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Polymorphisms in β-Adrenergic Receptors Are Associated with Increased Risk to Have a Positive Head-Up Tilt Table Test in Patients with Vasovagal Syncope

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ABSTRACT

Background: Vasovagal syncope (VVS) is a frequent clinical condition in which a genetic background seems to be implicated. Considering that the adrenergic receptors (ARs) may play a role in VVS, the present study has as principal aim to determine if the α- and β-AR (*ADRA* and *ADRB*) gene polymorphisms are associated with an increased risk to have a positive head-up tilt table (HUTT) test in patients with VVS. Methods: Nine polymorphisms in the *ADRA1A* (rs1048101, rs1383914, rs574584, and rs573542), *ADRB1* (rs1801252 and rs1801253), *ADRB2* (rs1042713 and rs1042714), and *ADRB3* (rs4994) genes were analyzed using the 5' exonuclease TaqMan genotyping assay in a group of 134 patients with VVS. **Results:** Under different models, the rs1801252 (OR = 8.63, 95% CI: 0.95-78.72, $P_{recessive}$ = 0.02), rs1042713 (OR = 1.94, 95% CI: 1.02-3.66, $P_{additive}$ = 0.04), and rs4994 (OR = 2.46, 95% CI: 1.01-6.01, $P_{dominant}$ = 0.042 and OR = 2.62, 95% CI: 1.04-6.63, $P_{over\text{-}dominant}$ = 0.03) polymorphisms were associated with increased risk for a positive HUTT. All models were adjusted for statistically significant covariates. **Conclusion:** These results suggest that some polymorphisms of the β-AR genes could contribute to a positive tilt test in patients with VVS. (REV INVEST CLIN. 2019;71:124-32)

Key words: Adrenergic receptors. Genetics. Head-up tilt testing. Polymorphism. Vasovagal syncope.

*The contributions by Manlio F. Márquez and José Manuel Fragoso are equal and the order of authorship is arbitrary.

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INTRODUCTION

Syncope is a frequent clinical condition, and the most common is vasovagal syncope (VVS) with an estimated rate of 1.31/1000 person-year[1]. It has been proposed that the autonomic nervous system (ANS) plays an essential role in the pathophysiology of this disease through an abnormal regulation of the baroreflex system. A sudden increase in sympathetic tone, preceding a robust vasovagal response, is considered a central mechanism responsible for systemic vasodilation (hypotension) and brain hypoperfusion[2]. The etiology of VVS is still unknown, but clinical observations suggest a multifactorial etiology, including a possible genetic background[3,4].

A dysfunction of the adrenergic receptors (ARs) has long been suspected in the pathophysiology of VVS. α-ARs are associated with the control of the peripheral resistance. The α(1A)-AR is highly expressed in human vasculature, and its stimulation is primarily responsible for adrenergic-mediated smooth muscle contraction[5]. β-ARs are also associated with smooth muscle relaxation; polymorphisms of the β2-AR are considered important determinants of the vascular response to stress[6].

Various studies around the world have attempted to document the association between single nucleotide polymorphisms (SNPs) in ARs and VVS with mixed results[7-11]. For example, the groups of Sorrentino et al.[11] and Żelazowska et al.[10] found no association in Arg389Gly β-1 AR polymorphism, but in Mexico, the groups of Márquez et al.[8] and Hernández-Pacheco et al.[9] did find an association with the same polymorphism.

Other studies have focused on different polymorphisms in the α-AR, obtaining mixed results. Sorrentino et al.[11] found no association with the Cys347Arg polymorphism for the alpha-1 AR (*ADRA1A*), but in Mexico, Hernández-Pacheco et al. found a positive association[7].

In an attempt to solve this controversy, we designed a new study to analyze whether these SNPs indeed predict responses to the head-up tilt table (HUTT) test. In this study, seven additional polymorphisms were included, four of which have participation in cardiovascular physiology[12] and three more SNPs that correlated with fibromyalgia in the Mexican Mestizo population. The reasons behind this choice were[1] the correlation of VVS and ANS dysfunction and the report of the participation of this same dysfunction as a critical element in fibromyalgia[13], and[2] the description of a positive association of α-AR SNPs with fibromyalgia[14].

This study had three main objectives. The first is an attempt to fill the gap concerning the discussion of the HUTT test and the genetics of VVS in the literature for the Mexican population. Second, to study the participation of nine polymorphisms located in four ARs and their association with increased risk to have a positive HUTT test in patients with VVS. And third, this study is a call for a consortium conformation for the genetic study of VVS.

SUBJECTS AND METHODS

Subjects

We enrolled 134 unrelated consecutive Mexican Mestizo patients, who were referred for an HUTT test to the Electrophysiology Department of the National Institute of Cardiology in a 5-year period, with more than one unexplained syncope or presyncope episode and clinical suspicion of VVS. The inclusion criteria for participants were: adult patients, with a history of recurrent episodes of syncope or pre-syncope - defined as more than two episodes in the previous 6 months - and who agreed to sign the informed consent. Exclusion criteria were signs or symptoms of other ANS-related diseases, pregnancy, lactation, or patients who were taking medication interfering with the ANS. Subjects with a negative HUTT test (n = 46) were compared with those with a positive test (n = 88). Mexican ancestry origin was defined as individuals who were born in Mexico and traced back to the third generation. This project was designed according to the declaration of Helsinki and the Institutional Ethical and Research Committees approved the protocol. All participants were informed about the objectives and benefits of the results of this study for the Mexican population with VVS. In this case–control study, all patients signed the informed consent before enrollment.

Table 1. Characteristics of the SNP examined.

Adrenergic receptor	Gene, Ref. SNP #	Location	Chromosome	Position	Variation	Amino acid
α-Adrenergic receptors	ADRA1A, rs1048101	Coding	8p21-p11.2	29770511	T > C	p. Cys347Arg
	ADRA1A, rs1383914	Promoter	8p21.2	26865532	A > G	−108 A > G
	ADRA1A, rs574584	Promoter	8p21.2	26866167	G > A	−743 G > A
	ADRA1A, rs573542	Promoter	8p21.2	26866301	G > A	−877 G > A
β-Adrenergic receptors	ADRB1, rs1801252	Coding	10q24-26	114044277	A > G	p. Ser49Gly
	ADRB1, rs1801253	Coding	10q24-26	114045297	G > C	p. Gly389Arg
	ADRB2, rs1042713	Coding	5q31-q32	148826877	A > G	p. Arg16Gly
	ADRB2, rs1042714	Coding	5q31-q32	148826910	C > G	p. Gln27Glu
	ADRB3, rs4994	Coding	8p12-p11.2	37966280	T > C	p. Trp64Arg

SNP: Single nucleotide polymorphism, Ref. SNP #, SNP reference number.

HUTT test protocol

The HUTT test was performed between 9:00 and 12:00 h, and all the subjects had fasted the previous night. A surface three-lead electrocardiogram was continuously displayed, and blood pressure measured with a sphygmomanometer by the same researcher at intervals of 5 min and every minute when symptoms of impending syncope developed. Then, after the subjects had rested in a supine position for a minimum of 10 min to obtain stable values, a 5 min baseline recording was captured. Phase I: The participants were tilted to 70° using a motorized table with footboard support located in a silent room with a constant temperature of 21°C, for 20 min or until syncope was imminent, associated with a clear precipitous fall in blood pressure or heart rate (positive HUTT). The imminence of syncope was recognized by a subjective sensation of impending fainting, dizziness, nausea, sweating, pallor, altered mental state, and visual changes plus hypotension (systolic blood pressure < 80 mmHg). If a favorable response was not detected during Phase I of the test, the subjects were returned to the supine position, received 5 mg of sublingual isosorbide dinitrate and were tilted at the same angle for a further 12 min or less (Phase II).

DNA extraction

A 5-ml blood sample was obtained from participants, and the DNA was extracted by the method proposed by Miller et al.[15]. Once obtained, the DNA was measured in a Thermo Scientific™ NanoDrop™ spectrophotometer (Waltham, Massachusetts, USA) with 1–2 µL of sample, verifying the integrity of the product through an agarose gel (1%) dyed with ethidium bromide. After verification of quality, the DNA was aliquoted and stored.

Genetic analysis

The *ADRA1A* (rs1048101 [Company identifier: C___2696454_30], rs1383914 [C___2696575_1_], rs574584 [C___2315104_10], and rs573542 [C_____903247_10]), *ADRB1* (rs1801252 [C___8898508_10] and rs1801253 [C___8898494_10]), *ADRB2* (rs1042713 [C___2084764_20] and rs1042714 [C___2084765_20]), and *ADRB3* ([rs4994 [C___2215549_20]) SNPs were genotyped using the 5' exonuclease TaqMan genotyping assays on a 7900HT Fast real-time polymerase chain reaction system following the manufacturer's instructions (Applied Biosystems, Foster City, USA) (Table 1). The thermocycling protocol was: denaturation at 95°C for 10 min followed by 40 cycles of 15 sec at 95°C (denaturation) and 1 min at 60°C (annealing/extension). Samples previously sequenced of the different genotypes of the polymorphisms studied were included as positive controls.

Statistical analysis

For statistical analysis purposes, SPSS version 18.0 (SPSS, Chicago, IL) statistical package was used.

Table 2. Baseline clinical characteristics of the studied individuals.

Clinical variable	Positive HUTT (*n* = 88)	Negative HUTT (*n* = 46)
Age (years)	28 (20-37.2)	22.5 (21-29)
Female	63 (72)	26 (57)
Syncope*	64 (73)	18 (39)
Pre-syncope	61 (69)	24 (53)
Dizziness*	67 (76)	26 (57)
Diaphoresis	46 (52)	17 (37)
Tremor	41 (47)	18 (39)
Dysesthesia	38 (43)	14 (30)
Blurred vision*	53 (60)	15 (33)

The data are presented in median (± IQR) for years and n (%) for the rest of the variables.
*$p < 0.05$, p values were calculated using the Chi-squared test.
HUTT: head-up tilt table; IQR: Inter-quantile range.

Means or medians with standard deviation or inter-quartile range were calculated for baseline clinical characteristics. For comparisons between groups, we used the Chi-squared test and Student´s *t*-test or Mann–Whitney U-test as necessary. The Hardy-Weinberg equilibrium was evaluated by the Chi-square test. For the comparison between our Minor Allele Frequency (MAF) and those of open international databases, the Chi-squared test was used when the allele counts for each SNP was available. Logistic regression analysis was used to test for associations of polymorphisms with the HUTTT result using the following inheritance models: codominant, dominant, recessive, over-dominant, and additive. The effect size was calculated by odds ratio transformation to the Cohen's d effect size estimator and considered as follows: small (0.2), medium (0.5), and a substantial effect (0.8). SNPstats (http://bioinfo.iconcologia.net/snpstats/start.htm) software for Windows® was used to analyze the genetic frequencies and to evaluate the linkage disequilibrium (LD, D'). Haplotype construction was made with Haploview version 3.32 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA).

RESULTS

From 134 cases with clinical criteria for VVS, 88 patients had positive HUTTT. 56 were positive in Phase I (63.6%), and 32 were positive in Phase II (36.4%). Table 2 shows the baseline clinical characteristics of the subjects. Some dysautonomic symptoms were more prevalent in the HUTT test positive group - syncope, dizziness, and blurred vision - than in the control group.

The SNPs investigated in the present study showed similar frequencies compared to international information from several databases, except for the rs1801252 (*ADRB1*), where the MAF was lower in both groups compared to the Latino population in the Exome Aggregation Consortium; rs4994 (*ADRB3*) showed an increased MAF in the HUTT-positive group compared to the Mexicans in the 1000 Genomes population (Table 3).

The studied polymorphisms were in Hardy-Weinberg equilibrium. *ADRA1A* polymorphisms showed similar prevalence between groups. The rs1801252 (*ADRB1*) increased the risk of a positive HUTT test under a recessive model (OR = 8.63, 95% CI: 0.95-78.72, p = 0.02). In *ADRB2*, the rs1042713 increased the risk of a positive HUTT test (OR = 1.94, 95% CI: 1.02-3.66, p = 0.04). Finally, the rs4994 (*ADRB3*) was more prevalent in the positive HUTT test group in two models: dominant (OR 2.46, 95% CI 1.01-6.01, p = 0.04) and over-dominant (OR 2.62, 95% CI 1.04-6.63, p = 0.03). None of the polymorphisms in *ADRA1, ADRB1,* and *ADRB2* were in linkage disequilibrium (data not shown). In *ADRB3*, it was not possible to make this analysis because in the present study only one SNP was analyzed (Table 4).

Table 3. Minor allele frequency for the different SNP compared with other populational studies.

Gene Ref. SNP # (counted allele)	MAF		1000 Genomes		TOPMED	ExAC		GO-ESP
	HUTT (+)	HUTT (-)	Global	MEX		Global	Latino	
ADRA1A rs1048101 (T)	0.49	0.49	0.35	0.48	0.42	0.48	0.51	0.45
ADRB1 rs1801252 (G)	0.35	0.25	0.18	0.26	0.17	0.30	0.49*, +	0.14
ADRB1 rs1801253 (G)	0.17	0.13	0.30	0.17	0.31	0.26	0.14	0.30
ADRB2 rs1042713 (A)	0.47	0.36	0.48	0.48	0.43	0.42	0.42	0.41
ADRB2 rs1042714 (G)	0.22	0.18	0.20	0.14	0.31	0.32	0.17	0.34
ADRB3 rs4994 (C)	0.26	0.16	0.12	0.13*	0.09	0.11	0.22	0.09
ADRA1A rs1383914 (G)	0.48	0.43	0.49	0.47	0.47	ND	ND	ND
ADRA1A rs574584 (G)	0.14	0.12	0.15	0.11	0.07	ND	ND	ND
ADRA1A rs573542 (T)	0.09	0.13	0.12	0.12	0.10	ND	ND	ND

*$p < 0.05$ HUTT (+) VS marked database
+, $p < 0.05$ HUTT (-) VS marked database, p values were calculated using the Chi-squared test.
SNP: single nucleotide polymorphism; Ref. SNP #, SNP reference number; MAF: minor allele frequency; HUTT: head-up tilt table; MEX: Mexican ancestry in Los Angeles, California; ExAC: Exome aggregation consortium; GO-ESP: Grant opportunities Exome sequencing project; TOPMED: Trans-OMICS for precision medicine; ND: no data available.
Information obtained from the dbSNP database of the National Institutes of Health and from the respective database website when available.

DISCUSSION

The present study shows that some SNPs located in the β-AR gene increased the risk for a positive HUTT test, but not those of the α-AR gene. Clinically, there was an increased frequency of some dysautonomic symptoms in patients with positive HUTT test in comparison with the negative HUTT group.

From this study, based on 134 consecutive Mexican mestizo patients, we found associations between the *ADRB1* rs1801252, *ADRB2* rs1042713, and *ADRB3* rs4994 polymorphisms and the positivity of a HUTT test. These results are different from those reported by Sorrentino et al.[11] in an Italian population. They analyzed polymorphisms of ANS-related genes, including three ARs (α1-A, β1, and β2), and did not find any relation with the result of the HUTT test.

Our research group was one of the first conducting an association study between HUTT test and polymorphisms in *ADRB1* gene. We found in Mexican Mestizos a positive association for Arg389Gly polymorphism; however, the sample size was limited to 50 patients with syncope, and the results were reported as a pilot study[8]. In the present work, the sample size was 134 patients, with the double participants than the past research, collected in a 5-year period, with the proposal to test nine polymorphisms in four different adrenergic genes. To date, the maximum sample size for a positive association study of this type is 214 patients and corresponded to the *GNB3* gene[16].

The potential role of α-ARs in VVS

Of the four polymorphisms located in the *ADRA1A* gene for this study, the rs1048101 was the only SNP with a nonsynonymous change; it has been associated to a greater effect of nifedipine among Chinese population[17]. This polymorphism was also related to the hemodynamic response in the treadmill exercise test, especially in men, being present in those with higher vasoconstriction (greater maximal systolic blood pressure)[18]. Hernández-Pacheco et al.[7] studied

Table 4. Association of the *ADRA-1A* and *ADRB1*, *ADRB2*, and *ADRB3* polymorphisms with HUTT result.

Gene	Polymorphism Ref. SNP # Group (n)	n (%)			Model	d	OR (95% CI)	p
ADRA1A	p. Cys347Arg							
	rs1048101							
		CC	CT	TT	Codominant	0.021	1.04 (0.34-3.21)	1.00
	HUTT (+) (88)	22 (25)	45 (51)	21 (24)	Dominant	0.010	1.02 (0.41-2.58)	0.96
					Recessive	0.016	1.03 (0.41-2.58)	0.95
	HUTT (-) (46)	11 (24)	25 (54)	10 (22)	Over-dominant	−0.005	0.99 (0.45-2.21	0.99
					Additive	0.01	1.02 (0.58-1.79)	0.94
	−108 A > G							
	rs1383914							
		AA	AG	GG	Codominant	0.276	1.65 (0.52-5.22)	0.61
	HUTT (+) (88)	26 (30)	40 (45)	22 (25)	Dominant	0.086	1.17 (0.49-2.78)	0.73
					Recessive	0.272	1.64 (0.61-4.42)	0.32
	HUTT (-) (46)	14 (30)	24 (52)	8 (17)	Over-dominant	−0.109	0.82 (0.37-1.80)	0.62
					Additive	0.127	1.26 (0.72-2.20)	0.42
	−743 G > A							
	rs574584							
		AA	AG	GG	Codominant	0.594	2.94 (0.20-43.71)	0.70
	HUTT (+) (88)	66 (75)	19 (22)	2 (3)	Dominant	0.127	1.26 (0.48-3.31)	0.64
					Recessive	0.577	2.85 (0.19-42.2)	0.42
	HUTT (-) (46)	36 (78)	9 (20)	1 (2)	Over-dominant	0.042	1.08 (0.40-2.95)	0.87
					Additive	0.153	1.32 (0.58-3.01)	0.51
	−877 G > A							
	rs573542							
		CC	CT	TT	Codominant	−0.578	0.35 (0.03-3.55)	0.15
	HUTT (+) (88)	75 (85)	11 (13)	2 (2)	Dominant	−0.594	0.34 (0.11-1.01)	0.05*
					Recessive	−0.465	0.43 (0.04-4.16)	0.47
	HUTT (-) (46)	36 (78)	8 (17)	2 (4)	Over-dominant	−0.254	0.36 (0.11-1.13)	0.08
					Additive	−0.440	0.45 (0.19-1.07)	0.06
ADRB1	Ser49Gly							
	rs1801252							
		AA	AG	GG	Codominant	1.169	8.34 (0.88-79.5)	0.06
	HUTT (+) (88)	38 (43)	39 (44)	11 (13)	Dominant	0.100	1.20 (0.53-2.75)	0.66
					Recessive*	1.188	8.63 (0.95-78.72)	0.02*
	HUTT (-) (46)	24 (52)	21 (46)	1 (2)	Over-dominant	−0.188	0.71 (0.31-1.62)	0.42
					Additive	0.245	1.56 (0.81-3.01	0.18
	Arg389Gly							
	rs1801253							
		CC	CG	GG	Codominant	−0.151	0.76 (0.06-10.20)	0.81
	HUTT (+) (88)	60 (68)	26 (30)	2 (2)	Dominant	0.131	1.27 (0.51-3.16)	0.61
					Recessive	−0.204	0.69 (0.05-9.03)	0.78
	HUTT (-) (46)	35 (76)	10 (22)	1 (2)	Over-dominant	0.161	1.34 (0.53-3.40)	0.54
					Additive	0.086	1.17 (0.51-2.66)	0.71

(Continua)

Table 4. Association of the *ADRA-1A* and *ADRB1, ADRB2,* and *ADRB3* polymorphisms with HUTT result. *(Continuación)*

Gene	Polymorphism Ref. SNP # Group (n)	n (%)			Model	d	OR (95% CI)	p
ADRB2	Arg16Gly							
	rs1042713							
		GG	GA	AA	Codominant	0.765	4.01 (1.00-16.04)	0.11
	HUTT (+) (88)	23 (26)	48 (55)	17 (19)	Dominant	0.406	2.09 (0.88-4.95)	0.09
					Recessive	0.567	2.80 (0.79-9.92)	0.09
	HUTT (-) (46)	18 (39)	23 (50)	5 (11)	Over-dominant	0.091	1.18 (0.53-2.63)	0.69
					Additive*	0.365	1.94 (1.02-3.66)	0.04*
	Gln27Glu							
	rs1042714							
		CC	CG	GG	Codominant	0.223	1.50 (0.12-19.02)	0.93
	HUTT (+) (88)	52 (59)	33 (38)	3 (3)	Dominant	0.062	1.12 (0.49-2.58)	0.78
					Recessive	0.204	1.45 (0.12-17.87)	0.77
	HUTT (-) (46)	30 (65)	15 (33)	1 (2)	Over-dominant	0.042	1.08 (0.47-2.50)	0.86
					Additive	0.072	1.14 (0.54-2.39)	0.74
ADRB3	Trp64Arg							
	rs4994							
		TT	TC	CC	Codominant	0.193	1.42 (0.22-9.10)	1.00
	HUTT (+) (88)	48 (54)	35 (40)	5 (6)	Dominant*	0.496	2.46 (1.01-6.01)	0.04*
					Recessive	−0.005	0.99 (0.16-6.09)	0.99
	HUTT (-) (46)	33 (72)	11 (24)	2 (4)	Over-dominant*	0.531	2.62 (1.04-6.63)	0.03*
					Additive	0.348	1.88 (0.88-4.01)	0.09

*$p < 0.05$, p values were calculated from logistic regression analysis and the inheritance models were adjusted for dizziness, syncope, and blurred vision.
SNP: single nucleotide polymorphism; Ref. SNP #, SNP reference number; d, Cohen´s d effect size calculated; OR: odds ratio; CI: confidence interval; HUTT: head-up tilt table.

89 patients with VVS and compared them with 40 healthy Mexican controls. They analyzed the association of the Arg/Arg genotypes of the rs1048101 polymorphism with the occurrence of syncope (codominant model OR = 13.21, 95% CI 3.69-54.99, $p < 0.001$ and additive model OR = 12.68, 95% CI 3.5-53.07 $p < 0.001$). As reported by Sorrentino et al.[11], in the present work, no association was found between the same polymorphism and a positive HUTT. The main difference between these reports is that Hernández-Pacheco's et al. group included healthy, sex- and age-matched controls, while in our study and Sorrentino's et al., there were no healthy controls, and the reference group was the negative HUTT group. Nevertheless, when compared to the healthy population from open databases (1000 Genomes project and Exome Aggregation Consortium) there were no differences between *ADRA1A* receptor polymorphisms and the positivity in the HUTT test.

The other α-AR SNPs (rs1383914, rs574584, and rs573542) are in no coding regions of the gene and were included in the present study because a previous study reported the association of these SNPs with fibromyalgia in Mexican and Spanish populations. However, we did not detect any association in this study[14].

The potential role of β-ARs in VVS

The rs1801252 and rs1801253 polymorphisms in *ADRB1* genes were previously studied in essential hypertension in a Chinese population[19]. The rs1801253 polymorphism (Arg389Gly) was found to be associated with the disease. *In vitro* studies demonstrated that the Arg389 variant of the *ADRB1* gene elicits an increased response to agonist stimulation compared with the Gly389 variant[20]. In our study, this polymorphism was associated with a positive HUTT test.

Our study included two *ADRB2* polymorphisms (rs1042713 and rs1042714) that have been previously analyzed by Diatchenko et al.[21]. This group examined 3 *ADRB2* polymorphisms and reported an association of one haplotype (rs1042713, rs1042714, and rs1042717) with the presence of low blood pressure, somatization, and temporomandibular joint syndrome. In the same way, Vargas-Alarcón et al.[14] found an association between β2-AR AC haplotype (rs1042713 and rs1042714) and fibromyalgia in Mexican and Spanish individuals. In our cohort, only the rs1042713 was associated with a positive HUTT test.

In the *ADRB3* gene, we studied the rs4994 polymorphism, which has been associated with increased cardiovascular risk in women without evidence of obstructive coronary artery disease[12]. In our study, under two different models, this polymorphism was associated with an increased risk to have a positive HUTT.

The rs4994 in the *ADRB3* was overexpressed in the positive HUTT test group compared with the Mexicans in the 1000 Genomes project; this may enhance the findings of a pathologic relationship between this polymorphism and the positivity of the test.

Resolving discrepancies in the literature among genetic studies of VVS

The findings herein reported contradicted some previous studies, even when they were performed in a Mexican Mestizo population[7-9], but also supported the results of others. For example, Zelazowska et al.[10] and Sorrentino et al.[11], with limited sample sizes and in populations with the different genetic background (Italian and Polish), found a negative association for rs1801253.

At this point, in the study of a genetic basis for VVS, there is still much confusion about the validity of the results so far reported. This confusion stems from three principal hurdles: all these studies, including the one presented here, have a limited sample size; therefore, studies in many individuals are necessary to establish the real effect of these polymorphisms. The second probable source of error is a subtle population stratification in self-reported ethnicity observed within the ethnically homogeneous population, which can also occur in ethnically mixed populations and can lead to detecting significant associations at loci that have nothing to do with the disease. The gold standard to avoid this problem is the use of matched case–control patients with ancestry markers. The third point to consider is that the frequency of syncope can vary from country to country and even in their borders. Furthermore, the genetic and relevant environmental factors within populations may not be balanced between initial and subsequent studies; the variations in effect size from gene-gene and gene-environment interactions could in part explain the inability to replicate the association[22].

In this study, we included five models of inheritance to be considered in future replication studies. There is no current consensus about the best model of inheritance to be chosen in association studies. However, in the last years, the additive model has been proposed as the preferred model to detect genetic association even when the real model of inheritance is different[23].

Other limitations are the increased cost and pitfalls for recruitment, which is reflected in a lack of a bigger sample size to detect a lower effect, as is usual in multifactorial disorders. However, in the results obtained here, an important effect could be detected in a few polymorphisms in this small sample because the calculated OR was high (OR=8.63, 95% CI 0.95-78.72). This can be seen in the polymorphism Ser-49Gly located in *ADRB1* under the recessive model with a d = 1.188, and also in the Trp64Arg located in *ADRB3*, under the dominant and over-dominant models with a medium effect size (d = 0.496 and 0.531) with ORs higher than 2. In the same context, the power of the study was low for the great part of polymorphisms studied but high in those polymorphisms where a high effect size was detected, and an allelic frequency was more prominent than 0.25. Another limitation of the study is the case–control approach because, due to the reduced sample size, a family approach is a better alternative to obtain best power for similar sample size and avoid the problem of subpopulation stratification[24].

One strategy to overcome these obstacles is to form a multiethnic consortium in the study of the genetics of VVS, to determine the participation of

polymorphisms in these four genes of *ADRA* and *ADRB* in patients with VVS.

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REFERENCES

1. Soteriades ES, Evans JC, Larson MG, et al. Incidence and prognosis of syncope. N Engl J Med. 2002;347:878-85.
2. Morillo CA, Eckberg DL, Ellenbogen KA, et al. Vagal and sympathetic mechanisms in patients with orthostatic vasovagal syncope. Circulation. 1997;96:2509-13.
3. Klein KM, Berkovic SF. Genetics of vasovagal syncope. Auton Neurosci. 2014;184:60-5.
4. Nordkamp LR, Wieling W, Zwinderman AH, Wilde AA, van Dijk N. Genetic aspects of vasovagal syncope: a systematic review of current evidence. Europace. 2009;11:414-20.
5. Lei B, Morris DP, Smith MP, et al. Novel human alpha1a-adrenoceptor single nucleotide polymorphisms alter receptor pharmacology and biological function. Naunyn Schmiedebergs Arch Pharm. 2005;371:229-39.
6. Dishy V, Sofowora GG, Xie HG, et al. The effect of common polymorphisms of the beta2-adrenergic receptor on agonist-mediated vascular desensitization. N Engl J Med. 2001; 345:1030-5.
7. Hernández-Pacheco G, González-Hermosillo A, Murata C, et al. Arg347Cys polymorphism of α1a-adrenergic receptor in vasovagal syncope. Case-control study in a Mexican population. Auton Neurosci. 2014;183:66-71.
8. Márquez MF, Hernández-Pacheco G, Hermosillo AG, et al. The arg389Gly beta1-adrenergic receptor gene polymorphism and susceptibility to faint during head-up tilt test. Europace. 2007;9:585-8.
9. Hernández-Pacheco G, Serrano H, Márquez MF, et al. Genetic study of the vasovagal syncope associated to the arg389Gly polymorphism of the beta1 adrenergic receptor. Arch Cardiol Mex. 2008;78:134-8.
10. Zelazowska M, Lelonek M, Fendler W, Pietrucha T. Arg389Gly β1-adrenergic receptor polymorphism and susceptibility to syncope during tilt test. Arch Med Sci. 2014;10:240-5.
11. Sorrentino S, Forleo C, Iacoviello M, et al. Lack of association between genetic polymorphisms affecting sympathetic activity and tilt-induced vasovagal syncope. Auton Neurosci. 2010; 155:98-103.
12. Pacanowski MA, Zineh I, Li H, et al. Adrenergic gene polymorphisms and cardiovascular risk in the NHLBI-sponsored women's ischemia syndrome evaluation. J Transl Med. 2008;6:11.
13. Vallejo M, Martínez-Martínez LA, Grijalva-Quijada S, et al. Prevalence of fibromyalgia in vasovagal syncope. J Clin Rheumatol. 2013;19:111-4.
14. Vargas-Alarcón G, Fragoso JM, Cruz-Robles D, et al. Association of adrenergic receptor gene polymorphisms with different fibromyalgia syndrome domains. Arthritis Rheum. 2009; 60:2169-73.
15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
16. Lelonek M, Pietrucha T, Matyjaszczyk M, Goch JH. Genetic insight into syncopal tilted population with severe clinical presentation. Auton Neurosci. 2009;147:97-100.
17. Zhang Y, Hong X, Liu H, Huo Y, Xu X. Arg347Cys polymorphism of alpha1A-adrenoceptor gene is associated with blood pressure response to nifedipine GITS in chinese hypertensive patients. J Hum Genet. 2009;54:360-4.
18. Nunes RA, Barroso LP, Ada CP, Krieger JE, Mansur AJ. Gender-related associations of genetic polymorphisms of α-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor with treadmill exercise test responses. Open Heart. 2014;1:e000132.
19. Peng Y, Xue H, Luo L, Yao W, Li R. Polymorphisms of the beta1-adrenergic receptor gene are associated with essential hypertension in chinese. Clin Chem Lab Med. 2009;47:1227-31.
20. La Rosée K, Huntgeburth M, Rosenkranz S, Böhm M, Schnabel P. The arg389Gly beta1-adrenoceptor gene polymorphism determines contractile response to catecholamines. Pharmacogenetics. 2004;14:711-6.
21. Diatchenko L, Anderson AD, Slade GD, et al. Three major haplotypes of the beta2 adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder. Am J Med Genet B Neuropsychiatr Genet. 2006;141B:449-62.
22. Li A, Meyre D. Challenges in reproducibility of genetic association studies: lessons learned from the obesity field. Int J Obes (Lond). 2013;37:559-67.
23. Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype – Phenotype associations. Eur J Hum Genet. 2001;9:301-6.
24. Laird NM. Family-Based Association Test (FBAT). Boston: ELS; 2001.