CYBA C242T and NOS3 G894T gene polymorphisms are positively associated with subclinical vascular disease in healthy women of reproductive age

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Introduction

Clinical data indicate that genetic modifications of nitric oxide (NO) production and function are related to the pathogenicity of several cardiovascular events. Accumulating evidence has described associations between the G894T mutation, with the occurrence of ischemic heart disease, myocardial infarction, essential hypertension, and coronary spasm, though data regarding the evaluation of its effect on NO production are debated. Moreover, the C242T genetic variation leads to altered production of ROS in the vascular wall, enhancing oxidative stress and increasing the risk for ischemic cerebrovascular disease.

Objectives

In this study, we aimed to evaluate the association of the NOS3 G894T (rs1799983) and CYBA C242T (rs4673) polymorphic variations with the presence of subclinical vascular disease in a sample of healthy premenopausal, normally menstruating women.

Materials & Methods

Seventy (70) healthy, normally ovulating, premenopausal women were recruited for this study. Venous blood samples were obtained for biochemical/hormonal assessment as well as for genotyping, using real-time PCR.

Sonographically assessed indices of vascular structure and function included carotid and femoral intima-media thickness (IMT), flow-mediated dilation (FMD), carotid-femoral pulse-wave velocity (PWV), and augmentation index.

Results

The prevalence of wild type, heterozygote, and homozygote genotype was 44.3% (31/70), 54.3% (38/70), and 1.4% (1/70) for the G894T polymorphism and 38.6% (27/70), 31.4% (22/70), and 30.0% (21/70) for the C242T polymorphism, respectively. After multivariable adjustment, the hC242T polymorphism was a predictor of both internal carotid IMT (b-coefficient − 0.119, p = 0.011) and combined-IMT (b-coefficient − 0.061, p = 0.015). Systolic blood pressure, lipids, and hC242T determined values of FMD (b-coefficient − 1.604, p = 0.034). Concerning the NOS3 G894T polymorphism, carriers of the polymorphic variant had higher values of IMT and PWV compared to the wild-type subgroup (carotid bulb-IMT and PWV, heterozygotes/homozygotes vs wild type 0.7 ± 0.2 vs 0.6 ± 0.1 mm; 7.1 ± 0.8 vs 6.6 ± 0.7 m/s; p = 0.048 and p = 0.029, respectively). These differences, however, were rendered non-significant in the multivariable analysis.

Table 1 Mean values of vascular function and structure indices according to the presence of CYBA polymorphism of the 70 women of our study. Differences were evaluated using t-test or Mann-Whitney U test.

1. Normally Distributed Parameters: Independent Samples T Test [a]			
	Wild type	*Heterozygotes/homozygotes*	*p-value*
Combined-IMT (mm)	0.58 (0.07)	0.63 (0.12)	0.201
CB-IMT (mm)	0.70 (0.18)	0.65 (0.16)	0.134
FA-IMT (mm)	0.72 (0.13)	0.74 (0.13)	0.434
PWV (m/s)	6.87 (0.68)	6.83 (0.86)	0.145
Aix (%)	18.9 (8.8)	19.7 (6.3)	0.660

2. Non-Normally Distributed Parameters: Mann- Whitney U Test [a]			
	Median (IQR) *Wild type*	Median (Min- Max) *Heterozygotes/homozygotes*	*p-value*
CCA-IMT (mm)	0.60 (0.54-0.63)	0.60 (0.56-0.65)	0.823
ICA-IMT (mm)	0.60 (0.46-0.62)	0.60 (0.50-0.77)	0.628
FMD (%)	3.33 (2.56-6.97)	3.57 (2.77-6.06)	0.058

IMT=intima media thickness; CCA=common carotid artery; CB=carotid bulb; ICA=internal carotid artery; FMD=flow mediated dilation; PWV=pulse wave velocity; AIx=heart-rate adjusted augmentation index; IQR=interquartile range
[a] Comparison refers to wild type vs heterozygotes/homozygotes combined; [b] Comparison between all available genotypes, namely wild type vs heterozygotes vs homozygotes; Statistical significance was set at the level of p-value<0.05

Table 2. Multivariable regression analysis evaluating the association between the NOS3 genetic polymorphism and indices of vascular function and structure.

	b-coefficient	95% CI	p-value
Combined – IMT (mm)			
eNOS polymorphism	0.024	-0.189 to 0.371	0.244
CCA-IMT (mm)			
eNOS polymorphism	0.017	-0.090 to -0.390	0.259
CB-IMT (mm)			
eNOS polymorphism	0.038	-0.111 to 0.210	0.214
ICA-IMT (mm)			
eNOS polymorphism	-0.019	-0.038 to 0.199	0.619
FA-IMT (mm)			
eNOS polymorphism	0.001	-0.073 to 0.096	0.980
FMD (%)			
eNOS polymorphism	0.832	0.278 to 1.890	0.267
PWV (m/s)			
eNOS polymorphism	0.305	0.199 to 0.589	0.141
AIx (%)			
eNOS polymorphism	2.693	-1.290 to 5.924	0.120

BMI=body mass index; SBP=systolic blood pressure; HDL=high density lipoprotein; **Multivariable regression analysis including vascular structure and function indices as dependent variables and the NOS3 genetic polymorphism as an independent variable in two categories. The models were adjusted for traditional cardiovascular risk factors (e.g. age, body mass index, systolic blood pressure, diastolic blood pressure, smoking, triglycerides, high density lipoprotein cholesterol, C-reactive protein)**
Reference group: wild type polymorphism
Statistical significant results, p-value<0.05

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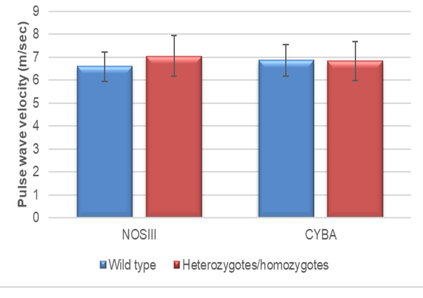

Fig. 1. Estimated values of pulse-wave velocity according to the presence of NOS3 and CYBA genetic polymorphisms, adjusting for the presence of the traditional cardiovascular risk factors. PWV values for heterozygotes/homozygotes vs wild type, NOS3 7.057 ± 0.89 vs 6.59 ± 0.64 m/s, p value 0.047; CYBA 6.84 ± 0.86 vs 6.86 ± 0.69 m/s, p value = 0.85, ANCOVA adjusted for age, BMI, systolic blood pressure, triglycerides, HDL-cholesterol

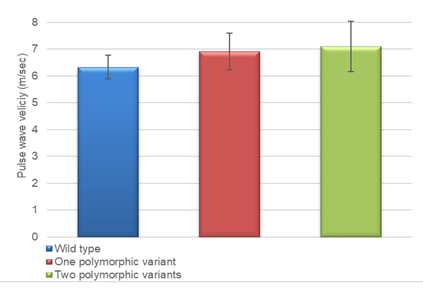

Fig. 2 Values of pulse-wave velocity differed significantly according to the presence of the assessed cardiovascular polymorphic variants. The results were adjusted for the presence of the traditional cardiovascular risk factors. PWV, wild type vs one polymorphic variant (CYBA and/or NOS3) vs two polymorphic variants (CYBA and NOS3): R2 = 15.9, 6.33 ± 0.44 vs 6.91 ± 0.69 vs 7.11 ± 0.94 m/s, p value = 0.037 adjusted for age, pulse pressure, body mass index, smoking, triglycerides, HDL-cholesterol, and HOMA-IR). Statistical significance was set at the level of p value < 0.05

Table 3. Multivariable regression analysis evaluating the link between the CYBA genetic polymorphism and indices of vascular structure and function

	b-coefficient	95% CI	p-value
Combined – IMT (mm)			
NADPH oxidase hetero	-0.007	-0.278 to 0.167	0.763
NADPH oxidase homo	-0.061	-0.201 to 0.013	0.015*
CCA-IMT (mm)			
NADPH oxidase hetero	0.015	-0.389 to 0.178	0.400
NADPH oxidase homo	-0.021	-0.278 to 0.189	0.257
CB-IMT (mm)			
NADPH oxidase hetero	-0.036	-0.296 to 0.018	0.342
NADPH oxidase homo	-0.034	-0.190 to 0.258	0.366
ICA-IMT (mm)			
NADPH oxidase hetero	-0.004	-0.078 to 0.138	0.920
NADPH oxidase homo	-0.119	-0.378 to 0.134	0.011*
FA-IMT (mm)			
NADPH oxidase hetero	-0.026	-0.311 to 0.089	0.506
NADPH oxidase homo	-0.049	-0.233 to 0.139	0.223
FMD (%)			
NADPH oxidase hetero	-1.604	-3.751 to 0.486	0.034*
NADPH oxidase homo	0.072	-1.543 to 2.493	0.639
PWV (m/s)			
NADPH oxidase hetero	0.403	-0.436 to 0.620	0.061
NADPH oxidase homo	-0.156	-0.794 to 0.281	0.342
AIx (%)			
NADPH oxidase hetero	0.445	-5.284 to 6.450	0.792
NADPH oxidase homo	-0.159	-8.691 to 2.304	0.249

Multivariable regression analysis including vascular function and structure indices as dependent variable and the CYBA genetic polymorphism as independent variables. The models were adjusted for traditional cardiovascular risk factors (e.g. age, body mass index, systolic blood pressure, diastolic blood pressure, smoking, triglycerides, high density lipoprotein cholesterol, C-reactive protein).
Reference group: wild type polymorphism
* indicates statistically significant results, i.e. p-value<0.05

Conclusions

In healthy premenopausal women, the CYBA C242T polymorphism is an independent determinant of endothelial function and subclinical atherosclerosis of the carotid arteries. The NOS3 G894T polymorphic variant also correlated with atherosclerosis, an association probably mediated by the traditional risk factors for CVD. The relevance of these findings in the clinical setting remains to be elucidated.

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