Association of Intron 4a/b VNTR NOS3 Gene Polymorphism with Arterial Stiffness in Russian Population

Abstract

Nitric oxide is one of the important modulators of arterial tone. It is still unclear whether arterial stiffness depends upon NOS3 gene polymorphism in intron 4. The aim of this observational study was to test an association of central hemodynamic and arterial distensibility with VNTR 4b/a NOS3 gene polymorphism determined by PCR, in Caucasian population.

The routinely ascertained tonometry data were studied by pulse wave analysis using SphygmoCor device in 61 healthy Russian miners (27 women) aged 27–65 years and residing in middle Kola Peninsula (68 degrees N).

Paired comparisons showed that male BB homozygotes had lower values of augmentation pressure (p=.005), and higher brachial-to-aortic pulse pressure amplification (p=.002) than A allele carriers (AB and AA genotypes). These associations remain significant after adjusting for age, heart rate, and systolic blood pressure in multiple regression analysis. Female AB carriers had higher aortic systolic blood pressure (p=.046) and lower subendocardial viability ratio (p=.049) compared to homozygous BB subsample.

Individuals carrying A allele thus have stiffer conduit arteries and seem to be at higher risk for cardiovascular diseases.

Key words: NOS3 gene; VNTR polymorphism; arterial stiffness; pulse wave analysis; central hemodynamics

1. Introduction

The synthesis of endothelium-derived NO from L-arginine is catalyzed by endothelial nitric oxide synthase (NOS3) which is encoded by *NOS3* gene on chromosome 7. A variety of studies have evaluated the association between *NOS3* gene polymorphisms and risk of fatal cardiovascular events and nonlethal disorders in different populations [1–3], and inconsistent results have been obtained. From one hand, an association has been observed [4–11]. From the other hand, the link could not be found in several studies [12,13].

The variable number of tandem repeats (VNTR) in intron 4 of this gene relates to adverse cardiovascular phenotypes in various populations with some exceptions [9,14]. In the majority of papers, the presence of rare *A* allele and especially homozygous *AA* genotype has been found to be a risk factor for the cardiovascular diseases [15–17] but this is not the case for Sudanese [18], Iranian [19], Greek [20], Chilean [10], and Siberian native [21] populations. No difference could be observed in arterial flow-mediated dilation and the response to glyceryl trinitrate between *A* allele carriers and *BB* homozygotes [22]. Cardiovascular, renal, metabolic parameters and forearm responses to acetylcholine have been found to be homogeneously distributed across b/a genotypes [23]. These results demonstrate population- and ethnic-dependent inconsistency in existed reports on association between 4b/a genotypes and cardiovascular phenotypes.

The contribution of genetic polymorphisms to the variance of arterial distensibility phenotypes has been addressed in many studies [24,25]. Although associations between arterial stiffness and genetic variants at the *NOS3* locus have been established in various populations [9,12,24,26–29,30], the VNTR 4b/a polymorphism is still not among the genotypes studied in this context. In the present population-based observational study we hypothesized that VNTR 4b/a *NOS3* gene polymorphism associates with central hemodynamics and elastic properties of conduit arteries.

2. Subjects and Methods

2.1. Cohort Details

The study population consisted of 60 apparently healthy normotensive nonsmoking Russian individuals of Caucasoid race (27-65 years, 28 women, 51 were born in Kola Peninsula). They worked at underground loparite mine in the middle Kola Peninsula (68 °N) and were examined in winter during regular yearly medical inspection. All participants provided written consent to participate in the study. The protocol was approved by the Ethical Committee of the Institute of Physiology.

2.2. Cardiovascular Phenotypes

Non-invasive measures of arterial stiffness and wave reflection were assessed in sitting position of participants as previously described for this sample [31, 32]. The following phenotypical traits were routinely ascertained by applanation tonometry and pulse wave analysis with use of SphygmoCor device (AtCor Medical, Australia): values of brachial and central systolic (SBP), diastolic (DBP), mean blood pressure (MBP) and pulse pressure (PP), aorta-to-brachial pulse pressure amplification (PP Ampl, $\%=PP_{brachial} / PP_{aortic} \times 100$), augmentation pressure (AP) and augmentation index (AIx@75, corrected to HR=75 beats/min), left ventricular ejection duration (ED) and time to reflective wave (Tr). The latter two timing parameters were expressed as percentage of the length of cardiac cycle. As described elsewhere, higher AP, AIx [33] and lower Ampl [34] and time to reflective wave [35] indicate greater arterial stiffness. The Buckberg subendocardial viability ratio (SEVR) was calculated as percentage of diastolic pressure-time integral/systolic pressure-time integral ratio and was considered a measure of myocardial O_2 demand/supply ratio.

2.3. Details of the SNP Studied

Genomic DNA was isolated from the venous blood using standard protocol and commercial kit (BioSilica, Novosibirsk) in the Genetic Laboratory at the Institute of Physiology & Basic Medicine. The intron 4 b/a VNTR *NOS3* polymorphism was selected and amplified by polymerase chain reaction followed by polyacrylamide gel electrophoresis.

2.4. Statistical Analysis

Since the *AA* genotypic subsample consisted of three individuals only, it was grouped together with the *AB* carriers and analyzed by paired comparing with homozygous (*BB*) subsample. The phenotypic traits were then comparatively examined for genotype differences via Mann-Whitney test. Univariate analysis of variance and multiple linear regression analysis (GLM option) were performed by using SPSS-19 package (IBM, USA) to ascertain relationships between phenotypic variables and genotypes after adjustment for covariates that are known to influence tonometric parameters (age, systolic blood pressure, and heart rate). A value of $p \leq .05$ was considered statistically significant but the value $\leq .1$ was also indicated to note a suggestive effect. Tonometric data were expressed as median and 25th and 75th percentiles.

3. Results

For both sexes, no significant differences in age, BMI, and HR were observed between two genotypes compared (Table 1). The *BB male* carriers had lower AP, AIx values and higher PPAmpl and hence more compliant peripheral arteries compared to *A* allele containing carriers. In *women*, the differences reached borderline level of significance for SBP and SEVR. In the *sex-pooled* analysis, the differences are significant for PP Ampl and AP in favor of *BB* genotype. These results report on the harmful effect of *A* allele on tonometric parameters in both sexes. For *men*, the genotype effect remains statistically significant after adjusting for age, HR, and SBP (Table 2) and explains 27.2% (p=0.026), 16.8% (p=0.034), and 21.3% (p=0.020) of AP, AIx and PPAmpl variances, respectively, in multiple regression analysis. Other phenotyping variables did not reach borderline level of significance while controlling the effects of these confounding factors.

4. Discussion

This study evaluated relations of *NOS3* gene intron 4 polymorphism with several tonometric measures of hemodynamics and arterial elasticity and found that homozygosity for the major (*B*) allele was associated in men with reduced augmentation pressure and increased aorta-to-brachial pulse pressure amplification, i.e. with

compliant conduit arteries. Women with this genotype had greater SEVR, i.e. better diastolic myocardial perfusion than their counterparts carrying the minor (*A*) allele.

The harmful effect of *A* allele, found in the present work, on tonometric measures is in consistence with studies having linked this allele with adverse cardiovascular phenotypes. Thus, the *A* allele frequency has been found to be high in Systemic Lupus Erythematosus patients [36] and in persons with coronary artery disease and renal disease [15,37]. However, conflicting data have been reported in the literature regarding the association of this allele with hypertension [13,38] and end-stage renal disease [39].

The present study thus adds indirect evidence to the set of data indicating the association between the minor VNTR allele and adverse cardiovascular phenotypes in view of arterial distensibility. However, the literature existed does not allow making definite conclusion on the cardiovascular effect of the described genetic locus. Gamil and co-authors [18] consider it unlikely that the VNTR itself has a functional role in the development of essential hypertension as it lies in intronic region and seems to act as a marker for other functional variants elsewhere in the gene.

The wild-type *B/B* homozygosity for *NOS3* intron 4 VNTR was found in 37 (60.7%) subjects and *A/B* allele combination presented in 24 (39.3%) [40]. The authors could not find an association of 4 b/a polymorphism with hypertension in type 1 diabetes.

The results obtained here are suggestive for modest relations between VNTR variant within intron 4 at the *NOS3* locus and arterial stiffness and allow suggesting that *A* allele might negatively determine either quantity, i.e. expression, or activity of NO synthase itself or other substances involved in the regulation of the arterial tone and/or elasticity, i.e. smooth muscle contractility or molecular structure of extracellular matrix. However, it seems unlikely that there is a specific major genetic factor determining arterial stiffness that exceeds all other known vascular risk factors probably mainly because of multiple causality of arterial rigidity and its weak association with an individual gene or allele.

To the best of our knowledge, there are no data in scientific literature on the genetic determinants of vascular properties in northern residents. Two papers by Fould et al. [41,42] report on the high vascular distensibility, assessed by pulse wave velocity, in native inhabitants of the Canadian North. The low prevalence of arterial hypertension

and metabolic syndrome among the aboriginal northerners [43,44] despite the predominance of fatty foods in their diet allows suggesting the compliance of peripheral arteries in Arctic Aboriginals compared to residents of mid-latitude regions.

Limitations. Unfortunately, this research does not answer the question of whether the established gene-vascular phenotype association is a general phenomenon or is characteristic feature of the northern inhabitants and whether the *BB* genotype is selected by the specific northern environment. The appropriate answer needs a comparative investigation.

Since the present study is exploratory, additional researches of larger sample size to confirm these findings are warranted.

5. Conclusions

This study established the relation of *NOS3* gene intron 4 polymorphism with several tonometric measures of central hemodynamics and arterial elasticity and found that homozygosity for the major (*B*) allele was associated in men with reduced augmentation pressure and increased aorta-to-brachial pulse pressure amplification, i.e. with compliant conduit arteries. Women with this genotype had greater SEVR, i.e. better diastolic myocardial perfusion than their counterparts carrying the minor (*A*) allele. It is concluded that individuals carrying *A* allele seem to be at higher risk for cardiovascular diseases.

Table 1. Physical characteristics and parameters of central hemodynamics and arterial compliance in healthy individuals by sex and 4b/4a *NOS3* genotype

Parameter	Genotypes		P (Mann-Whitney)
	b/b	a/b + a/a	
Men	***N=18***	***N=12+2***	
Age, years	36.0 (30.0-57.0)	49.5 (39.1-59.0)	.077
BMI, kg/m^2	25.7 (23.4-28.6)	25.3 (23.3-30.2)	NS
HR, bpm	80.5 (73.3-87.0)	75.5 (67.5-85.3)	NS

SBP, mmHg	116.0 (106.3-121.0)	118.5 (111.3-129.5)	NS
DBP, mmHg	88.0 (82.5-93.5)	87.0 (81.8-92.5)	NS
MBP, mmHg	100.0 (95.8-105.8)	101.5 (92.0-108.0)	NS
PP, mmHg	26.5 (21.5-33.5)	32.0 (23.8-40.8)	NS
PPAmpl, %	170.5 (141.3-179.5)	143.5 (115.8-159.3)	**.002**
ED, %	37.0 (33.8-38.3)	35.0 (32.5-40.3)	NS
Tr, %	19.1 (18.1-21.4)	17.8 (15.0-21.1)	NS
aAP, mmHg	0.00 (–1.25-5.00)	5.50 (1.75-15.3)	**.005**
aAIx, %	5.5 (–1.0-21.8)	16.5 (5.8-26.8)	.081
SEVR, %	156.5 (141.5-168.5)	164.5 (132.8-172.8)	NS
Women	***N=18***	***N=9+1***	
Age, years	44.0 (28.3-57.3)	49.0 (27.8-56.3)	NS
BMI, kg/m^2	25.3 (23.0-29.7)	31.4 (24.6-33.7)	NS
HR, bpm	74.0 (69.8-78.0)	76.5 (67.8-82.5)	NS
SBP, mmHg	116.5 (106.0-123.3)	124.0 (109.5-138.5)	**.049**
DBP, mmHg	83.5 (75.8-91.5)	84.0 (77.8-91.3)	NS
MBP, mmHg	99.0 (89.8-105.3)	102.5 (96.1-112.0)	NS
PP, mmHg	30.5 (24.8-34.0)	34.0 (25.0-51.3)	NS
PPAmpl, %	133.0 (120.8-160.5)	128.5 (119.5-148.0)	NS
ED, %	36.6 (35.5-37.3)	38.5 (35.3-41.3)	NS
Tr, %	16.6 (15.0-19.7)	17.4 (14.8-18.8)	NS
aAP, mmHg	7.5 (4.3-10.3)	10.0 (4.8-15.5)	NS
aAIx, %	25.0 (16.8-32.8)	30.0 (16.0-35.5)	NS
SEVR, %	153.0 (145.1-170.2)	133.5 (129.0-162.3)	.049
Both sexes	***N=36***	***N=21+3***	
Age, year	39.1 (30.3–57.7)	49.0 (37.0-58.8)	NS
BMI, kg/m^2	25.3 (23.6–29.2)	26.5 (24.0-32.3)	NS
HR, bpm	76.0 (70.3–84.5)	76.5 (68.3-83.5)	NS
SBP, mmHg	116.0 (107.0–122.8)	120.5 (112.2-131.4)	.094

DBP, mmHg	86.0 (78.5–93.0)	85.0 (81.3-91.0)	NS
MBP, mmHg	100.0 (94.0–104.8)	102.1 (92.3-111.8)	NS
PP, mmHg	29.5 (23.3–34.0)	32.4 (25.1-42.3)	NS
PPAmpl, %	153.0 (130.5–172.8)	134.4 (118.5-158.0)	**.019**
ED, %	37.0 (34.3–38.0)	37.0 (33.5-41.0)	NS
Tr, %	18.4 (15.7–20.6)	17.8 (15.1-20.0)	NS
aAP, mmHg	5.01 (0.02–8.04)	7.10 (4.18-14.50)	**.030**
aAIx@75, %	21.5 (4.3–27.3)	22.5 (12.5-30.8)	NS
SEVR, %	154.5 (144.5–168.5)	156.5 (131.3-167.8)	NS

Values are expressed as median (25th–75th percentiles). BMI, body mass index; HR, heart rate; SBP, DBP, MBP, PP, aortic systolic, diastolic, mean, and pulse blood pressure; PPAmpl, aorta-to-brachial pulse pressure amplification; ED, ejection duration; Tr, time to reflection; aAP, aortic augmentation pressure; aAIx, aortic augmentation index; SEVR, subendocardial viability ratio. NS, not significant. P-values ≤ 0.1 are indicated only, those in boldface are significant, P≤0.05.

Table 2. Results of univariate analysis of variance and multiple regression (GLM) between augmentation pressure, augmentation index, pulse pressure amplification (continued dependent variables), age, heart rate, systolic blood pressure (covariates), and 4b/4a *NOS3* genotype (fixed factor, two categories, bb and ab+aa genotypes) in healthy men (N=32)

Source of variation	F	p	Partial Eta squared (corrected R^2)	Standardized β
Dependent variable: Augmentation pressure				
Corrected model	21.6	<.001	0.824	–
HR	8.96	.006	0.280	–0.318
Age	6.07	.022	0.209	0.292
SBP	9.35	.006	0.289	0.403
NOS3 genotype	4.28	.026	0.272	0.351
Dependent variable: Augmentation index				
Corrected model	14.2	<.001	0.532	–
Intercept	8.6	.007	0.256	–
SBP	12.0	.002	0.324	0.546
NOS3 genotype	5.0	.034	0.168	0.395
Dependent variable: Pulse pressure amplification				
Corrected model	23.77	<.001	0.806	–
Intercept	53.2	<.001	0.698	–
HR	11.5	.003	0.333	0.323
Age	9.00	.006	0.281	–0.322
SBP	11.00	.003	0.323	–0.392
NOS3 genotype	6.2	.020	0.213	–0.371

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