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Polymorphism C1114G of Gene Encoding the Cardiac Regulator of G-Protein Signaling 2 May Be Associated with Number of Episodes of Neurally Mediated Syncope

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Background and Aims. The cardiac regulator of G-protein signaling 2 (RGS2) negatively regulates G-protein-coupled receptor signaling. The C1114G polymorphism reduces RGS2 gene expression. This molecular disorder may be one of the important factors influencing progress of neurally mediated syncope. The aim of the study was to evaluate the association between C1114G RGS2 polymorphism and tilting results and number of syncope episodes in patients with no other diseases.

Methods. Of 214 tilted patients (39% males, 39.7 ± 17.1 years of age), genomic DNA was extracted from cellular blood components. C1114G RGS2 polymorphism was diagnosed by designed primers. Clinical variables and genetic traits were introduced into multivariate stepwise regression. Analysis was performed as follows: positive tilting $n = 145$ vs. negative $n = 69$, positive passive $n = 49$ vs. nitroglycerin (NTG)-positive $n = 96$, dominant vasodepressive $n = 111$ vs. cardioinhibition $n = 34$; and in number of syncope groups with cut-off ≥ 10 vs. < 10.

Results. No relationship was found between the studied polymorphism and outcome of tilting ($p > 0.05$). In multivariate regression model, homozygosity G/G 1114 RGS2 was the only variable associated with a reduced number of episodes of syncope (95% CI 2.3–10.9; $p = 0.04$).

Conclusions. Our preliminary results suggest the association of G/G 1114 RGS2 genotype with the number of episodes of neurally mediated syncope. Detailed molecular mechanism of the influence of the studied polymorphism on syncopal number is probably associated with the reduced expression of RGS2 gene.

Key Words: Syncope, Tilting, RGS2, Gene polymorphism.

Introduction

Neurally mediated syncope is a frequent symptom and has wide clinical manifestations ranging from one or few episodes to numerous incidents. Recently, a positive vasovagal family history (1–4) and an association between certain single nucleotide polymorphisms (SNP) and neurally mediated syncope (5–7) have been described. For normal cardiovascular function, the heterotrimeric G-protein system is critical (8–10). The cardiac regulator of G-protein signaling 2 (RGS2) stimulates GTPase activity of Gα subunits of G proteins, thereby negatively regulating G protein-coupled receptor signaling (GPCRS) (11). RGS2 plays an important role also with direct interaction with cardiac adenylyl cyclase (12). In the cardiovascular system, activated β1-adrenergic receptors, which belong to the GPCRS family, stimulate the production of cyclic adenosine monophosphate (cAMP). A potential molecular pathway underlying vasovagal syncope susceptibility may be associated with the negative regulation of GPCRS. The

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C1114G polymorphism of the gene for RGS2 (GeneID: 5997) has been shown to be associated with cardiovascular regulation (13). This polymorphism reduces RGS2 gene expression (13). This phenomenon may in turn result in enhanced adenylyl cyclase activity and protein kinase A (PKA) activity. Activated PKA phosphorylates many proteins that results in, among other effects, an increased cAMP-mediated cardiac contractility and increased heart rate. It seems to be responsible for excessively enhanced inotropism, which has been described in the early phase of the upright posture as a susceptibility marker of vasovagal syncope (14). Thus, we hypothesized that the C1114G RGS2 gene polymorphism may underlie, via the decreased negative regulation of G-protein signaling, the predisposition to neurally mediated syncope or the number of syncopal incidents. Based upon this background, we investigated the importance of polymorphic variants 1114G of the candidate gene approaching RGS2 and tilting results or number of syncopal episodes among syncopal patients.

Patients and Methods

Two hundred fourteen syncopal subjects (mean age 39.7 ± 17.1 years, 39% males) with no history or symptoms of cardiovascular disease were enrolled after informed consent for the entire procedure was obtained. All subjects were Caucasian and inclusion criterion was a history of >3 episodes of syncope during the subjects' entire life. Exclusion criteria were 1) positive family history for sudden cardiac death; 2) history suggesting syncope in mechanism of carotid sinus hypersensitivity, catecholaminergic polymorphic ventricular tachycardia, long QT syndrome or short coupling variant of torsade de pointes; 3) ECG abnormalities and/or abnormal exercise testing; and 4) abnormal echocardiogram.

All patients were tilted according to the Italian protocol (15). The head-up tilt test (HUT) was performed in the morning and used the mechanical tilt table SP-1 with a foot support and straps. Before tilting, patients were in a supine position. The tilt table achieved the upright position with a 60° angle. If syncope did not occur during 20 min of passive tilting, it was followed by a 20-min pharmacological phase after the aerosol administration of sublingual nitroglycerin (NTG, 400 μg). Positive tilting was recognized when syncope occurred accompanied by marked reduction of blood pressure (systemic hypotension <80 mm Hg) and/or heart rate during passive titling (16) or in the first 5 min after NTG administration (15). The type of positive response to tilting was defined according to the Vasovagal Syncope International Study (VASIS) classification (16): VASIS1—mixed (blood pressure falls before the heart rate falls not less than 40 beat/min or <40 beats/min for <10 sec with or without asystole of <3 sec); VASIS 2A—cardioinhibition without asystole (significant bradycardia with heart rate fall to <40 beats/min for >10 sec but asystole of >3 sec does not occur, blood pressure falls before the heart rate fall); VASIS 2B—cardioinhibition with asystole (asystole occurs for >3 sec, blood pressure falls coincidently with or occurs before the heart rate falls); and VASIS 3—vasodepressor with hypotension without bradycardia (heart rate does not fall for >10% from its peak at the time of syncope).

VASIS2 groups A and B were analyzed together as VASIS2 with regard to the low number of patients.

After genotyping, patients were analyzed as regards to results of tilting: positive vs. negative, positive passive vs. NTG-positive, dominant vasodepressive (VASIS1 + VASIS3) vs. cardioinhibition and related to the number of syncope with cut-off ≥10 vs. <10. The criterion of syncopal number resulted from the median of incidents in the syncopal population.

The control group consisted of 40 subjects (mean age 37.7 ± 11.67 years, 56% males) without history of syncope and other disorders.

Study procedures were approved by the Local Bioethics Committee.

Genotyping

Genomic DNA was extracted from cellular blood components using an extraction kit (Chemagic DNA Blood 100 Kit, Chemagic AG, Baesweiler, Germany). Polymorphisms were detected by standard techniques such as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods with primer pairs designed in the Department of Medical Biotechnology, University of Lodz, Lodz, Poland. In the studied RGS2 gene polymorphism, two designed primers were specific for each allele, whereas the third one (antisense) is common for both variants. Oligonucleotide primer sequences were as follows: for C allele: 5′-AGTGAAGTGTTTACTATGTGCTAC-3′; for G allele: 5′-AGTGAAGTGTTTACTATGTGCTAG-3′; and common primer (antisense): 5′-TCAACACCATAGCAC TCATTCTAT-3′.

Statistical Analyses

Categorical variables were described as numbers and percentage. Association between analyzed parameters were examined using χ^2 Pearson test, Yates corrected χ^2 test for 2 × 2 contingency tables, and Fisher exact test for larger than 2 × 2 contingency tables. Non-normally distributed continuous variables were analyzed with the Mann-Whitney U test and presented as mean value ± standard deviation (SD) or 95% confidence intervals (95% CI). For post-hoc comparisons, Tukey test or Mann-Whitney U test with Bonferroni correction was used.

To test the impact of body mass index (BMI), age, and systolic and diastolic blood pressure on analyzed parameters, one-way analysis of variance (ANOVA) was performed. To identify the factors influencing tilting outcome, univariate