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Paraoxonase 2 Cys311Ser polymorphism and its association with the systolic blood pressure values in asymptomatic dyslipidemic individuals: a pilot study

Paraoxonase 2 Cys311Ser Genpolymorphismus und seine Verbindung mit den Konzentrationen des systolischen Blutdrucks bei asymptomatischen dyslipidämischen Personen: eine Pilotstudie

DOI 10.1515/labmed-2015-0009
Received January 16, 2015; accepted May 18, 2015; previously published online June 26, 2015

Abstract

Background: The study aimed to evaluate the relationship of paraoxonase 2 (*PON2*) gene Cys311Ser variants with blood pressure values, endothelial/hemostatic marker levels, and other laboratory parameters in asymptomatic dyslipidemic subjects. The same analyses were also performed with the common variants of *PON2* Cys311Ser and –T1131C apolipoprotein A5 (*ApoA5*) polymorphisms, and *PON2* Cys311Ser and apolipoprotein E (*ApoE*) polymorphisms.
Methods: Two hundred and sixty-four individuals were included in the study. The laboratory parameters were assessed by routine kit methods, while methods based on polymerase chain reaction were used for *PON2*, *ApoA5*, and *ApoE* genotyping.
Results: *PON2* 311 SS homozygous individuals had significantly lower systolic blood pressure values (SBP, $p<0.01$), C-reactive protein, and apolipoprotein A1 levels ($p<0.05$), as compared with C allele carriers. The analysis revealed no differences in the levels of endothelial/hemostatic markers, except for the increased adhesion molecule [soluble vascular cell adhesion molecule 1 (sVCAM-1)] concentrations in 311S/E2 carriers ($p<0.05$). *PON2* 311S subjects showed the lowest values of SBP in combination with "neutral" *ApoE3* allele ($p<0.05$).
Conclusions: The presence of the *PON2* 311 C variant could represent an elevated risk of atherosclerotic complications in asymptomatic dyslipidemic individuals. Nevertheless, considering the study limitations, these relationships are necessary to be confirmed in further research.

Keywords: apolipoprotein A5; apolipoprotein E; dyslipidemia; genetic polymorphism; paraoxonase 2.

Zusammenfassung

Hintergrund: Die Studie wurde durchgeführt mit dem Ziel die Beziehung von verschiedenen Varianten des Genpolymorphismus Paraoxonase 2 (*PON2*) Cys311Ser mit Blutdruckwerten, Endothelzellen/blutstillenden Markern und weiteren Laborparametern bei asymptomatischen dyslipidämischen Probanden zu bewerten. Die gleichen Analysen wurden sowohl mit den gemeinsamen Varianten *PON2* Cys311Ser und –T1131C Apolipoprotein A5 (*ApoA5*) Polymorphismen als auch *PON2* Cys311Ser und Apolipoprotein E (*ApoE*) Polymorphismen durchgeführt.
Methoden: Es wurden 264 Personen an der Studie beteiligt. Die Laborparameter wurden durch Routine kit Methoden bewertet, während für die *PON2*, *ApoA5*, und *ApoE* Genotypisierung Polymerase Chain Reaction-basierte Methoden verwendet wurden.

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Ergebnisse: Im Vergleich zu C-Allel Trägern hatten *PON2* 311 SS homozygote Probanden deutlich geringere systolische Blutdruckwerte (SBP, $p<0.01$) und deutlich geringere Konzentrationen an C-reaktivem Protein und Apolipoprotein A1 ($p<0.05$). Die Analyse der gemeinsamen Varianten zeigte den Einfluss von *ApoA5* C und *ApoE4* Allels auf eine erhöhte Konzentration an Triglyceriden. Es gab keine Unterschiede bei der Konzentration der endothelialen/blutstillenden Marker, lediglich bei Trägern der Variante 311C/E2 wurde eine erhöhte Konzentration des löslichen Adhäsionsmoleküls (sVCAM-1) beobachtet ($p<0.05$). *PON2* 311S Probanden zeigten die niedrigsten systolische Blutdruckwerte in Kombination mit "neutralem" *ApoE3*-Allel ($p<0.05$).

Schlussfolgerungen: Die Ergebnisse zeigen, dass die *PON2* 311C-Variante ein erhöhtes Risiko von atherosklerotischen Komplikationen bei asymptomatischen Personen mit Fettstoffwechselstörungen darstellen kann. Diese Ergebnisse müssen durch weitere Untersuchungen bestätigt werden.

Schlüsselwörter: Apolipoprotein A5; Apolipoprotein E; Fettstoffwechselstörungen; genetischen Polymorphismus; Paraoxonase 2.

Introduction

Enhanced coagulation as well as impaired fibrinolysis was observed in patients with dyslipidemia and metabolic syndrome (MetS). High levels of fibrinogen and plasminogen activator-inhibitor 1 (PAI-1), together with increased von Willebrand factor (vWF) and tissue plasminogen activator (tPA) concentrations, could reflect emerging endothelial dysfunction [1]. This process is regarded as an early step in the development of atherosclerosis and could be characterized by an increased permeability of endothelium and thrombosis.

Paraoxonase 2 (PON2) is a ubiquitously expressed intracellular protein. PON2 is present in many different tissue types including the vital organs, such as heart and lungs [2, 3]. In addition, the protein is constitutively expressed in both primary and immortalized human endothelial cells, human aortic smooth muscle cells, and macrophages, unlike PON1 and PON3 proteins [3–5]. While PON1 and PON3 are associated with high-density lipoproteins (HDL) in plasma, PON2 is undetectable in HDL and low-density lipoproteins (LDL), but appears to remain associated with plasma membrane fractions [3]. PON2 has been reported to possess antioxidant, as well as anti-atherogenic properties [6].

The *PON2* gene has several polymorphisms associated with a number of pathophysiological conditions, but only two with a prominent role: Ala148Gly (rs12026) and Cys311Ser (rs7493) [7]. The clinical studies showed a relationship of *PON2* Cys311Ser polymorphism to a risk of atherothrombosis [8], ischemic stroke [9], cardiovascular disease [10], myocardial infarction [11], and type 2 diabetes mellitus and its complications [12]. However, the results are inconsistent. No data are available concerning a relationship of *PON2* Cys311Ser variants to the markers of endothelial dysfunction and parameters of hemostasis.

Apolipoprotein A5 (ApoA5), a minor plasma apolipoprotein, has been documented to play a key role in triglyceride (TG) metabolism [13]. The *ApoA5* gene was identified 30 kb upstream of the well-characterized *ApoA1/C3/A4/A5* gene cluster on chromosome 11q23. The –1131C *ApoA5* allele of polymorphism (rs662799) was identified as a susceptibility variant for development of MetS in recent studies in different populations [14–17]. Higher plasma concentrations of TG in –1131C allele carriers may themselves reflect an increased risk of endothelial dysfunction, as shown in some experimental and clinical studies [18, 19].

Apolipoprotein E (ApoE) is a structural component of TG-rich lipoproteins. It serves as a ligand for LDL receptor and related proteins. *ApoE4* (Cys112Arg, rs429358) and *ApoE2* (Arg158Cys, rs7412) variants differ from the common *ApoE3* isoform by a single-amino-acid substitution. The variants vary on both structural and functional levels. In some studies, the *ApoE* gene has been suggested to play an important role as a factor influencing susceptibility to thromboembolic diseases [20, 21].

Thus, the presented study aimed to evaluate the relationship of *PON2* Cys311Ser (rs7493) variants alone, *PON2* variants in combination with –1131C *ApoA5* polymorphism (rs662799), and *ApoE* common polymorphisms (rs429358, rs7412), with anthropometrical parameters, blood pressure (BP) values, levels of blood lipids, endothelial/hemostatic markers, and parameters of insulin resistance in asymptomatic dyslipidemic subjects. Considering the endothelial expression of PON2 and its influence to vascular hemostasis, we hypothesized possible relationship of especially the endothelial/hemostatic marker levels with the *PON2* C311 variant, as well as the impact of the minor variants of *ApoA5* and *ApoE* polymorphisms on lipid and endothelial/hemostatic parameters.

Materials and methods

Study design and subjects

The study was performed with asymptomatic dyslipidemic subjects (i.e. in individuals without history of clinically manifest atherosclerosis – coronary artery disease, heart failure, cerebrovascular ischemic disease, and peripheral vascular disease, with total cholesterol (TC) ≥5.0 mmol/L and/or TGs ≥1.5 mmol/L, n=264, 129 men and 135 women). They had been examined consecutively for the first time in the Lipid Centre of the 3rd Department of Internal Medicine, University Hospital Olomouc, Czech Republic, during the period from January 2006 to March 2011. All subjects were tested for the signs of secondary hyperlipidemia: diabetes mellitus, hypothyroidism, renal or hepatic diseases, and nephrotic syndrome. Other exclusion criteria were as follows: history of clinically manifested atherosclerosis presented by coronary artery disease, cerebrovascular disease, and peripheral arterial disease, any hypolipidemic therapy in previous 8 weeks, hormone therapy, and clinical presence of acute infections. The subjects with E24 genotype of *ApoE* polymorphism were also excluded because of the opposite effects of E2 and E4 alleles on lipid levels (a total of seven individuals). All individuals filled out a questionnaire on their previous medical history, especially cardiovascular status, medication, and smoking habits. Body mass index (BMI) and systolic and diastolic blood pressures (SBP and DBP, respectively) were also determined. The study was approved by the Ethics Committee of Medical Faculty and University Hospital Olomouc and written informed consent was obtained from all participants. The authors have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Laboratory analysis

Venous blood samples were drawn in the morning after a 12-h fast. After centrifugation, the serum was used for other analyses. For the assessment of hemostatic markers, venous blood was collected in 3.8% sodium citrate tubes and plasma was obtained after centrifugation.

Routine serum biochemical parameters were analyzed on Modular SWA (Roche, Basel, Switzerland) on the same day of blood collection. Concentrations of other special analytes (i.e. insulin, C-peptide, adhesion molecules, and hemostatic markers) were measured in the sample aliquots stored at –20 °C, no longer than 6 months (see below).

TC, TG, and HDLc levels were determined enzymatically on Modular SWA system (Roche). LDLc levels were calculated using Friedewald formula (for TG <4.5 mmol/L). Other calculated parameters were as follows: non-HDL-cholesterol (non-HDLc=TC–HDLc), atherogenic index of plasma (AI=logTG/HDLc), and homeostasis model assessment (HOMA-R=glucose×insulin/22.5). Concentration of ApoB and ApoA1 were determined immunoturbidimetrically using Tina-Quant ApoB and ApoA-1 kits (Roche). Lipoprotein (a) [Lp(a)] was measured immunoturbidimetrically using Tina-Quant Lipoprotein(a) TQ kit (Roche). C-reactive protein (CRP) was assessed by an ultrasensitive immunoturbidimetric method using the kit Tina-Quant (Roche). Glucose was determined using GOD-PAP method (Roche). All tests were measured from fresh sera in the same day of blood collection.

Insulin was determined by the commercially available kit (Immunotech, Marseille, France) using specific antibodies by the IRMA method. C-peptide was determined using C-peptide Kit (Immunotech) by the IRMA method. Serum levels of the soluble adhesion molecules (soluble intercellular adhesion molecule 1 [sICAM-1] and soluble vascular cell adhesion molecule 1 [sVCAM-1]) were determined by immunoenzymatic assay using commercially available kits sICAM-1 and sVCAM-1 (both Immunotech). The sample aliquots were stored at –20 °C, no longer than 6 months.

The following hemostatic markers were examined from human plasma stored at –20 °C: fibrinogen (function coagulation method by Clauss; Technoclone, Vienna, Austria), vWF (immunoturbidimetric assay; Instrumentation Laboratory SpA, Milan, Italy), PAI-1, tPA (both ELISA; Technoclone), and soluble thrombomodulin (sTBM, ELISA Thrombomodulin; Diagnostica Stago, Asnieres sur Seine, France).

Genotyping

DNA was extracted from the peripheral leukocytes of all subjects using a standard commercial kit (QIAamp DNA Blood Mini kit, Qiagen, Hilden, Germany). After isolation, the extracts were stored at –20 °C, no longer than 3 months. *PON2* Cys311Ser variants were identified using polymerase chain reaction (PCR) method followed by the restriction fragment length polymorphism analysis [22]. Briefly, after DNA extraction, a 262-bp product was amplified by PCR using the pair of primers with following sequences: 5′-ACA TGC ATG TAC GGT GGT CTT ATA-3′ and 5′-AGC AAT TCA TAG ATT AAT TGT TA-3′. *PON2* Cys311Ser polymorphism was detected by digesting the PCR-amplified product with the *Dde*I restriction enzyme, followed by size fractionation in 3% agarose gel and visualization of bands with ethidium bromide. *ApoA5* genotypes were determined by a melting curve analysis after a real-time PCR method adapted from the work of Frances et al. [23] using LightCycler, v.2.0 (Roche). Genotyping of *ApoE* alleles was performed using commercially available ApoE LightMix Kit (Roche) by melting curve analysis after a real time PCR. The metod was performed using the same instrument.

Measurement of BP

The auscultatory method of the BP measurement with a properly calibrated and validated mercury sphygmomanometer was used. Persons were seated quietly for at least 5 min in a chair, with feet on the floor and arm supported at heart level. An appropriate-sized cuff was used to ensure accuracy. The first and fifth Korotkoff sounds were used to identify SBP and DBP. At least three sitting BP measurement with a 30-s interval were taken and the mean from the last two was calculated.

Statistical analysis

All values of quantitative parameters are expressed as means± standard deviation (SD), and parameters with skewed distribution

were also expressed as medians. The Kolmogorov-Smirnov test was used to check for normal distribution. Variables with skewed distribution [MetS signs, CRP, TG, AI, Lp(a), tPA, PAI-1, sTBM, sICAM-1, insulin, HOMA-R, C-peptide] were log transformed in order to normalize their distribution before statistical analysis. Differences in variables between individual groups were analyzed with ANOVA, after adjustment for age and sex. The results have been corrected by the Bonferroni correction for multiple testing. Statistical analysis was performed by SPSS for Windows, version 12.0 (SPSS, Chicago, IL, USA). Probability values of $p<0.05$ were considered as statistically significant.

The genotype and allele frequencies for *PON2*, *ApoA5*, and *ApoE* gene polymorphisms were determined by a gene counting method and then evaluated by performing the Pearson χ^2 statistical analysis to identify whether followed polymorphisms were consistent with the Hardy-Weinberg equilibrium expectation. To create the common variants of genetic polymorphisms, a model with two variants of *PON2* 311 (CC+CS, and SS), two variants of T–1131C *ApoA5* (TT, and CT+TT), and three variants of *ApoE* polymorphisms (E22+E23, E33, and E34+E44) was used.

Results

The clinical and laboratory characteristics of all dyslipidemic individuals, as well as those of subjects divided according to *PON2* Cys311Ser variants, are summarized in Table 1. We have not observed any significant differences in anthropometrical parameters, smoking habits, lipid levels, the number of characteristics of MetS, the presence of MetS, levels of endothelial/hemostatic parameters, and markers of insulin resistance between *PON2* Cys311Ser variants. Nevertheless, the *PON2* 311 SS homozygotes had significantly lower SBP values ($p<0.01$), CRP levels ($p<0.05$), and ApoA1 levels ($p<0.05$), in comparison with C allele carriers. The mild non-significant increase in TG, AI, non-HDLc, sVCAM-1, and sICAM-1 and a decrease in HDLc concentrations were seen in SS carriers as well.

In Table 2, *PON2* (rs7493) polymorphism genotype and allele frequencies are depicted in the study group. Genotype frequencies were shown to be in the Hardy-Weinberg equilibrium. The frequency of C and S alleles was 20.3% and 79.7%, respectively, which is in accordance with the studies in Caucasian general population. The distribution of *PON2* Cys311Ser genotypes were as follows: CC homozygotes 6.4%, CS heterozygotes 27.7%, and SS homozygotes 65.9%.

Detailed *PON2* (rs7493), *ApoA5* (rs662799), and *ApoE* (rs429358, rs7412) variant frequencies are introduced in Table 3.

The significant differences in variables according to the common variants of *PON2* Cys311Ser (rs7493) and *ApoA5* T–1131C (rs662799) polymorphisms are shown in Table 4. The carriers of the most frequent combination (311S/–1131T) had significantly lower levels of CRP ($p<0.01$), while elevated TG levels were observed in the 311S/–1131C variant subgroup ($p<0.01$), compared with the 311C/–1131T variant.

Similarly, the significant differences in variables according to the common variants of *PON2* Cys311Ser (rs7493) and *ApoE* polymorphisms (rs7412, rs429358) in dyslipidemic subjects are introduced in Table 5, in comparison with the 311C/E3 variant. The 311S/E4 variant carriers had elevated levels of TG ($p<0.05$). No differences were seen in levels of endothelial/hemostatic markers, except of increased sVCAM-1 levels ($p<0.05$) in the 311S/E2 carriers. In accordance with Table 1, the *PON2* 311S carriers showed the lowest values of SBP combined with "neutral" *ApoE3* allele ($p<0.05$).

Discussion

PON2 variants

The PON2 expression in arterial cells apparently plays an important role in the body resistance to vascular disease [8]. Considering the effect of PON2 to vascular hemostasis, we hypothesized possible relationship of the endothelial/hemostatic marker levels with the *PON2* Cys311Ser polymorphism even in asymptomatic individuals with dyslipidemia. In addition, the influence of the polymorphism on the level of these markers has not been evaluated in clinical studies so far. However, no significant differences between the PON2 subgroups were observed in the presented study, suggesting that the *PON2* 311C allele has probably no different effect on the expression of endothelial markers compared with S allele in asymptomatic dyslipidemic patients.

In contrast, the results showed significantly higher SBP values and CRP levels in the C allele carriers. Although the influence of *PON2* Cys311Ser polymorphism on the risk of cardiovascular and other diseases is controversial in recent studies, some of them point to a link with the 311C variant. Cozzi et al. [8] described the presence of C allele as an independent possible cofactor in determining the risk of atherothrombotic events, together with hypertension and HDLc, in an Italian subpopulation. The frequency of C allele was significantly higher in the group of subjects with history of atherothrombotic events. CC homozygotes of the *PON2* gene 311 polymorphism were more likely to have an increased risk of coronary artery disease in a Taiwanese population [24]. In the presence of MetS and

Table 1: Characteristics of all individuals and subjects according to *PON2* Cys311Ser (rs7493) variants.

	All individuals, n=264	*PON2* 311 (CC+CS), n=90	*PON2* 311 (SS), n=174
Males/females	129/135	40/50	89/85
Age, years	43.1±14.5 (32–55)	45.9±14.1 (34–57)	41.6±14.6 (31–55)
Smoking	36 (13.6%)	10 (11.1%)	26 (14.9%)
SBP, mm Hg	128.3±15.2 (120–140)	133.1±15.6 (120–140)	**125.8±14.5[a] (117–135)**
DBP, mm Hg	81.7±8.8 (80–85)	83.1±9.8 (80–90)	80.1±8.1 (80–85)
Presence of MetS	58 (22%)	21 (23%)	37 (21%)
MetS signs	1.50±1.24 [1.00] (0.00–2.00)	1.70±1.24 [1.00] (1.00–2.50)	1.41±1.23 [1.00] (0.00–2.00)
Waist, cm	85.1±12.2 (76–92)	86.3±12.7 (78–92)	84.4±11.9 (75–93)
BMI, kg/m^2	25.36±3.70 (22.67–27.46)	25.71±4.09 (22.90–27.50)	25.17±3.47 (22.58–27.44)
CRP, mg/L	1.99±2.31 [1.17] (0.60–2.62)	2.65±3.00 [1.40] (0.83–3.55)	**1.65±1.77 [1.00][b] (0.57–2.06)**
TC, mmol/L	6.29±2.10 (5.02–7.07)	6.19±1.44 (5.03–7.12)	6.34±2.37 (5.02–7.04)
TG, mmol/L	2.32±2.81 [1.63] (1.08–2.59)	1.99±1.41 [1.60] (1.03–2.20)	2.50±3.30 [1.63] (1.09–2.68)
AI	0.0747±0.3696 [0.0476] (–0.1868 to 0.3004)	0.0387±0.3576 [0.0420] (–0.2332 to 0.2425)	0.0936±0.3753 [0.0505] (–0.1594 to 0.3080)
Non-HDL, mmol/L	4.74±1.85 (3.57–5.64)	4.66±1.51 (3.50–5.65)	4.78±2.01 (3.58–5.63)
HDLc, mmol/L	1.48±0.40 (1.21–1.71)	1.54±0.44 (1.24–1.77)	1.45±0.37 (1.19–1.67)
LDLc, mmol/L	3.80±1.39 (2.80–4.62)	3.78±1.18 (2.92–4.63)	3.81±1.50 (2.79–4.59)
ApoA1, g/L	1.55±0.30 (1.37–1.72)	1.62±0.30 (1.46–1.75)	**1.52±0.30[b] (1.32–1.66)**
ApoB, g/L	1.19±0.33 (0.99–1.39)	1.19±0.33 (0.96–1.40)	1.19±0.33 (0.99–1.38)
Lp(a), g/L	0.365±0.454 [0.163] (0.077–0.548)	0.356±0.444 [0.162] (0.065–0.527)	0.370±0.461 [0.163] (0.083–0.557)
Fibrinogen, g/L	3.19±0.58 (2.87–3.55)	3.19±0.63 (2.81–3.64)	3.19±0.55 (2.87–3.47)
vWF,%	136±53 (101–160)	144±52 (113–170)	132±54 (100–152)
tPA, ng/mL	5.12±4.99 [3.45] (1.60–7.03)	5.28±4.54 [4.40] (1.70–7.50)	5.03±5.22 [3.20] (1.60–6.60)
PAI-1, ng/mL	90±37 [88] (64–109)	86±39 [84] (64–102)	92±36 [91] (65–110)
sTBM, ng/mL	61.0±79.5 [37.2] (20.0–80.8)	67.7±110.8 [37.2] (21.0–79.0)	57.6±57.2 [37.2] (19.1–81.0)
sICAM-1, ng/mL	593±177 [552] (495–650)	575±161 [554] (498–620)	602±184 [552] (494–656)
sVCAM-1, ng/mL	867±235 (714–996)	855±221 (699–972)	874±242 (721–1004)
Glucose, mmol/L	5.04±0.78 (4.60–5.38)	5.09±0.77 (4.69–5.46)	5.01±0.79 (4.56–5.30)
Insulin, mIU/L	8.1±4.5 [7.4] (5.3–9.8)	7.7±4.3 [7.4] (5.1–10.7)	8.3±4.6 [7.4] (4.9–9.8)
HOMA-R	1.870±1.236 [1.625] (1.132–2.243)	1.828±1.266 [1.625] (1.119–2.176)	1.893±1.224 [1.624] (1.151–2.286)
C-peptide, mg/L	2.4±1.2 [2.1] (1.5–3.0)	2.4±1.2 [2.1] (1.5–3.0)	2.4±1.2 [2.1] (1.6–2.9)

Data are expressed as means±SDs, in parameters with skewed distribution also as medians [in brackets]. Values of 25th and 75th percentiles are expressed in parentheses. Differences in variables between *PON2* 311 (CC+CS) and *PON2* 311 (SS) groups were analyzed by ANOVA after adjustment for age and sex. Variables with skewed distribution [MetS signs, CRP, TG, AI, Lp(a), tPA, PAI-1, sTBM, sICAM-1, insulin, HOMA-R, C-peptide] were log transformed to normalize their distribution before statistical analyses. Significant differences between *PON2* 311 (CC+CS) and *PON2* 311 (SS) groups (bold values): [a]$p<0.01$ and [b]$p<0.05$. PON2, paraoxonase 2; CC, CS, SS, *PON2* genotypes; SBP, systolic blood pressure; DBP, diastolic blood pressure, MetS signs, number of characteristics of MetS according to NCEP-ATPIII; BMI, body mass index; CRP, C-reactive protein; TC, total cholesterol; TG, triglycerides; AI, atherogenic index of plasma (log TG/HDLc); non-HDL, TC-HDLc; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein (a); vWF, von Willebrand factor; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; sTBM, soluble thrombomodulin; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; HOMA-R, homeostasis model assessment.

Table 2: *PON2* Cys311Ser (rs7493) polymorphism genotype and allele frequencies in dyslipidemic patients.

	All individuals, n=264
PON2 311 genotypes	
CC	17 (6.4%)
CS	73 (27.7%)
SS	174 (65.9%)
PON2 311 alleles	
C	107 (20.3%)
S	421 (79.7%)

PON2, paraoxonase 2; CC, CS, SS, *PON2* 311 variants; C, S, *PON2* 311 alleles.

diabetes, *PON2* 311 CC genotype was associated with an increased risk of significant coronary stenosis in Tunisian subjects, with no relationship of the *PON2* Cys311Ser polymorphism to lipid profile. The authors hypothesize that the R allele of the *PON1* 192 and the C allele of the *PON2* 311 polymorphisms may promote oxidative stress, and this might increase cardiovascular risk in diabetic or the MetS patients [25]. Similarly, no association of *PON2* Cys311Ser variants with lipid and lipoprotein parameters and C allele overrepresentation in subjects with diabetes and microvascular complications were observed in another study [12]. Thus, it appears that C allele might be a predisposing

Table 3: *PON2* (rs7493), *ApoA5* (rs662799), and *ApoE* (rs429358, rs7412) variant frequencies in dyslipidemic patients.

Variant frequency	All individuals, n=264
PON2 (rs7493) variants	
CC+CS	90 (34.1%)
SS	174 (65.9%)
ApoA5 (rs662799) variants	
CC+CT	54 (20.5%)
TT	210 (79.5%)
ApoE (rs429358, rs7412) variants	
E22+E23	51 (19.3%)
E33	138 (52.3%)
E34+E44	75 (28.4%)

PON2, paraoxonase 2; CC, CS, SS, *PON2* 311 variants; ApoA5, apolipoprotein A5; CC, CT, TT, *ApoA5* variants; ApoE, apolipoprotein E; E22, E23, E33, E34, E44, *ApoE* variants.

risk factor of adverse conditions in symptomatic patients, especially those with metabolic disturbances.

The study of Kokubo et al. [26] analyzed the association between 61 SNPs with BP variations and hypertension in a population-based study using randomly selected 1880 Japanese subjects. Analyses of covariance revealed that 17 polymorphisms in 16 genes were significantly associated with BP variations, including the *PON2* gene. In contrast, the large genome-wide association study (GWAS) with 200,000 individuals of European descent identified 29 independent genetic variants associated with BP. Subsequent analysis confirmed the association of several independent genetic variant in individuals of non-European ancestry. No association was found between BP and the *PON2* gene [27]. Previously, another GWAS performed with 34,433 subjects of European ancestry identified an association between SBP or DBP and common variants in eight regions, but not with the *PON2* gene [28]. Based on these conclusions, our observation (i.e. association of the *PON2* 311C variant with elevated SBP) could be considered a random phenomenon. This discrepancy might be the result of different study design, including a small number of participants in particular. Nevertheless, two recent experimental studies revealed that renal PON2 is involved in the inhibition of reactive oxygen species and NADPH oxidase activity and contributes to the maintenance of normal BP [29, 30]. Therefore, the relationship of *PON2* genotypes to the BP values should not be completely excluded. Similarly, because the expression of all three *PON* genes negatively correlates with a number of "inflammatory" diseases including atherosclerosis, it is highly probable that PON2 might play an important role in the process of inflammation. Thus, the relationship to CRP and other proinflammatory markers should be also evaluated.

The observed effect of *PON2* Cys311Ser polymorphism on the levels of ApoA1 was surprising. In contrast to PON1 and PON3, PON2 is not associated with HDL, and no similar information was found in recent literature. Formerly, two studies showed that *PON2* Ala148/Ser311 homozygotes exhibited significantly higher plasma TC, LDLc, and ApoB than subjects with the other two genotypes [31, 32]. Another study revealed that *PON2* Gly148 homozygotes had the highest plasma concentrations of TC, HDLc, and ApoA1; Ala148 homozygotes the lowest; and heterozygotes had an intermediate phenotype [33]. In our work, the association of higher ApoA1 levels with the *PON2* 311C allele was apparent in analysis of the common *PON2/ApoE* variants as well. But as the study was conducted as a pilot, one with a limited number of participants, we can speculate about a random phenomenon in this case.

Since our study was performed with individuals without the symptoms of cardiovascular disease, we had no clinical correlates to assess the impact of the *PON2* Cys311Ser variants. Nevertheless, higher levels of proinflammatory CRP and SBP in the C allele carriers suggest

Table 4: Significant differences in variables according to common variants of *PON2* Cys311Ser (rs7493) and *ApoA5* T–1131C (rs662799) polymorphisms in dyslipidemic patients compared with 311C/–1131T variant.

***PON2* C311S/ T–1131C *ApoA5* common variants**	**311C/–1131T (CC+CS/TT), n=74**	**311C/–1131C (CC+CS/TC+CC), n=17**	**311S/–1131T (SS/TT), n=136**	**311S/–1131C (SS/TC+CC), n=37**
CRP, mg/L	2.84±3.13 [1.54]	1.88±2.30 [1.21]	**1.62±1.69 [0.98]**[a]	1.71±2.06 [1.04]
TG, mmol/L	1.85±1.29 [1.49]	2.59±1.76 [1.93]	2.49±3.63 [1.59]	**2.52±1.75 [1.78]**[a]

Data are expressed as means±SDs, in parameters with skewed distribution also as medians (in parentheses). Differences in variables between the 311C/–1131T variant and other groups were analyzed with ANOVA after adjustment for age, sex, and by the Bonferroni correction for multiple testing. CRP and TG were log transformed to normalize their distribution before statistical analyses. Significant differences in variables between the 311C/–1131T variant and other variants of *PON2/ApoA5* (bold values): [a]$p<0.01$ and [b]$p<0.05$. PON2, paraoxonase 2; CC, CS, SS, *PON2* variants; T, C, alleles of the *ApoA5* gene at position –1131; CRP, C-reactive protein; TG, triglycerides.

Table 5: Significant differences in variables according to common variants of *PON2* Cys311Ser (rs7493) and *ApoE* (rs7412, rs429358) polymorphisms in dyslipidemic patients compared with 311C/E3 variant.

***PON2* C311S/*ApoE* common variants**	**311C/E3 (CC+CS/E33), n=57**	**311C/E2 (CC+CS/E22+E23), n=14**	**311C/E4 (CC+CS/E34+E44), n=19**	**311S/E2 (SS/E22+E23), n=37**	**311S/E3 (SS/E33), n=81**	**311S/E4 (SS/E34+E44), n=56**
SBP, mm Hg	132.4±16.5	128.9±13.8	139.9±12.8	127.3±13.3	**124.6±13.4**[a]	126.5±16.6
TG, mmol/L	1.79±1.28 [1.54]	2.40±1.90 [1.55]	2.28±1.34 [1.94]	3.81±5.96 [1.65]	1.67±0.93 [1.49]	**2.82±2.75 [2.05]**[a]
sVCAM-1, ng/mL	843±215	882±280	868±198	**988±299**[a]	849±218	836±215

Data are expressed as means±SDs, in parameters with skewed distribution also as medians (in parentheses). Differences in variables between the 311C/E3 variant and the other groups were analyzed with ANOVA after adjustment for age, sex, and by the Bonferroni correction for multiple testing. TG levels were log transformed to normalize their distribution before statistical analyses. Significant differences in variables between the 311C/E3 variant and other variants of *PON2/ApoE* (bold values): [a]$p<0.05$ and [b]$p<0.01$. PON2, paraoxonase 2; CC, CS, SS, *PON2* 311 genotypes; E22, E23, E24, E33, E34, E44, *ApoE* genotypes; SBP, systolic blood pressure; TG, triglycerides; sVCAM-1, soluble vascular cell adhesion molecule 1.

that this variant could represent the symptom of higher risk of vascular complications compared with SS genotype carriers.

PON2/ApoA5 common variants

As the majority of genetic polymorphisms exert their effects in accordance with other polymorphisms as haplogroups, we evaluated a relationship between the *PON2/ApoA5* combined variants to the levels of all measured parameters. Numerous studies have confirmed the association of *ApoA5* naturally occurring variants, including –1131C allele of the *ApoA5* gene, with an increased TG that may itself represent elevated risk of endothelial dysfunction [19]. Formerly, we have observed that the presence of the *ApoA5* –1131C variant was associated with higher TG and tPA levels in dyslipidemic patients [34]. In the present study, influence of the *ApoA5* –1131C variant was also confirmed. The presence of the –1131C minor allele has been associated with higher levels of TG in comparison with T allele carriers, regardless of *PON2* genotype (both in the 311C/–1131C and the 311S/–1131C common variant subgroups). In context of the above mentioned data, the carriers of the 311S/–1131T combination had also significantly lower CRP compared with the 311C/–1131T variant that probably represents the action of the *PON2* 311 polymorphism (see *PON2* variants section).

PON2/ApoE common variants

The *ApoE* gene has been suggested as a factor influencing susceptibility to atherothrombotic diseases. The minor alleles E2 and E4 of *ApoE* common polymorphism were found to be associated with higher plasma TG in comparison with E3 common variant, which we demonstrated previously in the cohort of dyslipidemic patients [35]. This effect, particularly of the E4 variant, was evident in the presented study, with elevated TG concentration in the 311S/E4 subgroup. As no elevation of TG was observed in *PON* 311S carriers alone, we hypothesize the action only of *ApoE* polymorphism on TG levels. No effects of combined *PON2/ApoE* variants were found on the level of endothelial/hemostatic markers and parameters of IR, but with one exception. The 311S/E2 variant carriers had increased sVCAM-1 compared with 311C/E3 subjects. According to some authors, sVCAM-1 levels may be related to the extent of atherosclerotic lesions and a prediction of subsequent cardiovascular progression in subject with coronary artery disease [36, 37]. A significant increase in the adhesive molecule levels was detected in hypertensive patients with coexisting impaired glucose tolerance, hyperlipidemia, obesity, and diabetes [38, 39]. Elevated sVCAM-1 levels in the 311S/E2 subgroup are surprising, as asymptomatic patients were included in the study. However, the small number of participants in subgroups has to be taken into account and any general conclusions should not be deduced.

Probably the most interesting finding was the detection of decreased values of SBP in all S allele subgroups, compared with the similar C allele cohorts (i.e. 311S/E2 vs. 311C/E2, 311S/E3 vs. 311C/E3, 311S/E4 vs. 311C/E4). The lowest SBP values were observed in the 311SS/E33 homozygotes, i.e. in the patients carrying the most frequent variant, and the significant decline was observed only in this subgroup in comparison with the 311C/E3 variant. This observation could support the above-mentioned speculation that the C allele carriers might be at higher risk of elevated SBP. However, it is necessary to

bear in mind the limited number of individuals in the followed subgroups.

Limitation of the study

The major limitation of the study is represented particularly by the small number of subjects in the *PON2/ApoA5* and the *PON2/ApoE* minor variant subgroups, which could reduce the power of significance of the observed relationships. For this reason, our results require validation in larger cohorts of individuals, both in symptomatic and healthy control subjects.

Conclusions

We demonstrated a weak association of the *PON2* Cys311Ser polymorphism with the levels of endothelial/hemostatic markers in our study. The influence of the minor alleles of especially –1131C *ApoA5* and *ApoE4* variants on TG levels was confirmed as well. The *PON2* 311 C allele was associated with higher levels of proinflammatory CRP. Probably the most interesting finding was the detection of decreased values of SBP in all S allele carrier subgroups as compared with the *PON2* 311 C allele. In light of the recent experimental studies involving PON2 in the maintenance of BP, the influence of *PON2* genotypes should not be completely excluded.

Thus, the C variant could represent a sign of the higher risk of vascular complications in comparison with S allele in asymptomatic individuals with dyslipidemia. As we are aware of the study limitations, the influence of the *PON2* Cys311Ser polymorphism on the SBP values and inflammatory markers should be analyzed in further research with symptomatic individuals and a healthy control group.

Acknowledgments: This work was supported by the Institutional Support of Ministry of Health, Czech Republic, Nr. 1 RVO-FNOL2013.
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Kostapanos M, Florentin M, Elisaf MS, Mikhailidis DP. Hemostatic factors and the metabolic syndrome. Curr Vasc Pharmacol 2013;11:880–905.
2. Primo-Parmo SL, Sorenson, RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics 1996;33:498–507.
3. Ng CJ, Wadleigh, DJ, Gangopadhyay A, Hama S, Grijalva VR, Navab M, et al. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. J Biol Chem 2001;276:44444–9.
4. Ng CJ, Shih DM, Hama SY, Villa N, Navab M, Reddy ST. The paraoxonase gene family and atherosclerosis. Free Radic Biol Med 2005;38:153–63.
5. Précourt LP, Amreb D, Denisa MC, Lavoieb JC, Delvinc E, Seidmand E, et al. The three-gene paraoxonase family: physiologic roles, actions and regulativ. Atherosclerosis 2011;201:20–36.
6. Horke S, Witte I, Wilgenbus P, Krüger M, Strand D, Förstermann U. Paraoxonase-2 reduces oxidative stress in vascular cells and decreases endoplasmic reticulum stress induced caspase activation. Circulation 2007;115:2055–64.
7. Mochizuki H, Scherer SW, Xi T, Nickle DC, Majer M, Huizenga JJ, et al. Human PON2 gene at 7q21.3: cloning, multiple mRNA forms, and missense polymorphisms in the coding sequence. Gene 1998;213:149–57.
8. Cozzi L, Campolo J, Parolini M, De Maria R, Patrosso MC, Marocchi A, et al. Paraoxonase 1 L55M, Q192R and paraoxonase 2 S311C alleles in atherothrombosis. Mol Cell Biochem 2013;374:233–8.
9. Li BH, Zhang LL, Yin YW, Pi Y, Yang QW, Gao CY, et al. Association between paraoxonase 2 Ser311Cys polymorphism and ischemic stroke risk: a meta-analysis involving 5008 subjects. Mol Biol Rep 2012;39:5623–30.
10. Shih DM, Lusis AJ. The roles of PON1 and PON2 in cardiovascular disease and innate imunity. Curr Opin Lipidol 2009;20:288–92.
11. Marchegiani F, Spazzafumo L, Provinciali M, Cardelli M, Olivieri F, Franceschi C, et al. Paraoxonase2 C311S polymorphism and low levels of HDL contribute to a higher mortality risk after acute myocardial infarction in elderly patiens. Mol Gen Metab 2009;98:314–8.
12. Mackness B, Mc Elduff P, Mackness MI. The paraoxonase-2-310 polymorphism is associated with the presence of microvascular complications in diabetes mellitus. J Intern Med 2005;258:363–8.
13. Sharma V, Ryan RO, Forte TM. Apolipoprotein A-V dependent modulation of plasma triacylglycerol: a puzzlement. Biochim Biophys Acta 2012;1821:795–9.
14. Grallert H, Sedlmeier EM, Huth C, Kolz M, Heid IM, Meisinger Ch, et al. APOA5 variants and metabolic syndrome in Caucasians. J Lipid Res 2007;48:2614–21.
15. Maasz A, Kisfali P, Horvatovich K, Mohas M, Marko L, Csongei V, et al. Apolipoprotein A5 T–1131C variant confers risk for metabolic syndrome. Pathol Oncol Res 2007;13:243–7.
16. Garelnabi M, Lor K, Jin J, Chai F, Santanam N. The paradox of ApoA5 modulation of triglycerides: evidence from clinical and basic research. Clin Biochem 2013;46:12–9.
17. Zaki M, Amr K. Apolipoprotein A5 T–1131C variant and risk for metabolic syndrome in obese adolescents. Gene 2014;634:44–7.

18. Sokup A, Goralczyk B, Goralczyk K, Rosc D. Triglycerides as an early pathophysiological marker of endothelial dysfunction in nondiabetic women with a previous history of gestational diabetes. Acta Obstet Gynecol Scand 2012;91:182–8.
19. Matsumoto S, Gotoh N, Hishinuma S, Abe Y, Shimizu Y, Katano Y, et al. The role of hypertriglyceridemia in the development of atherosclerosis and endothelial dysfunction. Nutrients 2014;6:1236–50.
20. Hoppe B, Tolou F, Dörner T, Kiesewetter H, Salama A. Gene polymorphisms implicated in influencing susceptibility to venous and arterial thromboembolism: frequency distribution in a healthy German population. Thromb Haemost 2006;96:465–70.
21. Gialeraki A, Politou M, Rallidis L, Merkouri E, Markatos C, Kremastinos D, et al. Prevalence of prothrombotic polymorphisms in Greece. Genet Test 2008;12:541–7.
22. Sanghera DK, Aston CE, Saha N, Kamboh MI. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. Am J Hum Genet 1998;62:36–44.
23. Frances F, Corella D, Sorli JV, Guillen M, Gonzalez JI, Portoles O. Validating a rapid method for detecting common polymorphisms in the APOA5 gene by melting curve analysis using LightTyper. Clin Chem 2005;51:1279–82.
24. Chen CC, Chen CC, Tu JD, Wu YL, Leu SJ. Associations between genetic polymorphisms of paraoxonase genes and coronary artery disease in a Taiwanese population. Clin Biochem 2013;46:1664–67.
25. Rejeb J, Omezzine A, Rebhi L, Boumaiza I, Mabrouk H, Rhif H, et al. Association of PON1 and PON2 polymorphisms with PON1 activity and significant coronary stenosis in a Tunisian population. Biochem Genet 2013;51:76–91.
26. Kokubo Y, Tomoike H, Tanaka C, Banno M, Okuda T, Inamoto N, et al. Association of sixty-one non-synonymous polymorphisms in forty-one hypertension candidate genes with blood pressure variation and hypertension. Hypertens Res 2006;29:611–9.
27. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 2011;478:103–9.
28. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet 2009;41:666–76.
29. Yang Y, Zhang Y, Cuevas S, Villar VA, Escano C, D Asico L, et al. Paraoxonase 2 decreases renal reactive oxygen species production, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of NADPH oxidase. Free Radic Biol Med 2012;53:437–46.
30. Cuevas S, Villar VA, Jose PA, Armando I. Renal dopamine receptors, oxidative stress, and hypertension. Int J Mol Sci 2013;14:17553–72.
31. Hegele RA. Paraoxonase genes and disease. Ann Med 1999;31:217–24.
32. Shin BS, Oh SY, Kim YS, Kim KW. The paraoxonase gene polymorphism in stroke patients and lipid profile. Acta Neurol Scand 2008;117:237–43.
33. Boright AP, Connelly PW, Brunt JH, Scherer SW, Tsui LC, Hegele RA. Genetic variation in paraoxonase-1 and paraoxonase-2 is associated with variation in plasma lipoproteins in Alberta Hutterites. Atherosclerosis 1998;139:131–6.
34. Novotny D, Karasek D, Vaverkova H, Slavik L. The influence of apolipoprotein A5 T–1131C and apolipoprotein E common genetic variants on the levels of hemostatic markers in dyslipidemic patiens. Clin Chim Acta 2014;436:11–17.
35. Novotny D, Vaverkova H, Karasek D, Malina P. Genetic variants of apolipoprotein A5 T–1131C and apolipoprotein E common polymorphisms and their relationship to features of metabolit syndrome in adult dyslipidemic patiens. Clin Biochem 2014;47:1015–21.
36. Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis 2003;170:191–203.
37. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol 2007;27:2292–301.
38. Ferri C, Desideri G, Baldoncini R, Bellini C, De Angelis C, Mazzocchi C, et al. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. Diabetes 1998;47:660–7.
39. Boulbou MS, Koukoulis GN, Makri ED, Petinaki EA, Gourgoulianis KI, Germenis EA. Circulating adhesion molecule levels in type 2 diabetes mellitus and hypertension. Int J Cardiol 2005;98:39–44.