

Association of Polymorphisms in Endothelin-1 and Endothelin Receptor A Genes With Vasovagal Syncope

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Received March 25, 2021

Accepted September 29, 2021

Epub Ahead of Print January 19, 2022

Summary

The endothelin system may play a role in the pathogenesis of vasovagal syncope (VVS) because it is implicated in blood pressure regulation. We hypothesized that endothelin-related genetic polymorphisms might modulate susceptibility to VVS. This study aimed to evaluate the possible influence of endothelin-1 (*EDN1*) and endothelin receptor A (*EDNRA*) gene variants on the occurrence of tilt-induced VVS and autonomic nervous system activity during the head-up tilt test (HUT). Results were expressed as mean \pm SEM. In 254 patients with recurrent syncope (age 45.33 ± 1.22 years, 94 males, 160 females), heart rate variability (HRV) was measured during HUT. *EDN1* rs5370 G>T and *EDNRA* rs5333 T>C gene polymorphisms were assessed using high-resolution melting analysis. There was no statistically significant association between polymorphisms *EDN1* rs5370 and *EDNRA* rs5333 and positivity of HUT or hemodynamic types of VVS. Patients with GT or TT genotypes at the rs5370 locus of the *EDN1* had significantly higher values of high-frequency (HF) and the standard deviation of the average NN intervals at the time of the syncope, and they tended to have lower low-frequency (LF) and LF/HF ratio when compared to homozygotes (GG). No statistically significant differences were found in HRV parameters concerning the *EDNRA* rs5333 genotypes. Our findings suggest the potential role of *EDN1* rs5370 variants in regulating autonomic nervous activity and pathogenesis of VVS.

Key words

Endothelin • Endothelin receptor • Syncope • Heart rate variability • Head-up tilt test

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Introduction

Vasovagal syncope (VVS) is a transient loss of consciousness caused by hypoperfusion of the brain due to sudden arterial hypotension, functional bradycardia, or even asystole; typically, it is caused by increased parasympathetic activity and decreased sympathetic tone. VVS is the most common type of syncope and typically occurs in young people and more frequently in females. The prevalence decreases with age. It is estimated that up to 10 % of the population may experience a single vasovagal presyncope or syncope during their lifetime; nevertheless, some people have a strong predisposition, and such events may occur very frequently. VVS outcomes are excellent; however, injuries during the loss of postural tone can be potentially dangerous.

Pathophysiological mechanisms of VVS are complex and remain unclear. Dysregulation of the autonomic nervous system plays a critical role. Other mechanisms are being studied, including neurohumoral background, autoimmunity, and (notably) genetics. Because the autonomic nervous system is regulated by numerous humoral substances (e.g. endothelin, adenosine, catecholamines, and the renin-angiotensin-aldosterone system), genetic alterations of these pathways

can affect the susceptibility to VVS [1]. Polymorphisms of susceptibility genes, especially genes for adenosine receptors, alpha and beta-adrenergic receptors, angiotensin-converting enzyme, angiotensin receptors, G-proteins, endothelin, and its receptors, have already been investigated with inconsistent results [1-5].

Among the neurohumoral systems that participate in the pathophysiology of syncope, the endothelin system is one of the most often studied. The endothelin system consists of three endothelin isopeptides (endothelin-1, endothelin-2, and endothelin-3), their activating peptidases, and converting enzymes. The effects of endothelins are mediated by three types of G-protein-coupled receptors (endothelin receptor types A, B1, B, and C) [6].

Endothelin-1 (EDN1) is the most critical member of the endothelin system. It is secreted predominantly by vascular endothelial cells; however, it is also produced by the heart, lungs, kidneys, gastrointestinal tract, CNS, and hypothalamic-pituitary complex. In addition to its physiological role in controlling vascular tone and blood pressure, it has inotropic and mitogenic properties, affects natriuresis, stimulates the renin-angiotensin-aldosterone system, potentiates central and peripheral sympathetic activity, affects platelet activation, and has corticotropin-releasing hormone-like activity. Its receptors mediate the effects of EDN1; type A (EDNRA) is located primarily in vascular smooth muscle cells, mediating vasoconstriction, whereas type B (EDNRB) is located on endothelial cells, causing vasodilation due to nitric oxide release [6,7].

To date, a few types of endothelin gene polymorphisms have been studied in connection with various diseases. The most commonly studied *EDN1* 3A/4A (rs10478694) polymorphism is associated with higher levels of ET-1 [8]. Therefore, we speculated that other genetic variations affecting EDN1 levels would modulate the susceptibility to VVS.

Aims

To determine the associations of *EDN1* rs5370 and *EDNRA* rs5333 gene variants with the occurrence of tilt-induced vasovagal syncope and autonomic nervous system activity during the head-up tilt test (HUT).

Subjects and Methods

Ethics

The Ethical Committee of Faculty of Medicine

of P.J. Safarik University in Kosice approved the study. All patients provided written informed consent.

Subjects

Of 254 patients with recurrent syncope (mean age 45.33 ± 1.22 years), we performed the HUT in 94 males (age 45.22 ± 2.0 years) and 160 females (age 45.39 ± 1.54 years). There were 6.1 ± 1.3 syncopal events and 12.6 ± 1.7 presyncopal events. In 114 patients, VVS was induced during HUT (HUT+ group). HUT+ and HUT- subgroups did not significantly differ in age (43.25 ± 2.6 years and 46.48 ± 2.4 years, respectively), sex (females: 78 and 82, respectively), in the prevalence of comorbidities, or the number of syncopal episodes on history (6.26 ± 0.85 and 6.02 ± 1.4 , respectively). The mean time of VVS during HUT was 22.7 ± 2.6 min.

All patients underwent standard evaluations consisting of clinical history, physical examination, 12-lead electrocardiography (ECG), echocardiography, and ECG Holter monitoring. If indicated, invasive electrophysiological studies, neurological examinations, and electroencephalography were also performed. Results of these examinations did not explain the etiology of syncope. Exclusion criteria were structural heart diseases, history of myocardial infarction, arrhythmias, neurological diseases, untreated thyroid disorders, anemias, oncological diseases, and alcoholism or any use of drugs of abuse.

The most common comorbidities were thyroid diseases (45 patients), arterial hypertension (34 patients, 27 of whom were on antihypertensive therapy), while patients with potent hypotensive treatment (three or more drugs) were excluded. Less prevalent comorbidities were anxiety and depression (22 patients), gastroesophageal reflux diseases (21 patients), and diabetes mellitus (eight patients) without diabetic autonomic polyneuropathy.

A group of 112 healthy individuals without a history of syncope was included to compare the distribution of alleles with the healthy population. This group consisted of 70 males and 42 females, with mean age 41.7 ± 16.3 years. Every participant provided written informed consent.

HUT

According to the Italian protocol, HUT was performed in the 60-degree position (20 min passive phase, followed by 15 min of the nitroglycerin-stimulated test). The result was classified according to The Vasovagal Syncope International Study classification [9].

Heart rate variability (HRV) was evaluated during the HUT in 5-minute intervals using commercially available software (CardioScan, DM Software, USA). Evaluated parameters were very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) bands of HRV and standard deviation of averages of normal-to-normal interval (SDANN).

Genotyping

Genotyping of *EDNI* rs5370 and *EDNRA* rs5333 polymorphisms were performed using real-time PCR. DNA was isolated from peripheral blood using the Wizard Genomic DNA Isolation kit (Promega Ltd., USA). Variants of interest were analyzed using high-resolution melting analysis after real-time PCR in the presence of Melt Doctor HRM Master Mix (Applied Biosystems™) on the Eco Real-Time PCR System (Illumina, Inc., San Diego, CA, USA).

Sequences of oligonucleotides were following:

EDNRA forward:

5'-CTCTTTGCTGGTTCCTATTC-3'

EDNRA reverse:

5'-ACAGTTTCTTCAATATACGGCT-3'

EDNI forward:

5'-CGCTCGCTGCCTTCTCTCT-3'

EDNI reverse:

5'-GGGTTTCCTCAGATCTCAAAGCG-3'

Genotypes were identified using Eco TM Software 4.1.

Statistical analysis

For statistical analysis, the JMP program version 13.0 (SAS Institute) was used. Continuous variables are expressed as mean \pm standard error of the mean. The chi-square test was used to compare nominal variables. Analysis of variance and the Student's *t*-test were used to compare continuous parametric variables. In the case of variables with unequal means, Welch's test was used. P-values less than 0.05 were considered statistically significant.

Results

HUT was positive in 114 patients and negative in 140 patients. The distribution of genotypes and alleles for *EDNI* rs5370 and *EDNRA* rs5333 gene polymorphisms in the patients' group according to positivity of HUT and in the control group of healthy subjects are presented in Table 1. The genotypes and

alleles distribution were consistent with Hardy-Weinberg equilibrium for both polymorphisms ($p=0.82$; $p=0.54$).

We found no significant association of *EDNI* and *EDNRA* polymorphisms with HUT positivity, nor were there any significant differences in genotype distribution among various hemodynamic types of VVS (according to VASIS classification) (Table 2).

We compared hemodynamic parameters (i.e. blood pressure, heart rate, and HRV) between the group of major homozygotes and a group of patients with at least one minor allele. There was a significantly higher basal heart rate (HR) (70.7 ± 1.0 vs. 74.5 ± 1.1 bpm (beat per minute), $p=0.0155$), HR after 5 min of active standing (81.0 ± 1.3 vs. 84.8 ± 1.3 bpm, $p=0.04$) and higher basal systolic blood pressure (BP) (121.97 ± 1.8 vs. 128.2 ± 1.8 mm Hg, $p=0.0155$) found in patients carrying at least one minor allele C of *EDNRA* rs5333 polymorphism. Other parameters were not statistically significant (Table 3). There were no differences in these parameters between major homozygotes and minor allele carriers in *EDNI* rs5370 gene polymorphism (Table 3).

HRV parameters during HUT are presented in Table 4. The most significant finding was higher values of SDANN during the entire tilt test in patients with genotypes GT or TT of *EDNI* polymorphism. We found significantly higher VLF activity 5 min before the end of HUT in minor allele carriers (1459.4 ± 230 vs. 2288.8 ± 294.7 , $p=0.0276$) and higher HF power at the end of the test in this subgroup (187.4 ± 22.2 vs. 261.5 ± 28.5 , $p=0.042$), respectively. LF values and LF/HF ratio tended to be lower at the time of syncope in this group (which can be interpreted as a decline in sympathetic tone corresponding to increased parasympathetic tone parameters as described above); however, the difference was not statistically significant. There were no significant differences in any of the HRV parameters between subgroups for *EDNRA* rs5333 gene variants.

Discussion

We found no significant association between *EDNI* rs5370 or *EDNRA* rs5333 gene variants and the prevalence of tilt-induced VVS. The distribution was comparable to the healthy population. Nevertheless, we identified higher basal HR and systolic BP in individuals carrying at least one minor allele in the studied *EDNRA* gene variant. In terms of HRV, patients with genotype GT or TT in *EDNI* rs5370 polymorphism presented significantly increased SDANN and VLF during the

HUT. Our findings suggest a possible role of polymorphisms in the endothelin gene and the gene encoding its receptor, modulating the activity or responses of the endothelin system, affecting hemodynamic parameters and the activity of the autonomic nervous system. The prevalence of various polymorphisms in endothelin system genes has been studied; however, no research has been done on *EDNI* rs5370 variant; therefore, there are no data to compare

our results.

There is a growing body of evidence to suggest the role of the neurohumoral background in the pathophysiology of VVS. A study showed the possible participation of the adrenergic and adenosinergic systems, antidiuretic hormone, natriuretic peptides, and the endothelin system directly affecting blood pressure and indirectly through regulation of the autonomic nervous system [10].

Table 1. Distribution of genotypes and alleles frequencies for *EDNI* rs5370 and *EDNRA* rs5333 polymorphisms.

Genotype/Alleles	Total n=254 (%)	HUT+ n=114 (%)	HUT- n=140 (%)	Healthy subjects n=112 (%)	P*
<i>EDNI</i> rs5370					
GG	160 (63.0)	75 (65.8)	85 (60.7)	69 (60.7)	NS
GT	80 (31.5)	32 (28.1)	48 (34.3)	37 (33)	NS
TT	14 (5.5)	7 (6.1)	7 (5.0)	6 (5.3)	NS
G	400 (78.7)	182 (79.8)	218 (77.9)	175 (78.1)	NS
T	108 (21.3)	46 (20.2)	62 (22.1)	49 (21.9)	NS
	HWE (p=0.345)				
<i>EDNRA</i> rs5333					
	Total n=252 (%)	HUT+ n=113 (%)	HUT- n=139 (%)	Healthy subjects N=112 (%)	
TT	132 (52.4)	57 (50.4)	75 (53.9)	71 (63.4)	NS
TC	101(40.1)	47 (41.6)	54 (38.9)	35 (31.2)	NS
CC	19 (7.5)	9 (7.96)	10 (7.2)	6 (5.4)	NS
T	365 (72.4)	161(71.2)	204 (73.4)	177 (79)	NS
C	139 (27.6)	65 (28.8)	74 (26.6)	47 (21)	NS
	HWE (p=0.958)				

* between HUT-positive, HUT-negative group, and control group of healthy subjects. HUT – head-up tilt test; *EDNI* – endothelin-1; *EDNRA* – endothelin receptor A.

Table 2. Distribution of genotype frequencies for *EDNI* rs5370 and *EDNRA* rs5333 polymorphisms in the HUT+ group, according to hemodynamic type of syncope.

<i>EDNI</i> rs5370	VASIS 1 n=42 (%)	VASIS 2 n=7 (%)	VASIS 3 n=65 (%)	P
GG	29 (69.0)	5(71.4)	41 (63.1)	NS
GT	11(26.2)	2 (28.6)	19 (29.2)	NS
TT	2 (4.8)	0 (0.0)	5 (7.7)	NS
<i>EDNRA</i> rs5333				
	VASIS 1 (n=42)	VASIS 2 (n=6)	VASIS 3 (n=65)	
TT	22 (52.4)	2 (33.3)	33 (50.8)	NS
TC	18 (42.9)	4 (66.7)	25 (38.4)	NS
CC	2 (4.7)	0 (0.0)	7 (10.0)	NS

VASIS 1 – a mixed type of VVS; VASIS 2 – a cardioinhibitory type of VVS and VASIS 3 – a vasodepressor type of VVS.

Table 3. Hemodynamic parameters in patients according to *EDN1* rs5370 and *EDNRA* rs5333 genotypes during HUT.

Gene	<i>EDN1</i> rs5370		P	<i>EDNRA</i> rs5333		P
	GG (n=156)	GT+TT (n=86)		TT (n=122)	CC+TC (n=119)	
Supine SBP (mm Hg)	126.45 ± 1.6	122.95 ± 2.2	NS	121.97 ± 1.8	128.2 ± 1.8	0.01
Supine DBP (mm Hg)	77.36 ± 0.9	76.02 ± 1.3	NS	76.1 ± 1.1	77.7 ± 1.1	NS
SBP min (mm Hg)	76.3 ± 3.4	79.4 ± 4.5	NS	73.6 ± 3.8	81.3 ± 3.8	NS
DBP min (mm Hg)	50.9 ± 2.6	49.8 ± 3.4	NS	48.4 ± 3.0	52.7 ± 2.9	NS
Max decline in SBP (mm Hg)	50.8 ± 3.1	45.3 ± 4.2	NS	49.5 ± 3.6	47.6 ± 3.6	NS
Max decline in DBP (mm Hg)	46.1 ± 3.1	54.7 ± 4.4	NS	48.6 ± 3.7	49.7 ± 3.7	NS
HR basal (bpm)	73.1 ± 0.95	71.6 ± 1.3	NS	70.7 ± 1.1	74.5 ± 1.1	0.01
HR after standing (bpm)	83.5 ± 1.15	81.8 ± 1.5	NS	81.0 ± 1.3	84.8 ± 1.3	0.04
HR max (bpm)	108.5 ± 1.8	111.6 ± 2.4	NS	107.9 ± 2.1	111.8 ± 2.0	NS
HR min (after NTG) (bpm)	76.5 ± 1.77	76.9 ± 2.4	NS	76.9 ± 2.0	76.9 ± 2.0	NS
Max decline in HR (bpm)	33.6 ± 1.97	35.7 ± 2.6	NS	33.3 ± 2.2	35.2 ± 2.2	NS

SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; NTG – nitroglycerin.

Endothelin is a potent vasoconstrictor; therefore, it may participate in arterial hypertension and orthostatic intolerance syndromes. However, published results are conflicting. Kaufmann *et al.* found a significant increase in endothelin levels during tilt test in patients with VVS, whereas patients with primary autonomic failure had decreased BP accompanied by no rise in endothelin concentrations [11]. Magerkurth *et al.* found elevated basal and stimulated plasma EDN1 levels in patients with VVS [12]. Few recent studies agree with this hypothesis that patients prone to vasovagal syncope have permanently higher blood levels of EDN1 [13,14]. Other studies did not confirm the previous results. Williford *et al.* did not demonstrate any differences in plasma EDN1 concentrations in patients with tilt-induced VVS [15]. Hammrefors *et al.* found significantly lower EDN1 levels in patients with asystolic cardioinhibitory VVS than in the control group [16]. Clinical research results remain controversial, and it remains unknown whether changes in endothelin levels cause or are caused by VVS (i.e. a compensatory mechanism of vasodilation and hypotension).

Endothelin affects vascular smooth muscle cells differently, depending on its levels. Very high levels of EDN1 cause vasoconstriction; however, only slightly increased levels have a vasodilatory effect [17,18]. There is concern about explaining the possible cause of elevated endothelin levels in patients suffering from VVS. EDN1 is encoded by a gene located on the short arm of chromosome 6. Gene polymorphisms causing

overexpression and, therefore, higher endothelin levels were suggested [1,2,19].

To date, many polymorphisms in the *EDN1* gene (i.e. the 3A/4A (rs10478694) polymorphism, rs1800541, rs5370, rs2070699, rs3087459, rs5369, rs2071942, rs1476046, rs2071943, rs9296345, and rs1800997) have been identified to be associated with various diseases, especially those related to the cardiovascular system, including hypertension, ischemic heart disease, atrial fibrillation, preeclampsia, asthma, hearing impairment, thyroid cancer, obesity, and sleep apnea [20,25].

To date, only one study has focused on endothelin gene polymorphisms and VVS. Sorrentino *et al.* demonstrated that patients carrying the 4A allele in 3A/4A *EDN1* polymorphism are significantly more likely to develop vasodepressor syncope in HUT [8]. According to an *in vitro* study of Popowski *et al.* cells with homozygous 4A/4A genotype showed higher expression of EDN1 mRNA and a higher level of EDN1 compared to 3A/4A and 3A/3A cells [26]. A Japanese *in vivo* study found significantly higher levels of plasma EDN1 in patients with at least one 4A allele present in their genotype [27]. Most probably, this polymorphism is associated with increased levels of endothelin.

Our study focused on the *EDN1* rs5370 G/T (Lys198Asn) polymorphism because previous investigators described its effect on serum and plasma endothelin levels; however, the data are not very clear, as in the previously mentioned polymorphism. Tousulis *et al.* and Barden *et al.* described significantly higher

Table 4. Parameters of heart rate variability during HUT according to genotypes.

	<i>EDN1</i> rs5370			<i>EDNRA</i> rs5333		
	GG (n=117)	GT+TT (n=71)	P	TT (n=89)	TC+CC (n=91)	P
VLF						
Min 0	1675.5 ± 188.6	1794.5 ± 242	NS	1840.8 ± 212.9	1540.6 ± 202.9	NS
Min 5	1478.7 ± 170.5	1860.8 ± 218.9	NS	1690.8 ± 182.5	1457.5 ± 173.9	NS
Min 15	1902.2 ± 282.7	1906.2 ± 362.9	NS	2154.2 ± 322.8	1624.8 ± 307.6	NS
5 min before the end	1459.42 ± 230	2288.8 ± 294.7	0.028	1851.2 ± 267.1	1717.7 ± 254.6	NS
Syncope/end	3153.5 ± 862	4796 ± 1102	NS	4639.2 ± 987	3062.9 ± 945.5	NS
LF						
Min 0	664.2 ± 56.3	756 ± 72.3	NS	661.8 ± 64.8	733.4 ± 61.8	NS
Min 5	722.1 ± 64.5	814.6 ± 82.8	NS	782.85 ± 73.6	717.5 ± 70.1	NS
Min 15	744.2 ± 61.8	735.9 ± 79.3	NS	750.1 ± 70.9	739.3 ± 67.6	NS
5 min before the end	758.8 ± 61	767.9 ± 80	NS	807.95 ± 70.6	719 ± 67.3	NS
Syncope/end	836.7 ± 70.9	761.9 ± 90.9	NS	812.7 ± 80.8	793.1 ± 77.04	NS
LF/HF						
Min 0	3.17 ± 0.23	3.63 ± 0.3	NS	3.36 ± 0.26	3.34 ± 0.25	NS
Min 5	3.34 ± 0.27	3.82 ± 0.36	NS	3.5 ± 0.32	3.6 ± 0.3	NS
Min 15	4.48 ± 0.4	3.6 ± 0.5	NS	4.1 ± 0.4	4.2 ± 0.4	NS
5 min before HUT end	5.56 ± 0.5	4.66 ± 0.7	NS	5.5 ± 0.6	5.0 ± 0.6	NS
Syncope/HUT end	6.02 ± 0.52	4.86 ± 0.67	NS	5.4 ± 0.6	5.8 ± 0.5	NS
HF						
Min 0	295 ± 34	336.2 ± 43.5	NS	279.7 ± 38.5	331.2 ± 36.5	NS
Min 5	288 ± 34.2	369.5 ± 44	NS	326.3 ± 38.9	303.7 ± 37.2	NS
Min 15	244.7 ± 30.9	319.1 ± 39.9	NS	268.1 ± 35.7	273.8 ± 33.8	NS
5 min before HUT end	218.7 ± 25.5	268.9 ± 32.8	NS	225.7 ± 29.5	247.1 ± 27.8	NS
Syncope/ HUT end	187.4 ± 22.2	261.5 ± 28.5	0.04	212.8 ± 25.3	209 ± 24.1	NS
SDANN						
Min 0	52.1 ± 2.1	53.9 ± 2.6	NS	53.7 ± 2.3	51.3 ± 2.2	NS
Min 5	50.6 ± 2.04	59.23 ± 2.62	0.0099	56.1 ± 2.4	52.15 ± 2.3	NS
Min 15	49.3 ± 1.99	57.1 ± 2.6	0.017	52.2 ± 2.3	52.4 ± 2.2	NS
5 min before HUT end	50.9 ± 2.3	59.1 ± 2.96	0.03	54.4 ± 2.7	53.6 ± 2.6	NS
Syncope/HUT end	60.4 ± 3.7	71.2 ± 4.7	0.07	67.5 ± 4.2	61.3 ± 4.0	NS

HUT – head-up tilt test, VLF – very low frequencies, LF – low frequencies, HF – high frequencies, SDANN – the standard deviation of the average NN intervals, min – minute, NS – not significant.

EDN1 levels in TT homozygotes [23,25]. On the other hand, an *in vitro* study by Tanaka *et al.* found no significant difference between EDN1 or big-EDN1 (preproEDN1) levels in the Asn-type and Lys-type transfectants.

In another small human *in vivo* association study in hypertensive patients, plasma levels of EDN1 were not different in those with minor alleles from major ones [27]. As already mentioned, EDN1 is a potent vasoconstrictor that acts as a modulator of vasomotor tone and vascular remodelling; therefore, most studies investigated the possible association of rs5370 polymorphism with arterial hypertension, systolic BP,

and some kidney diseases [28,29]. The rs5370 polymorphism was associated with a higher prevalence of arterial hypertension in other studies [28,29]. Dubovyk *et al.* found an increased risk of large vessel stroke in people carrying minor allele T [30]. A recently published study showed a possible disease-modifying role in atrial fibrillation in patients undergoing coronary bypass grafting (CABG) [22]. To the best of our knowledge, no studies about the rs5370 G/T polymorphism in the *EDN1* gene in patients with VVS have been published to date.

Although we found no significant associations of HUT results with *EDN1* rs5370 G/T polymorphism, our findings suggest that carriers of at least one minor allele

have greater parasympathetic activity during HUT than major homozygotes. Because decreased sympathetic tone and increased parasympathetic tone mediate vasovagal reactions, the potential pathogenic role of endothelin rs5370 G/T polymorphism in VVS cannot be excluded. There is some evidence that endothelin probably increases sympathetic activity. EDN1 primarily affects the ventral part of the medulla oblongata in the brain in very low doses, leading to direct activation of sympathetic activity and indirectly by modulation of baroreflex sensitivity [31]. Another possible explanation of elevated sympathetic activity could be that people with the GT or TT genotype have altered EDN1 levels, and increased sympathetic activity is only a compensatory mechanism.

The effects of EDN1 are mediated by types A and B endothelin receptors. Almost all tissue types have differing densities, mainly in the brain and vascular endothelial cells [32]. Changes in their sensitivity may influence the final effect of endothelin, although total endothelin levels remain normal. Activation of EDNRA causes vasoconstriction that usually leads to an increase in BP, whereas EDNRB mediates vasodilation and increased natriuresis, which finally leads to arterial hypotension [7,8]. The most commonly studied polymorphism is rs5333, also known as the H323H T/ polymorphism, in the *EDNRA* gene. There has been published only one study about this *EDNRA* polymorphism and syncope. Sorrentino *et al.* found no association between susceptibility to tilt-induced VVS and *EDNRA* rs5333 gene polymorphism [8]. Our results agree with the previously published study. We did not identify a more common occurrence of HUT positivity; however, we found a significantly higher basal systolic BP and higher basal HR in patients carrying at least one recessive allele. This finding suggests that the *EDNRA* gene polymorphism may be associated with altered

affinity or sensitivity for its primary ligand (EDN1). Because the data remain inconsistent and our study was a pilot, more studies are needed.

Limitations

The main limitation of our study was that we considered the absence of plasma endothelin level. Doing so would help determine whether the investigated gene polymorphisms caused changes in effective concentrations of EDN1.

Conclusions

Endothelin may participate in the pathophysiology of VVS. The altered function of the endothelin system may also have a genetic background in some cases. In the present study, we identified no relationship between specific genotypes at the *EDN1* rs5370 and *EDNRA* rs5333 gene loci and susceptibility to tilt-induced VVS. On the other hand, we found significantly higher parasympathetic activity during HUT in patients carrying at least one minor allele of the *EDN1* rs5370 polymorphism; therefore, the potential role of this polymorphism in the regulation of autonomic nervous activity and pathogenesis of VVS cannot be excluded. This is the first study investigating a relationship between this *EDN1* rs5370 variant and syncope to the best of our knowledge. Further studies are needed to validate our results.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

A grant from Slovak Scientific Agency – project VEGA (1/0769/19) supported this study.

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