Original Article

Paraoxonase 1 (PON1) L55M and Q192R Polymorphisms are Associated with Type 2 Diabetes Mellitus in Southern Thai Subjects

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Background: Paraoxonase 1 (PON1) plays a critical role in the prevention of cardiovascular disease, diabetes mellitus, and other chronic diseases.

Objective: The aim of the present study was to investigate the association of PON1 L55M and Q192R polymorphisms with type 2 diabetes mellitus (T2DM), and pre-diabetes.

Materials and Methods: The present study included 512 subjects (223 T2DM patients, 150 pre-diabetes, and 139 healthy controls) from Southern Thailand. The PON1 L55M and Q192R polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Results: PON1 Q192R polymorphism showed a statistical difference in genotype frequencies between T2DM patients and healthy controls (p<0.0001). Multiple logistic regression analyses after adjusting for age, gender, and BMI showed that LM and RR genotypes increased the risk for T2DM compared with LL and QQ+QR genotypes (OR 1.96; 95% CI 1.02 to 3.76, p=0.042, and OR 1.85; 95% CI 1.11 to 3.10, p=0.019, respectively). Whereas, QR, genotype was associated with decreased risk for T2DM (OR 0.240; 95% CI 0.13 to 0.45, p<0.0001), and pre-diabetes (OR 0.549; 95% CI 0.30 to 0.99, p=0.048) compared with QQ genotype.

Conclusion: PON1 LM and RR genotypes may be genetic risk factors for developing T2DM but QR genotype may prevent T2DM, and pre-diabetes in Southern Thai population.

Keywords: PON1, Polymorphisms, Type 2 diabetes, Pre-diabetes

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Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia resulting from insulin resistance[1]. A previous study has reported that the increased oxidative stress and low antioxidant were associated with T2DM[2]. Reduced paraoxonase-1 (PON1) activity was observed in T2DM, as well as other high oxidative stress diseases such as cardiovascular diseases (CVDs)[3], diabetes mellitus[3], non-alcoholic fatty liver disease (NAFLD)[3], Alzheimer's disease[3], and cancer[4].

Human serum PON1 is a 354-amino acid glycoprotein hydrolyzed organophosphate xenobiotics such as paraoxon (insecticide), nerve gases, carbamates, and aromatic carboxylic acid esters[5]. PON1 is synthesized in the liver and is localized on high-density lipoprotein (HDL)[6]. In vitro and in vivo studies have demonstrated that PON1 can prevent oxidation of low-density lipoprotein (LDL)[7,8]. This is suggesting that PON1 may play a critical role in the prevention of atherosclerosis[3]. PON1 enzyme is encoded by the PON1 gene, which is located on chromosome 7q21.3-22.1. The PON1 gene contains nine exons and eight introns[9]. There

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are more than 200 polymorphisms of the PON1 gene[10]. However, two common polymorphisms of the PON1 gene, L55M (rs854560) and Q192R (rs662) have been widely studied[11,12]. These polymorphisms were found to affect the serum concentrations and activities of PON1[13]. The paraoxonase activity is higher in RR genotype than in QR and QQ genotypes, respectively. In addition, such activity is higher in LL genotype than in LM and MM genotypes, respectively[13]. Nevertheless, RR genotype is less effective in protecting LDL from oxidation than that in the QQ genotype[13].

PON1 L55M or Q192R polymorphisms have been found to be the independent risk factors for T2DM in various populations[14-18]. However, the association between these polymorphisms and T2DM is still inconsistent in some studies[11,19]. In addition, there was no association study between PON1 L55M, and Q192R polymorphisms and pre-diabetes. The authors suggested that the association between these polymorphisms and pre-diabetes may predispose to early detection before the diagnosis of diabetes mellitus. Thus, the aim of the present study was to investigate the relationship between PON1 polymorphisms and T2DM and pre-diabetes in the Southern Thai population.

Materials and Methods

Subjects and sample collection

The study group included 512 individuals from Southern Thailand. The T2DM, impaired fasting glucose (IFG), and control groups consisted of 223 unrelated type 2 diabetic patients (59 men and 164 women), 150 pre-diabetes (50 men and 100 women), and 139 unrelated non-diabetic control subjects (49 men and 90 women), respectively. The diagnosis of T2DM was based on the World Health Organization (WHO) criteria[20]. T2DM was diagnosed as fasting glucose of 7.0 mmol/L or more (126 mg/dL) or the two hour glucose of 11.1 mmol/L or more (200 mg/dL) or were treated with oral hypoglycemic agents or insulin. Pre-diabetes was diagnosed as fasting glucose of 5.6 to 6.9 mmol/L (100 to 125 mg/dL). The control group was healthy subjects with no diabetes, CVDs, and other chronic diseases. Anthropometric measurements, such as body mass index (BMI) and waist circumference (WC), were recorded from each participant. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. Exclusion criteria for subjects included the presence of hepatic, thyroid, cardiac, autoimmune, and hematologic neoplastic diseases, or non-diabetic kidney disease. Written informed consent was obtained from all subjects before being included in the study. The study protocol was approved by the Ethics Committee of Walailak University (protocol no. WUEC-18-126-01).

Laboratory analysis

Blood samples were collected from the subjects after 12 hours of fasting. The serum and plasma were separated by centrifugation at 3,000 rpm for 10 minutes. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were measured using standard enzymatic method. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. Fasting blood glucose (FBG) was measured using the glucose oxidase method. All tests were performed by using the Konelab analyzer (KONELAB 20, Tokyo, Japan).

Genotyping

DNA was extracted from blood leukocytes using the Genomic DNA mini kit (GeneAid Biotech Ltd., Taiwan) according to manufacturer's instructions. The genotyping for PON1 L55M and Q192R polymorphisms was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis according to the previously reported protocol[17]. All polymorphisms detected by PCR-RFLP were confirmed by DNA sequencing.

Statistical analysis

The primary outcome was to investigate the association of PON1 L55M, and Q192R polymorphisms with T2DM and pre-diabetes in Southern Thai subjects. Sample size was calculated using G*Power 3.1.9.4[21], with the alpha level of 0.05, and statistical power of 0.95. All data were analyzed using SPSS Statistics software, version 16.0 (SPSS Inc., Chicago, Ill, USA). The distribution of L55M and Q192R polymorphisms were tested for the Hardy-Weinberg equilibrium (HWE) using the Chi-square test. The data were tested for normality. Continuous variables were expressed as mean and standard deviation (SD). For multiple comparisons of means among groups, the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test or Kruskal-Wallis test were performed. Differences between the two groups were tested using the Student t-test for parametric factors and the Mann-Whitney U test for non-parametric factors. Binary and multivariate logistic regression

Table 1. Clinical characteristics of patients with T2DM, pre-diabetes, and control groups in the present study

Variable	T2DM (n=223) Mean±SD	Pre-diabetes (n=150) Mean±SD	Control (n=139) Mean±SD	p-value
Sex: male/female	59/164[a]	50/100[b]	49/90	0.075[a]/0.731[b]
Age (years)	59.28±9.02[c,d]	57.17±11.64[e]	51.68±13.57	<0.0001
BMI (kg/m^2)	26.32±4.43[c,d]	24.46±3.67	24.15±4.07	<0.0001
SBP (mmHg)	139.75±20.85[c,d]	136.39±20.14	129.41±17.93	<0.0001
DBP (mmHg)	78.26±12.89[c,d]	82.40±10.04	81.23±10.04	0.002
TC (mg/dL)	251.49±41.60[c,d]	219.99±41.60	215.41±39.23	<0.0001
TG (mg/dL)	168.85±68.35[c,d]	136.17±57.77	127.95±63.85	<0.0001
HDL-C (mg/dL)	49.06±25.17[c,d]	65.15±20.08	66.30±18.56	<0.0001
LDL-C (mg/dL)	168.66±46.62[c,d]	127.57±40.28	126.52±36.25	<0.0001
FBS (mg/dL)	180.52±73.74[c,d]	107.85±6.47[e]	91.20±5.48	<0.0001

T2DM=type 2 diabetes mellitus; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglyceride; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; FBS=fasting blood sugar; SD=standard deviation

[a] T2DM vs. control, [b] pre-diabetes vs. control, p-value obtained in the chi-square test

[c] T2DM vs. pre-diabetes, [d] T2DM vs. control, [e] pre-diabetes vs. control, p-value obtained in the Tukey post-hoc test

$p<0.05$ was considered significant

analyses were used to analyze the odd ratio (OR) by comparing allele, and genotype frequencies between case and control groups. Covariates including gender, age, and BMI were included in the multivariate model. A p-value of less than 0.05 was considered statistically significant.

Results

Clinical characteristics of T2DM patients, pre-diabetes, and the healthy controls in the present study are summarized in Table 1. Age, BMI, SBP, DBP, TC, TG, LDL-C, and FBS levels were significantly higher but HDL-C was significantly lower in T2DM in comparison to the healthy controls ($p<0.05$). In addition, age, and FBS levels were significantly higher in pre-diabetes in comparison to healthy controls ($p<0.0001$). Moreover, age, BMI, SBP, DBP, TC, TG, LDL-C, and FBS levels were significantly higher, but HDL-C was significantly lower in T2DM patients than in pre-diabetes ($p<0.05$).

PON1 L55M and Q192R polymorphisms of T2DM patients, pre-diabetes, and healthy controls are shown in Table 2. Genotype frequencies of PON1 L55M were consistent with HWE in controls, and pre-diabetes, but not in T2DM patients. Whereas, genotype frequencies of PON1 Q192R were consistent with HWE in controls but not in T2DM, and pre-diabetes. PON1 Q192R polymorphism demonstrated a statistical difference in genotype frequencies between T2DM patients and healthy controls ($p<0.0001$). However, the allele and genotype frequencies of PON1 L55M polymorphism were not significantly different between T2DM patients and controls. Furthermore, the allele and genotype frequencies of PON1 L55M and Q192R polymorphisms were not significantly different between pre-diabetes and controls (Table 2).

The multiple logistic regression analysis for potential risk factors of T2DM is shown in Table 2. LM genotype was associated with an increased risk for T2DM compared with LL genotype after adjusting for age, gender, and BMI. Additionally, the analysis according to the recessive model demonstrated that RR genotype was associated with increased risk for T2DM when compared with QQ+QR genotypes. Co-dominant, dominant, and over-dominant models showed that QR, QR+RR, and QR genotypes were associated with decreased risk for T2DM when compared with QQ, QQ, and QQ+RR genotypes, respectively. Moreover, QR genotype was also associated with decreased risk for pre-diabetes compared with QQ genotype.

The metabolic parameters in T2DM patients, pre-diabetes, and healthy controls according to PON1 L55M and Q192R variants are presented in Table 3 and 4, respectively. LM genotype was associated with higher BMI in comparison to LL genotype in T2DM patients. In contrast, LM genotype was associated with lower TC, and LDL-C concentrations in comparison to LL genotype in pre-diabetes (Table 3).

Table 2. Genotype and allele frequencies of PON1 L55M, and Q192R polymorphisms in T2DM and control groups

	Control	T2DM				Pre-diabetes			
	n (%)	n (%)	p-value (χ^2)	Odds ratio (95% CI)	p-value	n (%)	p-value (χ^2)	Odds ratio (95% CI)	p-value
L55M genotype (rs854560)									
LL	118 (84.89)	171 (76.68)	0.058 (3.585)	1		135 (90.00)	0.189 (1.726)	1	
LM	21 (15.11)	52 (23.32)		1.959 (1.020 to 3.761)[a]	0.042	15 (10.00)		0.674 (0.314 to 1.445)[a]	0.310
MM	0 (0.00)	0 (0.00)				0 (0.00)			
Total	139	223				150			
L55M allele									
L	257 (92.45)	394 (88.34)	0.074 (3.183)	1		285 (95.00)	0.204 (1.611)	1	
M	21 (7.55)	52 (11.66)		1.615 (0.950 to 2.746)[b]	0.077	15 (5.00)		0.644 (0.325 to 1.276)[b]	0.207
Q192R genotype (rs662)									
Codominant model									
• QQ	40 (28.78)	100 (44.84)	<0.0001 (33.351)	1		53 (35.33)	0.144 (3.874)	1	
• QR	59 (42.45)	34 (15.25)		0.240 (0.129 to 0.448)[a]	<0.0001	47 (31.33)		0.549 (0.303 to 0.994)[a]	0.048
• RR	40 (28.78)	89 (39.91)		1.019 (0.566 to 1.833)[a]	0.950	50 (33.33)		0.788 (0.419 to 1.481)[a]	0.459
• Total	139	223				150			
Dominant model									
• QQ	40 (28.78)	100 (44.84)	0.002 (9.319)	1		55 (35.33)	0.154 (2.035)	1	
• QR+RR	99 (71.22)	123 (55.16)		0.541 (0.326 to 0.895)[a]	0.017	95 (64.67)		0.606 (0.356 to 1.032)[a]	0.065
Recessive model									
• QQ+QR	99 (71.22)	134 (60.09)	0.031 (4.627)	1		102 (66.67)	0.552 (0.354)	1	
• RR	40 (28.78)	89 (39.91)		1.853 (1.108 to 3.101)[a]	0.019	48 (33.33)		1.029 (0.602 to 1.759)[a]	0.917
Overdominant model									
• QQ+RR	80 (57.55)	189 (84.75)	<0.0001 (33.183)	1		103 (68.67)	0.050 (3.836)	1	
• QR	59 (42.45)	34 (15.25)		0.238 (0.136 to 0.416)[a]	<0.0001	47 (31.33)		0.617 (0.372 to 1.022)[a]	0.061
Q192R allele									
Q	139 (50.00)	234 (52.47)	0.518 (0.417)	1		153 (51.00)	0.810 (0.058)	1	
R	139 (50.00)	212 (47.53)		0.906 (0.671 to 1.223)[b]	0.518	147 (49.00)		0.961 (0.693 to 1.332)[b]	0.810

T2DM=type 2 diabetes mellitus; BMI=body mass index; χ^2=chi-square; CI=confidence interval

[a] Multivariate logistic regression analysis after adjustment for age, sex, and BMI; [b] Binary logistic regression analysis

Table 3. Differences in metabolic parameters in T2DM, pre-diabetes, and control groups according to PON1 L55M genotype

Variable	T2DM; mean±SD			Pre-diabetes; mean±SD			Control; mean±SD		
	LL	LM	p-value	LL	LM	p-value	LL	LM	p-value
No. of patients	171	52		135	15		118	21	
Age (years)	59.41±8.94	58.85±9.33	0.709	57.70±11.62	52.40±11.02	0.073	52.20±13.24	48.71±15.30	0.279
BMI (kg/m^2)	26.05±4.31	27.21±4.73	0.027	24.40±3.66	25.01±3.87	0.339	24.23±4.26	23.69±2.83	0.861
SBP (mmHg)	140.60±21.09	136.94±19.99	0.269	136.77±20.55	133.00±16.24	0.799	130.01±17.93	126.10±17.99	0.264
DBP (mmHg)	78.96±13.11	75.98±11.97	0.154	82.28±10.18	83.47±8.90	0.665	81.45±10.36	80.05±10.93	0.573
TC (mg/dL)	253.74±43.17	244.08±35.33	0.199	222.63±41.09	196.20±39.75	0.024	215.81±40.35	213.14±32.96	0.775
TG (mg/dL)	171.35±70.86	160.62±59.24	0.442	137.97±58.49	119.93±49.57	0.250	125.85±64.38	139.76±60.94	0.212
HDL-C (mg/dL)	49.25±28.02	48.46±11.84	0.407	65.12±20.29	65.47±18.76	0.829	66.14±18.73	67.19±17.99	0.813
LDL-C (mg/dL)	170.23±49.79	163.49±34.08	0.114	129.90±40.60	106.67±31.19	0.034	124.50±36.53	118.00±34.95	0.451
FBS (mg/dL)	180.89±73.44	179.33±75.42	0.543	107.92±6.55	107.27±5.87	0.712	91.35±5.42	90.38±5.88	0.492

T2DM=type 2 diabetes mellitus; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglyceride; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; FBS=fasting blood sugar; SD=standard deviation

Table 4. Differences in metabolic parameters in T2DM, pre-diabetes, and control groups according to PON1 Q192R genotype

Variable	T2DM; mean±SD				Pre-diabetes; mean±SD				Control; mean±SD			
	QQ	QR	RR	p-value	QQ	QR	RR	p-value	QQ	QR	RR	p-value
No. of patients	100	34	89		53	47	50		40	59	40	
Age (years)	58.91±9.58	60.59±8.48	59.19±8.61	0.757	55.23±12.67	57.74±11.50	58.68±10.51	0.183	50.80±14.27	51.98±12.93	52.10±14.09	0.890
BMI (kg/m^2)	26.50±4.24	25.40±3.48	26.47±4.92	0.459	24.86±3.69	23.59±3.48	24.85±3.76	0.155	24.38±3.65	24.12±4.69	23.96±3.56	0.554
SBP (mmHg)	136.98±18.04	140.32±22.65	142.64±22.83	0.174	139.23±23.41	131.40±15.85	137.88±19.40	0.291	129.42±19.33	129.09±16.24	129.87±19.27	0.995
DBP (mmHg)	76.49±11.67	80.15±14.36	79.54±13.49	0.191	84.87±9.65	79.84±11.10[a]	82.08±8.93	0.045	80.00±11.08	83.00±9.25	79.87±11.23	0.237
TC (mg/dL)	251.43±39.60	255.26±48.81	250.11±41.23	0.848	221.91±42.55	213.57±42.96	223.98±39.34	0.510	215.10±44.99	217.25±38.27	213.00±35.09	0.869
TG (mg/dL)	164.80±66.75	183.32±73.88	167.87±68.01	0.388	136.87±55.36	134.87±63.51	136.64±55.72	0.832	129.68±64.65	126.90±61.85	127.78±67.48	0.970
HDL-C (mg/dL)	51.53±35.22	46.97±10.96	47.09±12.07	0.809	64.13±20.38	64.70±18.18	66.66±21.7	0.772	64.68±19.74	69.44±18.46	63.30±17.20	0.220
LDL-C (mg/dL)	166.94±51.50	171.63±44.69	169.45±41.75	0.662	130.32±40.22	121.94±41.63	129.96±39.33	0.514	124.45±35.13	122.44±37.14	124.18±36.89	0.956
FBS (mg/dL)	179.20±70.01	182.88±66.86	181.11±80.77	0.891	107.98±6.59	109.34±6.60	106.32±5.98[b]	0.049	90.23±6.29	91.98±4.74	91.03±5.61	0.495

T2DM=type 2 diabetes mellitus; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglyceride; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; FBS=fasting blood sugar; SD=standard deviation

[a] QQ vs. QR, [b] QR vs. RR, p-value obtained in the Tukey post-hoc test; p<0.05 was considered significant

In addition, QR genotype had significantly lower DBP than QQ genotype, as well as, RR genotype had significantly lower FBS than QR genotype in pre-diabetes (Table 4).

Discussion

In Southern Thailand, only the association between APOE, and CETP polymorphisms with T2DM was studied[22]. In the present study, the authors further investigated the association between PON1 L55M, and Q192R polymorphisms with T2DM and pre-diabetes in Southern Thai population. The authors found that the minor allele frequencies (MAFs) of 55M were 5.00% to 11.66%. These findings were similar to previous reports in Asian populations in Korea (5.8%)[12], Thailand (5%)[12], Japan (5.9%)[11], and China (4.6% to 5.8%)[11]. Higher MAFs of 55M were observed in some Asian populations in Iran (41%)[12], Pakistan (21.7%)[12], in European populations in Austria (35.6%)[12], U.K. (37%)[12], Netherlands (37.3%)[12], Germany (36%)[12], Italy (39.9%)[12], Poland (36%)[12], Spain (37.4%)[12], and Switzerland (33.4%)[12], and in populations in U.S.A. (36.3%)[12], and Canada (36%)[12]. In addition, the MAFs of Q192R in the present study were 47.53% to 50.00%. The present results were similar to Asian population in China (40.9% to 75%)[11]. Higher MAFs of Q192R were observed in some Asian populations in Japan (68.8% to 73.6%)[11] and Thailand (61%)[11], but lower MAFs of Q192R were observed in Asian populations in another study in Thailand (29%)[12], and India (26% to 39.2%)[11], in European populations in U.K. (26.3% to 32.7%), Germany (25.2% to 28%), France (37.5%), and Italy (36.2%)[11], as well as in population in Canada (24%)[11]. The authors suggest that the difference of MAFs of L55M and Q192R polymorphisms among various studies may be due to the differences of ethnicities, and the number of study subjects.

The present study showed that PON1 L55M was associated with T2DM but not pre-diabetes after adjusting for age, gender, and BMI. The present findings were similar to some previous studies in which PON1 L55M was associated with T2DM in the South Indian[14], Pakistani[15], and South Iranian[16] populations. In contrast, there was no significant association of the PON1 L55M polymorphism with T2DM in the Asian populations under all genetic models. In addition, the 55M allele showed the significant protective effects on T2DM under the heterozygous and dominant genetic models in the European population[11]. The present study also

showed that recessive model (RR) was associated with increased risk for T2DM. Whereas, co-dominant (QR), dominant (QR+RR), and over-dominant (QR) models of Q192R were associated with decreased risk for T2DM, respectively. Codominant (QR) was also associated with decreased risk for pre-diabetes. Suggesting that, QR genotype may have a strongly protective effect against T2DM in the study population. The present results were similar to study in South Indian[14] that RR genotype was associated with T2DM. Moreover, PON1 Q192R polymorphism was found associated with T2DM in South Asian and East Asian populations in a meta-analysis[11]. In addition, PON1 Q192R under a heterozygous genetic model demonstrated protective effect for T2DM in the meta-analysis in European population[11]. However, there was no association between PON1 Q192R or L55M polymorphisms and T2DM in South Iranian[16], Iranian[17], Turkish[18], and Japanese[19] populations. Similarly, PON1 Q192R polymorphism was not associated with T2DM in European populations under the allelic, homozygous, recessive, and dominant genetic models in the meta-analysis[11]. Suggesting that, the inconsistent results between PON1 L55M and Q192R polymorphisms and T2DM may result from the different ethnicities among various populations.

The possible explanation for the effect of PON1 polymorphisms on the development of T2DM may be related to the antioxidant activity of PON1. Previous study has shown that paraoxonase activity was increased in the following order of PON1 genotypes (MM<LM<LL and QQ<QR<RR) in ACS patients and healthy subjects[23]. However, the antioxidant activity on prevention of HDL and LDL oxidation was decreased in the following order of PON1 genotypes (QQ>QR>RR), with almost no antioxidant activity for RR genotype[13]. Suggesting that, decreased antioxidant activity of PON1 may accelerate insulin resistance and T2DM. Previous study supported that PON1 MM, and RR carriers had higher HOMA-IR than PON1 L/Q allele carriers[14]. Moreover, an in vivo study also showed that PON1 knockout mice, fed with high fat diet developed insulin resistance[24]. PON1 was found to enhance GLUT4 mRNA expression, increase GLUT4 protein, glucose uptake, and cellular glycogen accumulation in muscle in mice fed with high fat diet[24].

Nevertheless, another study demonstrated that the RR genotype significantly retarded the oxidation of LDL compared to the QQ genotype[25]. This suggested that the relationships between PON1 antioxidant activity and its protection for HDL or LDL oxidation may be modulated by other environmental factors e.g., smoking, obesity, metabolic status, oxidative stress status, and, aging. A previous study demonstrated that PON1 protective effect seemed to be blunted with advancing age[26]. Another study showed that PON-para over HDL and PON-aryl over HDL were negatively correlated with age in the QR+RR group[27]. This could suggest that the presence of R allele may potentiate the effect of age on susceptibility to CVD in T2DM[28]. Thus, this may partly lead to the inconsistency results of the relationship between PON1 L55M and Q192R polymorphisms and T2DM.

In the present study, only LM genotype was associated with lower TC, and LDL-C levels in comparison to LL genotype in the pre-diabetes group. In addition, QR genotype had significantly lower DBP than QQ genotype, as well as, RR genotype had significantly lower FBS than QR genotype in the pre-diabetes group. Whereas, there were no association between PON1 L55M, and Q192R polymorphisms and lipid profiles in the control and the T2DM groups. The present results were similar to other studies in which there was no association between PON1 L55M, and Q192R polymorphisms and lipid levels in healthy subjects in Mexican[28], as well as, in patients with coronary artery disease (CAD) in populations in Germany[29], U.S.A.[30], and Switzerland[31]. In contrast, PON1 QQ and RR genotypes were found associated with highest and lowest values of HDL-C, respectively in healthy Mexican men[32]. In addition, the relationship between the PON1 RR genotype and reduced HDL-C and higher LDL-C concentrations was also observed in Brazilian population[33]. Moreover, QR and RR genotypes were associated with higher levels of TC, and TG compared with QQ genotype in South Indian population[14]. Other studies have also shown that PON1 QR or RR genotypes were associated with higher serum TG levels compared with QQ genotype in acute coronary syndrome (ACS) patients in North African[23], healthy Chinese subjects in Singapore[34], CAD patients in Italy[35], and Canadian populations[36]. For PON1 L55M polymorphism, PON1 LL genotype was associated with the lower LDL-C levels when compared to PON1 LM genotype in Mexican men[32]. Whereas, PON1 MM genotype was associated with higher TG and VLDL levels when compared to LL genotype in South Indian population[14]. In contrast, PON1 MM genotype was associated with lower TG and higher TC and LDL-C levels in Brazilian population[37]. Fewer studies have analyzed the relationship of PON1 L55M, and Q192R polymorphisms and

blood pressure. PON1 RR, and MM genotypes showed lower SBP, and DBP in CAD patients[38] and healthy subjects, respectively[39]. Whereas, SBP, and DBP were not significantly different among Q192R genotypes in essential hypertensive men[40], and in subjects in the Lipoprotein and Coronary Atherosclerosis Study (LCAS)[30]. The mechanisms in which PON1 polymorphisms affect the blood pressure and lipid levels were still unclear. The authors suggest that the inconsistent results between PON1 L55M or Q192R polymorphisms and blood pressure, and serum lipids may result from several factors e.g., age, gender, BMI, ethnicity, smoking, exercise, alcohol consumption, dietary fat, drug therapy, gene-gene interaction, gene-environmental interaction, diabetes complications in the study subjects, and the number of the study subjects.

The limitations of the present study result from a small sample size and a higher proportion of female than male. In addition, other biochemical parameters e.g., HbA1c, paraoxonase activity and insulin resistance parameters, as well as other PON1 polymorphisms were not determined. The authors suggested that PON1 L55M, and Q192R may have linkage disequilibrium with other polymorphisms, which may susceptible to T2DM. Further study in the larger sample size should be performed to confirm the present results.

Conclusion

PON1 LM and RR genotypes may be genetic risk factors for developing T2DM but QR genotype may protect T2DM, and pre-diabetes in Southern Thai population. In addition, these polymorphisms were not associated with serum lipids in control and T2DM groups.

What is already known on this topic?

PON1 L55M or Q192R polymorphisms have been found to be the independent risk factors for T2DM in various populations. However, the association between these polymorphisms and T2DM is still inconsistent in some studies.

What this study adds?

This study investigated the relationship between PON1 polymorphisms and T2DM and pre-diabetes in Southern Thai population. Our results showed that PON1 Q192R polymorphism showed a statistical difference in genotype frequencies between T2DM patients and healthy controls. Moreover, multiple logistic regression analyses showed that LM and RR genotypes increased risk for T2DM compared with LL and QQ+QR genotypes. Whereas, QR genotype was associated with decreased risk for T2DM, and pre-diabetes.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26 Suppl 1:S5-20.
2. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol 2019;11:45-63.
3. Meneses MJ, Silvestre R, Sousa-Lima I, Macedo MP. Paraoxonase-1 as a regulator of glucose and lipid homeostasis: Impact on the onset and progression of metabolic disorders. Int J Mol Sci 2019;20.pii:E4049.
4. Arenas M, Rodriguez E, Sahebkar A, Sabater S, Rizo D, Pallise O, et al. Paraoxonase-1 activity in patients with cancer: A systematic review and meta-analysis. Crit Rev Oncol Hematol 2018;127:6-14.
5. Li B, Sedlacek M, Manoharan I, Boopathy R, Duysen EG, Masson P, et al. Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. Biochem Pharmacol 2005;70:1673-84.
6. Tomás M, Latorre G, Sentí M, Marrugat J. The antioxidant function of high density lipoproteins: a new paradigm in atherosclerosis. Rev Esp Cardiol 2004;57:557-69.
7. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett 1991;286:152-4.
8. Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 1993;104:129-35.
9. Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 1993;3:73-6.
10. Richter RJ, Jarvik GP, Furlong CE. Paraoxonase 1 status as a risk factor for disease or exposure. Adv Exp Med Biol 2010;660:29-35.
11. Luo JQ, Ren H, Liu MZ, Fang PF, Xiang DX. European versus Asian differences for the associations between paraoxonase-1 genetic polymorphisms and

susceptibility to type 2 diabetes mellitus. J Cell Mol Med 2018;22:1720-32.
12. Shin BS. Paraoxonase gene polymorphism in south-western Korean population. J Korean Med Sci 2009; 24:561-6.
13. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett 1998;423:57-60.
14. Gomathi P, Iyer AC, Murugan PS, Sasikumar S, Raj NBAJ, Ganesan D, et al. Association of paraoxonase-1 gene polymorphisms with insulin resistance in South Indian population. Gene 2018;650:55-9.
15. Batool A, Jahan N, Sun Y, Hanif A, Xue H. Genetic association of IDE, POU2F1, PON1, IL1alpha and IL1beta with type 2 diabetes in Pakistani population. Mol Biol Rep 2014;41:3063-9.
16. Moasser E, Azarpira N, Ghorbani dalini A, Shirazi B. Paraoxonase 1 (PON1) gene polymorphism and haplotype analysis in type 2 diabetes mellitus: a case-control study in the south Iranian population. Int J Diabetes Dev Ctries 2018;38:62-8.
17. Mahrooz A, Hashemi-Soteh MB, Heydari M, Boorank R, Ramazani F, Mahmoudi A, et al. Paraoxonase 1 (PON1)-L55M among common variants in the coding region of the paraoxonase gene family may contribute to the glycemic control in type 2 diabetes. Clin Chim Acta 2018;484:40-6.
18. Ergun MA, Yurtcu E, Demirci H, Ilhan MN, Barkar V, Yetkin I, et al. PON1 55 and 192 gene polymorphisms in type 2 diabetes mellitus patients in a Turkish population. Biochem Genet 2011;49:1-8.
19. Sakai T, Matsuura B, Onji M. Serum paraoxonase activity and genotype distribution in Japanese patients with diabetes mellitus. Intern Med 1998;37:581-4.
20. World Health Organization, International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Geneva: WHO; 2006.
21. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 2009; 41:1149-60.
22. Srirojnopkun C, Kietrungwilaikul K, Boonsong K, Thongpoonkaew J, Jeenduang N. Association of APOE and CETP TaqIB polymorphisms with type 2 diabetes mellitus. Arch Med Res 2018;49:479-85.
23. Bounafaa A, Berrougui H, Ghalim N, Nasser B, Bagri A, Moujahid A, et al. Association between paraoxonase 1 (PON1) polymorphisms and the risk of acute coronary syndrome in a North African population. PLoS One 2015;10:e0133719.
24. Koren-Gluzer M, Aviram M, Hayek T. Paraoxonase1 (PON1) reduces insulin resistance in mice fed a high-fat diet, and promotes GLUT4 overexpression in myocytes, via the IRS-1/Akt pathway. Atherosclerosis 2013;229:71-8.
25. Kuremoto K, Watanabe Y, Ohmura H, Shimada K, Mokuno H, Daida H. R/R genotype of human paraoxonase (PON1) is more protective against lipoprotein oxidation and coronary artery disease in Japanese subjects. J Atheroscler Thromb 2003;10:85-92.
26. Cherki M, Berrougui H, Isabelle M, Cloutier M, Koumbadinga GA, Khalil A. Effect of PON1 polymorphism on HDL antioxidant potential is blunted with aging. Exp Gerontol 2007;42:815-24.
27. Zargari M, Sharafeddin F, Mahrooz A, Alizadeh A, Masoumi P. The common variant Q192R at the paraoxonase 1 (PON1) gene and its activity are responsible for a portion of the altered antioxidant status in type 2 diabetes. Exp Biol Med (Maywood) 2016;241:1489-96.
28. Gamboa R, Zamora J, Rodriguez-Perez JM, Fragoso JM, Cardoso G, Posadas-Romero C, et al. Distribution of paraoxonase PON1 gene polymorphisms in Mexican populations. Its role in the lipid profile. Exp Mol Pathol 2006;80:85-90.
29. Gardemann A, Philipp M, Hess K, Katz N, Tillmanns H, Haberbosch W. The paraoxonase Leu-Met54 and Gln-Arg191 gene polymorphisms are not associated with the risk of coronary heart disease. Atherosclerosis 2000;152:421-31.
30. Turban S, Fuentes F, Ferlic L, Brugada R, Gotto AM, Ballantyne CM, et al. A prospective study of paraoxonase gene Q/R192 polymorphism and severity, progression and regression of coronary atherosclerosis, plasma lipid levels, clinical events and response to fluvastatin. Atherosclerosis 2001;154:633-40.
31. Blatter Garin MC, Moren X, James RW. Paraoxonase-1 and serum concentrations of HDL-cholesterol and apoA-I. J Lipid Res 2006;47:515-20.
32. Pérez-Herrera N, May-Pech C, Hernández-Ochoa I, Castro-Mañé J, Rojas-García E, Borja-Aburto VH, et al. PON1Q192R polymorphism is associated with lipid profile in Mexican men with Mayan ascendancy. Exp Mol Pathol 2008;85:129-34.
33. Lima J, Alexandre P, Leão L, Rodrigues L, Almeida M, Leal D. Paraoxonase activity polymorphism relationships with lipid profiles in a Brazilian population. Epidemiology 2007;18 Suppl:S168.
34. Saha N, Roy AC, Teo SH, Tay JS, Ratnam SS. Influence of serum paraoxonase polymorphism on serum lipids and apolipoproteins. Clin Genet 1991;40:277-82.
35. Ombres D, Pannitteri G, Montali A, Candeloro A, Seccareccia F, Campagna F, et al. The gln-Arg192 polymorphism of human paraoxonase gene is not associated with coronary artery disease in italian patients. Arterioscler Thromb Vasc Biol 1998;18: 1611-6.
36. Hegele RA, Brunt JH, Connelly PW. A polymorphism of the paraoxonase gene associated with variation in plasma lipoproteins in a genetic isolate. Arterioscler Thromb Vasc Biol 1995;15:89-95.
37. Oliveira SA, Mansur AP, Ribeiro CC, Ramires JA,

Annichino-Bizzacchi JM. PON1 M/L55 mutation protects high-risk patients against coronary artery disease. Int J Cardiol 2004;94:73-7.
38. Aynacioglu AS, Kepekci Y. The human paraoxonase Gln-Argl92 (Q/R) polymorphism in turkish patients with coronary artery disease. Int J Cardiol 2000;74: 33-7.
39. Barbieri M, Bonafe M, Marfella R, Ragno E, Giugliano D, Franceschi C, et al. LL-paraoxonase genotype is associated with a more severe degree of homeostasis model assessment IR in healthy subjects. J Clin Endocrinol Metab 2002;87:222-5.
40. Dell'Omo G, Penno G, Pucci L, Lucchesi D, Del Prato S, Pedrinelli R. Q192R paraoxonase (PON)1 polymorphism, insulin sensitivity, and endothelial function in essential hypertensive men. Clin Med Insights Cardiol 2014;8:57-62.