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Association of C-344T Gene Polymorphism with Adrenal Incidentaloma in Uzbek Population

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ABSTRACT

The objective of the given research was to study distribution of C-344T polymorph marker of CYP11B2 gene and possible relation of that polymorphism to hormonal, biochemical, and multifunctional parameters of testing in patients with adrenal incidentalomas. We have checked 80 Uzbek patients with adrenal incidentalomas, who received out and inpatient therapy and were followed at the clinic of Turakulov RSSPMC of endocrinology. Verification of the diagnosis of adrenal incidentaloma was based on clinical biochemical, hormonal, and visual imaging tests. Eighty samples of whole blood were genotyped for C-344T polymorphism of CYP11B2 gene. Analysis of the obtained results showed dominance of CT-heterozygotes on C-344T polymorph marker of CYP11B2 gene in Uzbek people suffering adrenal incidentaloma. Analysis of the associations of CYP11B2 gene polymorph markers carriage with parameters of hormonal status, electrolyte balance, and blood lipid spectrum revealed several peculiarities. Carriage of CT- and TT-genotypes of CYP11B2 gene C-344T polymorph marker is associated with higher levels of aldosterone in blood serum of the patients with adrenal incidentalomas, whilst TT-homozygotes had reliably lower values of potassium and high level of sodium in blood serum; carriage of CT-genotype of CYP11B2 gene is associated with the risk of GTI in patients with adrenal incidentalomas. Analysis of correlations between AP parameters and hormonal status showed low-renin character of arterial hypertension associated with CYP11B2 gene C-344T polymorph marker T-allele carriage.

Introduction

Adrenal neoplasms have both clinical and morphological peculiarities related to complex histogenesis, and structure of the gland. Defects in zone-specific expression of steriodogenic enzymes can cause clinical disorders in steroidogenesis. Underlined in the description of steroidogenesis pathways, the key component of the functional division to zones is expression of cytochrome P450 (CYP) enzymes, catalyzing various modifications of predecessor steroids, specific for each zone. External glomerular area expresses aldosterone synthetase (CYP11B2), which catalyses three separate reactions for the conversion of 11-deoxycorticosterone to aldosterone: 11β-hydroxylation, 18-hydroxylation, and 18-hydroxyl oxidation to aldehyde part

[1].

There is no doubt that contribution of CYP11B2 gene to the development of arterial hypertension (AH), as well as frequently diagnosed concomitant adrenal incidentalomas (AI), is of great interest, as there are almost no researches of aldosterone synthetase gene polymorphism in patients with AI both in Uzbek population and the whole world.

There are descriptions of several types of aldosterone synthetase gene polymorphism such as C-344T, Lys-173Arg, introne-1, K173R, introne-2, T4986C and A6547G [2, 3]. Best studied polymorphism is in 5'-end of the gene replacement of cytosine by thymidine in 344th position, which participates in conjunction of SF-1 transcription factor, and by these means can effect gene's expression.

Several studies showed association of C-344T polymorphism of CYP11B2 gene with AH and its positive correlation with plasma aldosterone. According to E. Brand et al the prevalence of -344T-allele among patients with hypertension in French population was higher than among healthy people and -344T allele was associated with AH [4]. In his work E. Davies et al also noted a great accumulation of -344T-allele in patients with hypertension and showed, that carriers of the allele had a greater excretion of aldosterone compared to CC-homozygotes [5]. However, the study performed among 420 European students showed that -344C allele was an independent factor of SAP rise in men (TT-125,6, TC-128,4, CC-130.5 mmHg), p=0.03 [6], while the study by L. Pogoja [3] demonstrated that presence of -344C allele was associated with high plasma aldosterone (90±8pg/mL for TT-genotype, 110±6pg/mL for TC-genotype, 129±10pg/mL for CC-genotype). At the same time H.Schunkert et al did not find any association between C-344T polymorphism of CYP11B2 gene, AP and aldosterone [7].

Japanese researchers revealed a high prevalence rate of -344C allele among patients with AH. It was also noted, that -344C allele was associated with the development of AH, high aldosterone/plasma rennin ratio, which served the basis for making conclusion, that -344C allele was genetic marker of low-renin hypertension among Japanese people [8], while other authors reported low prevalence rate of -344C allele among patients suffering low-renin hypertension [9]. According to A.D.Tiago et al C-344T polymorphism of CYP11B2 gene in African population was associated with high SAP in compliance with the results of day monitoring of AP (APDM) and office tests [10]. Thus, the data on C-344T polymorphism of CYP11B2 gene demonstrate absence of common facts on the relation of analyzed polymorphism to AH even in the frames of ethnic groups.

The objective

To study distribution of C-344T polymorph marker of CYP11B2 gene and its possible link with hormonal, biochemical, and multifunctional test parameters in patients with AH.

Materials and methods

Our study was based on clinical follow up of 80 Uzbek patients with AI, who received out and in-patient therapy in the clinic of Turakulov RSSPMC of Endocrinology. Sixty healthy people of corresponding age and gender served the control. AI diagnosis was verified on the basis of clinical biochemical, hormonal, and visual imaging tests results. All the patients underwent common clinical tests, biochemical blood tests with the definition of serum potassium, sodium, chlorine, lipid spectrum, fasting glycemia and creatinine and urea within OGTT; tests, including serum aldosterone and plasma rennin activity in hormonal status, ACTH, cortisol, 11 OKC, and day excretion of urine catecholamines and 17- SC. Topical diagnostics was performed using ultra sound tests and CT of adrenals.

Genotyping of 80 samples of whole blood for C-344T polymorphism of CYP11B2 gene was

done in the laboratory of Arterial Hypertension in RSSPMC of Cardiology by a group of molecular-genetic researchers. Isolation of genome DNA was performed using DIAtom™ DNA Prep200 (IsoGen Laboratory, Russia).

Polymerase chain reaction was performed using a set for genotyping for polymorphic marker C-344T of CYP11B2 gene (GenTest, Russia) amplifier GeneAmp® PCR System 9700 (Applied Biosystems, USA).

The following primer sequences were used:

Direct primer: 5`- AGGGCTGAGAGGAGTAAAATG - 3`,

Reverse primer: 5`- TGACCACCAGGAGGAGAC - 3`

Amplification conditions:

First denaturation	35 cycles	Last chain synthesis
95°C – 2 min	94°C – 10 sec	72°C – 2 min
	60°C – 20 sec	
	72°C – 20 sec	

Detection of amplification results was done with the help of horizontal electrophoresis in 1% agarose gel with field voltage ~15V/cm for 30 minutes. Buffer 1xSB (GenTest, Russia) was used as an electrophoresis buffer. Gels were stained with the solution of ethidium bromide with further ultra violet visualization (λ =254 nm) using transilluminator ECX-15M (Vilber Lourmat, France). Results were documented in photos using Gel Imager-2 (Helicon, Russia) gel documentation system.

As a result of electrophoresis detection we revealed a 537 b.s. length fragment, corresponding to amplified DNA part, containing polymorphic sequence C-344T of CYP11B2 gene in the gel.

For the detection of the genotype we applied further restriction of amplification products with the help of restrictase BsuR and HaeIII using a set for genotyping for C-344T polymorphic marker of CYP11B2 gene (GenTest, Russia) in 37°C for 16 hours.

Detection of restriction results of amplified fragments was performed with the help of horizontal electrophoresis in 4% agarose gel with field voltage ~8V/cm for 2 hours. Buffer 1xSB (GenTest, Russia) was used as an electrophoresis one. Gels were stained with a solution of ethidium bromide with further visualization in ultra violet light (λ =254 nm) using ECX-15M (Vilber Lourmat, France) transilluminator. The obtained results were documented in photos using Gel Imager-2 (Helicon, Russia) gel documentation system.

As a result of electrophoresis division of restriction products in gel we revealed various sets of specific DNA fragments such as 274, 138, 126 b.s. long, corresponding to homozygous genotype T/T, 203, 138, 126 and 71 b.s. long corresponding to homozygous genotype C/C, and 274, 203, 138, 126 and 71 b.s. long corresponding to heterozygous genotype T/C.

Results and discussion.

Among tested patients distribution of the polymorphic marker C-344T of CYP11B2 gene was as follows: TT- genotype was revealed in 19 patients (23.75%), CT- genotype in 43 (53.75%), CC-genotype in 18 (22.5%), χ^2 =22.538, p=0.000. T-allele was revealed in 81 (50.6%), while C-allele in 79 (49.4%) cases, χ^2=0.013, p=0.911. analysis of the results showed dominance of CT-heterozygotes on C-344T polymorphic marker of CYP11B2 gene in Uzbek patients suffering adrenal incidentalomas (fig. 1).

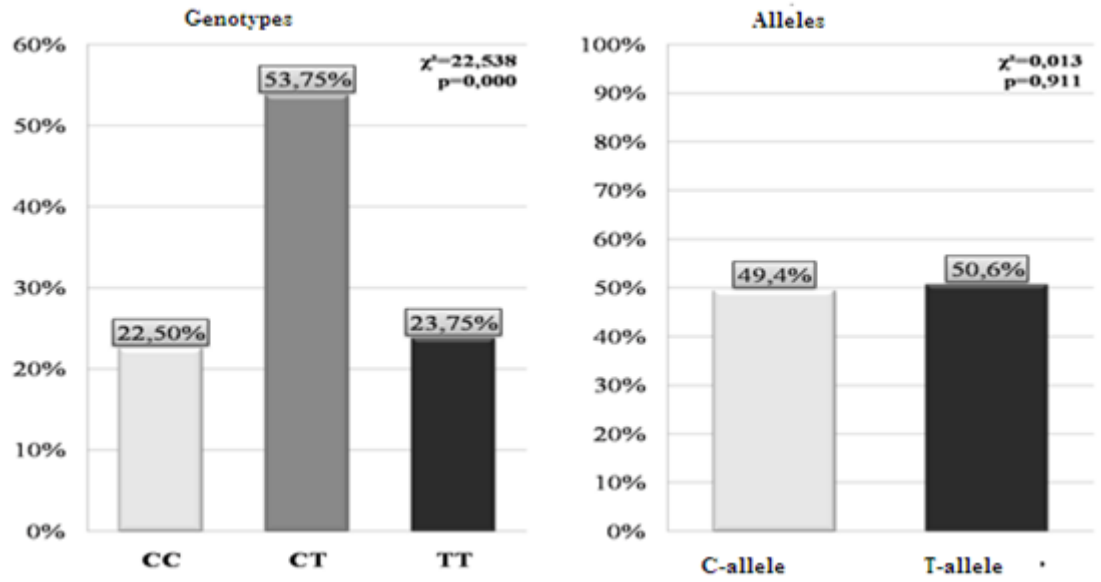

Figure 1. Distribution of genotypes and alleles of C-344T polymorphic marker of CYP11B2 gene in Uzbek patients with adrenal incidentalomas

In the group of healthy people similar test for distribution of genotypes of C-344T polymorphic marker of CYP11B2 gene also revealed high prevalence rate of CT-heterozygotes in 30 patients (51.7%) and equal distribution of homozygous forms: TT-genotypes in 15 patients (25.8%) and CC-genotype in 13 (22.4%), χ^2=13.4, p=0.000. it should be noted that, both in healthy and sick people the prevalence rates of the alleles of C-344T polymorphic marker of CYP11B2 gene were similar: T-allele was found in 60 (51.7%), C-allele in 56 (48.3%) of them, p=0.69, χ^2=0.15 (fig.2).

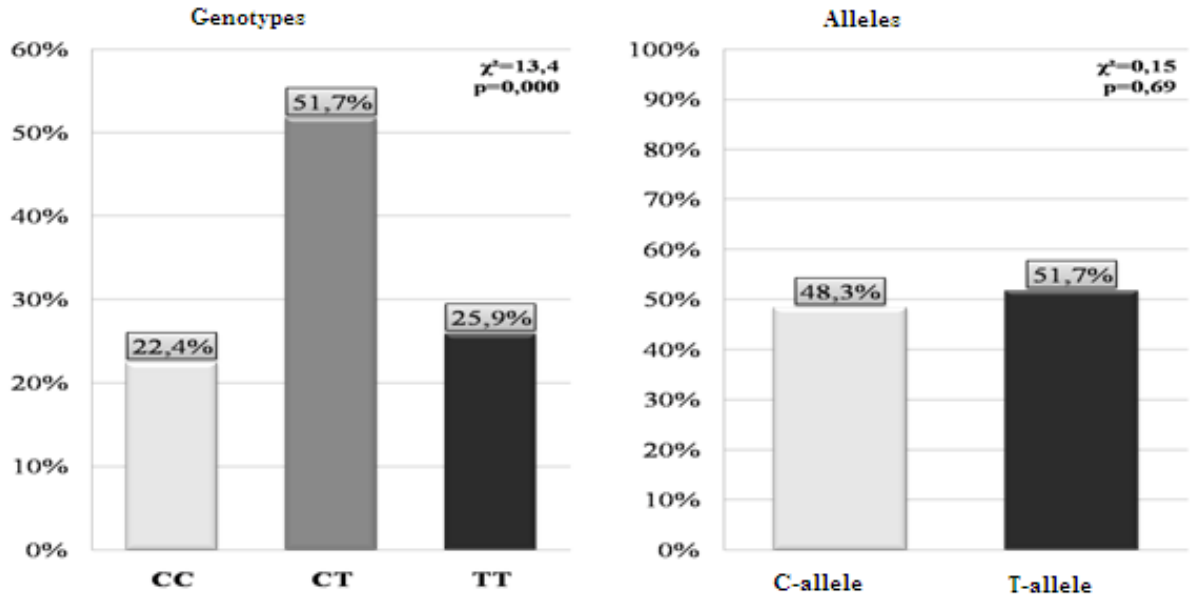

Figure 2. Distribution of genotypes and alleles of C344T polymorphic marker of CYP11B2 gene in healthy Uzbeks people

Described distribution of C-344T polymorphic marker of CYP11B2 gene in healthy people and patients corresponds to Hardy-Weinberg theoretical estimation of genotypes and alleles prevalence.

Results of electrophoresis of PCR products of CYP11B2 gene C-344T polymorphic marker amplification in the examined patients are presented in figure 3.

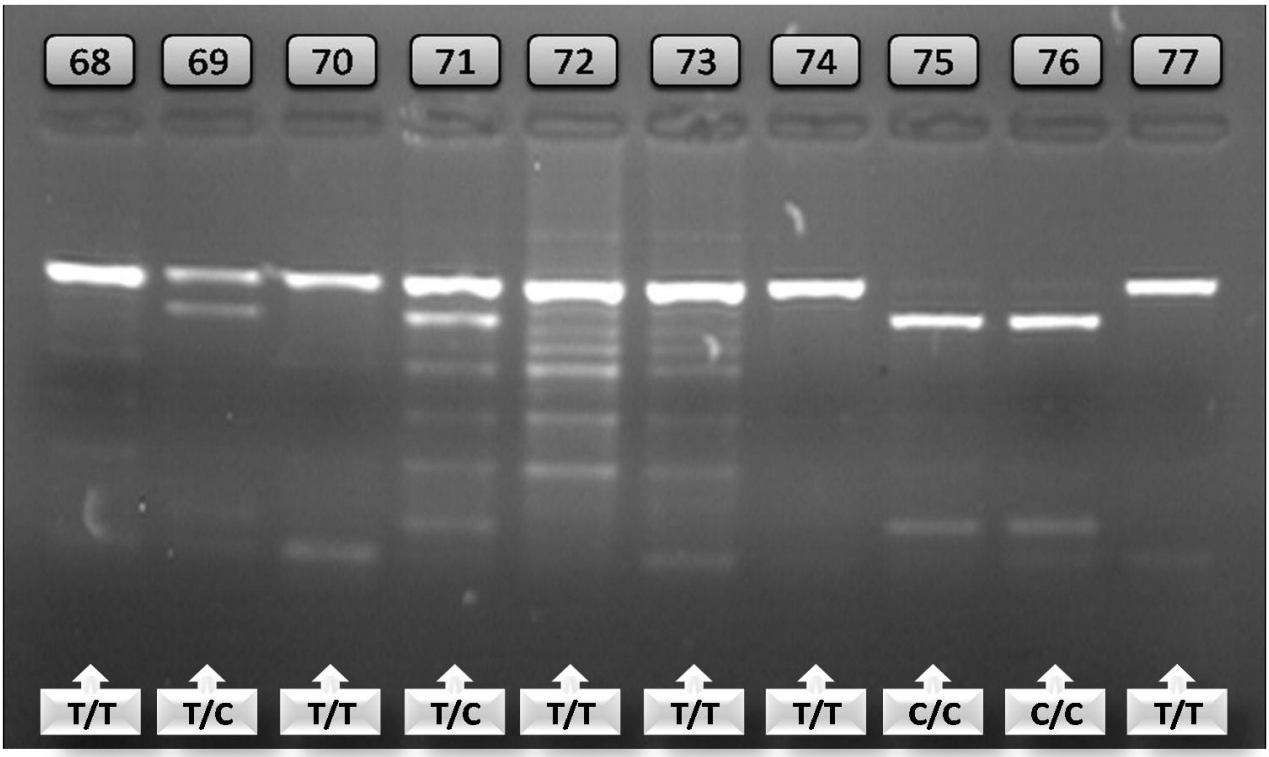

Figure 3. Results of electrophoresis of PCR products of CYP11B2 gene C-344T polymorphic marker amplification

Distribution of genotypes and alleles of CYP11B2 gene C-344T polymorphic marker in healthy people and patients from Uzbek population corresponded to that of Europeans. Apart from the aforesaid ones, Japanese differed by a greater prevalence rate of TT-homozygotes with no CC-homozygotes [11]. It is interesting that the greatest prevalence of TT-genotype and T-allele carriers was registered among African population [10].

Thus, performed tests showed some difference in carriage of CYP11B2gene polymorphic markers taking into account ethnic peculiarities.

For the assessment of hem dynamic specificity in the patients with adrenal incidentalomas taking into account C-344T polymorphism of CYP11B2 gene we divided the patients to the groups with CC-, CT- and TT-genotypes, and carriers of C- and T-allele.

As it is seen in Figure 4 carriage of these genotypes was not associated with the stage of AH and HBR.

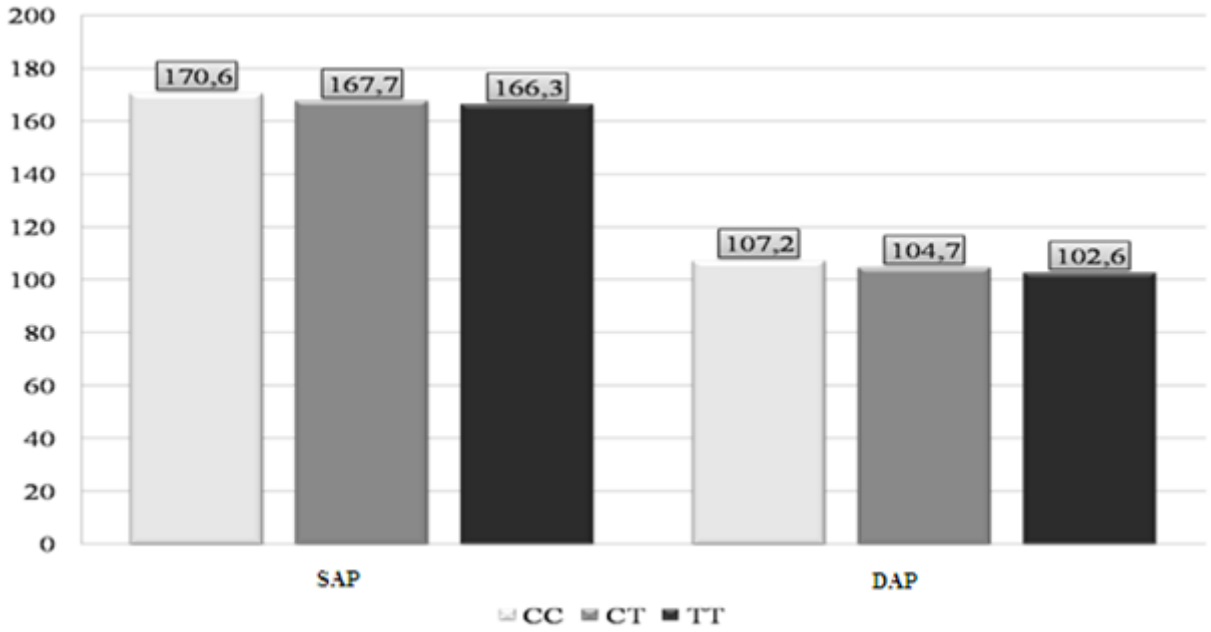

Figure 4. Parameters of AP in Uzbek people with adrenal incidentaloma taking into account CYP11B2gene C-344T polymorphism.

Analysis of the associations of carriage of gene CYP11B2 polymorphic markers with the parameters of hormonal status, electrolyte balance, and blood lipid spectrum revealed several characteristic features (Tables 1, 2).

For instance, compared to CC-homozygotes, TT-homozygotes had higher mean values of aldosterone: 85.22±54.8 pg/mL versus 55.94±24.17 pg/mL, p=0.045. the highest values of aldosterone were revealed in CT-heterozygotes: 111.4±62.94 pg/mL, which was more reliably different from CC-genotypes carriers (p=0.000). it should be noted, that the amount of 11-OKC – aldosterone predecessor was the least in the group with TT-homozygotes compared to CC-homozygotes (8.99±1.23 mkg% vs 9.91±1.03 mkg% (p=0.019)), which can be conditioned by its faster consumption. Blood serum potassium was reliably different with lower values in TT-genotype carriers compared to CC-homozygotes (4.09±0.39 mmol/L vs 4.47±0.59 mmol/L (p=0.026)). The amount of sodium in TT-homozygotes was reliably higher than that of CT-heterozygotes (143.05±9.52 mmol/L vs 138.02±7.56 mmol/L (p=0.03)). The results are explained by the characteristic features of potassium-sodium pump functioning and feedback regulating role of potassium in aldosterone synthesis, which was mostly expressed in TT-genotype carriers. Higher aldosterone levels in TT-homozygous and carriers of CT-heterozygous status according to C-344T-polymorphic marker of CYP11B2 gene was reflected in the character of AH, obviously low-renin, which can be described by the presence of reliable negative correlations between rennin and SAP and DAP parameters. Thus, carriers of T-allele had $r_{renin/SAP}$=-0.31 and $r_{renin/DAP}$=-0.22, CT-heterozygotes $r_{renin/SAP}$=-0.40 and $r_{renin/DAP}$=-0.36. Comparative analysis of carbohydrate exchange disorders prevalence taking into account carriage of C-344T polymorphic marker of CYP11B2 gene displayed reliably greater prevalence rate of GTD in patients with CT-genotype compared to CC one (10(23.6%) vs 1(5.55%), respectively, χ^2=7.324, p=0.026). It should be noted, that in the single study of C-344T polymorphism of CYP11B2 gene association with glycemia parameters, performed among 1368 Japanese and Chinese patients, compared to the carriers of T-allele CC-genotype ones had reliably higher fasting glucose level [12]. In that aspects, our controversial data can be explained by the differences in the distribution and function of C-344T polymorphism in European population (similarity in gene polymorphism distribution in rennin-angiotensin-aldosterone

system between Uzbek and European population was demonstrated in local literature [13]) and Japanese and Chinese ones.

Table 1. Parameters of AP, hormonal status, electrolyte balance, lipid spectrum and glycemia in people with different genotypes of C-344T polymorphic marker of CYP11B2 gene.

Parameter	**CC**	**p** (CC-CT)	**CT**	**p** (CT-TT)	**TT**	**p** (CC-TT)
SAP	170.56±26.89	0.758	167.67±35.45	0.888	166.32±33.37	0.674
DAP	107.22±21.91	0.664	104.65±20.63	0.711	102.63±17.27	0.482
Aldosterone	55.94±24.17	**0.001**	111.40±62.94	0.122	85.22±54.80	**0.045**
Renin	1.22±0.65	0.453	1.35±0.55	0.603	1.26±0.68	0.862
Cortisol	554.62±154.30	0.977	556.04±181.29	0.789	543.62±131.80	0.817
Adrenalin	12.03±6.00	0.569	11.08±5.84	0.570	12.00±5.88	0.990
Noradrenalin	22.48±7.27	0.998	22.47±10.10	0.848	21.99±5.59	0.821
Dopamine	276.00±149.69	0.578	296.69±123.91	0.574	276.27±146.53	0.996
17-SC	8.56±2.71	0.510	9.19±3.67	0.216	8.05±2.26	0.544
11-OCS	9.91±1.03	0.252	9.44±1.59	0.284	8.99±1.23	**0.019**
Potassium	4.47±0.59	0.353	4.28±0.75	0.296	4.09±0.39	**0.026**
Sodium	140.94±5.77	0.147	138.02±7.56	**0.030**	143.05±9.52	0.426
Chlorine	102.70±6.29	0.646	103.72±8.47	0.266	106.17±6.41	0.106
Total cholesterol	4.73±0.77	0.331	5.00±1.06	0.858	4.95±0.65	0.346
Triglycerides	1.18±0.58	0.163	1.39±0.52	0.245	1.23±0.43	0.750
C HDL	1.76±0.36	**0.012**	1.47±0.40	0.313	1.59±0.43	0.206
C LDL	2.47±0.86	0.199	2.78±0.86	0.935	2.80±0.73	0.211
BMI	25.48±4.95	0.286	26.85±4.33	0.569	26.23±2.69	0.569
Glycemia	5.02±1.42	0.732	4.89±1.34	0.763	5.01±1.44	0.971

Note: p – difference reliability between corresponding genotypes

Table 2. Parameters of AP, hormonal status, electrolyte balance, lipid spectrum, and glycemia in patients with various alleles of C-344T polymorphic marker of CYP11B2 gene.

Parameter	**C-allele**	**p**	**T-allele**
SAP	168.99±31.53	0.708	167.04±34.08
DAP	105.82±20.98	0.503	103.70±18.94
Aldosterone	86.13±56.22	0.160	99.11±60.03
Renin	1.29±0.59	0.864	1.31±0.61
Cortisol	555.39±167.55	0.841	550.21±158.46
Adrenalin	11.51±5.86	0.999	11.51±5.80
Noradrenalin	22.47±8.83	0.866	22.24±8.23
Dopamine	287.26±134.70	0.994	287.11±133.52
17-CS	8.90±3.25	0.628	8.66±3.12
11-OCS	9.65±1.37	0.058	9.23±1.43
Potassium	4.36±0.68	0.088	4.19±0.61
Sodium	139.35±6.89	0.413	140.38±8.79
Chlorine	103.26±7.49	0.178	104.87±7.59
Total cholesterol	4.88±0.94	0.483	4.98±0.88

Triglycerides	1.30±0.55	0.786	1.32±0.48
C HDL	1.60±0.40	0.245	1.53±0.41
C LDL	2.64±0.86	0.249	2.79±0.79
BMI	26.23±4.61	0.612	26.56±3.63
Glycemia	4.95±1.36	0.977	4.94±1.37

Note: p – difference reliability between alleles

Conclusion:

1. Patients with AI and healthy Uzbek people had high prevalence rate of CT-genotype with similar distribution of C and T-alleles of C-344T polymorphic marker of CYP11B2 gene.
2. Carriage of CT- and TT-genotypes of C-344T polymorphic marker of CYP11B2 gene was associated with higher levels of serum aldosterone in patients with AI, while TT-homozygous patients had reliably lower serum potassium and higher serum sodium levels; carriage of CT-genotype of CYP11B2 gene was associated with the risk of GTD development in patients with AI.
3. Analysis of correlation between AP and hormonal status showed low-renin AH, associated with T-allele of C-344T polymorphic marker of CYP11B2 gene.

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