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 95 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 chromosome17 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

Table I: Genotype and allele frequency of ACE I/D gene polymorphism in hypertensive and normotensive patients (control group)

	Hypertensive group	Control group
ACE I/D	n=38	n=40
Alleles		
I	36 (47.37%)	25 (31.25%)
D	40 (52.63%)	55 (68.75%)
Genotype		
II	5 (13.16%)	4 (10%)
ID	26 (68.42%)	17 (42.5%)
DD	7 (18.42%)	19(47.5%)

polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The sequences of the forward and reverse primers were: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT-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.

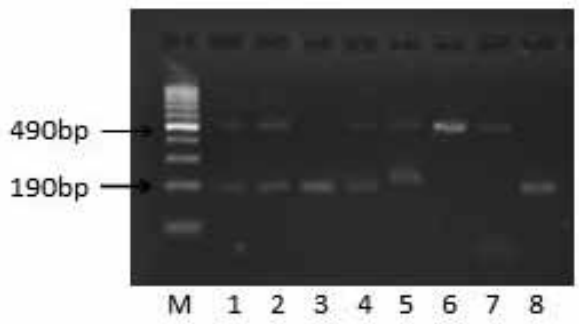

Fig. 1. Agarose gel electrophoresis of PCR products of angiotensin-converting enzyme gene. M: molecular marker 100 bp DNA ladder; lanes 6, 7: homozygous II cases; lanes 1, 2, 4, 5: hetrozygous ID and lanes 3, 8: homozygous DD case

Table II. Distribution of ACE genotypes between men and women

ACE genotypes	Hypertensive group		Control group	
	Female	Male	Female	Male
DD	2 (11.76 %)	5 (23.81%)	8 (40%)	11 (55%)
ID	13 (76.47%)	13 (61.90%)	11 (55%)	6 (30%)
II	2 (11.76 %)	3 (14.29%)	1(5%)	3 (15%)

The ACE genotype and allele frequencies distribution of control and hypertensive subjects are shown in table I. It has been observed that the ACE ID genotype was significantly (p<0.05) higher in hypertensive subjects, whereas, DD genotype was significantly (p<0.05) higher in control subjects.

The frequency of the I allele is also more frequent but not quite significant in hypertensive subjects than in controls (OR 1.980; 95% CI: 1.031–3.804; p=0.057).

The distribution of genotype frequencies associations of ACE gene polymorphisms between male and female among control and hypertensive subjects are given in Table II.

The results showed that among three genotypes within control group, DD genotype was more prevalent but not significantly in male as compared to other two genotypes (p>0.05), whereas, among female, ID genotype is comparatively more prevalent but not significantly differ (p>0.05).

In the hypertensive group, both male and female are more associated with ID genotype than compared to other two genotypes. The results showed that among three genotypes within hypertensive group, ID genotype was more prevalent in female as compared to other two genotypes (p<0.05).

Comparing the three genotypes between the two study groups we didn't find a significant difference regarding distribution in males, while in females we observed a significant difference in genotype distribution (p=0.028, Chi square 7.144; degree of freedom 2).

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 young patients, aged 20–40 years, than in normotensive controls [23].

Similar with our results two studies from Australia [24] and Pakistan [23] recorded the association of I allele with hypertension.

A different study had shown that I allele is associated with hypertension, insulin resistance, metabolic syndrome, mitral valve prolapse syndrome, and atrial fibrillation with hypertrophic cardiomyopathy, rheumatic heart disease [25,26,27].

There are also previous reports showing negative association between ACE genotypes and hypertension [28].

Conclusion

It is concluded that 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.

However, the data need validation in a large cohort study.

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References

1. The National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. Pediatrics 2004, 114: 555–576.
2. Sinaiko AR – Hypertension in Children. N Engl J Med 1996, 335: 1968–1973
3. Sipahi T, Budak M, Sen S, Ay A, Sener S – Association between ACE gene Insertion (I)/Deletion (D) polymorphism and primary hypertension in Turkish patients of Trakya region. Biotechnol & Biotechnol Eq 2006, 20(2):104–108.
4. Fahmy ME, FatTouh AM, Hegazy RA, Essawi ML – ACE gene polymorphisma in Egyptian children with idiopathic nephritic syndrome. Bratisl Lek Listy 2008, 109(7): 298–301.
5. Stephens JW, Dhamrait SS, Cooper JA, Acharya J, Miller GJ, Hurel SJ, Humphries SE – The D allele of the ACE I/D common gene variant is associated with type 2 diabetes mellitus in Caucasian subjects. Mol Genet Metab 2005, 84: 83–89.
6. Singh PP, Naz I, Gilmour A, Singh M, Mastana S – Association of APOE (Hha1) and ACE (I/D) gene polymorphisms with type 2 diabetes mellitus in North West India. Diabetes Res Clin Pract 2006, 74: 95–102.
7. Movva S, Alluri RV, Komandur S, Vattam K, Eppa K, Mukkavali KK, et al. – Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with type 2 diabetes mellitus in Asian Indians. J Diabetes Complications 2007, 21: 237–241.
8. Lehmann DJ, Cortina-Borja M, Warden DR, Smith AD, Sleegers K, Prince JA, et al. – Large meta-analysis establishes the ACE insertion-deletion polymorphism as a marker of Alzheimer's disease. Am J Epidemiol 2005, 162: 305–17.
9. Fahmy ME, Fattouh AM, Hegazy RA, Essawi ML – ACE gene polymorphism in Egyptian children with idiopathic nephrotic syndrome. Bratisl Lek Listy. 2008, 109(7): 298–301.
10. Hohenfellner K, Wingen AM, Nauroth O, Wühl E, Mehls O, Schaefer F – Impact of ACE I/D gene polymorphism on congenital renal malformationsPediatr Nephrol 2001, 16: 356–361.
11. Szadkowska A, Pietrzak I, Klich I, Młynarski W, Bodalska-Lipińska J, Bodalski J – Polymorphism I/D of the angiotensin-converting enzyme gene and disturbance of blood pressure in type 1 diabetic children and adolescents. Przegl Lek 2006, 63: 32–36.
12. Freitas SRS, Cabello PH, Moura-Neto RS, Dolinsky LC, Bóia MN – Combined Analysis of genetic and environmental factors on essential hypertension in a Brazilian rural population in the Amazon Region. Arq Bras Cardiol 2007, 88: 393–397.
13. Feng Y, Niu T, Xu X, Chen C, Li Q, Qian R, Wang G, Xu X – Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. Diabetes 2002, 51: 1986–1988.
14. Hsieh MC, Lin SR, Hsieh TJ, Hsu CH, Chen HC, Shin SJ, Tsai JH – Nephrol Dial Transplant 2000, 15: 1008–1013.
15. O'Donell CJ, Lindpainter K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Mayers RH, Levy D – Circulation 1998, 97: 1766–1772.
16. Akra-Ismail M, Makki R, Chmaisse H, Kazma A, Zgheib N – Association Between Angiotensin-Converting Enzyme Insertion/Deletion Genetic Polymorphism and Hypertension in a Sample of Lebanese Patients. Genetic Testing and Molecular Biomarkers 2010, 14(6): 787–792.
17. Higaki J, Baba S, Katsuya T, et al. – Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita Study. Circulation 2000, 101: 2060–2065.
18. Hsieh MC, Lin SR, Hsieh TJ, Hsu CH, Chen HC, Shin SJ, Tsai JH – Increased Frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes mellitus. Nephrol Dial Transplant 2000, 15: 1008–1013.

19. El-Mahdy MIM, Morsy MM – Angiotensin-converting enzyme gene polymorphism in patients with Essential hypertension. The Egyptian Journal of Hospital Medicine 2002, 9 : 65–73.
20. Das M, Pal S, Ghosh A – Angiotensin converting enzyme gene polymorphism (insertion/deletion) and hypertension in adult Asian Indians: a population-based study from Calcutta, India. Hum Biol 2008, 80: 303–312.
21. Nápoles OC, Castellanos MS, Leal L, Casalvilla R, Camacho H, Ferrer A, et al. – ACE I/D polymorphism study in a Cuban hypertensive population. Clin Chim Acta 2007, 378: 112–116.
22. Barbalic M, Skaric-Juric T, Cambien F, Barbaux S, Poirier O, Turek S, Vrhovski-Hebrang D, Cubrilo-Turek M, Rudan I, Rudan P, et al. – Gene polymorphisms of the renin-angiotensin system and early development of hypertension. Am J Hypertens 2006, 19(8): 837–842.
23. Ismail M, Akhtar N, Nasir M, Firasat S, Ayub Q, Khaliq S – Association between the Angiotensin-converting Enzyme Gene Insertion/Deletion polymorphism and essential Hypertension in Young Pakistani Patients. Journal of Biochemistry and Molecular Biology 2004, 37(5): 552–555.
24. Zee RY, Lou YK, Griffiths LR, Morrise BJ – Association of a polymorphism of angiotensin–I converting enzyme gene with essential hypertension. Biochem Biophys Res Commun 1992, 184: 9–15.
25. Chou HT, Chen YT, Shi YR, Tsai FJ – Association between angiotensin I-converting enzyme gene insertion/deletion polymorphism and mitral valve prolapse syndrome. Am Heart J 2003, 145: 169–173.
26. Ogimoto A, Hamada M, Nakura J, Miki T, Hiwada K – Relation between angiotensin-converting enzyme II genotype and atrial fibrillation in Japanese patients with hypertrophic cardiomyopathy. J Hum Genet 2002, 47: 184–189.
27. Chou HT, Tsai CH, Tsai FJ – Association between angiotensin I-converting enzyme gene insertion/deletion polymorphism and risk of rheumatic heart disease. Jpn Heart J 2004, 45(6): 949–57.
28. Companioni Nápoles O, Sautié Castellanos M, Leal L, Casalvilla R, Camacho H, et al. – ACE I/D polymorphism study in a Cuban hypertensive population. Clin Chim Acta 2007, 378(1–2): 112–116.w