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The association of *PLA2G2A* single nucleotide polymorphisms with type IIa secretory phospholipase A2 level but not its activity in patients with stable coronary heart disease

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ABSTRACT

Background: Single nucleotide polymorphisms (SNPs) of the secretory phospholipase A2 type IIa (sPLA-IIa) gene (*PLA2G2A*) affect sPLA2-IIa level and activity in patients with diabetes mellitus, acute coronary syndrome or recent cardiovascular surgical interventions. Our study examined the effects of *PLA2G2A* SNPs on sPLA2-IIa levels and activity in patients with stable CHD.
Methods and results: The study included a total of 396 patients (30% women). Six SNPs of *PLA2G2A*: rs1774131, rs11573156, rs3753827, rs2236771, rs876018, and rs3767221, sPLA2-IIa level and activity were determined for all patients. Four SNPs (rs1774131, rs11573156, rs3753827, rs3767221) correlated with sPLA2-IIa level but not activity with the strongest correlation observed for rs11573156 ($r = 0.49$, $p = 3.7 \cdot 10^{-13}$). All partial correlations controlling for rs11573156 became insignificant, whereas, the partial correlation of rs11573156 with sPLA2-IIa level controlling for other SNPs remained significant. Only rs11573156 showed association with sPLA2-IIa level in multiple regression analysis. Haplotype CGGGTT was associated with a significantly higher sPLA2-IIa level but not activity compared with all other haplotypes after adjustment for gender, age, diabetes mellitus and statin use ($p = 0.0023$).
Conclusions: According to our results the examined SNPs affect the sPLA2-IIa level to a greater extent than its activity in patients with stable CHD. It seems that, the impact of these SNPs on sPLA2-IIa level is caused by their linkage to rs11573156 whose minor alleles were associated with higher sPLA2-IIa level. At the same time haplotype CGGGTT, which includes the minor allele of rs11573156, was the dominant haplotype and was associated with the highest sPLA2-IIa level.

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1. Introduction

Secretory phospholipase A2 type IIa (sPLA2-IIa) is an enzyme capable of hydrolyzing bonds of phospholipid cell membranes, generating lysophospholipids and free fatty acids. sPLA2-IIa takes part in the formation of eicosanoids, thus it has a significant role in the process of inflammation and thrombosis (Dennis, 1994). Also, the presence of sPLA2-IIa in the atherosclerotic plaques suggests its participation in atherogenesis (Elinder et al., 1997; Hurt-Camejo et al., 1997).

In large clinical trials sPLA2-IIa was identified as a marker of coronary heart disease (CHD) (Lind et al., 2012), the increase in its level and activity was associated with an increase in the risk of CHD in healthy individuals (Boekholdt et al., 2005; Mallat et al., 2007) and an increase in the risk of major adverse cardiovascular events in patients with unstable angina pectoris (Kugiyama et al., 2000), severe acute coronary syndrome (ACS) (Mallat et al., 2005; Simon et al., 2008), undergoing percutaneous coronary intervention (Liu et al., 2003) and coronary artery bypass grafting (Koenig et al., 2009).

In other clinical trials, it was shown that single nucleotide polymorphisms (SNPs) of secretory phospholipase A2 type IIa gene (*PLA2G2A*) significantly affect sPLA2-IIa level and activity (Mallat et al., 2010). However, it is worth noting that these studies included subjects with the following factors that may influence the level and activity of sPLA2-

Abbreviations: A, adenine; ACS, acute coronary syndrome; C, cytosine; CHD, coronary heart disease; DM, diabetes mellitus; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; G, guanine; *PLA2G2A*, secretory phospholipase A2 type IIa gene; RT-PCR, Real-Time polymerase chain reaction; SNP, Single nucleotide polymorphism; sPLA-IIa, secretory phospholipase A2 type IIa; T, thymine.

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Table 1
The frequency of the studied *PLA2G2A* gene SNP genotypes and alleles.

	rs1774131	rs11573156	rs3753827	rs2236771	rs876018	rs3767221
Homozygotes for the major allele	0.37	0.53	0.39	0.77	0.73	0.44
Heterozygotes	0.46	0.41	0.46	0.17	0.24	0.42
Homozygotes for the minor allele	0.18	0.06	0.16	0.07	0.04	0.14
Major allele	0.59	0.73	0.61	0.85	0.85	0.65
Minor allele	0.41	0.27	0.39	0.15	0.15	0.35
Deviation from H–W equilibrium (p_{HW})	0.46	0.58	0.65	**0.0001**	0.27	0.34

IIa: type 2 diabetes mellitus (DM) (Wootton et al., 2006), ACS or recent cardiovascular surgical interventions (Breitling et al., 2011; Simon et al., 2009). Thus, in our study we tried to avoid these limitations and studied the influence of the same previously identified SNPs affecting the level and activity of sPLA2-IIa in patients with stable CHD.

2. Materials and methods

2.1. Population

The study included male and female patients with stable CHD documented by the results of exercise tests. Patients who had suffered ACS or undergone surgical intervention less than 6 months before the beginning of the study, patients with inflammatory diseases (in severe chronic or acute state at the time of inclusion), patients with oncological diseases and patients with familial hypercholesterolemia were excluded from the study. All patients signed written informed consent for genetic research.

2.2. Genetic methods

Venous blood was collected with EDTA as an anticoagulant and stored at 70 °C until analysis. Genomic DNA was isolated from whole blood using the “DNA-EXTRAN-1” reagent kit (Synthol, Russia). DNA concentration was measured using a nanophotometer IMPLEN (GmbH, Germany), and adjusted to a concentration of 10 ng/μl. Determination of *PLA2G2A* SNPs was performed by Real-Time polymerase chain reaction (RT-PCR) using a 7500 Fast Real-Time PCR System amplifier (Applied Biosystems, USA). SNPs of *PLA2G2A* (dbSNP-polymorphism repository: http://www.ncbi.nlm.nih.gov/SNP/): rs1774131, rs11573156, rs3753827, rs2236771, rs876018 and rs3767221 were determined. Primers and probes were synthesized by a company Synthol, Russia. The primer and probe sequences used for amplification are shown in online Supplemental materials.

In addition to primers (10 pmol) and probes (5 pmol) the reaction mixture contained 200 μM of each dNTP; Taq-polymerase 2.5 U; 10-fold buffer of Taq-polymerase 2.5 ml; DNA solution (10 ng/μl) 5 μl and 18.2 mQ water to 25 μl. The temperature profile of the reaction included denaturation (95 °C 20 s), 50 cycles of amplification (95 °C 15 s, then 55 °C 30 s), primer annealing, and elongation (30 s; 59 °C for rs1774131, rs3753827, rs3767221; 57 °C for rs11573156, rs876018; 55 °C for rs2236771). In the case of major allele presence only (homozygous genotype for the major allele), growth curves of amplification were observed only in the VIC channel. In the case of minor allele presence only (homozygous genotype for the minor allele), growth curves of amplification were observed only in the ROX channel. Visualization of the amplification curves in both channels VIC/ROX indicated the existence of both major and minor alleles and their corresponding heterozygous genotype. In order to ensure quality control, 10% of the samples were blind duplicates by RT-PCR method for every SNP. Cohen's kappa coefficients of agreement were computed to evaluate the inter-methodology variability in genotyping. Kappa coefficient for different SNPs ranged from 0.93 to 1.

2.3. Biochemical methods

Plasma level of sPLA2-IIa was determined with the use of monoclonal antibodies — $sPLA_2$-IIa human synovial enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, MI, USA). This antibody is specific for sPLA2-IIa and does not cross react with types I, IV, V, VI and X sPLA2. The limit of detection was 15.6 pg/ml. The results obtained are presented in ng/ml.

sPLA2-IIa activity was determined with fluorescence-labeled phosphatidyl choline in a VICTOR™ *X*3 Multilabel PlateReader (Perkin Elmer, Finland). The reader settings corresponded to those for fluorescence (excitation — 485 nm, emission — 535 nm). Measurements were carried out in a 96-well plate. The final volume in a well was 200 μl. The reaction mixture contained 20 μl serum, 0.9 μM fluorescence-labeled substrate,

	rs11573156	rs3753827	rs2236771	rs876018	rs3767221
rs1774131	0.12248 0.3185 <2e-16	-0.09354 -0.1553 <2e-16	-0.00966 -0.0034 0.100929	-0.04227 -0.0658 8.41e-13	-0.05062 -0.0466 2.10e-09
rs11573156		-0.09763 -0.1996 <2e-16	-0.02447 -0.0253 8.27e-06	-0.03007 -0.0392 2.56e-08	-0.08082 -0.1401 <2e-16
rs3753827			0.00751 0.0019 0.210643	0.05986 0.1301 <2e-16	-0.02507 -0.0113 0.002963
rs2236771				-0.01582 -0.0183 0.000147	0.07266 0.1909 <2e-16
rs876018	D r² p-value				-0.04729 -0.0832 7.77e-16

Fig. 1. Linkage disequilibrium within the *PLA2G2A*. D — the deviation between the expected haplotype frequency (under the assumption of no association) and the observed frequency; r^2 — Pearson's correlation coefficient between alleles.

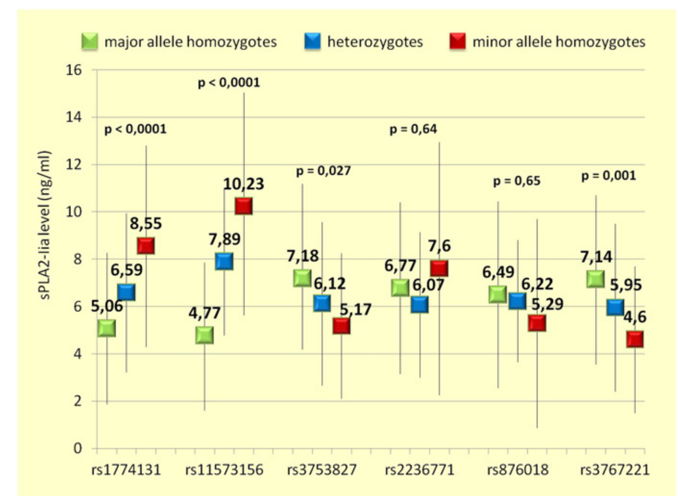

Fig. 2. SNP genotypes of *PLA2G2A* and sPLA2-IIa level adjusted for gender, age, type 2 DM and statin use. Multiple linear regression. Data are presented as Mean ± SD.

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