

# THE IMPACT OF THE GENETIC FACTOR IN THE REALIZATION OF THE ARTERIAL HYPERTENSION AND METABOLIC DISORDERS AT CHILDREN WITH OVERWEIGHT OR OBESITY

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## ABSTRACT

Essential arterial hypertension is a multifactor pathology and in its realization genetic and environmental factors play a very important role. It is estimated that 30-60% of the variation of the arterial hypertension among people is due to genetic factor. Its investigation is argued at children especially, because from age it can be changed under the influence of the environment.

**Objectives.** Estimation of the polymorph variants frequency of the candidate genes from RAS (ACE, AGTR1) and NOS3 at children with arterial hypertension, obesity or overweight. Appreciation of the possible relations between carrying genotypes of the genes studied and the risk of arterial hypertension, overweight and metabolic disorders to appear. Interaction of the genetic factors with the modifiable ones in the appearance of these pathologies.

**Material and methods.** The investigation included 120 children with obesity and overweight 62 (51.67%) children with hypertension and 58 (48.33%) with normal tension) at the age of 10-18. The polymorphism of the candidate genes was identified through the analysis of the length of the amplified fragments and restriction fragment length polymorphism. The products of restriction were placed in 2% agarose gel and dyed with ethidium bromide. The protocol of the studies was approved by the Commission in Medical Ethics and the signed consent for participation in studies was obtained.

**Results.** Children-carriers of ACE DD and AGTR1 CC showed increased values of BMI, CA and SBP. At the carriers of GG and AG genotypes of NOS3 AG were also revealed statistically significant higher values of SBP in comparison with the carriers of AA genotype. At the same time, children with ACE DD genotype were distinguished by the increased serum level of triglycerides ( $1.90 \pm 0.122$  mmol/l), insulin ( $27.27 \pm 2.557$   $\mu$ U/ml) and reduced of HDL-C ( $1.24 \pm 0.048$  mmol/l). Simultaneously, there were found statistically significant differences between small gestational age and genotype DD of ACE and between big gestational age and genotype ID of ACE. DD carriers of the ACE and CC carriers of the AGTR1 also proved to be more often passive smokers, consumers of the products with higher concentration of salt and fat.

**Conclusion.** Identification of the children with obesity/overweight carriers of genotypes ACE DD, AGTR CC and NOS GG could facilitate early detection of the children with the risk of appearance of arterial hypertension and metabolic disorders to add to taking early measures for prophylaxis and treatment.

**Keywords:** arterial hypertension, overweight/obesity, gene polymorphism, genotype, candidate gene

## INTRODUCTION

In the last decade there are known more than 150 candidate genes with pleiotropic effect which determine genetic predisposition to arterial hypertension (AHT), obesity, metabolic syndrome (MS) and risk of the realization of the cardio-vascular complications (1,2). Among these there can be found the genes that encode the components of the

renin- angiotensin system (RAS) and nitric oxide synthase (NOSe). One of the first, it was described the polymorphism of the gene angiotensin converting enzyme (ACE) located on the chromosome 17 (17q23) determined by the presence or the absence (insertion/deletion) of one block made up of 287pb in the intron16. ACE transforms angiotensin I from the inactive form into the active one – angiotensin II, the main effector peptide of RAS (3). Cellular

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effects of angiotensin II are mainly mediated by the type I receptors of angiotensin II that were studied because of their impact in developing of AHT. Stimulation of the receptor AT1 induces secretion of aldosterone by the adrenal cortex, retention of sodium and water, vasoconstriction, expression of the factor of growth and proliferation of smooth vascular muscles and, as a consequence, increase of the arterial tension (3). The polymorphism of the gene (AGTR1) is located on the chromosome 3q21-Q25 and is due to variability of adenine and cytosine in position 1166 from the sequence of nucleotides. Allele 1166 C interrelate with AHT, but allele A (adenine) and genotype AA reduce the risk (4,5). But some factors indicate the association between the polymorphism of the gene ACE I/D and the risk the overweight/obesity to appear (6). ACE being present in the adipose tissue can be involved in growing and metabolism of the adipocytes. It is considered that angiotensin II inhibit the differentiation of the adipocytes, promote ectopic lipid deposition that leads to lipotoxicity (6).

Endothelial dysfunction, another chain in AHT pathogenesis occurs through the influence of the peripheral vascular resistance from the paracrine viewpoint (secretion of endothelin, nitric oxide, prostaglandins) along with neurogenic regulation (the sympathetic nervous system) and hormonal regulation (renin-angiotensin-aldosterone axis, catecholamines, insulin). The most important vasodilators substance is nitric oxide (NO). It acts through the medium of the guanylate cyclase, determines the relaxation of the smooth muscles cells with vasodilatation, inhibit aggregation, platelet adhesiveness and myointimal proliferation of vascular muscle (7,8). NO synthetase constitutively NO through conversion of L-arginine into L-citrulline that involve the transfer of five electrons provided by NADPH. In people, NO synthetase is coded by the gene  $NOS_3$ , located on the chromosome 7q35-36. Production of NO can be modified by the polymorphism of the gene  $NOS_3$ . Great attention was focused on the variant Glu298Asp (8). At the same time, the existing studies often provide with contradictory or inconclusive results, because the majority of them included small amount of children and were not enough informative to demonstrate association, so there is necessity of supplementary investigations (4-6,8-14). This fact determined the initiation of this research with the following **objective**:

Estimation of the frequency of the polymorph variants of candidate genes from RAS (ACE,

AGTR1) and  $NOS_3$  at children with arterial hypertension, overweight or obesity. Appreciation of possible relations between carrying of genotypes of the studied genes and the risk of appearing of AHT, extra weight and metabolic disorders. Interaction of the genetic factors with the modifiable ones in the appearance of these pathologies.

## MATERIAL AND METHODS

General group of the investigation included 120 children with overweight and obesity at the age of 10-18. According to the tension children with obesity/overweight were subdivided so: 62 (51.67%) hypertensive children and 58 (48.33) normotensive. Children with the secondary forms of AHT and obesity were not included in the investigation. Disagreement to participate in the investigation was also considered the reason for excluding from the research. The degree of the obesity was determined through the calculation of the Body Mass Index (BMI). Arterial tension was appreciated through the auscultation method ( $AHT \geq$  percentile 95 depending on age, sex and stature). Glucose a jeun, lipid parameters (total cholesterol (T-C, LDL-C, HDL-C, triglycerides (TR)) were determined through the optical colorimetric method. Serum insulin through chemiluminescent method. HOMA-IR was calculated according to the formula: fasting insulin ( $\mu$ U/ml) x fasting glucose (mmol/l)/22.5. The polymorphism of the candidate genes was identified through the analysis of the length of the amplified fragments and restriction fragment length polymorphism. The products of restriction were placed in 2% agarose gel and dyed with ethidium bromide.

## RESULTS

### Relations between the polymorphism of the genes RAS (ACE, AGTR1) and $NOS_3$ with tension values, BMI and AC

From 120 children with overweight and obesity - 41 turned out to be carriers of the genotype ACE DD, 15 children- carriers of t ACE II and 64 children - carriers of ACE ID. The carriers of DD showed the body mass index (BMI) higher ( $32.33 \pm 0.940$ ), statistically significant ( $p < 0.001$ ) in comparison with the carriers of genotype II ( $28.43 \pm 0.731$ ) and the carriers of the genotype ID ( $28.11 \pm 0.239$ ). Statistically significant differences between the genotypes compared ( $p < 0.001$ ) were found depending on abdominal circumference (AC) also higher at the carriers of the genotype DD

(100.91±2.247 cm), in comparison with the values obtained at the carriers of ID (88.55±1.229 cm) and the carriers of the genotype II (85.60±2.244 cm). The highest average values of the systolic blood pressure (SBP) statistically significant ( $p<0.001$ ) were found at the carriers of the genotype DD (151.83±856 mmHg), followed by the carriers of ID (129.45±3.50 mmHg), but the carriers of the genotype II showed the values at the limit of the norm for the age (120.87±6.00 mmHg).

Analyses of the polymorphism A/C of the gene AGTR-1 depending on the degree of obesity revealed that the carriers of the genotype CC had BMI higher (31.63±1.00) compared with the carriers of the genotype AC (28.88±0.409) and AA (28.27±0.618), the differences that are statistically significant ( $p<0.01$ ). The value of AC turned out to be statistically significant ( $p<0.001$ ). It was higher at the carriers of the genotype CC being 98.94±2.448 cm compared to 91.02±1.417cm at the carriers of the genotype AC and 84.83±2.11cm at the carriers of the genotype AA. The similar tendency was also viewed in the SBP values. Statistically significant ( $p<0.001$ ) higher values were registered at the carriers of the genotype CC (153,00±4,11 mmHg and AC (130.52±3.41 mmHg) as compared to the carriers of the genotype AA (123.50±6.11 mmHg).

Analyses of the polymorphism of the gene NOS<sub>3</sub>A/G depending on BMI (GG-30.71±1.178; AA-28.26±0.854; AG-29.52±0.453) showed no statistical veracity ( $p>0.05$ ). The same tendency ( $p>0.05$ ) is for AC (GG-94.86±2.804 cm, AA-88.64±2.737 cm, AG-92.38±1.453 cm). Increased values of SBP were registered at the carriers of GG (149.55±5.659 mmHg) and AG (134.02±3.146

mmHg) genotypes in comparison with AA carriers (126.79±6.83 mmHg). So these differences are statistically significant ( $p<0.05$ ) (Fig. 1).

### Relations between the polymorphism of the genes RAS (ACE, AGTR1) and NOS<sub>3</sub> with the metabolic parameters and other risk factors

Analysis of the lipid metabolic disorders depending on the polymorphism ACE I/D revealed higher average values of total cholesterol (5.19±0.130 mmol/l) at the carriers of the genotype DD as compared to the carriers of the genotype II (5.00±0.207 mmol/l) and ID (4.88±0.090 mmol/l). However, the differences between the genotypes compared did not reach the statistic significance ( $p>0.05$ ). Also, the carriers of the genotype DD had the values of LDL-C higher (2.83±0.131 mmol/l) than the carriers of the genotype II (2.75±0.202 mmol/l) and ID (2.48±0.100 mmol/l) the differences that are still not statistically significant between the genotypes ( $p>0.05$ ). At the same time there were revealed lower serum levels of HDL-C (1.24±0.048 mmol/l) at the carriers of the genotype DD as compared to the carriers of II (1.33±0.086 mmol/l) and ID (1.39±0.035 mmol/l), the differences that are statistically significant ( $p<0.05$ ). Also, at the carriers of the genotype DD the serum level of the triglycerides was statistically significant higher (1.90±0.122) in comparison with the carriers of II (1.80±0.157) and ID (1.47±0.068) ( $p<0.01$ ).

Analyzing the lipid metabolic disorders depending on the polymorphism of AGTR1 we found out that the concentrations of total cholesterol, though statistically insignificant ( $p>0,05$ ), had the similar tendency of being higher at children with the geno-

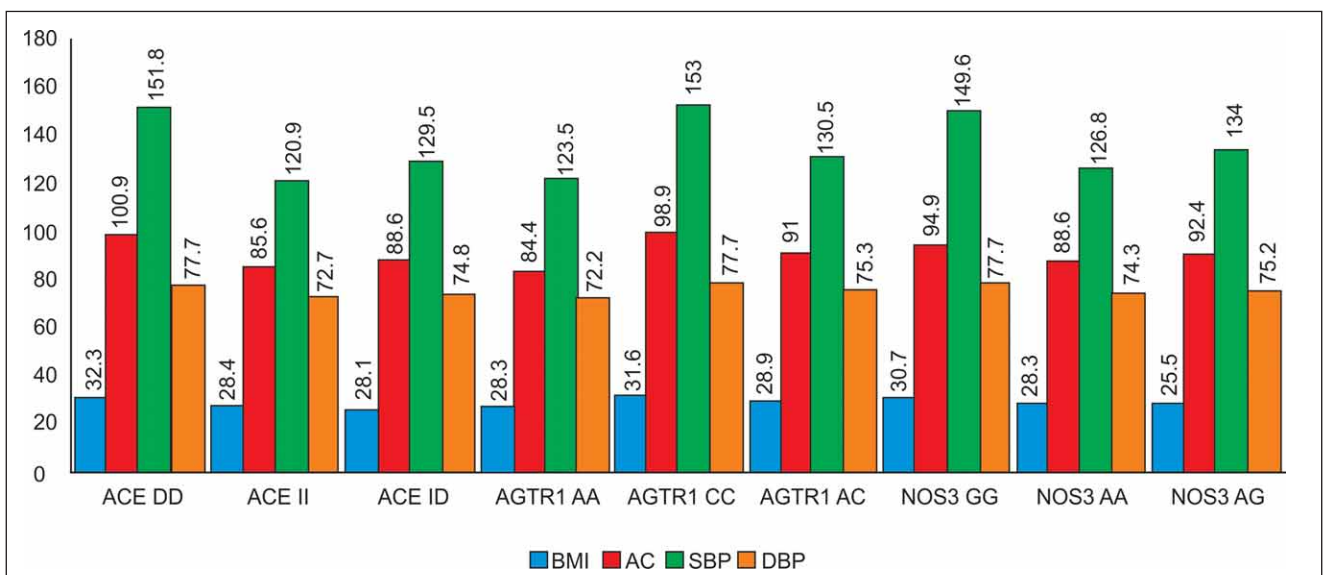


FIGURE 1. Relation between the polymorphism of the genes studied with BMI, AC and tension values

type CC ( $5.21 \pm 0.151$  mmol/l) as compared to the children with the genotype AC ( $4.92 \pm 0.086$  mmol/l) and AA ( $4.86 \pm 0.180$  mmol/l). Analysis of the serum levels of HDL-C and LDL-C depending on the genotype also revealed statistically insignificant differences ( $p > 0.05$ ). Serum level of the triglycerides was increased at children with the genotype CC  $1.82 \pm 0.128$ , the children with genotype AA  $1.73 \pm 0.141$  mmol/l showed the border line level and the genotype ID  $1.56 \pm 0.076$  showed the level at the limits of the norm that is still statistically insignificant ( $p > 0.05$ ) (according to IDF 2007, hypertriglyceridemia  $\geq 1.7$  mmol/l). The same tendency ( $p > 0.05$ ) was revealed for the polymorphism of NOS<sub>3</sub> according to the concentration of the total cholesterol with the values  $4.92 \pm 0.171$  mmol/l,  $4.96 \pm 0.180$  mmol/l and  $5.02 \pm 0.086$  mmol/l at the genotype carriers GG, AA and AG respectively. There were not revealed statistically significant differences ( $p > 0.05$ ) neither for LDL-C nor HDL-C for according to the studied genotypes. What concerns the level of serum triglycerides there were higher values at the carriers of the genotype GG  $1.72 \pm 0.181$  mmol/l as compared to the genotypes AA  $1.67 \pm 0.178$  mmol/l and AG  $1.64 \pm 0.068$  mmol/l but still without statistics significance (Fig.2).

According to glucose a jeun were not revealed statistically significant differences among the carriers of the polymorph variants of the genes studied ( $p > 0.05$ ). However the carriers of the genotype ACE DD distinguished themselves statistically significant ( $p < 0.01$ ) through the increased level of se-

rum insulin ( $27.27 \pm 2.557$   $\mu$ U/ml) as compared to the carriers of the genotype ECA II ( $18.50 \pm 2.542$   $\mu$ U/ml) and ECA ID ( $18.70 \pm 1.290$   $\mu$ U/ml). Children carriers of the polymorphism AGTR1 A/C did not distinguish themselves statistically ( $p > 0.05$ ) according to the serum insulin level, while the children with the genotype CC ( $25.94 \pm 2.800$   $\mu$ U/ml) and AC ( $20.27 \pm 1.426$   $\mu$ U/ml) showed higher levels in comparison with the children carriers of AA ( $18.15 \pm 2.192$   $\mu$ U/ml). Also, there were not revealed statistically significant differences ( $p > 0.05$ ) between carrying of the genotypes of the enzyme NOS<sub>3</sub> and the level of the serum insulin (GG- $20.67 \pm 2.688$   $\mu$ U/ml; AA- $21.22 \pm 2.385$   $\mu$ U/ml; AG- $21.91 \pm 1.533$   $\mu$ U/ml) (Fig. 3).

Analyzing the relation between the polymorphism of the studied genes and other risk factors, there was found a statistically significant association between the small gestational age (term  $< 37$  weeks) and genotype DD, as well as between the big gestational age (term  $> 42$  weeks) and genotype ID ( $\chi^2 = 13.003$ ;  $p < 0.05$ ). There were also found statistically significant differences ( $\chi^2 = 8.067$ ;  $p < 0.05$ ) between the genetic polymorphism of the gene ACE I/D and the status of the passive smoker (DD-51.22%, II-26.67%, ID-25%). At the same time, children carriers of the genotype DD turned out to be more frequent consumers of the more salted products (salted – 39.02%, medium salted – 53.66%, less salted – 7.32%), as compared to the children carriers of the genotypes II (salted – 13.33%, medium salted – 80.00%, less salted – 6,67%) and ID

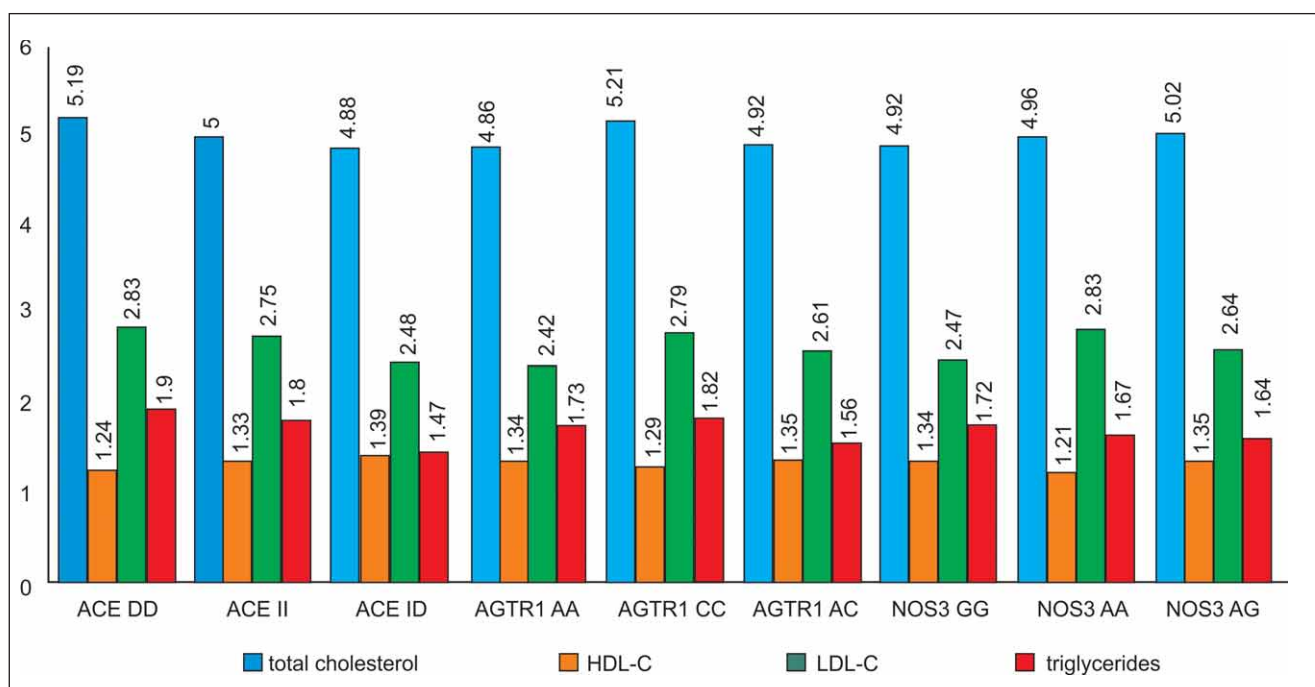
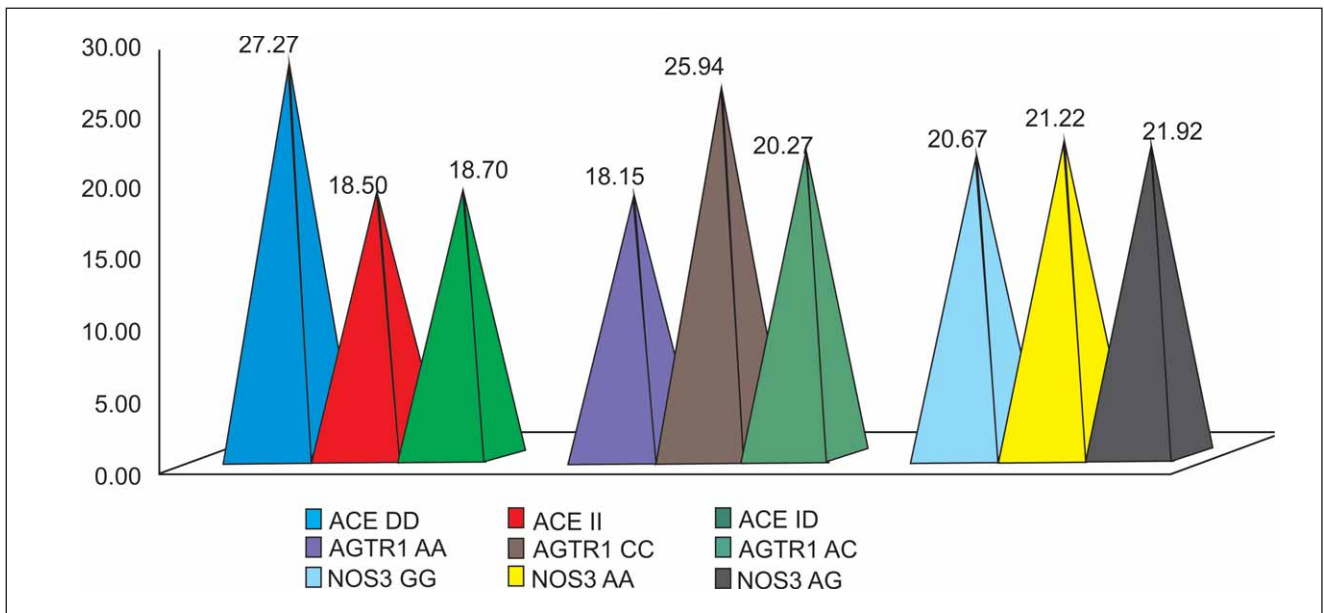


FIGURE 2. The relation between the polymorphism of the studied genes and the parameters of the lipid metabolism



**FIGURE 3.** Relation between the polymorphism of the studied genes and serum insulin

(salted – 10.94%, medium salted – 75.00%, less salted – 14.06%) ( $\chi^2=13.257$ ;  $p<0.05$ ). Also, among the carriers of the genotype DD are consumed more animal fats (DD – 39.02% compared to II – 13.33% and ID – 12.50%) and less vegetal fats (DD – 60.98% as compared to II – 86.67%, ID – 87.50%) ( $\chi^2=11.061$ ;  $p<0.01$ ). Statistically significant differences between the genotypes depending on the frequency of the fats consuming ( $\chi^2=7.208$ ;  $p<0.05$ ) and consuming of salted products ( $\chi^2=6.373$ ;  $p<0.05$ ) were also found at the carriers of the polymorphism AGTR1 A/C. Thus, 3.14% of the children with the genotype CC consume animal fats in comparison with 11.11% children with the genotype AA and 16.42% with AC. Vegetal fats are consumed by 62.86% of children with the genotype CC as compared to 88.89% – genotype AA and 83.58% – genotype AC. It is consumed more salt at the carriers of the genotype CC (salted – 34.29%, medium salted – 60.00%, less salted – 5.71%) in comparison with the carriers of the genotype AC (salted – 16.42%, medium salted – 70.15%, less salted – 13.43%) and AA (salted – 11.11%, medium salted 77.78%, less salted – 11.11%). What concerns the influence of the chronic stress, status of the active smoker, consuming energy drinks or alcohol, there were not revealed significant differences between the genotypes of the genes ACE I/D and AGTR1 A/C ( $p<0.05$ ). There were not also revealed statistically significant differences between the carrying of the genotypes of the gene NOS<sub>3</sub>A/G and modifiable risk factors such as: active and passive smoking, chronic stress, consuming energy drinks or any other type of alcohol as well as salt or fats.

## DISCUSSIONS

Renin angiotensin system plays an important role in the homeostasis of the arterial tension and fluid balance, but genetic polymorphism of their code components can be implied in the predisposition to AHT, obesity and metabolic disorders (1-5). These associations formed the subject of the great number of investigations (4-6, 8-14). At the same time, when some reported the genotype D/D of ACE and the genotype CC of AGTR1 as an independent risk factor for realization of such pathologies, others did not get any proofs of the association. Wu and his colleges, in a research that included 105 children with hypertension and 105 children with normal tension, found the following genotypes in the group of children with increased values of the tension: DD – 30.5%, ID – 47.6% and II – 21.9% as compared to control group – DD – 14%, ID – 46.7% and II – 39.1%. At the same time the frequency of D allele was significantly higher. In the group of children with AHT (54.3% as compared to 37.6%), but the frequency of I allele was significantly lower in comparison with the control group (45.7% to 62.4%) ( $p<0.01$ ) (9). Simultaneously, it should be mentioned the fact that the identification of the genetic factor of cardio-vascular risk is more important at children with obesity/overweight where prevalence of AHT and MS is much higher, but their presence considerably multiply the risk of the realization of cardio-vascular complications. The meta analyses of 14 studies ( $n=3.371$  with obesity,  $n=4.490$  control group) showed a significant association between the geno-

type ACE DD and the risk of the realization of obesity/overweight (6). In our research that included 120 children with overweight and obesity at the age of 10-18-62 (51.67%) hypertensive and 58 (48.33%) normotensive, DD carriers of ACE outlined themselves statistically significant with increased values of BMI, AC and SBP. Similar results were obtained by Joey and his colleges in their research (n=150 children) (10). At the same time, in another investigation where 199 children with obesity participated (44 with hypertension), the prevalence of AHT at the ones with obesity, carriers of the genotype DD, II and ID was similar but not showing any difference between the group with hypertension compared to the one with normal tension in accordance with the genotype ACE I/D (11). Kotaska and her colleges stated in the research they made that ACE D allele and the genotype ACE DD were the most frequent genetic variants met in the group of patients with dyslipidemia (12). In accordance with these findings were also our results. At the carriers of the genotype DD we got medium values of total cholesterol, higher values of LDL-C in comparison with the carriers of the genotype II and ID. However, the differences between the genotypes compared did not reach the degree of statistics significance ( $p > 0.05$ ). At the same time, at the carriers of the genotype DD were found lower serum levels of HDL-C as compared to the carriers of the genotypes II and ID, the differences of statistics significance ( $p < 0.05$ ). Also, at the carriers of the genotype DD serum level of triglycerides was statistically significant higher in comparison with the carriers of the genotype II and ID ( $p < 0.01$ ). Investigating the interrelation between the polymorphism ACE I/D with the perinatal risk factors we found a statistically significant association between the small gestational age (term  $< 37$  weeks) and the genotype DD and large gestational age (term  $> 42$  weeks) and the genotype ID ( $\chi^2 = 13.003$ ;  $p < 0.05$ ). Aline and his colleges in the research that included 167 children (60 with light weight at birth, and 107 with normal weight) stated the activity of the ACE enzyme greatly increased at the children with light weight at birth in comparison with the group with normal weight ( $p < 0.001$ ). Children with a light weight at birth had a higher frequency of D allele and the genotype DD as compared to the children with normal weight ( $p = 0.036$ ) (13).

The polymorphism of the gene AGTR1 A1166C was also identified as a potential genetic risk factor for AHT, overweight obesity and MS. Moreover, there is a limited amount of the investigations at children, but the results of the existent ones give

contradictory or inconclusive results, because most of them included small amount of children and there not informative enough to show association. Thus, a research that included 40 korean teenagers with hypertension stated the frequency of the genotype AA – 87.5%, AC – 12.5%, but the genotype CC was not detected (4). In our research the values of SBP were statistically significant higher ( $p < 0.001$ ) at the carriers of the genotype AGTR1 CC ( $153.00 \pm 4.11$  mmHg) and AGTR1 AC ( $130.52 \pm 3.41$  mmHg) as compared to the carriers of the genotype AGTR1 AA ( $123.50 \pm 6.11$  mmHg). Similar to our results were the results of another investigation that analyzed the polymorphism of the genes AGTR1 A/C at 250 patients with hypertension and 250 with normal tension and revealed a significant association between the genotypes AGTR1 (AC+CC) with AHT ( $\chi^2 = 22.48$ ;  $p = 0.0001$ ). The people with the genotypes CC had the chance 2.4 times higher ( $p = 0.001$ ) to have AHT developed as compared to the genotypes AC and AA. Statistically significant intergenotype variation was found at patients with CC ( $169.4 \pm 36.3$  mmHg) compared with the ones of genotypes AA ( $143.5 \pm 28.1$  mmHg) and AC ( $153.9 \pm 30.5$  mmHg) ( $p = 0.0001$ ) (5).

The polymorphism of one single nucleotide identified in the gene NOS<sub>3</sub> can be also associated with the increased risk of the realization of cardio-metabolic diseases, including AHT and MS (8). In a study that included 175 healthy children (control group), 110 with obesity and normal tension and 73 with obesity and hypertension the combination of the haplotypes of the variants 4b, C and Glu for the three polymorphism variants of endothelial synthase (eNOS) were more frequent at the patients with obesity and hypertension as compared to the ones with obesity and normal tension or the control group (14). In our research higher values of SAT were found at the carriers of NOS<sub>3</sub>GG ( $149.55 \pm 5.659$  mmHg) and NOS<sub>3</sub>AG ( $134.02 \pm 3.146$  mmHg) as compared to the NOS<sub>3</sub>AA ( $126.79 \pm 6.83$  mmHg), these differences being of statistics significance ( $p < 0.05$ ).

## CONCLUSIONS

Carrying genotypes ACE DD, AGTR1 CC and NOS<sub>3</sub> GG was associated with a higher degree of obesity (mainly, abdominal), AHT, hyperinsulinemia and metabolic disorders. Identification of the children with obesity/overweight carriers of these genotypes could facilitate early detection of the children with the risk of appearance of arterial hypertension and metabolic disorders to add to taking early measures for prophylaxis and treatment.

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