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Association between ins4436A in 11β-hydroxysteroid dehydrogenase type 1 gene and essential hypertension in Polish population

Zależność między występowaniem ins4436A w genie kodującym dehydrogenazę 11β-hydroksysteroidową typu 1 a pierwotnym nadciśnieniem tętniczym w populacji polskiej

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Essential hypertension (EH) is the most common cardiovascular disease worldwide, and it has a strong genetic component. Cortisol homeostasis is an important factor in controlling blood pressure, and the availability of this hormone is regulated by 11βhydroxysteroid dehydrogenase type 1 enzyme (11βHSD1), which converts cortisone into cortisol.

Materials and Methods: We investigated the correlation between EH and the single nucleotide polymorphism (SNP) ins4436A located on the hydroxysteroid (11-beta) dehydrogenase 1 gene among the Polish population. The study included a total of 268 patients with confirmed EH and 151 unrelated controls. All studied polymorphisms were detected using the restriction fragment length polymorphism (RFLP) method.

Results: The carriage of ins4436A (rs45487298) polymorphism in intron 3 of the *HSD11B1* gene was more frequent among patients with EH than among controls (p=0.013). The analysis of association of ins4436A with the risk of EH indicated an odds ratio (OR) of 2.44 (95% confidential interval: 1.24-4.82). Moreover, essential hypertension occurred less frequently in males than in females. Results of multivariate analysis in the study group showed that ins4436A is a strong predictor of diabetes mellitus type 2 and ins4436A may lead to a decrease of the high-density lipoprotein (HDL) cholesterol level.

Discussion: The cause of essential hypertension has not been fully established, but genetic factors seem to play a very important role. In our study we found that ins4436A in the *HSD11B1* gene was associated with essential hypertension in a Polish population. Nevertheless, the impact of ins4436A in the *HSD11B1* gene on the occurrence of essential hypertension requires further investigations.

Key words: hypertension • ins4436A • 11β-hydroxysteroid dehydrogenase type 1

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Introduction

Essential hypertension (EH) is the most common cardiovascular disease, affecting 20 to 50% of adults of the developed countries' populations [16]. Moreover, EH accounts for 95% of all cases of hypertension [6] and is a consequence of the coexistence of both environmental and genetic factors [26]. Numerous epidemiological studies have shown that elevated blood pressure (BP) is a risk factor for coronary artery disease, heart failure, stroke, peripheral artery disease and renal failure, in both women and men [14,21,25]. The number of adults with hypertension in 2025 was predicted to increase by about 60% to a total of 1.56 billion (1.54-1.58 billion) worldwide [15]. What is more, in Poland, the same as in other countries, the costs of hypertension treatment are very high [18]. Studies of families, including studies conducted on monozygotic and dizygotic twins, indicated that hypertension has a strong genetic component and the coefficient of hypertension heritability is in the range 20-55% [3,20].

Cortisol homeostasis is an important factor in controlling blood pressure. The availability of cortisol is regulated by two isoenzymes of 11β-hydroxysteroid dehydrogenase: 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) and 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). These enzymes catalyze the oxidation and reduction reactions within a hydroxyl group of cortisol and the cortisone carbonyl group at position C11 [8,17]. The 11βHSD2-dependent NAD/NADH enzyme is responsible for the conversion of cortisol (F) to the 300 times less active metabolite cortisone (E). Decreased activity of 11βHSD2 causes inefficient conversion of F to E, which may result in hypertension occurrence as a result of mineralocorticoid receptor activation by F [5]. In vitro 11βHSD1 enzyme has bidirectional activity (F↔E), but under in vivo conditions this enzyme predominantly exhibits reductase activity, converting E to F in the presence of NADPH [23]. 11βHSD1 is widely distributed in various tissues, but it is highly expressed in the liver and adipose tissue, where it may increase the level of the active form of glucocorticoids, including cortisol [19]. Moreover, in the case of essential hypertensive patients, the excretion of urinary free cortisol was greater than among normotensive subjects [15].

Based on this, the aim of the study was to examine whether there is a correlation between essential hypertension and the occurrence of ins4436A in the *HSD11B1* gene among the Polish population.

Materials and methods

Study population: The study group consisted of 268 patients with confirmed essential hypertension (information from medical records and independent ambulatory measurements) in accordance with the applicable guidelines of the European Society of Hypertension and European Society of Cardiology (2013 ESH/ESC Guidelines for the management of arterial hypertension). All patients were white Caucasians with a mean age of 65 years, standard deviation ± 11.8 years. The additional data obtained from hospital records included: total cholesterol (mg/dl), HDL (mg/dl), low-density lipoprotein (LDL) (mg/dl), triglycerides (mg/dl) and glucose level (mg/dl).

The control group consisted of 151 healthy and unrelated subjects who were selected from the Polish population (mean age 64 years, standard deviation ± 10.8 years). The inclusion criterion for the control group was no symptoms of hypertension (information from medical records and independent ambulatory measurements). All control group participants were recruited from the same geographic region as the patients and were selected randomly.

Hypercholesterolemia (elevated total serum cholesterol levels > 200 mg/dl) was not an exclusion criterion for the control or study groups. Patients and controls with diabetes mellitus type 2 were included in the study – in the case of the study group – only when patients had confirmed essential hypertension and diabetes mellitus was diagnosed at least one year after the first incidence of hypertension. The Institutional Local Ethics Committee approved the study protocol and sample size. All participants and patients were obliged to sign an informed consent form.

Genotype Determination: Venous blood from all individuals was collected in vials containing 3.2% sodium citrate. Samples were stored at –20°C until the DNA isolation process. Genomic DNA was isolated from blood leukocytes by using a commercially available kit: Chemagic DNA Blood250 Kit (PerkinElmer Chemagen Technologie GmbH, Germany). In this study a specific type of polymerase chain reaction was used: restriction fragment length polymorphism (PCR-RFLP). Amplification was performed using a pair of primers: 5'AGACTGATGCCATTTCTGCTGT 3' and 5' GGT GATGTGGTTGAGAATGAGC3'. Polymerase chain reaction (PCR) was carried out in a 25 µl

Table 1. Demographic and biochemical characteristic of the study group and control group

Parametr	Study group N=268	Control group N=151	p - value
Sex (male)	105 (39.18%)	84 (55.63%)	0.0015
Essential Hypertension	268 (100%)	0 (0%)	p<0.05
Diabetes mellitus type 2	34 (12.7%)	19 (12.6%)	0.9026
Hipercholesterolemia (>200 mg/dl)	70 (26.12%)	47 (31.13%)	0.3254
HDL (male<40 mg/dl; female<45 mg/dl)	20 (7.45)	-	
LDL (>115mg/dl)	57 (21.27%)	-	
Triglicerydes (>150 mg/dl)	50 (18.66%)	-	

Table 2. Distribution of genotypes in control group

SNP	wt/wt (%)	wt/ins 4436A (%)	ins 4436A / ins 4436A (%)	2	P - value HW
ins4436A (rs45487298)	139 (92%)	11 (7.4%)	1 (0.6%)	2.02	0.15

Table 3. Distribution of genotypes in study group

SNP	wt/wt	wt/ins4436A	ins4436A / ins4436A (%)
ins4436A (rs45487298)	222 (82,8%)	40 (15%)	6 (2.2%)

Table 4. Results of univariate analysis of distribution of minor alleles and clinical parameters between study and control group

Parameter	minor allele in study group (%)	minor allele in control gropu (%)	p - value	OR	95% CI
ins4436A (rs45487298)	46 (17.2%)	12 (7.9%)	0.013	2.41	1.22-4.70
Male	105 (39.18%)	84 (55.63%)	0.0016	0.51	0.34-0.71

volume containing 2.5 µl of 10x PCR buffer, 2 µl of 2 mM dNTP, 0.2 µl of each primer, 1 U of TAQ polymerase, 3 µl of extracted DNA and 16.3 µl of distilled water. PCR reactions were conducted under the following conditions: initial denaturation – 95°C for 5 minutes, 30 cycles of the following conditions: denaturation cyclic – 95°C for 30 seconds, annealing – 57°C for 30 seconds, elongation – 72°C for 30 seconds, final extension – 72°C for 10 minutes and cooling to 16°C. Digestion was performed at 37°C for 3 hours using the restriction enzyme XcmI. Separation of digestion products was carried out by electrophoresis on polyacrylamide gel. Digestion products were as follows: for wild type 301 bp and for ins4436A 201 bp and 101 bp.

Statistical analysis: Age was expressed as median and interquartile range due to a non-normal distribution. The frequency of alleles was tested against Hardy-Weinberg equilibrium. Pearson chi-square and Yates corrected chi-square exact tests were used for data comparisons, depending on the number of cases. Logistic regression analysis was performed to determine the association of particular factors with occurrence of essential hypertension. Univariate comparisons were performed for all analyzed factors, and factors with p values < 0.15 were entered into a multivariate backward, stepwise model. A final p value below 0.05 was deemed statistically significant in the multivariate analysis. Statistica 8.0 and MedCalc 9.36 statistical packages were used for all computations.

Results

Of the 419 subjects included in the study, 151 formed the control group and 268 constituted the study group (Table 1).

Table 5. Results of univariate analysis of clinical parameters and ins4436A in study group

Parameter	Carriers of ins4436A in study group	
	Ins 4436A (N=46) [%]	p - value
Total cholesterol (>200 mg/dl)	16 (34.8)	0.04
Cholesterol LDL	17 (37)	0.008
Cholesterol HDL (M <40 mg/dl; K< 45 mg/dl)	7 (15.0)	0.017
Trigliceryde (>150 mg/dl)	8 (17.4)	0.97
Diabetes mellitus	1 (2.17)	0.09

Table 6. Results of multivariate analysis of clinical parameters and ins4436A in study group

Parameter	p - value	OR	95% CI
Diabetes mellitus	0.035	9.40	1.15-76.22
Cholesterol HDL	0.008	0.24	0.08-0.69

The frequency of analyzed genotypes was compatible with Hardy-Weinberg equilibrium (Table 2). Distribution of genotypes and minor alleles in the study group is provided in Table 3.

Because of the very low frequency of polymorphic alleles (Table 2, 3), a comparison of the polymorphism carrier state between analyzed groups was made (Table 4).

The multivariate analysis included clinical and genetic parameters such as essential hypertension, sex and the carrier state of rs45487298 polymorphism. The multivariate analysis reveal that ins4436A (rs45487298) was a strong predictor of essential hypertension among Polish population (OR 2.41; 95%Cl: 1.22-4.70) and essential hypertension occurred less frequently in males than in females (OR=0.51; 95%Cl: 0.34-0.71).

Analysis within the study group – correlation between ins4436A carrier state and clinical parameters in the study group

Univariate comparisons were performed for all analyzed factors, and factors with p values <0.15 were entered into a multivariate backward, stepwise model. Univariate analysis carried out in the study group showed that ins4436A, cholesterol, LDL cholesterol, HDL cholesterol and diabetes mellitus regardless of gender fulfill inclusion criteria for multivariate logistic regression (Table 5). For this reason these parameters were entered into a multivariate backward, stepwise model.

Results of multivariate analysis (Table 6) showed that ins4436A is strong predictor of diabetes mellitus (OR=9.40; 95%Cl: 1.15-76.22). Moreover, ins4436A may lead to a decrease of cholesterol HDL level (OR=0.24; 95%Cl: 0.08-0.69).

Discussion

Essential hypertension has been the most common cardiovascular disease worldwide for many years. In the present study, we have demonstrated that ins4436A in the *HSD11B1* gene is correlated with the occurrence of essential hypertension among the Polish population (OR 2.44; 95%Cl: 1.24-4.82).

11βHSD1 enzyme plays an important role in regulation of the amount of cortisol. Moreover, the results of Campino et al. showed that 15.7% of essential hypertensive patients had a high F/E ratio [4].

Insertion of adenine in position 4436 of the *HSD11B1* gene (rs45487298) is localized in intron 3. Draper et al. found that changes in intron 3 of *HSD11B1*may influence the transcriptional activity of the gene, suggesting that this region of the gene acts as an intronic enhancer of *HSD11B1* expression [9]. This insertion might be associated with higher activity of the 11βHSD1 enzyme [12] and reflected in increased cortisol level. It is known that cortisol binds to the mineralocorticoid receptors with higher affinity than aldosterone and through this mechanism may induce essential hypertension.

Essential hypertension occurred less frequently in males than in females (OR 0.51; 95%Cl: 0.34-0.71). This study involved mainly people over 55 years of age. Before age 50 years, women have a lower prevalence of hypertension than men, while after 55 years the incidence of hypertension increases and is higher in women than in men [13].

Increased incidence of essential hypertension in women may be explained by the end of the protective effect of estrogen [2]. For this reason, women over 55 years of age will suffer from cardiovascular disease more often [22].

The multivariate analysis among the study group showed that ins4436A is a strong predictor of diabetes mellitus (OR=9.40; 95%Cl: 1.15-76.22). This is consistent with the results of Alberti et al., who demonstrated that type 2 diabetes is associated with increased expression of 11βHSD1 [1]. However, Valsamakis et al. reported that 11βHSD1 enzyme activity does not differ among patients with diabetes when compared to BMI-matched controls [24]. Studies by Dube et al. confirmed that there is conversion of cortisone to cortisol via the 11βHSD 1 enzyme pathway among participants with type 2 diabetes mellitus. Moreover, they pointed out that this observation has significant implications for development of tissue-specific 11βHSD inhibitors in type 2 diabetes mellitus, emphasizing the role of 11βHSD enzyme in diabetes pathogenesis [10].

Moreover, multivariate analysis among the study group showed that ins4436A leads to a decrease of cholesterol HDL level (OR=0.24; 95%Cl: 0.08-0.69). Studies by Fraser et al. indicated that the cortisol excretion rate correlates negatively with HDL cholesterol [11]. This is consistent with the results of Chang et al., who demonstrated that a high cortisol level may have adverse effects on cardiovascular disease through decreasing serum HDL cholesterol concentration [7].

Conclusions

To the best of our knowledge the present study is the first one to analyze the correlation between the ins4436A polymorphism and essential hypertension worldwide. The results of this study confirm the necessity to thoroughly examine the effect of the presence of this polymorphism in *HSD11B1* expression profile. Moreover, further studies are needed to precisely define the impact of this insertion among patients with essential hypertension.

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