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ASSOCIATION OF LIPIDS' METABOLISM DISORDERS WITH ALDOSTERONE SYNTHASE CYP11B2 (-344C/T) GENE POLYMORPHISM IN HYPERTENSIVE PATIENTS DEPENDING ON GLOMERULAR FILTRATION RATE

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Abstract

The cytochrome 11B2 aldosterone synthase gene (CYP11B2) one of the possible encoding genes that relates to changes of aldosterone and blood pressure regulation. The aim is to analyse the lipids profile changes in arterial hypertensive patients (EAH) depending on glomerular filtration rate (GFR) and gene CYP11B2 (-344C/T) polymorphism.

One-hundred hypertensive patients with hypertensive-mediated target-organ damaging (2nd stage), moderate, high or very high cardiovascular risk were enrolled in the case-control study. Mean age 59.87±8.02 y.o. 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/1,73m^2 for ≥3 months (measured by CKD-EPI equations). Lipids profile assessed by total cholesterol level (TC), triglycerides (TG) and low / high density level cholesterol (LDL-C, HDL-C) in serum. Also, calculated waist-hip ratio (WHR) for abdominal obesity evaluation. Control group included 48 practically healthy persons of relevant age. Gene's nucleotide polymorphism CYP11B2 (-344C/T) was examined by polymerase chain reaction.

TC, LDL-C level in hypertensive patients do not relate directly to polymorphic variants of CYP11B2 (rs1799998) gene. Though, dyslipidemia is more intensively manifested in the T-allele carriers by elevation of TG and atherogenic index (AI) 22.61-56.21% (p<0.01) as much, with lower HDL-C concentration – by 12.23% (p=0.043) and 12.95% (p=0.039), respectively, particularly in men by 25.84 (p=0.031) and 35.76% (p=0.042) higher than in women. CKD evolution in hypertensive patients follows by higher TC, TG and LDL-C that causes an atherogenic index increase (AI) by 13.54% (p=0.028). Polymorphic site of CYP11B2 (rs1799998) gene is associated with TG and AI elevation in general population (F=13.98 and F=13.25; p<0.001), both in women (F=22.99 and F=15.21; p<0.001) and men particularly (F=5.09; p=0.018 and F=4.44; p=0.027) and reduced HDL-C content (F=5.28; p=0.007), especially in men (F=9.57; p=0.001). Furthermore, it associates with WHR increase (F=13.09; p=0.003), especially in the TT-genotype carriers' men (F=12.74; p<0.001).

Polymorphic site of CYP11B2 (rs1799998) gene associates with dyslipidemia: TG and AI elevation, as well as WHR increase in general population, particularly in TT-genotype carriers' men. CKD in hypertensive patients is more related to lipids misbalance, than polymorphic site of CYP11B2 (rs1799998) gene.

Keywords: *gene CYP11B2 (rs1799998), pharmacogenetics, cholesterol, triglyceride, lipids, chronic kidney disease, hypertension, gender.*

Introduction

Metabolic disorders are insidious health problem worldwide and widely prevalent in patients with cardio-vascular (CV) diseases. Moreover, they are associated with kidney impairment and chronic kidney disease (CKD) progression [1-3]. It has been recognized that decreased estimated glomerular filtration rate (GFR) elevates cardiovascular morbidity and mortality risk. Approximately one out of ten persons worldwide have CKD, since the number of patients with chronic non-communicable diseases such as diabetes, Arterial Hypertension (AH), dyslipidemia and obesity has increased intensively in the last two decades. However, the underlying pathogenic mechanisms of CKD development are complicated, crossed and includes endothelial dysfunction, mild chronic inflammation in vessels wall, increased arterial blood pressure (BP), chains of renin-angiotensin-aldosterone system (RAAS) dysregulation, decreased baroreflex sensitivity, advanced oxidative stress, enhanced fibrotic activity, lipids and glucose metabolism disorders, with possible genetic predispositions [4-6]. Furthermore, among possible causative predictors are aldosterone and cytochrome 11B2 aldosterone synthase gene CYP11B2 expression. Obviously, that aldosterone is synthesized mainly from cholesterol. Therefore, it could be suggested that aldosterone content is linked to cholesterol level as well as to CYP11B2 gene polymorphism. Nevertheless, some pathogenic relationships between cholesterol, CYP11B2 gene and CKD development have not been proven and need more studies.

Thereby we focus our research on analysis of cholesterol profile changes in arterial hypertensive patients depending on GFR and aldosterone synthase gene CYP11B2 -344C/T polymorphism.

Methods

Inclusion / Exclusion criteria

The study included essential arterial hypertensive (EAH) patients with hypertension-mediated organ damage (HMOD) estimated according to European Societies of Hypertension and Cardiology recommendation (ESH/ESC 2018) [7]: target-organs damage – 2nd stage (asymptomatic EAH), from the 1st through to the 3rd grade of BP; moderate-high cardiovascular (CV) risk; age above 30 years old.

Exclusion criteria have been described in previous publications [8-10]: we excluded patients with EAH stage 3 (established CV disease, chronic kidney diseases (CKD) – with estimated glomerular filtration rate (eGFR) decline <30 ml/min/1,73m2); secondary arterial hypertension; chronic heart failure (CHF) higher than II functional class (NYHA III-IV), EAH patients with complications of HMOD; 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; 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.

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. All enrolled subjects signed a consent form to participate in the study.

All participants underwent a complex of basic examinations: general clinical analyses of complete blood count, total cholesterol level (TC), triglycerides (TG) and low / high density level cholesterol (LDL-C, HDL-C), serum uric acid, body

mass index (BMI, kg/m2) for evaluation of overweight and obesity; the waist-hip ratio (WHR) – for determination of abdominal obesity (AO), office measurement of systolic and diastolic BP (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 National (2016) and European recommendations ESC/ESH (2018) [7].

Essential Arterial Hypertension and Renal Function Assessment

Hypertension was defined as office SBP values ≥140 mmHg and/or DBP values ≥90 mmHg at least for three measurements during a month, according to National (2016) and European Societies of Hypertension and Cardiology ESH/ESC (2018) recommendations' requirement [7].

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) [11] and CysC alone (CKD-EPI CysC) [12] depending on 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 m2 for over 3 months with or without other signs of kidney damage, according to KDIGO recommendations [13]. CKD was diagnosed in 29 EAH persons.

Cholesterol profile assessment

Blood lipids' profile included TC, TG, HDL-C and LDL-C assessment in serum samples using BioSystem S.A. reagents (Spain), on a spectrophotometer "FP" (Finland), with a wavelength of 500 ± 20 nm. The Atherogenity Index (AI) was calculated by the formula: TC – HDL-C / HDL-C.

As "target" lipids' levels were taken as follows, according to the European and National Guidelines recommendation: TC <5.0 mmol/l for persons with moderate Cardiovascular (CV) risk, <4.5 mmol/l for persons with high CV risk, <4.0 mmol/l for persons with extremely high CV risk; LDL-C <3.0 mmol/l for persons with moderate CVR, <2.5 mmol/l – for high CV risk, <1.8 mmol/l – for extremely high CVR; TG generally <1.7 mmol/l; HDL-C >1.02 mmol/l for men, >1.2 mmol/l for women [14, 15]. AI "target" level was for persons under 30 years old <2.5 conventional Unit (CU), over 30 years old <3.5 CU.

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

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.

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.

Statistical analysis

Statistical analysis was performed using Statistica v. 7.0 (StatSoft, USA) software. For the genotypes distribution comparison we used Pearson's ($\chi 2$) criterion. Analysis of independent quantitative samples were calculated using Student's t-test, two-tail distribution and equal/unequal variances between two samples (M±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 ($\chi 2$), df=1. One-way ANOVA analysis was used to confirm the association of CYP11B2 (rs1799998) gene polymorphism with diagnostic clinical and laboratory parameters. P-values <0.05 were considered statistically significant.

Results

Dyslipidemia in EAH patients is characterized by hypercholesterolemia due to LDL-C expense, TG elevation, and certain HDL-C decrease. Therefore, it resulted in higher AI particular in patients with GFR ≤60 ml/min/1.73m2 by 13.54% (P=0.028) than in those with maintained GFR (Table 1). One-way ANOVA confirmed the association of GFR with TG content elevation (F=11.75; p=0.001), AI (F=47.20; p<0.001) and decreased HDL-C (F=13.36; p<0.001).

There was no clear association found of TC and LDL-C content changes with polymorphic variants of CYP11B2 (rs1799998) gene (Table 2). Though in T-allele carriers the AI was higher with a lower HDL-C content than in the CC-genotype patients: for AI – by 22.93% (p=0.009) and 22.61% (p=0.014), for HDL-C – by 12.23% (p=0.043) and 12.95% (p=0.039), respectively. Moreover, TG level in the TT-genotype carriers was higher than in CC-genotype patients by 56.21% (p=0.011). A similar tendency of lipidogram changes was observed in the control group depending on CYP11B2 (rs1799998) gene's alleles state: TG concentration in the T-allele carriers was higher by 36.23% (p=0.019) and 31.16% (p=0.046), likewise LDL-C – 23.23% (p=0.011) and 13.03% (p=0.047) and AI –77.42% (p<0.001) and 62.67% (p=0.007), with contrary lower content of HDL-C – by 27.22% (p=0.002) and 18.34% (p=0.053), respectively. One-way ANOVA confirmed the association of CYP11B2 gene's (rs1799998) polymorphism with increase of TG (F=13.98; p<0.001), AI (F=13.25; p<0.001) and diminished HDL-C (F=5.28; p=0.007).

TG level is higher in T-allele women of CYP11B2 (rs1799998) gene than that in the CC-genotype carriers by 40.0% (pCC=0.049) and 60.71% (pCC=0.019) as much (Table 3). At the same time, in T-allele men the HDL-C level is lower than that in CC-genotype – by 15.67% (pCC=0.02) and 23.88% (pCC=0.032) as much. On the other hand, the AI in T-allele carriers is higher than in CC-genotype patients regardless of gender: in women – by 21.54% (pCC=0.02) and 14.47% (pCC=0.055), in men – by 21.82% (pCC=0.052) and 35,76% (pCC=0.042), but AI was higher by 25.84% in men than women (pF=0.031). One-way ANOVA (Table 3) confirmed the association of CYP11B2 gene's (rs1799998) polymorphism with TG increase in women and men (F=22.99; p<0.001 and F=5.09; p=0.018), HDL-C decrease in men (F=9.57; p=0.001) and AI elevation in women (F=15.21; p<0.001) and men (F=4.44; p=0.027), respectively.

Discussion

Combination of AH with abdominal obesity (AO), or metabolic syndrome (MS) occurs in around 30-35% of population and causes a high CV risk of complications [16-19]. The adipose tissue is proved to be an active endocrine organ in addition to the source of energy. Its excess is accompanied by RAAS hyperactivity, intensification of the local and systemic AS synthesis, secondary aldosteronism, that was confirmed partially by our former results [8, 9]. AS influences directly on the adipose tissue through the increased density of mineral corticoid receptors (MCR), expressed on the surface of the

adipocytes, resulting in acceleration of their maturation with following adipose tissue increase [20, 21]. MCR activation plays a crucial role in kidneys' sodium reabsorption, BP control, as well as in differentiation of pre-adipocytes into mature adipocytes, inflammation induction and excessive cytokines production, such as tumor-necrotic factor alpha (TNF-α), monocyte chemotactic protein (MCP-1) and interleukin-6 (IL-6) in the white adipose tissue, reduced thermogenic activity and transcription of the brown fat uncoupling protein-1 (UCP-1) [22]. An excessive AS production and activation of MCR was found in obese mice (db/db mice), as well as association with increased BMI, human cholesterol elevation, insulin resistance development, obesity, CV risk enhancement and increased expression of CYP11B2 gene [23, 24]. Apart from this, the AS associated with kidney impairment in hypertensive patients, type 2 DM and dyslipidemia as it was proved in our former and current researches [8]. Furthermore, adipocytes are able to synthesize and secrete AS manifesting autocrine and paracrine effects influencing on the adipose tissue and vascular remodelling.

The mechanisms of AS production regulation by adipocytes include calcineurin/Nuclear factor of activated T-cells (NFAT) – a signal cellular pathway depending on free O2 radicals. Angiotensin II (AII) activates aldosterone-synthase (CYP11B2) through the angiotensin 1 receptor (AT1R), whereas transcription factor NFAT – through the calcineurin signalling pathway [25, 26]. The stimulator of adenosine monophosphate kinase (AMPK) mitochondrial biosynthesis activates steroidogenesis and is released during starvation. Cholesteryl ester transfer protein (CETP) mediates transportation of cholesterol ethers from high density lipoproteins on the atherogenic apoB lipoprotein particles, via AS expression and active O2 forms increase, as well as peroxisome proliferator activated receptor-γ (PPAR-γ) activation, various transcriptional factors such as hypoxia-inducible factor-1, nuclear factor-κB and the Signal Transducer and Activator of Transcription-3 (STAT-3) related to inflammation and tumorigenesis [27, 28]. CETP inhibitors (CETPI) like Dalcetrapib, primarily manufactured for HDL-C blood level increase, provoke hyperaldosteronism and AH. Therefore, the adipose tissue cells are able to regulate AS production both local and suprarenal. In case of abdominal obesity, the AS induces both insulin resistance and low intensity inflammation inside of the adipose tissue, and it could be a connective link between these factors. Therefore, MCR blockade in obese patients with AH might not only decrease BP but prevent possible metabolic disorders and heart failure [29].

On the other hand, some studies evidence that T-allele of CYP11B2 (rs1799998) gene is associated with higher SBP and DBP values, more frequent MS development and higher CV risk [30]. Though some of them found no changes in the plasma renin and AS levels or activity, as well as lipids and carbohydrate metabolism deviations depending on genotypes of CYP11B2 gene. Other studies (other populations) determined such kind of dependence [31-33], when presence of T-allele of CYP11B2 gene associated with insulin resistance, higher AS levels, increased activity of aldosterone-synthase, dyslipidemia, elevated sodium concentration with lower content of potassium [34]. Moreover, P. Purkait et al. and G.J. Ko et al. independently did not confirm association of T-allele of CYP11B2 (rs1799998) gene in the Indian and Chinese populations suffering from type 2 DM with diabetic nephropathy development or progression, but contrary it clearly associated with AH in diabetic patients [35, 36]. Thus, there is a close relation between AH, abdominal obesity, AS production and dyslipidemia as well as kidney failure development, which was partially confirmed by our study.

However, the gene-environment interactions of CYP11B2 with kidney impairment and lipids profile disorders in hypertensive patients for possible prediction of renal failure progression and CV risk enhancement as well as influence on pharmacologic corrections still need to be provided and extended.

Conclusions

TC, LDL-C blood level content in hypertensive patients do not relate directly to polymorphic variants of CYP11B2 (rs1799998) gene. Though, dyslipidemia is more intensively manifested in the T-allele carriers with higher atherogenic index (AI) and

TG by 22.61-56.21% ($p<0.05$), lower HDL-C concentration – by 12.23% and 12.95% ($p<0.05$), particularly in men.

CYP11B2 gene's (rs1799998) polymorphism in EAH patients associates with increase of TG and AI in general population ($F=13.98$ and $F=13.25$; $p<0.001$), both in women ($F=22.99$ and $F=15.21$; $p<0.001$) and men particularly ($F=5.09$; $p=0.018$ and $F=4.44$; $p=0.027$) with following HDL-C content reduce ($F=5.28$; $p=0.007$), especially in men ($F=9.57$; $p=0.001$). Furthermore, it associates with WHR increase ($F=13.09$; $p=0.003$), especially in the TT-genotype carriers' men ($F=12.74$; $p<0.001$).

CKD in hypertensive patients is more related to lipids misbalance, than polymorphic site of CYP11B2 (rs1799998) gene. Reduced GFR (≤60 ml/min/1.73m2) in EAH patients is accompanied by TC, LDL-C and TG elevation that causes an atherogenic index increase by 13.54% ($p=0.028$) as much.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Lipids blood content depending on the glomerular filtration rate, M±SD

Parameters	Control group	Hypertensive patients	
		GFR >60 ml/min/1.73m^2	GFR ≤60 ml/min/1.73m^2
TC, mmol/l	5.60±0.37	5.61±0.67	5.72±0.42
TG, mmol/l	1.64±0.24	1.89±0.33	2.19±0.41 P=0.014
LDL-C, mmol/l	3.95±0.36	4.13±0.42	4.26±0.59
HDL-C, mmol/l	1.42±0.15	1.28±0.11 P=0.029	1.19±0.09 P=0.004
AI, U	3.18±0.54	3.47±0.25	3.94±0.33 P=0.011 P_1=0.028
BMI, kg/m^2	25.86±2.14	30.86±1.88 P<0.001	32.35±2.20 P<0.001

GFR - glomerular filtration rate; TC – total cholesterol; TG – triglycerides; HDL-C and LDL-C - high and low density lipoprotein cholesterol; AI - Atherogenity Index; BMI – body mass index; P – significance of differences with control group; P_1 – significance of differences with group of patients with GFR >60 ml/min/1.73m^2.

Table 2. Lipids profile in hypertensive patients depending on genotypes of gene *CYP11B2* (rs1799998), M±SD

Parameters	Genotypes of *CYP11B2* gene in Control group		Genotypes of *CYP11B2* (rs1799998) gene in patients		
			CC	***TC***	***TT***
TC, mmol/l	***CC***	5.30±0.62	5.62±0.81	5.79±0.53	5.72±0.78
	TC	5.80±0.64			
	TT	5.60±0.52			
TG, mmol/l	***CC***	1.38±0.27	1.53±0.48	1.92±0.35	2.39±0.66 P_{CC}=0.011
	TC	1.88±0.41 P_{CC}=0.019			
	TT	1.81±0.38 P_{CC}=0.046			
HDL-C, mmol/l	***CC***	1.69±0.15	1.39±0.18 P=0.029	1.22±0.20 P_{CC}=0.043	1.21±0.14 P_{CC}=0.039
	TC	1.23±0.19 P_{CC}=0.002			
	TT	1.38±0.22 P_{CC}=0.053			
LDL-C, mmol/l	***CC***	3.53±0.36	4.10±0.68	4.36±0.50	4.25±0.36
	TC	4.35±0.62 P_{CC}=0.011			
	TT	3.99±0.39 P_{CC}=0.047			
AI, U	***CC***	2.17±0.22	3.14±0.50 P=0.006	3.86±0.44 P_{CC}=0.009	3.85±0.49 P_{CC}=0.014
	TC	3.85±0.61 p_{CC}<0.001			
	TT	3.53±0.54 P_{CC}=0.007			
BMI, kg/m²	***CC***	23.05±1.99	34.01±3.80 p<0.001	31.79±2.08 p=0.006	32.21±4.44 p=0.034
	TC	27.13±1.82 P_{CC}=0.002			
	TT	27.05±2.48 P_{CC}=0.042			
WHR, U	***CC***	0.74±0.05	0.89±0.04 p<0.001	0.91±0.05 p=0.013	0.97±0.06 P_{CC}=0.03 P_{TC}=0.04
	TC	0.84±0.04 P_{CC}=0.01			
	TT	0.91±0.08 P_{CC}=0.01			

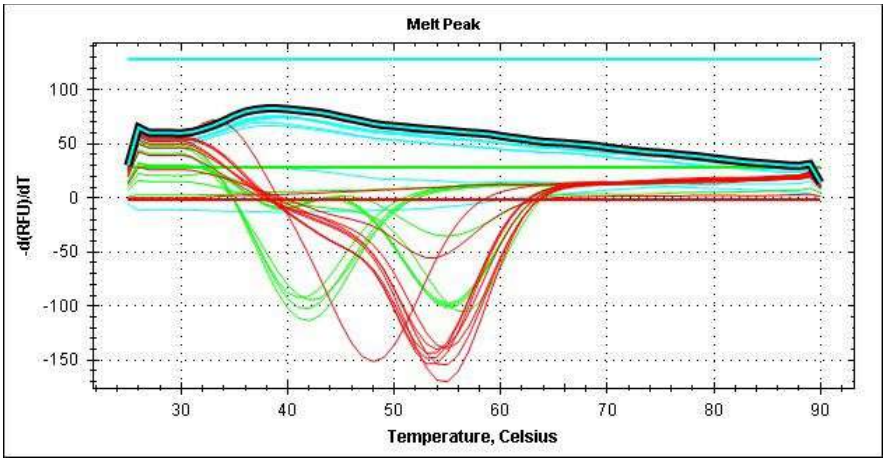
WHR – waist-hip ratio; P – significance of differences with control group according to appropriate genotype; P_{CC} – significance of differences with *CC*-genotype carriers in particular group (control/patients); P_{TC} – significance of differences with *TC*-genotype carriers in particular group.

Table 3. Lipids profile in hypertensive patients depending on gender and *CYP11B2* (rs1799998) gene's polymorphic variants, M±SD

Parameters	Gender	Genotypes of *CYP11B2* (rs1799998) gene		
		CC	***TC***	***TT***
TC, mmol/l	F	5.62±0.85	5.94±0.50	5.82±0.68
	M	5.65±0.36	5.50±0.64	5.50±0.87
TG, mmol/l	F	1.40±0.33	1.96±0.28 P_{CC}=0.049	2.25±0.39 P_{CC}=0.019
	M	2.36±0.39 P_F=0.028	1.85±0.55	2.69±0.60
HDL-C, mmol/l	F	1.40±0.19	1.27±0.18	1.29±0.14
	M	1.34±0.09	1.13±0.09 P_{CC}=0.02	1.02±0.10 P_{CC}=0.03 P_F=0.008
LDL-C, mmol/l	F	4.10±0.69	4.44±0.43	4.30±0.56
	M	4.13±0.40	4.20±0.61	4.15±0.54
AI, U	F	3.11±0.38	3.78±0.35 P_{CC}=0.02	3.56±0.30 P_{CC}=0.055
	M	3.30±0.57	4.02±0.54 P_{CC}=0.052	4.48±0.46 P_{CC}=0.042 P_F=0.031
BMI, kg/m²	F	31.44±4.05	32.19±3.47	32.11±4.40
	M	28.39±0.14	30.99±4.61	32.42±3.92
WHR, U	F	0.89±0.04	0.88±0.03	0.90±0.03
	M	0.91±0.01	1.0±0.04 P_{CC}=0.005 P_F<0.001	1.06±0.04 P_{CC}=0.006 P_F<0.001

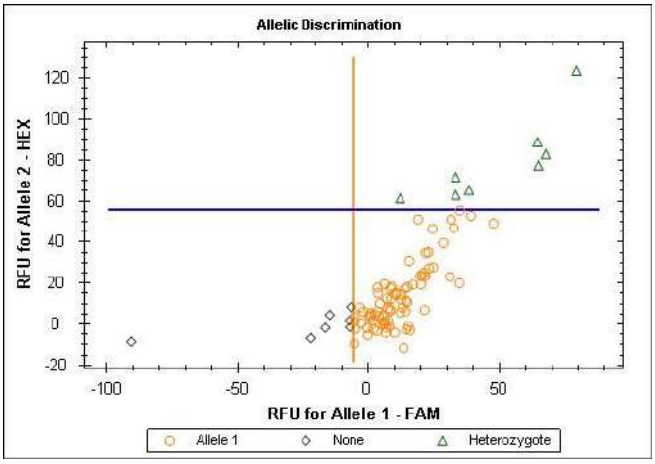
F – Female; M – Male; P_{CC} – significance of differences with *CC*-genotype carriers; P_{TC} – significance of differences with *TC*-genotype carriers; P_F – significance of differences with female according to appropriate genotype.

Figure 1. Temperature bars in analysis of CYP11B2 344C>T gene's polymorphism in observed population



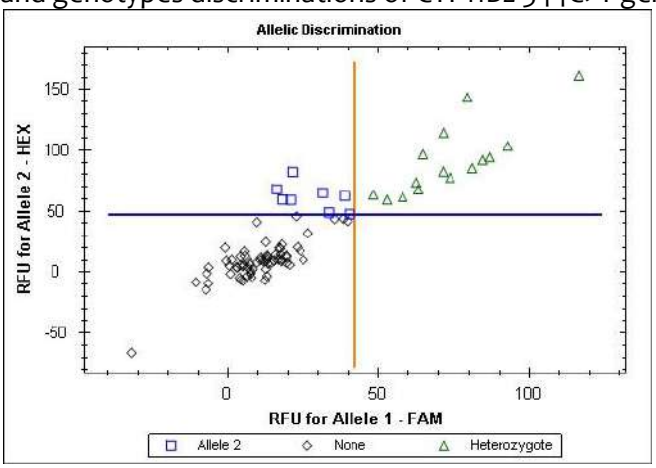
Note: Blue colour shows the samples homozygous for the C-allele of the CYP11B2 gene (344C>T), determined by the Fam channel; Greens – samples homozygous for Hex channel (T-allele); Reds – heterozygous (TC) specimens; Yellows – questionable and unreliable results.

Figure 2. Alleles and genotypes discriminations of CYP11B2 344C>T gene's polymorphism



Note: oAllele 1 – CC genotype carriers; ΔHeterozygote – CT genotype carriers; ◊None – non-determined

Figure 3. Alleles and genotypes discriminations of CYP11B2 344C>T gene's polymorphism



Note: □Allele 2 – TT genotype carriers; ΔHeterozygote – CT genotype carriers; ◊None – non-determined