Paraoxonase 1 polymorphism Q192R affects the pro-inflammatory cytokine TNF-alpha in healthy males

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Abstract

Background: Human paraoxonase 1 (PON1) is an HDL-associated enzyme with anti-oxidant/anti-inflammatory properties that has been suggested to play an important protective role against coronary heart diseases and underlying atherogenesis. The common PON1 Q192R polymorphism (rs662, A>G), a glutamine to arginine substitution at amino acid residue 192, has been analyzed in numerous association studies as a genetic marker for coronary heart diseases, however, with controversial results.

Findings: To get a better understanding about the pathophysiological function of PON1, we analyzed the relationships between the Q192R polymorphism, serum paraoxonase activity and serum biomarkers important for atherogenesis. Genotyping a cohort of 49 healthy German males for the Q192R polymorphism revealed an allele distribution of 0.74 and 0.26 for the Q and R allele, respectively, typical for Caucasian populations. Presence of the R192 allele was found to be associated with a significantly increased paraoxonase enzyme activity of 187.8 ± 11.4 U/l in comparison to the QQ192 genotype with 60.5 ± 4.9 U/l. No significant differences among the genotypes were found for blood pressure, asymmetric dimethylarginine, LDL, HDL, triglycerides, and cholesterol. As expected, MIP-2 alpha a cytokine rather not related to atherosclerosis is not affected by the PON1 polymorphism. In contrast to that, the pro-inflammatory cytokine TNF-alpha is enhanced in R192 carriers (163.8 ± 24.7 pg/ml vs 94.7 ± 3.2 pg/ml in QQ192 carriers).

Conclusions: Our findings support the hypothesis that the common PON1 R192 allele may be a genetic risk factor for atherogenesis by inducing chronic low-grade inflammation.

Introduction

Paraoxonase 1 (PON1) is a calcium-dependent enzyme exhibiting esterase, lactonase and peroxidase activity. It accepts a broad range of substrates including organophosphates, diverse lactones and lipid peroxides and has been studied for its ability to breakdown pesticides and nerve gases. PON1 is a glycoprotein of about 45 kDa that is predominantly synthesized by the liver, from where it is distributed to other tissues, mainly to serum [1,2]. In serum, PON1 is associated with high-density-lipoprotein (HDL) particles [3]. HDL-associated PON1 has been frequently shown to have anti-oxidant and anti-inflammatory potential mainly by protecting lipids of HDLs and low-density lipoproteins (LDL) from oxidative modifications [1,2]. Most likely, these protective effects depend on the peroxidase and esterase activity of PON1 allowing the detoxification of oxidized molecules such as phospholipids and lipid hydroperoxides [4,5]. Cardiovascular diseases and underlying atherosclerosis are associated with oxidative stress and inflammation. Hence, serum PON1 is suggested to contribute to the established anti-atherogenic function of HDLs which is, at least partly, attributable to their anti-oxidative properties [1,2,6].

This notion is further supported by animal model studies using PON1 knock out and transgenic PON1 over-expressing mice. HDLs of PON1−/− knock-out mice were
found to prevent LDL oxidation less efficient than LDLs from control mice [7]. On the other hand, increased PON1 content in transgenic mice overexpressing murine or human PON1 resulted in HDLs that were more protected from lipid peroxidation [8,9]. Moreover, PON1 deficiency in mice resulted in elevated levels of oxidative stress and endothelial adhesion molecules [10]. Accordingly, PON1−/− animals exhibited increased susceptibility to the development of large atherosclerotic lesions on a high-fat diet [7], whereas mice overexpressing human PON1 exhibited decreased atherosclerotic lesion sizes when fed an atherogenic diet [8,9].

The human PON1 gene is located on the long arm of chromosome 7 between q21 and q22. Two common coding region polymorphisms occur: a glutamine to arginine substitution at position 192 (Q192R) which affects PON1 enzyme activity and is analyzed in this study, and a leucine to methionine substitution at position 55 (L55M) [11]. PON1 gene polymorphisms have been examined with respect to their association to various human diseases including coronary heart disease (CHD), Parkinson’s disease, type 2 diabetes and inflammatory bowel disease [12,13]. Most studies focused on the anti-oxidant/anti-inflammatory properties of PON1 in association with the development of atherosclerosis and the role of the Q192R polymorphism as a genetic marker for CHD. However, the results reported so far are controversial, some indicating an association between the Q192R polymorphism and atherosclerosis and CHD risk, while others do not as reviewed in [12,13].

In the present study, we have analyzed the influence of the PON1 Q192R polymorphism on serum lipids and inflammatory biomarkers in a cohort of 49 healthy male individuals to get a better understanding of the role of the PON1 Q192R polymorphism in the development of atherosclerosis and CHD.

**Materials and methods**

**Participants and study design**

The cohort of 53 healthy males investigated in the present study has been recently described [14]. In short, based on clinical laboratory tests, the participants aged between 21 and 48 had an average Body Mass Index (BMI) of 24.1 ± 2.5 and fulfilled four criteria: (i) no history of gastrointestinal, hepatic, cardiovascular or renal disease, (ii) no supplemental vitamin use for ≥ 2 weeks before the start of the study, (iii) non- or occasional smoking (≤ 3 cigarettes/day), and (iv) perpetuation of usual nutrition habits. Fasting blood samples were taken from each participant for genotyping, PON1 enzyme activity determination and inflammatory biomarker analyses. The study was approved by the ethics committee of the Medical Faculty of Kiel University, Germany, (permission number A121/07) and was conformed to Helsinki Declaration. All volunteers gave written informed consent prior to participation.

**Genotyping**

Genomic DNA was isolated from whole blood samples. Genotyping of the PON1 Single Nucleotide Polymorphism (SNP) A/A, A/G, G/G (rs662) responsible for the Q192R substitutions was performed by using the TaqMan system. Fluorescence was measured with ABI Prism 7900 HT sequence detection system (ABI, Foster City, USA).

**PON enzyme activity assay**

PON enzyme activity was determined spectrophotometrically in plasma samples following the protocol described in [15]. Briefly, the rate of hydrolysis of paraoxon (diethyl-p-nitrophenyl phosphatate; Supelco) was measured by monitoring the increase of absorbance at 405 nm using 100 mM Tris-HCl (pH 8.0), 1 mM paraoxon and 2 mM CaCl2. One unit of PON activity is defined as 1 nmol of 4-nitrophenol formed per minute at 20 °C under standard assay conditions (ε = 17600 M−1 cm−1).

**Serum biomarkers**

Supernatants of whole blood samples were measured with commercially available ELISA kits for TNF-alpha, MCP-1 (R&D Systems, Minneapolis, MN), oxLDL (KAMIYA Biomedical Company, Seattle, USA), asymmetric dimethylarginine (ADMA) (DLD Diagnostika, Hamburg, Germany) and MIP-2-alpha (Promocell, Heidelberg, Germany). Optical density was read on a microplate reader (Spectramax® 190, Molecular Devices). Laboratory measurements including serum lipid concentrations have been described previously [16].

**Statistical analysis**

Results are displayed as means ± SEM. Data were analyzed by an unpaired two-sided Student’s t-test (Microsoft Excel Version 2003 or GraphPad Prism 4.0 software). P-values < 0.05 were considered statistically significant.

**Results**

**Genotype distribution and basic characteristics**

Genotype analysis of the PON1 Q192R polymorphism (rs662, A>G) of 53 male volunteers revealed 25 homozygous for Q/Q (51%), 23 heterozygous for Q/R (47%) and 1 homozygous for R/R (2%), while 4 probes failed genotyping. Because of the small incidence, in further studies the G/G genotype (phenotype R/R) was combined with the A/G genotype (phenotype Q/R) group. Basic characteristics of the cohort have been recently...
reported by our group [14]. Briefly, the values for age (30.13 ± 6.71 years), weight (79.11 ± 10.17 kg), height (1.81 ± 0.06 m), BMI (24.12 ± 2.50 kg/m²) and fasting glucose level (86.47 ± 10.68 mg/dl) as well as for the kidney and liver parameters creatinine (1.05 ± 0.10 mg/dl), aspartate aminotransferase (30.09 ± 8.67 U/l), glutamate pyruvate transaminase (37.79 ± 14.73 U/l) and γ-glutamyl transpeptidase (20.49 ± 10.36 U/l) were in accordance with the inclusion criteria of the study and show values within the physiological range for healthy men.

**Effect of the Q192R polymorphism on PON1 activity**

PON1 enzyme activity was found to be significantly elevated in the serum of Q/R and R/R individuals with a mean value of 187.8 ± 11.4 U/l, when compared to the Q/Q group having a mean value of 60.5 ± 4.9 U/l (p < 0.001; unpaired two-sided Student’s t-test) (Figure 1). These data confirm previous studies that have demonstrated a similar effect of the Q192R polymorphism on serum PON1 activity [4,15].

**Effect of the Q192R polymorphism on blood pressure and ADMA**

Subsequently we analyzed a possible association between the Q192R polymorphism and blood pressure. However, no significant differences for blood pressure and ADMA levels, a blood pressure biomarker, were found between the Q/Q and the combined Q/R + R/R group with mean values of 82.5 ± 2.0 versus 82.9 ± 1.9 for diastolic and 125.0 ± 2.4 versus 128.0 ± 2.6 for systolic pressure as well as 0.96 ± 0.05 versus 0.98 ± 0.07 μmol/l for ADMA.

**Effect of the Q192R polymorphism on serum lipoproteins and lipids**

The Q192R polymorphism had no significant effect on HDL (51.7 ± 2.8 versus 50.8 ± 2.4 mg/dl), LDL (95.8 ± 5.7 versus 92.8 ± 6.1 mg/dl), triglycerides (TG) (91.0 ± 8.1 versus 102.9 ± 11.6 mg/dl) and cholesterol (165.7 ± 5.1 versus 164.1 ± 6.7 mmol/l). In addition, we found a slight but not significant increase in the oxLDL levels for the Q/R + R/R group with a mean of 21.7 ± 2.8 U/ml compared to Q/Q individuals with a mean value of 17.8 ± 3.0 U/ml.

**Effect of the Q192R polymorphism on inflammatory biomarkers**

Finally, we analyzed the effect of the Q192R polymorphism on the inflammatory biomarkers TNF-alpha, MCP-1 and MIP-2-alpha. As shown in Figure 2A, the mean level of TNF-alpha for the Q/Q group was found to be 94.7 ± 3.2 pg/ml. In the sera of Q/R and R/R individuals the respective level was significantly enhanced with a mean value of 163.8 ± 24.7 pg/ml (p = 0.007; unpaired two-sided Student’s t-test). Furthermore, the inflammatory biomarker MCP-1 was slightly but not significantly enhanced in Q/R and R/R individuals when compared with the Q/Q group (202.0 ± 30.7 versus 173.0 ± 11.2 ng/ml) (Figure 2B). In contrast to that, MIP-2-alpha levels were not affected by the Q192R polymorphism (258.2 ± 13.7 versus 248.1 ± 12.9 pg/ml; Figure 2C).

**Discussion**

The role of the PON1 Q192R polymorphism in cardiovascular diseases is still under debate [1,2,12,13]. In the present study, genotyping of a cohort of 49 German males revealed a frequency of 0.74 for the Q192 allele confirming previous reports on Western populations with Caucasian origin [17-20]. Moreover, we found that the occurrence of the R192 allele led to an elevated serum paraoxonase activity which is also in good accordance with previously published data on the Q and R allozymes [21,22]. In this regard it is noteworthy that paraoxon represents a non-natural substrate and that the natural substrate(s) of PON1 has/have not been identified so far [2]. Consequently, it is not a contradiction that elevated specific paraoxonase activities of PON1 allozymes have been demonstrated to be negatively correlated with their antioxidant capacity in HDLs, i.e. protecting LDLs against oxidation, reversing the biological effects of oxidised LDLs and preserving the function of HDLs [13,21,22]. Since the oxidation of LDL and the accompanied formation of foam cell layers are thought to represent crucial steps in the initiation process of atherosclerosis [23,24], an enhanced antioxidant activity of HDLs has been suggested to prevent atherosclerosis and CHD [1,2]. Accordingly, a low-active paraoxonase allele such as PON1 Q192 should protect

![Figure 1: Effect of the PON1 Q192R polymorphism on serum PON1 enzyme activity](image-url)
against atherosclerosis when compared with the corres-
ponding high-active R192 allele. Although such an
association has been found in some studies
[17-19,25-31], no relationship has been revealed by
others [20,32-38]. Recent meta-analyses suggested a
weak association between the \textit{PON1} Q192R polymor-
phismand CHD risk [39], however, no or only a popula-
tion-specific effect of the R192 allele on human
longevity [40]. Here we addressed the question whether
the Q192R polymorphism and the related differences in
\textit{PON1} activity are linked to changes in biomarkers indi-
cative for a pro-atherogenic status.

Our analyses revealed that \textit{PON1} genotypes are not
associated with alterations in blood pressure and
ADMA levels, elevation of both are linked with athero-
sclerosis [41]. Moreover, there were no differences in
the serum lipid profiles including TG, HDL, LDL and
cholesterol, except for slightly but not significantly
enhanced oxLDL levels. Similar results for TG, HDL,
LDL and cholesterol have been reported previously
[19,20,25,29,31-34,36-38], whereas a few studies found a
more pro-atherogenic serum lipid and/or lipoprotein
pattern in association with the R192 allele [35,42]. The
principal finding of this study is that the frequency of
the low-antioxidant R192 allele is associated with signifi-
cantly increased levels of the pro-inflammatory cytokine
\textit{TNF-alpha}. Since chronic low grade alterations of
inflammatory markers are known to be associated with
increased atherogenic risk [43], elevated R192 allele-
dependent \textit{TNF-alpha} levels may thus represent a puta-
tive risk marker.

Only recently, it has been demonstrated that adeno-
virus-based overexpression of human \textit{PON1} in apolipo-
protein E knock-out mice caused enhanced serum anti-
oxidative and anti-inflammatory capabilities reflected
among other factors by decreased \textit{TNF-alpha} levels [44].
Hence, enhanced \textit{PON1} antioxidant capacity was found
to be associated with reduced \textit{TNF-alpha} levels, prob-
ably protective against atherosclerosis. Considering the
anti-oxidant property of HDL-associated \textit{PON1}, it is
intriguing to speculate that low amounts of serum
\textit{PON1} or low-anti-oxidant \textit{PON1} alleles such as R192
lead to elevated levels of reactive oxygen species in this
way triggering the redox-sensitive NfκB signaling
pathway that is known to stimulate \textit{TNF-alpha} expres-
sion. However, further mechanistic investigations are
necessary to decipher this proposed \textit{PON1} \textit{TNF-alpha}
relationship. It is remarkable that, in turn, enhanced
\textit{TNF-alpha} levels have been shown to down-regulate
\textit{PON1} expression in murine and human hepatoma cell
lines as well as \textit{in vivo} in mice, most probably via an
NF-kappaB- and nuclear receptor peroxisome prolifera-
tor-activated receptor-alpha (PPAR-alpha)-dependent
mechanism thereby diminishing the antioxidant and
anti-atherogenic activity of HDLs [45,46]. Moreover, a
\textit{TNF-alpha} antagonist therapy in rheumatoid arthritis
patients led to enhanced \textit{PON1} levels concurrent with
elevated anti-oxidative capacities of HDLs and lowered
inflammatory status [47]. Interestingly, anti-\textit{TNF-alpha}
therapy response was found to be associated with SNPs
in the \textit{PON1} locus [48] and a recent case-control study
on atherosclerosis in rheumatoid arthritis found a
correlation between PON-1 activity and serum TNF-alpha and IL-6 levels [49], emphasizing the close regulatory interrelation between PON1 activity and TNF-alpha levels. In addition to TNF alpha, we found that levels of a second pro-inflammatory cytokine MCP-1 were slightly although not significantly enhanced in R192 carriers that usually express PON1 allozymes with less antioxidant capacity. MCP-1 expression and secretion of endothelial cells are known to be induced by oxidized LDL and accordingly, are thought to represent a crucial step in the initial phase of the inflammatory processes in atherosclerosis [43]. Consistent with its anti-oxidant function that leads to reduced lipid peroxidation, PON1 has been demonstrated to attenuate MCP-1 expression of cultured endothelial cells [50]. In good accordance with that are data on HDLs isolated from wild type and PON1 knock-out mice. In a human endothelial cell culture model PON1+/−: HDLs exhibited significantly less antioxidant capacity accompanied with elevated MCP-1 levels, hence, linking PON1 deficiency to lipid hydroperoxide-triggered expression of pro-inflammatory MCP-1 [7]. Since the amount of MIP-2 alpha, a cytokine rather not related to atherosclerosis, is not affected by PON1 polymorphism, the observed changes of TNF-alpha and MCP-1 levels observed in the current study are most likely specific.

Conclusion
Our data indicate that the low-antioxidant PON1 R192 allele is associated with increased pro-inflammatory cytokines known to be involved in the initiation process of atherosclerosis. However, our finding needs to be confirmed in further studies and especially in larger study population.

Abbreviations used
ADMA: asymmetric dimethylarginine; CHD: coronary heart disease; PON1: paraoxonase 1; SNP: single nucleotide polymorphism; TG: triglycerides.

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Authors’ contributions
KL analysed the data and wrote the manuscript. CS participated in the design of the study, acquired and analysed data. CBS performed enzyme assays. CK carried out genotyping studies. GR participates in the design of the study and critically revised the manuscript. FD conceived and designed the study, analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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