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Genetically determined enlargement of carotid body evaluated using computed angiotomography

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ABSTRACT

It has recently been established that carotid bodies play a significant role in the regulation of activities of the cardiovascular system as well as in the pathogenesis of arterial hypertension, heart failure and diabetes. Aim of study was to determinate the influence of polymorphisms within genes of the renin-angiotensin-aldosterone system (RAAS) on the volume of the carotid bodies (CB) in patients with hypertension (HTA).

The study group consisted of 77 patients with HTA. All patients were genotyped for single-nucleotide polymorphisms of genes coding for: angiotensinogen: rs4762, rs5049, rs5051 and rs699; angiotensin-converting enzyme: rs4343; angiotensin receptor type 1 gene (AGTR1): rs5182 and rs5186; and the aldosterone synthase: rs1799998. The estimation of volumes of CB ($V_{rCB+lCB}$) was based on computed tomography angiography.

Among individuals with essential hypertension certain relationships were documented between rs5182 and rs5186 polymorphisms of AGTR1 gene and rs1799998 polymorphism of CYP11B2 gene on one hand and the volume of carotid bodies on one other. Patients carrying the C alleles within the rs5182 and rs5186 of AGTR1 gene was associated with higher values of $V_{rCB+lCB}$. The carriage of the T allele in the rs5182 locus of the AGTR1 gene determine lower values of $V_{rCB+lCB}$.

In summary, in patients with HTA a higher volume of CB may be resulted from the presence of specific genotypes in RAAS.

1. Introduction

The renin-angiotensin-aldosterone system (RAAS) is the essential mechanism regulating the water-electrolyte balance of the organism (Bisping et al., 2014). According to the current knowledge, inappropriate regulation of the RAAS connected with its hyperreactivity is a crucial factor in the pathogenesis of arterial hypertension, atherosclerosis, coronary artery disease, cardiomyopathy, renal failure and diabetes (Ma et al., 2010; Chrysant, 2010; Te Riet et al., 2015; Patel and Mehta, 2012; Favre et al., 2015).

A numerous group of polymorphisms of genes coding particular elements of the RAAS has been identified. The description includes: polymorphisms of the angiotensin gene, angiotensin-converting enzyme gene, angiotensin II receptor type 1 gene, as well as polymorphisms of the aldosterone synthase gene. Moreover, relationships have been established between the occurrence of particular polymorphisms and the degree of expression of RAAS genes, corresponding with concentrations of RAAS components (Miller and Scholey, 2015; Staessen et al., 1997).

The carotid bodies (CB), small-sized anatomical structures located on both sides in the region of the bifurcation of common carotid artery, are the main constitutive forms of the system of chemical control of the regulation of respiratory action (Fitzgerald et al., 2009; Ponikowski and Banasiak, 2001). It has been established that an increased activity of the carotid bodies play a significant role in the regulation of activities of the cardiovascular system as well as in the pathogenesis of arterial hypertension, heart failure and diabetes (Del Rio et al., 2015). The main mechanisms stimulating the activity of the carotid bodies include a lowered concentration of nitric oxide and increased concentration of angiotensin II, one of the RAAS components (Schultz and Li, 2007). A part of the study also documents a positive relationship between blood pressure and the size of carotid bodies (Cramer et al., 2014; Kato et al., 2012; Bee et al., 1989), although results of examinations conducted on

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rats and published in the past most of all indicate a dominating impact of hypoxia on carotid bodies (Barer et al., 1987; Behm et al., 1986; Habeck et al., 1986).

The objective of the work was to estimate the influence of selected polymorphisms of the genes of the renin-angiotensin-aldosterone system on the volume of the carotid bodies (CB) in patients with essential hypertension.

2. Material and methods

The research group consisted of 77 consecutive persons fulfilling the following qualifying criteria: age over 18 years, essential hypertension treated with hypotensive medication with the duration of the illness of at least five years and clinical indication for computed tomography angiography (CTA) of the carotid arteries, i.e. headaches and dizziness with probable vascular origins, a pre-existing transient ischemic attack (TIA), recent ischemic cerebrovascular incident or inconclusive USG result of the cephalad arteries. The next stage excluded 18 persons diagnosed with: secondary hypertension (1 person), diabetes (2), ischemic heart disease (8), hypercholesterolemia (8), hypertriglyceridemia (9), renal failure (2), hyperthyroidism (3) and hypothyroidism (3). As a result, 59 essential hypertension patients were identified who fulfilled the assumed research inclusion and exclusion criteria. In the resulting group, CTA examinations of the carotid arteries were carried out, with a retrospective estimation of the sizes of the carotid bodies. Both carotid bodies were exposed in 49 cases. Table 1 presents the clinical characteristics of the research group.

In all patients, an analysis was carried out of selected single-nucleotide polymorphisms (SNPs) of the angiotensinogen (AGT) gene: rs4762, rs5049, rs5051 and rs699; angiotensin-converting enzyme (ACE) gene: rs4343; angiotensin receptor type 1 gene (AGTR1): rs5182 and rs5186; and the aldosterone synthase gene (CYP11B2): rs1799998. DNA was extracted from peripheral blood taken on EDTA using silica membranes (QiAmp Blood kit, Qiagen, Hilden, Germany) following the recommendation of the manufacturer. All patients were genotyped by real-time PCR amplifications using LightSNiP typing assays designed by TIB MOLBIOL (Germany). The reaction was performed following the recommendation of the manufacturer and with the usage of the Roche LightCycler 480 instrument. The characteristics of selected SNPs is given in Table 2.

Table 1
Clinical characteristics and characteristics of hypotensive treatment in the study group.

	X	SD
Age (years)	70.71	8.59
Height (cm)	167.31	8.05
Body mass (kg)	72.08	12.02
BMI (kg/m^2)	25.65	3.20
	n	%
Overweight/obesity (%)	28	57.1
Gender (%)		
Men	26	53.1
Women	23	46.9
Grades of arterial hypertension according to ESH/ECS (%)		
Mild	21	42.8
Moderate	23	46.9
Severe	5	10.2
Hypotensive Treatment (%)		
Monotherapy	15	30.6
Combination Therapy	34	69.4
Hypotensive drugs (%)		
ACE inhibitors	25	51.0
β-blockers	19	38.8
Diuretics	15	30.6
Calcium channel blockers	18	36.7
Angiotensin receptor blockers	12	24.5
Other hypotensive drugs	4	8.2

ACE – angiotensin-cenverting enzyme inhibitors; BMI – body mass index; ESC – European Society of Cardiology; ESH – European Society of Hypertension; SD – standard deviation; X – mean.

Table 2
Selected single nucleotide polymorphism of renin-angiotensin-aldosterone system genes.

SNP	Chromosome location	Gene	Polymorphic site
rs4762	1q42.2	angiotensinogen (AGT)	C3889T
rs5049	1q42.2	angiotensinogen (AGT)	G-217A
rs5051	1q42.2	angiotensinogen (AGT)	G-6A
rs699	1q42.2	angiotensinogen (AGT)	T4072C
rs4343	17q23.3	angiotensin-converting enzyme (ACE)	G2350A
rs5182	3q24	angiotensin receptor type 1 (AGTR1)	C573T
rs5186	3q24	angiotensin receptor type 1 (AGTR1)	A1166C
rs1799998	8q22	aldosterone synthase (CYP11B2)	C-344T

The CTA examination of the carotid arteries was performed by means of a SOMATOM Definition Dual-Source CT scanner (Siemens Healthcare, Germany) in accordance with the standard protocol of carotid artery assessment with the acquisition of images in two phases, arterial and delayed. The following technical parameters of image acquisition were used: the option of following the test bolus, pre-monitoring and monitoring on the level of the aortic arch, the spiral method of image acquisition, 0.6 mm collimation, cephalad direction of the scan, the range of examination from the aortic arch level to the level of the base of the brain including the vessels of the circle of Willis, the kilovoltage of exposure at the level of 120 units, variable levels of mAs resulting from the usage of the Care Dose function (Siemens Medical Solutions, Germany). All the examinations were carried out with the use of a contrast medium with the following parameters of infusion: 100 ml of the non-ionic iodinated contrast medium Iomeprol (Iomeron 400, Bracco UK Ltd, Great Britain), intravenous infusion with an automatic syringe in the vein of the cubital fossa, infusion rate of 4.5 ml/s.

The retrospective assessment of the summative volume of the carotid bodies ($V_{rCB+lCB}$) was made by means of axial scans and MPR reconstruction in frontal and sagittal projections obtained in CTA examinations of the carotid arteries on the basis of the following mathematical formula assuming an elliptic shape of the carotid bodies: $V_{rCB+lCB} = 4/3 \times \pi \times$ half of the transverse dimension of the CB in the axial projection x half of the longitudinal dimension of the CB in the axial projection x half of the craniocaudal dimension of the CB in the sagittal/frontal projection.

The research was conducted in accordance with the principles of Good Clinical Practice and Helsinki Convention, and approved by the Local Bioethical Committee. The written informed consent was obtained from all persons taking part in the study.

The statistical analysis was carried out by means of the STATISTICA 12 statistical programme (StatSoft). The distribution of variables was checked by means of the Shapiro-Wilk Tests. In case of normal-distribution quantitative variables, the ANOVA single-factor parametric analysis of variance was used for further statistical analysis. In case of variables with the distribution other than normal, the non-parametric ANOVA equivalent − Kruskal-Wallis analysis of variance test was used for quantitative variables. Statistically significant differences between arithmetic means were marked by means of post hoc tests. The results for qualitative (nominal) variables were expressed as percentages. For qualitative variables, the chi-squared test of highest reliability was used in further statistical analysis. The statistically relevant results were assumed to be those at the level of $p < 0.05$.