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Original Article

Paraoxonase-1 Q192R polymorphism and its association with hs-CRP and fasting blood glucose levels and risk of coronary artery disease

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ABSTRACT

Aims: Paraoxonase-1 (PON1) has been shown to protect low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) against oxidative-modification and thereby might protect against coronary-artery-disease (CAD). Here we explored the relationship of a genetic variant (a substitution (R) Arg with (Q) Gln at position 192) of PON1 in 250 patients with/without CAD.
Materials and methods: Genotyping of PON1 Q192R was carried out using Real-Time-PCR TaqMan-based-probe. Demographic-characteristics and biochemical-analyses, including fasting blood sugar (FBS), HDL, LDL, triglycerides (TG) and C-reactive protein (CRP) were evaluated. Univariate/multivariate analyses were performed to determine the association of the genetic polymorphism and CAD as well as with clinical-characteristics of population.
Results: Our findings showed that RR-genotype was more frequent in CAD-patients, compared to the wild-type genotype. Moreover, CAD patients with RR-genotype had an odd ratio of 5.0 (95% CI: 1.3–18.6; $p = 0.017$), versus wild-type genotype, in multivariate-analysis. Of note we also observed that CAD-patients with QQ-genotype had a significantly lower Hs-CRP level, compared to the RR-genotype.
Conclusion: we demonstrate that PON1-Q192R-polymorphism was associated with CRP and FBS levels; R-allele of PON1-Q192R may be an independent risk factor for CAD. Further studies are warranted to determine the value of this marker as a surrogate marker in CAD patients.

1. Introduction

Coronary artery disease (CAD) is one of the major causes of death and morbidity worldwide [1,2]. In addition to common risk factors for CAD such as hypertension, hypercholesterolemia, diabetes, and smoking, it has been shown that genetic factors are involved in susceptibility to coronary atherosclerosis [3]. Paraoxonase 1 (PON1) is a glycoprotein with a molecular weight of 43 kDa, which is composed of 354 amino acids [4]. It is synthesized in the liver and associated with high-density lipoprotein (HDL) [5].

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It has been proposed that PON1plays an important role in lipoprotein metabolism and perhaps cardio vascular disease via preventing the oxidation of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [6]. The paraoxonase gene family comprises of three genes, PON1, PON2 and PON3 that are located on chromosome 7q21.3-q22.1. It has been suggested that a PON1gene polymorphism, substitution of (leucine vs. methionine) at position 55 and (glutamine vs. arginine) at position 192 is related to high risk of CAD [7]. Some of studies have displayed that the Q192R polymorphism is related to an increased risk of CAD [8–12]. However, this has been an inconsistent finding [13–18]. In particular, Antikainen., et al. found no association between the Gln-Arg 192 polymorphism of PON1 gene and coronary artery disease in Finn [6]. Another study investigated the association of the polymorphism of rs662 PON1 in 134 patients with myocardial infarction (MI), and 252 healthy controls in a Japanese population. They showed no association between genotypes and risk of CHD [6]. This may be justified for several reasons including ethnicity, life style, and environmental factors. Therefore in this study, we surveyed the association of PON1 rs662 polymorphism with CAD in a pilot study including 150 CAD patients and 100 control subjects in an Iranian population.

2. Materials and Methods

2.1. Population

In this study 250 subjects, including 150 CAD subjects, and 100 healthy controls were enrolled from Mashhad University of Medical Science [19,20], who subjects with stroke, and diabetes were excluded. CAD patients had signs or symptoms of cardiac disease (e.g., chest pain, ECG changes, unstable angina, angina of effort), and were validated by coronary angiography at Ghaem Medical Educational Hospital. Subjects had ≥50% occlusion in at least one coronary artery, as detected by angiography. Healthy control subjects that had no history of cardiovascular symptoms were enrolled. Informed consent was obtained from all participants according to protocols approved by the Ethics Committee of the MUMS.

2.2. Anthropometric and biochemical measurements

Anthropometric parameters such as height, body weight, waist and hip circumference were measured as described previously [21]. TC, HDL, LDL and TG, CRP, and FBG concentrations were measured as described previously [22].

2.3. Genotyping

Peripheral blood was obtained from the individuals, and genomic DNA was extracted using a QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) according to the manufacturer's protocol at the VU University Medical Center Amsterdam. The concentration and purity of DNA samples was measured with the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). The forward, probe and reverse primers of Q192R polymorphism were GAGCACTTTTATGGCACAAA, TAGTAGACAACATACGACCAC and FAM TCTCCCAGGATTGTAAGTAGGGGT-BHQ1,TET–TCTCCCAGGATCGTAA GTAGGGGT-BHQ1, respectively. Genotyping was carried out by Taqman®-probes-based assay and the ABIPRISM-7500 instrument equipped with the SDS version-2.0 software was used to evaluate the allelic content of the samples [23,24].

2.4. Statistical analysis

All statistical analyses were performed using SPSS-11.5 software (SPSS Inc., IL, USA). The Kolmogorov-Smirnov test was used to determine the normality of distribution. Clinical data were presented as means ± SD where appropriate or median and interquartile range. The *t*-student test was utilized to survey the clinical and baseline characteristics between the groups for normally distributed variables and continuous variables with not normal distribution; the Mann-Whitney *U* test was used. A Bonferonni correction was used for multiple comparisons Categorized variables were analyses by the Chi-square or Fisher exact tests. Logistic regression was used to determine the odds ratios for the association between the polymorphism and CAD. A two-sided P-value <0.05 was considered statistically significant.

3. Result

3.1. Clinical and baseline characteristics of population

The baseline characteristics of the controls and CAD patients are shown in Table 1. In particular subjects with CAD had a significantly higher FBS, HC, and HsCRP ($p < 0.05$), while no differences were detected for Age, gender, WC, serum TC, LDL-C, HDL-C, TG, SBP and DBP between the groups (Table 1).

3.2. PON1 polymorphism and CAD

To investigate whether there was any relationship between CAD and Q192R polymorphism, we performed genotyping using genomic DNA extracted from peripheral blood samples. As shown in Table 2, the wild-type Q192R genotype (QQ) had a frequency of 8% whereas the QR and RR genotypes were found in 68% and 24% of the CAD group, respectively. The corresponding frequencies in the control group were 31% (QQ), 60% (QR), and 9% (RR). Moreover, individuals with RR genotypes were overrepresented in the CAD group, with an OR of 3.2 (95% CI: 1.5–7.0; $p = 0.003$). Further adjustment for age, gender, FBG, LDL-C,HDL-C, Systolic Blood Pressure, Diastolic Blood Pressure reduced the magnitude of the association with an OR of 5.0 (95% CI: 1.3–18.6; $p = 0.017$) (Table 2).

3.3. Association of polymorphisms with lipid profile

We also investigated the association of the genotypes with LDL-C, HDL, FBS, HsCRP and TG levels. This analysis showed that the

Table 1
Clinical and demographic characteristics of population.

Characteristics	Control (n = 100)	CAD (n = 150)	P Value
Age, year	54.12 ± 9.128	59.08 ± 10.73	0.306
Gender, male no (%)	M: %62 و F: %38	M: %62 و F: %38	0.999
Weight, kg	70.09 ± 11.553	69.384 ± 12.18	0.434
FBS (mg/dl)	82.17 ± 17.059	143.286 ± 73.44	0.001
TC (mg/dl)	191.57 ± 40.27	158.18 ± 47.80	0.126
TG (mg/dl)	129.5 ± 74	136.50 ± 78	0.374
HDL (mg/dl)	41.691 ± 9.388	37.052 ± 17.695	0.133
LDL (mg/dl)	123.361 ± 36.252	93.66 ± 47.655	0.276
BMI (kg/m2)	25.947 ± 3.451	26.213 ± 4	0.132
WC (cm)	87.856 ± 10.184	96.853 ± 11.418	0.766
HC (cm)	97.17 ± 5.952	101.6 ± 9.276	0.001
HsCRP(mg/dl)	1.285 ± 1.19	4.47 ± 8.59	0.001
DBP (mm Hg)	70.293 ± 9.265	75.31 ± 9.578	0.189
SBP (mm Hg)	122.829 ± 14.831	119.54 ± 18.394	0.180

Values are expressed as mean ± SD, or median and interquartile range for normally and non normally distributed variables, respectively. BMI: body mass index; WC: waist circumference, TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBS: fasting blood sugar; HC: hip circumference, SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2
Association between PON1 Q192R genetic polymorphism and CAD.

		Control	CAD	OR	95%CI	P value	OR[a]	95%CI[a]	P[a] value
PON1	QQ (n)	31	12	1			1		
	QR	60	102						
	RR (n)	9	36						
	RR-QR	69	138	3.2	1.5–7.0	0.003	5.0	1.3–18.6	0.017
	Q	42%	58%	1					
	R	61%	39%	2.6	1.5–3.1	0.0001			

[a] OR after adjustment (Age- Sex- FBS- LDL-C- HDL-C- Systolic Blood Pressure- Diastolic Blood Pressure).

PON1 rs662- RR + QR genotypes were significantly associated with an increased concentrations of Hs-CRP, in the CAD group, compared to the same genotypes in control group ($p > 0.001$) (Fig. 1), whilst CAD subjects with RR + QR had a significantly ($P = 0.04$) higher level of FBS compared to the wild type QQ genotype in CAD group (Fig. 1).

4. Discussion

In this study we investigated the association between PON1 Q192R polymorphism and CAD. Our results showed that PON1 Q192R polymorphism was associated with CAD and R allele of PON1 Q192R polymorphism can be considered as an independent risk factor for CAD. In agreement with our observation a recent study on 129 patients with CAD and 125 control, showed the higher rate of RR and QQ genotypes in CAD and control groups, respectively, and RR genotype was as a genetic risk factor for CAD [25]. Similarly another study by Agrawal and colleagues showed that RR genotype was significantly associated with CAD in Southeast Indian population [26]. Also Mackness et al., in a meta-analysis observed a higher frequency of the PON1 192R allele in CAD patients [27]. In line with these results, Gupta et al., found that 192R allele frequency was significantly higher in CAD patients, and 192QR and 192RR variants were associated with the increased risk of CAD [28]. Furthermore Liu and colleagues investigated PON1 expression by immunohistochemistry in 2456 unrelated Chinese Han individuals in order to investigate its polymorphisms and plasma status, with the risk of CAD. They showed that low PON1 expression was correlated with CAD. Also a significant association was detected for PON1 Q192R polymorphism with susceptibility of CAD and 192R allele was identified as an independent predictor for CAD [29]. Similar study by Al-Hakeem et al., in 2014 performed a case-control study in 500 pregnant women, including 200 Gestational diabetes mellitus (GDM) cases and 300 non-GDM women. They revealed the association of 192R polymorphism with Gestational diabetes mellitus in a Saudi population [30]. Consistent with these observations, another study explore the role of PON1 Q192R gene polymorphism in 242 ischemic stroke, 231 myocardial infarction, and 310 healthy control group in a Chinese population. They suggested the association of this genetic variant with stroke and MI. In particular patients with R genotypes were related with vascular disease with OR of 1.5 ($P = 0.03$) [31]. Similar results were also found in a Tunisian population [32]. Another study in obese subject illustrated the influence of Q192R polymorphisms on PON1 activity in Portuguese Caucasian premenopausal women [24]. Khoshi et al., assessed the association of PON1 polymorphisms with LDL/HDL

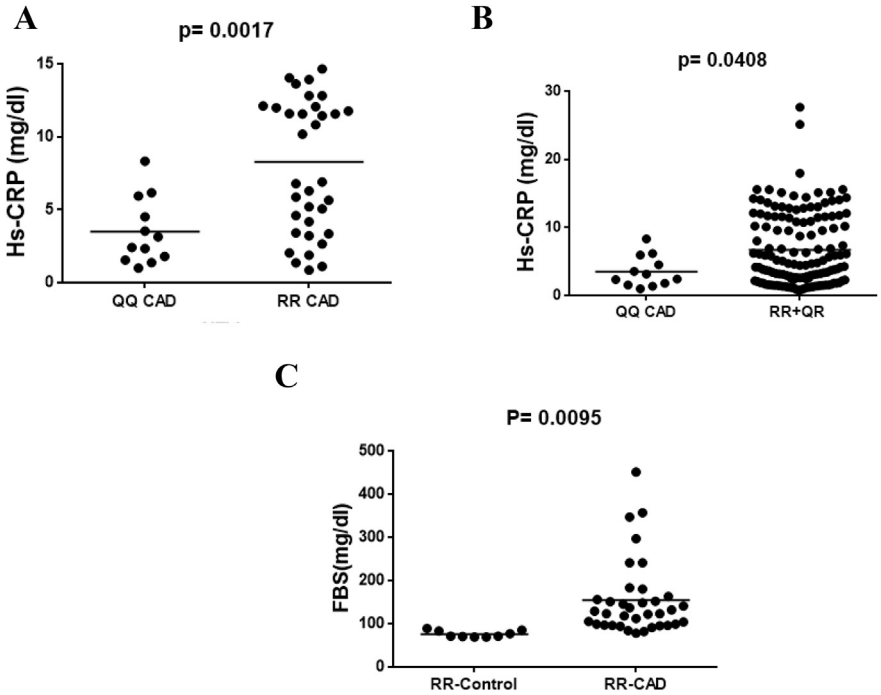

Fig. 1. Association of PON1 polymorphisms with HsCRP and FBS. A) HsCRP levels in QQ versus RR genotype in CAD group. B) HsCRP levels in QQ versus RR + QR genotypes in CAD group. C) FBS levels in RR genotype in CAD group versus control group.

ratios as an important risk factors to develop coronary heart diseases. This data showed that PON1 L55M polymorphism can affect lipid metabolism [33]. Conversely, several other studies have illustrated conflicting results [18,25,34]. In particular Taşkiran et al., performed a genetic study on 120 patients with CAD and 102 healthy subjects. They showed that PON1 192R allele frequency did not differ between groups and this genetic polymorphism was not associated with CAD [25]. Additionally, no association was observed between the Gln192Arg polymorphism of paraoxonase and CAD in a Turkish population [35]. Similarly Ko et al., in Taiwan population, found no relationship between genotype distribution and CAD patients [18]. Furthermore several studies have been shown the association of PON1 polymorphism or activity with plasma lipid profile and CRP [36,37]. In particular Charles-Schoeman and colleagues investigated the relationship between genetic and biochemical determinants of paraoxonase 1 activity in carotid plaque development as a surrogate marker of cardiovascular risk in patients with rheumatoid arthritis. This data revealed that, patients with the RR genotype demonstrated decreased risk of carotid plaque, CRP levels, compared to patients with QQ or QR genotype. Another study by Scherrer et al, investigated the correlation between p.Q192R SNP of PON1, biochemical parameters and carotid atherosclerosis in an asymptomatic, normolipidemic Brazilian population sample. They found that p.192Q variant was related with plasma lipid profile but not with carotid atherosclerosis [38]. In line, we observed an association between PON1 polymorphisms and CRP and FBG in our population. In particular our data showed that the level of HsCRP was lower in QQ genotype versus RR genotype in CAD group. Patients with RR + QR genotypes had also a significantly higher level of CRP, compared to wild type. Similar results we also observed for the level of FBG in CAD group.

The main limitations of this study were the cross-sectional study design and modest sample size. Additionally, it is possible that other lifestyle characteristics such as diet have an influence on the outcome, supporting further studies on the role of this polymorphism with CAD in a larger population.

In conclusion, we demonstrated the significant association of Q192R polymorphism with CAD and increased the level of HsCRP and FBS, supporting further studies on evaluating the role of these polymorphisms with CAD in a larger population.

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Conflicts of interest

The authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.01.010.

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