Vasovagal syncope patients and the C825T GNB3 polymorphism

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ABSTRACT

Objective: The G protein is responsible for signal intracellular transduction and participates in cardiovascular reflexes. C825T polymorphism of the gene encodes the B3 subunit of G protein (GNB3) and causes the increased intracellular signal transduction. The aim was the evaluation GNB3 C825T polymorphism manifestation in vasovagal patients with no other diseases.

Methods: In 68 positive tilted patients genomic DNA was extracted from blood using an extraction kit. The GNB3 C825T polymorphism was diagnosed by restriction of the PCR amplicon with BseDI (MBI Fermentas). All patients were genotyped and next analyzed in regard to typical vasovagal history.

Results: The prevalence of genotype CC was 38%. Genotypes CT and TT were found equally in 31% of cases. The C allele in comparison to the T allele appeared in 54% vs 46% ($p>0.05$). Typical vasovagal history was present in 83% of patients. The frequency of GNB3 825T allele was significantly higher in patients with non-typical vasovagal history than in group with typical history ($p<0.001$).

Conclusions: Genotype CC GNB3 is the most popular in vasovagal patients. The predisposition to vasovagal syncope seems to be not associated with the GNB3 825T allele. Further studies are planned to clarify the genotype/phenotype relationship in vasovagal patients. *(Anadolu Kardiyol Derg 2007: 7 Suppl 1; 206-8)*

Key words: syncope, tilting, gene polymorphism

Introduction

Most of people have one or few episodes of syncope in their lives, while others subjects have frequent syncope (1). The classical vasovagal syncope (VVS) is generally not associated with other diseases and is benign and common (2). The most common age at it's first presentation is 13 years (3). The pathophysiology of VVS is not completely understood. To date some familial cases of VVS have been described (4-7). These observations have suggested that the predisposition to VVS could be associated with the genetic factors.

The molecular mechanism of signal intracellular transmission plays an important role in cardiovascular reflexes. The heterotrimeric G protein is responsible for signal transduction with a major receptors class G-protein-coupled receptors (GPCR), an integral membrane proteins that have seven amphipathic helices (7TM) (8), and participates in cardiovascular reflexes. Integral membrane proteins that have structure of 7TM are e.g. adrenergic receptors. A model of GPCR activation postulates that GPCR are in equilibrium between an activated and an inactivated state. G protein consisting of three subunits referred to as α, β and γ (9). The α-subunit contains the guanine nucleotide binding site and in inactivated form GDP is bound to $G\alpha$. Activation of G protein by an agonist-occupied 7TM receptor by e.g. catecholamines, disrupts the heterodimer $G\beta\gamma$ and $G\alpha$ resulting the GDP dissociation (10). Further transmission of the signal may take place via $G\alpha$ or via the $G\beta\gamma$.

The effector molecules of the heterodimer $G\beta\gamma$ are the specific subtypes of phospholipase C, β-adrenergic receptor kinase, and K^+ and Ca^{2+}-specific ion channels (11). There are 5 genes that code for GB subunits (GB1-GB5) (8). The gene encodes the $\beta 3$ subunit of the human G protein (GNB3) is located on chromosome 12p (12). A single nucleotide polymorphism C825T, located in exon 10, leads to an exchange at position 825 in the cDNA of the GNB3 the amino acid cytosine by thymidine (13), which causes the increased intracellular signal transduction and e.g. enhanced vascular reactivity (14). The hypothesis was the predisposition to VVS can be associated with this molecular mechanism. The aim was the evaluation C825T polymorphism in GNB3 in VVS patients.

Methods

In research were involved 68 consecutive patients with history of repeated syncope (more than 2 syncope in last 3 months) after the positive outcome of head-up tilt testing (HUTT) and with no other diseases. Typical vasovagal history (1) was assessed. The main demographic and clinical characteristics of studied patients are shown in Table 1. The HUTT was performed using the tilt table SP-1 with a foot support and straps. During the procedure the electrocardiogram (ECG) and blood pressure were supervised continuously. The HUTT consisted of the 20-min supine pre-tilt phase and passive phase with the patient tilted to 60 degrees. The passive phase of Italian protocol went on for 20 min (15). If syncope did not occur in the passive phase of HUTT, the upright position was continued with a 20-min drug challenge phase. The

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provocation was performed by the administration of 400 micrograms of nitroglycerine in spray. The results were considered positive in the case of syncope associated with marked reduction of blood pressure and / or heart rate (2).

Everyone signed up the voluntary agreement form before enrollment to the study. Used protocol obtained the Local Bioethics Committee's acceptation. All patients were genotyped for GNB3 C825T polymorphism and next analyzed in regard to typical vasovagal history.

Genotyping

DNA extraction from cellular blood components was performed with the Chemagic DNA Blood 100 kit. The polymorphism was detected by restriction fragment length polymorphism (RFLP). Genomic DNA was amplified with the usage of the following primer pair:

forward 5'-TGA CCC ACT TGC CAC CCG TGC-3'

and reverse 5'-GCA GCA GCC AGG GCT GGC-3' (synthesized by Eurogentec).

The polymerase chain reaction was carried out in a final volume of 25 µl containing 2 mM of $MgCl_2$, 0.25 µM of each dNTP and 10 µM of each primer. After an initial denaturation step at 94 °C for 5 min, the samples were subjected to 35 cycles at 94 °C for 1 min, 60 °C for 45 seconds, and 72 °C for 1 minute, followed by a final extension of 5 minutes at 72 °C. The PCR amplicon was restricted with BseDI (Fermentas) at 55 °C for 3 hours. The DNA fragments were separated on the polyacrylamide gel (40%) and visualized under UV light after ethidium bromide staining. The undigested 268-bp product represents the T allele, whereas a C allele results in two bands (116 bp and 152 bp).

Statistical analyses

Data are presented as mean value±standard of the mean. The significance of differences between groups was assessed using Student's t-test. Qualitative data expressed as a percentage were compared by Chi-square test with Yates correction. Statistically significant difference was defined as $p<0.05$. All analyses were performed using the Statistica version 5.0 PL (StatSoft Poland) statistical software.

Table 1. Patients' characteristics

Age, years	35.7±17.8
Male, n (%)	14 (21)
Mean number of syncope	19±34.2
Duration of syncopal history, years	7.9±9.5
Typical vasovagal history, n (%)	56 (83)
Data are expressed as n (%) or mean±SD.	

Results

In positive tilted patients, there was a prevalence of genotype CC (Table 2). Typical vasovagal history was present in 83% of patients ($p<0.001$). The strong association between the genotype and typical vasovagal history ($\chi^2=14.92$, C-Pearson=0.42, $p<0.001$) was found. All homozygotes CC had the typical history contrary to patients with non-typical history (Table 2). The manifestation of alleles is shown in Table 2. The strong association between the alleles and typical vasovagal history ($\chi^2=18.64$, $P<0.001$) was found. The frequency of GNB3 825C allele was significantly higher in patients with typical vasovagal history than in patients with non-typical history and the manifestation of GNB3 825T allele prevailed in patients with non-typical history ($p<0.001$) (Table 2).

Discussion

We aimed to examine the association between the molecular background and vasovagal syncope. To date there were no publications about genetic mechanism and predisposition to vasovagal syncope. In vasovagal patients, we found strong association between the GNB3 C825 allele and typical vasovagal history. The GNB3 825T allele was predominant in patients with non-typical history. To date this polymorphism of GNB3 has been investigated e.g. in hypertension (14), left ventricular hypertrophy (16), obesity (14) and orthostatic hypotension (17). The present study is the first detailed characterization of the C825T polymorphism manifestation in the gene encodes the β3 subunit of the human G protein in vasovagal patients. It provides new insight but raises several important questions. The GNB3 825T allele favors the generation of the split variant G β3s, which causes increased signal transmission and ultimately enhanced vascular reactivity (18), and proliferation of the smooth muscle cells and cardiac myocytes (19). The detailed molecular mechanism of influence the studied polymorphism on syncopal effect in vasovagal patients is unknown.

In conclusion, we have characterized vasovagal patients in regard to GNB3 C825T polymorphism and typical history. Further population-based and molecular studies could lead to an enhanced precision in the prediction of the risk for vasovagal syncope in individuals with these nucleotide exchanges in GNB3.

Table 2. Manifestation of genotypes and alleles in studied patients

Parameters	Total group (n=68)	Typical VVS history (n=56)	Non-typical VVS history (n=12)	p for typical vs non-typical VVS history
Genotype CC, n (%)	26 (38)	26 (47)**	0	-
Genotype CT, n (%)	21 (31)	18 (32)	3 (25)*	>0.05
Genotype TT, n (%)	21 (31)	12 (21)	9 (75)	<0.01
*p<0.05 for CT vs TT, **-p<0.01 for CC vs TT				
Allele C, n (%)	73 (54)	71 (63)	3 (12.5)	<0.001
Allele T, n (%)	63 (46)	41 (37)	21 (87.5)	<0.001
p for allele C and T comparisons	>0.05	<0.001	<0.001	
VVS- vasovagal syncope				

Conclusions

Genotype CC in the gene that encodes the β3 subunit of the human G protein is the most popular in patients with vasovagal syncope. The predisposition to vasovagal syncope seems to be not associated with the GNB3 825T allele. Further studies are planned to clarify the genotype/phenotype relationship in vasovagal patients.

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References

1. Alboni P, Brignole M, Menozzi C, Raviele A, Del Rosso A, Dinelli M, et al. Clinical spectrum of neurally mediated reflex syncopes. Europace 2004; 5: 55-62.
2. Brignole M, Alboni P, Benditt D, Bergfeldt L, Blanc JJ, Bloch Thomsen PE, et al. Guidelines on management (diagnosis and treatment) of syncope-update 2004. Executive summary. Eur Heart J 2004; 25: 2054-72.
3. Sheldon RS, Sheldon AG, Connolly SJ, Morillo CA, Klingenheben T, Krahn AD, et al. Age of first faint in patients with vasovagal syncope. J Cardiovasc Electrophysiol 2006; 17: 49-54.
4. Newton JL, Kenny R, Lawson J, Frearson R, Donaldson P. Prevalence of familial history in vasovagal syncope and haemodynamic response to head up tilt in first degree relatives. Clin Auton Res 2003; 13: 22-6.
5. Marquez MF, Urias KI, Hermosillo AG, Jardon JL, Iturralde P, Colin L, et al. Familial vasovagal syncope. Europace 2005; 7: 472-4.
6. Newton JL, Kerr S, Pairman J, McLaren A, Norton M, Kenny RA, et al. Familial neurocardiogenic (vasovagal) syncope. Am J Med Genet 2005; 133: 176-9.
7. Camfield PR, Camfield C. Syncope in childhood: a case control clinical study of the family tendency to faint. Can J Neurol Sci 1990; 17: 306-8.
8. Bourne HR. How receptors talk to trimeric G proteins. Curr Opin Cell Biol 1997; 9: 134-42.
9. Hepler JR, Gilman AG. G proteins. Trends Biochem Sci 1992; 17: 383-7.
10. Hamm HE. The many faces of G protein signaling. J Biol Chem 1998; 273: 669-72.
11. Sprang SR. G protein mechanisms: insights from structural analysis. Annu Rev Biochem 1997; 66: 639-78.
12. Ansari-Lari MA, Muzny DM, Lu J, Lu F, Lilley CE, Spanos S, et al. A gene-rich cluster between the CD4 and triosephosphate isomerase genes at human chromosome 12p13. Genome Research 1996; 6: 314-26.
13. Rosskopf D, Busch S, Manthey I, Siffert W. G protein B3 gene. Structure, promoter, and additional polymorphisms. Hypertension 2000; 36: 33-41.
14. Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, et al. Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet 1998; 18: 45-8.
15. Del Rosso A, Bartoli P, Bartoletti A, Brandinelli-Geri A, Bonechi F, Maioli M, et al. Shortened head-up tilt testing potentiated with sublingual nitroglycerin in patients with unexplained syncope. Am Heart J 1998; 135: 564-70.
16. Poch E, Gonzalez D, Gomez-Angelats E, Enjuto M, Pare JC, Rivera F, et al. G-protein beta (3) subunit gene variant and left ventricular hypertrophy in essential hypertension. Hypertension 2000; 35: 214-8.
17. Tabara Y, Kohara K, Miki T. Polymorphisms of genes encoding components of the sympathetic nervous system but not the renin-angiotensin system as risk factors for orthostatic hypotension. J Hypertens 2002; 20: 651-6.
18. Baumgart D, Naber C, Haude M, Oldenburg O, Erbel R, Heusch G, et al. G protein beta3 subunit 825T allele and enhanced coronary vasoconstriction on α2-adrenoceptor activation. Circ Res 1999; 85: 965-9.
19. Lindemann M. Virchow S, Ramann F. The G beta3 subunit 825T allele is a genetic marker for enhanced T cell response. FEBS Lett 2001; 495: 82-6.