

Angiotensin-converting enzyme gene polymorphisms in pulmonary arterial hypertension in children

Polimorfismul genei enzimei de conversie a angiotensinei în hipertensiunea arterială pulmonară la copil

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Abstract

Introduction. Angiotensin converting enzyme (ACE) plays an important role in the pathogenesis of pulmonary arterial hypertension. In this study we determined whether the deletion (D)/insertion (I) polymorphism in the ACE gene may be associated with pulmonary arterial hypertension in children. **Methods and Results.** We performed a case-control study involving 29 patients with pulmonary arterial hypertension and 26 healthy controls. Genomic DNA extracted from peripheral blood was amplified using the polymerase chain reaction to detect the polymorphic marker. In patients, ACE genotype distribution of DD, ID, and II was 34.5%, 58.6%, and 6.9%, respectively, whereas in controls it was 11.5%, 69.3%, and 19.2%, respectively. **Conclusion.** The ACE DD genotype is significantly increased in patients with pulmonary hypertension compared with normal controls, suggesting that certain individuals may be genetically predisposed to developing pulmonary hypertension.

Keywords: pulmonary hypertension, gene polymorphisms, angiotensin converting enzyme.

Rezumat

Introducere. Enzima de conversie a angiotensinei (ACE) joacă un rol important în patogeneza hipertensiunii arteriale pulmonare. În acest studiu am analizat posibila asociere a polimorfismului genei ACE deleție (D) / inserție (I) cu hipertensiunea arterială pulmonară la copii. **Metodă și rezultate.** Am efectuat un studiu caz-control cu 29 pacienți cu hipertensiune arterială pulmonară și 26 martori sănătoși. ADN-ul genomic extras din sângele periferic a fost amplificat prin reacția polimerizării în lanț pentru a detecta markerul polimorfic. Frecvența genotipului DD, ID, și II în gena ACE la pacienți a fost de 34.5%, 58.6%, respectiv 6.9%, iar la lotul control a fost de 11.5%, 69.3%, respectiv 19.2%. **Concluzii.** Genotipul DD în gena ACE este semnificativ crescut la pacienți comparativ cu lotul control, sugerând faptul că anumiți indivizi au o predispoziție genetică în dezvoltarea hipertensiunii pulmonare.

Cuvinte cheie : hipertensiune pulmonară, polimorfism genic, enzima de conversie a angiotensinei

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Introduction

Pulmonary arterial hypertension (PAH) consists of a group of vascular abnormalities with elevated pulmonary arterial pressure and pulmonary vascular resistance. PAH is defined as a mean pulmonary artery pressure of over 25mmHg at rest or over 30mmHg with exercise in the absence of increased left heart blood pressure. PAH is a life-threatening disease characterized by a progressive increase of pulmonary vascular resistance (PVR), which, if left untreated, progresses rapidly to right ventricular failure. Cardiac disorders, pulmonary disorders, or both in combination are the most common causes of secondary pulmonary hypertension. The current nosology of pulmonary hypertension (PH) has been provided by consensus of the Venice classification in 2003. There are five groups of PH arranged according to pathogenetic, clinical and therapeutic criteria. The first group (PAH) includes the idiopathic (IPAH), familial (FPAH) forms and PAH associated with different conditions such as congenital heart defects, portal hypertension, connective tissue disease and others that share similar clinical and hemodynamic aspects and comparable pathological changes of the lung microcirculation. The other four groups include the PH that develops secondary to left-heart diseases, hypoxic lung diseases, pulmonary embolism, and miscellaneous conditions. Moreover, the functional classification of PH is performed in agreement with classification of the New York Heart Association according to the World Health Organization (1998).

There are generally four main alterations in the walls of the pulmonary vessels. These include vasoconstriction, rarefaction of vessels, vascular remodeling and the occlusion of vascular lumen by a thrombus with subsequent structural remodeling of the vascular and extracellular matrix (1).

A lot of studies suggest that the renin-angiotensin system (RAS) has a fundamental role in the onset and progression of cardiovascu-

lar diseases and in regulating blood pressure (2). Genes encoding components of RAS are possible candidate genes for hypertension. Among the candidate genes of the RAS, the angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensinogen II type-1 receptor (AGTR1), and aldosterone synthase (CYP11B2) genes have been widely investigated as genetic determinants of hypertension (3 - 5).

Angiotensin converting enzyme (ACE) plays an important role in the pathogenesis of pulmonary arterial hypertension. ACE is a key enzyme in renin-angiotensin system (RAS) and widely distributed in human tissues including the lung, vascular endothelium, kidney and heart. ACE converts angiotensin I to angiotensin II, and inactivates bradykinin through the kallikrein-kininogen system. Angiotensin II may play a role in vascular diseases through vascular smooth muscle cell contraction and proliferation, monocyte adhesion, platelet aggregation, mediated either directly or via various factors such as endothelin, nitric oxide, and prostacyclin (6).

The ACE gene contains a polymorphism based on the presence (insertion, I) or absence (deletion, D) within an intron of a 287-bp non-sense DNA domain, resulting in three genotypes (DD and II homozygotes, and DI heterozygotes) (7).

To date, the ACE DD genotype has been associated with increased circulating and cardiac tissue ACE activity (8), the risk for coronary artery disease and myocardial infarction (9), the risk for either ischemic or idiopathic dilated cardiomyopathy (10), hypertrophic cardiomyopathy (11), and possibly the risk of left ventricular hypertrophy (2, 12). Since these syndromes are associated with abnormal vasoconstriction and vascular smooth muscle proliferation, Abraham et al. postulated a role for the RAS and the ACE genotype in the pathophysiology of pulmonary hypertension (PH) (2).

Also, Tanabe et al. identified this polymorphism as risk factor for chronic thrombo-embolic pulmonary hypertension (13). According to Oztürk et al. ACE gene I/D polymorphism D allele may modulate the relationship between an-

terior acute myocardial infarction and pulse pressure (14). Another study suggests that I/D ACE gene polymorphism is linked to pulmonary artery pressure in Caucasian patients with chronic obstructive pulmonary disease (15).

Although there are some studies which reported a positive association between allele and coronary artery disease susceptibility (9), this result was not replicated in a recent study by Shafiee SM et al. The study showed that DD genotype does not increase the coronary artery disease susceptibility in the studied population and may not be a risk factor (16).

The relation between ACE gene polymorphism and pulmonary arterial hypertension in children has not been previously reported. Therefore, the objective of this study was to investigate the relation between ACE gene polymorphism and pulmonary arterial hypertension in children.

Methods

Subjects

The study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy Tg. Mures and Institute of Cardiovascular Diseases Tg. Mures. Written informed consent was obtained from each subject's parent included in the study.

The study included 55 children: 26 healthy individuals as a control group and 29 patients with pulmonary hypertension. Among them, 2 patients were diagnosed with idiopathic pulmonary arterial hypertension (IPAH) and 27 with PAH associated with CHD. They were included in NYHA functional classes: 8 patients pertained to the NYHA II class, 16 patients to the NYHA III class, and 5 patients to the NYHA IV class.

The patients were evaluated by echocardiography, using an IE33 Philips echocardiograph. The echocardiographic technique to evaluate the pulmonary hypertension consisted in: indirect assessment of pulmonary arterial hypertension, measurement of pulmonary artery pressure from tricuspid valve regurgitation and from pulmonary regurgitation, the impact of

pulmonary arterial hypertension on the right ventricle. Estimation of right atrial pressure is most often done by assessing the IVC diameter and its degree of collapse with inspiration. Tricuspid annular plane systolic excursion (TAPSE) was used to evaluate right ventricular longitudinal function. This parameter is measured using an M-mode cursor passed through the tricuspid lateral annulus in a four – chamber view. This parameter measures the extent of systolic motion of the lateral portion of the tricuspid ring towards the apex. The tricuspid inflow pattern was used to evaluate the right ventricular diastolic function. The tricuspid inflow pattern is obtained in a four- chamber view by placing a cursor at the tips of the tricuspid valve. The Tei index, which measures global ventricular function, has been used to assess the impact of increased pulmonary artery pressure. It is calculated as the ratio between the sum of the times of the isovolumetric periods and the ejection time for the right ventricle.

Genotyping

Genomic DNA was extracted from peripheral blood as described previously (7). Briefly, genomic DNA was extracted from whole blood containing ethylenediamine-tetraacetic acid (EDTA) as an anticoagulant, by using the Genomic DNA Purification Kit (ZymoResearch). The sequences of the forward and reverse primers used were: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGCCATCACATTGGTCAGAT-3' (Fermentas). 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.

Detection of ACE Polymorphism by Electrophoresis

The reaction products were separated by electrophoresis in 2% agarose gels and stained with ethidium bromide. Under ultraviolet light

two bands, insertion (I allele; 490 bp) and deletion (D allele; 190 bp) were visible (*Figure 1*).

Statistical analysis

Data analysis was performed using the statistical analysis software system SPSS.15. Chi square tests according to Pearson were performed to compare the frequency of ACE genotype between patients and control groups. A p value less than 0.05 was considered significant. Allele frequencies were estimated by the gene counting method.

Results

Since no reports are available regarding the association between ACE I/D polymorphism and pulmonary hypertension in children in Romania, the present case control study examined the possible association of ACE I/D polymorphism in the pulmonary hypertensive patients.

The patient group comprised 15 boys and 14 girls. The mean age of the patients was 10.83 years. In the control group there were 12 girls and 14 boys. The mean age of the group was 12.1 years.

The frequencies of II and ID genotypes of ACE gene polymorphism were not significantly different between patients and controls. ACE-DD genotype was significantly higher in patients compared with controls (0.046). The frequency of I/D heterozygotes as compared to homozygotes, was higher both in the patient and control group (*Table 1*). Both groups were in Hardy-Weinberg equilibrium.

We have also analyzed the association of all the three genotypes with the phenotypic

variables such as gender and severity of pulmonary hypertension. Genotype frequencies were not different between NYHA functional classes (*Table 2*) and we did not find a significant difference between boys and girls.

As summarized in *Table 2*, I allele frequencies analysis revealed a significant difference between the two groups ($p=0.046$).

Discussion

Pulmonary arterial hypertension (PAH) is a debilitating chronic disease of the small pulmonary arteries that is characterized by vasoconstriction and vascular remodeling. Endothelial dysfunction is believed to occur early in disease pathogenesis, leading to endothelial and smooth muscle cell proliferation and structural changes or remodeling of the pulmonary vascular bed resulting in an increase in pulmonary vascular resistance (17). The renin-angiotensin system components appear to play an important role in the pathogenesis of pulmonary hypertension (18).

Angiotensin-converting enzyme (ACE), the key enzyme mainly responsible for the conversion of angiotensin I to angiotensin II, is believed to contribute to the development of pulmonary hypertension, because angiotensin II stimulates the growth and proliferation of human vascular smooth muscle cells (19). ACE expression was found to be elevated in patients with pulmonary hypertension (20).

The expression of ACE and angiotensin II are partially genetically determined, with the D allele of ACE associated with higher blood ACE levels and activity (19).

We determined the prevalence of insertion/deletion (I/D) polymorphism of the ACE gene in children with pulmonary hypertension. ACE DD genotype was significantly higher in patients compared with controls. Our findings in this study suggest that patients with DD genotype may have a greater increase in tissue ACE activity than those with the II and ID genotypes. The increased local production of ACE could contribute to elevated pulmonary vascu-

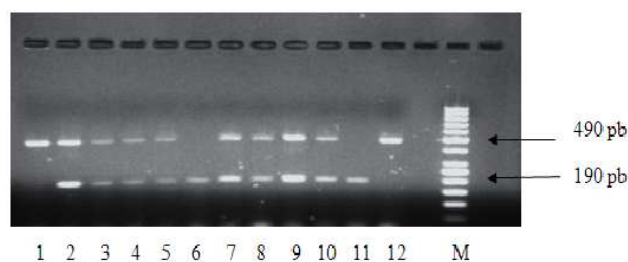


Figure 1. Agarose gel electrophoresis of PCR products of angiotensin-converting enzyme gene. M: marker, lanes 1,12: homozygous II cases, lanes 2-5, 7-10 heterozygous ID and lane 6,11: homozygous DD case

lar tone and pulmonary vascular remodeling by increasing the local production of angiotensin II, because angiotensin II is known to stimulate the growth of human vascular smooth muscle cells. The association between the D allele and pulmonary hypertension in patients suggests a potential role for renin-angiotensin system in the pathogenesis of PH.

There are a few reports that have found an association between the D allele and pulmonary hypertension. Kanazawa H et al suggested that I/D polymorphism in the ACE gene may be associated with pulmonary hypertension evoked by exercise challenge in patients with chronic obstructive pulmonary disease (21). Abraham et al. demonstrated an increased incidence of the ACE DD genotype in patients with primary pulmonary hypertension, and an association of the ACE DD genotype with preserved right ventricular function in these patients (2). Solari et al. suggested that D

allele of the ACE gene insertion/deletion polymorphism may be associated with primary pulmonary hypertension in newborns with congenital diaphragmatic hernia (19). In contrast to the aforementioned studies, Hoepfer et al. found no evidence for an association between ACE genotype and ACE serum activity, respectively, and right ventricular performance in patients with primary pulmonary hypertension (22).

The present investigation provides evidence of a role for the RAS in the pathogenesis of pulmonary hypertension, by demonstrating a high incidence of the ACE DD genotype in PAH patients. The frequency of the ACE DD genotype in our patients is smaller (34.5%) than that reported by Abraham et al (45%) (2).

In our study, the incidence of I allele and II genotype was higher in controls than in children with pulmonary hypertension. These findings may suggest that the I allele and II genotype of the ACE gene could be protective against PAH in children.

To our knowledge, this is the first study that evaluates the association of ACE I/D polymorphism with pulmonary hypertension in children in Romania. The number of patients in this study was relatively small for a genetic association study, and our results should be enlarged upon in wider future studies.

In conclusion, our results showed that pulmonary hypertension is associated with a higher incidence of DD genotype.

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Table 1. Distribution of ACE genotype and allele frequencies in patients and controls

	<i>Patients</i> <i>n=29 (%)</i>	<i>Controls</i> <i>n=26 (%)</i>	<i>p value</i>
<i>Genotype frequency</i>			
<i>II</i>	2 (6.9)	5 (19.2)	0.426
<i>ID</i>	17 (58.6)	18 (69.3)	0.414
<i>DD</i>	10 (34.5)	3 (11.5)	0.046
<i>Allele frequency</i>			
<i>I</i>	19 (39.6)	23 (52.3)	0.171
<i>D</i>	29 (60.4)	21 (47.7)	0.046

ACE- angiotensin converting enzyme; I- Insertion; D- deletion

Table 2. Frequency of Genotypes of ACE-I/D polymorphism according to NYHA functional class in PH

	<i>ACE genotype</i>		
	<i>II</i>	<i>ID</i>	<i>DD</i>
<i>NYHA I</i> <i>n= 0 (%)</i>	0 (0)	0 (0)	0 (0)
<i>NYHA II</i> <i>n= 7 (%)</i>	1 (14.3)	5 (71.4)	1(14.3)
<i>NYHA III</i> <i>n= 16 (%)</i>	1 (6.3)	7 (43.7)	8 (50)
<i>NYHA IV</i> <i>n= 4 (%)</i>	0 (0)	3 (75)	1(25)

NYHA - New York Heart Association functional class

Abbreviations

ACE= angiotensin converting enzyme
 I= insertion
 D= deletion
 DNA= deoxyribonucleic acid
 NYHA = New York Heart Association functional class
 PCR= polymerase chain reaction
 PAH= pulmonary arterial hypertension
 RAS= renin-angiotensin system
 bp= base pairs

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