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ACE Insertion/Deletion polymorphism (rs4646994) affects body composition in middle-aged premenopausal women with essential hypertension

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ABSTRACT: This study assesses the association between angiotensin converting enzyme (*ACE*) I/D (rs4646994) polymorphism and body composition parameters in essential hypertension (HT) and menopausal status in Slovak women. The entire study sample comprised 575 women in two groups: 255 with HT and 320 without. Body composition parameters were measured by bioelectric impedance analyzer and *ACE* I/D polymorphism genotypes were detected by polymerase chain reaction. Premenopausal HT women with *ACE* II genotype had significantly lower body cell mass (p=0.004), extra- and intracellular water (p=0.027; p=0.004), fat free mass and muscle mass (p=0.006; P = 0.003), fat free mass index (p=0.006) and body cell mass index (p=0.003) than their ID/DD counterparts. These associations were not determined in normotensive and/or postmenopausal women. This study confirmed that *ACE* I/D gene polymorphism affects body composition in HT premenopausal women.

KEY WORDS: hypertension, ACE, body composition, SNP

Introduction

Angiotensin converting enzyme (ACE) is a key zinc metalloenzyme in the renin angiotensin system (RAS) which converts angiotensin I (Ang I) to the angiotensin II (Ang II) vasoconstrictor molecule involved in controling fluid electrolyte balance and systemic blood pressure (Salem and Batzer 2009). Similarly, body fat and body weight can be raised or lowered by stimulating or inhibiting Ang II production. This suggests a possible link between ACE, hypertension and obesity (Wang and Staessen 2000). The *ACE* gene is located on the long arm of chromosome 17 (17q23.3). Rigat et al. (1990) first described the *ACE* gene polymorphism characterized by the presence

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(I) or absence (D) of a 287 bp Alu repeat sequence in intron 16.

ACE I/D polymorphism could also affect the development of obesity and hypertension because these are closely linked to cardiovascular risk factors (Chandel, Doza and Digvijay 2017). *ACE* polymorphisms were found to be associated with body weight, body mass index (BMI), overweight and obesity in white, black and Chinese subjects (Riera-Fortuny et al. 2005; Bordoni et al. 2017) and the *ACE* DD genotype was associated with body weight gain and adiposity in middle-aged men (Strazzullo et al. 2003) and higher abdominal adiposity (Riera-Fortuny et al. 2005) and with greater amounts of subcutaneous fat in adolescent females (Moran et al. 2005). However, a controversial study indicates that it is not- the DD- but the II-genotype which is related to the prevalence of obesity (Bienertova-Vasku et al. 2009). The varying associations of the *ACE* I/D polymorphism with obesity could result from differences in ethnic background and subject characteristics such as age, gender and disease (Strazzullo et al. 2003). Therefore, we conducted a cross-sectional study to identify the association between *ACE* I/D gene polymorphism and body composition in essential hypertension incidence in midlife Caucasian women.

Materials and methods

The sample comprised 575 Slovak women ranging in age from 39 to 65 years. Women recovering from acute disorders such as cancer, myocardial infarction or stroke were excluded from the survey. The women were divided into two groups on blood pressure status; 255 with essential hypertension and 320 without; as diagnosed by WHO criteria. Each participant provided written informed consent for this study which adhered to the Declaration of Helsinki principles. All women were interviewed during their regular check-up by a medical doctor; and all anthropometric measurements were conducted in the morning after at least 12 hours fasting. From the entire sample, 440 women fulfilled study criteria and provided blood or saliva samples for DNA analysis. Women were divided according to their menopausal status into pre-, peri- and postmenopausal groups. Due to the low number of perimenopausal women, this group was amalgamated with premenopausal women.

Body composition measurements were performed using a bioelectric impedance analyzer (BIA 101, Akern S.r.l.) at 50 kHz signal frequency and 800μA constant excitation current in a four-electrode arrangement. Detailed body composition variables were then obtained by the two specific measurements of resistance and reactance revealed by Bodygram programme software (Version 1.21, Akern S.r.l).

Resting systolic and diastolic blood pressures were obtained after a 5-minute rest, with the participant in a semi-recumbent position. Incident hypertension was defined by SBP$\geq$140 or DBP$\geq$90 mmHg at follow-up health examinations or a self-report of receiving treatment for high BP and/or a physician's diagnosis of hypertension during the follow-up period (WHO 2013).

DNA was extracted from peripheral blood samples, or saliva samples, using the SiMax TM Genomic DNA Extraction Kit; and the *ACE* I/D rs4646994 SNP variant was detected by polymerase chain reaction using the method previously described by Dankova et al. (2009). The presence of the insertion (I) and deletion

(D) alleles on 3% agarose gel provided the I allele which formed an uncut PCR product of 490 bp and the D allele 190 bp fragment.

All statistical analyses were performed with IBM SPSS for Windows (Statistical Package for the Social Science, version 20.0, Chicago, IL) and statistical significance was defined as $P \leq 0.05$. General linear models (GLMs) were used to determine differences in body composition between HT and non-HT women, with age as the covariate. We also used GLMs with age, smoking, and regular sport activity as covariates to evaluate the relationships between *ACE* I/D polymorphism and body composition in HT presence and menopausal status in the study subjects. The genotype frequencies were tested for deviation from the Hardy-Weinberg equilibrium by chi-square goodness of fit and the contingency tables with the chi-square independence test were used to analyze the differences in genotype distribution in the compared groups.

Results

The study participants' variable bioelectric impedance values, obesity indices, life

Table 1. Baseline characteristics of the study women

Variable	Total n=575 Mean±SD	Hypertensive women (HT) 255 (44.4%) Mean±SD	Normotensive women (NT) 320 (55.6%) Mean±SD
Age (y)	49.7±6.2	52.4±6.1	47.4±5.3
FM (kg)	28.5±11.8	33.5±12.7[a]	24.6±9.4
FFM (kg)	44.7±4.7	45.8±5.1[a]	43.8±4.1
BCM (kg)	21.2±2.8	21.8±3.1[a]	20.7±2.3
BMI (kg/m^2)	27.1±5.6	29.5±6.1[a]	25.1±4.3
WHR	0.8±0.1	0.9±0.1[a]	0.8±0.1
WHtR	0.5±0.1	0.6±0.1[a]	0.5±0.1
SBP (mmHg)	126.3±16.7	134.7±15.7[a]	119.6±14.3
DBP (mmHg)	79.3±10.2	82.8±10.4[a]	76.5±9.2
	n (%)	n (%)	n (%)
Smokers			
Yes	174 (30.3)	76 (29.8)	98 (30.6)
No	401 (69.7)	179 (70.2)	222 (69.4)
Menopausal status			
Premenopause	305 (53.0)	94 (36.9)	211 (65.9)
Postmenopause	270 (47.0)	161 (63.1)	109 (34.1)
Regular sport activity			
Yes	67 (11.7)	39 (12.2)	28 (11.0)
No	508 (88.3)	281 (87.8)	227 (89.0)
ACE I/D (rs4646994), n = 440			
II	90 (20.5)	45 (50.0)	45 (50.0)
ID	180 (40.9)	96 (53.3)	84 (46.7)
DD	170 (38.6)	102 (60.0)	68 (40.0)

Abbreviations: y – years, FM – fat mass, FFM – fat free mass, BCM – body cell mass, BMI – body mass index, WHR – waist to hip ratio, WHtR – waist to height ratio, SBP – systolic blood pressure, DBP – diastolic blood pressure, [a] – Significant difference in the adjusted means for age between HT and NT women.

style characteristics and the distribution and frequency of *ACE* I/D genotype are summarised in Table 1. The HT (hypertensive) and NT (normotensive) women's groups differed significantly in their mean ages ($p<0.001$, 47.4±5.29 years; and 52.4±6.10 years) and therefore all statistical comparisons of the quantitative variables were adjusted for age. The mean values of the studied variables differed significantly in the compared groups even after adjustment for age; and our results revealed that HT women have statistically significantly more fat mass (kg) (FM) and body cell mass (kg) (BCM) and less fat free mass (kg) (FFM) than NT women ($p<0.001$). The frequency of smokers and the practice of regular sport activity were almost the same in HT and NT women, and the frequency

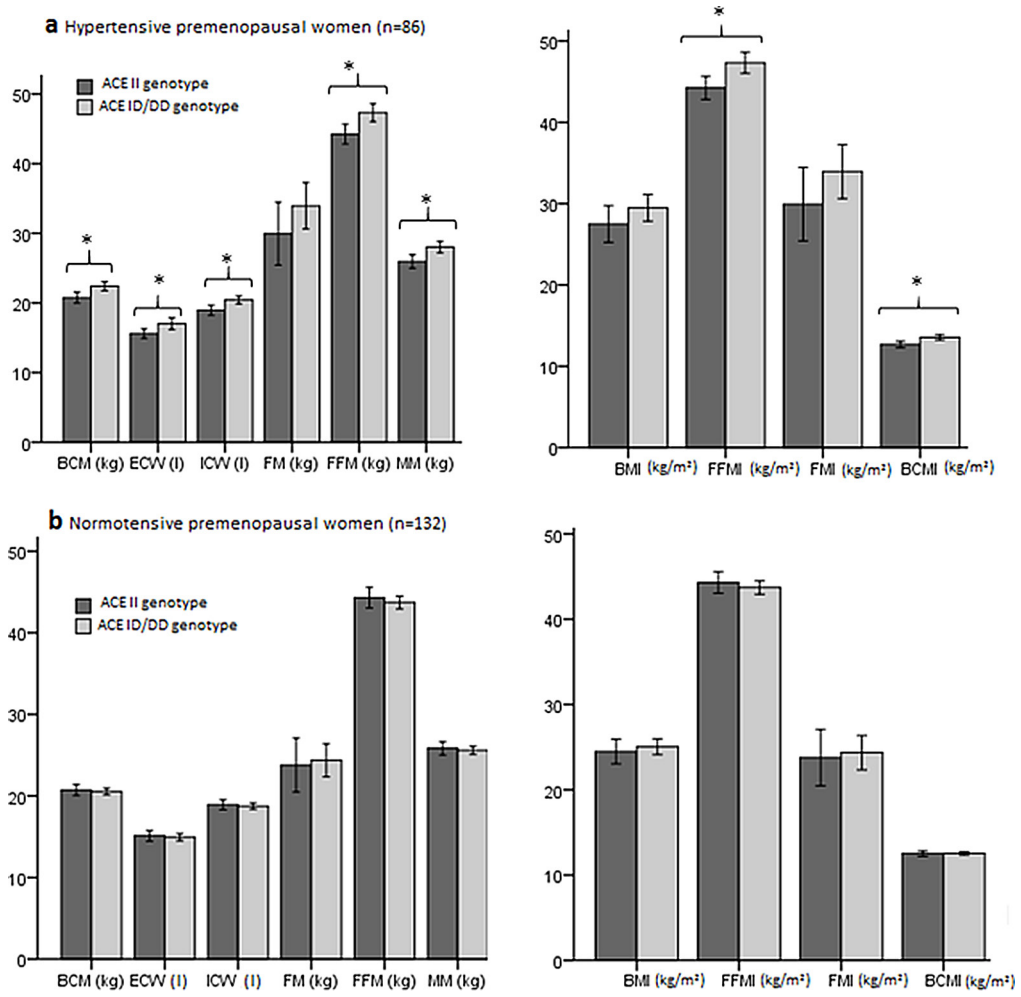

Fig. 1. Body composition comparison between ACE II genotype and ACE ID/DD genotype in (a) hypertensive premenopausal women and (b) normotensive premenopausal women. Error bars represent standard errors; *$p<0.05$

of postmenopausal women was higher in the HT women's group (63.1%) than in NT subjects (36.9%). The distribution of genotypic polymorphism frequencies in the study population fell within the Hardy-Weinberg equilibrium in the NT women's group (χ^2 = 3.613, df = 1, p=0.06), but the HT cohort deviated from the Hardy–Weinberg equilibrium (χ^2 = 6.525, df = 1, p=0.011). Herein the *ACE* genotype distribution did not differ in HT and NT women (p=0.244). Although the D allele frequency was higher in HT women (0.62) than in NT women (0.56), the differences were not statistically significant (p=0.523); thus indicating no *ACE* D allele association with HT.

Figure 1 highlights body composition variable differences between *ACE* II and ID/DD genotype groups in premenopausal HT and NT women. When adjusted for age, smoking and sport activity covariates, our results showed that HT premenopausal women with II genotype had significantly lower BCM (Mean±S.E.; 20.8±0.4 vs. 22.4±0.3 kg; p=0.004), extracellular wat p=P=0.004), FFM (44.2±0.7 vs. 47.3±0.6 kg; p=0.006), muscle mass (MM) (26.0±0.5 vs. 28.0±0.4 kg; p=0.003), FFM index (44.2±0.7 vs. 47.3±0.6 kg/m^2; p=0.006) and BCM index (12.7±0.2 vs. 13.6±0.2 kg/m^2; p=0.003) than their ID/DD counterparts.

The *ACE* II HT carriers also had also lower FM (29.9±2.3 vs. 34.0±1.7 kg), BMI (27.5±1.1 vs. 29.5±0.8 kg/m^2) and FM index (29.9±2.3 vs. 34.0±1.7 kg/m^2) than ID/DD HT carriers, however the difference in mean values fell just beyond the statistical significance level (p=0.054, p=0.073 and p=0.054, respectively). Further adjusted analyses revealed that NT premenopausal women with *ACE* II genotype had similar body composition values to their *ACE* ID/DD counterparts, and the differences in means were not statistically significant (p>0.05). Finally,we compared the body composition between *ACE* II and *ACE* ID/DD postmenopausal women for HT incidence (HT postmenopausal women, n=157; NT postmenopausal women, n = 64) and no significant difference was established for any body composition parameter (p>0.05) (data not shown).

Discussion

Herein, we established that homozygosity for the human *ACE* gene I allele, which is a marker for low tissue ACE activity, is consistently associated with lower BIA measured body composition variables in hypertensive premenopausal women. To the best of our knowledge, this is the first study focused on *ACE*/body composition associations in Central European midlife women in regard to their menopausal status and presence of hypertension. However, Wacker et al. (2008) recorded that Delhi resident women with the DD homozygote had higher risk of both general and central obesity, body fat percentage, waist circumference and WHR and WHtR than ID heterozygotes. Lima et al. (2011) reported that older Brazilian women with a mean age of 66.6 years and carrying the D/D genotype presented higher appendicular FFM than the I-allele carriers. Montgomery et al. (1999) determined that young I/I genotype carriers had greater FFM increase in response to training. Bordoni et al. (2017) found out that Italian children and adolescents with the I/I allele had higher BCM index values. The study of Kim et al. (2009), however, found no significant differences in *ACE* genotypes in body composition in Korean adult women. There, the ab-

dominal and visceral fat area, and ratio of visceral fat area to subcutaneous fat area had a high trend in the DD genotype, but without significant difference; and importantly, similar results were determined herein where our D carriers had higher FM and FM indices, but the difference was above 0.05 significance.

In conclusion, an important limitation to this study is that some study women had prescribed hypertension treatment, and this could have affected body composition parameters. It is therefore paramount that future detailed analysis is conducted on greater study sample size; and this is particularly pertinent to the number of perimenopausal women. Despite these limitations, our study results contribute to evidence demonstrating association between *ACE* I/D gene polymorphism and body composition in hypertensive premenopausal middle-aged women. Finally, while our results revealed that ACE II genotype carriers had almost all body composition parameters lower than D allele carriers, this impact was not established in normotensive and/or postmenopausal women.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

DF was involved in the conception, design, and performance of the study, and the writing of the manuscript; LV participated in collection of data, analysis and interpretation of data, and the writing of the manuscript; VCČ participated in analysis and interpretation of data; RB was responsible for the statistical analysis, and writing of the manuscript; DS was innovator for the project, participated in the conception, design, data collection, analysis and interpretation of data, performance of the study. All authors read and approved the final version of the manuscript.

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