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Arg347Cys polymorphism of α_{1a}-adrenergic receptor in vasovagal syncope. Case–control study in a Mexican population

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ABSTRACT

Background: Vasovagal syncope is a common clinical condition, consequential to reduced cerebral blood flow resulting from a failure in cardiovascular homeostasis during orthostasis. Blood pressure regulation is the basis for syncope development. In this regulation, the α_{1a}-adrenergic receptor plays a major role. Some studies have found a positive correlation between the Arg347Cys polymorphism of the α_{1a}-adrenergic receptor to hypertension and heart autonomic control. The goal of this study is to evaluate the possible association between the Arg347Cys α_{1a}-adrenergic receptor polymorphism and vasovagal syncope in a Mexican population.
Methods/major findings: A sample of 89 vasovagal syncope patients and 40 healthy controls were studied. Arg347Cys α_{1a}-adrenergic receptor polymorphism was determined by the PCR-RFLP method. We found an increased frequency of genotype ArgArg in vasovagal syncope patients. In a logistic regression model significant associations were found in two genetic models, in codominant model (OR = 13.21: CI 95% 3.69–54.99, $p < 0.001$) and in additive model (OR = 12.68: CI 95% 3.5–53.07, $p < 0.001$) for ArgArg genotype with CysCys as reference.
Conclusions: Our data suggests an important participation of Arg347Cys polymorphism as susceptibility factor in patients with vasovagal syncope. ArgArg genotype could be a marker for vasovagal syncope susceptibility in the Mexican population.

1. Introduction

Vasovagal syncope (VVS) is the commonest cause of transient loss of consciousness, accounting for 1.6% of all admissions to the emergency department (Cárdenas et al., 2009). It is associated with a transient failure of the autonomic mechanisms that regulate cardiovascular function. The pathophysiology of VVS remains incompletely understood. Nevertheless, one consistent observation is that evolving vasovagal episodes are preceded by an apparent transient period of increased sympathetic activity (Moya et al., 2009). The initial phase of sympathetic stimulation occurs in response to a stressor, which may either be orthostatic, physical or emotional. VVS has been observed more frequently on those in the 10 to 30 year range (Ganzeboom et al., 2003; Sheldon et al., 2006; Moya et al., 2009). The evaluation of this condition is difficult due to the transitory state and its diagnosis is based on clinical history. From its introduction in 1986, the head-up tilt test (HUT) is used to study the hemodynamic changes and symptom reproduction of vasovagal syncope (Kenny et al., 1986). In its familial form, it is commonly observed that more than one family member is affected (Camfield and Camfield, 1990; Mathias et al., 1998; Newton et al., 2003; Márquez et al., 2005) suggesting a possible genetic component (Azevedo et al., 2009; OldeNordkamp et al., 2009).

According to cardiovascular homeostasis some studies of gene polymorphism have been conducted in renin–angiotensin and serotonin system (Newton et al., 2007; Mudraková et al., 2009), in endothelin system (Sorrentino et al., 2009), in genes encoding G-proteins (Lelonek et al., 2009; Huang et al., 2010) and sympathetic nervous system (Sorrentino et al., 2010).

Cardiac output and sympathetic vasoconstrictor responses are crucial for the cardiovascular homeostasis during orthostasis. Short-time adjustments in the regulation of circulation produced by heart rate changes, cardiac contractility, vasoconstriction and ultimately, cerebral blood flow depend on the autonomic nervous system. In previous studies from our group, we found an association between Arg389Gly polymorphism β_1-adrenergic receptor and positive HUT response in

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patients with VVS (Hernández-Pacheco et al., 2008). The α_{1a}-adrenergic receptor has been proven to be important in blood pressure regulation in knock-out mice (Rokosh and Simpson, 2002), and it is expressed predominantly in the human vascular system (Rudner et al., 1999).

The human ADRA1A gene is located in chromosome 8q21.2, and it bears a single base polymorphism (SNP rs1048101) at the 1441 position where the change of Thymidine instead of Cytosine originates the change of Arginine to Cysteine as the 347 amino acid residue (Arg347Cys) in the carboxy-terminal end of the receptor protein (Hoehe et al., 1992). This polymorphism has been associated with hypertension and autonomic heart control in some populations (Snapir et al., 2003; Jiang et al., 2005, Gu et al., 2006; Iacoviello et al., 2006; Freitas et al., 2008; Zhang et al., 2009).

The ultimate cause of syncope is the reduction in brain blood flow secondary to decreased cardiac output and low blood pressure. In this scenario, blood pressure regulation is determinant of syncope development, due to the vast expression of the α_{1a}-adrenergic receptor in the vascular system along with its polymorphic character. The goal of this study was to explore the possible association between the Arg347Cys polymorphism of α_{1a}-adrenergic receptor and the occurrence of vasovagal syncope in the Mexican population.

2. Methods

We carried out a case–control study according to the Declaration of Helsinki. All participants gave a written consent after the study characteristics were explained. When participants were younger than 18 years old, the responsible adult gave his/her consent as above. The "Bioethics Commission of Instituto Nacional de Cardiología Ignacio Chávez" approved this study. All chemicals were from Sigma Chemicals unless otherwise indicated or of the highest quality available.

2.1. Participants

All cases were patients attending the syncope unit at the Instituto Nacional de Cardiologia, aged 10 to 50 years old, both sexes with at least 2 syncope or presyncope episodes with a probable vasovagal origin, recruited within the previous 12 months. Presyncope was determined clinically as the experience of weakness, diaphoresis, chills, dizziness and nausea without loss of consciousness. The cases that were considered were those that met the criteria mentioned above, without any previous disease or condition that could explain syncope.

Controls were healthy volunteers with no familial relationship to cases, with no syncope or presyncope history. They usually were the guardians of the patients attending diverse services of the Instituto Nacional de Cardiologia. Both cases and controls came from diverse regions of the country.

2.2. Head-up Tilt Fainting Protocol (HUT)

The HUT in both cases and controls was performed according to the previously reported Institutional protocol (Hermosillo et al., 2000). All participants were tested after a 10 to 12 h fasting period in a specially adapted room with facilities for blood pressure and heart monitoring. Prior to baseline recording, there was a stabilization period that consisted in at least 20 min of rest in supine position. The protocol comprised three stages: supine, 70° head up tilt and recovery in supine position. The head-up tilt period was performed under two conditions: passive (in the absence of any provocative drugs), and pharmacological (with drug challenge). In the passive phase, upright posture was maintained until either development of syncope or intolerable near syncope symptoms or completion of maximum 45 min tilt duration. If syncope occurred the table was promptly returned to the supine position. If syncope or presyncope did not occur, subjects were given a single sublingual 5 mg isosorbide dinitrate dose (Hermosillo et al., 2000) and upright posture was maintained for 15 min or earlier if syncope or presyncope occurs. Cases were studied in both passive and pharmacological phases, whereas control individuals were submitted to the passive phase only if they completed a 45 min , 70° upright tilt without syncope or presyncope, to ensure that they were true controls (Hermosillo et al., 2010). The test was considered positive when syncope or near-syncope symptoms were developed (dizziness, diaphoresis or nausea) with arterial hypotension (systolic blood pressure <80 mm Hg or a fall larger or equal to 30% of basal values) and/or bradycardia (heart rate lower than 50 beats per minute or a reduction higher than 20% of the basal level). A test was considered negative when subjects did not show vasovagal symptoms.

2.3. DNA isolation

Before the HUT test, a peripheral blood sample was taken in an EDTA-containing Vacutainer. Samples were maintained at 4 °C for up to 2 days before processing. DNA was isolated in a guanidine-phenol free protocol (Lahiri and Nurnberger, 1991). In short, erythrocytes underwent lysis in a low salt solution and chromatin complexes were freed in a high salt buffer. After protein removal, DNA was ethanol precipitated and washed by centrifugation; concentration and integrity were determined by spectrophotometry and electrophoresis.

2.4. Determination of the α_{1a}-adrenergic receptor polymorphism

Samples for genotyping were handled in a blind code with no particular order for case or controls. The polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) (Shibata et al., 1996). A 502 bp fragment was obtained by using 12.5 μl JumpStartREDtaq (SIGMA), 100 ng DNA and 2 μl [10 μM] primers P1: 5′-ATGCTCCAGCCAAGAGTTCA-3′ and P2: 5′-TCCCAAGAAG AGCTGGCCTTC-3′ in a GeneAmp PCR System 2700 (Applied Biosystems) programmed with 30 cycles of 94 °C 1 min, 30 s 55 °C annealing and 1 min extension at 72 °C. After amplification, amplicons were evaluated by 1.5% agarose gel electrophoresis. Amplified material was then digested with PstI (New England Biolabs) for 1 h at 37 °C. Fragments were identified in an 8% polyacrylamide gel and visualized by ethidium bromide stain. Allele identification was registered and then associated to the corresponding code.

3. Statistical analysis

Categorical variables were expressed as percentages. The age distribution was not normal; therefore it was expressed as median and quartile one and three. Association of genotype and vasovagal syncope was assessed by a logistic regression model adjusted for age and sex. We used three genetic models of inheritance; recessive, codominant and additive form. Genotype and hemodynamic response was evaluated by correspondence analysis.

Supplemental analysis was made, from the group of cases and controls, we randomly formed subgroups of 15 men and 15 women in each group, with age greater than 18 years. In these subgroups, logistic regression analysis was made in two models of inheritance, codominant and additive. Results from the logistic regression were expressed as odds ratio (OR) with a 95% confidence interval (CI 95%). Statistical significance was taken at $p < 0.05$. Analyses were conducted by using the JMP9 facility software (SAS Institute, Inc., Cary, NC).

4. Results

4.1. General characteristics of participants

Fig. 1 shows the strategy for participant selection. We were able to collect a sample of 241 participants attending the syncope until an 18 month period. From them, 89 non-related cases were identified, as diagnosed VVS with a positive HUT test. Also, in this period, 81