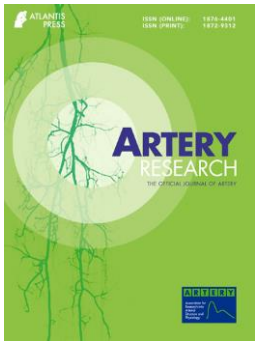

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Association between endothelial NO synthase polymorphism (rs3918226) and arterial properties

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KEYWORDS
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Abstract *Background:* Recently, rs3918226 polymorphism in the promoter region of endothelial NO synthase (eNOS) was strongly associated with arterial hypertension in a large genome-wide association study. We investigated whether this polymorphism was associated with arterial phenotypes in a Czech general population.
Methods: In a pilot study, we genotyped 101 untreated subjects (mean age, 54.0 years). Arterial properties were measured using SphygmoCor. We used robust multivariate analysis to assess whether rs3918226 was associated with peripheral or central blood pressure, carotid-femoral pulse wave velocity (PWV) and aortic augmentation index (AIx). As independent covariates we considered sex, age, MAP, heart rate and smoking.
Results: Frequencies of rs3918226 genotypes were CC 85.2%, CT 14.8%, and TT 0%. Current smokers carrying mutated T allele had marginally higher PWV (10.0 ± 0.8 vs. 8.7 ± 0.4 m/s; $P = 0.051$) and significantly higher AIx (172.2 ± 6.8 vs. 153.2 ± 3.8%; $P = 0.024$) compared to CC homozygotes. In non-smokers we did not find any association between rs3918226 and arterial properties ($P \geq 0.62$). Moreover, we did not observe any association between either peripheral or central blood pressure and the polymorphism under study ($P \geq 0.58$).
Conclusions: This is the first study to explore the association of rs3918226 polymorphism in eNOS gene with arterial properties. Mutated T allele was associated with higher PWV and AIx in smokers. We hypothesize that genetic modulation of intermediate arterial phenotypes might lead to higher blood pressure. As the prevalence of T allele is low, further study with a sufficient number of subjects is warranted.

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Introductions

Measurement of arterial stiffness have gained increased attention in recent decades.[1] One of the methods is a measurement of carotid-femoral pulse wave velocity, which is a surrogate measurement of aortic stiffness.[2] Increased PWV is associated with an increasing risk for cardiovascular outcome in several populations, in both general population[3,4] and in subjects with several pathological conditions[5,6] and has been added to the 2007 European Guidelines for the Management of Hypertension.[7] Augmentation index, on the other hand, is a more complex parameter, reflecting both arterial stiffness and pulse wave reflection.

Recently, in a large genome-wide association study rs3918226 polymorphism in the promoter region of endothelial NO synthase was strongly associated with arterial hypertension.[8] Moreover, the same authors discovered a potential binding site for ETS (E-twenty six) transcription factors directly next to rs3918226, suggesting a potential modulation of endothelial NO synthase expression.[8] Biological evidence links endothelial NO synthase with hypertension, because it is an important mediator of cardiovascular homeostasis and blood pressure control via vascular tone regulation.[9] This finding supports the hypothesis that there may be a causal genetic variation at this locus.

The aim of the present pilot study is to investigate the effect of this polymorphism of eNOS gene with a known pathophysiological role in arterial hypertension and arterial properties in untreated subjects from the Czech general population.

Methods

Study population

The Czech post-MONICA study is a population survey studying trends and determinants of cardiovascular risk factors in a 1% random sample of the Czech population in nine districts of the country. Methods of the Czech post-MONICA study are described elsewhere.[10] Our study included individuals aged over 25 years from the City of Pilsen. The overall response rate in this district was 68.0%. From 1126 participants with arterial measurements, we selected 101 subjects untreated for hypertension for a pilot genotype study.

Research protocol included an administration of a standardized questionnaire to obtain information on each subject's medical history, smoking and drinking habits, and use of medications. Blood pressure was the average of three consecutive readings. Mean arterial pressure (MAP) was diastolic pressure plus one third of pulse pressure. Furthermore, blood samples were obtained for biochemical analyses. Height and weight were determined for all participants. Body mass index (BMI) was calculated as body weight (kg)/height^2 (m^2).

Arterial measurement

We measured PWV by means of SphygmoCor device (AtCor Medical Ltd, Sydney, Australia). We computed the PWV from recordings of the arterial pressure wave at the carotid and femoral arteries.[11] We measured the distance between the site of the carotid recordings and the suprasternal notch and between the suprasternal notch and the site of the femoral recordings. We subtracted these two distances to obtain travel distance. To express PWV according to the international standard[12,13] we then proceeded according to the proposed equations,[12,13] and we report PWV as the ratio of the travel distance using calculated direct measurement*0,80 to the transit time in seconds. The aortic augmentation index was derived from radial pulse wave and defined as the ratio of the second to the first peak of the pressure wave expressed as a percentage. Central blood pressures were derived from radial pulse wave measurements.

Table 1 General characteristics according eNOS rs3918226 genotype.

	CC homozygotes(n = 86)	T allele carriers (n = 15)	P
Women, n (%)	46 (53.5)	6 (40.0)	0.41
Age, years	53.8 ± 5.8	55.3 ± 3.4	0.17
Systolic blood pressure, mm Hg	128.5 ± 17.2	127.0 ± 12.8	0.74
Diastolic blood pressure, mm Hg	83.4 ± 8.7	81.4 ± 6.5	0.40
Mean arterial pressure, mm Hg	98.4 ± 10.8	96.6 ± 8.3	0.53
Heart rate, bpm	70.3 ± 8.3	65.2 ± 6.3	0.024
Central systolic pressure, mm Hg	123.8 ± 19.0	124.5 ± 23.8	0.90
Central pulse pressure, mm Hg	40.8 ± 11.8	44.6 ± 14.9	0.28
Aortic pulse wave velocity, m/s	9.1 ± 2.4	9.7 ± 2.8	0.38
Augmentation index, %	148.0 ± 20.2	157.3 ± 32:2	0.14
BMI, (kg/m^2)	26.5 ± 4.2	25.0 ± 3.3	0.20
Serum total cholesterol, mmol/l	5.4 ± 0.8	5.6 ± 1.4	0.66
Serum glucose, mmol/l	5.1 ± 0.6	5.2 ± 0.4	0.68
Serum creatinine, μmol/l	80.0 ± 10.0	80.5 ± 12.5	0.86
Smoking, n (%)	23 (26.7)	8 (53.3)	0.065
Hypolipidemic treatment, n (%)	4 (4.6)	0	1.00
Diabetes mellitus, n (%)	1 (1.2)	0	1.00

Data are means ± standard deviation or number (percentage), respectively.

Genotyping

All participants provided 1 ml blood samples for DNA extraction. The blood was collected in 2 ml tube and immediately stored at −20 °C until use. DNA was extracted with an extraction kit DNeasy Blood &Tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol, and quantified by a spectrophotometer.

Detection of rs3918226 was performed by PCR and followed by High-Resolution Melting (HRM) analysis with probes. Sequences of the forward and reverse primers and probes were following: 5′-GAAGCGTGCGT CACTGAATG-3′, 5′-AGCCCCAATTTCCTGGAACC-3′, and 5′-CACTGGAAGGTAGCTTCCTGCT/3SpC3/-3′, respectively (Catalogue number HRLSSRV0004 LUNAPROBE CUSTOM DESIGN, Idaho Technology Inc., Salt Lake City, Utah, USA). The HRM analysis was performed on high - resolution melting instrument Light Scanner (Idaho Technology Inc., Salt Lake City, Utah, USA).

Statistical methods

For database management and statistical analyses, we used the SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA). Data are presented as mean ± SD or proportions. A Student *t*-paired test and Fisher test were used to compare differences between genotypes. To minimize the influence of outlying data we used robust multiple regression model applying the SAS procedure

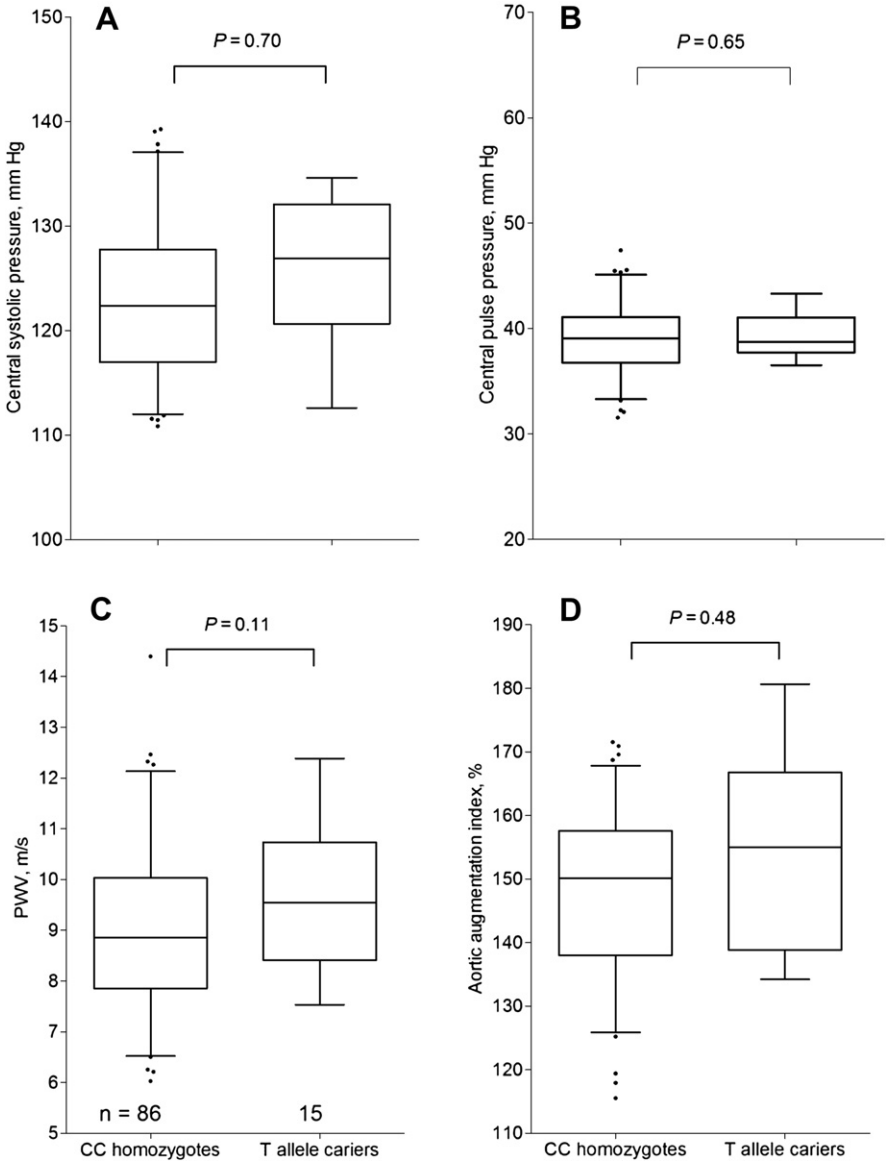

Figure 1 Central blood pressure, pulse wave velocity and augmentation index in relation to rs3918226 in eNOS gene. The analyses were adjusted for sex and age (central SBP; panel A); sex, age and heart rate (central PP; panel B); age, mean arterial pressure and heart rate (PWV; panel C); and sex, age, MAP, heart rate and smoking (AIx; panel D), respectively. Values are expressed as box plots with whiskers standing for 95 percentile. The number of subjects contributing to each group is given. *P*-values for difference between CC homozygotes and T allele carriers.

PROC ROBUST for estimating the effect of genotypes on pressure and arterial phenotypes. We carefully selected potential covariates using three steps analysis. First, we used a non-parametric regression matrix with the *P* value < 0.20 as a threshold. In a second step, we included selected parameters into a robust multivariate analysis followed by stepwise regression procedure with the *P*-values for independent variables to enter and to stay in the model set at 0.15. As covariates we considered age, sex, MAP (not for blood pressure parameters), heart rate, and smoking. Furthermore, we searched for possible interaction with smoking by implementing interaction term into regression models.

Results

Characteristics of participants

General characteristics of subjects are listed in Table 1. Of the 101 participants included in the study, 52 (51.5%) were women, 24 (23.8%) had arterial hypertension, and 31 (30.7%) were current smokers. Mean age was 54.0 years (range 40.7—71.1 years). Genotypes frequencies of rs3918226 were CC 86 (85.2%), CT 15 (14.8%) and TT 0, respectively. CC homozygotes had slower heart rate (65.2 ± 6.3 vs. 70.3 ± 8.3 bpm; $P = 0.024$) than carriers of mutated T allele. Otherwise, the two genotype groups did

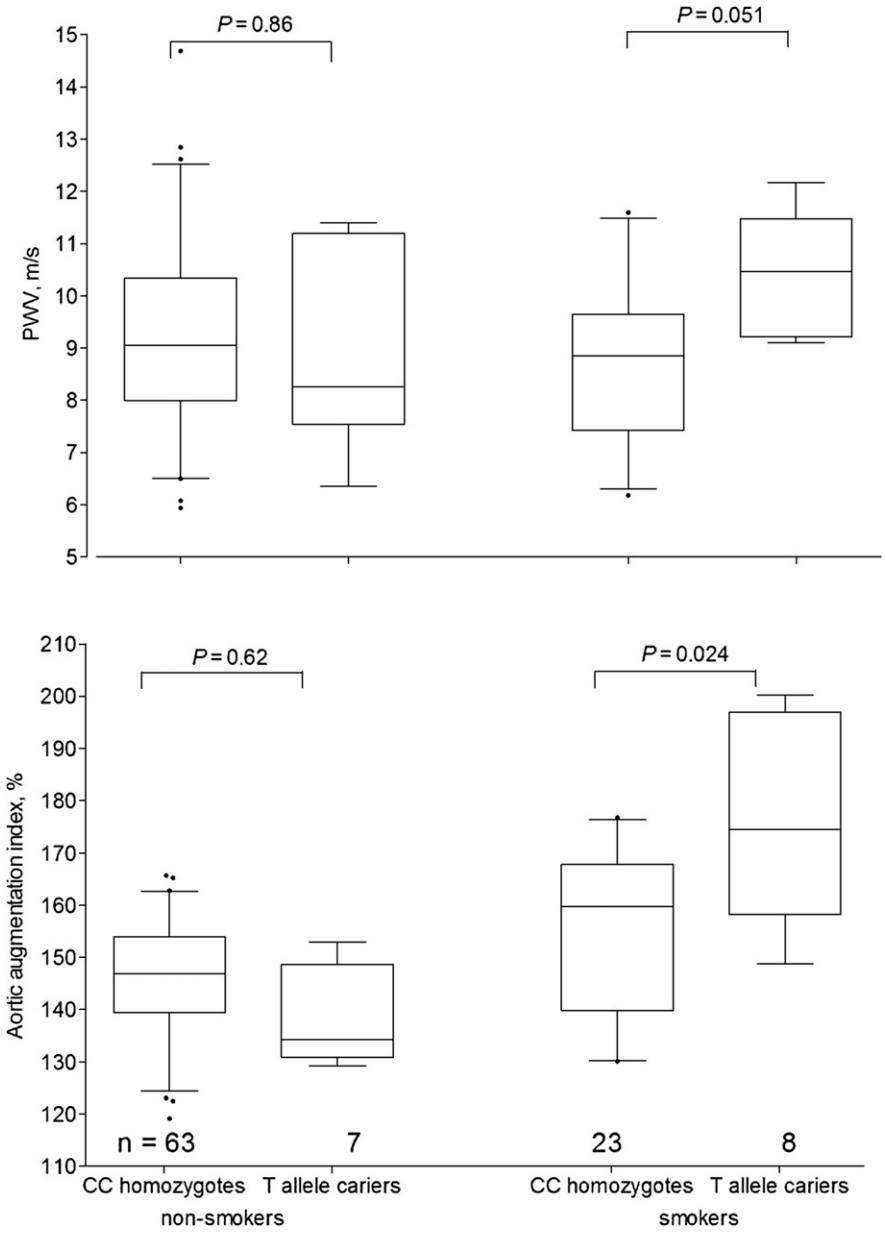

Figure 2 Pulse wave velocity and augmentation index in relation to rs3918226 in eNOS gene according to smoking status. The analyses were adjusted for age, mean arterial pressure and heart rate (PWV) and sex, age, MAP, and heart rate (AIx), respectively. For further explanation see Fig. 1.

not differ in age, blood pressure, biochemical parameters and arterial properties.

Association between eNOS genotype and arterial properties

Figure 1 shows the relationships between rs3918226 and blood pressure, PWV and augmentation index after adjustment for sex and age (central SBP), sex, age and heart rate (central PP), age, mean arterial pressure, heart rate (PWV), and sex, age, MAP, heart rate and smoking (AIx), respectively. Carriers of mutated T allele tended to have higher PWV (9.9 ± 0.5 vs. 9.0 ± 0.2 m/s; $P = 0.11$). Interaction term between rs3918226 and current smoking was significant for PWV ($P = 0.032$) and borderline significant for AIx ($P = 0.078$). Therefore in further step we analysed smokers and non-smokers separately (Fig. 2). In smokers we observed a similar trend for PWV as in the whole population. Current smokers carrying T allele had marginally higher PWV (10.0 ± 0.8 vs. 8.7 ± 0.4 m/s; $P = 0.051$) and significantly higher AIx (172.2 ± 3.8 vs. $153.2 \pm 3.8\%$; $P = 0.024$), while in non-smokers we did not find any association with rs3918226 polymorphism ($P \geq 0.62$). We did not observe any association between peripheral and central blood pressure and the polymorphism under study ($P \geq 0.58$). In analysis, where subjects using statins were not included, confirmed our results.

Discussion

Our study is the first to examine the relationship between polymorphism rs3918226 in endothelial nitric oxide synthase gene and arterial properties. In a pilot study we observed the possible association between this genotype and arterial properties. This association was modified by smoking.

Endothelial NO synthase is the main source of nitric oxide in the vascular wall, a molecule with anti-inflammatory, antithrombotic, vasorelaxant, antioxidant and anti-atherogenic properties.[9] Several candidate gene studies were performed to find an association between eNOS polymorphisms and arterial hypertension but the results are inconsistent as shown by recent meta-analysis.[14] With respect to arterial properties, only a few studies investigated eNOS gene polymorphisms. In the Bogalusa heart study,[15] the T allele of the eNOS G894T polymorphism has been associated with arterial wall stiffness of the carotid artery in 403 young participants. We have previously shown[16] that current smokers had increased stiffness of muscular arteries in carriers of both T786C and G894T mutations. In non-smokers we did not observe any associations between these polymorphisms and arterial properties.[16] The effect of smoking on the regulation of arterial tone and reduction of eNOS protein expression was demonstrated in an animal study.[17] Indeed, exposure to smoking increased the elastic modulus, decreased stress and strain, and increased the wall thickness of coronary arteries compared with those of control mice. Exposure to smoking led to reduction of eNOS protein expression. Moreover, the NO metabolite was markedly decreased in mice exposed to smoking.[17] These findings suggest that smoking might possibly be an important confounding factor of eNOS regulation. Our observation that carriers of mutated T allele of rs3918226 had higher PWV and AIx is in line with these findings.

The present study must be interpreted within the context of its limitations and strengths. First, our study was a pilot study with a limited number of subjects. This fact and rare prevalence of mutated variant of studied genotype increased the possibility of a false negative finding. Our results need to be confirmed in a larger study. However, low prevalence of T allele in our settings is in accordance with the findings from Hyper Genes study.[8] Second, we did not confirm smoking status information obtained in the questionnaire by measuring the concentration of CO in exhaled air or by measuring the cotinine concentration in blood.

This is the first study to explore the association of rs3918226 polymorphism in eNOS gene with arterial properties. Smokers carrying the mutated T allele had marginally higher PWV and significantly higher AIx in this pilot study. We hypothesize that genetic modulation of intermediate arterial phenotypes might lead to higher blood pressure. As the prevalence of T allele is low, further study with sufficient number of subjects is warranted.

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Conflict of interest

None of the authors has a conflict of interest with regard to the data presented in this paper.

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