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Combined renin–angiotensin system gene polymorphisms and outcomes in coronary artery disease — a preliminary report

Zofia Dzielińska[1], Łukasz A. Małek[2], Marta Roszczynko[3], Małgorzata Szperl[3], Marcin Demkow[1], Jacek Kądziela[2], Aleksander Prejbisz[4], Tomasz Zieliński[5], Andrzej Januszewicz[4], Witold Rużyłło[6]

[1]Department of Coronary Artery Disease and Structural Heart Diseases, Institute of Cardiology, Warsaw, Poland
[2]Department of Interventional Cardiology and Angiology, Institute of Cardiology, Warsaw, Poland
[3]Laboratory of Molecular Biology, Institute of Cardiology, Warsaw, Poland
[4]Department of Hypertension, Institute of Cardiology, Warsaw, Poland
[5]Department of Heart Failure and Transplantology, Institute of Cardiology, Warsaw, Poland
[6]Institute of Cardiology, Warsaw, Poland

Abstract

Background: Common variants of the renin–angiotensin system (RAS) genes have been linked to a higher risk of coronary artery disease (CAD) and its complications.

Aim: To determine the prognostic significance of a combination of three common polymorphisms of RAS genes (angiotensin converting enzyme — *ACE* Ins/Del, angiotensin receptor type 1 — *AGT1R* A1166C and angiotensinogen — *ATG* M235T) in patients with CAD.

Methods: The study included 216 patients (mean age 58 ± 9 years, 74% male) prospectively followed for a mean 41 ± 17 months. The end-points were all-cause mortality, myocardial infarction, stroke or the need for coronary revascularisation.

Results: An end-point occurred in 41 (19%) patients. None of the polymorphisms analysed separately was associated with the end-point. Odds ratios were calculated for different combinations of analysed alleles to determine their relation to outcomes. Based on the cut-off points of odds ratios, the study group was divided into three subgroups: 55 patients without *ATG* 235T allele (T– subgroup); 100 patients with *ATG* 235T allele alone or *ATG* 235T allele combined with *ACE* Del allele or *AGT1R* 1166C allele (T+ or T+1 subgroup); and 61 patients with all three variants (T+2 subgroup). Multivariate analysis showed that the only independent predictor of the endpoint was an increasing number of variant genes (HR = 2.6, 95% CI 1.4–4.9, p = 0.002).

Conclusions: Co-existing angiotensinogen M235T *AGT* polymorphism and two other common polymorphisms of the RAS genes are related to adverse events in patients with CAD.

Key words: coronary artery disease, polymorphisms, renin–angiotensin system, outcomes

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INTRODUCTION

Various polymorphisms have been shown to influence the risk of coronary artery disease (CAD) or myocardial infarction (MI) [1, 2]. Although the list of genetic variants associated with cardiovascular (CV) diseases is still expanding, renin–angiotensin system (RAS) genes belong to the group of polymorphisms which has been studied more than any other to date [3–5]. This is related to the fact that RAS exerts many effects on the CV system. It plays a key role in the stimulation of vascular smooth muscle cell proliferation, intimal fibrosis, in-

Address for correspondence:
Zofia Dzielińska, MD, PhD, Department of Coronary Artery Disease and Structural Heart Diseases, Institute of Cardiology, ul. Alpejska 42, 04–628 Warszawa, Poland, tel: +48 22 34 34 340, fax: +48 22 613 38 19, e-mail: zdziel@wp.pl
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flammatory reactions, prothrombotic processes and plaque calcification [6–9]. Three common polymorphisms of different RAS genes have been most frequently linked to the risk of CAD or MI in single polymorphism studies [10]. Those variants are the insertion/deletion (Ins/Del) polymorphism of the angiotensin-converting enzyme gene (*ACE,* GeneBank accession no. EU332840); the A1166C polymorphism of the angiotensin II type 1 receptor gene (*AGTR1,* GeneBank accession no. AY436325); and the M235T polymorphism of angiotensinogen gene (*AGT,* GeneBank accession no. EU326304). Because of the high frequency of RAS genetic variants in the general population, it is likely that two or more of them are found in one patient. As those polymorphisms affect RAS function at different points by increasing the plasma levels of angiotensin converting enzyme and angiotensinogen, or by modifying the number/function of angiotensin II type 1 receptors, their co-existence may increase the risk of CV disease beyond that found for single polymorphisms. However, most data on co-existing RAS polymorphisms come from case control studies comparing frequencies of genotypes/alleles in patients with CAD or after MI and controls at a single time point [10–17]. Therefore, the aim of our analysis was to determine the prognostic significance of a combination of three common polymorphisms of the RAS genes (angiotensin converting enzyme — *ACE* Ins/Del, angiotensin receptor type 1 — *AGT1R* A1166C, and angiotensinogen — *ATG* M235T) in patients with CAD.

METHODS

Study group and end-points

The analysis included 216 consecutive patients (mean age 58 ± 9 years, 74% males) with CAD confirmed by coronary angiography (presence or history of at least one stenosis > 50%) who did not require revascularisation at the beginning of the study. Before catheterisation, clinical examination was performed to determine demographics, cardiac history, CV risk factors, features of extracoronary vascular disease and related comorbidities.

Hypertension was defined as systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg or current antihypertensive treatment. Significant coronary artery stenosis was defined as > 50% stenosis of the left main coronary artery or > 70% stenosis of any of the other coronary arteries. Patients with significant stenosis of more than two arteries or left main coronary artery were considered as having multivessel disease. Hyperlipidaemia was diagnosed if the total cholesterol level was > 5.2 mmol/L or low-density lipoprotein (LDL) cholesterol level was > 3.4 mmol/L, or if the patient was taking a lipid-lowering drug because of hyperlipidaemia. Obesity was defined as body mass index (BMI) > 30. Diabetes mellitus type 2 was diagnosed according to WHO criteria. Estimated creatinine clearance (CrCl) was measured from the baseline serum creatinine using the Cockroft-Gault formula. All subjects underwent standard transthoracic echocardiography at baseline with estimation of the left ventricular ejection fraction. Atheromatous renal artery stenosis was defined as > 50% stenosis of the renal artery. Peripheral artery disease was defined as the presence of > 50% narrowing in the carotid or femoral artery on duplex Doppler ultrasonography.

All patients were prospectively followed for a mean 41 ± 17 months. The end-point was all-cause death, MI, stroke or the need for coronary revascularisation (percutaneous coronary intervention [PCI] or coronary artery bypass grafting [CABG]). The decision to perform PCI/CABG was based on signs and/or symptoms of ischaemia. An effort was made to contact all patients after their discharge from hospital. Information obtained from patients was supported by medical records where available. Data on the survival of patients who had not been contacted directly was checked in the Polish Electronic National Death Registry. Those collecting the clinical data and information on outcomes were blinded to the results of genetic analyses.

The study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Cardiology, Warsaw, Poland. Written informed consent was obtained from each patient.

Genetic analysis

A 5 mL venous blood sample was drawn into an EDTA sample tube. Genomic DNA was extracted from peripheral leukocytes using the phenol method.

The *ACE* Ins/Del polymorphism was estimated according to the nested polymerase chain reaction (PCR) method, using the following primers: sense 5′-GCCCTGCA-GGTGTGTG-CAGCATGT-3′, anti-sense 5′-GGATGGCTCTCCCCGCCT-TGT-CTC-3′, and the Del/Del genotype was confirmed also by nested PCR using the following starters: sense 5′-TGG-GACCACAGCGCCCGCCACTAC-3′, anti-sense 5′-TCG-CCAGCCCTCCCATGCCCATAA-3′. DNA fragments obtained as a result of the amplifying reaction were separated electrophoretically in a 2% agarose gel.

For the *AGT1R* A1166C polymorphism, we used a forward starter 5′-GCAGCA-CTTCACTACCAAATGGGC-3′ and reverse starter 5′-CAGGACAAAAGCAGG-CTAGGGAGA-3′ and Hae III restriction enzyme as described previously [18]. Digestion products were than separated electrophoretically.

The *AGT* M235T polymorphism was assessed using the previously desribed method [19]. In brief, the following primers were used: sense 5′-CCGTTTGTGCAGGGCCTGCTCTCT-3′ and antisense 5′-GCCAGGGTGCTG-TCCACACTGACTCCC-3′. The PCR products were subjected to Box I digestion and DNA fragments were separated electrophoretically.

Statistical analysis

Baseline characteristics of study patients are summarised in terms of frequencies and percentages for categorical variables, and by means and SD for continuous variables. Categorical variables were compared by either Fisher exact or χ^2 test

for trend and continuous variables by student t-test, Mann-Whitney U test for unpaired samples or Kruskal-Wallis test, as appropriate. To test for Hardy-Weinberg equilibrium for each polymorphism, the expected genotype numbers were calculated from the allele frequencies, and deviation from the observed genotype numbers was determined using χ^2 test. Odds ratios were calculated for different combinations of analysed alleles to determine their relation to outcomes. The occurrence of the primary end-point in relation to hyplotypes was analysed using Kaplan-Meier curves and compared by the log-rank test. Multivariate Cox model incorporating all variables with $p < 0.1$ from the univariate analysis was performed. A p value (two-tailed) of < 0.05 was considered statistically significant and confidence intervals (CI) were 95%. All data analyses were performed using MedCalc software software version 9.4.2.0 (MedCalc, Mariakerke, Belgium).

RESULTS

Analysed polymorphisms consisted of the following numbers and frequencies of homozygous reference as well as heterozygous and homozygous variants: *ACE* Ins/Del – Ins/Ins 60 (28%), Ins/Del 104 (48%), Del/Del 52 (24%); *AGT1R* A1166C – AA 96 (44%), AC 104 (48%), CC 16 (8%); *AGT* M235T – MM 54 (25%), MT 97 (45%), TT 65 (30%). All of the polymorphisms were in Hardy-Weinberg equilibrium ($p = 0.59$ for *ACE* Ins/Del, $p = 0.09$ for *AGT1R* A1166C, and $p = 0.14$ for *ATG* M235T). No differences in terms of patient baseline characteristics or pharmacological treatment were observed between carriers and non-carriers of the studied polymorphisms.

Primary end-point

Patients who achieved the primary end-point were more likely to have multivessel CAD and peripheral artery disease and showed a trend towards older age, higher prevalence of diabetes and more frequent history of previous MI and PCI (Table 1). None of the genetic variants analysed separately was associated with the end-point. However, a trend was observed towards a higher prevalence of 235T *ATG* allele in patients with events compared to those without events (Table 2). This relationship was not observed for other polymorphisms. Subsequently, an individual risk of events for each

Table 1. Baseline characteristics and pharmacological treatment according to outcome

Parameter	Without events N = 175 (81%)	With events N = 41 (19%)	Univariate p	Multivariate HR (95% CI), p
Male gender	127 (72.6)	32 (78.0)	0.60	
Mean age [years]	57.5 ± 9.2	60.5 ± 9.5	0.06	1.2 (0.85–1.8), 0.29*
Diabetes	24 (13.7)	11 (26.8)	0.07	1.7 (0.8–3.5), 0.18
Hypertension	175 (100)	41 (100)	1.00	
Hyperlipidaemia	134 (76.6)	35 (85.4)	0.40	
Obesity	53 (30.3)	9 (22.0)	0.36	
Former or current smoking	110 (62.9)	31 (75.6)	0.16	
Familial history of CAD	73 (41.7)	16 (39.0)	0.85	
Multivessel CAD	97 (55.4)	32 (78.0)	0.01	2.0 (0.9–4.4), 0.10
Previous MI	86 (49.1)	27 (65.9)	0.07	1.4 (0.7–2.8), 0.29
Previous PCI	62 (35.4)	21 (51.2)	0.09	1.8 (1.0–3.2), 0.05
Previous CABG	10 (5.7)	3 (7.3)	0.72	
LVEF [%]	60.9 ± 10.3	58.4 ± 12.1	0.24	
eGFR [mL/min]	62.8 ± 13.8	60.6 ± 14.7	0.37	
PAD	12 (6.9)	8 (19.5)	0.03	1.7 (0.8–3.8), 0.20
ARAS	18 (10.3)	7 (17.0)	0.33	
SBP [mm Hg]	136.4 ± 23.2	137.1 ± 17.4	0.90	
DBP [mm Hg]	83.2 ± 14.3	83.6 ± 12.2	0.86	
Aspirin	153 (87.4)	39 (95.1)	0.18	
Statins	113 (64.6)	26 (63.4)	0.97	
Fibrats	25 (14.3)	5 (12.2)	1.00	
Beta-blockers	149 (85.1)	35 (85.4)	0.90	
ACEI or ARB	140 (80.0)	28 (68.3)	0.16	

*For every ten year increase; ACEI — angiotensin-converting enzyme inhibitor; ARAS — atheromatous renal artery stenosis; ARB — angiotensin receptor blocker; CABG — coronary artery bypass grafting; CAD — coronary artery disease; CI — confidence interval; DBP — diastolic blood pressure; eGFR — estimated glomerular filtration rate; HR — hazard ratio; LVEF — left ventricular ejection fraction; MI — myocardial infarction; PAD — peripheral artery disease; PCI — percutaneous coronary intervention; SBP — systolic blood pressure

Table 2. Distribution of analysed polymorphisms according to outcome

Polymorphism	Genotype/allele	Without events N = 175 (81%)	With events N = 41 (19%)	P
ACE	Ins/Ins	51 (29.2)	9 (22.0)	
	Ins/Del	83 (47.4)	21 (51.2)	
	Del/Del	41 (23.4)	11 (26.8)	0.80*
	Ins/Del + Del/Del	124 (70.8)	32 (78.0)	0.46**
	Ins	185 (52.9)	39 (47.6)	
	Del	165 (47.1)	43 (52.4)	0.46***
AGT1R	A/A	82 (46.8)	14 (34.1)	
	A/C	81 (46.3)	23 (56.1)	
	C/C	12 (6.9)	4 (9.8)	0.76
	A/C + C/C	93 (53.2)	27 (65.9)	0.19
	A	245 (70.0)	51 (62.2)	
	C	105 (30.0)	31 (37.8)	0.22
ATG235	M/M	48 (27.4)	6 (14.6)	
	M/T	79 (45.2)	18 (43.9)	
	T/T	48 (27.4)	17 (41.5)	0.13
	M/T + T/T	127 (72.6)	35 (85.4)	0.12
	M	175 (50.0)	30 (36.6)	
	T	175 (50.0)	52 (63.4)	0.04

For each polymorphism: *p-value for homozygote variant *vs* heterozygote + homozygote reference according to end-point; **p-value for homozygote variant + heterozygote vs. homozygote reference according to end-point; ***p-value for variant alleles vs. reference alleles according to end-point

Table 3. Risk of end-point for each combination of alleles

Haplotype			OR (95% CI)	P
***ACE* Ins/Del**	***AGT1R* A1166C**	***ATG* M235T**		
Ref.				
Ins	A	M		
Single polymorphism				
Del	A	M	1.57 (0.06–40)	0.78
Ins	C	M	1.94 (0.06–57)	0.70
Ins	A	T	2.66 (0.1–60)	0.54
Dual polymorphism				
Del	C	M	1.97 (0.1–44)	0.67
Del	A	T	2.25 (0.1–44)	0.59
Ins	C	T	2.47 (0.1–52)	0.56
Triple polymorphism				
Del	C	T	4.68 (0.2–89)	0.30

CI — confidence interval; OR — odds ratio

combination of analysed alleles was calculated (Table 3). Based on the cut-off points of the effect size (odds ratio intervals 1 to 2, 2 to 3 and so on) the study group was divided into three subgroups: 55 patients without *ATG* 235T allele (T– subgroup); 100 patients with *ATG* 235T allele or *ATG* 235T allele combined with *ACE* Del or *AGT1R* 1166C alleles (T+ or T+1 subgroup); and 61 patients with all three variants (T+2 subgroup). No differences in terms of patient baseline characteristics or pharmacological treatment were observed between subgroups (Table 4). The number and frequency of events included in the end-point in each of the three subgroups is shown in Table 5. The Kaplan-Meier curves for the end-point in the three subgroups are plotted on Figure 1 (log--rank p = 0.01). Multivariate analysis incorporating mean age,

Table 4. Baseline characteristics and pharmacological treatment in each of the subgroups

Parameter	T– subgroup N = 55 (26%)	T+ or T+1 subgroups N = 100 (46%)	T+2 subgroup N = 61 (28%)	P for trend
Male gender	41 (75)	72 (72)	46 (75)	0.90
Mean age [years]	55.4 ± 8.9	59.0 ± 9.7	58.2 ± 8.6	0.08
Diabetes	7 (13)	19 (19)	9 (15)	0.76
Hypertension	55 (100)	100 (100)	61 (100)	1.00
Hyperlipidaemia	41 (75)	78 (78)	50 (82)	0.33
Obesity	16 (29)	30 (30)	16 (26)	0.81
Former or current smoking	36 (65)	69 (69)	36 (59)	0.45
Familial history of CAD	25 (45)	41 (41)	23 (38)	0.35
Multivessel CAD	29 (53)	65 (65)	35 (57)	0.65
Previous MI	30 (55)	51 (51)	32 (52)	0.83
Previous PCI	23 (42)	39 (39)	21 (34)	0.71
Previous CABG	3 (5)	8 (8)	2 (3)	0.46
LVEF [%]	58.0 ± 11.1	61.0 ± 10.5	60.7 ± 11.6	0.38
eGFR [mL/min]	64.2 ± 14.6	61.3 ± 15.0	62.8 ± 12.4	0.65
PAD	5 (9)	10 (10)	5 (8)	0.88
ARAS	6 (11)	12 (12)	7 (11)	0.93
SBP [mm Hg]	134.6 ± 22.8	137.2 ± 24.7	137.1 ± 16.1	0.49
DBP [mm Hg]	86.3 ± 16.3	83.1 ± 13.6	82.1 ± 11.2	0.36
Aspirin	45 (82)	90 (90)	57 (93)	0.05
Statins	31 (56)	69 (69)	39 (64)	0.42
Fibrats	10 (18)	12 (12)	8 (13)	0.45
Beta-blockers	44 (80)	90 (90)	50 (82)	0.81
ACEI or ARB	43 (78)	75 (75)	50 (82)	0.59

Abbreviations as in Table 1

Table 5. Combined end-point and its components in each of the subgroups

	T– subgroup (n = 55)	T+ or T+1 subgroups (n = 100)	T+2 subgroup (n = 61)
Combined end-point	6 (10.9%)	17 (16.8%)	18 (29.5%)*
Death	2 (3.6%)	5 (5.0%)	5 (8.2%)
Myocardial infarction	2 (3.6%)	8 (7.9%)	7 (11.5%)
Stroke	0	2 (2.0%)	1 (1.6%)
PCI	4 (7.2%)	8 (7.9%)	7 (11.5%)
CABG	2 (3.6%)	8 (7.9%)	8 (13.1%)

*T+2 *vs* T–: p = 0.02, T+2 *vs* T+ or T+1: p = 0.09, T+ or T+1 *vs* T–: p = 0.45, p for trend = 0.0099; abbreviations as in Table 1

diabetes, multivessel CAD, previous MI, previous PCI, peripheral artery disease and subgroup coding showed that the only independent predictor of the end-point was an increasing number of variant genes (HR = 2.6, 95% CI 1.4–4.9, p = 0.002).

DISCUSSION

Activation of the RAS increases the risk of adverse CV events in patients with CAD. This may be related to atherosclerotic plaque instability due to increased expression of adhesion molecules and increased oxidative stress caused by local pro-

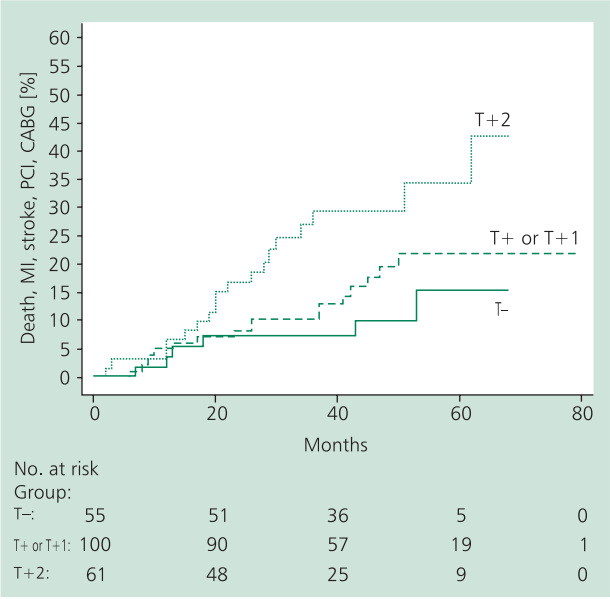

Figure 1. Kaplan-Meier curves for the end-point in each of the subgroups (log-rank p = 0.01); abbreviations as in Table 1

duction of angiotensin II [20]. Angiotensin II can also promote atherosclerosis by activation of genes related to coronary calcifications in the vascular smooth muscle cells of coronary arteries [8, 9]. In addition, RAS influences other mechanisms of CAD progression such as proliferation of smooth muscle cells, inflammatory and prothrombotic processes or lipid accumulation [6, 7].

Some common polymorphisms of the RAS genes may influence its function. Carriage of the *ACE* Del allele leads to increased serum levels of circulating enzyme [21]. Similarly, T allele of *AGT* was related to increased concentrations of angiotensinogen in plasma [22, 23]. Finally, A1166C *AGTR1* polymorphism may be engaged in post-transcriptional receptor modification which alters cell signalling [24, 25]. Other investigators have shown that the 1166C allele may also lead to elevation of AGTR1 levels by abrogating miRNA regulation and gene expression [26].

The important role of RAS in the pathophysiology of atherosclerosis and functional relevance of common RAS polymorphisms described above are responsible for the fact that these polymorphisms may be associated with CAD and/or MI [4, 5, 27]. However, only a few studies have addressed the issue of the coexisting effects of RAS polymorphisms. In most of them, coexisting RAS gene variants were linked to CAD or MI, but information about those events was collected retrospectively from patients' clinical records [10–17].

To the best of our knowledge, there has only been one prospective study on the relation between the *ACE* Ins/Del polymorphism and the *AGTR1* A1166C polymorphism and outcomes [20]. The study included 885 male subjects and analysed the occurrence of combined CV events similar to those studied in our study, including death, MI, non-scheduled PCI or CABG and stroke or transient ischaemic attack during two years of observation. The authors found that carriers of combined *ACE*-Del/Del and *AGT1R*-CC genotypes had more ischaemic events during follow-up compared to those carrying other genotype combinations. The *ATG* M235T variant was not analysed in this study. We have prospectively confirmed in patients with CAD that co-existence of the 235T *AGT* allele and two other genetic variants of RAS increases the risk of long-term CV events compared to other combinations of the analysed polymorphisms. Our results are supported by the findings of other studies on the M235T *AGT* polymorphism, which showed that this polymorphism is the strongest determinant of progression of coronary artery calcifications [9] and, in contrast to other polymorphisms, is related to the extent of angiographic lesions on coronary angiography [22].

A possible mechanism of the increase in coronary events in patients with CAD and coexisting polymorphisms is a potential acceleration of RAS function over the one observed in carriers of single or double polymorphism, leading to faster atherosclerotic plaque growth and/or a higher risk of plaque instability.

Our study has potential clinical implications, as the prevalence of all three genetic variants is not uncommon (it was found in more than 25% of our patients). Theoretically, knowledge of the genotype of renin–angotensin genes could be used to direct treatment with RAS inhibitors [28]. So far, the relationhip between common RAS polymorphisms and response to treatment in CAD remains unresolved, as single studies often show conflicting results [29–32].

Limitations of the study

Our study had some limitations. Firstly, the studied group was not very large, but the power of the study calculated for this sample size and more than 4.5 higher risk of events in the T+2 subgroup in comparison to reference was calculated to exceeded 95%. We are aware that the sample size might have been too low to fully exclude the association of single polymorphisms or some well established risk factors (such as diabetes or peripheral artery disease) with clinical events. However, this does not negate the main finding of the study.

Secondly, in the final analysis we have not included information on another *AGT* polymorphism, a T174M substitution previously linked to the extent of CAD [33]. This was due to the fact that the frequency of MM genotype in our group was low (3.2%) and there was almost equal distribution of MT and MM genotypes between patients with and without events (p = 0.94).

Finally, we did not analyse the activity of angiotensin converting enzyme and plasma levels of angiotensinogen. Although single genotype-phenotype associations were confirmed in earlier studies [21–26], there is no report regarding the activity of angiotensin converting enzyme and plasma levels of angiotensinogen in combined RAS gene variants.

CONCLUSIONS

In summary, we conclude that co-existing angiotensinogen M235T *AGT* polymorphism and two other genetic variants of RAS (A1166C of *AGT1R* and Ins/Del of *ACE*) increase the risk of adverse events in patients with CAD.

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Współwystępowanie polimorfizmów genów układu renina–angiotensyna a rokowanie u osób z chorobą wieńcową — doniesienie wstępne

Zofia Dzielińska[1], Łukasz A. Małek[2], Marta Roszczynko[3], Małgorzata Szperl[3], Marcin Demkow[1], Jacek Kądziela[2], Aleksander Prejbisz[4], Tomasz Zieliński[5], Andrzej Januszewicz[4], Witold Rużyłło[6]

[1]Klinika Choroby Wieńcowej i Strukturalnych Chorób Serca, Instytut Kardiologii, Warszawa
[2]Klinika Kardiologii i Angiologii Interwencyjnej, Instytut Kardiologii, Warszawa
[3]Klinika Biologii Molekularnej, Instytut Kardiologii, Warszawa
[4]Klinika Nadciśnienia Tętniczego, Instytut Kardiologii, Warszawa
[5]Klinika Niewydolności Serca i Transplantologii, Instytut Kardiologii, Warszawa
[6]Instytut Kardiologii, Warszawa

Streszczenie

Wstęp: Często występujące warianty genów układu renina–angiotensyna (RAS) są związane z większym ryzykiem wystąpienia choroby wieńcowej i jej powikłań.

Cel: Celem pracy była ocena znaczenia rokowniczego skojarzonego występowania trzech polimorfizmów genów RAS (enzymu konwertującego angiotensynę — *ACE* Ins/Del, receptora dla angiotensyny typu 1 — *AGT1R* A1166C oraz angiotensynogenu — *ATG* M235T) u osób z chorobą wieńcową.

Metody: Do badania włączono 216 pacjentów (średni wiek 58 ± 9 lat, 74% mężczyzn), których obserwowano prospektywnie przez średnio 41 ± 17 miesięcy. Punkt końcowy obejmował: śmiertelność całkowitą, zawał serca, udar mózgu i potrzebę rewaskularyzacji wieńcowej.

Wyniki: Punkt końcowy wystąpił u 41 (19%) chorych. Badane polimorfizmy oceniane oddzielnie nie były związane z punktem końcowym. W celu oceny związku poszczególnych kombinacji allelów z punktem końcowym dla każdego z nich obliczono iloraz szans jego wystąpienia. Na podstawie tak uzyskanych punktów odcięcia grupę podzielono na 3 podgrupy: 55 pacjentów bez allelu *ATG* 235T (podgrupa T–), 100 nosicieli allelu *ATG* 235T osobno lub w skojarzeniu z allelem *ACE* Del lub z allelem *AGT1R* 1166C (podgrupa T+ or T+1) i 61 nosicieli wszystkich 3 wariantów genowych (podgrupa T+2). Analiza wieloczynnikowa wykazała, że jedynym niezależnym czynnikiem rokowniczym była zwiększająca się liczba obecnych u pacjentów wariantów genowych (HR = 2,6; 95% CI 1,4–4,9; p = 0,002).

Wnioski: Współwystępowanie polimorfizmu M235T *AGT* i dwóch innych polimorfizmów genów RAS wiąże się z gorszym rokowaniem u osób z chorobą wieńcową.

Słowa kluczowe: choroba wieńcowa, polimorfizm, układ renina–angiotensyna, zdarzenia końcowe

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Adres do korespondencji:
dr n. med. Zofia Dzielińska, Klinika Choroby Wieńcowej i Strukturalnych Chorób Serca, Instytut Kardiologii, ul. Alpejska 42, 04–628 Warszawa, tel: +48 22 34 34 340, faks: +48 22 613 38 19, e-mail: zdziel@wp.pl
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