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# Association between endothelial NO synthase polymorphisms and arterial properties in the general population

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ABSTRACT

*Objective:* Nitric oxide plays an important role in vascular biology. Several single nucleotide polymorphisms (SNP) in the endothelial nitric oxide gene (NOS3) have been previously associated with arterial hypertension. We investigated whether these SNPs might be associated with arterial phenotypes in the Czech general population.

*Methods:* We genotyped three NOS3 SNPs in 426 subjects not treated for arterial hypertension (mean age, 49.1 years; 55.9% women). Arterial properties were measured using applanation tonometry. In multivariate-adjusted analyses, we assessed the gene effects of *rs3918226* (*–665 C > T*), *rs1799983* (*glu298asp G > T*) and *rs2070744* (*786 T > C*) on augmentation index (AIx), central augmentation pressure (AP) and aortic pulse wave velocity (PWV).

*Results:* Carriers of *rs3918226* mutated *T* allele had marginally higher AIx (145.3 ± 2.5 vs. 140.2 ± 1.1%; $P = 0.064$) and significantly higher AP (12.7 ± 0.7 vs. 11.1 ± 0.3 mm Hg; $P = 0.033$). These associations were independent of potential confounding factors. Aortic PWV was not different in the two *rs39182226* genotypes groups ($P = 0.35$). In single gene analyses, we did not observe any association between measured phenotypes and *rs1799983* or *rs2070744* ($P \geq 0.11$). In haplotype analysis, we observed trend for higher PWV in haplotypes containing *rs3918226* mutated *T* allele compared with other allelic combination ($P \leq 0.079$).

*Conclusion:* Mutated *T* allele of *rs3918226* polymorphism in NOS3 gene was associated with parameters reflecting central arterial stiffness and wave reflection. We hypothesize that genetic modulation of intermediate arterial phenotypes might lead to higher blood pressure.



## 1. Introduction

Nitric oxide (NO) is an important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life of a few seconds in the blood. It is biosynthesized endogenously from L-arginine by various nitric oxide synthase (NOS) enzymes, including the endothelial NOS. The endothelial cells use nitric oxide to signal the surrounding smooth muscle to relax, which results in vasodilation and increasing blood flow. Moreover, NO inhibits vascular smooth muscle cell migration and proliferation [1].

Reduced NO formation is related to increased blood pressure [2]. Several single nucleotide polymorphisms of endothelial NOS gene (*NOS3*) were associated with hypertension, particularly the *rs3918226* (*–665 C > T*) located at a binding site of E26 transformation-specific (ETS) transcription factors [3,4], *rs1799983* (*glu298asp G > T*) in exon 7 [5,6] and *rs2070744* (*786 T > C*) in the promoter region [7,8]. Moreover, several studies linked the *NOS3* haplotypes containing the latter two mentioned SNPs with hypertension [9–11]. The observed results were independent of significant differences in haplotype frequency distribution among different ethnic groups [9].

Only few studies focused on the association between *NOS3* genotypes and arterial properties. In our previous pilot study, we observed possible association between *NOS3 rs3918226* and aortic pulse wave velocity and augmentation index [12]. In the Bogalusa heart study, the African Americans *G* allele carriers of *rs1799983* had significantly higher distal arterial stiffness than those with wild-type genotype [13]. In the Framingham study, *rs1799983* was related to central pulse pressure and forward wave amplitude in women [14]. Moreover, we have previously shown the interaction between genes for angiotensin II type 1 receptor (*AGTR1, A1166C, rs5186*) and *NOS3 rs2070744* and stiffness of muscular-type arteries [15]. In the same subjects [16], we observed that current smokers had increased stiffness of muscular arteries in carriers of both *rs2070744* and *rs1799983*

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mutations. In non-smokers we did not observe any associations between these polymorphisms and arterial properties [16].

The aim of the present study was to investigate the possible relation of the three *NOS3* genotypes connected with arterial hypertension with the arterial properties in a large sample of subjects untreated for arterial hypertension from the Czech general population.

## 2. Methods

### 2.1. 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 [17]. Our study included individuals aged over 25 years from the City of Pilsen. The overall response rate in this district was 68.0%. From 952 participants with complete data on arterial measurements, we excluded 321 subjects treated for arterial hypertension and 205 subjects with missing genotypes. Thus the number of participants statistically analyzed totaled 426. All participants gave their written informed consent. The study was approved by the local ethics committee of the University Hospital in Pilsen and was in accordance with the Declaration of Helsinki.

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$^2$ ($m^2$).

### 2.2. 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 [18]. 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. Pulse wave velocity was calculated as the ratio of the travel distance in meters to the transit time in seconds. The aortic augmentation index (AIx) 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. The augmentation pressure (AP) was the difference between second and first peak of the pressure wave.

### 2.3. Genotyping

All participants provided 1 ml whole 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. The method of *rs3918226* detection was described previously [12]. Detection of *SNPs rs1799983* and *rs2070744* were performed by PCR, followed by High-Resolution Melting (HRM) analysis with probes. Sequences of the forward and reverse primers and probes for *rs1799983* were the following: 5′-TACGTGGACATTTTCTGCAGTTT-3′, 5′-CCAGGGCTGAGAGGAGTAA-3′, and 5′-TATTAAAAGAATCCAAGG CCCCCTCTCATCTCaaa/3SpC3/-3′, respectively. The corresponding sequences for *rs2070744* were '5′-CAGAACTACAAACCCCAGCAT-3′, 5′-CACCCTGTCATTCAGTGAGG-3′, and 5′-GGCATCAAGCTCTTCCCT/I/G/I/TGGCTGACC/3SpC3/-3′, respectively (Catalogue number HRLSSRV0004 LUNAPROBE CUSTOM DESIGN, Idaho Technology Inc., Salt Lake City, UT, USA). The HRM analysis was performed on LightScanner instrument (Idaho Technology Inc.).

### 2.4. Statistical methods

For database management and statistical analyses, we used the SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA). We tested linkage disequilibrium and we reconstructed haplotypes, using the SAS procedures PROC ALLELE and PROC HAPLOTYPE, as implemented in the genetics module of the SAS software. In our analyses, we included only those *NOS3* haplotypes that were unambiguously determined. Data were presented as mean ± SD or proportions. A Student *t*-paired test and Fisher test were used to compare differences between genotype groups. We used multivariate linear regression to estimate effect of genotypes and haplotypes on blood pressure and arterial properties. As covariates we considered age, sex, MAP (not for blood pressure), heart rate, and smoking. Furthermore, we searched for possible interaction with smoking by implementing interaction term into regression models.

## 3. Results

### 3.1. Characteristics of participants

General characteristics of subjects are listed in Table 1. Of the 426 participants included in the study, 238 (55.9%) were women and 140 (32.9%) were current smokers. Mean age was 49.1 years (range 24.8–73.6 years). As regards the r*s3918226* genotype, *CC* homozygotes were more frequently women (58.9 vs. 39.4; $P = 0.0044$) compared with *T* allele carriers. Otherwise, the two genotype groups did not differ in age, blood pressure, biochemical parameters and arterial properties. Table 2 gives allele and genotypes frequencies. The frequencies of the *rs3918226* and *rs2070744* did not deviate from Hardy–Weinberg equilibrium ($P \geq 0.18$).

### 3.2. Association between eNOS genotypes and arterial properties

Out of the three genotypes under study, we observed significant association with the arterial properties only for *rs3918226* (*–665 C > T*). Figure 1 shows the relationships between *rs3918226* and

**Table 1**
General characteristics of the whole study population and according to *rs3918226* genotype.

| | All subjects | *rs3918226 (–665 C > T)* | | |
|---|---|---|---|---|
| | *n* = 426 | *CC* (*n* = 360) | *CT + TT* (*n* = 66) | *P* |
| Women, *n* (%) | 238 (55.9) | 212 (58.9) | 26 (39.4) | 0.0044 |
| Age, years | 49.1 ± 13.3 | 48.9 ± 13.2 | 50.2 ± 13.6 | 0.47 |
| Systolic BP, mm Hg | 124.2 ± 15.7 | 124.1 ± 15.9 | 124.8 ± 14.7 | 0.77 |
| Diastolic BP, mm Hg | 80.3 ± 8.9 | 80.3 ± 9.0 | 80.1 ± 8.8 | 0.86 |
| Mean arterial pressure, mm Hg | 94.9 ± 10.3 | 94.9 ± 10.4 | 95.0 ± 9.9 | 0.96 |
| Heart rate, bpm | 69.7 ± 9.3 | 70.0 ± 9.3 | 68.2 ± 9.4 | 0.15 |
| PWV, m/s | 7.3 ± 2.1 | 7.2 ± 2.1 | 7.6 ± 2.1 | 0.17 |
| Augmentation index, % | 140.2 ± 27.2 | 139.7 ± 26.5 | 143.0 ± 31.0 | 0.36 |
| Augmentation pressure, mm Hg | 11.2 ± 7.9 | 10.9 ± 7.5 | 12.5 ± 9.4 | 0.12 |
| BMI, (kg/$m^2$) | 26.3 ± 4.6 | 26.2 ± 4.6 | 26.4 ± 4.5 | 0.85 |
| Total cholesterol, mmol/l | 5.2 ± 1.0 | 5.1 ± 1.0 | 5.3 ± 1.1 | 0.095 |
| Glycemia, mmol/l | 5.0 ± 0.5 | 5.0 ± 0.5 | 5.1 ± 0.5 | 0.40 |
| Serum creatinine, μmol/l | 78.6 ± 11.4 | 78.5 ± 11.3 | 79.2 ± 11.7 | 0.64 |
| Smoking, *n* (%) | 140 (32.9) | 119 (33.1) | 21 (31.8) | 0.89 |

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