

Original Article

Amendment of amino acid in Q192R genetic polymorphism of paraoxonase 1 is a conventional risk factor for type 2 diabetes mellitus in the Saudi population

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Abstract: Background: The paraoxonase 1 (*PON1*) gene polymorphism Q192R has been found to be consistent with multiple metabolic diseases comprising type 2 diabetes mellitus (T2DM). The R allele has been found to be associated with coronary artery disease and gestational diabetes in a Saudi population. Therefore, we attempted to determine the association between Q192R and T2DM in a Saudi population. Materials and methods: Eight hundred subjects were enrolled in this case-control study, including T2DM patients ($n = 400$) and control individuals ($n = 400$). Epidemiological, clinical, and Q192R genotype data were obtained from all the subjects included in this study. Genotyping was performed by PCR-RFLP analysis followed by 2.5% agarose gel electrophoresis. Results: The clinical characteristics and metabolic variables were significantly higher in T2DM compared with controls, and also with allele and genotype frequencies [R vs. Q: odds ratio (OR), 1.659; 95% confidence interval (95% CI), 1.344-2.048; $P = 0.0002$; RR vs. QQ; OR, 2.1; 95% CI, 1.3-3.2; $P = 0.001$; QR+RR vs. QQ; OR, 2.101; 95% CI, 1.583-2.788; $P = 0.0002$]. Multiple regression analysis showed positive correlation of lipid profile with genotype ($P < 0.05$). Conclusion: The present findings provide robust evidence of *PON1* Q192R polymorphism being associated with T2DM in a Saudi population.

Keywords: PON1, Q192R, T2DM, Saudi population

Introduction

Type 2 diabetes mellitus (T2DM) is generally recognized as a multi-factorial disease characterized by insulin resistance and reduced glucose-stimulated insulin secretion [1]. This disease is the result of exposure to both genetic and environmental risk factors, particularly western lifestyle. The disease pathogenesis involves a combination of β -cell insufficiency and insulin resistance [2]. Genome-wide association studies have identified new genetic variants with reproducible associations with susceptibility to T2DM, the majority of which were found in genes that have not even been consid-

ered as candidate genes [3]. Paraoxonase 1 (*PON1*) is a calcium-dependent esterase that catalyzes reactive oxygen species produced under oxidative stress during atherogenesis and contributes to the prevention of low-density lipoprotein (LDL) from oxidation. This enzyme also hydrolyzes the active metabolite of an insecticide, parathion [4, 5]. *PON1* maps to human chromosome 7q21-22, and several polymorphisms in the promoter and coding regions have been identified. It contains a coding region polymorphism at position 192 (glutamine [Q] to arginine [R] substitution) [6]. *PON1* activity is reduced in high oxidative stress diseases such as coronary heart disease (CHD), dyslipidemia,

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Table 1. Clinical characteristics of T2DM patients and control subjects in a Saudi population

	T2DM (n = 400)	Controls (n = 400)	P
Age (Years)	53.6±10.8	46.0±7.7	< 0.001
Sex: Male/Female	232/168	211/189	0.13
Body mass index (kg/m ²)	30.7±6.3	29.2±5.5	0.001
Waist (cms)	94.5±22.2	91.6±19.9	0.02
Hip (cms)	110.8±18.2	101.4±7.8	0.001
SBP (mmHg)	124.3±11.2	114.9±7.7	< 0.001
DBP (mmHg)	78.4±6.9	75.6±6.0	< 0.001
FBS (mmol/L)	12.9±4.6	5.2±0.6	< 0.001
Triglycerides (mmol/L)	2.2±1.2	1.6±0.8	< 0.001
Cholesterol (mmol/L)	5.6±1.2	5.1±1.0	< 0.001
HDL- cholesterol (mmol/L)	0.8±0.4	0.6±0.2	< 0.001
LDL- cholesterol (mmol/L)	3.8±1.0	3.6±0.8	0.18
Glucose (mmol/L)	9.4±1.5	8.7±1.8	0.001
Insulin (μU/mL)	16.2±2.2	12.3±1.7	0.006
Homa-IR	7.1±2.4	2.8±1.7	< 0.0001
Family History	354 (88.5%)	210 (52.5%)	< 0.0001

inflammatory processes, diabetes, gestational diabetes mellitus (GDM), and certain neuropathies [3, 7, 8]. Several prospective studies have shown that low PON1 activity is an independent risk factor for new coronary events, independent of all other risk factors, including high-density lipoprotein (HDL) [9]. In earlier studies from Saudi Arabia, *PON1* was found to be associated with GDM [3] and coronary artery disease (CAD) [10]. In the present study, *PON1* polymorphism Q192R was studied in the same population. The aim of this study was to evaluate, for the first time, allele and genotype frequencies of Q192R polymorphism in *PON1* in relation with T2DM in a Saudi population.

Materials and methods

Ethics statement

This study was approved by the institutional review board at the King Khalid University Hospitals, King Saud University, Riyadh, Kingdom of Saudi Arabia. Written informed consent was obtained from all the participants of the study.

Selection of subjects

In this case control study, 800 subjects were recruited. Four hundred subjects were diagnosed with T2DM and had been monitored for

a minimum of 6 years after diagnosis. Four hundred healthy control participants were selected from the general Saudi population. The details of the selection of all the subjects were included in an earlier publication [11].

Blood sampling

Venous blood samples (5 mL) were obtained from all the subjects. Serum samples (3 mL each) were collected in plain vacutainers and used to measure the biochemical profile, and blood-EDTA samples (2 mL) were used for studying genotype and allele frequencies of the Q192R polymorphism.

Clinical analysis

Anthropometric parameters, including, weight (in kilograms), height (in meters), waist circumference (in centimeters), hip circumference (in centimeters), systolic blood pressure (SBP) (mmHg), and diastolic blood pressure (DBP) (mmHg) were calculated as mentioned in our earlier publication [12]. Body mass index (BMI) was calculated as weight/height² (kg/m²). Subjects with a BMI ≥ 25 kg/m² were considered overweight and those with a BMI ≥ 30 kg/m² as obese. Clinical details of all the subjects were recorded, and subjects were classified as T2DM patients and healthy controls. The blood pressure of the subjects was measured in a sitting position, and the mean of 2 readings, 30 min apart, was taken. Hypertension was defined as mean SBP of 140 mmHg and/or a DBP of 90 mmHg.

Biochemical analysis

Plasma glucose samples were collected from all the subjects after overnight fasting for at least 12 h. Fasting blood sugar (FBS), and lipid profile, comprising total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C), were measured. Insulin resistance index [homeostasis model assessment-Insulin resistance (HOMA-IR)] was calculated as fasting insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5, and β-cell function (HOMA-β) was calculated as fasting insulin ×

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Table 2. Genotype and allele distribution of Q192R polymorphism in T2DM patients and control subjects

rs662 (Q192R)	T2DM Cases (n = 400)	Controls (n = 400)	X ²	Odds ratio (95% CI)	p value ^a
Genotype and allele	N (%)	N (%)			
QQ	149 (37.25)	222 (55.5)	-	1.0	0.0
QR	193 (48.25)	136 (34)	23.8	2.1 (1.5-2.8)	0.0001
RR	58 (14.5)	42 (10.5)	10.1	2.0 (1.3-3.2)	0.001
QR+RR	251 (62.75)	178 (44.5)	26.7	2.1 (1.5-2.7)	0.0002
Q	491 (0.61)	580 (0.725)			
R	309 (0.39)	220 (0.275)	22.3	1.6 (1.3-2.0)	0.0002

^aChi-square p value.

20/(fasting plasma glucose-3.5), as described previously [13].

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using the Norgen DNA extraction kit (Norgen Biotek Corp, Canada). DNA samples were stored at -80°C. Genotyping of the Q192R (rs662) polymorphism was performed by polymerase chain reaction (PCR) using the Norgen 2 × master mix, followed by restriction fragment length polymorphism (RFLP) analysis. PCR amplification of Q192R polymorphisms of the *PON1* gene was performed with the primers described by Al-Hakeem [3]. PCR amplification of Q192R polymorphism was carried out in 20-μL reactions, each containing 75 ng of genomic DNA (1.0 μL), 15.0 μL of 2× Norgen master mix, 1.0 μL of both forward and reverse primers, and 2.0 μL of sterile water. Thermal cycling was as follows: DNA denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 45 s; and a final extension at 72°C for 5 min. The PCR products were digested by restriction endonuclease *Mbol* (G¹ATC) at 37°C for 4 h and analyzed by 2.5% agarose gel electrophoresis.

Statistical analysis

The expected and observed frequencies of categorical variables were measured at a significance threshold of $P < 0.05$ (two-tailed), and data were analyzed by using a statistical software package, SPSS version 19.0 (IBM Corp., Chicago, IL, USA). Genotype frequency difference between T2DM patients and control subjects were tested by the chi-square test. Odds

ratios (ORs) and 95% confidence interval (95% CI) were calculated by binomial logistic regression for the allele, genotype, and haplotype frequencies, and departures from Hardy-Weinberg equilibrium were identified. The data for 3 or more independent groups were analyzed by one-way analysis of variance (ANOVA). $P < 0.05$ was used as the criterion of significance. Multinomial logistic

regression was used to test for association of disease severity and genotypes, assessing independence from potential confounders [10]. OR and 95% CI values were calculated to estimate the strength of the association between polymorphisms and T2DM.

Results

Participant characteristics

Basic demographic and clinical characteristics of the T2DM cases and control subjects are provided in **Table 1**. The mean age was 53.6 years for T2DM patients and 46.0 years for the control group. There was a significant difference in age, BMI, waist circumference, hip circumference, SBP, DBP, FBS, TC, TG, and HDL-C between the T2DM patients and healthy controls. HOMA-IR was found to be significantly associated with T2DM ($P < 0.0001$). Sex and LDL-C were not found to be associated ($P > 0.05$). In this study, 88.5% of the T2DM cases had a family history of T2DM; however, only 52.5% of the control group had a family history of T2DM.

Molecular analysis of Q192R polymorphism

Distribution of alleles and genotype frequencies of Q192R polymorphism in T2DM patients and healthy controls fulfilled the Hardy-Weinberg equilibrium ($\chi^2 = 9.9$; $P = 0.23$). The genotype and allele distribution of Q192R polymorphism is concisely presented in **Table 2**. The frequencies of Q192R genotypes were as follows: QQ (37.25%), QR (48.25%), and RR (14.5%) in patients and QQ (55.5%), QR (34%), and RR (10.5%) in control individuals. The genotype distribution between the T2DM patients and he-

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Table 3. Multiple logistic regression analysis association of the Q192R polymorphism with T2DM risk

Parameters	β	SE	ORs (95% CI)	<i>p</i> -value
Age	-0.0096	0.017	1.0 (0.95-1.0)	0.58
Gender (Male vs. Female)	0.185	0.36	1.20 (0.59-2.45)	0.61
BMI	0.0072	0.028	1.0 (0.95-1.0)	0.80
SBP	0.0019	0.015	1.0 (0.97-1.0)	0.90
DBP	0.012	0.025	1.0 (0.96-1.0)	0.63
FBS	-0.042	0.040	0.95 (0.88-1.0)	0.29
TG	-593.3	44.0	< 0.0001 (< 0.0001-< 0.0001)	< 0.0001
TC	1305.0	96.8	< 0.0001 (< 0.0001-< 0.0001)	< 0.0001
HDL-C	0.42	0.17	1.42 (1.1-2.0)	0.04
LDL-C	-1304	96.8	< 0.0001 (< 0.0001-< 0.0001)	< 0.0001
Q192R polymorphism				
QR	0.15	0.36	3.85 (1.4-3.7)	0.0001
RR	0.48	0.51	2.61 (1.2-3.6)	0.0001

A *p* value significant at < 0.05, β coefficient, SE Standard error, *Reference to QQ genotype.

Table 4. Distribution of patient characteristics according to Q192R genotypes

	QQ (<i>n</i> = 149)	QR (<i>n</i> = 193)	RR (<i>n</i> = 58)	<i>P</i>
Age (Years)	54.2±10.7	53.0±10.8	53.5±11.0	0.96
Body mass index (kg/m ²)	29.4±6.6	29.5±4.8	30.1±5.4	0.0001
Sex: Male/Female	101 (67.8)/48 (32.2)	102 (52.8)/91 (47.2)	28 (48.2)/30 (51.8)	0.001
SBP (mmHg)	123.1±12.1	124.1±10.5	126.1±11.0	0.22
DBP (mmHg)	78.1±6.4	79.1±7.6	78.1±6.4	0.35
Waist (cms)	93.1±22.1	96.3±23.7	94.3±20.8	0.42
Hips (cms)	110.1±6.1	112.6±6.2	110.1±6.1	0.003
FBS (mmol/L)	12.7±5.0	13.1±4.4	12.9±4.3	0.58
TG (mmol/L)	2.1±1.1	2.6±1.3	2.2±1.2	0.005
TC (mmol/L)	5.0±0.9	5.1±1.0	4.9±0.9	0.32
HDL-C (mmol/L)	0.6±0.2	0.6±0.2	0.6±0.2	1.0
LDL-C (mmol/L)	3.6±0.8	3.7±0.8	3.6±0.9	0.47

althy controls were significantly different ($P < 0.05$). The frequency of the R allele in T2DM patients was higher in control subjects (OR, 1.6; 95% CI, 1.3-2.0; $P = 0.0002$). There was a significant difference between RR vs. QQ (OR, 2.0; 95% CI, 1.3-3.2; $P = 0.001$) and QR+RR vs. QQ genotypes (OR, 2.1; 95% CI, 1.5-2.7; $P = 0.0002$) between the cases and controls. The co-dominant model (RR vs. QR+QQ) was found to yield the same result with risk of T2DM (OR, 1.4; 95% CI, 0.9-2.2; $P = 0.08$).

Multiple logistic regression analysis

The association of Q192R polymorphism with T2DM risk was further tested by multiple logistic regression analysis for its independence from other risk factors (Table 3). Lipid profile

features such as TG, TC, LDL-C, and HDL-C were found to be associated with T2DM risk ($P < 0.05$). Association of QR (OR, 3.8; 95% CI, 1.4-3.7; $P = 0.0001$) and RR (OR, 2.6; 95% CI, 1.2-3.6; $P = 0.0001$) genotypes with T2DM risk remained significant even after accounting for these risk factors.

Characteristics of genotype distribution

The distribution of clinical and anthropometric parameters based on the QQ, QR, and RR genotypes of the Q192R polymorphism was also analyzed to study the effects of this polymorphism on such parameters. BMI, sex, waist circumference, and TG values were found to be significantly associated ($P < 0.05$) when compared with the 3 different genotypes, namely,

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QQ, QR, and RR. BMI was found to be high in the QR genotype ($P < 0.05$), whereas sex, hip circumference, and TG were found to be high in RR genotypes ($P < 0.05$) (Table 4).

Discussion

Diabetes is a risk factor for cardiovascular diseases, associated micro- and macrovascular complications include retinopathy, neuropathy, nephropathy, CAD, cerebrovascular disease, and peripheral vascular disease, leading to death in the diabetic population [14]. The aims of this study were to investigate the association between the *PON1* polymorphism Q192R and T2DM in a Saudi population and to discuss this association with regard to clinical and biochemical factors. To the best of our knowledge, this is the first study to investigate the association of the Q192R polymorphism and T2DM in a Saudi population. Several studies have shown conflicting results regarding the association between specific biomarkers and the prediction of T2DM. Genetic polymorphisms that correlate with specific phenotypes can sometimes be associated with the development of human diseases in different ethnic groups [15].

PON1 was first investigated for its ability to hydrolyze highly toxic oxon forms of the organophosphorus pesticides parathion, chlorpyrifos, and diazinon, as well as the nerve agent's sarin and soman [16]. *PON1* is a 26,857-bp gene with 9 exons, localized on chromosome 7q21.3 (gene ID: 5444), along with *PON2* and *PON3*, which share ~65% similarity at the amino acid level [17]. It encodes a glycoprotein located on the surface of HDLs and plays a pivotal role in preventing LDL oxidation [18]. The enzyme *PON1* present in the serum of mammals is responsible for resistance to organophosphate toxicity [19]. Q192R is a common polymorphism wherein glutamine is substituted by arginine, affecting the hydrolytic activity of *PON1* isoenzymes with respect to certain substrates, such as paraoxon and lipid peroxides [14]. The analysis of *PON1* 192QR polymorphism has revealed that the high-activity allele (R) is associated with a more atherogenic lipid profile than the low-activity allele (Q). In the present study, the *PON1* 192R allele frequency in T2DM patients was found to be higher when compared with control subjects ($P < 0.0001$).

There are different reports on the distribution of alleles Q and R with regard to wild and mutant

allele states in various ethnic populations. The present control subjects with Q and R alleles were similar with Saudi subjects with CAD and GDM [3, 10]; the control subjects were closer with Caucasians [16, 20, 21], Asian Indians [22], Turks [23], and Egyptians [24], but differed from Japanese [25, 26], Chinese [18], and Hispanic [27] populations where the R allele was predominant. Importantly, Q and R allele distributions observed in this study confirm a previous, independent study carried out in a Saudi population, where the phenotypic frequencies (paraoxonase/arylesterase activity ratio) suggested that the low-activity phenotype (Q allele) was the major allele and the high-activity phenotype (R allele) the minor allele [28]. However, we did not find the common associations with the alleles in the case of subjects with different diseases.

Thus far, no meta-analyses have been carried out on T2DM with regard to Q192R polymorphisms. However, limited meta-analysis studies have been carried out with different diseases, and among them, stroke was positively associated according to Banerjee [29] and negatively associated according to Dahabreh [30]. Alzheimer disease, breast cancer, and Parkinson disease have not shown any such positive association [31-33]. Two meta-analyses on CHD differed with regard to statistical association [34, 35]. Lescai et al [36] conclude a positive association with the longevity gene. From all the meta-analysis studies, stroke and cardiovascular events were found to be significantly associated with specific alleles. *PON1* inhibits the peroxidation of LDL and it is the main antioxidant enzyme of HDL and it. The earlier studies have been correlated the Q192R polymorphism with the lipid levels in T2DM subjects. The lipid profiles were significantly associated with [37, 38] and without [39, 40] Q192R polymorphisms in T2DM subjects and our results is also accordance with the significantly associated studies.

The present study had several limitations. First, this was a case-control study, where casual associations can be frequent when the sample size is relatively small for the association analysis of complex diseases with genetic variants of multifactorial traits. Secondly, this case-control study was not age-matched ($P < 0.001$), but sex-matched ($P = 0.13$). Third, these results should be interpreted with caution because the

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population was only from Saudi Arabia, which increases the possibility of confounding due to ethnicity. We have opted only the single snip, which could be our fourth limitation of our study. Therefore, the present findings might not be extrapolated to other ethnic groups. Finally, paraoxonase levels were not measured.

This study found an association between T2DM risk and the Q192R polymorphism in Saudi subjects. Further studies using next-generation sequencing of exomes and targeted genes could be employed for identifying the genetic modifiers proposed from the findings of this study and previous studies. Molecular and genetic studies in different populations with larger sample sizes evaluating additional *PON1* polymorphisms, haplotypes, and importantly, *PON1* activity, may comprehensively provide the role of genotype and phenotype applications in determining and predicting T2DM risk in this population.

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Disclosure of conflict of interest

None.

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References

- [1] Song JF, Wang T, Zhu J, Zhou XY, Lu Q, Guo H, Zhang F, Wang Y, Li W, Wang DD, Cui YW, Lv DM, Yin XX. PPAR δ rs2016520 polymorphism affects repaglinide response in Chinese Han patients with type 2 diabetes mellitus. *Clin Exp Pharmacol Physiol* 2015; 42: 27-32.
- [2] Khan IA, Poornima S, Jahan P, Rao P, Hasan Q. Type 2 Diabetes Mellitus and the Association of Candidate Genes in Asian Indian Population from Hyderabad, India. *J Clin Diagn Res* 2015; 9: GC01-5.
- [3] Al-Hakeem MM, Abotalib Z, Alharbi KK, Khan IA. Relationship between the paraoxonase 1 gene glutamine 192 to arginine polymorphism and gestational diabetes mellitus in Saudi women. *Clin Biochem* 2014; 47: 122-125.
- [4] Unur M, Demirez E, Agachan B, Gormus U, Ergen A, Dalan B, Isbir T. The relationship of oral disturbances of diabetes mellitus patients with paraoxonase gene polymorphisms. *Cell Biochem Funct* 2008; 26: 870-873.
- [5] Murata M, Maruyama T, Suzuki Y, Saruta T, Ikeda Y. Paraoxonase 1 Gln/Arg polymorphism is associated with the risk of microangiopathy in Type 2 diabetes mellitus. *Diabet Med* 2004; 2: 837-844.
- [6] Fekih O, Triki S, Hellara I, Neffati F, Rejeb J, Ommezine A. Can paraoxonase 1 polymorphisms (L55 M and Q192 R) protect children with type 1 diabetes against lipid abnormalities? *J Clin Lipidol* 2014; 8: 249-255.
- [7] Nowak M, Wielkoszynski T, Marek B, Kos-Kudła B, Swietochowska E, Sieminska L, Karpe J, Kajtaniuk D, Głogowska-Szeląg J, Nowak K. Antioxidant potential, paraoxonase 1, ceruloplasmin activity and C-reactive protein concentration in diabetic retinopathy. *Clin Exp Med* 2010; 10: 185-192.
- [8] Tomas M, Latorre G, Senti M, Marrugat J. The antioxidant function of high density lipoproteins: a new paradigm in atherosclerosis. *Rev Esp Cardiol* 2004; 57: 557-569.
- [9] Mackness B, Marsillach J, Elkeles RS, Godsland IF, Feher MD, Rubens MB, Flather MD, Humphries SE, Cooper J, Mackness M. Paraoxonase-1 is not associated with coronary artery calcification in type 2 diabetes: results from the PREDICT study. *Dis Markers* 2012; 33: 101-112.
- [10] Hassan MA, Al-Attas OS, Hussain T, Al-Daghri NM, Alokail MS, Mohammed AK, Vinodson B. The Q192R polymorphism of the paraoxonase 1 gene is a risk factor for coronary artery disease in Saudi subjects. *Mol Cell Biochem* 2013; 380: 121-128.
- [11] Alharbi KK, Hussain T, Alharbi FK, Tabassum SN, Mohammed AA, Gambhir D, Khan IA. Apolipoprotein C3 Gene Variants and Risk of Developing Type 2 Diabetes in Saudi Subjects. *Metab Syndr Relat Disord* 2015; 13: 298-303.
- [12] Alharbi KK, Khan IA, Syed R. Association of apolipoprotein E polymorphism with type 2 diabetes mellitus in a Saudi population. *DNA Cell Biol* 2014; 33: 637-641.
- [13] Alharbi KK, Khan IA, Al-Sheikh YA, Alharbi FK, Alharbi FK, Al-Nbaheen MS. Lack of association between UBE2E2 gene polymorphism (rs7612463) and type 2 diabetes mellitus in a Saudi population. *Acta Biochim Pol* 2014; 61: 769-772.

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- [14] Elattar N, Swelam EE, Hamed E, Elnahal A, Mostafa E. PARAOXONASE 1 Gene Polymorphism Relationship with Type 2 Diabetes Mellitus. *Life Science Journal* 2012; 9: 1742-1751.
- [15] Bhaskar S, Ganesan M, Chandak GR, Mani R, Idris MM, Khaja N, Gulla S, Kumar U, Movva S, Vattam KK, Eppa K, Hasan Q, Pulakurthy UR. Association of PON1 and APOA5 gene polymorphisms in a cohort of Indian patients having coronary artery disease with and without type 2 diabetes. *Genet Test Mol Biomarkers* 2011; 15: 507-512.
- [16] Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet* 2001; 68: 1428-1436.
- [17] Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multi-gene family. *Genomics* 1996; 33: 498-507.
- [18] Wang X, Fan Z, Huang J, Su S, Yu Q, Zhao J, Hui R, Yao Z, Shen Y, Qiang B, Gu D. Extensive association analysis between polymorphisms of PON gene cluster with coronary heart disease in Chinese Han population. *Arterioscler Thromb Vasc Biol* 2003; 23: 328-334.
- [19] Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, Miller JE, Boulton AJ, Durrington PN. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. *Atherosclerosis* 1998; 139: 341-349.
- [20] Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, Roberts C, Durrington PN, Mackness MI. Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? *Arterioscler Thromb Vasc Biol* 2001; 21: 1451-1457.
- [21] Scacchi R, Corbo RM, Rickards O, De Stefano GF. New data on the world distribution of paraoxonase (PON1 Gln 192 → Arg) gene frequencies. *Hum Biol* 2003; 75: 365-373.
- [22] Sanghera DK, Saha N, Aston CE, Kamboh MI. Genetic polymorphism of paraoxonase and the risk of coronary heart disease. *Arterioscler Thromb Vasc Biol* 1997; 17: 1067-1073.
- [23] Bayrak A, Bayrak T, Tokgozoglu SL, Volkan-Salanci B, Deniz A, Yavuz B, Alikasifoglu M, Demirpençe E. Serum PON-1 activity but not Q192R polymorphism is related to the extent of atherosclerosis. *J Atheroscler Thromb* 2012; 19: 376-384.
- [24] Mohamed RH, Mohamed RH, Karam RA, Abd El-Aziz TA. The relationship between paraoxonase1-192 polymorphism and activity with coronary artery disease. *Clin Biochem* 2010; 43: 553-558.
- [25] Zama T, Murata M, Matsubara Y, Kawano K, Aoki N, Yoshino H, Watanabe G, Ishikawa K, Ikeda Y. A 192Arg 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* 1997; 17: 3565-3569.
- [26] Suehiro T, Nakamura T, Inoue M, Shiinoki T, Ikeda Y, Kumon Y, Shindo M, Tanaka H, Hashimoto K. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. *Atherosclerosis* 2000; 150: 295-298.
- [27] Rojas-Garcia AE, Solis-Heredia MJ, Pina-Guzman B, Vega L, Lopez-Carrillo L, Quintanilla-Vega B. Genetic polymorphisms and activity of PON1 in a Mexican population. *Toxicol Appl Pharmacol* 2005; 205: 282-289.
- [28] Nogueira CP, Evans DA, La Du BN. The paraoxonase polymorphism in a Saudi Arabian population. *Pharmacogenetics* 1993; 3: 144-149.
- [29] Banerjee I. Relationship between Paraoxonase 1 (PON1) gene polymorphisms and susceptibility of stroke: a meta-analysis. *Eur J Epidemiol* 2010; 25: 449-458.
- [30] Dahabreh IJ, Kitsios GD, Kent DM, Trikalinos TA. Paraoxonase 1 polymorphisms and ischemic stroke risk: A systematic review and meta-analysis. *Genet Med* 2010; 12: 606-615.
- [31] Pi Y, Zhang L, Chang K, Li B, Guo L, Fang C, Gao C, Wang J, Xiang J, Li Je. Lack of an association between Paraoxonase 1 gene polymorphisms (Q192R, L55M) and Alzheimer's disease: a meta-analysis. *Neurosci Lett* 2012; 523: 174-179.
- [32] Saadat M. Paraoxonase 1 genetic polymorphisms and susceptibility to breast cancer: a meta-analysis. *Cancer Epidemiol* 2012; 36: e101-3.
- [33] Liu YL, Yang J, Zheng J, Liu DW, Liu T, Wang JM, Wang CN, Wang MW, Tian QB. Paraoxonase 1 polymorphisms L55M and Q192R were not risk factors for Parkinson's disease: a HuGE review and meta-analysis. *Gene* 2012; 501: 188-192.
- [34] Lawlor DA, Day IN, Gaunt TR, Hinks LJ, Briggs PJ, Kiessling M, Timpson N, Smith GD, Ebrahim S. The association of the PON1 Q192R polymorphism with coronary heart disease: findings from the British Women's Heart and Health cohort study and a meta-analysis. *BMC Genet* 2004; 23: 5:17.
- [35] Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J. Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. *Lancet* 2004; 363: 689-695.
- [36] Lescai F, Marchegiani F, Franceschi C. PON1 is a longevity gene: results of a meta-analysis. *Ageing Res Rev* 2009; 8: 277-284.

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- [37] 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.
- [38] Ruiz J, Blanché H, James RW, Garin MC, Vaisse C, Charpentier G, Cohen N, Morabia A, Passa P, Froguel P. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. *Lancet* 1995; 346: 869-872.
- [39] Agachan B, Yilmaz H, Karaali Z, Isbir T. Paraoxonase 55 and 192 polymorphism and its relationship to serum paraoxonase activity and serum lipids in Turkish patients with non-insulin dependent diabetes mellitus. *Cell Biochem Funct* 2004; 22: 163-168.
- [40] Gupta N, Binukumar BK, Singh S, Sunkaria A, Kandimalla R, Bhansali A, Gill KD. Serum paraoxonase-1 (PON1) activities (PONase/AREase) and polymorphisms in patients with type 2 diabetes mellitus in a North-West Indian population. *Gene* 2011; 487: 88-95.