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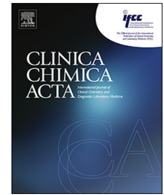

Paraoxonase 1 (PON1)-L55M among common variants in the coding region of the paraoxonase gene family may contribute to the glycemic control in type 2 diabetes

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A R T I C L E I N F O

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A B S T R A C T

Objective: Genome studies have shown that the genes encoding paraoxonase 1 (PON1) and PON2 are associated with glucose metabolism. The goal of this study was to simultaneously evaluate the association between functional variants in PON1 and PON2 genes and susceptibility for type 2 diabetes (T2D) and determine whether they can affect glycemic control.
Methods: We performed a case-control study with 145 newly diagnosed patients with T2D and 148 controls. The common variants including PON1-Q192R, PON1-L55M and PON2-S311C were genotyped by PCR-based RFLP. A mismatch-PCR/RFLP was applied for genotyping the PON2-A148G variant.
Results: The variant PON1-Q192R in males (OR = 2.55, 95%CI 1.16–5.69, $p = 0.023$) and PON2-A148G in females (OR = 1.56, 95%CI 1.00–2.44, $p = 0.059$) were associated with T2D. Compared with the LL genotypes of PON1-L55M, HbA1c levels were significantly lower in the LM genotypes ($p = 0.01$) and MM genotypes ($p = 0.032$) in patients. Multiple linear regression analyses showed that among the study variants only the PON1-L55M variant as an independent variable significantly associated with glycemic control. This variant significantly influenced glycemic control in patients with poor glycemic control so that it was better with the following order: LL < LM < MM. Based on gamma correlation, there was a significant inverse association between the number of M alleles of the PON1-L55M and HbA1c levels ($r = -0.261$, $p = 0.001$).
Conclusions: Sex should be considered a confounding variable in association studies on the variants PON1-Q192R and PON2-A148G in T2D. Patients sharing the 55 M allele were prone to having good glycemic control. Our findings provide genetic evidence that the PON1-L55M variant may be a factor contributing to glycemic control.

1. Introduction

The paraoxonase (PON) gene family is composed of three members (PON1, PON2, PON3) that are located adjacent to each other on the long arm of chromosome 7q21–22 in humans [1,2]. PON1 and PON3 are predominantly located in the circulation associated with high density lipoprotein (HDL) particles, while PON2 is an intracellular enzyme and is not present in the circulation [2,3]. All three members of the family are important players in retarding the oxidative modification of low density lipoprotein (LDL) and cell membranes and, therefore, prevention of atherosclerosis [1,2].

PON1 (EC 3.1.8.1), as an atheroprotective and antioxidative enzyme can prevent oxidized-LDL (ox-LDL) production [4,5]. Oxidized phospholipids accumulate in PON1-deficient mice, and the animals are more prone to accelerate atherosclerotic lesions than control mice [4]. There are two common and functional single nucleotide variants in the PON1 gene: glutamine (Q) to arginine (R) substitution at codon 192 (Q192R; rs662) and leucine (L) to methionine (M) substitution at codon 55

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Table 1
Primers, annealing temperatures, amplified product sizes and restriction enzymes for the study variants.

Variant	Rs number	PCR primer (5′ → 3′)	Annealing temperature (°C)	Amplified product size (bp)	Restriction enzyme
PON1-Q192R	662	Forward: TATTGTTGCTGTGGGACCTGAG Reverse: CCTGAGAATCTGAGTAAATCCACT	58	238	*AlwI*
PON1-L55M	854560	Forward: GAAGAGTGATGTATAGCCCCAG Reverse: TTTAATCCAGAGCTAATGAAAGCC	53	170	*NlaIII*
PON2-A148G	11545941	Forward: AGTGGAAATTTTTAAATTTGAAGCAG Reverse: TTGTTTGCAAATGCTGGGGAT	53	130	*SatI*
PON2-S311C	6954345	Forward: AATCAGTGTGTCATTGTGG Reverse: GAGGCTACAGAACTTCC	52	190	*DdeI*

(L55M; rs854560) [6]. According to studies, genetic variability of the PON1 position 192 and 55 and PON1 phenotype affect risk for cardiovascular disease (CVD) and cerebrovascular disease [3,7]; however, there are inconsistencies [8]. A number of studies have addressed the role of PON1 in diabetes mellitus (DM) [7,9,10]. Accordingly, PON1 protein and paraoxonase activity in HDL from patients with diabetes are significantly reduced by 2.8- and 1.7-fold, respectively, when compared to controls' HDL [9]. PON1 has a cytoprotective effect on beta cells against high glucose cytotoxicity and may play a role in insulin secretion from these cells [11]. Moreover, the importance of PON1 coding region polymorphisms in DM and its complications has been highlighted in several studies [10,12].

PON2 is widely expressed in mammalian tissues and has the greatest lactonase activity among PON family members [13,14]. The enzyme is a critical antioxidant in various tissues and its gene contains two common nonsynonymous coding variants: Alanine (A) to glycine (G) substitution at codon 148 (A148G; rs11545941) and serine (S) to cysteine (C) substitution at codon 311 (S311C; rs6954345) [3,15]. Studies have shown that the variant PON2-A148G was independently associated with diabetic nephropathy, and the variant may affect fasting plasma glucose in type 2 diabetes (T2D) [15,16]. Another important variant, PON2-S311C, was associated with cardiovascular complications in diabetes and ischemic stroke [12,13].

According to studies, further association studies of the new variants will need to evaluate their role in the complex disease, T2D and in different populations, particularly populations with a high prevalence of diabetes [17]. Reports have shown that the prevalence of T2D in Iran seems to be among the highest in developing countries [18]. PON1 and PON2 have been shown to play important roles in protecting against CVD, which is the principal cause of mortality in patients with T2D, and so it seem worthy of further investigation. Studies that have simultaneously evaluated the functional variants in two gene of PON family in T2D are limited. It seems that there is a need for determining the genetic variants of these enzymes in different ethnic populations, because some variants were associated with diabetes, its cardiovascular complications, and could potentially influence beta cell function [12,15,19]. Therefore, the aim of the present study was to analyze associations between the genotypes and alleles of four functional variants in PON1 and PON2 genes with susceptibility for T2D according to sex, age and BMI, and to assess the effects of these variants on glycemic control.

2. Materials and methods

2.1. Study population and design

The case-control study comprised of 145 newly diagnosed T2D patients diagnosed based on the WHO criteria (age range 52.86 ± 10.67 years; 30 men and 115 women) and 148 control subjects (age range 51.63 ± 3.62 years; 50 men and 98 women). Individuals in the control group were selected from a volunteer population with no diabetes and with no cardiovascular disease. All patients had no type 1 diabetes, no liver diseases, no renal failure, and no chronic diseases and were not pregnant. Assessments included a questionnaire to assess demographic characteristics, medical history and personal habits. The rate of current smoking was 5.9% in controls and 7.3% in patients. Some of patients were taking antihypertensive medication including losartan, an angiotensin-converting enzyme (ACE) inhibitor, or a beta blocker. None of the patients were receiving insulin therapy or oral antidiabetic drugs prior to their diabetes diagnosis. The study was planned based on the ethical criteria detailed in the Declaration of Helsinki and was approved by the university local ethics committee. Participants gave informed consent after explaining the purpose of the investigation.

2.2. Biochemical analyses

Venous whole blood was obtained from the participants after overnight fasting. The blood samples were collected into vacutainer tubes containing EDTA. Fasting blood glucose (FBG), alanine aminotransferase (ALT), total cholesterol (TC), triglycerides (TGs), HDL-C levels were determined by standard laboratory methods. The HbA1c levels were measured by a commercially available kit using affinity technique (Axis-Shield, Oslo, Norway; accuracy, failure < 5%). The LDL-C levels were calculated according to the Friedewald method.

2.3. Genotyping

2.3.1. Primer design

The primers used in the present study are listed in Table 1. The primers of the variants PON1-Q192R, PON1-L55M and PON2-A148G were obtained from previous studies [10,16,20]. The primer for PON2-S311C was designed using the program Gene Runner (version 3.05, Hastings Software, Inc). The information considering the PON2 gene and the variant PON2-S311C (rs6954345) was obtained from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). The specificity of the designed primer was confirmed through BLAST on the NCBI.

2.3.2. Selection of variants and polymerase chain reaction (PCR) conditions

The study variants were known common variants and have a functional impact on enzymatic efficacy [3,6,13]. According to studies, there is moderately strong linkage disequilibrium between the four variants [8,21]. For example, the reported values for pairwise linkage disequilibrium coefficients as follows: $D' = 0.68$ between PON1-L55M and PON1-Q192R, $D' = 0.56$ between PON1-L55M and PON2-S311C, and $D' = 0.51$ between PON1-Q192R and PON2-S311C ($p < 0.001$ for all pairwise comparisons) [21].

Standard PCR protocols using a thermocycler, followed by restriction fragment length polymorphism (RFLP), were used to screen the four PON single nucleotide variants. A PCR mix reaction incorporated 300–400 ng genomic DNA, 200 mmol/L of each dNTP, 1.5 mmol/L MgCl2, 0.3 mmol/L of each primer, and 1 unit of Taq polymerase. DNA was amplified with an initial melting temperature of 94 °C for 3 min, followed by 35 cycles. Each cycle consisted of denaturation at 94 °C for

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