Volume 10, Issue 3, 2020, 5406 - 5411 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

https://doi.org/10.33263/BRIAC103.406411

Original Research Article **Open Access Journal**

Received: 26.01.2020 / Revised: 02.03.2020 / Accepted: 05.03.2020 / Published on-line: 09.03.2020

The cytochrome 11B2 aldosterone synthase gene CYP11B2 (RS1799998) polymorphism associates with chronic kidney disease in hypertensive patients

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ABSTRACT

Renin-angiotensin aldosterone system (RAAS) holds a crucial role in blood pressure regulation. Aldosterone is encoded by the cytochrome 11B2 aldosterone synthase gene (CYP11B2). The study aim was to analyze the association of Chronic Kidney Disease (CKD) with allelic polymorphism of the CYP11B2 at position -344 (-344C/T) in the promoter in patients with essential arterial hypertension (EAH). 72 subjects with EAH and target-organ damaging (2nd stage), moderate, high or very high cardiovascular risk were involved in the case-control study. Among them, 70.83% (51) females and 29.17% (21) males, mean age 59.87±8.02 yo; disease duration from 6 to 25 years. CKD was determined by the National Kidney Foundation recommendations (Kidney Disease: Improving Global Outcomes (KDIGO), 2012) after glomerular filtration rate (GFR) decline <60 ml/min/1,73 m^2 for over 3 months (by Cockroft-Gault formula and CKD-EPI for Cystatin-C and Creatinine serum levels depending on gender). CKD was diagnosed in 29 persons. Control group consisted of fourty-eight practically healthy individuals of relevant age. Gene polymorphism of aldosterone synthase gene CYP11B2 (-344C/T) was examined by polymerase chain reaction (PCR). The probability of EAH in the observed population increased 1.49 times in T-allele carriers of CYP11B2 gene, but only in females [OR=1.90; 95%CI:1.02-3.54; p=0.029], with contrary decreasing in C-allele women (p=0.041). Moreover, T-allele increased probability of CKD (GFR<60 ml/min/1,73m^2) in hypertensive population 1.48 times [OR=1.86; 95%CI:1.01-3.58; p=0.049], especially in T-allele females 1.53 times [OR=6.51; 95%CI:1.39-30.60; p=0.007] with low CKD risk in T-allele males [OR=0.15; 95%CI:0.03-0.72; p=0.009], respectively. Furthermore, some predictors like Diabetes, the 2nd and 3rd grades Obesity, and the 3rd grade of Blood Pressure elevation escalated the risk of CKD 2.4, 2.08-2.32 and 2.91 times, accordingly (p<0.05). Thus, aldosterone synthase gene CYP11B2 (-344C/T) associated with EAH. T-allele increased risk of CKD in hypertensive population, especially in females.

Keywords: *CYP11B2 gene (-344C/T); Chronic Kidney Disease; Arterial Hypertension; Risk; Aldosterone.*

1. INTRODUCTION

Essential Arterial Hypertension (EAH) and Chronic Kidney Disease (CKD) nowadays are becoming a global public health care problem worldwide [1]. The EAH and CKD incidence are spreading steadily preferably due to the rising burden of obesity and type 2 diabetes (DM2). Both hypertension and metabolic disoders are widely prevalent among patients with CKD and play conciderable role in the renal damage progression and end-stage renal disease development (ESRD) [1-4]. In the American population CKD is registered among 23% patients suffering from Arterial Hypertension (AH) [5], in Italian population – in 42% hypertensive subjects [6]. Apparently, Blood Pressure (BP) elevation influence independently to cardiovascular (CV) and renal events and their relationship is continuous (like haemorrhagic or ischaemic strokes, sudden death, myocardial infarction, heart failure, atrial fibrillation, peripheral artery disease, as well as ESRD) [1]. EAH infrequently befall in isolation, and often combines with other cardiovascular risk factors such as dyslipidaemia and glucose intolerance, or other evidence of metabolic disorders [1, 3, 7]. Whether CV risk factors contribute independently to the onset of CKD in EAH patients is at present unclear.

In numerous studies it was found a great amount of mineralocorticoid receptors on surface of different tissues and cells: like kidneys, cardiomyocytes, fibroblasts, vascular endotheliocytes, cells of smooth muscles, monocytes, and especially macrophages in the state of active phagocytosis, cerebral cells astrocytes etc. Therefore, stimulation of these receptors by an appropriate hormone stipulates a number of pathogenic effects [8]. Aldosterone may foster infiltration of the endothelial intima by macrophages followed by an intensified expression of cyclooxygenase-2 genes, osteopontin, focal necrotic changes, stimulates myocardial left ventricular hypertrophy (LVH), sclerotic changes and apoptosis of cardiomyocytes. Moreover, it has been revealed that aldosterone mediates

metabolic disorders: its levels correlate with the concentration of blood glucose, insulin and C-peptide concentration, dyslipidemia and insulin resistance onset [9-11]. In spite of pathogenic effects of aldosterone on renin-angiotensinogen system is well known, the concerns of CKD mechanisms development in hypertensive patients depending on genetic factors related to aldosterone requires more investigations to precisely elucidate possible linking pathways. Due to these facts, the issue of early diagnostics of CKD in EAH patients with the aim of early secondary prevention is extremely topical and needs further research.

The primary aim of this study is to analyze the association of aldosterone synthase gene (CYP11B2) biallelic polymorphism in the promoter position -344 (-344C/T) with CKD in patients with EAH.

2. MATERIALS AND METHODS

2.1. Compliance with bioethics.

Study was performed in compliance with the European Convention on Human Rights and Biomedicine, GCP, EUC directive #609 and other EU and international legislation on bioethics. All enrolled patients have been treating in Family Medicine Department of Chernivtsi (Western Ukraine, Bukovina region) since the 2016 year. Genetic study performed in the laboratory of Zaporizhzhia State Medical University (Ukraine). After screening fo matching inclusion and exclusion criteria, 100 patients were selected for further examination, among them genetic examination was performed in 72 cases. The control group consisted of 48 practically healthy individuals who were not relatives of the patients and without reliable differences of gender distribution and mean age with a study group .

2.2. Inclusion / Exclusion criteria.

Inclusion criteria. EAH patients were included in the current study with hypertension-mediated organ damage (target-organs damage – 2nd severity stage, asymptomatic disease), from the 1st through to the 3rd grade of BP values; moderate-high CV risk; age above 30 y.o. All enrolled subjects signed a consent form to participate in the study.

Exclusion criteria. We excluded patients with EAH stage 3 (established CV disease, CKD – with estimated glomerular filtration rate (eGFR) decline <30 ml/min/1,73m^2); chronic heart failure (CHF) higher than II functional class (NYHA III-IV), EAH patients with complications of hypertension-mediated organ damaging; secondary arterial hypertension; diabetes mellitus type I (DM 1), sub- and decompensated DM type 2 (with diabetes target-organ damage); malignant or uncontrolled arterial hypertension; sub- and decompensated diseases of the liver (three times over the norm level of aspartate aminotransferase, alanine aminotransferase); bronchial asthma, chronic obstructive pulmonary disease of III-IV stage with C or D risk value (GOLD 2019); exacerbated infectious diseases or during unstable remission; psychological disorders; oncologic problem of any location; taking oral corticosteroids or contraceptives; pregnancy or lactation period.

2.3. Diagnosis of Arterial Hypertension and Chronic Kidney Disease.

Hypertension was defined as office systolic BP (SBP) values ≥140 mmHg and/or diastolic BP (DBP) values ≥90 mmHg at least for three measurements during a month, according to national and European Societies of Hypertension and Cardiology (ESH / ESC, 2016, 2018) recommendations requirement [1, 12, 13]. Left ventricular hypertrophy was confirmed by electrocardiography (ECG) and/or echocardiography (EchoCG).

CKD was determined by the National Kidney Foundation recommendations (Kidney Disease: Improving Global Outcomes [KDIGO], 2012) after glomerular filtration rate (GFR) decline ≤60 ml/min/1.73 m^2 for over 3 months (by Cockroft-Gault formula and CKD-EPI for Cystatin-C and Creatinine serum levels depending on gender) with or without other signs of kidney damage, according to KDIGO recommendations [4]. CKD was diagnosed in 29 EAH persons.

Screening of the patients and their distribution into groups depending on GFR level (>60 or ≤60 ml/min/1.73m^2) was performed according to the Recommendations of Ukrainian Societies of Nephrology and Cardiology, ESC/ESH, KDIGO, and Orders of the Ministry of Health of Ukraine [1, 4, 12, 13]. All enrolled patients underwent a complex of basic examinations: general clinical analyses of complete blood count, total cholesterol level and low/high density level cholesterol (LDL-, HDL-C), serum uric acid, body mass index (BMI, kg/m^2) for evaluation of overweight and abdominal obesity (AO), office measurement of SBP, DBP, heart rate (HR), ECG in 12 leads, ultrasound examination of the kidneys, EchoCG and Daily Holter BP monitoring in undetermined conditions, consultations of ophthalmologist and neurologist according to Ukrainian (2016) and European recommendations ESC/ESH (2018) [1, 12, 13].

2.4. Genotyping of the Aldosterone synthase *CYP11B2* (*C-344T*) gene polymorphism.

DNA extraction.

Venous blood was collected in a sterile vacutainer, stabilized by K2-EDTA. DNA was extracted from the whole venous blood lymphocytes' nuclei of participants. Isolation and purification of DNA from the obtained material was performed according to Thermo Scientific GeneJET Genomic DNA Purification Kit Manufacturer's Guidance (Thermo Fisher Scientific, USA).

DNA amplification and genotyping.

Quantitative Real-Time polymerase chain reaction (RT-PCR) was used for DNA fragments of *CYP11B2* gene amplification and performed on CFX96 Touch™ (Bio-Rad Laboratories, Inc., USA). Genotyping performed with specific TaqMan catheters/probe by CFX96 RT-PCR Detection System.

The amplification mixture compounded PCR buffer, Taq-AT polymerase and mineral oil. Further, the TaqMan signal probe, containing fluorescent labels *Fam* (samples homozygous for *C* allele of the *CYP11B2* gene (344C> T) on the *Fam* channel) and *Hex* (samples homozygous for the *T* allele of the *CYP11B2* gene on the *Hex* channel), was added to amplification mixture with the aim to detect duplexes formed by amplicons and signal probes during PCR melting. The melting point of the TaqMan signal probes was fixed by the software of the CFX96 Thermocycler according to the partial (lower temperature) or full (higher temperature) complementarity of the TaqMan probe to the target DNA of the amplicon, resulting in different levels of fluorescence and corresponding temperature graphs (Fig. 1).

The DNA fragments amplification (amplicons) analysis of *CYP11B2 (344C>T)* gene polymorphism was performed by the licensed CFX96 RT-PCR Detection System Software (Microsoft, USA). The obtained images are presented in Figures 2-3.

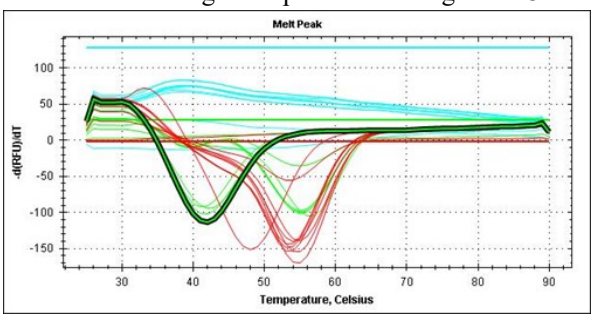

Figure 1. Temperature bars in analysis of *CYP11B2 344C>T* gene's polymorphism in observed population.
Note: Blue color shows the samples homozygous for the C-allele of the *CYP11B2* gene (*344C>T*), determined by the Fam channel; Greens – samples homozygous for Hex channel (*T*-allele); Reds – heterozygous (*TC*) specimens; Yellows – questionable and unreliable results.

2.5. Statistical analysis.

Statistical analysis was performed using StatSoft Statistica v. 7.0 (USA) software. For the genotypes distribution comparison used Pearson's criterion (χ^2). Analysis of qualitative data (categorical variables), risk of pathology development was assessed by a binary logistic regression model using relative risk (RelR); risk ratio (RR) was estimated by odds ratio (OR) with 95% confidence interval [95% CI] using a chi-square test (χ^2) (df=1). P values <0.05 were considered statistically significant.

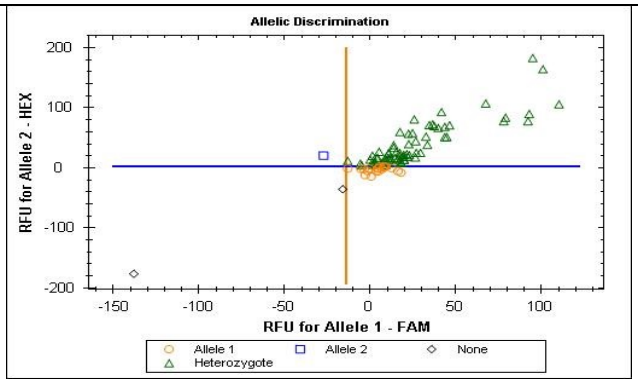

Figure 2. Alleles and genotypes discriminations of *CYP11B2 344C>T* gene's polymorphism. Note: o Allele 1 – *CC* genotype carriers; □ Allele 2 – *TT* genotype carriers; Δ Heterozygote – *CT* genotype carriers; ◊ None – non-determined.

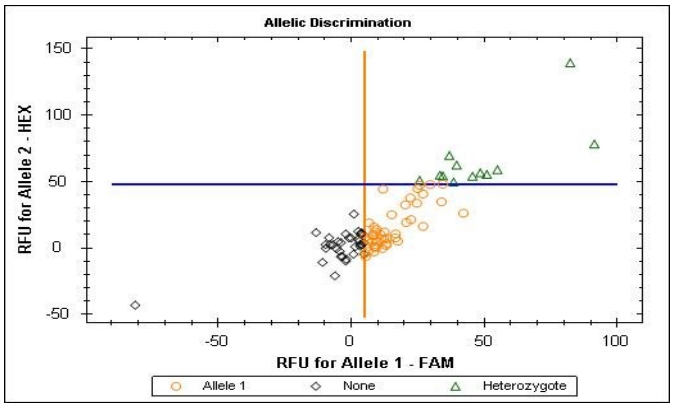

Figure 3. Alleles and genotypes discriminations of *CYP11B2 344C>T* gene's polymorphism. Note. oAllele 1 – *CC* genotype carriers; ◊ None – non-determined; ΔHeterozygote – *CT* genotype carriers.

3. RESULTS

3.1. *CYP11B2 344C>T* gene polymorphism association with Essential Arterial Hypertension and Chronic Kidney Disease.

Distribution of genotypes and alleles of *CYP11B2 344C>T* gene polymorphism in patients suffering from EAH and in the control group did not significantly differ (Table 1). Out of 144 isolated alleles of the study group the mutant T-allele dominated the C-allele by 11.12% (χ^2=3.56; p=0.038), with a parity distribution in the control group (p>0.05).

Table 1. Genotypes and alleles distribution of *CYP11B2* (*344C>T*) gene polymorphism in observed population

Polymorphic variants of *CYP11B2* gene		Study group, n=72 (%)	Control group, n=48 (%)	OR [95% CI]	χ^2 p
***CYP11B2* (*344C>T*), n (%)**	*CC*	14 (19.44)	14 (29.17)	0.59 [0.25-1.38]	χ^2=1.52 p>0.05
	TC	36 (50.0)	20 (41.67)	1.40 [0.67-2.92]	χ^2<1.0 p>0.05
	TT	22 (30.56)	14 (29.17)	0.93 [0.42-2.10]	χ^2<1.0 p>0.05
χ^2 ; p		χ^2=1.61; p>0.05		-	-
***CYP11B2* (*344C>T*), n (%)**	*C*-allele	64 (44.44)	48 (50.0)	0.80 [0.48-1.34]	χ^2<1.0 p>0.05
	T-allele	80 (55.56)	48 (50.0)	1.25 [0.74-2.10]	χ^2<1.0 p>0.05
χ^2 ; p		χ^2<1.0; p>0.05		-	-

Glomerular filtration rate (GFR) estimated after creatinine CKD-EPI was lowered (≤60 ml/min/1.73m^2) in 18 patients with EAH (25.0 %), while by Cockroft-Gault formula in 8 individuals only (11.11 %). Relative frequency of women in the group with GFR ≤60 ml/min/1.73m^2 prevailed over the matched one in the group with preserved GFR by 31.48 % as much: 94.44% vs 62.96% (p=0.011). On the contrary, among men, a relative frequency of those with preserved GFR prevailed over those with decreased GFR (p=0.025).

Distribution of EAH patients depending on polymorphic variants of CYP*11B2* (*344C>T*) gene considering sex and GFR level estimated after creatinine (CKD-EPI) is presented in Table 2. Relative frequency of women with GFR ≤60 ml/min/1.73m^2, who were *TC*-genotype and *T*-allele carriers, prevailed over those with

GFR >60 ml/min/1.73m^2 by 29.63% (χ^2=5.33; p=0.021) and 24.08% (χ^2=6.68; p=0.009) respectively.

Table 2. Distribution of polymorphic variants of *CYP11B2* (*344C>T*) gene in hypertensive patients depending on gender and glomerular creatinine filtration rate (CKD-EPI)

Genotypes, alleles n (%)			Patients, n=72 (%)		χ^2	p
			GFR ≤60 ml/min/1.73m^2, n=18	GFR >60ml/min/1.73m^2, n=54		
CYP11B2 (*344C>T*), n (%)	*CC*	M	0	2 (3.70)	-	-
		F	2 (11.11)	10 (18.52)	<1.0	>0.05
	χ^2 ; p		χ^2<1.0; p>0.05			
	TC	M	0	12 (22.22)	-	-
		F	10 (55.56)	14 (25.93)	5.57	0.018
	χ^2 ; p		P=0.008			
	TT	M	1 (5.55)	6 (11.11)	<1.0	>0.05
		F	5 (27.28)	10 (18.52)	<1.0	>0.05
	χ^2 ; p		χ^2<1.0; p>0.05			
Alleles of *CYP11B2* (*344C>T*) gene polymorphism						
CYP11B2 (*344C>T*), n (%)	*C*-allele	M	0	16 (14.81)	-	-
		F	14 (38.39)	34 (31.48)	<1.0	>0.05
	χ^2 ; p		P=0.01			
	T-allele	M	2 (5.55)	24 (22.22)	<1.0	>0.05
		F	20 (55.56)	34 (31.48)	6.68	0.01
	χ^2 ; p		χ^2=7.58; p=0.006			

Note. M – males; F – Females

Analysis of GFR estimated by cystatin-C (CKD-EPI) determined its decrease <60 ml/min/1.73m^2 in 29 (40.28%) hypertensive patients (26.39% – women, 13.89% – men, p=0.018). As well as among patients with preserved GFR (59,72% individuals) where women dominated men threefold: 44.44% vs 15.28% (p<0.001). Though, the relative frequency of individuals with preserved and decreased GFR did not differ reliably between the groups by their gender.

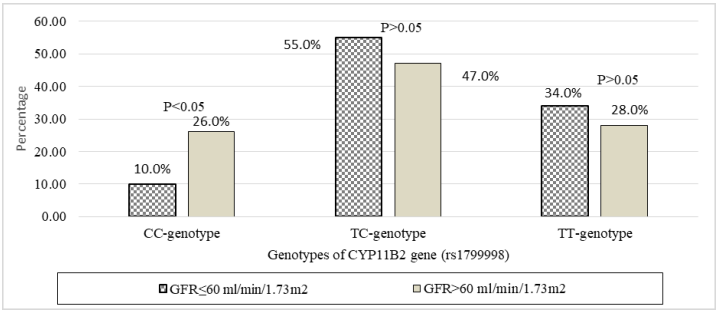

Figure 4. Distribution of patients depending on polymorphic variants of CYP*11B2* (*344C>T*) gene and glomerular filtration rate by Cystatin-C (CKD-EPI). Note. P – significance of differences between groups for each genotype.

Relative frequency of EAH patients depending on polymorphic variants of *CYP11B2* (*344C>T*) gene, sex and GFR estimated by Cystatin-C (CKD-EPI) did not differ significantly. Though, among the *CC*-genotype carriers, relative amount of patients with preserved kidneys' functions (GFR >60 ml/min/1.73m^2) was over 2.5 times more than those with decreased GFR: 25.58% vs 10.34% (p<0.01) (Fig. 4). No reliable differences were observed considering GFR among the *TC*- and *TT*-genotypes carriers.

The genotypes and alleles of the analyzed gene were not found to be additional risk factors of EAH occurrence or type 2 DM in the examined population in general. Though, *T*-allele of *CYP11B2* (rs1799998) gene presence among women increases the probability of EAH almost 1.49 times as much [OR=1.90; 95%OR:1.02-3.54; p=0.029] (Table 3). At the same time, *C*-allele contrary decreases chances of EAH occurrence among women [OR=0.53; 95%OR: 0.28-0.98; p=0.041].

Epidemiological analysis of the polymorphic variants of *CYP11B2* (rs1799998) gene as risk factors of GFR decrease in EAH patients indicated that the risk of CKD (after GFR decreased by the Cystatin-C content) 1.48 times increases, but only in the *T*-allele carriers generally [OR=1.86; 95%OR: 1.01-3.58; p=0.049]. Moreover, *T*-allele increases the chances of CKD estimated by creatinine level (CKD-EPI) over 6.5 times as much [95%OR:1.39-30.60; p=0.007] but among women only, with the lowest probability of such changes in *T*-allele carriers' men [OR=0.15; p=0.009]. Furthermore, GFR decrease by Cystatin-C is associated with borderline increased risk of CKD among *T*-allele women as well [OR=2.23; 95%OR:0.98-5.90; p=0.054].

Type 2 DM presence in EAH patients increases the risk of CKD 2.4 times [OR=3.29; 95%OR:1.06-10.19; p=0.034], in case of obesity (BMI >30 kg/m^2) this risk increases 2.08 and 2.32 times as well [OR=3.30; 95%OR:1.33-8.16; p=0.009 and OR=3.58; 95%OR:1.02-9.34; p=0.048, respectively], in case of increased SBP ≥180 Hg mm or DBP ≥110 Hg mm (3rd grade) the probability of CKD elevates almost threefold [RR=2.91; OR=5.06; 95%OR:1.94-13.23; p<0.001].

3.2. Discussion.

Molecular level of Aldosterone synthesis directly depends on the mitochondrial aldosterone synthase enzyme (ASS) activity.

The latter is coded by *CYP11B2* gene (Cytochrome P450, family 11, subfamily B, polypeptide 2), located on the 8th chromosome. ASS belongs to the superfamily of P450 cytochrome and regulates the synthesis of hormone aldosterone. The *CYP11B2* gene in humans is very polymorphic with 227 single nucleotide polymorphisms (SNP) described in different populations. Bi-allele polymorphism in the *CYP11B2* gene promoter in the position -344 (*-344C/T*) is associated with EAH [14, 15], especially *T*-allele *(-344T*) [16], or salt-dependent hypertension [17], development of aldosterone-producing adenoma [18-20], decreased GFR in patients with CKD [21], idiopathic hyperaldosteronism [22],

higher BP levels and development of LVH in transgenic mice [16], etc., which corresponded to the obtained results. Our studies found relations between *-344C/T* polymorphism of *CYP11B2* gene and EAH occurrence (especially in *T*-allele carriers' women). The risk of CKD estimated by creatinine level (CKD-EPI) 6.5 times increased, and by Cystatin-C blood level – 1.5 times increased mainly in *T*-allele carriers' women. Moreover, 2 DM increased the risk of CKD 2.4 times, obesity (BMI >30 kg/m^2) – 2.08 and 2.32 times as much. Furthermore, the 3rd grade of BP elevation (SBP ≥180 Hg mm and/or DBP ≥110 Hg mm) increased the CKD probability threefold, which partially correlates with multiple studies and confirms our certain results published earlier [14-16, 23-26].

In addition, the frequency of wild and mutated alleles obtained by us corresponded to the prevailing majority of Caucasian populations (European race and Americans): P_C=0.44-0.50 vs P_C=0.44-0.49; and P_T=0.50-0.56 vs P_T=0.51-0.56, respectively (p>0.05). Whereas, the wild *C*-allele frequency is considerably higher in our research, than in the Equatorial or Asian races representatives, and African Americans as well (p<0.05), with lower *T*-allele frequency, respectively: P_C=0.19-0.39, P_T=0.61-0.81 (p<0,05) [27].

Table 3. Alleles of *CYP11B2* (rs1799998) gene as risk factors of Essential Arterial Hypertension depending on gender

Potential risk factor		Parameters				
		RR	95% CI RR	OR	95% CI OR	p
Females	*C*-allele	0.75	0.57-0.98	0.53	0.28-0.98	0.041
	T- allele	1.42	1.0-2.03	1.90	1.02-3.54	0.029
Males	*C*-allele	0.98	0.56-1.72	0.97	0.39-2.41	>0.05
	T-allele	1.01	0.71-1.44	1.03	0.41-2.58	>0.05

Note. RR – risk ratio; OR – odds ratio; 95%CI - Confidence Intervals

4. CONCLUSIONS

Mutation of *CYP11B2* (rs1799998) gene in homozygous condition in hypertensive patients and practically healthy individuals does not differ reliably. Distribution of polymorphic variants of *CYP11B2* (rs1799998) gene in observed population corresponds to the populations of European race.

Presence of *T*-allele of *CYP11B2* (*344C>T*) gene in women increases risk of EAH 1.5 times. Whereas, *C*-allele promotes the lowest chances of EAH occurrence (OR=0.53; p=0.041). The risk of CKD appearance increases in *T*-allele hypertensive women 1.5 times and 6.5 times according to GFR CKD-EPI calculated by Cystatin-C and creatinine blood level respectively with the lowest probability of CKD in *T*-allele men. Moreover, type 2 diabetes mellitus in hypertensive patient, likewise obesity and 3rd degree blood pressure elevation increases the CKD risk 2.4, 2.08, 2.32 and 3.0 times accordingly.

Our investigation results suggest that Aldosterone synthase (*CYP11B2*) gene *C-344T* polymorphism associated with Chronic Kidney Disease development in hypertensive patients and may affect the disease clinical course.

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