Association Between Angiotensin Converting Enzyme Gene Insertion (I)/Deletion (D) Polymorphism and Secondary Arterial Hypertension in a Romanian Children Population

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Introduction: Arterial hypertension is defined as systolic or diastolic blood pressure measurements higher than 95th age-gender-height percentile of the adopted reference values. Angiotensin-converting enzyme (ACE) is a component of renin-angiotensin system. ACE insertion/deletion (I/D) gene polymorphisms have been associated with the risk of various cardiovascular anomalies.

Aim: The purpose of our study was to assess the possible association of ACE I/D polymorphism gene and secondary hypertension in children.

Material and method: We genotyped 40 healthy and 38 hypertensive children and adolescents. The ACE I//D gene polymorphism was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism technique utilizing specific primers. We compared the distribution of ACE I/D genotypes in the two study groups.

Results: The results of the study showed that the frequency of I/D ACE genotype distribution in patients with hypertension (DD = 18.42%, ID = 68.42%, II = 13.16%) did differ significantly from genotype distribution in controls (DD = 47.5%, ID = 42.5%, II = 10%), and the DD genotype was not associated with secondary hypertension.

Conclusion: In conclusion we demonstrate that ACE gene polymorphisms are genetic markers for secondary arterial hypertension in children.

Keywords: ACE gene, polymorphism, children, hypertension.

Introduction

Arterial hypertension in children is defined as systolic and/or diastolic blood pressure based on repeated measurements (more than three occasions) above the 95th percentile for age, sex, and height, according to The National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents [1].

Hypertension is usually described as primary (essential) or secondary due to a definable cause. The secondary cause will be found more frequent when the patient is younger and hypertension is more severe. Most acute hypertension in childhood is due to glomerulonephritis and it is transitory. Chronic or persistent hypertension is commonly associated with renal parenchymal disease and only a small proportion has renovascular hypertension, pheochromocytoma or coarctation of the aorta [2].

The renin-angiotensin system (RAS) is one of the most important factors that affect the control of blood pressure. This system has been implicated in the pathological changes of organ damage through modulation of gene expression, proliferation and inflammatory response [3]. ACE is the key enzyme that converts inactive angiotensin I into a vasoactive and aldosterone-stimulating peptide angiotensin II. There are two forms of ACE in humans, encoded by a single gene located on chromosome 17 q23. It is a 21kb length gene and contains 26 exons and 25 introns [4]. The polymorphism consists of the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence resulting in 3 genotypes (DD and II homozygote, and ID heterozygote) [3].

The influence of ACE I/D polymorphism on pathophysiological conditions mediated through ACE activity has generated a lot of data showing its association with several diseases such as: diabetes mellitus [5,6], diabetic nephropathy [7], diabetic retinopathy, atherosclerosis, Alzheimer’s disease [8], nephrotic syndrome [9], progression of chronic renal failure in congenital renal malformation [10], cardiovascular anomalies [11,12].

The aim of our study is to investigate whether genetic polymorphisms in the insertion/deletion (I/D) polymorphism of the angiotensin-1 converting enzyme (ACE) gene are associated with secondary hypertension in children.

Materials and methods

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy from Targu Mures and has been performed in accordance with ethical standards of the declaration of Helsinki. Written informed consent was obtained from each subject’s parent after they were informed about the nature and the object of the study.

A total of 78 children were examined, 40 normotensive respectively 38 hypertensive children. There were 37 girls and 41 boys; age range from 2 months to 17 years. The subjects from control group were without cardiovascular or kidney disease and were comparable for gender and age with patient group.

Genomic DNA was extracted from whole blood using ZymoBead Genomic DNA Kit (ZymoResearch). Analyses of ACE I/D polymorphism were performed using the
polymmerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The sequences of the forward and reverse primers were: 5'-CTGGAGACCCTCCATCCATTTCT-3' and 5'-GATGTGGCCATCATTCGTCAGAT-3', respectively. DNA amplifications were performed with a Mastercycler Gradient Thermal Cycler (Eppendorf) with 5 min of denaturation at 94°C, followed by 30 cycles with denaturation for 1 min at 94°C, annealing for 1 min at 58°C, and extension for 2 min at 72°C, followed by 5 min of extension at 72°C. The PCR products were visualized on a 2% agarose gel.

Homozygosity for the D allele (DD) was identified by the presence of a single 190-bp PCR product, homozygosity for the I allele (II) was identified by the presence of a single 490-bp PCR product, and heterozygosity (ID) was identified by the presence of both 190- and 490-bp PCR products.

Chi square tests according to Pearson were performed to compare the frequency of ACE genotype between the groups. A p value <0.05 was considered significant. Allele frequencies were estimated by the gene counting method.

### Results

The frequencies of II, ID and DD genotype among the control group were 10% (n=4), 42.5% (n=17) and 47.5% (n=19) respectively, whereas, in hypertensive group the same genotypes were found to be 13.16% (n=5), 68.42% (n=26) and 18.42% (n=7) respectively. There is significant difference (p<0.05) observed in the distribution of ACE genotype polymorphism between the two groups.

### Discussion

This is the first study investigating the association of ACE I/D genetic polymorphism with hypertension in a sample of Romanian children patients.

There are few data in the literature about ACE I/D gene polymorphism in pediatric hypertension, most studies referring to essential hypertension.

Studies have demonstrated that ACE I/D gene polymorphism is not only associated with diabetes, coronary heart disease, and diabetic nephropathy, but have also demonstrated that ACE I/D polymorphism is associated with hypertension [5,6,7,13,14,15].

In the study conducted by Akra-Imail et al., ACE I/D polymorphism was not associated with hypertension as we found in our study; and age was found to be the most significant risk factor for hypertension and this was even more prominent when accounting for the D allele of the ACE genotype [16].

In our study ACE ID genotype was more frequent in hypertensive group, especially in females; while the DD genotype was more frequent in controls.
In contrast to our results, few studies have shown that hypertension is more prevalent in men when compared with women, and hence, investigators have adjusted for sex in their analysis. This is the case, for example, of Higaki et al. who showed a higher OR of the DD genotype in young men [17].

Several studies have shown a high prevalence of the DD genotype among patients with primary hypertension [3, 18], while in our study the DD genotype seems to be protective against hypertension regarding secondary causes of high blood pressure.

El-Mahdy et al. showed that ACE gene DD genotype had significantly higher frequency in patients with essential hypertension and was associated with higher blood pressure as compared to the other genotypes ID and II [19].

A significant association between ACE DD genetic polymorphism and hypertension was reported by Das et al. [20] in contrast to our results. However, this association was not found in a different study [21].

In our hypertensive group, both male and female are more associated with ID genotype as compared to other two genotype (II, DD). Sipahi et al found that the frequencies of the DD genotype in female with hypertension group were statistically significantly higher than that in female control group. Their study revealed a relationship between the ACE DD genotype and the development of primary hypertension in female population of Turkey [3], while in our study this genotype seems to be protective.

A case-control study in adults found that the DD genotype had 2.5-fold odds of hypertension compared to the homozygous for I allele (II genotype) group [22].

In the present case controlled study, we found an association between ACE ID genotype and secondary hypertension. The increased risk of secondary hypertension associated with ACE I allele was observed.

In a study conducted by Ismail et al. the frequency of the ACE I/I genotype was significantly higher in hypertensive patients as compared to the other genotypes ID and II [19].

Several studies have shown a high prevalence of the DD genotype in determining the risk of hypertension among the Romanian children. However, the data need validation in a large cohort study.

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References


The present study demonstrates a significant difference in the frequencies of ACE ID genotype and I allele between normal controls and children with secondary arterial hypertension, thereby supporting a role for ACE I/D polymorphism in determining the risk of hypertension among the Romanian children.


