sciendo

ENDOCRINE REGULATIONS, Vol. 54, No. 3, 217–226, 2020

doi:10.2478/enr-2020-0024

The cytochrome 11B2 aldosterone synthase gene rs1799998 single nucleotide polymorphism determines elevated aldosterone, higher blood pressure, and reduced glomerular filtration, especially in diabetic female patients

Larysa Sydorchuk[1], Valentina Dzhuryak[1], Andrii Sydorchuk[2], Svitlana Levytska[1], Volodymyr Petrynych[1], Ruslan Knut[1], Anna Kshanovska[1], Oksana Iftoda[1], Olexii Tkachuk[1], Petro Kyfiak[1], Andrii Popovich[1], Oleh Khomko[1], Ruslan Sydorchuk[1]

[1]Bukovinian State Medical University, Chernivtsi, Ukraine; [2]St. Anna Hospital, Herne, Germany
E-mail: lsydorchuk@bsmu.edu.ua

Objective. The cytochrome *11B2* aldosterone synthase gene (*CYP11B2*) that links to aldosterone synthase enzyme synthesis changes and blood pressure regulation is of particular interest among the renin-angiotensin-aldosterone system encoding genes.

Methods. One-hundred hypertensive patients with target-organ damaging (2nd stage), moderate, high or very high cardiovascular risk were involved in the case-control study. Mean age was 59.87±8.02 years. Diabetes Mellitus type 2 (DM2) was in 28 persons. Chronic kidney disease (CKD) was diagnosed in 29 persons according to the National Kidney Foundation recommendations (2012) after glomerular filtration rate (GFR) decline <60 ml/min/1.73m2 for ≥3 months (measured by CKD-EPI equations). Aldosterone, cystatin-C, and creatinine levels were measured in serum. Control group included 48 practically healthy persons of relevant age. Gene's nucleotide polymorphism *CYP11B2* (-344C/T) was examined by polymerase chain reaction.

Results. CKD evolution in hypertensive patients followed by higher systolic and diastolic blood pressure (SBP, DBP) values increased creatinine, cystatin-C, and aldosterone serum concentrations by 28.76%, 28.41% and 29.43% ($p<0.05$), respectively. Polymorphic site of *CYP11B2* (rs1799998) gene is associated with SBP and DBP increase ($p<0.05$), reduced GFR preferably calculated by CKD-EPI-cystatin C ($F=10.79–14.45$; $p<0.001$) and elevated aldosterone content ($F=55.84$; $p<0.001$), creatinine and cystatin-C as well ($F=4.16–5.08$; $p<0.05$) mainly in the *TT*-genotype female carriers ($p<0.001$). Hypertensive women with DM2 demonstrated stronger relations of *CYP11B2* gene polymorphic site with the increased aldosterone content ($F=47.52$; $p<0.001$), than women without DM2 ($p<0.001$) and male patients ($p=0.014$).

Conclusions. Genetic variations involving *CYP11B2* might influence the kidney function, hypertension course, and severity via aldosterone secretion upregulation.

Key words: CYP11B2 (rs1799998) gene, aldosterone, chronic kidney disease, hypertension, diabetes

Aldosterone (AS) is a crucial mineralocorticoid hormone synthesized from cholesterol mainly in the adrenal cortex *zona glomerulosa* influencing the electrolyte and fluid balances. However, synthesis and release of AS is controlled by the renin-angiotensin-aldosterone system (RAAS), especially angiotensin II

Corresponding author: Larysa P. Sydorchuk, MD, PhD, DSc; Department of Family Medicine, Bukovinian State Medical University (BSMU), 58002, 2, Theatre sq., Chernivtsi, Ukraine; Scopus ID 8428375700; phone: +380678784456; e-mail: lsydorchuk@bsmu.edu.ua.

(ATII), potassium ions concentration, and the hypothalamic-hypophysial system: adrenocorticotropic hormone, dopamine level, arterial blood pressure (BP) value via vascular baroreceptors as well as the sodium (Na^+) and magnesium (Mg^{2+}) blood concentrations. Besides, AS effects are mediated by endocrine, autocrine, and paracrine impacts. Moreover, the particular mineralocorticoid receptors (MCR) are extensively located not only in the vascular endothelium, but also distributed on the surface of many organs and cells, such as kidneys, brain, liver, myocardiocytes, smooth muscles, astrocytes, adipocytes, macrophages, monocytes, etc. (Whaley-Connell et al. 2010; Sia et al. 2013).

The molecular level of AS synthesis depends directly on the activity of mitochondrial aldosterone synthase enzyme, a steroid hydroxylase cytochrome P450 enzyme. The latter is coded by *CYP11B2* gene (cytochrome P450, family 11, subfamily B, polypeptide 2). Mitochondrial aldosterone synthase enzyme belongs to the cytochrome super-family P450 and regulates aldosterone synthesis. It catalyzes the key reactions in aldosterone synthesis from precursors: conversion of 11-deoxycorticosterone into corticosterone, conversion of corticosterone into 18-hydroxycorticosterone, and finally conversion of 18-hydroxycorticosterone into aldosterone. *CYP11B2* gene codes a key enzyme of the final phase aldosterone synthesis, 18-hydroxylase, which attaches the hydroxyl group (OH-) to C18 residue and converts deoxycorticosterone into aldosterone (Stocco 2001; Garg and Adler 2015).

Therefore, we focus our research on analysis of secondary hyperaldosteronism and chronic kidney disease in essential arterial hypertensive patients' determination by the cytochrome 11B2 aldosterone synthase gene CYP11B2 (rs1799998) nucleotide polymorphism with considerations to gender and diabetes.

Materials and methods

Inclusion/exclusion criteria. The study included essential hypertensive patients with hypertension-mediated organ damage (HMOD) estimated according to European Societies of Hypertension and Cardiology recommendation (ESH/ESC 2018): target-organs damage – 2nd stage (asymptomatic AH), from the 1st through to the 3rd grade of BP; moderate-high cardiovascular (CV) risk; age above 30 years.

Exclusion criteria have been described in previous publications (Sydorchuk et al. 2015a, 2017; Dzhuryak et al. 2020): we excluded patients with EAH stage 3 (established CV disease, chronic kidney diseases (CKD) – with estimated glomerular filtration rate (eGFR) decline <30 ml/min/1.73 m^2); secondary arterial hypertension; chronic heart failure (CHF) higher than II functional class (NYHA III–IV), EAH patients with complications of HMOD; diabetes mellitus type 1 (DM1), 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; administration of oral corticosteroids or contraceptives; pregnancy or lactation period.

After screening of matching inclusion and exclusion criteria, 100 patients were selected for further examination. The genetic examination was performed in 72 cases. The control group included 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. All enrolled subjects signed a consent form to participate in the study.

Research was performed in compliance with the European Convention on Human Rights and Biomedicine, GCP, EUC directive #609 and other EU and international legislations on bioethics. The Study Protocol was approved by the Ethics' Committee of Bukovinian State Medical University.

Essential arterial hypertension and renal function assessment. 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) recommendations' requirement (Williams et al. 2018).

All enrolled patients underwent kidney ultrasound. Serum and urine sample were collected in the beginning of the research. Serum sample was used to measure creatinine (Cr) and cystatine C (CysC) levels with following GFR calculating using several estimating formulae: Cockroft-Gault and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations with Creatinine alone (CKD-EPI Cr) (Levey et al. 2009) and CysC alone (CKD-EPI CysC) (Inker et al. 2012) depending on the gender. CKD was determined according to the National Kidney Foundation recommendations

(Kidney Disease: Improving Global Outcomes – KDIGO 2012) after GFR decline ≤60 ml/min/1.73 m^2 for over 3 months with or without other signs of kidney damage, according to KDIGO recommendations (Leevin et al. 2013). CKD was diagnosed in 29 EAH individuals.

Aldosterone level detection. Aldosterone serum level was determined by immune-enzyme assay (ELISA) according to the Manufacturer's Guidelines (Aldosterone®, DRG, Germany) on the "StatFax 303" device (USA). This assay employed a polyclonal antibody (of a rabbit) specific for human aldosterone coated on a micro-titrating 96-well plate. Standards and samples were pipetted into the wells and endogenous Aldosterone present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human aldosterone antibody was added. After washing out unbound biotinylated antibody horseradish peroxidase-conjugated streptavidin was pipetted to the wells. After incubation unconjugated conjugate was washed out. Then tetramethylbenzidine substrate solution was added to the wells and color developed in proportion to the amount of aldosterone bound. The Stop Solution contained 0.5 M H_2SO_4 changed the color from blue to yellow. The intensity of the color was inversely to aldosterone concentration and measured in optic unit (450 nm).

Evaluation of serum cystatin C. Cystatin-C content in the blood serum was determined by the immune chemiluminescence analysis on "Maglumi 1000 Plus" ("Snibe", China) device according to the Manufacturer's reagents set instruction (CysC® "Dialab", Austria). We used calibrator "Cystatin C Dialab" to build a calibrated curve on the base of human serum as a reference sample. The principle of the method was based on immune chemiluminescence measuring concentration of insoluble immune complexes antigen-antibody formed when a serum sample is introduced on the CysC latex sensitized by goat antibodies on the surface of micro-titrating wells. Intensity of staining (turbidity) with formation of insoluble immune complexes antigen-antibody was registered by chemiluminescence analyzer.

Genotyping of the aldosterone synthase CYP11B2 (C-344T) gene polymorphism. DNA extraction and amplification. 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). 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 consists of 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 (Figure 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 of amplification cycles and full RT-PCR protocol of *CYP11B2 344C>T* gene's polymorphism detection are given in Figures 2 and 3.

Statistical analysis. Statistical analysis was performed using Statistica v. 7.0 (StatSoft, USA) software. For the genotypes distribution comparison

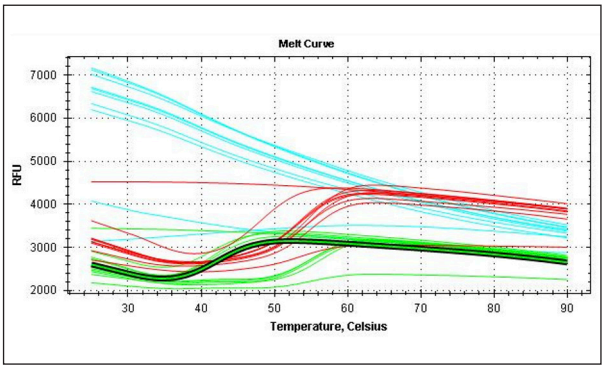

Figure 1. Temperature bars in analysis of CYP11B2 344C>T gene's polymorphism in observed population. Blue color shows the samples homozygous for the C-allele of the CYP11B2 gene (344C>T), determined by the Fam channel; green color – samples homozygous for Hex channel (T-allele); red color – heterozygous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

we used Pearson's (χ^2) criterion. Analysis of independent quantitative samples were calculated using Student's t-test, two-tail distribution and equal/unequal variances between two samples (mean±SD) if Kolmogorov-Smirnov test or Shapiro-Wilk W-test proved an even/close to the normal distribution, or Wilcoxon-Mann-Whitney U-test in case of uneven distribution. Analysis of qualitative data (categorical variables), risk of pathology development was assessed by odds ratio (OR), with 95% confidence interval (CI) using a chi-square test (χ^2), df=1. One-way ANOVA analysis was used to confirm the association of *CYP11B2* (rs1799998) gene polymorphism with diagnostic clinical and laboratory parameters. A p-value <0.05 was considered statistically significant.

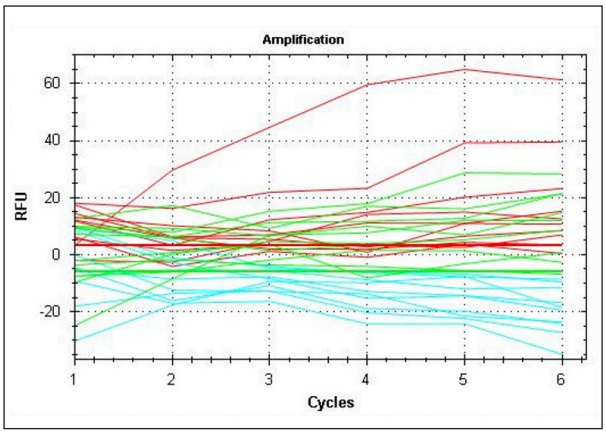

Figure 2. Amplification results of CYP11B2 344C>T gene's polymorphism. Blue color – CC-genotypes carriers; green color – TT-genotypes carriers; red color – TC-heterozygous specimens.

Results

SBP and DBP values depending on GFR were reliably higher in hypertensive patients with lowered GFR (≤60 ml/min/1.73 m^2) than in preserved GFR patients by 5.88% (p=0.025) and 7.74% (p=0.003), respectively (Table 1). Creatinine and cystatin-C concentrations were higher in patients with GFR ≤60 ml/min/1.73 m^2 likewise by 28.76% and 28.41% (p<0.001), respectively. Eventually, it resulted in lower GFR according to creatinine and cystatin-C levels (CKD-EPI) by 36.40% and 39.80% (p<0.001), respectively. Moreover, a serum aldosterone in synergic bound with creatinine and cystatin-C was higher in patients with GFR ≤60 ml/min/1.73 m^2 by 27.82% (p=0.048) and 29.43 (p=0.043), respectively. Furthermore, uric acid prevailed in patients with GFR ≤60 ml/min/1.73 m^2 over control group and patients with preserved kidney function by 35.48% (p<0.01) and 21.76% (p=0.048), correspondingly.

SBP and DBP values in hypertensive patients depending on the polymorphic variants of *CYP11B2* (rs1799998) gene are presented in Table 2. SBP and DBP levels are reliably higher in patients with *TT*-genotype than those in *C*-allele carriers: for SBP by 5.52% and 6.17% (p<0.05), for DBP by 7.48% and 5.13% (p<0.05), respectively. One-way ANOVA confirmed the association of *CYP11B2*) gene's rs1799998 polymorphism with SBP (F=4.33; p=0.017) and DBP (F=6.81; p=0.002) increase.

Gender-depending distribution indicated that SBP and DBP values in *TT*-genotype women carriers with EAH are slightly higher than those in the *C*-allele carriers: for SBP by 4.53% (p=0.051) and 5.70% (p=0.025), for DBP by 5.57% (p=0.035) and 3.67%

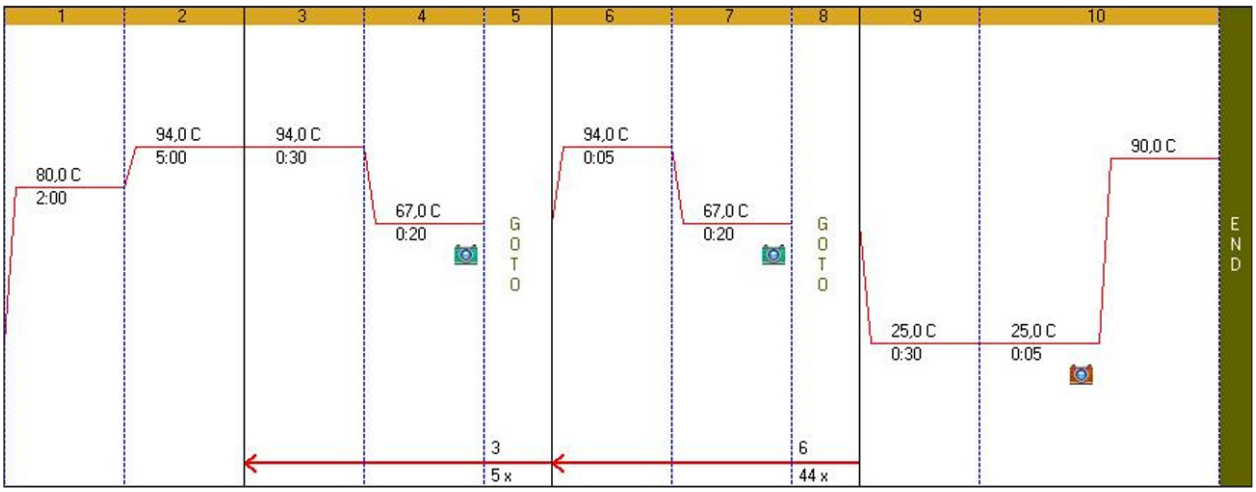

Figure 3. Full RT-PCR protocol of CYP11B2 344C>T gene's polymorphism detection.

Table 1
Baseline clinical characteristics of the enrolled subjects depending on the glomerular filtration rate.

Parameters	Control group	Hypertensive patients	
		GFR >60 ml/min/1.73m²	GFR ≤60 ml/min/1.73m²
Age (years)	50.36±7.22	59.44±6.28	60.93±5.88
SBP (mmHg)	116.33±4.73	150.23±3.94 p<0.001	159.07±5.32 p<0.001; p_1=0.025
DBP (mmHg)	76.0±5.16	92.27±1.94 p<0.001	99.41±2.59 p<0.001; p_1=0.003
BMI (kg/m²)	25.86±2.14	30.86±1.88 p<0.001	32.35±2.20 p<0.001
Uric acid (mmol/l)	4.81±0.48	5.86±0.97	7.49±0.85 p<0.01; p_1=0.048
ALT (μmol/h/l)	0.48±0.14	0.59±0.22	0.56±0.11
AST (μmol/h/l)	0.34±0.10	0.40±0.15	0.41±0.09
Total bilirubin (μmol/l)	15.41±3.44	14.28±2.48	15.60±2.68
Creatinine (μmol/l)	73.13±5.45	68.67±3.43	88.42±2.62 p, p_1<0.001
GFR by CKD-EPI-Cr (ml/min/1.73 m²)	102.93±10.74	89.10±5.60 p=0.011	58.67±4.83 p, p_1<0.001
Cystatin C (mg/L)	0.93±0.07	0.88±0.04	1.13±0.05 p, p_1<0.001
GFR by CKD-EPI-CysC (ml/min/1.73m²)	94.18±7.98	88.35±5.99	54.19±3.52 p, p_1<0.001
Aldosterone (pg/ml)	65.48±3.31	135.99±22.05 p<0.001	176.01±19.34 p<0.001, p_1=0.043

Data are presented as mean ± SD. Abbreviations: SBP, DBP – systolic, diastolic blood pressure; BMI – body mass index; ALT, AST – alanine, aspartate aminotransferase enzymes; GFR – glomerular filtration rate; CKD-EPI – Chronic Kidney Disease-Epidemiology Collaboration estimating equations; p – significance of differences with control group; p1 – significance of differences with group of patients with GFR >60 ml/min/1.73 m².

Table 2
Blood pressure values in hypertensive patients depending on CYP11B2 (rs1799998) gene's genotypes.

Parameters	Groups	Genotypes of *CYP11B2* (rs1799998) gene		
		CC	TC	TT
SBP (mmHg)	Control	115.71±4.28	117.0±3.82	117.14±3.57
	Patients	151.07±7.81 p<0.001	150.14±12.06 p<0.001	159.41±9.76; p<0.001; p_{CC}=0.03; p_{TC}=0.01
DBP (mmHg)	Control	75.71±4.29	76.0±5.09	77.14±3.17
	Patients	91.43±3.57 p<0.001	93.47±5.27 p<0.001	98.27±5.13; p<0.001; p_{CC}=0.008; p_{TC}=0.04

Data are presented as mean ± SD. Abbreviations: SBP, DBP – systolic, diastolic blood pressure; p – significance of differences with control group according to appropriate genotype; p_{CC} – significance of differences with CC-genotype patients; p_{TC} – significance of differences with TC-genotype patients.

(p=0.054), respectively (Table 3). Similar dependence was not observed among men. One-way ANOVA analysis (Table 3) confirmed association of *CYP11B2* (rs1799998) gene polymorphism in hypertensive women with increasing SBP (F=5.81; p=0.005) and DBP (F=4.78; p=0.013).

Creatinine, cystatin-C and aldosterone serum content and GFR indices depending on polymorphic variants of *CYP11B2* (rs1799998) gene are presented in Table 4. GFR calculated by CKD-EPI-Cr and CKD-EPI-CysC in *TT*-genotype patients are lower than those in *C*-allele carriers by 17.44%, 16.75 (p<0.05) and 20.62%, 18.12% (p<0.05), respectively. On the contrary, the GFR parameters in the control group in the *TT*-genotype carriers are higher than those in the *C*-allele carriers: for CKD-EPI-Cr by

37.36% (p<0.001) and 24.06% (p=0.048), for CKD-EPI-CysC by 26.95% (p=0.01) and 15.18% (p>0.05), respectively. Aldosterone content in patients was higher than in the control group regardless genotypes: for *CC*-, *CT*- and *TT*-variants – 2.14, 2.45 and 2.72 times as much (p<0.001), respectively. Moreover, within every group aldosterone concentration in the *T*-allele carriers prevailed over one in the *CC*-genotype persons: in the control group by 11.09% (p=0.02) and 21.46% (p=0.009), in EAH patients by 26.90% (p=0.015) and 53.62% (p<0.001), respectively. One-way ANOVA analysis (Table 4) confirmed the association of *CYP11B2* (rs1799998) gene polymorphism with reduced GFR calculated by CKD-EPI-Cr and CKD-EPI-CysC (F=10.79; p<0.001 and F=14.45; p<0.001), as well as with aldosterone content increase (F=55.84; p<0.001).

In *TT*-genotype women the levels of creatinine and cystatin-C prevailed those in the *CC*-genotype carriers by 11.99% (p=0.046) and 11.58% (p=0.048), which caused (Table 5) reliably lower GFR (CKD-EPI-CysC) by 22.73% (p=0.02). Similar tendency among men was not found, though GFR (CKD-EPI-Cr) was higher than in women, particularly in the

Table 3
Blood pressure values depending on gender and polymorphic variants of the CYP11B2 (rs1799998) gene's genotypes.

Parameters	Gender	*CYP11B2* gene's genotypes		
		CC	TC	TT
SBP (mmHg)	F	150.83±7.05	149.17±8.80	157.67±6.12 p_{CC}=0.05; p_{TC}=0.025
	M	152.50±8.33	152.08±13.46	160.0±7.50
DBP (mmHg)	F	91.25±4.04	92.92±5.23	96.33±3.17 p_{CC}=0.035; p_{TC}=0.054
	M	92.50±5.0	94.58±7.31	99.29±4.64

Data are presented as mean ± SD. Abbreviations: SBP, DBP – systolic, diastolic blood pressure; F – females; M – males; p_{CC} – significance of differences with CC-genotype patients; p_{TC} – significance of differences with TC-genotype patients.

Table 4
Glomerular filtration rate and aldosterone levels in observed population regarding to CYP11B2 (rs1799998) gene's genotypes.

Parameter	Group	CYP11B2 gene's genotypes		
		CC	TC	TT
Creatinine (μmol/l)	Control	80.28±8.11	75.50±7.67	67.61±6.88 p_{CC}=0.02
	Patients	73.84±7.46	76.14±8.59	80.10±8.19 p=0.044
GFR by CKD-EPI-Cr (ml/min/1.73 m²)	Control	79.17±10.50	87.66±12.94	108.75±7.85 p_{CC}<0.001; p_{TC}=0.048
	Patients	83.12±9.88	82.43±12.98	68.62±10.76 p<0.001; $p_{CC,TC}$<0.05
Cystatin C (mg/L)	Control	1.05±0.11	0.99±0.10	0.89±0.09 p_{CC}=0.02
	Patients	0.97±0.10	1.0±0.12	1.03±0.13 p=0.048
GFR by CKD-EPI-CysC (ml/min/1.73 m²)	Control	74.06±9.08	81.63±10.55	94.02±10.56 p_{CC}=0.01
	Patients	78.84±8.49	76.43±13.07	62.58±6.64 p<0.001; p_{CC}=0.03
Aldosterone (pg/ml)	Control	58.07±2.33	64.51±1.89 p_{CC}=0.02	70.53±6.32 p_{CC}=0.009
	Patients	124.56±12.85 p<0.001	158.07±17.44 p<0.001; p_{CC}=0.015	191.35±23.28 p, p_{CC} <0.001

Data are presented as mean ± SD. Abbreviations: GFR – glomerular filtration rate; CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration estimating equations; Cr – Creatinine; CysC - Cystatin C; p, p – significance of differences with control group according to appropriate genotype; p_{CC} – significance of differences with CC-genotype carriers; p_{TC} – significance of differences with TC-genotype carriers.

TC-genotype carriers, by 14.88% (p=0.008). Moreover, aldosterone concentration in T-allele carriers appeared to be higher than that in the *CC*-genotype patients irrespective of the gender: for women by 27.01% (p<0.05) and 49.64% (p<0.001), for men by 20.93% and 43.11% (p<0.05). Besides, the aldosterone content is reliably higher in men than in women, regardless the *CYP11B2* (rs1799998) gene's polymorphic variants, by 12.38%, 18.04% and 17.50% (p<0.05), respectively.

One-way ANOVA analysis confirmed the association of *CYP11B2* (rs1799998) gene's polymorphism in hypertensive women with increased creatinine (F=5.08; p=0.01) and cystatin-C (F=4.16; p=0.022), reduced GFR calculated by CKD-EPI-CysC (F=13.85; p<0.001) and elevated aldosterone both in women (F=49.65; p<0.001) and men (F=13.61; p<0.001).

Aldosterone level in *T*-allele carriers' women is higher than in men (Table 6), especially in presence of the DM2. Moreover, aldosterone level among *T*-allele women with comorbid DM2 is higher than in those without type 2 DM: for *TC*-genotype by 13.06% (p_{DM2}=0.002), for *TT*-genotype by 16.77% (p_{DM2}=0.003). Furthermore, in *T*-allele female carriers (particularly with *TT*-genotype) aldosterone level was higher than that in *CC*-genotype women: with comorbid DM2 by 24.66% and 50.82% (p_{CC}<0.001), without DM2 by 19.17% and 39.60% (p_{CC}<0.001), respectively. Apart from that, higher aldosterone levels were found in *T*-allele men without DM2, than in *CC*-genotype carriers by 16.81% (p_{CC}=0.037) and 32.01% (p_{CC}=0.057). In addition, one-way ANOVA analysis confirmed an association of *CYP11B2* (rs1799998) gene's polymorphism in EAH patients with increased aldosterone in women with comorbid DM2 (F=47.52; p<0.001) and without DM2 (F=28.74; p<0.001), as well as in hypertensive men without DM2 (F=6.10; p=0.014).

Discussion

The major AS effects include regulation of the water-salt metabolism, increase blood volume circulation, and arterial BP (Freel and Connell 2004; Abdel Ghafar 2019a). On the cellular level, AS intensifies kB nuclear factor (NF-kB) expression's transcription with increase of superoxide radical production in the vascular wall. Apart from that mediates endothelial dysfunction and vasoconstriction, AS induces low intensive inflammation in the vascular wall, myocardium, and kidneys, resulting in the development of myocardiosclerosis, nephrosclerosis, and fibrinolysis

Table 5
Glomerular filtration rate and aldosterone levels in hypertensive patients depending on gender and CYP11B2 (rs1799998) gene's polymorphic variants.

Parameters	Gender	*CYP11B2* gene's genotypes		
		CC	TC	TT
Creatinine (μmol/l)	F	72.22±5.11	73.94±10.43	80.88±3.21 p_{CC}=0.046
	M	83.50±1.01 p_F=0.037	80.54±8.95	85.0±6.0
GFR by CKD-EPI-Cr (ml/min/1.73 m²)	F	81.46±9.61	78.94±7.07	83.31±11.19
	M	93.11±1.35	90.69±5.55 p_F=0.008	90.92±6.94
Cystatin C (mg/L)	F	0.95±0.08	0.97±0.14	1.06±0.07 p_{CC}=0.048
	M	1.10±0.01 p_F=0.046	1.06±0.12	1.12±0.11
GFR by CKD-EPI-CysC (ml/min/1.73 m²)	F	80.26±9.62	75.68±10.41	62.02±8.50 p_{CC}=0.02
	M	70.32±1.12 p_F=0.051	73.92±8.45	68.43±9.42
Aldosterone (pg/ml)	F	130.41±10.24	165.64±16.50 p_{CC}<0.05	195.14±20.86 p_{CC}<0.001
	M	116.04±8.60 p_F<0.05	140.33±12.74 p_F<0.05; p_{CC}<0.05	166.07±15.53 p_F<0.05; p_{CC}<0.001

Data are presented as mean ± SD. Abbreviations: p_F – significance of differences with female according to appropriate genotype; p_{CC} – significance of differences with CC-genotype carriers; p_{TC} – significance of differences with TC-genotype carriers.

Table 6
Aldosterone levels in hypertensive patients depending on gender, type 2 diabetes mellitus and CYP11B2 (rs1799998) gene's polymorphic variants.

Parameter	Gender/DM		*CYP11B2* gene's genotypes		
			CC	TC	TT
Aldosterone (pg/ml)	F	with DM2	136.35±9.64	169.97±10.82 p_{CC}<0.001	205.64±14.62 $p_{CC,TC}$<0.001
		without DM2	126.15±7.45	150.33±12.61 P_{CC}<0.001 p_{DM2}=0.002	176.11±16.47 $p_{CC,TC}$<0.001 p_{DM2}=0.003
	M	with DM2	–	149.02±9.45 p_{F+DM2}=0.052	172.33±12.67 p_{F+DM2}=0.007
		without DM2	116.04±8.60	135.55±10.67 p_{CC}=0.037 p_{F-DM2}=0.004	153.19±18.01 p_{CC}=0.057 p_{TC}=0.039; p_{F-DM2}=0.052

Data are presented as mean ± SD. Abbreviations: F – females; M – males; DM2 – type 2 diabetes mellitus; p_{DM2} – significance of differences with patients with type 2 DM according to appropriate genotype; p_{F+DM2} – significance of differences with hypertensive females with type 2 DM according to appropriate genotype; p_{F-DM2} – significance of differences with hypertensive females without type 2 DM according to appropriate genotype; p_{CC} – significance of differences with CC-genotype carriers; p_{TC} – significance of differences with TC-genotype carriers.

disorders etc. Moreover, in the case of AH, ischemic heart disease and heart failure AS synthesis is activated in the vascular walls and heart (Connell and Davies 2005; Tomaschitz et al. 2010; Schutten et al. 2017). Expression of aldosterone gene in these tissues is only 1/15 of expression in the adrenal glands, and high-salt diet increases AS synthesis. Concentration and AS activity are determined by *CYP11B2* (rs1799998) gene expression, located on the long arm of 8th chromosome in the position 24.3 (8q24.3) (MacKenzie et al. 2017). Nowadays, 227 single-nucleotide polymorphisms (SNP) of *CYP11B2* gene are identified in different populations. SNP-344 *C/T* variant in 5`-promoter region of *CYP11B2* gene is the most studied, it can associate with the development of AH. Though, the available studies are confusing and disputable. The majority of researchers found that the *T*-allele is associated with AH (Ye et al. 2013), that confirms our certain results published earlier (Sydorchuk et al. 2015a, b; Dzhuryak et al. 2020). Other studies confirm the *C*-allele role in the AH development and its association with an increased aldosterone synthesis (Fernandes-Rosa et al. 2014). Moreover, the -344*C* allele binds transcription steroidogenic factor-1 (SF-1) 4 times more potent than *T*-allele, resulting in AS level increase (Nicod et al. 2003). On the other hand, this kind of polymorphism is suggested to enhance its action via a tight linkage disequilibrium with *CYP11B1* gene polymorphism of *T*- to *C*- substitution in the codon 75 and *G*- to *A*- in the intron 6, resulting in 11β-hydroxylase deficiency (Ganapathipillai et al. 2005). It causes a sustained adrenocorticotropic hormone elevation with further expression increase of numerous genes responsible for aldosterone synthesis such as *StAR*, *CYP11A* and *CYP21* and resulting in an intensified aldosterone synthesis (Connell et al. 2003). The *C*-allele of *CYP11B2* gene in Chinese population was found to be mostly associated with EAH (Cheng and Xu 2009). Though, other researchers did not find a reliable relation between this polymorphism and EAH, BP values, AS level or HMOD as well as salt sensitivity (Tsujita et al. 2001; Chen et al. 2015; Abdel Ghafar 2019b).

Other researchers have suggested that AS appears to play a pivotal role in the left ventricle hypertrophy progression and hypertrophic cardiomyopathy (HCM) advance since AS is synthetized in the heart locally and *CYP11B2* mRNA levels demonstrate seven-fold AS increase in the myocardium of HCM patients than in healthy cardiac tissue (Tsybouleva et al. 2004). Our results concerning AS level conform partially to the data obtained by Chai et al. (2006) who have found that HMOD and an interventricular septum thickness was bigger in the *T*-allele carriers' men of *CYP11B2* gene and associated with an increased AS plasma concentration. In addition, adipocytes stimulate AS synthesis in case of CKD as well. They are responsible for the development of insulin resistance (Hall et al. 2015). It confirms our results, where patients with EAH and DM2, particularly *T*-allele carriers' women of *CYP11B2* gene had reliably higher AS levels.

Therefore, genetic variations involving *CYP11B2* gene might influence the structure and function of kidney tissue, adipocytes activities, worsening the metabolic disorders, kidney failure, and hypertension course via increasing of AS secretion, as we have suggested in our research. The research perspective of this study may be supplemented by evaluating the linkage between lipid metabolism and CYP11B2 (rs1799998) gene's polymorphic variants, as well as some non-specific immunological profile and kidney function in hypertensive diabetic patients.

In conclusion, Chronic Kidney Disease evolution in essential hypertensive patients is followed by higher SBP and DBP values, increased creatinine, cystatin-C and aldosterone serum concentration by 28.76%, 28.41% and 29.43% ($p<0.05$), respectively. Polymorphic site of *CYP11B2* (rs1799998) gene is associated with the increase of SBP ($F=4.33$; $p=0.017$) and DBP ($F=6.81$; $p=0.002$), reduced GFR preferably calculated by CKD-EPI-CysC ($F=10.79–14.45$; $p<0.001$) and elevated AS content ($F=55.84$; $p<0.001$), Creatinine and cystatin-C as well ($F=4.16–5.08$; $p<0.05$) mainly in the *TT*-genotype women carriers ($p<0.001$).

Hypertensive women with DM2 demonstrate stronger relations of *CYP11B2* gene's polymorphic site with the increased aldosterone content ($F=47.52$; $p<0.001$) than in women without DM2 ($F=28.74$; $p<0.001$) and men as well ($F=6.10$; $p=0.014$).

References

Abdel Ghafar MT. Aldosterone synthase gene (CYP11B2) polymorphisms and enhanced cardiovascular risk. In The recent topics in genetic polymorphisms (Eds. Çalişkan M, Erol O, Oz GC), IntechOpen Limited, London, 2019a.

Abdel Ghafar MT. Association of aldosterone synthase CYP11B2 (–344C/T) gene polymorphism with essential hypertension and left ventricular hypertrophy in the Egyptian population. Clin Exp Hypertens 41, 779–786, 2019b.

Chai W, Hoedemaekers Y, van Schaik RH, van Fessem M, Garrelds IM, Saris JJ, Dooijes D, ten Cate FJ, Kofflard MM, Danser AH. Cardiac aldosterone in subjects with hypertrophic cardiomyopathy. J Renin Angiotensin Aldosterone Syst 7, 225–230, 2006.

Chen JF, Jing J, Tan H, Song MB, Yu SY, Huang L. Lack of association of CYP11B2-344C/T polymorphism with essential hypertension: A meta-analysis. Int J Clin Exp Med 8, 9162–9167, 2015.

Cheng X, Xu G. Association between aldosterone synthase CYP11B2 polymorphism and essential hypertension in Chinese: a meta-analysis. Kidney Blood Press Res 32, 128–140, 2009.

Connell JM, Fraser R, MacKenzie S, Davies E. Is altered adrenal steroid biosynthesis a key intermediate phenotype in hypertension? Hypertension 41, 993–999, 2003.

Connell JM, Davies E. The new biology of aldosterone. J Endocrinol 186, 1–20, 2005.

Dzhuryak V, Sydorchuk L, Sydorchuk A, Kamyshnyi O, Kshanovska A, Levytska S, Knut R, Sheremet M, Ivashchuk S, Petrynych O, Kazantseva T, Nikyfor L, Melnychuk L, Sokolenko A, Yarynych Y, Semianiv M, Repchuk Y, Voroniuk K, Sydorchuk R, Sokolenko L, Iftoda O, Kushnir O. The cytochrome 11B2 aldosterone synthase gene CYP11B2 (RS1799998) polymorphism associates with chronic kidney disease in hypertensive patients. Biointerface Research in Applied Chemistry 10, 5406–5411, 2020.

Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, Strom TM, Monticone S, Amar L, Meatchi T, Mantero F, Cicala MV, Quinkler M, Fallo F, Allolio B, Bernini G, Maccario M, Giacchetti G, Jeunemaitre X, Mulatero P, Reincke M, Zennaro MC. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. Hypertension 64, 354–361, 2014.

Freel EM, Connell JM. Mechanisms of hypertension: The expanding role of aldosterone. J Am Soc Nephrol 15, 1993–2001, 2004.

Ganapathipillai S, Laval G, Hoffmann IS, Castejon AM, Nicod J, Dick B, Frey FJ, Frey BM, Cubeddu LX, Ferrari P. CYP11B2-CYP11B1 haplotypes associated with decreased 11 beta-hydroxylase activity. J Clin Endocrinol Metab 90, 1220–1225, 2005.

Garg R, Adler GK. Aldosterone and the mineralocorticoid receptor: risk factors for cardiometabolic disorders. Curr Hypertens Rep 17, 52, 2015.

Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res 116, 991–1006, 2015.

Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS; CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 367, 20–29, 2012.

Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 150, 604–612, 2009.

Leevin A, Stevens PE, Bilous RW, Coresh J, deFrancisco ALM, deJong P, Griffith KE, Hemmelgarn BR, Iseki K, Lamb EJ, et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Inter Suppl 3, 19–62, 2013.

MacKenzie SM, Davies E, Alvarez-Madrazo S. Analysis of the aldosterone synthase (CYP11B2) and 11β-hydroxylase (CYP11B1) genes. Methods Mol Biol 1527, 139–150, 2017.

Nicod J, Bruhin D, Auer L, Vogt B, Frey FJ, Ferrari P. A biallelic gene polymorphism of CYP11B2 predicts increased aldosterone to renin ratio in selected hypertensive patients. J Clin Endocrinol Metab 88, 2495–2500, 2003.

Schutten MT, Houben AJ, de Leeuw PW, Stehouwer CD. The link between adipose tissue renin-angiotensin-aldosterone system signaling and obesity-associated hypertension. Physiology (Bethesda) 32, 197–209, 2017.

Sia SK, Chiou HL, Chen SC, Tsai CF, Yang SF, Ueng KC. Distribution and phenotypic expression of mineralocorticoid receptor and CYP11B2 T-344C polymorphisms in a Taiwanese hypertensive population. Mol Biol Rep 40, 3705–3711, 2013.

Stocco DM. StAR protein and the regulation of steroid hormone biosynthesis. Annu Rev Physiol 63, 193–213, 2001.

Sydorchuk LP, Sokolenko AA, Sydorchuk AR, Kryklyvets LG, Biryuk IG, Fliundra IG, Sokolenko MA. Insulin resistance in patients with arterial hypertension and abdominal obesity depending on ace and ppar-γ2 genes polymorphism: A new opinion concerning an old problem. New Armenian Medical Journal 9, 43–51, 2015a.

Sydorchuk LP, Ursuliak YV. Genes allele status of angiotensinconverting enzyme (I/D) and endothelial nitric oxide synthase (894 G > T) in patients with acute coronary syndrome. Likarska sprava/Ministerstvo okhorony zdorovia Ukrainy (Ukraine) 5–6, 24–34, 2015b.

Sydorchuk LP, Serdulets YI, Fediv OI, Havrysh LO, Teleki YM, Sydorchuk AR, Kshanovska AI, Turubarova-Leunova NA, Lekhai DO. The polymorphism of matrilin-3 (rs77245812) and interleukin-10 (rs1800872) genes in osteoarthritis patients with arterial hypertension, obesity and type 2 diabetes mellitus. Arch Balk Med Union 52, 422–429, 2017.

Tomaschitz A, Pilz S, Ritz E, Obermayer-Pietsch B, Pieber TR. Aldosterone and arterial hypertension. Nat Rev Endocrinol 6, 83–93, 2010.

Tsujita Y, Iwai N, Katsuya T, Higaki J, Ogihara T, Tamaki S, Kinoshita M, Mannami T, Ogata J, Baba S. Lack of association between genetic polymorphism of CYP11B2 and hypertension in Japanese: the Suita study. Hypertens Res 24, 105–109, 2001.

Tsybouleva N, Zhang L, Chen S, Patel R, Lutucuta S, Nemoto S, DeFreitas G, Entman M, Carabello BA, Roberts R, Marian AJ. Aldosterone, through novel signaling proteins, is a fundamental molecular bridge between the genetic defect and the cardiac phenotype of hypertrophic cardiomyopathy. Circulation 109, 1284–1291, 2004.

Whaley-Connell A, Johnson MS, Sowers JR. Aldosterone: role in the cardiometabolic syndrome and resistant hypertension. Prog Cardiovasc Dis 52, 401–409, 2010.

Williams B, Mancia G, Spiering W, Rosei EA, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen SE, Kreutz R, Laurent S, Lip GYH, McManus R, Narkiewicz K, Ruschitzka F, Schmieder RE, Shlyakhto E, Tsioufis C, Aboyans V, Desormais I, Authors/Task Force Members. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension [published correction appears in J Hypertens 37, 226, 2019]. J Hypertens 36, 1953–2041, 2018.

Ye WJ, Zheng L, Wang ZH, Chen HH. [Meta analysis on the association of CYP11B2 gene polymorphism and essential hypertension in Chinese Han population.] Zhonghua Xin Xue Guan Bing Za Zhi 41, 795–799, 2013.