Association between single nucleotide polymorphism rs342286 near the *PIK3CG* gene and acute coronary syndromes

Introduction Acute coronary syndromes (ACS) are a major cause of morbidity and mortality in the western world.[1-3]

Phosphoinositide-3-kinase is an important modulator of extracellular signals; it plays an important role in the maintenance of the structural and functional integrity of the epithelia.[4] The *PIK3CG* gene is located in a commonly deleted segment of chromosome 7. This gene encodes an enzyme that phosphorylates phosphoinositides on the 3-hydroxyl group of the inositol ring, a protein that belongs to the pi3/pi4-kinase family of proteins. PI3Kγ is expressed in cardiomyocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells. It modulates cell survival, hypertrophy, contractility, metabolism, and mechanotransduction. The PI3Kγ signaling pathway is involved in a wide variety of diseases including myocardial hypertrophy and contractility, heart failure, and preconditioning.[5]

Johnson et al.[6] indicated the association between single nucleotide polymorphism (SNP) rs342286 and increased platelet aggregation. rs342286 is located 140kb upstream of the *PIK3CG* gene and affects its expression most likely through cis- or trans-regulatory elements. The aim of this study was to examine the association between the rs342286 polymorphism and ACS.

Patients and methods A total of 203 patients with first acute coronary event defined as acute myocardial infarction (AMI, 115 patients) or unstable angina (88 patients) confirmed by coronary angiography (defined as >70% stenosis in at least 1 major coronary artery) and 204 healthy controls were recruited into the study. Patients and controls were matched for age, sex, and cardiovascular risk factors. The diagnosis of unstable angina included the presence of typical angina at rest associated with acute or transient ST-segment or T-wave changes without an increase in cardiac ischemia markers. AMI was diagnosed based on the occurrence of chest pain, increased myocardial enzymes (total and creatine kinase-MB and troponin twice the upper normal limit), and electrographic changes. Patients were analyzed during the acute coronary episode. Controls were subjects with negative findings on coronary angiography, free of cardiovascular disease, cancer, or inflammatory diseases.

All genetic analyses were performed in the Department of Genetics, Faculty of Biology, University of Szczecin, Poland. DNA was extracted from blood samples using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, United States) according to the manufacturer's protocol. All samples were genotyped using an allelic discrimination assay on a Rotor-Gene real-time polymerase chain reaction instrument (Corbett, Australia). For the discrimination of the SNP rs342286 G and A alleles, the TaqMan® Pre-Designed SNP Genotyping Assay was used (Applied Biosystems, United States). Genotypes were assigned using all of the genotyping data from the study simultaneously.

The Fisher exact test was used to compare genotype and allele frequencies between the study groups. Age at ACS onset was compared between the genotype groups with the Mann–Whitney test. A P value of less than 0.05 was considered statistically significant.

Results The distribution of *PIK3CG* genotypes and alleles in patients with ACS, AMI, and controls is shown in the TABLE. There were no statistically significant differences in the distribution of these genotypes and alleles between patients and controls. Nevertheless, the age at ACS onset in patients with the GG genotype was lower compared with patients with the AA genotype (59.93 ±11.78 years vs. 64.76 ±11.53 years, P = 0.017). A similar association was observed for AMI. In patients with the GG genotype, AMI occurred at a younger age compared with those with the AA genotype (58.78 ±10.57 years vs. 64.38 ±10.32 years, respectively; P = 0.014).

Discussion According to our knowledge, this is the first study examining the association between

TABLE Distribution of single nucleotide polymorphism rs342286 genotypes and alleles in patients with acute coronary syndromes, acute myocardial infarction, and in the control group

		ACS patients, n = 203	AMI patients, n = 115	control group, n = 204
rs342286 genotypes, n (%)				
AA		53 (16.1)	32 (27.8)	65 (31.9)
AG		106 (52.2)	60 (52.2)	105 (51.5)
GG		44 (21.7)	23 (20.0)	34 (16.6)
AA + AG vs. GG	OR (95% CI)	0.72 (0.43–1.18)	0.67 (0.37–1.22)	–
	P value	0.27	0.21	1.00
AA vs. AG + GG	OR (95% CI)	1.32 (0.86–2.03)	1.21 (0.73–2.00)	–
	P value	0.23	0.52	1.00
AA vs. GG	OR (95% CI)	1.58 (0.89–2.82)	1.37 (0.36–1.43)	–
	P value	0.14	0.39	1.00
rs342286 allele frequency, n (%)				
A		212 (52.2)	124 (53.9)	235 (57.6)
G		194 (47.8)	106 (46.1)	173 (42.4)
A vs. G	OR (95% CI)	1.24 (0.94–1.63)	1.16 (0.83–1.60)	–
	P value	0.14	0.40	1.0

Abbreviations: ACS – acute coronary syndromes, AMI – acute myocardial infarction, CI – confidence interval, OR – odds ratio

the rs342286 polymorphism located upstream of the *PIK3CG* gene and ACS. So far, the polymorphisms in or near the *PIK3CG* gene have not been widely investigated. Soranzo et al.[7] examined the association between another SNP located upstream of the *PIK3CG* gene, rs342293, and mean platelet volume (MPV) and reactivity. The G allele at rs342293 was associated with increased MPV, and homozygotes for the G allele were correlated with higher MPV levels as well as with decreased platelet reactivity measured as the proportion of activated platelet binding annexin V and the level of fibrinogen binding compared to GC and CC genotypes. Paul et al.[8] identified an open chromatin region at chromosome 7q22.3 in megakaryocytes, which harbors the same sequence variant rs342293 known to be associated with platelet volume and function. They demonstrated that the C and G alleles differentially bind the transcription factor EVI1 affecting *PIK3CG* gene expression in platelets and macrophages. The protein–protein interaction network including up- and down-regulated genes in *PIK3CG* knockout mice indicated that *PIK3CG* is associated with gene pathways with an established role in platelet membrane biogenesis and thrombus formation. In the GWAS study, Johnson et al.[6] showed that the rs342286 polymorphism near the *PIK3CG* gene is associated with epinephrine-induced platelet aggregation. Since the rs342286 and rs342293 polymorphic sites are in high linkage disequlibrium (r^2 = 0.87, Phase II HapMap CEU) and common haplotypes are rs342286A : rs432293C (51.9%) and rs342286G : rs342293G (45%), while the recombinant haplotype rs342286A : rs342293G is rare (3.1%), it may be assumed that individuals with the GG genotype for rs342293 are GG for rs342286. Based on the above data, subjects homozygous for the G allele at rs342286 would presumably be characterized by increased MPV.

The reports regarding the association between platelet volume and reactivity are controversial; however, the majority of the studies have shown increased MPV to be a strong, independent predictor of postevent outcome in coronary disease and myocardial infarction.

The association between the rs342286 polymorphism near the *PIK3CG* gene and ACS may be caused by other factors than increased thrombogenicity. PI3Kγ plays an important role in myocardial metabolism and mechanotransduction.[9] Enhanced glucose uptake following PIK3CG activation as a consequence of increased myocardial contraction may increase cardiac glycogen synthesis. Activation of PI3Kγ is sufficient to increase myocardial fatty acid oxidation, a characteristic response seen in physiological hypertrophy.

Activation of PI3Kγ in the heart during chronic exercise training is important for maintaining cardiac structure and function in the setting of pathological hypertrophy and may underlie the beneficial effects of exercise in patients with heart failure.[10] PI3Kγ activation has been linked with left ventricular enlargement and decompensation in pressure-overload-induced heart disease, suggesting that impaired PI3Kγ signaling may have therapeutic benefits in experimental models of heart failure. The above studies suggest that the influence of PI3Kγ on ACS is complex and may be associated with platelet function and thrombogenicity as well as myocardial metabolism.

The present study suggests the association between the rs342286 polymorphism near the *PIK3CG* gene and younger age at ACS onset; however, this issue requires further research.

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