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CLINICAL STUDY

Gene polymorphisms of renin angiotensin system and serotonin transporter gene in patients with vasovagal syncope

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Abstract: *Objective* of this study was to compare the distribution frequencies of gene polymorphisms of renin--angiotensin and serotonin system in patients with positive and negative head- up tilt test (HUT).
Methods: DNA from 191 patients (mean age 44+ 18 years, 61 men) was collected. HUT was positive in 117 and negative in 74 patients. Following gene polymorphisms were determined by the PCR method: ACE insertion/deletion (I/D ACE), angiotensinogen (AGT) (M 235), angiotensin II receptor (ATR1) (A 1166C) and serotonin transporter (SERT) polymorphism (5HTTLPR).
Results: No significant differences in the distribution of gene polymorphisms between syncopal patients with positive and negative HUT were dectected. Distribution of polymorphisms included: I/D ACE: II 19 vs 20 %, ID 55 vs 52 %, DD 26 vs 28 %. Angiotensinogen gene polymorphism MM 27 % vs 30 %, MT 48 % vs 46 %, TT 25 % vs 24 %. ATR1 polymorphism AA 44 vs 32 %, AC50 vs 60 %, CC 6 vs 8 %, 5HTTLPR serotonin transporter gene polymorphism LL 42 vs 43 %, SL 41 vs 39 %, SS 17 vs 18 %.
Conclusions: An association between polymorphisms of ACE, AGT, ATR1 and SERT gene, and predisposition to VVS was not proven by the present study *(Tab. 2, Ref. 22)*. Full Text (Free, PDF) *www.bmj.sk.*
Key words: familial vasovagal syncope, gene polymorphism, renin angiotensin system, serotonin transporter.

In literature, several cases of familiar vasovagal syncope are described (1–5).

It is presumed that genetic predisposition to vasovagal syncope (VVS) exists. However, specific character of this genetic predisposition is unknown. Multifactor heredity type with participation of several interacting genes combined with environmental influences (infection, drugs, nutrition) is suspected. Genes that might be related to the genetic background of VVS still remain unidentified.

Pathogenetic mechanism of VVS syncope has not been clarified in details. It is a reflex mediated abrupt drop of blood pressure with a variable degree of bradycardia. During orthostasis, baroreceptors are activated and subsequently sympathetic activity rises reflexively. An increased symphatetic activity leads to a vigorous heart-chambers contraction thus stimulating chamber afferent paths. The process might lead to an inhibitive response similar to that of Bezold-Jarish reflex resulting in hypotension and bradycardia (6).

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There is evidence that VVS pathogenesis is possibly related to the malfunction of the extracellular sodium regulation and excretion. VVS patients are marked with a low sodium excretion within 24 hours in urine (7) with sodium excretion correlated with the clinical severity and VVS symptoms (8, 9).

Renin-angiotensin system is one of the primary mechanisms that participate in regulation of sodium homeostasis in organism and influences blood pressure regulation. Several gene polymorphisms of the renin–angiotensin system have been described so far, as well as their role in cardiovascular and metabolic diseases.

An insertion/deletion (I/D) gene polymorphism of angiotensin converting enzyme (ACE) is the most studied one and has been described in relation to the hypertension, left ventricular hypertrophy and coronary artery disease (10–12).

The angiotensinogen gene polymorphism (M 235) resides in substitution of methionin for threonin at position 235. It is responsible for an increased level of circulating angiotensinogene. This polymorhism has also been described in relation to hypertension and coronary artery disease (13, 14).

Angiotensin II receptor type 1 (ATR1) is an effector of renin- angiotensin system. A1166C genetic polymorphism is related to low blood pressure and strong vasomotoric activity of coronary arteries (15). AGT1 gene polymorphism A1166C is a high-risk factor for hypertension (16), mitral valve prolapse (17) and left- ventricular hypertrophy (18).

A role of central nervous system neurotransmitters in the pathogenesis of vasovagal syncope was suggested, serotonin and endogenous opioids being the most frequently studied. Given

the fact that a direct intracerebral administration of serotonin in experimental animals leads to sympathetic withdrawal accompanied with hypotension, the role of serotonin in pathogenesis of VVS is speculated (19).

An indirect evidence of the role of serotoninergic system in the development of VVS comes from chronic administration of selective-serotonin reuptake inhibitors (SSRI). Chronic administration of SSRI decreases concentration of serotonin in synaptic cleft. Chronic SSRI administration leads to down-regulation of central serotoninergic receptors due to their longterm exposition to increased serotonin levels and finally leads to diminished serotoninergic activity. SSRI were shown to be effective in the treatment of VVS (fluoxetine, sertralien, paroxetine) (20). The intrasynaptic level of serotonin depends on the activity of serotonin transporter (SERT). Serotonin transporter gene polymorphism (5HTTLPR) has two allelic forms, short – S and long – L. L allele results in two or three times higher gene transcriptions compared to S allele. In literature, S allele was associated with psychiatric diagnoses, namely obsessive-compulsive disease, depression, anxiety and alcohol abuse (21, 22). The relationship of SERT polymorphism and orthostatic intolerance has yet not been described.

The aim of the present study was to study the association of gene polymorphisms in the renin–angiotensin system and serotonin transporter with the vasovagal syncope by comparing distribution frequencies of genotypes in patients with positive and negative head-up tilt test.

Methods

One hundred and ninety one patients with the history of syncope were included into study (61 men, 130 women, mean age 44±18). Head-up tilt test (HUT) was performed as a part of standard evaluation of syncope, which consisted of history, physical examination, electrocardiogram, carotid sinus massage, echocardiography, 24 ambulatory ECG monitoring and head-up tilt test. If needed, a neurological evaluation with EEG and CT of brain as well as an invasive electrophysiology study was carried out.

HUT was performed by Italian protocol. Patient was positioned upright at an angle of 60 degree for 20 minutes. If syncope with hypotension and/or bradycardia developed during the tilt, patient was lowered immediately to supine position and the test was terminated. If the passive test was negative, it was followed by nitroglycerine stimulated HUT without lowering patient to supine position. Nitroglycerine stimulated HUT was performed for additional 15 minutes after a sublingual administration of 0.4 mg nitroglycerine in a spray form.

Detection of gene polymorphisms

Blood sample of 4ml of venous blood was taken before the HUT from the cubital vein.

Genomic DNA was extracted from blood lymphocytes. All samples were immediately centrifuged, frozen and stored at minus 80 degrees Celsius until determination. Following gene polymorphisms were detected: ACE I/D genetic polymorphism, angiotensinogen gene polymorphism (M 235), angiotensin II receptor polymorphism (A 1166C) and serotonin transporter gene polymorphism (5HTTLPR).

Standard polymerase chain reaction was used to determine genetic polymorphisms.

Statistic analysis

Differences in respective genetic polymorphisms in the group of HUT-positive and HUT-negative patients were tested by the chi square test and Fischer exact test.

Differences in age and number of syncope episodes between HUT-positive and HUT-negative groups were tested by the unpaired Student t-test. p value <0.05 was considered statistically significant.

Tab. 1. Clinical characteristics of HUT positive and HUT negative patients.

	HUT positive (n=117)	HUT negative (n=74)	Statistical significance
Subjects	117	74	
Men	37	24	p=0.90
Women	80	50	p=0.90
Mean age	43±19	46±18	p=0.27
Cardiovascular diseases	14 (12%)	41 (55%)	p<0.001
Mean number of syncope episodes	4.2±1.8	2.8±1.2	p<0.,001

Results

Head-up tilt test (HUT) was positive in 117 patients (37 men, 80 women, average age of 43±19) and negative in 74 patients (24 men, 50 women, average age of 46±18).

No significant differences in age and gender were observed between HUT-positive and HUT-negative patients. The mean number of syncopal episodes was higher in HUT-positive patients. The presence of organic heart disease was higher in HUT-negative group of patients. This corresponds with the well-known fact that the presence of organic heart disease increases the probability of arrhythmogenic syncope (Tab. 1).

In 74 patients with the negative HUT, following etiology of syncope was diagnosed: sinus node disease (19 patients), atrioventricular block (16 patients), supraventricular tachycardia (14 patients), ventricular tachycardia (2 patients), situational reflex syncope (7 patients), orthostatic hypotension (2 patients). In 14 patients, the etiology of syncope remained unknown.

No significant differences in distribution of ACE, angiotensinogen, angiotensin II receptor and serotonine transporter genotypes were detected between the HUT-positive and HUT--negative patients (Tab. 2).

Tab. 2. Distribution of genotypes in HUT positive and HUT negative patients.

Polymorphism	Genotype (n=117)	HUT positive (n=74)	HUT negative significance	Statistical
ACE I/D	I/I	22 (19%)	14(20%)	p=0.99
	I/D	64 (55%)	39 (52%)	p=0.90
	D/D	31 (26%)	21(28%)	p=0.90
AGT M355 T	M/M	32 (27%)	22 (30%)	p=0.84
	M/T	56 (48%)	34 (46%)	p=0.91
	T/T	29 (25%)	18 (24%)	p=0.99
ART1 A1166C	A/A	52 (44%)	32 (48%)	p=0.77
	A/C	58 (50%)	30 (45%)	p=0.63
	C/C	7 (6%)	5 (7%)	p=0.45
SERT 5HTTLPR	L/L	50 (42%)	32 (43%)	p=0.99
	S/L	49 (41%)	29 (39%)	p=0.82
	S/S	18 (17%)	13 (18%)	p=0.84

Discussion

Familiar occurrence of VVS has been observed mostly in children and adolescents with the first manifestation of syncope prior to 20th year of age. Siblings and parents of probands are most commonly affected. Mathias et al. described this phenomenon with as many as 27 out of 30 children with VVS (73 %). The occurrence in other relatives was more rare (27 %) (5).

When studying familiar occurrence, it is necessary to take into account not only the genetic background but also the possibility of environmental influence that might be of a similar nature when speaking about a family. Thus environmental factors might contribute to the familiar occurrence of the disease.

In this respect, Camfield & Camfield observed the VVS occurrence in 30 children and their friends. Friends of the probands were free of syncope episodes. Syncope, however, was diagnosed significantly more often in a first-degree relative of syncopal children than in friends of these children (27 from 30 cases versus 8 from 24 friends) (3).

Similarly, Mathias described a case of VVS occurrence in several siblings of the proband but not in their adopted siblings (4). These findings show that VVS familiar occurrence cannot be interpreted exclusively as a result of environmental impacts but it is inevitable to consider also a genetic predisposition within the scope of multifactorial heredity.

This fact is supported also by the VVS occurrence in twins. Márquez has observed the VVS occurrence in two couples of monozygotic twins (one of boys, another of girls) as well as the occurrence of syncope in other members of the affected families (1).

A large patient group of 603 patients with VVS was published by Newton. Familiar occurrence was reported in 19 % of cases. First-degree relatives were affected in 37 % of cases (145 out of 389 cases). Eleven first-degree relatives of VVS patients have undergone HUT. HUT was positive in all with a previous history of a spontaneous syncope. These findings can be interpreted as a fact that the first-degree relatives had a predisposition to vasovagal reaction which, however, was not manifested as a syncope (23).

In 62 medical students, a familiar occurrence of syncope was observed in 32 % of cases. The probability of having the syncope was higher when syncope was manifested in both parents as compared to one of the parents. A syncope occurrence in mother only increased its occurrence with both sexes. A syncope manifestation in father increased the probability of syncope only in sons (24).

Polymorphisms of certain genes were suggested to be responsible for the genetic predisposition to VVS. Among them, renin angiotensin system polymorphism and polymorphisms of genes encoding the sympathetic nervous system are the most commonly examined in patients with various forms of orthostatic intolerance and blood pressure disorders.

Newton studied the ACE I/D genetic polymorphisms in patients with the history of VVS in 165 subjects. Results were compared to a large national control population (more then 6000 subjects) without history of VVS. There were no significant differences in distribution frequencies of ACE genotypes in vasovagal patients compared to the control population. Control group consisted of subjects without the history of syncope (other than vasovagal) was declared as a limitation of the study. Control group composed of subjects with non- vasovagal syncope was suggested to be more suitable for further research (25).

In our study, the control group consisted of patients with syncope, which etiology was different from vasovagal etiology. Negativity of HUT was considered as an evidence for such etiology. A limitation of our study is relatively small group of subjects. Similarly as in the study of Newton, no association between gene polymorphisms of renin–angiotensin system and serotonin trenasporter and the results of head up tilt test was found in our study.

Distribution frequencies of genotypes reported in our study in patients with VVS were similar to distribution frequencies in normal population described in literature. For the I/D ACE polymorphism, following frequencies were described by Newton in control population: II 23 %, ID 50 % and DD 27 %. Frequencies of MM 31 %, MT 50 %, TT 19 % for M235T angiotensinogen polymorphism and AA 50 %, AC 45 %, CC 5 % for A1166C ATR1 polymorphism were reported in literature (14, 26). Distribution frequencies of SERT polymorphism in normal popula-

tion were also reported to be similar to our findings in vasovagal patients (LL 34 %, SL 50 %, SS 16 %) (27). Thus it seems that polymorphisms of renin–angiotensin and serotonin transporter gene are not associated with the predisposition to VVS.

Other gene polymorphisms (especially those genes encoding sympathetic nervous system activity) may be more suitable for further research in the area of genetic background of VVS.

Tabara observed genetic polymorphisms of renin angiotensin system and sympathetic nervous system in 415 patients with orthostatic hypotension. There was no significant association between the ACE I/D polymorphism, angiotensinogen M235T polymorphism and orthostatic hypotension. On contrary, polymorphism of the Gs protein alfa subunit (GNAS1) was associated with changes of blood pressure in orthostasis (28).

Recently, an association of positive tilt table testing to a beta 1 adrenergic receptor polymorphism with a Gly to Arg switch at position 389 was reported in literature (29).

In conclusion, polymorphisms of ACE, AGT, ATR1 and SERT gene are not associated with the predisposition to vasovagal syncope.

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