Arg389Gly β1-adrenergic receptor polymorphism and susceptibility to syncope during tilt test

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Abstract

Introduction: Numerous hormones, neurotransmitters, and other stimuli exert their biological effect on cellular functioning through heptahelical receptors coupled to G proteins (GPCR – G protein-coupled receptors). Adrenergic receptors that belong to this superfamily of receptors are components of the sympathetic nervous system. They play a pivotal role in blood pressure regulation and myocardial contractility. Alterations of the adrenergic receptor pathway have been suggested to be involved in the pathophysiology of vasovagal syncope (VVS). The aim of the present study was to evaluate the distribution of Arg389Gly polymorphism within the *ADRB1* gene among patients with recurrent syncope.

Material and methods: Arg389Gly single nucleotide polymorphism was analyzed in 205 patients with recurrent syncope. Ninety-five patients (46%) had a positive head-up tilt test (HUT) result. The control group comprised 143 non-fainting subjects. Genotyping was performed by restriction fragment length polymorphism (RFLP) with *BstNI* enzyme.

Results: Both analyzed groups had similar distribution of the 389Gly allele. Sixty percent of polymorphic 389Gly carriers belong to the group of syncopal patients, while 40% belong to the control group of healthy subjects.

Conclusions: An association between syncopal incidence and Arg389Gly polymorphism within the *ADRB1* gene was not found. The analyzed polymorphism affecting sympathetic activity does not influence vasovagal syncope in Polish patients.

Key words: syncope, genetic polymorphism, Tilt test, adrenergic receptors.

Introduction

Syncope is considered as a common and potentially dangerous clinical problem [1]. Vasovagal syncope (VVS) is one the most frequently observed types of this ailment [2, 3]. The principal pathophysiological mechanism that underlies VVS is an abnormal, excessive reaction of cardiac mechanoreceptors that leads to hypotonia and/or bradycardia. Overstimulation of the autonomic nervous system that precedes syncope may be a result of alterations of cardiac adrenergic receptors' (AR) structure and/or function. The Arg389Gly polymorphism of the β1-AR gene (ADRB1) is a functionally important alteration, associated with lower stimulation of adenylyl cyclase activity [4]. Moreover, this polymorphism was previously linked to

a positive result of the head-up tilt test (HUT) [5] in the Mexican population. The results however are not unanimous, as Sorrentino *et al.* [6] have shown that the Arg389Gly genetic variant should not be considered as a risk factor for vasovagal syncope in the Italian population.

The objective of this report was to determine the distribution of Arg389Gly polymorphism in the β1-AR gene among Polish patients with recurrent syncope who were examined by HUT.

Material and methods

Study population

The study population consisted of 205 adult patients suffering from syncope and free of any other diseases diagnosed in the Department of Cardiology at the Medical University of Lodz. Exclusion criteria were as follows: ECG abnormalities, positive family history of sudden cardiac death, syncope due to cardiac disease. Patients were tilted according to the Italian protocol [7, 8]. The second group of analyzed subjects was composed of 143 healthy volunteers without any prior incidents of syncope. The clinical and demographic characteristics of study and control groups are presented in Table I. The study was approved by the Local Bioethics Committee and the patients' informed consent was obtained in all cases prior to any interventions related to the study protocol.

Tilt test protocol

The HUT according to the Italian protocol was performed using the tilt table SP-1 with a foot support and straps as described previously [7, 8]. Continuous ECG-curve registration was performed using a cardiomonitor and a 3-lead digital ECG recorder. Blood pressure, respiration rate and blood saturation were measured using a pulse oximeter throughout the HUT according to the protocol. The HUT consisted of a 20-min supine pre-tilt phase and a passive phase with the patient tilted upright to 60°. This passive phase of the Italian protocol was maintained for 20 min. If syncope did not occur during the passive phase, 400 µg of sublingual nitroglycerine in spray was administered in the upright position. The drug challenge phase was maintained for 20 min. Syncope of patients with positive HUT was classified on the basis of the modified Vasovagal Syncope International Study (VASIS) classification as:

a) VASIS 1 (mixed),
b) VASIS 2 A (cardioinhibition without asystole),
c) VASIS 2 B (cardioinhibition with asystole),
d) VASIS 3 (vasodepressive).

Similarly to earlier studies by the authors [9], due to the small number of patients in VASIS 2 A and B subgroups, both types were analyzed jointly. For statistical calculations, VASIS 1 and VASIS 3 were grouped together to be compared with VASIS 2.

Analysis of Arg389Gly polymorphism

Genomic DNA was isolated from peripheral blood drawn from the patients to EDTA-coated vials using the Chemagic DNA Blood100 kit (Chemagen, Baesweiler, Germany). The Arg389Gly polymorphism genotype (rs1801253) was detected using the restriction fragment length polymorphism (RFLP) method. The DNA fragment was amplified using site-specific oligonucleotide primers spanning the rs1801253 polymorphic site: P1: 5'-TGGGCTACGCCAACTCGG-3' and P2: 5'-GGCCCCGACGACATCGTC-3'. The polymerase chain reaction (PCR) was performed in the final volume of 25 µl containing 1 µM of $MgCl_2$, 0.2 mM of equimolar deoxynucleotide mix, 0.8 µM of each primer and 5% dimethyl sulfoxide. An initial denaturation step (94°C for 5 min) was followed by thirty cycles of: denaturation (94°C for 1 min), annealing (59°C for 1 min) and an extension phase (72°C for 1 min). Final extension was performed at 72°C for 8 min. Digestion of

Table I. General characteristics of study and control group

Parameter	Control	Fainting	
		Negative HUT	Positive HUT
Number of patients	143	110	95
Mean age [years]	26	41	42
Males, *n* (%)	77 (54)	37 (34)	37 (39)
BMI, mean ± SD [kg/m^2]	22.32 ±2.17	25.00 ±4.28	25.10 ±4.37
Systolic blood pressure at rest, mean ± SD [mm Hg]	–	132.8 ±18.2	126.8 ±14.7
Diastolic blood pressure at rest, mean ± SD [mm Hg]	–	83.5 ±12.1	81.0 ±10.4
Heart rate at rest, mean ± SD [bpm]	–	68.5 ±12.2	68.0 ±13.7
Saturation at rest, mean ± SD (%)	–	97.4 ±1.2	97.7 ±1.3

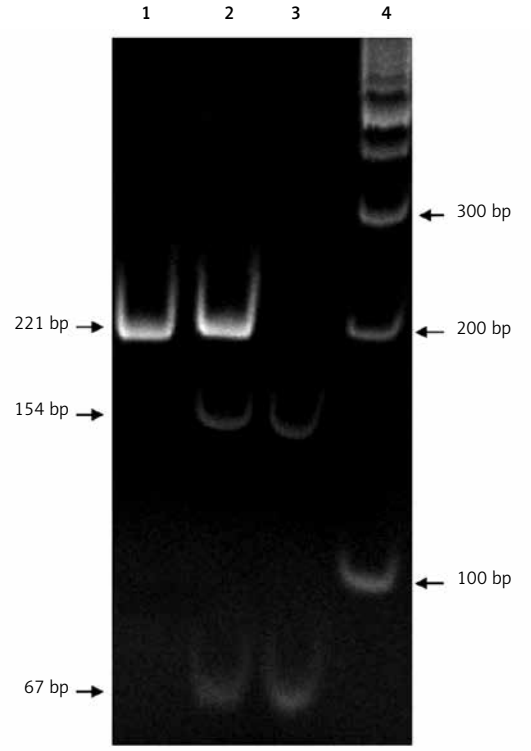

Figure 1. Genotyping results for Arg389Gly polymorphism of β1-adrenergic receptor gene. Shown is pattern of bands on ethidium bromide-stained gel electrophoresis after *BstNI* digestion of PCR product. Lane 1, homozygous Arg/Arg; Lane 2, heterozygous Arg/Gly; Lane 3, homozygous Gly/Gly; Lane 4, GeneRuler™ 100 bp DNA Ladder (Fermentas). The position and size of bands are indicated by arrows

the 221-bp PCR product with restriction enzyme *BstNI* (New England Biolabs, Ipswich, UK) yielded a 221-bp product for Arg/Arg genotype, three (221-, 154- and 67-bp) products for heterozygous Arg/Gly genotype and two (154- and 67-bp) products for Gly/Gly homozygotes. Digested fragments were scrutinized on 40% polyacrylamide gel and visualized under UV light after ethidium bromide staining (Figure 1).

Statistical analysis

As a measure of association odds ratios (ORs) with 95% confidence interval (95% CI) were used. The χ^2 tests were performed to test for deviations from the Hardy-Weinberg equilibrium. Categorical variables were compared using the Pearson's χ^2 test or Fisher's exact test, depending on the number of observations and variables. Statistica version 8.0 PL (StatSoft Inc., Tulsa, USA) was used for data analysis. Values of $p < 0.05$ were considered as statistically significant.

Results

Mean age of fainting patients in the study group was 41.5 ±15.7 year; 36% were males. Ninety-five patients (46%) had positive HUT: 21 during the passive phase and 65 during nitroglycerine administration. VASIS 1 response was observed in 63 patients, VASIS 2 response in 10 and VASIS 3 response in 19. Negative result of HUT was observed in a group of 110 patients (54%). The control group comprised 143 patients (54% males) (Table I); mean age equalled 28.7 ±12.00 years (22.0–30.0). The control group was significantly younger than the study group ($p < 0.0001$). Sex distribution differed significantly due to a greater percentage of males in the control group ($p = 0.003$).

The frequencies of Arg389Gly genotypes in the control group did not show significant deviations from the Hardy-Weinberg equilibrium ($p = 0.13$). The distribution of all genotypes is presented in Table II. No significant differences were observed between the study and control group ($p = 0.52$). Allele distributions in the control group and syncopal patients were very similar ($p = 0.81$). Carriers of the polymorphic 389Gly allele were present in both groups and were distributed similarly in both groups. Therefore the carriage of the polymorphic allele did not increase the odds of syncope (OR = 1.06, 95% CI: 0.69–1.63) (Table III). An association between carriage of this allele and negative result of the tilt test in the group of patients with syncope was observed, but did not reach statistical significance ($p = 0.09$; Table III). There was no association between the VASIS classification type of positive tilt response and genotype ($p = 0.76$) or polymorphic 389Gly allele carriage ($p = 1.00$).

Table II. Genotype distribution of Arg389Gly polymorphism in patients with syncope and control group

Group	Genotype		
	AA	AG	GG
Fainting	110 (59%)	82 (59%)	13 (72%)
Negative HUT	53 (28%)	49 (35%)	8 (44%)
Positive HUT	57 (31%)	33 (24%)	5 (28%)
Control	77 (41%)	58 (41%)	5 (28%)

p = 0.52 for all fainting patients and control group; p = 0.37 for negative HUT, positive HUT and control group

Table III. Distribution of alleles in patients with syncope and control group

Group	Genotype	
	Arg/Arg	Gly+
Fainting	110 (59%)	95 (60%)
Negative HUT	53 (28%)	57 (36%)
Positive HUT	57 (30%)	38 (24%)
Control	77 (41%)	63 (40%)

p = 0.23 for negative HUT, positive HUT and control group; p = 0.81 for all fainting patients and control group

Discussion

Numerous recent studies support the hypothesis that vasovagal syncope may be in fact a disease of strong genetic background [10]. Still, the association of various genetic variations with VVS is not as evident as initially expected, due to the limited number of relevant research papers focused on the matter [11] and further overshadowed by small sample sizes. In a study of French patients with unexplained syncope, a strong association between syncopal incidence, positive HUT and CC variant in the adenosine A_{2A} receptor (*ADORA*$_{2A}$) gene was found [12]. Recently, Sorrentino and Forleo *et al.* investigated the 3A/4A polymorphism of the endothelin-1 gene (*EDN1*). A strong association between 4A variant carriage and susceptibility to syncope was elucidated [13]. In a group of Polish patients with syncopal events, polymorphic variants of *GNAS1*, *GNB3* and *RGS2* were examined and showed mixed effects [7, 9, 14–17]. Although the molecular mechanism of VVS is still under investigation, preliminary results of aforementioned studies are promising.

Genetic alterations in the sympathetic system may be involved in pathophysiological mechanisms leading to VVS. Signal transduction pathway β1-adrenergic receptors regulate critical aspects of heart muscle contractility and blood pressure control [18]. They mediate the actions of two neurotransmitters (noradrenaline and adrenaline) through activation of heterotrimeric G proteins, and belong to the G protein-coupled receptor (GPCR) family. In vertebrates, this family of proteins contains from 1000 to 3000 members coded by genes that comprise 1–3% of the whole genome [19]. The GPCRs are built of seven transmembrane α-helices that are buried in cellular membrane preceded by an extracellular N-terminal domain and followed by an intracellular C-terminal domain [20]. The studied Arg to Gly polymorphism at position 389 of β1-AR is located close to the carboxyl terminus that is predicted to be an α-helix located on the cytoplasmic site of the cellular membrane. This region is evolutionally conserved as it is ubiquitous for G_s protein interaction [4, 21, 22]. Arg389Gly variants respond differently to catecholamine treatment [23]. The Arg variant exhibits increased responsiveness to agonist-induced stimulation *in vitro* [24] while the Gly variant is believed to disrupt α-helical structure of the region and lead to reduced responsiveness [25]. Moreover, Arg389Gly polymorphism may influence the GRK-dependent desensitization process [26]. The study of Rathz *et al.* revealed that desensitization for the Arg389 variant was in fact 50% greater than that of the 389Gly genotype [27].

The distribution of Arg389Gly polymorphism was reported in dbSNP. According to data gathered in this database, the frequencies of Arg389 and 389Gly alleles in the Caucasian population are equal to 0.68 and 0.32, respectively. The OMIM database gives the frequencies of 0.74 and 0.26 for Arg389 and 389Gly alleles, respectively. In our study, the proportion of genetic variants is similar and in line with data of healthy subjects (Table IV).

So far, the main limitation of conducted studies is the lack of a control group composed of a normative population representative of non-fainting subjects. In the present report, a group of 143 healthy volunteers was enrolled and genotyped. The distribution of Arg389Gly variants of the *ADRB1* gene in controls was close to HapMap data (0.76 for Arg389 variant and 0.24 for 389Gly variant; for further data see Table IV). A limiting factor

Table IV. Allele frequencies of Arg389Gly polymorphism in *ADRB1* gene in different populations

Population	Number of subjects	Allele frequency		Reference
		Arg	Gly	
Non-fainting subjects	143	0.760	0.240	Present study
Syncopal patients with negative HUT	110	0.700	0.300	
Syncopal patients with positive HUT	95	0.770	0.230	
HapMap CEU ss38568848	60	0.683	0.317	[32]
OMIM	50	0.740	0.260	[32, 33]
US White	316	0.720	0.280	[34]
Syncopal patients with negative HUT	17	0.970	0.030	[6]
Syncopal patients with positive HUT	33	0.697	0.303	
All syncopal patients	129	0.690	0.310	[7]
Syncopal patients with negative HUT	56	0.660	0.340	
Syncopal patients with positive HUT	73	0.710	0.290	

here may be the significant age difference, as controls were younger than fainting patients. Taking into consideration the fact that the effect of a genetic polymorphism does not generally increase during an individual's lifetime and vasovagal syncope manifests mainly at early age [1], selection of the control group seems to be proper despite the age difference.

Marquez *et al.* [4] showed that in patients of Mexican origin the Arg389Gly polymorphism may be associated with susceptibility to fainting. The 389Gly variant was found to be more frequent in patients with syncope and positive HUT (30.3%) in comparison with fainters with negative HUT (3%). Marquez stated that a decreased inotropic response of the Gly allele may alter adrenergic stimulation that takes place during orthostatic challenge. However, the distribution of the 389Gly allele in the positive HUT group is similar to that in the general healthy population of Caucasian descent (Table IV). The 389Gly variant is likely to be important as a key determinant of susceptibility to fainting during HUT, but cannot be considered as a main risk factor of vasovagal syncope.

Sorrentino *et al.* [6] in a cohort of Italian patients did not find evidence for a genetic background of vasovagal syncope. In this study, a broad range of genetic polymorphisms affecting sympathetic activity were scrutinized, i.e. Arg492Cys (ADRA1A gene), Ser49Gly and Arg389Gly (ADRB1), Arg16Gly and Gln27Glu (ADRB2), 825C/T (GNB3), –1021C/T (DBH) and S/L (SLC6A4). None of them seemed to influence the result of positive HUT.

Previous studies demonstrated that vasovagal syncope is more frequent in females than in males (42% vs. 31%) [28, 29]. However, in the aforementioned study the individuals were diagnosed exclusively based on their questionnaire without performing HUT or other medical investigations. Thus, females are more susceptible to fainting and/or are more likely to volunteer the information than males. In the group of fainting patients the result of HUT does not depend on gender as shown by Pietrucha *et al.* [30] but females experience syncopal incidents for longer periods of their lives [31]. In the present report, the male population was overrepresented in the control group (54% in control vs. 36% in study group). This parameter may slightly influence the results of the research, but there is no strong scientific evidence that susceptibility to vasovagal syncope depends on gender.

The main limitation of the present study was the small number of healthy subjects enrolled in the control group. Due to the low abundance of homozygotes for the Gly allele and small sample size, the results should be confirmed in a larger population.

In conclusion, an association between syncopal incidence and Arg389Gly polymorphism within the *ADRB1* gene was not found. The analyzed polymorphism affecting sympathetic activity does not influence tilt-induced vasovagal syncope in Polish patients.

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