Autonomic Neuroscience: Basic and Clinical 147 (2009) 97–100

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Genetic insight into syncopal tilted population with severe clinical presentation

Malgorzata Lelonek [a,*], Tadeusz Pietrucha [b], Monika Matyjaszczyk [b], Jan Henryk Goch [a]

[a] Department of Cardiology, Chair of Cardiology and Cardiac Surgery, Medical University of Lodz, Poland
[b] Department of Medical Biotechnology, Medical University of Lodz, Poland

ARTICLE INFO

Article history:
Received 8 September 2008
Received in revised form 26 January 2009
Accepted 28 January 2009

Keywords:
Malignant syncope
Tilting
Gene polymorphism
G-proteins

ABSTRACT

An impairment of cardiovascular reflexes may be the result of functional alterations in the G proteins intracellular signaling produced by functional genes' polymorphisms. The aim was to evaluate the relationships between single nucleotide polymorphisms in genes encoding G-proteins signaling pathways and syncopal patients with severe clinical manifestation.

Methods and results: From 307 syncopal patients free from any other diseases 83 (27%) had at least one malignant episode of syncope with a significant injury as fractures. There was 1.9 malignant spells per patient. All patients were tilted and genotyped by polymerase chain reaction followed by restriction fragment length polymorphism method. 74 healthy volunteers with negative history of syncope constituted the control group were also genotyped. Following polymorphisms were detected: C393T in gene encoding the alfa-subunit of Gs-protein (GNAS1), C825T of gene for G-protein beta 3 subunit (GNB3) and C1114G for the gene of cardiac regulator of G-protein signaling (RGS2).

We found an association with lower risk of malignant syncope in positive tilting patients during passive phase of the test compared to NTG-enhanced (OR 0.38; 95% CI 0.15–0.95; $P=0.04$). No difference between healthy controls and patients in the alleles frequency was found ($P>0.05$). Neither the 393T allele of GNAS1 and 825T allele of GNB3 nor 1114G allele of RGS2 was associated with enhanced risk of severe clinical manifestation ($P>0.05$).

Conclusions: The studied single nucleotide polymorphisms of genes encoding G-proteins signaling pathways seem to be not connected with the severe clinical manifestation of syncope.

1. Introduction

A malignant syncope is defined as an episode of syncope with little or no warning and results in a significant injury or property damage (Strickberger et al., 2006). It is well known that the predisposition to neurally-mediated syncope can run in families (Camfield and Camfield, 1990, Newton et al., 2003, 2005; Marquez et al., 2005). Furthermore, the genetic studies suggest the association between certain single nucleotide polymorphisms (SNPs) and vasovagal syncope (Marquez et al., 2007, Lelonek et al., 2007, 2008a; Hernandez-Pacheco et al., 2008). Genes encoding the proteins of the intracellular G-signaling pathways, that are critical for cardiovascular reflex control, are prime suspect. We hypothesized that, the predisposition to severe clinical manifestation of syncope as malignant episode could be associated with the genetic variation in proteins of G-system signaling. We investigated three SNPs in genes encoding the proteins of intracellular G signaling system, which have the studies of the importance in the regulation of arterial pressure or it's disturbances (Siffert et al., 1998, Jia et al., 1999, Tabara et al., 2002, Semplicini et al., 2006). Based upon this background, we investigated the association between polymorphic variants of the proteins involved in the intracellular G-system signaling and clinical manifestation or tilting results among syncopal patients.

2. Materials and methods

In the research 307 syncopal subjects with no history or symptoms of cardiovascular diseases were enrolled after informed consent for the entire procedure. The inclusion criteria were: a number of syncope ≥ 3 incidents in last 2 years with history suggesting neurally-mediated syncope. Eighty three (27%) patients had at least one malignant episode of syncope with a significant injury as fractures (1.9 malignant spells per patient — IQR 1–3). The baseline demographic and clinical characteristics of the patients are shown in Table 1.

All subjects were tilted under Italian protocol (Del Rosso et al., 1998). The positive tilting was recognized when syncope occurred accompanied by marked reduction of blood pressure (systemic hypotension< 80 mm Hg) and/or heart rate (Brignole et al., 2004). 74 healthy volunteers with negative history of syncope constituted the control group.

Genotyping of the studied polymorphisms was performed in all subjects. After genotyping the patients were analyzed regard to

* Corresponding author. Department of Cardiology, Sterling Str. 1/3, 91-425 Lodz, Poland. Tel./fax: +48 42 6364471.
E-mail address: mlelonek@poczta.fm (M. Lelonek).

1566-0702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2009.01.011

Table 1
Patients' characteristics regard to clinical manifestation: malignant vs. mild syncope.

Clinical manifestation of syncope	Mild $n=224$	Malignant $n=83$
Age (years)	38.5 ± 16.4	43.3 ± 17.6
Men	73 (33)	32 (39)
No. of syncope	10 (3–45)	10 (3–100)
Syncopal history, years	3 (1–12)	5 (1–13)
Systolic blood pressure at rest, mm Hg	121.1 ± 14.1**	126.7 ± 17.4**
Diastolic blood pressure at rest, mm Hg	78.8 ± 9.2	80.7 ± 9.8
Heart rate at rest beats/min^{-1}	69.3 ± 12.76	69 ± 11.6
BMI, kg/m^2	23.9 ± 4.2	24.7 ± 4
Positive result of tilt test	158 (71)	49 (59)
Positive passive tilt test	53 (24)*	8 (9.6)*

Data are expressed as n (%), mean ± SD or median with IQR.
BMI — body mass index.
*$P<0.05$, $P<0.01$.

clinical manifestation of syncope and results of tilting: positive vs. negative and positive passive vs. NTG-positive. We have also analyzed patients according to the other criteria as: number of syncope, duration of syncopal history, a type of vasovagal reaction and genetic abnormality (polymorphic homozygotes, heterozygotes).

2.1. Genotyping

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 by standard techniques as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods with primer pairs previously described for C393T (Jia et al., 1999) in gene encoding the alfa-subunit of Gs-protein (GNAS1) (GeneID:2778) and for C825T (Rosskopf et al., 2000) of the gene for the G-protein beta 3 subunit (GNB3) (GeneID:2784), and synthesized by Eurogentec. In the studied RGS2 gene (GeneID:5997) for C1114G polymorphism the primer pairs were designed in Department of Medical Biotechnology in Lodz. Two designed primers are specific for each allele whereas the third one (antisense) is common for both variants. The sequence of the oligonucleotide primers was as follows:

primer for C allele: 5′-AGTGAAGTGTTTACTATGTGCTAC-3′
primer for G allele: 5′-AGTGAAGTGTTTACTATGTGCTAG-3′
and common primer (antisense): 5′-TCAACACCATAGCACTCATT CTAT-3′.

The exclusion criteria were: a number of syncope <3 incidents in last 2 years, a positive family history for sudden cardiac death; history suggesting syncope in mechanism of: carotid sinus hypersensitivity, catecholaminergic polymorphic ventricular tachycardia, long QT syndrome or short coupling variant of torsade de pointes; ECG abnormalities and/or abnormal exercise testing; and abnormal echocardiogram. The study procedures were approved by the Local Bioethics Committee.

2.2. Statistical analyses

Continuous variables that did not show a normal distribution were analyzed with the Mann–Whitney U test and presented as mean value ± standard deviation (SD) or 95% Confidence Intervals (95% CI). Categorical variables were described as numbers and percentage. Association between analyzed parameters were examined using Chi^2 Pearson test, Yates corrected Chi^2 test for 2×2 contingency tables, and exact Fisher test for larger than 2×2 contingency tables. To test the impact of systolic and diastolic blood pressure at rest on analyzed parameters a one way analysis of variance (ANOVA) was performed. To identify the factors influencing clinical manifestation and tilting outcome univariate logistic regression followed by multivariate backward stepwise analysis were performed. Into the mutlivariate model were introduced the genetic traits (homozygocity and allele carriage) and clinical variables, those associated with the outcome of tilting with $P<0.15$ in the univariate comparisons. The results of regression analysis were presented as odds ratios (OR) with 95% CI. Data were analyzed with Statistica version 7.0 software (StatSoft, Tulsa, Oklahoma, USA), Medcalc 8.1 (Medcalc, Mariekerke, Belgium). P-value<0.05 was considered statistically significant.

3. Results

In baseline characteristics analyzed groups differed in systolic blood pressure at rest and in frequency of syncope during passive phase of tilt test (Table 1). Analysis of total population in this study showed similar allele frequencies to the healthy subjects: in C393T GNAS1 polymorphism allele C 46% vs. 54% respectively and allele T 54% vs. 46% ($P>0.05$), in C825T GNB3 polymorphism allele C 65% vs. 61% and allele T 35% vs. 39% ($P>0.05$), and in C1114G RGS2 polymorphism allele C 78% vs. 81% and allele G 22% vs. 19% ($P>0.05$).

In patients with mild and malignant syncope the frequencies of 825T allele carriers were 55% vs. 54% ($P>0.05$) and of 1114G allele carriers 56% vs. 55% ($P>0.05$), respectively. The carriage of 393T allele of GNAS1 allele also did not differentiate the clinical manifestation of syncope (37% vs. 41%, $P>0.05$). Neither the 393T allele of gene GNAS1 and 825T allele of the gene GNB3 nor 1114G allele of gene for RGS2 was associated with enhanced risk of severe clinical manifestation of syncope (all $P>0.2$). In other analyses (according to number of syncope, duration of syncopal history and a type of vasovagal reaction) we found no differences in genetic parameters. Table 2 presented the relevant demographic data related to genotypes.

There was found the association with lower risk of malignant syncope in positive tilting patients during passive phase of the test compared to NTG-enhanced (OR 0.38; 95% CI 0.15–0.95; $P=0.04$). From clinical variables into multivariate stepwise regression were introduced: age, the systolic and diastolic blood pressure at rest and

Table 2
General characteristics of the study participants according to the genotypes.

Genotype	C/C 393	C/T 393	T/T 393	C/C 825	C/T 825	T/T 825 825	C/C 1114	C/G 1114	G/G 1114
	$n=122$	$n=76$	$n=48$	$n=122$	$n=76$	$n=16$	$n=122$	$n=76$	$n=16$
Number of syncope	9 (3–50)	10 (3–50)	9 (3–30)	10 (3–50)	9 (3–50)	10 (3–35)	9 (3–45)	10 (3–40)	8 (3–30)
Syncopal history, years	4(1–12)	6(1–11)	7(1–10)	6(1–11)	5(1–10)	6(1–9)	5(1–11)	4(1–10)	4(1–9)
Systolic blood pressure at rest, mm Hg	110.9 ± 11.42**	106.6 ± 9.75*, **	113.5 ± 6.2*	110.9 ± 11.42**	106.6 ± 9.75*, **	113.5 ± 6.2*	110.9 ± 11.42**	106.6 ± 9.75*, **	113.5 ± 6.2*
Diastolic blood pressure at rest, mm Hg	67.3 ± 6.72	66,2 ± 8.7	69.5 ± 7.6	67.3 ± 6.72	66,2 ± 8.7	69.5 ± 7.6	67.3 ± 6.72	66,2 ± 8.7	69.5 ± 7.6
Heart rate at rest, beats × min^{-1}	68.2 ± 8.9	69.7 ± 9.1	68.8 ± 8.3	68.2 ± 8.9	69.7 ± 9.1	68.8 ± 8.3	68.2 ± 8.9	69.7 ± 9.1	68.8 ± 8.3
BMI, kg/m^2	23.0 ± 4.7	23.9 ± 3.7	21.9 ± 6.2	23.0 ± 4.7	23.9 ± 3.7	21.9 ± 6.2	23.0 ± 4.7	23.9 ± 3.7	21.9 ± 6.2

Data are expressed as n (%), mean ± SD or median with IQR.
*$P<0.05$, **$P<0.01$.