were distributed according to the age at diagnosis and age at recruitment, respectively; <40 years, 40–49 years, 50–59 years and ≥60 years old (Table 1). The mean ages of breast cancer patients and normal controls were 53.05± 11.717 and 52.56±11.263, respectively.

Genotyping L55M and Q192R Polymorphisms of PON1 Gene

High molecular weight genomic DNA was isolated according to the protocol provided by the manufacturer with some modification using PUREGENE Genomic DNA Purification kit (Gentra, USA) as described previously [27].

The L55M and Q192R genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described previously [6, 28]. The primer sequences used to amplify L55M and Q192R polymorphic regions were as follows: L55Mfwd: 5′-GAAGAGTGATGTATAGCCCCAG-3′; L55Mrev: 5′-TTTAATCCAGAGCTAATGAAAGCC-3′ (170 bp) and Q192Rfwd: 5′-TATTGTTGCTGTGGGACCTGAG-3′; Q192Rrev: 5′-CACGCTAAACCCAAATACATCTC-3′ (99 bp) [6, 28]. Each PCR amplification was performed in a 50 μl reaction mixture containing 100 ng genomic DNA, 1X PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$], 250 μM each of dGTP, dCTP, dTTP and dATP, 0.2 μM of each primers and 2.5 U of Ampli*Taq* DNA polymerase (Fermentas, Lithuania). The samples were amplified in a thermalcycler (MyCycler, Bio-Rad, USA). After initial denaturation at 94°C for 4 min., the PCR reaction was carried out for 35 cycles of 94°C for 40 sec., 56°C for 45 sec., and 72°C for 40 sec., and final

Table 1 Clinical characteristics of breast cancer patients and the normal controls

Characteristics	Cases (*N*=387)	Controls (*N*=252)
Age at diagnosis/recruitment		
<40 years	44 (11.4)	29 (11.5)
40–49 years	120 (31.0)	78 (30.9)
50–59 years	112 (28.9)	75 (29.8)
≥60 years	111 (28.7)	70 (27.8)
Ethnicity		
Malay	107 (27.6)	80 (31.8)
Chinese	219 (56.6)	111 (44.0)
Indians	61 (15.8)	61 (24.2)
Estrogen Receptor (ER) status		
ER+	200 (51.7)	–
ER-	183 (47.3)	–
N/A	4 (1.0)	–
Progesterone Receptor (PgR) Status		
PgR+	182 (47.0)	–
PgR-	201 (52.0)	–
N/A	4 (1.0)	–
Lymph Node (N) Status		
N+	191 (49.4)	–
N-	194 (50.1)	–
N/A	2 (0.5)	–
Histological Grade		
Grade I	33 (8.5)	–
Grade II	173 (44.7)	–
Grade III	155 (40.1)	–
N/A	26 (6.7)	–
Tumor Staging		
Stage 1	80 (20.7)	–
Stage 2a	111 (28.7)	–
Stage 2b	83 (21.4)	–
Stage 3a	30 (7.8)	–
Stage 3b	45 (11.6)	–
Stage 4	19 (4.9)	–
N/A	19 (4.9)	–

N/A not available

cycle of 72°C for 7 min. In each PCR run, DNA was substituted with sterile deionized water as a negative control. The PCR products were resolved on 2% agarose gel and identified by ethidium bromide staining. For genotyping L55M and Q192R polymorphisms, 10 μl of the PCR products were digested for 3 hrs at 37°C with 3 U of *Nla*111 and 6 U of *Alw*I (New England BioLabs, Beverly, MA, USA), respectively. The cleaved products were separated on 3% agarose gel. The L allele (leucine) is represented to the nondigested 170 bp fragment and the M allele (methionine) is corresponded to the digested 126 and 44 bp fragments. The restriction digest reveals 63 and 36 bp fragments in the presence of R allele (arginine) and nondigested 99 bp fragment in the presence of Q allele (glutamine).

Immunohistochemistry

The immunohistochemistry of ER (Clone SP1, rabbit monoclonal antibody) and PgR (Clone PgR 636, mouse monoclonal antibody) was performed using the DAKO EnVision™ System (Dako, Denmark) according to the manufacturer's instructions. The immunostaining and the scoring methods have been described earlier [27, 29].

Statistical Analysis

Statistical analysis was performed using SPSS (version 17.0, SPSS). The significance of association between the observed and expected number of the genotypes for a population in the Hardy-Weinberg equilibrium was analyzed using the Pearson's two-sided chi-square test. The significant difference of genotype or allele frequencies between the breast cancer cases and normal controls was evaluated using the Pearson's two-sided chi-square test. The odds ratio (OR) and its 95% confidence interval (CI) was obtained by logistic regression method to determine the correlation between the genotypes or alleles of L55M and Q192R polymorphisms and breast cancer risk. The crude odds ratio was determined through univariate logistic regression with only the genotype or allele factor taken into consideration. The adjusted odds ratio was calculated using the multivariate logistic regression method with an adjustment for age and ethnicity. For the purpose of statistical analysis patients homozygous and heterozygous for M and R alleles were combined into a single group, respectively, to determine association between the genotype frequencies and clinico-pathological parameters. Multivariable logistic regression analysis was performed to determine association between the genotype frequencies and clinico-pathological covariates such as age at diagnosis, ER status, PgR status, nodal status, tumor grade, tumor staging and ethnicity. The results were considered as statistically significant at $P<0.05$ (5%).

Results

Table 2 shows the genotype and allele frequencies of PON1 polymorphisms in the breast cancer cases and normal controls in a hospital-based Malaysian population. The genotype frequencies of L55M was conformed to the Hardy-Weinberg equilibrium in both the cases ($P=0.418$) and controls ($P=0.264$). The distribution of Q192R

Table 2 Distribution of PON1 L55M and Q192R allele and genotype frequencies in breast cancer cases and the control group

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	χ^2 P*-value	Adjusted OR (age and ethnicity) (95% CI)	χ^2 P*-value
PON1 L55M	(*n*=387)	(*n*=252)				
LL	159 (41.1)	126 (50.0)	1.00 (reference)	*P*=0.016^{+}	1.00 (reference)	*P*=0.023^{+}
LM	178 (46.0)	109 (43.3)	1.294 (0.927–1.807)	*P*=0.130	1.301 (0.928–1.823)	*P*=0.127
MM	50 (12.9)	17 (6.7)	2.331 (1.282–4.238)	*P*=0.006	2.229 (1.219–4.075)	*P*=0.009
M Carrier (LM+MM)	228 (58.9)	126 (50.0)	1.434 (1.042–1.973)	*P*=0.027	1.429 (1.035–1.974)	*P*=0.030
PON1 L55M Allele	No. of alleles (*n*=774)	No. of alleles (*n*=504)				
L	496 (64.1)	361 (71.6)	1.00 (reference)		1.00 (reference)	
M	278 (35.9)	143 (28.4)	1.415 (1.110–1.804)	*P*=0.005	1.397 (1.093–1.785)	*P*=0.008
PON1 Q192R	(*n*=387)	(*n*=252)				
QQ	200 (51.7)	115 (45.6)	1.00 (reference)	*P*=0.325^{+}	1.00 (reference)	*P*=0.333^{+}
QR	158 (40.8)	115 (45.6)	0.790 (0.567–1.101)	*P*=0.164	0.793 (0.567–1.110)	*P*=0.177
RR	29 (7.5)	22 (8.8)	0.758 (0.416–1.381)	*P*=0.365	0.746 (0.407–1.370)	*P*=0.345
R Carrier (QR+RR)	187 (48.3)	137 (54.4)	0.785 (0.571–1.079)	*P*=0.136	0.785 (0.569–1.084)	*P*=0.142
PON1 Q192R Allele	No. of alleles (*n*=774)	No. of alleles (*n*=504)				
Q	558 (72.1)	345 (68.5)	1.00 (reference)		1.00 (reference)	
R	216 (27.9)	159 (31.5)	0.840 (0.657–1.073)	*P*=0.163	0.838 (0.654–1.074)	*P*=0.163

'$^{+}$' represents chi-square analysis between breast cancer cases and normal controls for PON1 genotypes

'*' represents significance at $P<0.05$

genotypes was also consistent with Hardy-Weinberg equilibrium in cases (*P*=0.808) and controls (*P*=0.721).

The genotype (*P*=0.023) and allele (*P*=0.008) frequencies of L55M polymorphism were significantly different between the cancer cases and normal subjects. The frequency of M allele was higher in the patients (35.9%) than in the normal individuals (28.4%). Increased frequencies of LM (46.0%), MM (12.9%) and M allele genotype (LM+MM) (58.9%) was observed in breast cancer cases compared to the frequencies of LM (43.3%), MM (6.7%) and M allele genotype (50.0%) in normal individuals. No significant association was noted between LM genotype and breast cancer risk (OR^{adj}=1.301; 95% CI, 0.928–1.823). However, women who were homozygous for MM genotype (OR^{adj}=2.229; 95% CI, 1.219–4.075), and carriers of M allele (OR^{adj}=1.397; 95% CI, 1.093–1.785) or M allele genotype (OR^{adj}=1.429; 95%, 1.035–1.974) showed significant increased risk of breast cancer.

The distribution of genotype (*P*=0.333) and allele (*P*=0.163) frequencies of Q192R polymorphism were not significantly different between the cases and the controls. The frequencies of QR (40.8%), RR (7.5%) and R allele genotype (QR+RR) (48.3%) were lower in cancer patients than the frequencies of QR (45.6%), RR (8.8%) and R allele genotype (QR + RR) (54.4%) in the control groups. Women who were QR heterozygotes (OR^{adj}=0.793; 95% CI, 0.567–1.110) or RR homozygotes (OR^{adj}=0.746; 95% CI, 0.407–1.370), and carriers of R allele genotype (OR^{adj}=0.785; 95%, 0.569–1.084) or R allele (OR^{adj}=0.838; 95% CI, 0.654–1.074) were not associated with breast cancer risk.

Tables 3 and 4 summarizes the relationship between the L55M or Q192R genotypes and clinico-pathological parameters, respectively. Patients who were carriers of M allele genotype showed significant association with nodal metastases (*P*=0.004) and absence of ER (*P*=0.046) but not with other clinico-pathologic parameters. However, R allele genotype carriers were not significantly correlated with clinico-pathologic characteristics. The frequencies of the M allele genotype were higher in the ER negative (55.5%) and node positive (58.4%) than in the ER positive (44.5%) and node negative (41.6%) tumors.

Discussion

Our present data demonstrated that the frequencies of MM genotypes and M allele were significantly higher in the breast cancer patients than in the normal individuals which is in agreement with other findings [13, 14]. Compared with the wild-type LL homozygotes, women who were homozygous for M allele was at increased risk of developing breast cancer by 2.2-fold whereas individuals who were carriers of M allele genotype or M allele showed an increased risk by 1.5-fold and 1.4-fold, respectively. Our results suggest that individuals carrying both of the M

Table 3 Association between genotype frequencies of PON1 L55M polymorphism and clinico-pathological parameters of the breast cancer patients

Clinico-pathological parameters	Total no. of cases (n=387)	LL Genotype (%) (n=159)	M allele genotype (LM+MM) (%) (n=228)	χ^2 P*-value
Age at Diagnosis				
<40 years	44	15 (9.5)	29 (12.7)	P=0.834
40–49 years	120	49 (30.8)	71 (31.1)	
50–59 years	112	46 (28.9)	66 (29.0)	
≥60 years	111	49 (30.8)	62 (27.2)	
Estrogen Receptor (ER) Status[+]				
ER+	200	99 (63.5)	101 (44.5)	P=0.046
ER-	183	57 (36.5)	126 (55.5)	
Progesterone Receptor (PgR) Status[+]				
PgR+	182	89 (57.1)	93 (41.0)	P=0.307
PgR-	201	67 (42.9)	134 (59.0)	
Lymph Node (N) Status[+]				
N+	191	59 (37.1)	132 (58.4)	P=0.004
N-	194	100 (62.9)	94 (41.6)	
Histological Grade[+]				
Grade I	33	18 (12.2)	15 (7.0)	P=0.966
Grade II	173	76 (51.7)	97 (45.3)	
Grade III	155	53 (36.1)	102 (47.7)	
Tumor Staging[+]				
Stage 1	80	44 (28.8)	36 (16.7)	P=0.105
Stage 2a	111	44 (28.8)	67 (31.2)	
Stage 2b	83	31 (20.3)	52 (24.2)	
Stage 3a	30	6 (3.9)	24 (11.2)	
Stage 3b	45	20 (13.0)	25 (11.6)	
Stage 4	19	8 (5.2)	11 (5.1)	
Ethnicity				
Malays	107	44 (27.7)	63 (27.6)	P=0.991
Chinese	219	89 (56.0)	130 (57.0)	
Indians	61	26 (16.3)	35 (15.4)	

'[+]' values that are not available (N/A) for each parameter is not included in the statistical analysis. The N/A values are shown in Table 1

'*' represents significance at P<0.05

alleles have significantly greater risk of developing breast cancer compared to individuals who were carriers of at least one allele. To the best of our knowledge, only three papers were published on PON1 polymorphisms in breast cancer [12–14]. In a recent published data, Antognelli and coinvestigators reported that LM and MM genotypes, and M allele were associated with significant breast cancer risk [14]. On the other hand, Steven et al. [13] found individuals with MM genotype were at higher risk of developing breast cancer. Furthermore the authors also noted that the risk of breast cancer was elevated even among women who had only one M allele suggesting that even small changes in the PON1 activity may be important. Interestingly the findings of the present study with regard to MM genotype and M allele association with breast cancer risk were consistent with these studies.

In contrast to L55M polymorphism, our data failed to detect statistical significance in the distribution of allele and genotype frequencies of Q192R between breast cancer patients and normal women. The frequencies of QR and RR genotypes, and R allele were overrepresented in the control group than in the cancer patients. Similar results were noted by Steven et al. [13] and Gallicchio et al. [12] but significant association was observed by Antognelli and coresearchers [14]. In the present study, there was no significant evidence that Q192R polymorphism was associated with breast cancer risk and the findings were consistent with the observation made by Steven et al. [13]. However, Antognelli et al. [14] and Gallicchio et al. [12] reported that R polymorphic variant was associated with decreased risk of developing breast cancer with reference to normal women and benign breast disease

Table 4 Association between genotype frequencies of PON1 Q192R polymorphism and clinico-pathological parameters of the breast cancer patients

Clinico-pathological parameters	Total no. of cases (n=387)	QQ Genotype (%) (n=200)	R allele genotype (QR+RR) (%) (n=187)	χ^2 P*-value
Age at Diagnosis				
<40 years	44	20 (10.0)	24 (12.8)	P=0.723
40–49 years	120	62 (31.0)	58 (31.0)	
50–59 years	112	55 (27.5)	57 (30.5)	
≥60 years	111	63 (31.5)	48 (25.7)	
Estrogen Receptor (ER) Status[+]				
ER+	200	113 (57.4)	87 (46.8)	P=0.851
ER-	183	84 (42.6)	99 (53.2)	
Progesterone Receptor (PgR) Status[+]				
PgR+	182	105 (53.3)	77 (41.4)	P=0.081
PgR-	201	92 (46.7)	109 (58.6)	
Lymph Node (N) Status[+]				
N+	191	91 (45.5)	100 (53.4)	P=0.125
N-	194	109 (54.5)	85 (46.6)	
Histological Grade[+]				
Grade I	33	20 (10.6)	13 (7.5)	P=0.632
Grade II	173	97 (51.6)	76 (43.9)	
Grade III	155	71 (37.8)	84 (48.6)	
Tumor Staging[+]				
Stage 1	80	49 (25.5)	31 (17.6)	P=0.222
Stage 2a	111	54 (28.1)	57 (32.4)	
Stage 2b	83	42 (21.9)	41 (23.3)	
Stage 3a	30	11 (5.7)	19 (10.8)	
Stage 3b	45	27 (14.1)	18 (10.2)	
Stage 4	19	9 (4.7)	10 (5.7)	
Ethnicity				
Malays	107	59 (29.5)	48 (25.6)	P=0.424
Chinese	219	111 (55.5)	108 (57.8)	
Indians	61	30 (15.0)	31 (16.6)	

'[+]' values that are not available (N/A) for each parameter is not included in the statistical analysis. The N/A values are shown in Table 1

'*' represents significance at P<0.05

patients, respectively. Although conflicting observations have been reported, our data suggest that the polymorphic allele may not be a suitable marker for breast cancer susceptibility. One of the differences could be the sample saiz of the controls and cases used in these studies. Antognelli et al. [14] recruited 547 cases and 544 controls, and Gallicchio and coinvestigators [12] included 61 cancer and 933 benign cases. The other possible reasons might be the selection bias with regard to recruitment of patients for the study such as patients' characteristics (i.e postmenopausal and premenopausal women), heterogenous ethnic background and patients with different histopathological types of the tumors (IDC, *in situ* carcinomas, invasive lobular carcinomas etc.). These differences could alter the distribution of the genotype and allele frequencies in the patient and control groups. As a result this might influence the statistical power to determine association between the polymorphisms and cancer risk.

Our data demonstrated a significant association between M allele genotype and absence of ER or lymph node metastases which has not been documented previously. However, R allele genotype was not correlated with any of the clinico-pathological parameters. Although the age specific incidence rate is the highest in the 50–59 age group, no statistical association was observed between M or R polymorphism and age at diagnosis suggesting that breast cancer risk by these alleles may be independent of patients' age. Based on our study we noted an interesting observation which indicates increased frequencies of the M allele genotype in the ER negative and node positive tumors than in the ER positive and node negative patients. To our knowledge this is the first study that investigates association with clinic-pathological parame-

ters. The findings suggest that M variant may play an important role in tumor progression.

Earlier studies have reported that L allele was associated with significantly higher PON1 serum concentrations but the M variant decreases the stability of this enzyme thus lowers the concentration of PON1 in the blood which subsequently affects the activity of the enzyme [30, 31]. Individuals with LM genotype were found to have PON1 activity levels between those of LL and MM genotypes [31]. Lower levels of PON1 may increase the breast vulnerability to genomic damage by reducing the ability to detoxify inflammatory oxidants as well as dietary carcinogens. On the other hand, the enzyme coded by the R variant of Q192R polymorphism hydrolyzes various substrates more rapidly than the ones coded by wild-type Q allele [17, 32]. This suggests that R allele may lead to the production of PON1 enzyme with higher detoxification activity against potentially carcinogenic products of oxidative stress and lipid peroxidation. These experimental evidences further supports our observation that individuals with MM genotype and carriers of M allele were at higher breast cancer risk. However, our findings did not provide evidence of a significant association between Q192R polymorphism and breast cancer risk. The mechanism by which these polymorphisms could influence susceptibility to breast cancer is unknown. Observations made by Ferre et al. [33] also suggest that in addition to genetic factors other contributors such as nutrition and lifestyle do play an important role in determining PON1 enzyme activity.

In conclusion, our data shows that the polymorphic variant of L55M polymorphism could be a useful genetic marker for tumor prognosis and to identify women who might be at higher risk of developing breast cancer in a hospital-based Malaysian population. The R allele of Q192R polymorphism may not be a suitable marker for breast cancer susceptibility and tumor prognosis in our population. Our findings probably indicate that M variant allele may have a significant role in our hospital-based population as compared to R allele. Further studies with larger sample size are required to confirm whether PON1 polymorphisms could be potential genetic markers to identify women who might be at greater risk of developing breast cancer and for tumor prognosis.

Acknowledgements This study was financially supported by E-ScienceFund grant (02-02-10-SF0016) from Ministry of Science, Technology and Innovation, Malaysia.

References

1. Mackness B, Durrington PN, Mackness MI (1998) Human serum paraoxonase. Gen Pharmacol 31:329–336
2. La Du BN, Adkins S, Kuo CL et al (1993) Studies on human serum paraoxonase/arylesterase. Chem Biol Interact 87:25–34
3. Mackness MI, Mackness B, Durrington PN et al (1998) Paraoxonase and coronary heart disease. Curr Opin Lipidol 9:319–324
4. Teiber JF, Billecke SS, La Du BN et al (2007) Estrogen esters as substrates for human paraoxonases. Arch Biochem Biophys 461:24–29
5. Mackness MI, Arrol S, Mackness B et al (1997) Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. Lancet 349:851–852
6. Humbert R, Adler DA, Disteche CM et al (1993) The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 3:73–76
7. Brophy VH, Jampsa RL, Clendenning JB et al (2001) Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet 68:1428–1436
8. Aviram M, Hardak E, Vaya J et al (2000) Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation 101:2510–2517
9. Akcay MN, Yilmaz I, Polat MF et al (2003) Serum paraoxonase levels in gastric cancer. Hepatogastroenterology 50:cclxxiii–v
10. Akcay MN, Polat MF, Yilmaz I et al (2003) Serum paraoxonase levels in pancreatic cancer. Hepatogastroenterology 50:ccxxv–vii
11. Elkiran ET, Mar N, Aygen B et al (2007) Serum paraoxonase and arylesterase activities in patients with lung cancer in a Turkish population. BMC Cancer 7:48
12. Gallicchio L, McSorley MA, Newschaffer CJ et al (2007) 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
13. Stevens VL, Rodriguez C, Pavluck AL et al (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
14. Antognelli C, Buono CD, Ludovini V et al (2009) YP17, GSTP1, PON1, and GLO1 gene polymorphisms as risk factors for breast cancer: an Italian case-control study. BMC Cancer 9:115–128
15. Lee CH, Lee KY, Choe KH et al (2005) 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
16. Lurie G, Wilkens LR, Thompson PJ et al (2008) Genetic polymorphisms in the paraoxonase 1 gene and risk of ovarian epithelial carcinoma. Cancer Epidemiol Biomarkers Prev 17:2070–2076
17. Antognelli C, Mearini L, Talesa VN et al (2005) Association of CYP17, GSTP1, and PON1 polymorphisms with the risk of prostate cancer. Prostate 63:240–251
18. Kerridge I, Lincz L, Scorgie F et al (2002) Association between xenobiotic gene polymorphisms and non-Hodgkin's lymphoma risk. Br J Haematol 118:477–481
19. Lincz LF, Kerridge I, Scorgie FE et al (2004) Xenobiotic gene polymorphisms and susceptibility to multiple myeloma. Haematologica 89:628–639
20. van der Logt EMJ, Janssen CHJM, van Hooijdonk Z et al (2006) No association between genetic polymorphisms in NAD(P)H oxidase p22phox and Paraoxonase 1 and colorectal cancer risk. Anticancer Res 25:1465–1470
21. Kafadar AK, Ergen A, Zeybek U et al (2006) Paraoxonase 192 gene polymorphism and serum paraoxonase activity in high grade gliomas and meningiomas. Cell Biochem Funct 24:455–460
22. Lim GCC, Halimah Y (2004) Second Report of the National Cancer Registry. Cancer Incidence in Malaysia 2003. National Cancer Registry. Kuala Lumpur

23. Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer I. Histopathology 19:403–410
24. Bloom HI, Richardson WW (1957) Histological grading and prognosis. Br J Cancer 11:359–377
25. Breast. AJCC Cancer Staging Manual (2002) In: American Joint Committee on Cancer. 6th ed. Springer, New York, pp 171–180
26. Singletary E, Allred C, Ashley P et al (2002) Revision of the American Joint Committee on Cancer staging system for breast cancer. J Clin Oncol 20:3628–3636
27. Naidu R, Har YC, Taib NA (2007) P27 V109G Polymorphism is associated with lymph node metastases but not with increased risk of breast cancer. J Exp Clin Cancer Res 26:133–140
28. Zama T, Murata M, Matsubara Y et al (1997) A ^{192}Arg Variant of the human paraoxonase (HUMPONA) gene polymorphism is associated with an increased risk for coronary artery disease in the Japanese. Arterioscler Thromb Vasc Biol 17:3565–3569
29. Naidu R, Har YC, Taib NA (2009) Polymorphism of FGFR4 Gly388Arg does not confer an increased risk to breast cancer development. Oncol Res 18(2–3):65–71
30. Leviev I, Deakin S, James RW (2001) Decreased stability of the M54 isoform of paraoxonase as a contributary factor to variations in human serum paraoxonase concentrations. J Lipid Res 42:528–535
31. Brophy VH, Jarvik GP, Richter RJ et al (2000) Analysis of paraoxonase (PON1) L55M status requires both genotype and phenotype. Pharmacogenetics 10:453–460
32. Durrington PN, Mackness B, Mackness MI (2001) Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 21:473–480
33. Ferrè N, Camps J, Fernandez-Ballart J et al (2003) Regulation of serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population. Clin Chem 49:1491–1497