Genetic Aspects of Arterial Hypertension: How Gene Polymorphisms Determine the Risk of Developing Hypertension

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Abstract: Arterial hypertension (AH) is one of the leading causes of cardiovascular diseases and premature death. Recently, special attention has been paid to genetic markers that may predispose to the development of AH and its complications. The presented study, conducted with the participation of 392 people (100 without AH and 292 with AH), was focused on the analysis of genetic factors influencing the development of hypertension, myocardial hypertrophy and obesity. Genetic markers, such as polymorphisms of the AGTR1, AGTR2, AGT, ADD1, CYP11B2, NOS3 and GNB3 genes, were studied using PCR and other molecular methods. The results showed that the AGTR1 and AGTR2 genes did not have statistically significant differences between the groups. However, the ADD1 gene was associated with a family history of hypertension, and the CYP11B2 mutation was associated with obesity, as confirmed by the WC/HR indicator. The GNB3 gene showed a direct relationship with the presence of left ventricular hypertrophy and the WC/HR level. These data confirm the importance of genetic testing for assessing the risks of developing hypertension and its complications, as well as the need for further research to develop personalized approaches to the treatment and prevention of hypertension.

1 INTRODUCTION

Arterial hypertension (AH) remains one of the main causes of cardiovascular diseases and premature death worldwide. In particular, more than 1.1 billion people suffer from hypertension, which is associated with lifestyle changes, population aging and an increase in risk factors such as obesity, stress and lack of physical activity (Shabalov et al., 2019). Genetic predisposition to hypertension has become an important area of research in recent years, as it can significantly affect the development of the disease and its complications. The influence of genetic factors on the development of hypertension, myocardial hypertrophy and obesity has been confirmed by a number of international and Russian studies. For example, Sato et al. (2008) note the association of gene polymorphisms, such as AGT and CYP11B2, with the risk of hypertension and its progression (Sato et al., 2008). In Russia, genetic markers associated with predisposition to hypertension are being studied. In the works of Shabalov V.M. (2019) emphasizes the importance of genetic testing for the early diagnosis of hypertension. Also, as shown in the studies of Kawarazaki et al. (2016), certain mutations in genes regulating sodium balance may be associated with the development of hypertension and obesity. An important topic is also a genetic predisposition to left ventricular hypertrophy (LVH), which is confirmed by the works of Weiss et al. and Nazarov N.I. et al.. Thus, the study of genetic markers such as ADD1 G1378T, CYP11B2 C344T and GNB3 C825T remains an urgent task for improving the methods of prognosis, early diagnosis and creating individualized approaches to the treatment of arterial hypertension and its complications, which served as the goal of our scientific work.

2 MATERIAL AND METHODS

The study included 392 people, of whom 100 were individuals without hypertension (control group) and 292 had arterial hypertension (AH) of varying severity (main group). The ratio of men to women was 266/126, i.e. the number of men was 2.1 times greater than the number of women. The gender ratio in the groups was as follows: in the main group 198/94 (i.e. 2.1/1.0) and in the control group – 68/32 (i.e. 2.1/1.0). The average time from the diagnosis of hypertension to inclusion in the study was 2.2 years. The clinical parameters of the examined patients included: measurement of systolic and diastolic blood pressure (SBP and DBP, mmHg) of the maximum, usual and at the time of examination, as well as measurement of heart rate (HR, bpm).

From the anamnestic data, the presence of concomitant pathologies was analyzed: chronic obstructive pulmonary disease (COPD), cerebrovascular pathology, anemic syndrome (with a blood Hb level < 100 g / l), oncology, previous covid-19, chronic kidney disease (CKD), visual impairment.

In addition to a general clinical examination, their taste sensitivity to table salt was studied using a modified method of R.J. Henkin. Salt sensitivity was determined using the method of M.H. Weinberger. Genetic studies were performed using peripheral blood collected on ethylenediaminetetraacetic acid (EDTA). Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (QIAGEN, Germantown, MD, USA). We investigated the A1166C polymorphism of the AGTR1 gene and the G1675A polymorphism of the AGTR2 gene using real-time polymerase chain reaction (RT PCR) performed using the ViiA 7 Real Time PCR System (Life Technologies, USA). We used the TaqMan Pre-Designed SNP Genotyping Assay. The following candidate genes were analyzed:

angiotensin I receptor type 1 gene AGTR1 (A1166C),

angiotensin II receptor type 2 gene AGTR2 (G1675A),

angiotensinogen gene AGT (C521T and T704C),

ADD1 gene G1378T was determined to determine the genetic predisposition to salt-sensitive hypertension,

aldosterone synthase gene CYP11B2 C344T,

GNB3 gene C825T,

as well as NOS3 genes G894T and NOS3 T786C.

Standard chi-square (x2) and Student's t-test methods were used for statistical analysis; calculations were performed using the Statistica 6.0 statistical program to determine the probability of differences in the distribution of genotypes during the development of hypertension. Differences were considered reliable at $p<0.05$.

3 RESEARCH RESULTS

We studied in detail the frequency of certain candidate genes and their genotypes in the analyzed sample (Table 1). As can be seen from Table 1, the studied genetic markers AGTR2 G1675A, AGTR1 A1166C, AGT C521T, AGT T704C, as well as NOS3 G894T and NOS3 T786C did not differ significantly between the groups (all $p>0.05$).

Direct analysis of the isolated genotypes established that the homozygous AA genotype of the genetic marker AGTR1 A1166C, the CC genotype of the marker AGT C521T and the TT genotype of the marker AGT T704C were comparable in frequency of occurrence and turned out to be the predominant genotypes in general in all individuals of the studied sample, regardless of the presence/absence of AH (Table 1).

A similar situation was observed for the homozygous GG genotype of the genetic marker NOS3 G894T and the TT genotype of the marker NOS3 T786C, which made up the majority of these genes, both in patients with and without AH (Table 1).

In the cohort examined by us, the prevalence of the homozygous GG genotype was revealed for the ADD1 G1378T gene in both groups of patients, both in the control group (87.0%) and in patients with AH (56.5%). However, in the main group, this advantage was not so pronounced and was 30.5% less in comparison with the control group (p < 0.00001). In the group of patients with AH, in comparison with the control group, the heterozygous GT genotype prevailed (32.9% versus 9.0%, respectively, in the main and control groups; p < 0.00001). Also, among patients with AH, the homozygous TT genotype was noted 6.6% more often than in patients without AH. That is, the last two genotypes are heterozygote GT and homozygote TT, although they did not constitute the majority of genotypes, nevertheless, their frequency prevailed among individuals with AH (about 43.5%), while in the control group these genotypes constituted, in total, 13.0%.

Table 1: Frequency of the candidate genes in question in the analyzed sample and depending on the presence/absence of AH.
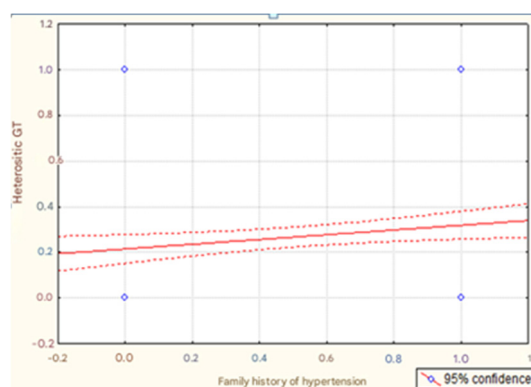
Genes, genetic markers and their genotypes	The entire sample (n=392)	Without AH (n=100)	With AH (n=292)	p	χ2
AGTR2 G1675A					
A	129 (32,9%)	32 (32,0%)	97 (33,2%)	0,920	0,010
G	137 (34,9%)	36 (36,0%)	101 (34,6%)	0,894	0,018
AA	38 (9,7%)	10 (10,0%)	28 (9,6%)	0,940	0,006
GA	40 (10,2%)	10 (10,0%)	30 (10,3%)	0,910	0,013
GG	48 (12,2%)	12 (12,0%)	36 (12,3%)	0,929	0,008
AGTR1 A1166C	392				
AA	266 (67,9%)	69 (69,0%)	197 (67,5%)	0,874	0,025
AC	85 (21,7%)	20 (20,0%)	65 (22,3%)	0,740	0,111
CC	41 (10,5%)	11 (11,0%)	30 (10,3%)	0,988	0,000
AGT C521T					
CC	309 (78,8%)	82 (82,0%)	227 (77,7%)	0,449	0,575
CT	62 (15,8%)	14 (14,0%)	48 (16,4%)	0,676	0,175
TT	21 (5,4%)	4 (4,0%)	17 (5,8%)	0,660	0,195
AGT T704C					
CC	40 (10,2%)	9 (9,0%)	31 (10,6%)	0,778	0,073
TC	84 (21,4%)	22 (22,0%)	62 (21,2%)	0,984	0,000
TT	268 (68,4%)	69 (69,0%)	199 (68,2%)	0,974	0,001
ADD1 G1378T					
GG	252 (64,3%)	87 (87,0%)	165 (56,5%)	**0,000**	**28,854**
GT	105 (26,8%)	9 (9,0%)	96 (32,9%)	**0,000**	**20,454**
TT	35 (8,9%)	4 (4,0%)	31 (10,6%)	0,072#	3,238
CYP11B2 C344T					
CC	269 (68,6%)	83 (83,0%)	186 (63,7%)	**0,000**	**12,007**
CT	69 (17,6%)	12 (12,0%)	57 (19,5%)	0,121	2,409
TT	54 (13,8%)	5 (5,0%)	49 (16,8%)	**0,006**	**7,740**
GNB3 C825T					
CC	241 (61,5%)	71 (71,0%)	170 (58,2%)	**0,032**	**4,612**
CT	114 (29,1%)	20 (20,0%)	94 (32,2%)	**0,029**	**4,794**
TT	37 (9,4%)	9 (9,0%)	28 (9,6%)	0,981	0,001
NOS3 G894T					
GG	259 (66,1%)	69 (69,0%)	190 (65,1%)	0,553	0,353
GT	92 (23,5%)	20 (20,0%)	72 (24,7%)	0,417	0,659
TT	41 (10,5%)	11 (11,0%)	30 (10,3%)	0,988	0,000
NOS3 T786C					
CC	45 (11,5%)	11 (11,0%)	34 (11,6%)	0,995	0,000
TC	89 (22,7%)	24 (24,0%)	65 (22,3%)	0,826	0,048
TT	258 (65,8%)	65 (65,0%)	193 (66,1%)	0,939	0,006

Note: n – number of patients; AH – arterial hypertension; p – significance of differences between groups with/without AH at p<0.05; # – tendency to significance

Since the ADD1 gene encodes the alpha subunit of the adducin protein, one of the regulators of sodium-potassium adenosine triphosphatase (Na+, K+-ATPase), the latter, in turn, is involved in the transport of these ions through the membrane of the renal epithelium, this gene is used in assessing the genetic predisposition to salt-sensitive hypertension.
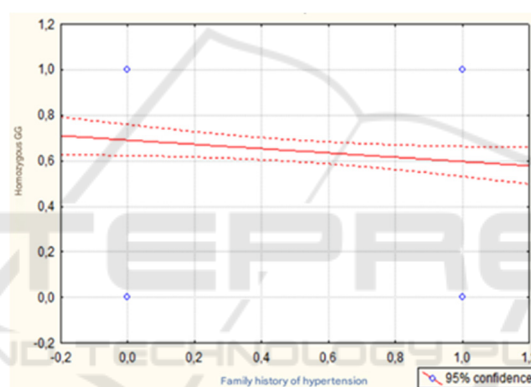
Based on this fact, we conducted a parallel analysis with family history data. In this aspect, it was found that in general, 201 (51.3%) people in the entire sample of subjects indicated a burdened heredity for AH, and in most cases (175 people) these were patients from the main group, i.e. with the presence of AH, the remaining 26 were from the control group. In percentage terms of the number of groups, this indicator also prevailed among people with AH, amounting to 59.9% versus 26.0% in the control group (p = 0.0000 and χ2 = 32.983). When conducting a correlation analysis between the frequency of the isolated genotypes of the ADD1 G1378T gene (GG, GT and TT) and family history, the following dependencies were revealed (Fig. 1A

Note: On the X-axis, under the number "0" - the absence of an aggravated family history and under the number "1" - the presence of an aggravated heredity for AH; on the Y-axis, under the number "0" - the absence of GT heterozygote and under the number "1" - the presence of GT heterozygote of the ADD1 G1378T gene.

Figure 1A: Correlation graph between the heterozygous GT genotype of the ADD1 gene and the presence of a burdened family history of AH. **p=0,020**; r=0,117 и t=2,328.

p=0.052#; r= -0.098 and t= -1.947

Note: On the X-axis, under the number "0" - the absence of an aggravated family history and under the number "1" - the presence of an aggravated heredity for AH; on the Y-axis, under the number "0" - the absence of a GG homozygote and under the number "1" - the presence of a GG homozygote of the ADD1 G1378T gene.

Figure 1B: Correlation graph between the homozygous GG genotype of the ADD1 gene and the presence of a burdened family history of AH.

and 1B). Namely, the prevalence of the heterozygous GT genotype was characterized by a direct relationship with an aggravated family history of AH, and the identified relationship reached the level of reliability (Fig. 1A). No significant interdependence was found between a family predisposition to AH and the homozygous TT genotype (p=0.738; r= -0.016 and t= -0.334). On the contrary, an inverse correlation with a tendency toward reliability was established between the homozygous GG genotype and an indication of an aggravated family history (Fig. 1B).

That is, a heredity burdened by AH, or a family predisposition to the development of AH, had a clear association with the heterozygous GT genotype of the ADD1 gene, and given its connection with a

predisposition to salt-sensitive hypertension, we can conclude that the patients we examined had, one way or another, a nutritional disorder. On the contrary, the homozygous GG genotype of the ADD1 gene, according to the results of our analysis, was more associated with healthy (free from AH) individuals.

Analysis of the genetic marker C344T of the CYP11B2 gene also established the prevalence of the homozygous CC genotype. Namely, in the sample as a whole, its frequency of occurrence was 68.6%, and in the compared groups 83.0% in the control group, i.e. in individuals without AH and 63.7% in patients with AH.

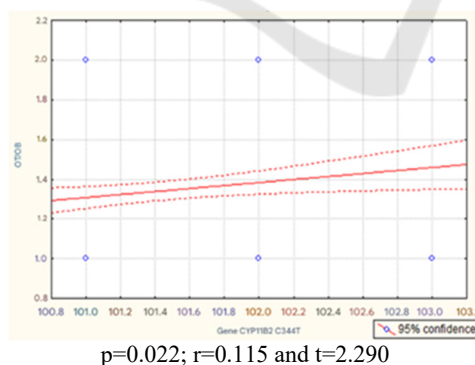The heterozygous CT genotype accounted for 17.6% of cases in the entire sample (Table 1). In the

group of patients with AH, this indicator was 7.5% higher than in the control group (p=0.121 and χ2=2.409).

A similar trend was observed for the homozygous TT genotype. In particular, in the sample as a whole, this genotype was recorded in 13.8% of cases, and in the compared groups in 5.0% and 16.8% of cases (p<0.05), respectively, in the control group and in the main group of patients (Table 1).

It is widely known that the CYP11B2 gene encodes aldosterone synthetase, which ensures the synthesis of aldosterone from deoxycorticosterone. Detection of the C344T mutation in the regulatory region of the gene is associated with an increase in aldosterone synthesis. The latter is known to be responsible for the functioning of the renal sodium-potassium pump and maintenance of water-electrolyte balance. In this regard, we conducted a correlation analysis between the isolated genotypes (CC, CT and TT) of the CYP11B2 gene and the presence of CKD in the examined patients. In this aspect, no significant relationships were revealed. Namely, the presence of renal pathology was characterized by an inverse relationship with the heterozygous CT genotype (p = 0.329; r = -0.057 and t = -0.976) and a positive correlation with homozygous CC (p = 0.900; r = 0.006 and t = 0.000) and TT genotypes (p = 0.854; r = 0.010 and t = 0.183), however, the identified trends did not reach the level of reliability. The obtained results were probably due to the small number of patients with CKD: 28.1% of the total sample, and in the selected groups their number was 12.0% in the control group and 33.6% in
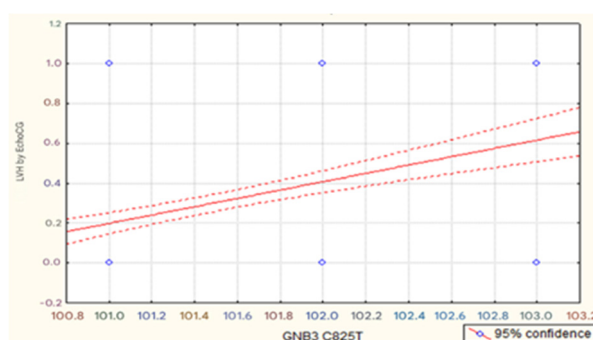
the group of patients with AH (p=0.0000 and χ2=16.104). According to the literature [3], some variants of genes associated with sensitivity to salt affect the risk of obesity, and together with salt consumption, their combination may be associated with the development of hypertension in obese people. In this regard, we tried to study the relationship of the isolated genetic marker CYP11B2 C344T with obesity, or rather its indirect indicator - the waist-to-hip ratio (WHR). We found a positive correlation (Fig. 2) between the studied genetic marker and the WHR (p<0.05).

That is, this fragment of our work indicates a relationship between the genetic marker CYP11B2 C344T and obesity (p<0.05), mediated by the presence of a mutation in the regulatory region of the gene responsible for the synthesis of aldosterone. At the same time, its relationship with the potassium-sodium renal pump and water-electrolyte balance did not reach the level of reliability (all p>0.05), which was probably due to the small number of diagnosed renal pathology in the analyzed sample of patients. In the sample of patients examined by us, another genetic marker attracts attention - this is GNB3 C825T. Detection of the C825T mutation in the GNB3 gene indicates changes in the differentiation of lymphoblasts, fibroblasts and proliferative activity. In scientific research, the study of this genetic marker is carried out with the aim of identifying a genetic predisposition to AH, as well as assessing the relationship with LV myocardial hypertrophy (LVH), the development of obesity and diabetes mellitus.

p=0.022; r=0.115 and t=2.290

Notes: on the X-axis – the selected genotypes of the CYP11B2 C344T marker and on the Y-axis – under the number "1" – WC/HR < 1 and under the number "2" – WC/HR > 1.
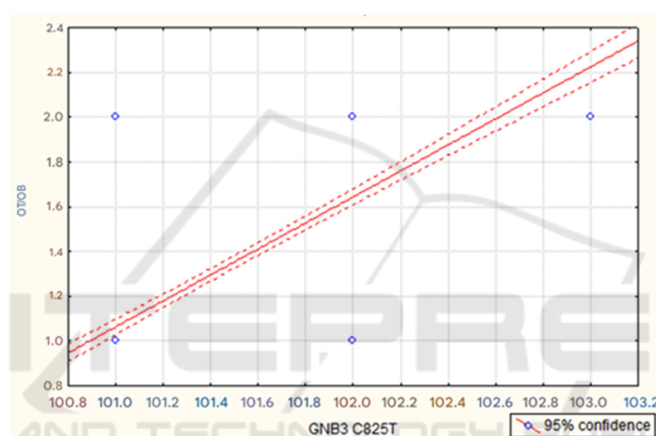
Figure 2: Graph of the correlation between the genetic marker CYP11B2 C344T and the WC/HR ratio.

p=0.000; r=0.302 and t=6.260

Notes: X-axis – isolated genotypes of the marker GNB3 C825T and Y-axis – left ventricular hypertrophy (LVH) detected by echocardiography.

Figure 3A: Correlation graph between the isolated genetic marker GNB3 C825T and the presence of LV myocardial hypertrophy.

p=0.000; r=0.810 and t=27.292

Notes: on the X-axis – isolated genotypes of the marker GNB3 C825T and on the Y-axis – under the number "1" - WC/HR < 1 and under the number "2" - WC/HR > 1.

Figure 3B: Correlation graph between the isolated genetic marker GNB3 C825T and the WC/HR ratio.

Based on the above, we conducted a correlation analysis between the selected genotypes (CC, CT and TT) of the genetic marker GNB3 C825T and the presence of LVH according to echocardiography, an indirect indicator of obesity - WC/OB and postprandial blood glucose values. From this position, a direct positive correlation was revealed between the genetic marker GNB3 C825T and the presence of LVH according to echocardiography - on the one hand (Fig. 3A) and the level of WC/OB - on the other hand (Fig. 3B), while both correlations were highly reliable (both p < 0.0000). A direct relationship was also established between the genetic marker GNB3 C825T and the level of postprandial blood glucose, although it did not reach the level of reliability (p=0.082; r=0.087 and t=1.738).

A study of the frequency of occurrence of homo- and heterozygous genotypes for the GNB3 C825T gene revealed a picture that, in the sample as a whole, regardless of the presence/absence of AH, the homozygous CC genotype prevailed (61.5%), while in patients with AH it was recorded in 58.2%, and in individuals without AH - in 71.0% of cases. The presence of the heterozygous CT genotype was present in 114 (29.1%) patients, of which 94 (82.5% of 114 people with this genotype or 32.2% of the main group n=292) were people with AH and 20 people (17.5% of 114 or 20.0% of the control group n=100) were from the control group (p<0.05). The homozygous TT genotype was found in the smallest number of cases, amounting to 9.4% of the total sample, and 9.0% and 9.6% in the analyzed control groups and in people with AH, respectively (Table 1). Thus, a detailed analysis of the genetic marker CYP11B2 C344T established its relationship with the presence of LVH (according to echocardiography)

and obesity, or rather with its indirect indicator – the WC/OB ratio, while both dependencies were highly reliable (p<0.0001). There was also a positive correlation with the level of postprandial blood glucose, but this trend did not reach the level of reliability. The genotypic picture of the GNB3 gene revealed the prevalence of the homozygous CC genotype, regardless of the presence/absence of AH, but the heterozygous CT genotype was predominant among patients with AH (82.5%), while the homozygous TT genotype occurred with almost the same frequency in the compared groups (9.0% and 9.6%).

4 DISCUSSION

The study found that among the genetic markers studied (AGT, CYP11B2, GNB3, ADD1 and NOS3), only some of them demonstrate a significant association with the development of arterial hypertension (AH) and its complications, such as myocardial hypertrophy and obesity. This is consistent with the results of many international studies that emphasize the role of genetic predisposition in the pathogenesis of AH.

The AGT gene (angiotensin I converting enzyme gene) and its polymorphisms, such as AGT C521T, AGT T704C, have long attracted the attention of researchers as key markers of AH risk. The works of Curb et al. (2007) and Gagliardi et al. (2009) show that variations in genes encoding components of the renin-angiotensin-aldosterone system (RAAS) significantly affect the development of hypertension, including the difficulty in its treatment [8,9]. Our results, which did not show statistically significant differences in these markers in the sample with AH and the control group, are generally consistent with similar studies, where polymorphisms in these genes do not always have a clear effect on the severity of the disease. The CYP11B2 gene, encoding aldosterone synthetase, is also of interest as a factor influencing the development of hypertension. The works of Kawarazaki and Fujita (2016), as well as Wang et al. (2018) demonstrated an association of mutations in this gene with increased sensitivity to salt and excessive secretion of aldosterone, which in turn contributes to an increase in blood pressure. Our findings of the prevalence of the homozygous CC genotype in the control group and the prevalence of the heterozygous CT genotype in the AH group confirm the results of similar studies. However, as in some studies, for example, Yang et al. (2015), we did not observe a significant correlation with the

development of chronic kidney disease, which may be due to differences in the sample or a lower incidence of kidney disease in the study group. The GNB3 C825T gene has been actively studied in recent years in the context of hypertension and its complications. Studies by Cohen et al. (2005) and Weiss et al. (2003) show that a mutation in this gene may be associated with myocardial hypertrophy, obesity, and diabetes. Our findings on the high prevalence of the heterozygous CT genotype among patients with AH are also confirmed by international data, which may indicate the importance of this marker for predicting the risk of developing hypertension and its cardiovascular complications. The ADD1 gene, involved in the regulation of sodium-potassium adenosine triphosphatase (Na+,K+-ATPase), showed a significant association with salt-sensitive hypertension, which is consistent with the opinion of other researchers. According to the work of Sowers et al. (2013), mutations in this gene can increase the body's sensitivity to excess salt, which leads to an increase in blood pressure. The results of our study, where the heterozygous GT genotype was more common among patients with AH and was associated with a family predisposition, are fully consistent with these findings. Overall, our findings confirming the role of genetic markers such as AGT, CYP11B2, ADD1 and GNB3 in the development of AH are consistent with international data and highlight the need for further research to identify the exact mechanisms of their action. At the same time, the lack of significant association with chronic kidney disease in the case of CYP11B2 and AGT requires additional studies aimed at clarifying their role in the pathogenesis of AH.

5 CONCLUSION

The prevalence of certain genotypes in the group of patients with AH (e.g., homozygous AGTR1 A1166C, AGT C521T, and NOS3 G894T genotypes) suggests that these markers do not have a significant difference in frequency between the groups with and without AH. However, the ADD1 G1378T gene demonstrated an association with a familial predisposition to AH, especially in the case of the heterozygous GT genotype, which is associated with malnutrition and salt sensitivity.

The results of the CYP11B2 C344T marker analysis showed a positive correlation with an indirect indicator of obesity (WC/HC), which confirms its possible role in the regulation of metabolism and the development of obesity.

However, its association with renal pathology in this sample was not statistically significant, which is probably due to the small number of patients with CKD. The GNB3 C825T marker showed a strong positive correlation with left ventricular hypertrophy (LVH) and obesity level, confirming its potential role in the development of metabolic disorders and hypertension. At the same time, the dependence on the level of postprandial blood glucose was not significant, which may indicate more complex interactions between genes and metabolic processes.

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