Genetic Variation in Gsα Protein as a New Indicator in Screening Test for Vasovagal Syncope

Malgorzata Lelonek, MD, PhD; Monika Zelazowska, BSc; Tadeusz Pietrucha, MD, PhD

**Background:** A quantitative history using Calgary syncope syndrome score (CSSS) is able to define the likely cause of syncope, but there is still a lack of diagnostic screening tests for vasovagal syncope (VVS). The aim of the present study was to develop a screening test for VVS on the basis of CSSS and the relationship between polymorphic variants of the G-system signaling protein genes and tilting results.

**Methods and Results:** From 730 syncopal patients, 307 consecutive subjects without structural and electrical abnormalities were genotyped and examined on blood pressure (BP) and tilt testing. Genotyping was performed using polymerase chain reaction—restriction fragment length polymorphism in genes encoding Gsα-protein GNAS1 (rs7121), G-protein β3 subunit (rs5443) and the cardiac regulator of G-protein signaling RGS2 (rs4606). The control group consisted of 100 healthy volunteers with a negative history of syncope. From multivariate regression analysis, being a carrier of 393T GNAS1 (odds ratio [OR], 2.29) and systolic BP (OR, 0.98) remained as independent factors associated with positive tilt results. The resultant screening test for VVS consisted of the following: carrier of 393T GNAS1; systolic BP <131 mmHg (from the receiver operating characteristic [ROC] curve); and CSSS ≥–2. Using ROC curve analysis for systolic BP and CSSS, 2 final models for the screening test were constructed: highest sensitivity (89%) and highest specificity (99%).

**Conclusions:** The novel screening test including the variation of Gsα protein gene seems to be helpful to identify tilt-induced vasovagal patients.

**Key Words:** Gsα protein gene polymorphism; Screening test; Tilting; Vasovagal syncope

Vasovagal syncope (VVS) is common in the general population and has a prevalence of 35–39%. A careful history is able to define the likely cause of syncope, but there is still a lack of diagnostic screening tests for VVS. Hereditary aspects of VVS are described in detail. The frequency of a positive family history of VVS ranges from 19% to 90%. Moreover, the genetic studies suggest a relationship between certain single nucleotide polymorphisms (SNPs) and VVS. For cardiovascular reflex control, α/β-adrenergic receptors and intracellular G-signaling pathways are critical. Recently we hypothesized that the predisposition to VVS could be associated with genetic variation in the proteins of G-system signaling, which have great importance in the regulation of arterial pressure or its disturbances. Based on this, the aim of the present study was to develop a screening diagnostic score for VVS in syncopal patients with structural and electrical normal hearts, on the basis of a history point score (Calgary syncope syndrome score, CSSS) and a relationship between polymorphic variants of the proteins involved in the intracellular G-system signaling and tilting results.

**Methods**

From 730 syncopal patients 307 consecutive subjects with no other history or symptoms of cardiovascular diseases were enrolled in the study after informed consent for the entire procedure. The inclusion criterion was ≥3 incidents of syncope in the last 2 years. All subjects were white Caucasian. According to CSSS, a quantitative history point score described by Sheldon et al. a patient probably had VVS if CSSS was ≥–2 or the other cause of syncope if CSSS was <–2.

Head-up tilt test was performed in all patients under the Italian protocol (20 min passive tilting, tilt angle 60°), prolonged with the active phase using nitroglycerin (400 μg in spray). Positive tilting was confirmed when syncope occurred accompanied by marked reduction of blood pressure (BP) (systemic hypotension <80 mmHg) and/or heart rate. Positive response to tilting was defined according to the Vasovagal Syncope International Study (VASIS) classification: VASIS 1 (mixed), BP falls before the heart rate falls to ≥40 beats/min or <40 beats/min for <10 s with or without asystole of <3 s; VASIS 2A (cardioinhibition without asystole),

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Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>No. patients</th>
<th>Tilt positive</th>
<th>Tilt negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F, n (%)</td>
<td>69/138 (33/67)</td>
<td>36/64 (36/64)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.6±16.9</td>
<td>40.5±16.8</td>
</tr>
<tr>
<td>No. syncope episodes</td>
<td>51.3±21.6** (10)</td>
<td>18.8±49.6** (10)</td>
</tr>
<tr>
<td>History of syncope (years)</td>
<td>8.6±10.9 (4.5)</td>
<td>7±10.8 (3)</td>
</tr>
<tr>
<td>CSSS ≥–2</td>
<td>137 (66)</td>
<td>55 (55)</td>
</tr>
<tr>
<td>Systolic BP at rest (mmHg)</td>
<td>121.2±13.7*</td>
<td>125.6±17.5*</td>
</tr>
<tr>
<td>Diastolic BP at rest (mmHg)</td>
<td>79.1±9.6</td>
<td>79.7±10.9</td>
</tr>
<tr>
<td>Heart rhythm at rest (beats/min)</td>
<td>69.2±12.5</td>
<td>69.3±12.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8±3.97</td>
<td>23.24±4.42</td>
</tr>
<tr>
<td>Positive tilting, n (%)</td>
<td>207 (100)</td>
<td>–</td>
</tr>
<tr>
<td>Positive passive tilting, n (%)</td>
<td>61 (29.5)</td>
<td>–</td>
</tr>
<tr>
<td>VASIS 1, n (%)</td>
<td>122 (59)</td>
<td>–</td>
</tr>
<tr>
<td>VASIS 2, n (%)</td>
<td>40 (19)</td>
<td>–</td>
</tr>
<tr>
<td>VASIS 3, n (%)</td>
<td>45 (22)</td>
<td>–</td>
</tr>
</tbody>
</table>

Data given as n (%), mean ± SD and median. CSSS, Calgary syncope syndrome score; BP, blood pressure; BMI, body mass index; VASIS, Vasovagal Syncope International Study. *P<0.05, **P<0.001.

significant bradycardia with heart rate fall to <40 beats/min for >10 s but asystole of >3 s does not occur, BP falls before the heart rate fall; VASIS 2B (cardioinhibition with asystole), asystole occurs for >3 s, BP falls with or occurs before the heart rate fall; and VASIS 3 (vasodepressor), hypotension without bradycardia (heart rate falls ≤10% from its peak at the time of syncope). A total of 100 healthy age-, body mass index (BMI)- and sex-matched volunteers with a negative history of syncope constituted the control group. In all cases the BP was measured at rest using a random-zero sphygmomanometer with the participant seated and after a 5-min rest. Systolic and diastolic pressures were recorded as phase I and phase V Korotkoff sounds. The average of 3 measurements of BP was used. Moreover, information on participant age, gender, BMI, number and history of syncope (in years), and heart rhythm at rest was collected.

Genotyping

Genotyping of the studied polymorphisms was performed in all subjects. Genomic DNA was extracted from cellular blood components using an extraction kit (Chemagic DNA Blood 100). In the candidate gene approach the SNPs were detected using standard techniques such as polymerase chain reaction and restriction fragment length polymorphism with primer pairs previously described for C393T (rs7121)\(^15\) in genes encoding the α-subunit of Gs-protein (GNAS1; GeneID: 2778) and for C825T (rs5443)\(^20\) of the gene for the G-protein β3 subunit (GNB3; GeneID: 2784), and synthesized by Eurogentec. In the studied RGS2 gene (GeneID: 5997) for C1114G polymorphism (rs4606) the primer pairs were designed in the Department of Medical Biotechnology in Lodz as described in our previous study.\(^\text{21}\) Moreover, analysis of secondary mRNA using a dynamic algorithm for prediction of RNA secondary structure\(^\text{22,23}\) was also done (mfold version 3.2).

Patients were analyzed with regard to tilting results (positive vs. negative), VASIS response and genetic abnormality (polymorphic homozygotes, heterozygotes).

The exclusion criteria were: <3 syncope incidents in last 2 years; a positive family history for sudden cardiac death; history suggesting syncope caused by another mechanism than VVS (carotid sinus hypersensitivity, catecholaminergic polymorphic ventricular tachycardia, long QT syndrome or short coupling variant of torsade de pointes); electrocardiogram abnormalities and/or abnormal exercise testing; and abnormal echocardiogram.

The study procedures were approved by the Bioethics Committee at Medical University of Lodz.

Statistical Analysis

The continuous variables that did not show a normal distribution were analyzed using the Mann–Whitney U-test and expressed as mean±SD or 95% confidence interval (95% CI). The categorical variables were described as numbers and percentage. Association between analyzed parameters was examined using the chi-square Pearson test, Yates corrected chi-square test for 2×2 contingency tables, and Fisher’s exact test for larger than 2×2 contingency tables. To test the impact of systolic and diastolic BP at rest on analyzed parameters a 1-way analysis of variance was performed. To identify the factors influencing tilt outcome, univariate logistic regression followed by multivariate backward stepwise analysis was performed.

Genotype frequencies were estimated by gene counting. Agreement of the genotype frequencies with Hardy–Weinberg equilibrium was tested using a chi-square goodness-of-fit test. The results underwent Bonferroni correction. The present study was designed to detect a difference of test results between respective alleles (or genotypes) of at least 25% with a statistical power of 80%.

Into the multivariate model were introduced those genetic traits (homoygosity and allele carriage) and clinical variables that were associated with tilt outcome with P<0.15 on univariate analysis. The results of regression analysis are presented as odds ratios (OR) with 95% CI. Data were analyzed using Statistica version 7.0 (StatSoft, Tulsa, OK, USA), and Medcalc 8.1 (Medcalc, Mariekerke, Belgium). P<0.05 was considered statistically significant.
Results

Patient characteristics stratified according to tilt results are listed in Table 1. In the tilt positive-patients there were more incidents of syncope (P<0.001) and lower systolic BP at rest (P<0.05). On multivariate analysis of clinical variables only systolic BP at rest (OR, 0.98; 95%CI: 0.97–0.99; P<0.05) was associated with positive tilting and had a protective activity above 132.7 mmHg (from receiver operating characteristic [ROC] curve analysis; sensitivity, 92%; specificity, 34%; positive predictive value [PPV], 46%; negative predictive value [NPV], 87%; area under the curve [AUC], 0.61; 95%CI: 0.53–0.69; P=0.013). For vasodepresssive reactions (VASIS 1 + VASIS 3) we found a correlation with CSSS ≥2 (OR, 2.28; 95%CI: 1.03–4.98; P<0.05) but not for VASIS 2. In VASIS 2 only 52% of patients had CSSS ≥2. The present syncopal subjects had similar allele frequencies to the control subjects: in C393T GNAS1 polymorphism, the frequency of the C allele was 65% vs. 61%, and for the T allele it was 35% vs. 39% (P>0.05); in C825T GNB3 polymorphism, the frequency of the C allele was 65% vs. 61%, and for the T allele it was 54% vs. 46% (P>0.05); in C1114G RGS2 polymorphism, the C allele frequency was 78% vs. 81%, and that of the G allele was 22% vs. 19% (P>0.05). Genotype frequency distributions were in accordance with Hardy–Weinberg equilibrium expectations (P>0.05).

From the investigated polymorphisms, multivariate logistic regression showed that being a carrier of only 393T GNAS1 enhanced the risk of syncope during tilting (OR, 2.37; 95%CI: 1.03–4.98; P<0.05) and only the homozygous TT genotype of the GNAS1 C393T enhanced the chance of CSSS ≥2 (OR, 2.28; 95%CI: 1.03–4.98; P<0.05). The incidence of GNAS1 polymorphism with regard to tilting results is given in Table 2. We did not find an association between the variation of 393 GNAS1 and type of VASIS response. From analysis of secondary mRNA the 393T allele in GNAS1 creates a new structure resulting in lower mRNA free energy (En C393, –27.8; En T393, –29.1; P<0.05).

On multivariate regression analysis of genetic and clinical variables (Table 3), being a carrier of 393T GNAS1 (P=0.004), and systolic arterial BP (P=0.03) remained as independent factors associated with positive tilt results in syncopal patients. No sex-based differences were present.

Based on these results we developed a diagnostic screening test for VVS that contained the following variables: being a carrier of 393T GNAS1; systolic arterial BP <131 mmHg (from ROC curve analysis); and CSSS ≥2 (for the screening test: sensitivity, 63%; specificity, 70%; PPV, 77%; NPV, 40%; AUC, 0.60; 95%CI: 0.53–0.65).

From the ROC curve analyses for systolic arterial BP and CSSS we constructed 2 final models of the diagnostic screening test for VVS (Table 4) with the highest sensitivity (model I: carrier of 393T GNAS1; systolic arterial BP ≤141 mmHg and CSSS ≥4), and with the highest specificity (model II: carrier of 393T GNAS1; systolic arterial BP >106 mmHg and CSSS ≥3).

Discussion

The main finding of this study is the novel view of VVS, including the genetic variant of the Gs-protein signaling system genes. From studying candidate genes of sympathetic signal transduction only for the α-subunit of Gs (s-stimulated) protein (which produces coupling of the β-adrenergic receptors and stimulation of cyclic adenosine monophosphate production), we found an association between the SNP C393T GNAS1 and tilting outcome. Individuals with at least 1 polymorphic allele, 393T GNAS1, had 2-fold the risk of syncope during tilting (OR, 2.297; 95%CI: 1.29–4.06) compared to those without this allele. The SNP C393T GNAS1 results in an enhanced activation of adenylyl cyclase, which leads to enhanced chronotropism and inotropism. This phenomenon is typical for the early phase of upright posture as an indicator of susceptibility to VVS. The hypothetical molecular mechanism of VVS related to 393T GNAS1 SNP we have described in detail recently. It is concluded that the presence of the 393T allele located in an untranslated region of the mRNA may be associated with greater mRNA stability, which in turn results in more efficient protein translation. Hence, the higher frequency of the GNAS1 393T allele among the patients with positive tilt results may be related to an accelerated adenylyl cyclase production, which was recently described. The main limitation of the present study, however, was the lack of laboratory examination of the activity of adenylyl cyclase.

A structured CSSS questionnaire reported by Sheldon et al, although having high sensitivity and specificity for VVS, seems to be not sufficient for cardioinhibition response to tilting (VASIS 2). We found a correlation with CSSS ≥2 (OR,
2.28; P<0.05) only for vasodepressory reactions (VASIS 1 + VASIS 3), indicating that the clinical importance of CSSS in the present total syncopal population was not so high: in the positive tilt group only 66% of patients had CSSS ≥2, and only 74% in positive passive tilting. So far, however, CSSS has been analyzed related to positive vs. negative response to tilting without VASIS analysis. But in the present study CSSS ≥2 did not differentiate the response to tilting. Interestingly, the systolic BP at rest was independently associated with syncrone during tilt test (OR, 0.98; 95%CI: 0.97–0.99). We found an inverse association: a rise of 1 point of systolic BP resulted in a decrease of the risk of positive tilting by 2%. We did not find any studies on the relationship between systolic BP at rest and tilt test results in the literature. The present results, therefore, suggest impairment of BP regulation at rest in VVS patients, but further studies are needed. Extension of diagnostic method to include hemodynamic measurements such as cephalic blood flow could be potentially useful in VVS patients.

We developed 2 models of this test: a model with the highest sensitivity, to identify all VVS patients within false-positive subjects; and a model with the highest specificity, to identify only VVS patients without false-positive subjects.

The detailed molecular mechanism of the influence of SNPs on VVS is still being investigated. Genetic alterations in the pathways involved in controlling vascular and heart reflex seem to affect susceptibility to VVS. We have previously hypothesized a mechanism of C825T GNB3 silent polymorphism and C1114G RGS2 in VVS. In comparison to CSSS ≥2, GNB3 C825T polymorphism had no discriminant value for prediction of tilt outcome. In contrast, we found an association of G/G C825T and C1114G RGS2 genotype with the number of VVS episodes and no relationship between SNPs in G-protein signaling pathways and severe clinical manifestation of syncope. Recently Sorrentino et al did not find any relationship between gene polymorphisms affecting sympathetic activity and tilt-induced syncope in a group of 129 Italian patients. That study group, however, was small and they analyzed a different location of G-protein signaling: mainly in the β-adrenergic receptor. In the present study the relationship between GNAS1 polymorphism and tilt-induced VVS has been demonstrated. This finding could be used to develop a new therapy target or to predict therapy responses in VVS.

The distribution of the analyzed polymorphisms in GNAS1 and RGS2 have been reported in Reference (SNP Report (www.ncbi.nlm.nih.gov/SNP?rs7211) and www.ncbi.nlm.nih.gov/SNP?rs4606), and that of C825T GNB3 polymorphism has been previously described by Siffert et al. To date, there are no reports on the distributions of the studied polymorphisms in Polish healthy individuals. To our knowledge, there is no fine mapping linkage analysis concerning RGS2 and GNB3 loci. The present study did not include linkage analysis of these genes. Linkage studies have shown that region 20q13.3 containing the GNAS1 locus was associated with pseudohypoparathyroidism. Genetic mutations that cause defective expression, however, are still undefined.

We have described a novel view of VVS, including the genetic variant of the intracellular Gαq protein of the signaling system gene, which facilitates a better understanding of the physiology underlying VVS. The present study is the first to report on a diagnostic screening test in vasovagal patients, which included such simple diagnostic criteria as quantitative history and systolic BP, in addition to genetic variation in sympathetic signal pathway genes. Other known and unknown factors, however, contribute to VVS. Results from molecular genetic association studies need to be interpreted with caution. Genomic-wide association studies analyzing thousands of SNPs might offer new opportunities in this regard.

**Conclusion**

The present novel screening test, including the genetic variation of the α subunit of the Gs protein gene signaling pathway, seems to be helpful in clinical practice to identify tilt-induced vasovagal patients.

**References**

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