Angiotensin-converting enzyme DD genotype in patients with primary pulmonary hypertension: increased frequency and association with preserved haemodynamics

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Hypothesis/introduction
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Methods and results
The incidence of the ACE DD genotype was evaluated in 60 patients with severe PPH compared with two normal control populations, a group of healthy population-based controls (n=158) and subjects found suitable for cardiac organ donation (n=79). Genomic DNA extracted from peripheral leukocytes was amplified using the polymerase chain reaction to detect polymorphic markers. Haemodynamics were determined by right heart catheterisation in a subset of the PPH patients. The frequency of the ACE DD genotype was 45% in the patients with PPH, compared with 24% in the organ donors, and 28% in population-based healthy controls (p<0.01 for chi-square test). Of the 32 PPH patients with baseline haemodynamics, 12 exhibited the ACE DD genotype and 20 were non-DD. While the mean pulmonary artery pressure and the duration of symptoms were similar between the DD and non-DD groups, cardiac output was significantly lower (3.29±0.27 vs 5.07±0.37 l/minute, p=0.002) and the mean right atrial pressure tended to be higher (8.85±1.29 vs 4.92±1.27 mmHg, p=0.08) in the non-DD group. The reduction in cardiac output seen in the non-DD group was not due to a difference in heart rate, but to a significant reduction in stroke volume, consistent with a decreased contractile state. In addition, non-DD patients exhibited a significantly worse functional capacity (NYHA Class 3.14±0.12 vs 2.40±0.28, p=0.02).

Conclusions
1) The ACE DD genotype is significantly increased in patients with severe PPH compared with normal controls, suggesting that certain individuals may be genetically predisposed to developing pulmonary hypertension. 2) The ACE DD genotype is associated with preserved right ventricular function in PPH patients, supporting a compensatory myocardial or inotropic role for Ang II in the pressure overloaded right ventricle.

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Materials and methods

Study populations

Three study populations were examined: 1) Patients with severe PPH referred to the Pulmonary Hypertension Program at the University of Colorado Health Sciences Center (secondary pulmonary hypertension was excluded in all cases), 2) brain-dead subjects determined suitable for cardiac organ donation on the basis of normal ventricular function and the absence of coronary artery disease, and 3) population-based controls from a local corporate-sponsored screening programme. The assessment of ACE genotype in these subjects was approved by the Institutional Review Board of the University of Colorado, and written informed consent was obtained from the subjects screened. In PPH patients, the average age (±SD) was 37±11 years, and there were 50 women and 10 men.

Genomic DNA preparation

Genomic DNA was extracted from peripheral blood as described previously. Briefly, genomic DNA was extracted from leukocytes in dried blood spots collected on filter paper. Spots were excised using sterile biopsy punches, placed into microfuge tubes, and incubated at 56°C for 48 hours in lysis buffer (154 mM NaCl, 10 mM Tris-HCl pH 7.5, 1 mM Na EDTA pH 8.0, 5% proteinase K, 1% SDS). Proteins were removed by sequential phenylchloroform, and chloroform extraction. DNA was precipitated from the aqueous phase by addition of 1/10th volume M sodium acetate (pH 5.0) and an equal volume of ice-cold isopropanol. Samples were held at -20°C overnight, and genomic DNA was collected by centrifugation.

Polymerase chain reaction amplification of the ACE polymorphism

The polymorphic region of the ACE exon 16, consisting of the present (I) or absence (D) of a 287 base pair (bp) alu repeat sequence, was amplified from genomic DNA by polymerase chain reaction (PCR). The oligonucleotide primers used in the reaction were as previously published. Approximately 500 ng to 1000 ng of genomic DNA was used per reaction, and the reaction conditions were as previously published. The ACE genotype of each study participant was scored based on resolution of PCR products by gel electrophoresis. Amplification of the D allele resulted in a 190 bp DNA fragment and amplification of the I allele resulted in a 490 bp fragment. Homozygotes had a single 190 (DD) or 490 (II) bp band. Random replication of PCR amplification was used to verify all of the results.

Assessment of haemodynamics

Thirty-two of the 60 patients with PPH underwent baseline assessment of right heart haemodynamics prior to pharmacological treatment. Briefly, a 7 French, balloon-tipped, flow-directed, thermodilution catheter was advanced into the pulmonary artery via either a right internal jugular or femoral vein, using pressure waveform and fluoroscopic guidance. Standard right heart haemodynamics were determined, including right atrial pressure, pulmonary artery systolic pressure, pulmonary artery diastolic pressure, pulmonary capillary wedge pressure, and cardiac output was determined by the thermodilution method. Pulmonary artery mean pressure, pulmonary vascular resistance and stroke volume were calculated using standard formulae. In addition, the duration of symptoms attributable to pulmonary hypertension (n=23) and the severity of symptoms at the time of catheterisation (n=24) were determined.

Statistical analysis

Data analysis was performed using the statistical analysis software system (SAS Institute, Cary, NC). Unless otherwise specified, data are presented as mean±SEM. Statistical significance was taken as two-sided p<0.05. Comparison of the distribution of ACE genotype across populations was by chi-square test. Within the PPH population, comparison of haemodynamic variables across DD and non-DD groups was by ordinary t-test and, in the case of unequal variances, Wilcoxon Rank Sum test. The general linear model was used to control the effects of age, gender, and ethnicity (white, Hispanic, and black). All analyses were pre-planned, precluding the need for multiple comparison procedures.

Results

ACE genotype

The distribution of ACE genotype by study population is shown in Table 1 and in Figure 1. The frequency of the ACE DD genotype was 45% in the
patients with PPH, compared with 24% in the
organ donors, and 28% in population-based
healthy controls (p=0.01 for chi-square test).

Haemodynamic results
Mean right heart haemodynamics, duration of
symptoms, and severity of disease according to
the NYHA Functional Classification are shown in
Table 2 and selected haemodynamic variables are
also shown in Figure 2. Of the 32 PPH patients
with baseline haemodynamics, 12 exhibited the
ACE DD genotype and 20 were non-DD. As shown
in Table 2, the mean pulmonary artery pressure
was similar in the DD and non-DD groups.
Moreover, the duration of symptoms attributable
to pulmonary hypertension was not different
between the two groups. Despite this, the cardiac
output was significantly lower in the non-DD
patients (3.29±0.27 vs. 5.07±0.37 L/minute,
p=0.002) and the mean right atrial pressure
tended to be higher in non-DD patients
(8.85±1.29 vs. 5.46±1.96 mmHg, p=0.08) (see
Figure 2). Fourteen of 20 patients in the non-DD
group had a mean right atrial pressure greater than
5 mmHg, but only 3 of 12 patients in the DD group
exhibited such an elevation of right atrial pressure.

The reduction in cardiac output seen in the
non-DD group was not due to a difference in heart
rate (80±3 vs. 83±4, p=NS), but to a reduction in
stroke volume (see Table 2), consistent with a
decreased contractile state. This was associated
with significantly worse functional capacity in the
non-DD group (NYHA Class 3.14±0.12 vs.
2.40±0.28, p=0.02).

Discussion
The RAS may be involved at a fundamental level in
the pathogenesis of a variety of forms of cardio-
vascular disease.1-15 The present investigation pro-
vides evidence of a role for the RAS in the patho-
genesis of PPH, by demonstrating a remarkably
high incidence of the ACE DD genotype in PPH
patients. The 45% incidence of the ACE DD geno-
type in these patients is higher than that reported
for any other group of subjects examined to date
in the published literature.1-15 An association
between the ACE DD genotype and PPH is
perhaps not surprising, given the known propen-
sity of Ang II to induce vasoconstriction and vas-
cular smooth muscle cell proliferation.16 A patho-
genic role for the RAS in PPH is also supported by
the observation that ACE inhibition reduces pul-
monary vascular resistance in some patients with
severe pulmonary hypertension.17-19
In 1983, Schmengler and Stumpe evaluated two groups of pulmonary hypertensive patients treated with an ACE inhibitor (ACE-I). One group of nine patients had precapillary pulmonary hypertension, while the second group consisted of patients with valvular heart disease. In each group of patients, a significant reduction of pulmonary vascular resistance was seen following the acute administration of 50 mg of the ACE-I, captopril. Niarchos et al. demonstrated a similar effect of a single-dose of captopril in patients with chronic pulmonary hypertension secondary to collagen vascular disease. Moreover, in one patient with severe pulmonary hypertension reported by our group, ACE inhibition resulted in a marked and sustained decrease in pulmonary artery pressures, while prior treatment with the vasodilator, hydralazine, did not.

The above observations demonstrate an association between the ACE DD genotype and the incidence of PPH and a pathogenic role for RAS activation in this disease. However, the present results also suggest that the ACE DD genotype is associated with preserved right ventricular function in PPH patients. Specifically, the ACE DD genotype was not related to pulmonary artery pressures in these patients, since both DD and non-DD patients exhibited a mean pulmonary artery pressure of about 55 mmHg. Significantly, cardiac output was substantially higher in the ACE DD group, related to a significantly higher stroke volume. This observation suggests a compensatory myocardial or inotropic role for Ang II in severe PPH, which is not surprising, perhaps, given the known effects of Ang II on stimulation of myocardial cell hypertrophy and ventricular remodelling.

In summary, the present results demonstrate for the first time the association between a genetic polymorphism and the incidence of PPH. Specifically, the data show an increased incidence of the ACE DD genotype in patients with PPH, and an association of the ACE DD genotype with preserved right ventricular function in these patients. These observations support a pathogenic role for the RAS in PPH and suggest a compensatory role for Ang II in the pressure-overloaded right ventricle.

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References

Lack of association between angiotensin converting enzyme (ACE) genotype, serum ACE activity, and haemodynamics in patients with primary pulmonary hypertension

M M Hoeper, A Tacacs, U Stellmacher, R Lichtinghagen

Primary pulmonary hypertension (PPH) is caused by progressive obliteration of the pulmonary vascular bed that leads to a right ventricular adaptive response—that is, right ventricular hypertrophy and dilatation—and eventually results in right heart failure. In patients with PPH, low cardiac output and elevated right atrial pressures are indicators of poor survival and both variables are closely related to right ventricular systolic and diastolic performance. It is unknown why some patients seem to adapt quite well to elevated pulmonary vascular resistances while others develop right heart failure early in the course of their disease. Apparently, adaptation of the right heart varies substantially among individuals. The mechanisms involved in the right ventricular response to elevated pulmonary vascular resistance are unknown. Earlier studies have suggested that the angiotensin converting enzyme (ACE) genotype may be closely related to right heart function in patients with pulmonary hypertension. The cloning of the ACE gene has led to the identification of a deletion (D)-insertion (I) polymorphism that affects the level of serum and tissue ACE activity. The D/D genotype is associated with the highest ACE level of activity and has been linked to left ventricular hypertrophy. This association has been attributed to increased formation of angiotensin II. However, other investigators were unable to duplicate these results.

To the best of our knowledge, only one study has yet addressed ACE activity and cardiac function in patients with pulmonary hypertension. In 20 patients with PPH, Abraham and colleagues found the D/D genotype to be more common (50%) than in the normal population (23%). More importantly, it appeared that patients who carried the D/D genotype had a more preserved cardiac function than those with the I/D or I/I genotype, suggesting that increased ACE activity might permit a greater hypertrophic adaptation of the pressure overloaded right ventricle. Despite the potential therapeutic implications of these findings, there have been no studies addressing this issue in a larger number of patients. We therefore initiated a clinical study to compare haemodynamic variables with ACE serum activity and ACE genotype in a larger group of patients with PPH.

METHODS
We studied 51 consecutive patients (38 women and 13 men) with PPH who underwent diagnostic evaluation including right heart catheter studies at our institution. The diagnosis of PPH was established in accordance with the World Health Organization criteria. All patients suffered from New York Heart Association (NYHA) functional class III or IV disease. None of them was treated with ACE inhibitors. For the purpose of this investigation, venous blood samples were obtained during the catheter studies for ACE genotyping and assessment of ACE serum activity. Serum ACE concentrations were measured utilising a COBA MIRA automated system (Roche Diagnostics, Mannheim, Germany) and commercially available ACE reagents (Sigma Diagnostics, St Louis, Missouri, USA). ACE I/D polymorphism was determined by polymerase chain reaction (PCR) and conventional separation of amplified DNA by gel electrophoresis. This protocol has been approved by the ethics committee of Hannover Medical School and all patients gave informed consent. The data were compared with those from 200 healthy blood donors studied at our centre.

The results are expressed as mean (SD). The Wilcoxon rank sum test was used to compare haemodynamic variables in patients with the I/I genotype and those with the I/D or D/D genotype. Pearson’s bivariate correlation analysis was performed to compare haemodynamic variables and ACE serum activity. The significance level was set at p < 0.05.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>ACE genotype and hemodynamic variables in 51 patients with primary pulmonary hypertension</th>
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<tr>
<td>ACE genotype</td>
<td>D/D mean (SD) n=5</td>
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<tr>
<td>Serum ACE (U/l)</td>
<td>45 (26)</td>
</tr>
<tr>
<td>RA (mm Hg)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>PAPm (mm Hg)</td>
<td>49 (9)</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>1.7 (0.4)</td>
</tr>
<tr>
<td>PVR (dynes*cm⁻⁵)</td>
<td>1.008 (514)</td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; CI, cardiac index; I/D, insertion/deletion; NYHA, New York Heart Association; PCR, polymerase chain reaction; PPH, primary pulmonary hypertension.
RESULTS
In a normal population of 200 healthy blood donors studied at our centre, the I/I genotype was found in 25%, the I/D genotype in 50%, and the D/D genotype in 25%. In this control group the 95% confidence intervals for serum ACE activities were 7 to 33 U/l in the I/I group, 13 to 66 U/l in the I/D group, and 24 to 89 U/l in the D/D group (data not shown). In our group of 51 PPH patients the I/I genotype was found in 31%, the I/D genotype in 59%, and the D/D genotype in 10%. As shown in table 1, serum ACE activities were higher in patients with the I/D and D/D genotype compared with the I/I genotype. However, there were no differences between these genotypes with respect to haemodynamic variables and indices of right ventricular performance (table 1). In addition, our data did not reveal any significant correlations between serum ACE activity and right atrial pressure ($r = 0.43$), mean pulmonary arterial pressure ($r = 0.25$), cardiac index ($r = 0.01$), and pulmonary vascular resistance ($r = 0.18$).

DISCUSSION
In contrast to a previous study by Abraham and colleagues, we found no evidence for an association between ACE genotype and ACE serum activity, respectively, and right ventricular performance in patients with PPH. In our study, there were no significant differences in mean pulmonary artery pressure, right atrial pressure, cardiac index or pulmonary vascular resistance between patients with the I/I, I/D, or D/D genotype. However, there were no differences between these genotypes with respect to haemodynamic variables and indices of right ventricular performance (table 1). In addition, our data did not reveal any significant correlations between serum ACE activity and right atrial pressure ($r = 0.43$), mean pulmonary arterial pressure ($r = 0.25$), cardiac index ($r = 0.01$), and pulmonary vascular resistance ($r = 0.18$).

Despite these limitations, our data do not support the hypothesis that ACE activity plays a major role in adaptation of the pressure overloaded right ventricle. Further studies should address other mechanisms that may affect cardiac function in patients with PPH.

REFERENCES
ABSTRACT

Objective: Evidence shows that an elevated pulse pressure (PP) may lead to an increased risk of cardiovascular morbidity and mortality. The aim of the present study was to determine the effects of polymorphism of the angiotensin-converting enzyme (ACE) gene on the PP after a first anterior acute myocardial infarction (AMI).

Methods: Overall 116 patients with a first anterior AMI were included in this cross-sectional study. DNA was isolated from peripheral leukocytes. The ID status was determined by polymerase chain reaction by a laboratory staff member who was unaware of the clinical details. Based on the polymorphism of the ACE gene, they were classified into 3 groups: Deletion/Deletion (DD) genotype (Group 1, n=45), Insertion/Deletion (ID) genotype (Group 2, n=58), Insertion/Insertion (II) genotype (Group 3, n=13). Blood pressure measurements were performed in all patients within 10 minutes admitted to coronary care unit. The PP was calculated by subtraction of diastolic blood pressure (DBP) from systolic blood pressure (SBP). Echocardiographic examinations were performed using the parasternal longitudinal axis and apical 4-chamber windows in accordance with the recommendations of the American Echocardiography Committee. One-way analysis of variance (ANOVA) and Chi-square analyses were used to compare differences among subjects with different genotypes.

Results: There were no significant differences among clinical parameters of patients. Pulse pressure was significantly higher in patients who have ACE DD and ID genotypes than in patients who have ACE II genotype (47±16, 47±14 and 39±12, F=3.4, p<0.05). But SBP, DBP and heart rate were not significantly different among ACE DD, ACE ID and ACE II genotypes.

Conclusion: Our results suggested that, ACE Gene I/D polymorphism D allele may affect PP in patients with a first anterior AMI.

(Anadolu Kardiyol Derg 2009; 9: 9-14)

Key words: Angiotensin converting enzyme, gene, polymorphism, pulse pressure, myocardial infarction

ÖZET

Amaç: Kan basıncındaki artış kardiyovasküler morbidite ve mortalite riskinde artışa yol açtığı göstermektedir. Bu çalışmamız amacı, ilk kez anteriyor akut miyokard infarktüs geçiren hastalarda, nabız basıncı üzerinde anjiyotensin dönüştürücü enzim (ACE) gen polimorfizminin etkilerini belirlemekti.

Yöntemler: Bu enine kesitli çalışmaya ilk kez anteriyor akut miyokard infarktüs geçiren 116 hasta alındı. DNA periferik lökositlerden izole edildi. ID durumu, klinik bulgularından habersiz laboratuvar üyesi tarafından, polimeraz zincir reaksiyonuya belirlendi. ACE gen polimorfizmine göre hastalar 3 gruba ayrıldı. Delesyon/Delesyon (DD) Genotip (Grup 1, n=45), Insertsiyon/Delesyon (ID) Genotip (Grup 2, n=58), Insertsiyon/Insertsiyon (II) Genotip (Grup 3, n=13). Kan basıncı ölçümleri hastalar koroner yoğun bakım ünitesine yatırılduktan sonraki ilk 10 dk içerisinde ölçülüldü. Nabız basıncı, sistolik kan basıncından diyastolik kan basıncının çıkarması ile elde edildi. Ekokardiyografik inceleme Amerikan Ekokardiyografi Komitesi önerilerine uygun olarak, parasternal uzun aks ve apikal dört boy Aşırı penceler kullanılarak yapıldı. Farklı genotiplere sahip örnekler arasından farklılık kirlaştırılmak için ANOVA ve Ki-kare testleri kullanıldı.

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Bulgular: Klinik özellikler bakımından hastalar arasında fark yoktu. Nabız basıncı ACE DD ve ID genotipli hastalarda ACE II genotipli hastalara göre anlamlı derecede yüksek bulundu (sırası ile 47±16, 47±14 ve 39±12, F=3.4, p<0.05). Fakat sistolik kan basınç, diastolik kan basınç ve kalp hızı bakımından gruplar arasında anlamlı fark saptanmadı.

Sonuç: Yapmış olduğumuz bu çalışmada ACE Gen I/D polymorfizmi D alleli ilk kez anteriyor akut miyokard infarktüsü geçiren hastalarda nabız basıncını etkilebilebileceği gösterildi. (Anadolu Kardiyol Derg 2009; 9:9-14)

Anahtar kelimeler: Anjiyotenzin dönüştürücü enzim, gen, polymorfizm, nabız basıncı, miyokard infarktüsü

Introduction

Cardiovascular (CV) mortality is not only positively correlated to the level of systolic blood pressure (SBP), but also at any given value of SBP, CV mortality is higher when diastolic blood pressure (DBP) is lower, particularly for subjects over 60 years of age (1). In recent years, evidence has accumulated that increased pulse pressure (PP) predicts CV mortality and coronary artery disease, myocardial infarction (MI) and congestive heart failure, independent of DBP and SBP, and other risk markers (2, 3).

Pulse pressure (PP), defined as the difference between the arterial SBP and DBP, is determined both by cardiac and vascular factors. Pulse pressure has been found to be a heritable trait and there is evidence that genetic factors influence the interindividual variation in PP. Genetic factors play a greater role in determining PP (4, 5).

Previous research findings on PP suggest that: (1) PP is an important predictor of CV disease and mortality, (2) genes appear to influence PP and (3) gender may be an effect modifier in the relation between PP and CV disease and mortality (5). In 1991, Hubert et al. (6) reported that the human angiotensin-converting enzyme (ACE) gene is localized in the long arm of chromosome 17 and that it consists of 26 exons and 25 introns. There is an I/D polymorphism in the Alu-like arrangement at intron 16 and this polymorphism correlates with the blood ACE concentration. The ACE gene explains 30-50% of its variance (6, 7). The ACE DD genotype has been associated with an increased risk of high blood pressure and MI in some studies, though this association is not consistently present (8, 9).

The relation between ACE gene polymorphism and PP in patients with a first anterior acute myocardial infarction (AMI) has not been reported previously.

Therefore, the objective of this study was to investigate the relation between ACE gene polymorphism and PP in patients with a first anterior AMI.

Methods

Subjects

This cross-sectional study included 125 consecutive patients (100 men, 25 women) who were admitted to the coronary care unit with anterior AMI, defined as (1) creatine kinase (CK) ≥210 IU/L and CK-MB ≥20 IU/L or (2) electrocardiographic evidence of MI (ST elevation ≥1 mm), and (3) typical chest pain. Patient’s mean age was 59±12 years. All patients were in sinus rhythm. Exclusion criteria were valvular heart diseases, atrial fibrillation, old MI, previous antihypertensive treatment, inadequate Doppler recordings and chronic obstructive pulmonary disease. Nine patients were excluded because of aortic stenosis (n=1), atrial fibrillation (n=2), old MI (n=3), previous antihypertensive treatment (n=2) and inadequate Doppler recordings (n=1), leaving a total of 116 patients. Based on the results of ACE gene polymorphism analysis, the patients were classified into 3 groups: group 1 (n=45)-DD genotype; group 2 (n=58)-ID genotype; group 3 (n=13)-II genotype. Patient characteristics are summarized in Table 1. The study protocol was approved by the ethics committee of our institution and informed consent was obtained from all patients.

Blood Pressure Measurement

Blood pressure measurements were performed in all patients within 10 minutes admittance to coronary care unit. Blood pressure was measured with a mercury sphygmomanometer after 5 minutes of rest, as recommended by the 6th Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (10). Patients were seated with their arm bared and supported at heart level. Two readings, separated by 2 minutes, were obtained and averaged. Additional readings were obtained if these readings differed by >5 mm Hg. Pulse pressure was calculated by subtraction of diastolic blood pressure from systolic blood pressure. Mean blood pressure was calculated by the addition of two thirds of the PP to diastolic blood pressure. Body mass index was calculated by dividing the weight in kilograms by the square of the height in meters.

Treatment

Patient’s blood pressure was measured after admittance to coronary care unit and then all patients were treated with thrombolytic therapy (streptokinase 1.5 million units/30 min or tissue type plasminogen activator 100 mg according to the accelerated protocol), acetylsalicylic acid-100, β-blocker (metoprolol 50-100 mg po) and intravenous nitroglycerin. The ACE inhibitor (cilazapril 2.5-5 mg) or angiotensin-receptor blocker (valsartan 80-160 mg) was added to the treatment in the first 24 h, if there was no contraindication. Patients who have antihypertensive treatment at time of MI were excluded from the study.

DNA Analysis

DNA was isolated from peripheral leukocytes by the method described previously (11). The ID status was determined by polymerase chain reaction by a laboratory staff member who was unaware of the clinical details. The DD genotype of the ACE gene was reconfirmed by a second PCR using a Taq extender (Fig. 1) (12).

Echocardiography

Echocardiography was performed by one examiner (O.O.) within 24 h of arrival at the coronary care unit with a VingMed CFM 800 (Vingmed Sound, Norway) ultrasonographic machine with a 2.5 and 3.25-MHz transducer. Analyses were done blinded for all clinical data. All examinations were performed using the
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Although the ACE gene I/D allelic variant in humans has been implicated in arteriosclerotic CV disease, cardiac hypertrophy, restenosis and progression of diabetic renal disease, its role in the mechanism of PP in MI has remained difficult to evaluate (1). O’Donnell et al. (32) found evidence for association and genetic linkage of the ACE gene with hypertension and blood pressure in men, but not in women, when they analyzed over 3000 participants from the Framingham Heart Study.

The finding that the DD genotype might influence arterial pulsatility is difficult to interpret. From association and linkage studies, there is strong evidence that the ACE D allele accounts for almost half of the variance in ACE plasma levels (33). Our results provide an interesting contribution to this problem because the D allele might contribute to the increase of pulsatility in patients with a first AMI. Pharmacological studies indicate that the ACE Gene I/D polymorphism influences not only angiotensin II generation but also the cross-talk of this hormone with bradykinin and even nitric oxide (34). It seems likely that the combination of all these vasoactive compounds changes with age and contributes in turn to the age-related changes in arterial stiffness and thus, in PP in subjects with DD genotype. Furthermore, the present findings agree with reports suggesting the influence of the ACE gene polymorphism on the mechanisms of blood pressure (35).

**Study Limitations**

Our study is the first to investigate the relationship between ACE gene I/D polymorphism and pulse pressure in patients with a first anterior AMI. For this reason, there are no data in the literature to compare our results. In this study, invasive hemodynamic measurements were not available. Angiography with assessment of artery patency was not performed routinely. Reperfusion rates were not measured.

**Conclusions**

Finally, the present investigation has shown that the **ACE Gene I/D polymorphism D allele may modulate the relationship between AMI and PP**. This finding might play a role in the mechanism of CV risk. Clearly these results require further investigation involving long-term follow-up.

**References**

The link between angiotensin-converting enzyme genotype and pulmonary artery pressure in patients with COPD*

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Summary. Objective: The insertion (I) / deletion (D) polymorphism of the angiotensin-converting enzyme (ACE) gene has been associated with an increased risk of cardiovascular diseases. In patients with primary pulmonary hypertension, the homozygous ACE DD genotype is more prevalent than the non-DD genotype. However, the relationship of ACE gene polymorphism to secondary pulmonary hypertension remains unclear, and ethnicity may be one of the factors that can modulate the effects of ACE genotypes reported in different studies. We hypothesized that in patients with chronic obstructive pulmonary disease (COPD) the presence of the D allele in the ACE gene polymorphism is associated with increased pulmonary artery pressure (Ppa).

Patients and methods: Bodyplethysmography was used to assess lung function in 66 consecutive patients with COPD; pulmonary artery pressures were determined using echocardiography. ACE gene I/D polymorphism was identified with the polymerase chain reaction. 118

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healthy persons served as the control group. All patients and controls were Caucasian. Genotype II was identified in 15 patients with COPD, genotype ID in 31 and genotype DD in 20. In the control group, genotype II was identified in 19 persons, genotype ID in 68 and genotype DD in 31. The distribution of ACE gene polymorphism did not differ between patients and the control group.

Results: In patients with COPD, no differences were seen between the three genotype groups in mean age, smoking history, hemoglobin concentrations or ventilatory or blood gas variables. Both systolic and mean PPa differed significantly between the II, ID and DD groups (Systolic PPa: 24.4 ± 2.2 versus 31.3 ± 2.5 and 36.7 ± 3.9 mm Hg, respectively, ANOVA, p < 0.05; Mean PPa: 13.0 ± 1.5 versus 17.5 ± 1.4 and 21.2 ± 2.8 mm Hg, respectively, ANOVA, p < 0.05). In multiple linear regression analysis, the I/D ACE gene polymorphism (p < 0.05), SaO2 (p < 0.05) and the duration of COPD (p < 0.02) were independent predictors of systolic and mean PPa.

Conclusion: The results of this study suggest that I / D ACE gene polymorphism is linked to pulmonary artery pressure in Caucasian patients with COPD.

Key words: Pulmonary hypertension, chronic obstructive pulmonary disease, ACE gene polymorphism.

Introduction

Pulmonary hypertension is a frequent complication of chronic obstructive pulmonary disease (COPD) with negative impact on the prognosis in such patients [1]. Mechanisms involved in sustained increases in pulmonary artery pressures include hypoxia-induced vasoconstriction and pulmonary vascular remodeling. In animal studies, the renin-angiotensin system was shown to play an important role in both of these pathological processes [2, 3]. Local expression of angiotensin-converting enzyme (ACE) is increased in the walls of small pulmonary arteries in rats exposed to chronic hypoxia [2, 3]. On the other hand, ACE inhibitors attenuate the development of structural changes of arterioles in the pulmonary vasculature under hypoxic conditions [4].

The insertion (I)/deletion (D) polymorphism at intron 16 of the ACE gene has been associated with increased risk of systemic arterial hypertension [5] and atherosclerosis [6]. Carriers of the D allele in I/D ACE gene polymorphism have higher serum and tissue activities of ACE [7, 8]. A positive correlation between the DD genotype and right ventricular hypertrophy was seen in patients with primary pulmonary hypertension [9]. In contrast, Van Suylen et al. [10] observed less right ventricular hypertrophy among the D-allele carriers with COPD. In a recent study in 19 Japanese patients with COPD, Kanazawa et al. [11] demonstrated higher pulmonary artery pressure (PPa) during exercise among those who carried the D allele. The aim of the present study was to analyze the link between ACE gene polymorphisms and pulmonary artery pressures at rest in a group of Caucasian patients with COPD. Since the presence of the D allele is associated with higher serum and tissue concentrations of ACE [7, 8], we hypothesized that pulmonary artery pressure would be higher in the D-allele carriers with COPD.

Methods

This was a cross-sectional study in clinically stable patients with a diagnosis of COPD as defined by the GOLD guidelines [12]. Exclusion criteria were primary pulmonary hypertension [13], thromboembolic disease, left ventricular systolic or diastolic dysfunction due to ischemic heart disease, valvular heart disease, systemic hypertension or primary myocardial disease. None of the patients had radiologic or clinical evidence of pulmonary congestion. Echocardiography was used to exclude left ventricular dysfunction in all patients. The control group comprised 118 healthy persons, and none was known to have acute or chronic illness. All patients and controls were Caucasian; both groups had a homogenous ethnic background of east Slovakia. The study was approved by the local ethics committee, and all participants gave written consent to the study.

Pulmonary function was evaluated with bodyplethysmography (Jaeger, Germany); all testing was performed according to the European Respiratory Society standards in patients in a sitting position. In order to ensure consistency of the technique, the measurements were made by the same technician at the same time of day under standard conditions of body temperature and pressure. Three technically acceptable measurements were made in each patient, and the highest one was included in analysis of the data. For arterial blood gas analysis, a sample was obtained by puncture of the radial artery.

Mean and systolic PPa were assessed with Doppler echocardiography [14] by a single investigator blinded to the results of the ACE genotype analyses. Continuous Doppler wave assessment of the peak velocity of the tricuspid regurgitation jet and pulsed Doppler recording of time-to-peak velocity curves of pulmonary artery blood flow and right ventricular outflow tract were used to assess pulmonary artery pressures. Tricuspid regurgitant flow was identified with color-flow Doppler techniques, and the maximum jet velocity was measured in continuous-wave Doppler recording. Estimation of right ventricular systolic pressure was based on the modified Bernoulli equation. The diameter of the inferior vena cava and its respiratory variations were used to estimate right atrial pressure, which was added to the right ventricular systolic pressure in order to calculate the systolic pulmonary artery pressure. The acceleration times of the systolic blood flow in the pulmonary artery (i.e. the time between onset and peak systolic flow) and the right ventricular outflow tract were determined with pulsed Doppler recordings from the parasternal short axis, with the 2-D-guided Doppler probe placed at the level of the pulmonary valve. Time-to-peak velocity curves of the systolic blood flow in the pulmonary artery and right ventricular outflow tract were used to assess the mean pulmonary artery pressure [14]. These estimations of PPa have been shown to be highly accurate and correlate extremely well (up to 97%) with hemodynamic measurements during right heart catheterization [15].

I/D polymorphism of the ACE gene was determined according to the method of Rigat et al. [16]. Genomic DNA, extracted from peripheral blood lymphocytes by a salting-out process, was amplified in a polymerase chain reaction (PCR) with primers 5′–CTG GAG ACC ACT CCC ATC CTT TCF-3′ and 5′–GAT GTG GCC ATC ACA TTC GTC AGA T-3′. PCR products were further analyzed using electrophoresis in 2% agarose gel and visualized directly with ethidium-bromide staining. The insertion allele (I) was detected as a 490-bp band and the deletion allele (D) as a 190-bp band. DMSO at 5% was included in the PCR to prevent underestimation of heterozygotes and overestimation of genotype DD. Each DD type was
Angiotensin converting enzyme DD genotype not associated with increased risk of coronary artery disease in the Iranian population

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Abstract

The coronary artery disease (CAD) is of the main causes of heart failure and there is evidence supporting the association of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and the susceptibility to CAD. Therefore, the relevance of ACE polymorphism for CAD was determined in the Iranian population.

487 age-matched individuals including 224 patients with >50% angiographically established coronary stenosis and 263 healthy subjects genotyped for ACE gene I/D polymorphism by a standard method. Paraclinical characteristics including lipid profile were also determined for both groups. While the systolic ($p < 0.0001$) and diastolic ($p < 0.0001$) blood pressure, serum cholesterol ($p < 0.005$) and LDL-C ($p < 0.05$) were significantly increased in CAD patients, our results show that there was no increased risk of CAD in association with DD genotype in Iranian population. Allele frequencies were also similar in both groups. Although, we found a significant difference in ID ($p < 0.005$) and II ($p < 0.05$) genotype between patients and healthy subjects. The present study showed that DD genotype does not increase the CAD susceptibility in the studied Iranian population and may not be as a risk factor. Therefore, further studies together with the other polymorphisms of ACE gene may be required to determine the relation between cardiovascular disease susceptibility and ACE genetic variations in Iranian population.

Keywords: Coronary artery disease; ACE gene; I/D polymorphism

1. Introduction

Coronary artery disease (CAD) is the leading cause of heart failure. The role of the rennin–angiotensin–aldosterone system in heart failure is well known and angiotensin converting enzyme (ACE) has a major role in this system. ACE is a zinc-metalloproteinase that converts angiotensin I to the potent vasoconstrictor angiotensin II by removing His-Leu from C-terminal, ACE also degrades bradykinin, a powerful vasodilator that plays critical roles in regulation of vascular tone and cardiac functions [1]. Repeated measurement of plasma ACE in adults has shown that while the enzyme is very stable under physiological conditions, the plasma level of this peptide varies greatly among individuals [2]. This variation has been shown to be independent of a large number of environmental, metabolic, and hormonal factors [3]. Therefore, these findings suggest that plasma ACE level is determined genetically for the most part [4].

Human ACE gene is 21 kb long, contains 26 exons and is located on chromosome 17q23. There exist several gene polymorphisms related to the ACE gene, but I/D polymorphism is the most studied and clinically important one [5]. It consists of the insertion (allele I) or deletion (allele D) of a 287-bp Alu repetitive sequence within intron 16 of the ACE
Angiotensin converting enzyme DD genotype not associated with increased risk of coronary artery disease in the Iranian population

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Deletion Polymorphisms in the Angiotensin Converting Enzyme Gene Are Associated with Pulmonary Hypertension Evoked by Exercise Challenge in Patients with Chronic Obstructive Pulmonary Disease

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Pulmonary hypertension is a manifestation of a wide variety of cardiac and pulmonary diseases and a consequence of profound structural alterations in the pulmonary vasculature, commonly called pulmonary vascular remodeling (1). This process is known to involve medial smooth-muscle cell hypertrophy and hyperplasia, fibroblast proliferation, and matrix protein synthesis (2–4). The mechanism governing these architectural changes is unknown, although angiotensin II (AII), among other mediators, may be involved (5). In the systemic circulation, AII formed by the action of angiotensin converting enzyme (ACE) and AII contributes to the development of pulmonary hypertension via its vasoconstrictor action or via effects on vascular smooth-muscle cell migration and growth (7). Moreover, increasing evidence that ACE plays an important role in systemic vascular pathology led us to question whether ACE participates in the structural remodeling associated with pulmonary hypertension. Indeed, ACE is present in very high concentrations in the lungs, and its activity is further increased by chronic hypoxia (8).

The ACE gene has been considered a candidate gene for contributing to the development of hypertension and cardiovascular diseases. The ACE gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within an intron of a 287-bp nonsense DNA domain, resulting in three genotypes (DD and II homozygotes, and DI heterozygotes) (9). The ACE DD genotype is associated with increased circulating and cellular concentrations of ACE (10, 11), and the observed codominant association between D-I polymorphism and ACE activity could be consistent with the reported increase in cardiovascular risk associated with the DD genotype (12).

Recent studies have shown that the renin-angiotensin system is not only a hormonal system of the circulation, but also a tissue system, widespread in cardiovascular organs, that has been implicated in vascular remodeling accompanying various cardiovascular diseases (13). However, substantive evidence of a role for locally increased ACE activity in pulmonary hypertension resulting from chronic obstructive pulmonary disease (COPD) is lacking. We hypothesized that though pulmonary hypertension may develop at some point in most patients with COPD, a genetic predisposition to pulmonary vascular remodeling may exist. In the present study we investigated whether D-I polymorphism in the ACE gene is a genetic factor for the development of pulmonary hypertension in patients with COPD, using exercise challenge during breathing of room air or oxygen.

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with the ID genotype (40.5 ± 5.9 mm Hg, p = 0.0049) or the II genotype (37.7 ± 5.9 mm Hg, p = 0.0027) (Figure 2). In addition, the Rpv in patients with the DD genotype (8.7 ± 0.3 mm Hg/L/min/m²) was higher than that in patients with the ID genotype (7.6 ± 0.4 mm Hg/L/min/m²) or the II genotype (6.9 ± 0.4 mm Hg/L/min/m²).

DISCUSSION

In this study we found that the significant increase in Ppa and Rpv evoked by exercise challenge is associated with the ACE DD genotype in patients with COPD. The 12 subjects with baseline $P_{Pa}$ > 20 mm Hg were evenly distributed (II: n = 5; ID: n = 3; DD: n = 4). Thus, greater severity of disease, as reflected by a higher baseline Ppa and Rpv, did not occur in patients with the DD genotype, and could not have caused greater increases in Ppa and Rpv with exercise. In normal subjects, there is only a minimal increase in Ppa during exercise, due to recruitment of collateral vessels and distension of pulmonary vessels themselves (17). In contrast, in our patients, Ppa increased markedly with exercise despite a slightly elevated Ppa at rest. Thus, pulmonary hypertension became particularly pronounced in patients with COPD during exercise, indicating that the increase in pulmonary blood flow during exercise resulted in a steep increase in Ppa. In this study, the patients with the DD genotype did not have an increased baseline Ppa or Rpv. For that reason, we thought that our patients had the ability to accommodate pulmonary blood flow at rest, but that they had lost the ability to accommodate increased pulmonary blood flow through distension of pulmonary vessels during exercise. This loss is due principally to pulmonary vascular remodeling.

Pulmonary vascular remodeling is known to occur as a consequence of persistent pulmonary vasoconstriction in response to chronic hypoxia, in addition to following pulmonary vascular destruction secondary to emphysematous change (18). In the present study, the three subgroups of COPD patients did not differ significantly with regard to their blood gas data, diffusing capacity of carbon monoxide, HRCT emphysema score, or exercise capacity on incremental exercise testing, suggesting that the patients had the same degree of emphysematous change and pulmonary vascular destruction. Moreover, it is well known that one of the most important determinants of Ppa and Rpv is hypoxia. The three subgroups in the study were similar in their degree of exercise-induced hypoxia. Therefore, these findings suggested that the D-I polymorphism in the ACE gene may be associated with pulmonary hypertension evoked by exercise challenge in patients with COPD.

Considerable controversy has recently surrounded the role of the D-I polymorphism in pulmonary hypertension. For example, Abraham and coworkers found that the DD genotype was associated with right-ventricular hypertrophy in patients with primary pulmonary hypertension (19). In contrast, van Suylen and associates found less right-ventricular hypertrophy in COPD patients who had the DD genotype (20). However, a limitation of their study was that evidence of right-ventricular hypertrophy was based on electrocardiographic criteria, and this technique is rather insensitive compared with hemodynamic and echocardiographic measurements.

Chronic hypoxia is a well-characterized experimental model of pulmonary hypertension, which is caused by vasoconstriction and pulmonary vascular remodeling (21). In recent studies, messenger RNA for ACE and ACE antigen expression were found to be increased locally in the walls of small pulmonary arteries in rat hypoxic pulmonary hypertension (22). Our findings in the present study suggested that patients with the DD genotype might have a greater increase in tissue ACE activity than those with the II and ID genotypes, and that increased production of AII causes proliferation and hypertrophy of vascular smooth-muscle cells in small pulmonary arteries. Moreover, to enable testing with a decreased degree of acute hypoxic pulmonary vasoconstriction, we performed another exercise challenge in which our subjects breathed oxygen. Induction of pulmonary hypertension by exercise challenge with breathing of oxygen was also greater in patients with the DD genotype than in those with the II or ID genotype.

We have also examined other possible ACE phenotypes. However, the D-I genotypes examined in the present study did not affect the attenuation of the increase in Ppa following exercise with breathing of oxygen, or the magnitude of the increase in $P_{Pa}$ following exercise, or the magnitude of the increase in exercise-induced systemic hypertension. On the other hand, several additional possible explanations exist for the observed association between ACE polymorphism genotypes and pulmonary hypertension with exercise in COPD patients. Population stratification based on ethnicity or other factors could have contributed to the differences in $P_{Pa}$ across the genotypes examined in our study. However, all of the subjects in this study were Japanese, and population stratification is therefore less likely to have occurred. In addition, it is also possible that the deletion polymorphism is associated with

<table>
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<th>TABLE 2</th>
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<tr>
<td><strong>PULMONARY VASCULAR RESISTANCE</strong>&lt;sup&gt;※&lt;/sup&gt; AT REST AND AFTER EXERCISE</td>
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<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>Rest</th>
<th>Exercise</th>
<th>Rest</th>
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<tbody>
<tr>
<td>II</td>
<td>6.9 ± 0.4</td>
<td>7.1 ± 0.3</td>
<td>6.8 ± 0.3</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>ID</td>
<td>6.7 ± 0.5</td>
<td>8.0 ± 0.3</td>
<td>6.6 ± 0.4</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>DD</td>
<td>7.1 ± 0.3</td>
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</tr>
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</table>

<sup>※</sup> Pulmonary vascular resistance (mm Hg/L/min/m²) = $P_{Pa}$ − $P_{Paw}$/CI.
<sup>‡</sup> p < 0.05 versus II group.
<sup>‡</sup> p < 0.01 versus ID group.
<sup>‡</sup> All values represent mean ± SD.

Figure 2. $P_{Pa}$ before and after exercise in patients with the ACE II, ID and DD genotypes during breathing of oxygen.
Ppa because this polymorphism is in linkage disequilibrium with another causative variant in or near the ACE gene. Further research will be required to clarify this issue.

In conclusion, the number of patients in this study was very small for a genetic association study, and our results should be examined in larger studies. However, since invasive physical monitoring such as that used in this study is difficult to perform on large numbers of subjects, further studies will probably be required to use noninvasive methods, including echocardiographic measurements. Identification of the D-I polymorphisms in the ACE gene may be valuable in the management of COPD, since there could be a genotype-based variation in response to ACE inhibitors that would make genotyping for these polymorphisms a clinically useful exercise. The effects of ACE genotypes on the response to therapy with ACE inhibitors or AII receptor antagonists should be studied.

References
Pulmonary hypertension--clinical aspects, pathophysiology, diagnostic and therapy
[Article in German]

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Abstract

Pulmonary hypertension (PH) is a disease which is characterised by an increase in the mean pulmonary arterial pressure (mPAP) in the lung circulation of over 25 mmHg in rest and over 30 mmHg in movement. Due to the chronic overload of the right ventricle, the heart is always affected by a PH and often develops a so-called cor pulmonale chronicum which can lead to right-heart failure. There are five groups in the clinical WHO Venice classification which are arranged according to pathogenetical, clinical and therapeutical criteria. In addition, an adjusted NYHA classification helps to grade the significance of the disease stages. Principally, one classifies a mostly isolated form of the pulmonary arterial hypertension (PAH) and other secondary forms of the PH which develop on the grounds of existing problems such as left-heart diseases, hypoxic lung diseases, pulmonary embolism and infections. The pathophysiological reasons for a PH are just as various as the different manifestations. Yet there are generally four main alterations in the walls of the pulmonary vessels. This includes vasoconstriction, rarefaction of vessels, vascular remodelling and the occlusion of vascular lumen by a thrombus with subsequent structural remodelling of the vascular and mounted extracellular matrix. The diagnostic procedure should be algorithm-oriented and includes anamnesis, physical examination, electrocardiogram (ECG), thoracic x-ray and echocardiography. To confirm the diagnosis and for a better measuring of the prognosis, an examination with a right-heart flow-directed balloon-tipped catheter is favourable. Because of the change in the pathophysiological concepts of the PH from a vasoconstrictive to a vasoproliferative genesis, additional pharmacological targets are developed for therapeutic treatment. Today the former regime of therapy with high-dosed calcium-channel blockers such as vasodilatators only finds application after pharmacological testing at so-called responders. The current scheme of therapy is focused on the synergic effects of different drugs, such as prostacyclines, endothelial-receptor blockers and phosphodiesterase-5 inhibitors. After the failure of pharmacological treatments, the endarteriection remains as the last therapy option, although it is accompanied by poor survival rates.
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**Background/Purpose:** The renin-angiotensin system plays an important role in pulmonary artery remodelling. Several polymorphisms of genes encoding for components of the renin angiotensin system such as the angiotensin converting enzyme (ACE), the angiotensinogen (AGT) gene, and the angiotensin II type 1 receptor (ATIR) have been associated with the development of pulmonary hypertension. The aim of this study was to investigate the ACE I/D genotype, the M235 T polymorphism of the AGT gene and the A1166 C polymorphism of AT1R in the lungs of congenital diaphragmatic hernia (CDH) complicated by persistent pulmonary hypertension (PPH) in the newborn.

**Methods:** Genomic DNA was extracted from archival paraffin-embedded lung tissue from 13 newborns with CDH complicated by PPH and from 9 controls. Genotyping for the I/D-ACE, the M235 T-AGT, and the A1166 C-ATIR gene polymorphisms were determined by a polymerase chain reaction–based method with appropriate restriction digest when required.

**Results:** In controls, ACE genotype distribution of DD, ID, and II was 11%, 33%, and 55%, respectively, whereas in CDH it was 70%, 15%, and 15%, respectively. The ACE-DD genotype was significantly higher in CDH compared with controls \((P < .05)\). In CDH samples, the prevalence of AGT-MM genotype was lower (8% vs. 33%; \(P < .05)\), whereas the AGT-TT genotype was higher (61% vs. 22%; \(P < .05)\) compared with controls. There were no differences in allele frequencies of AT1R between CDH patients and controls.

**Conclusions:** These data suggest that D allele of the ACE gene insertion/deletion polymorphism and angiotensinogen M235 T polymorphism may be associated with PPH in newborns with congenital diaphragmatic hernia.

**INDEX WORDS:** Congenital diaphragmatic hernia, pulmonary hypertension, angiotensin system gene polymorphisms.

*Severely Affected Infants with Congenital Diaphragmatic Hernia (CDH) Die of Hypoxemia Caused by Pulmonary Hypoplasia and Pulmonary Vascular Abnormalities Resulting in Elevated Pulmonary Vascular Resistance and Persistent Pulmonary Hypertension (PPH).*1,3 Persistent pulmonary hypertension in CDH is characterized by vascular remodelling with thickening of medial and adventitial layer and extension of smooth muscle into previous nonmuscularised arteries.1,3 The mechanisms regulating these architectural changes are unknown. The renin angiotensin system is reported to play an important role in the development of pulmonary artery remodelling in hypoxia-induced pulmonary hypertension.4

Angiotensin-converting enzyme (ACE) is a key enzyme in the generation of angiotensin (AT)-II from AT-I. Local production of AT II by ACE in the pulmonary arterial wall is believed to contribute to hypertensive pulmonary vascular remodelling through the growth stimulation of human pulmonary arterial smooth muscle.3 Furthermore, ACE is increased during the process of pulmonary vascular remodelling in small pulmonary arteries in hypoxia-induced pulmonary hypertension.6,7

The human ACE gene contains a polymorphism consisting of the presence (insertion, I) or absence (deletion, D) within an intron of a 287 base pair nonsense DNA domain, resulting in 3 genotypes (DD and II homozygotes, and ID heterozygotes). Plasma and tissue ACE levels and activity are reported to be higher in individuals homozygous for the deletion allele DD than in those of II genotype,8,9 and increased production of angiotensin II causes proliferation and hypertrophy of vascular smooth muscle cells in small pulmonary arteries.10 Increasing evidence in the literature supports the associations between the D allele and the development of pulmonary hypertension and the pathogenesis various pulmonary diseases.10,11

Similarly, polymorphisms in other genes of the renin angiotensin system such as the M235 T polymorphism in the angiotensinogen (AGT) gene and the A1166 C poly-
CDH (61% vs. 22%; \(P < .05\)) compared with controls, whereas AGT-MT distribution was similar (31% vs. 45%), (Table 1). There was no significant difference in the ATR1 A1166C genotype distribution between CDH and controls.

**DISCUSSION**

The most striking structural changes in pulmonary arteries in CDH patients complicated by persistent pulmonary hypertension include increased adventitial and medial thickness.2,3 In PPH, an exaggerated vasoconstrictive response of an anatomically abnormal vascular bed leads to an elevation of pulmonary vascular resistance resulting in the often fatal hypoxemia. The renin-angiotensin system components appear to play an important role in the pathogenesis of pulmonary hypertension.4,5,7 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 hypoxia-induced pulmonary hypertension, because angiotensin II stimulates the growth and proliferation of human vascular smooth muscle cells.3,8,19 ACE expression was found to be elevated in the walls of small pulmonary arteries undergoing remodelling and in patients with primary and secondary pulmonary hypertension.6 Additionally, ACE inhibitors and ATR1 antagonist attenuated the hemodynamic and structural changes of hypoxia-induced pulmonary hypertension by reducing vascular resistance.21,22 The association between the D allele and PPH in CDH patients suggests a potential role for rennin-angiotensin system in the pathogenesis of PPH.

In this study we also examined the association between polymorphisms in the genes encoding for components of angiotensinogen (AGT) with PPH in CDH patients. The AGT M235T polymorphism was more often observed in PPH patients compared with controls. To date, no study has analyzed the prevalence and distribution of M235T-AGT in patients with pulmonary hypertension or any other lung disease. The data described here suggest that AGT235T polymorphism may contribute to the development of PPH in CDH patients.

No significant association was found between the angiotensin II type 1 receptor (A1166C) and PPH. In the systemic circulation, angiotensin II type I receptor CC genotype has been reported to be associated with essen-

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CDH (n = 13)</th>
<th>Controls (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>2 (16%)</td>
<td>5 (56%)</td>
</tr>
<tr>
<td>ID</td>
<td>2 (15%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>DD</td>
<td>9 (70%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>22.5%</td>
<td>72.5%</td>
</tr>
<tr>
<td>D</td>
<td>77.5%</td>
<td>27.5%</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>1 (8%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>MT</td>
<td>4 (31%)</td>
<td>4 (45%)</td>
</tr>
<tr>
<td>TT</td>
<td>8 (61%)</td>
<td>2 (22%)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>23.5%</td>
<td>5.5%</td>
</tr>
<tr>
<td>T</td>
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<td>44.5%</td>
</tr>
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</tr>
<tr>
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<td>7 (78%)</td>
</tr>
<tr>
<td>AT</td>
<td>4 (31%)</td>
<td>2 (22%)</td>
</tr>
<tr>
<td>TT</td>
<td>1 (7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>77.5%</td>
<td>89%</td>
</tr>
<tr>
<td>T</td>
<td>22.5%</td>
<td>11%</td>
</tr>
</tbody>
</table>

ACE activity than those with the II and ID genotypes. The increased local production of ACE could contribute to elevated pulmonary vascular tone and pulmonary vascular remodelling by increasing the local production of angiotensin II, because angiotensin II is known to stimulate the growth of human vascular smooth muscle cells. In the lung, the pulmonary circulation is a potentially important target for rennin-angiotensin system activation. ACE inhibitors attenuate pulmonary vasoconstriction in normal humans and patients with con pulmonale as do type-I angiotensin receptor antagonists.22 The association between the D allele and PPH in CDH patients suggests a potential role for rennin-angiotensin system in the pathogenesis of PPH.

This is the first description of a specific allele association with the development of persistent pulmonary hypertension in patients with CDH. ACE-DD genotype was significantly higher in CDH samples compared with controls. Our findings in this study suggest that patients with DD genotype may have a greater increase in tissue

**Fig 2.** PCR-based restriction analysis of the ACE I/D, AGT M235 T and ATR1 A1166C gene polymorphism shown on a 4% agarose gel stained with ethidium bromide.

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**Table 1. Genotype and Allele Frequencies of the Angiotensin-Converting Enzyme Gene, Angiotensinogen Gene, and Angiotensin II Type 1 Receptor Gene in Patients With CDH and Persistent Pulmonary Hypertension and Controls**

<table>
<thead>
<tr>
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<th>CDH (n = 13)</th>
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<td></td>
</tr>
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<td>I</td>
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</tr>
<tr>
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</tr>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
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</tr>
</tbody>
</table>
CONGENITAL DIAPHRAGMATIC HERNIA

P. Frykman (Birmingham, AL): This is a very interesting paper and suggests that certainly ACE is involved in pulmonary hypertension of the newborn. My question is, is there a plan in the future to look at ACE levels in newborns with CDH in your center?

V. Solari (response): Yes. We are still continuing to collect samples from other CDH patients, especially patients who were not so severely affected and did not die.

R. Burd (New Brunswick, NJ): One problem with genetic association studies is that confirmation of their findings has been difficult. Only about 15% of genetic associations have been found to be significant and able to be replicated in follow-up studies without evidence of bias. In addition, I am concerned that your study has a small number of patients and that you may have a type I statistical error. Can you comment on these 2 issues and on where you are going next with these studies? Finally, did you control for racial distribution in your study?

V. Solari (response): Yes, that is a very interesting question. I think our study was hypothesis driven based on the findings of previous studies. Our association study, and our results should be examined in larger studies. Analysis of polymorphism of the ACE and AGT genes may, in the future, provide valuable information regarding risk assessment and medical management of severely affected newborns with CDH.

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17. Hingorani AD, Brown MJ: A simple molecular assay for the C1166 T polymorphism does not significantly contribute to the development of PPH.
Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism in Korean Patients with Systemic Sclerosis

Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism in Korean Patients with Systemic Sclerosis

INTRODUCTION

Systemic sclerosis (SSc) is a systemic autoimmune disease of unknown etiology, characterized by microcirculatory dysfunction and accumulation of excessive extracellular matrices. Among suggested mechanisms, endothelial derangement is important in the pathogenesis of SSc, resulting in some features of vasculopathy such as Raynaud phenomenon and pulmonary hypertension (1).

Vascular injury is associated with increased local production of angiotensin-converting enzyme (ACE) and angiotensin II (2). ACE is a key enzyme in renin-angiotensin system (RAS) and widely distributed in human tissues including the lung, vascular endothelium, kidney, heart, and testes (3). 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 (4, 5).

The ACE gene is located in the long arm of chromosome 17 (17q 23), and an insertion/deletion (I/D) polymorphism, determined by the presence or absence of a 287 base pair (bp) Alu repeat within intron 16, was identified (6). The DD genotype has about two-fold higher serum level of ACE than the II genotype, and the ID genotype has intermediate level (7). The DD genotype was associated with endothelial dysfunction, blunting the stimulated endothelial or donated nitric oxide response in normal population (8). Some epidemiologic studies have found that the D allele was associated with increased cardiovascular and renal risk (9).

The association of ACE I/D genes has been studied in other rheumatic diseases with vascular involvement, and showed inconsistent data in systemic lupus erythematosus (3, 10), association of II genotypes in Kawasaki disease with coronary aneurysm (11), and irrelevance in Korean Behcet’s disease (12). Although ACE D genotype was reported as a risk factor of SSc in Italian (13), it has not been evaluated in other ethnic groups. We studied the association of ACE I/D genotypes with the development and clinical features of SSc in Korean.

MATERIALS AND METHODS

Seventy two patients (68 women, 4 men) with SSc fulfilling the criteria proposed by the American College of Rheumatology (14) were enrolled. The age at diagnosis was 43.5 ± 12.6 yr old (mean ± SD). The controls were 114 healthy, disease free Koreans (41 women, 73 men). The age at enrollment was 37.2 ± 4.6 yr old. The medical records of patients were

8. Joung2006 (condensed)
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Secondary Pulmonary Hypertension


Nader Kamangar, Kelvin Chan, Sat Sharma, (Chief Editor Zab Mosenifar)

Background

Pulmonary arterial hypertension (PAH), defined as a mean pulmonary arterial pressure greater than 25 mm Hg at rest or greater than 30 mm Hg during exercise, is characterized by a progressive and sustained increase in pulmonary vascular resistance that eventually leads to right ventricular (RV) failure. It is a life-threatening condition if untreated; treatment success rates vary according to the specific cause.

Cardiac disorders, pulmonary disorders, or both in combination are the most common causes of secondary PAH (SPAH) (see the images below). Cardiac diseases produce PAH via volume or pressure overload, though subsequent intimal proliferation of pulmonary resistance vessels adds an obstructive element. Perivascular parenchymal changes, along with pulmonary vasoconstriction, are the mechanism of PAH in respiratory diseases.

![Gross pathology](image1.jpg)

Gross pathology on patient who died of severe pulmonary arterial hypertension secondary to persistent patent ductus arteriosus.

![Close-up view](image2.jpg)

Close-up view of gross pathology on patient who died of severe arterial pulmonary hypertension secondary to persistent patent ductus arteriosus.

Therapy for PAH is targeted at the underlying cause and its effects on the cardiovascular system. Novel therapeutic agents, such as prostacyclin and others undergoing clinical trials, have led to the possibility of specific therapies for these once untreatable disorders.

Clinical guidelines have been formulated by the American College of Chest Physicians (ACCP),[1,2] the European Society of Cardiology,[3] and the American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA).[4]

For patient education resources, see the Lung and Airway Center, as well as Chronic Obstructive Pulmonary Disease (COPD).
ACE gene deletion/deletion polymorphism may be a protective factor for respiratory distress in preterm infants

Ercan Sivaslı, Murat Yurdakök, Elif Babaoğlu, Halil Karabulut, Şule Yiğit, Melih Babaoğlu, Gülsevin Tekinalp, Ajlan Tükün

Departments of Pediatrics, and Pharmacology, Hacettepe University Faculty of Medicine, and Departments of Pharmacology, and Medical Genetics, Ankara University Faculty of Medicine, Ankara, Turkey


The objective in this study was to evaluate the angiotensin converting enzyme (ACE) insertion/deletion (I/D) gene polymorphism in premature infants with and without respiratory distress within the first 24 hours of life.

Totally, 87 premature babies who were followed up in the neonatal unit were included in the study. Of these babies, 41 had respiratory distress, and constituted the patient group. The remaining 46 babies who did not have respiratory distress constituted the control group. Blood samples were obtained from the babies within the first few days of life prior to administration of any blood product. The ACE gene insertion (I) and deletion (D) polymorphism was investigated using polymerase chain reaction method.

The I/I polymorphism was frequent in the patient group and the D/D polymorphism was frequent in the control group (p<0.05). There was no relationship between the ACE gene polymorphism and hospital stay, ventilation or oxygen consumption duration of the patients. In addition, taking into consideration the gestational age, no association was found between ACE gene polymorphism and birth weights of the babies. The I/I genotype was considered a risk factor for pulmonary disorders in neonates as the I/I variant was more frequent in the neonates with respiratory distress than in healthy newborns.

The ACE I/I genotype is associated with an increased risk of respiratory disorders among premature infants and the D/D genotype is a protective factor for respiratory disorders, but these infants with ACE D/D genotype might be at risk for the development of cardiovascular disorders later in life.

Key words: ACE gene polymorphisms, preterm infant, respiratory distress.
Table II. Frequency (%) of Angiotensin-Converting Enzyme (ACE) Gene I/D Polymorphism Genotypes in Premature Newborns with RD and in Non-RD Controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient group n (%)</th>
<th>Control group n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/D</td>
<td>11 (26.8)</td>
<td>20 (43.5)</td>
</tr>
<tr>
<td>D/I</td>
<td>17 (41.5)</td>
<td>23 (50.0)</td>
</tr>
<tr>
<td>I/I</td>
<td>13 (31.7)</td>
<td>3 (6.5)</td>
</tr>
<tr>
<td>Total</td>
<td>41 (100)</td>
<td>46 (100)</td>
</tr>
</tbody>
</table>

$\chi^2=9.507$, p=0.009


Table III. Frequency (%) of Angiotensin-Converting Enzyme (ACE) Gene D and I Alleles in Premature Newborns with RD and in Non-RD Controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patient group (n=41)</th>
<th>Control group (n=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>39/82 (47.6%)</td>
<td>63/92 (68.5%)</td>
</tr>
<tr>
<td>I</td>
<td>43/82 (52.4%)</td>
<td>29/92 (31.5%)</td>
</tr>
</tbody>
</table>

$\chi^2=7.820$, p=0.005.


Table IV. Frequency (%) of Angiotensin-Converting Enzyme (ACE) Gene I/D Polymorphism Genotypes According to Disease Severity in Premature Newborns with RD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>One-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F value</td>
</tr>
<tr>
<td>DD</td>
<td>(n=11)</td>
</tr>
<tr>
<td></td>
<td>ID</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
</tr>
<tr>
<td></td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
</tr>
</tbody>
</table>

|          | F value | P value |
| Hospitalization (days) | 0.559 | 0.577 |
| Mechanic ventilation (days) | 0.709 | 0.499 |
| Oxygen use (days) | 0.737 | 0.485 |
| Mean arterial pressure in first day (mmHg) | 0.921 | 0.407 |
| No. patients requiring vasopressor drug in first day | 0.761 | 0.475 |


Discussion

We determined the prevalence of insertion/deletion (I/D) polymorphism of the ACE gene in premature infants with RD. Our data indicate that the I/I genotype was considerably overrepresented in RD cases compared to non-RD cases (p<0.05).

Activation of a pulmonary RAS might influence the pathogenesis of lung injury via a number of cellular impacts, like changes in vascular permeability via modulation of endothelial intracellular calcium ion levels; changes in vascular tone via calcium influx and contraction of vascular smooth muscles and fibroblast activity via the AT1 receptor; and autocrine action of transforming growth factor β (TGF β)19-21. Wang and colleagues22 identified AT-II as a pro-apoptotic factor for alveolar epithelial cells.

Idell and colleagues23 demonstrated that ACE is elevated in bronchoalveolar lavage fluid in ARDS. These data suggest that RAS activation is important for both initiation and progression of lung injury. However, these studies were usually performed in adults. In neonates, the findings of the limited number of studies are controversial24.

Harding and colleagues24 demonstrated that the ACE polymorphism has a role in the development of preterm cardiorespiratory disease and that the D/D genotype may adversely influence the early health status of preterm infants. Another study demonstrated that the ACE I/D polymorphism did not significantly influence the development of bronchopulmonary dysplasia (BPD) in ventilated...
premature infants. But in another study, Kazzi and colleagues demonstrated that the D allele of ACE is associated with an increased risk and severity of BPD among preterm infants. In our study, the incidence of I allele and I/I genotype was higher in neonates with RD than in controls. These findings may suggest that the I allele and I/I genotype of the ACE gene increased the risk for RD, and the D allele and D/D genotype could be protective against RD in neonates. However, in our study, there was no relation between the ACE gene polymorphism and primary disorders, duration of hospital stay, ventilation, oxygen consumption, mean arterial pressure and number of patients who required vasopressor in the first day of life.

Pitt and colleagues demonstrated that lung endothelial cells were rich sources of circulating ACE, and therefore the increased activities in infants might be related to rapid lung development that occurred during the last trimester and the immediate postpartum period. These data were supported by the adverse findings of growth retardation and pulmonary hypoplasia with ACE inhibitor medication when prescribed antenatally. Lumbers and colleagues demonstrated that maternal use of ACE inhibitor caused decreased fetal lung liquid flow and oligohydramnios. AT-II has a specific growth factor-like effect on target tissue, and serum ACE activities are higher in preterm infants, suggesting that ACE may be more active in the immature lung. This strong association between pulmonary development and serum ACE suggests that ACE or the RAS may be linked to mechanisms controlling pulmonary growth. Taking into consideration that serum ACE activity is increased in the presence of D/D genotype, and the DD genotype is higher in healthy newborns than in neonates with RD, it may be suggested that increased ACE activity is a protective factor from respiratory disorders in the neonatal period.

In our study, we concluded that ACE I/I phenotype increased the risk of RD; however, the D/D genotype may be a protective factor for RD in premature infants. Several genetic polymorphisms have been described in genes of the RAS. These include polymorphisms in the angiotensinogen, ACE, and type I angiotensin II receptor (AT1) genes. The ACE I/D polymorphism is the most frequently studied in this respect. The ACE I/D polymorphism influences tissue and plasma ACE activity. The ACE D/D genotype is associated with increased circulating ACE levels, which are generally twice as high as those found for I/I genotypes; I/D or heterozygote genotype is associated with intermediate ACE levels. However, there is no evidence of an association of ACE genotypes with circulating AT-II levels. In our study, we could not measure the ACE activity in serum and tissue. Although serum and tissue ACE activity is stipulated to change RAS variably, in the conclusions of our study we speculated that ACE activity in serum and/or bronchoalveolar lavage liquid should be measured in the same patient group.

In conclusion, we found that significant association exists between the development of RD in neonates and the ACE gene I/I genotype. We speculate that ACE D/D genotype is a protective factor for respiratory disorders, but that these neonates with ACE D/D genotype might be at risk for the development of a cardiovascular system disorder later in life. However, the lack of a relationship between the ACE gene polymorphism and the severity of the disease suggests that some factors other than ACE gene polymorphism might be involved in determining the disease severity. Further studies are required for confirmation of our results.

Acknowledgement
The authors thank Ümit Yasar, MD, for his valuable discussion during the preparation of the project proposal.

REFERENCES
Pulmonary Arterial Hypertension (PAH) and PAH in Systemic Sclerosis (SSc)

Comments

PAH is a syndrome characterised by a progressive increase in pulmonary vascular resistance (PVR), which leads to right ventricular overload and eventually to right ventricular failure and premature death. Increased PVR is related to a number of progressive changes in the pulmonary arterioles, including vasoconstriction, obstructive remodelling of the pulmonary vessel wall through proliferation in the various layers of the blood vessel wall (smooth muscle cell and endothelial cell proliferation), inflammation and in-situ thrombosis. If untreated, PAH carries a very poor prognosis with a median survival of 2.8 years after diagnosis. This survival figure is comparable with some malignancies.

The main histological features of PAH include medial hypertrophy, intimal thickening, adventitial thickening, plexiform lesions and in-situ thrombosis. The plexiform lesion represents a focal proliferation of endothelial and smooth muscle cells resulting in a complex 3-Dimensional deformation of the vessel and is pathognomonic of PAH. PAH is defined as a sustained elevation of mean pulmonary arterial pressure (mPAP) of > 25 mmHg at rest or > 30 mmHg while exercise in the presence of a normal pulmonary capillary wedge pressure (PCWP) of ≤ 15 mmHg. The earliest symptom of PAH is often dyspnoea experienced on physical exertion. Other symptoms may include syncope or near syncope and fatigue. Chest tightness and pain similar to angina may occur, particularly on physical exertion. In severe disease, signs of right heart failure (RHF) may emerge. Pulmonary arterial hypertension represents Group 1 within the pulmonary hypertension (PH) WHO clinical classification system (Venice 2003 revision). Idiopathic PAH, which by definition has no identifiable underlying cause, is one of the more common types of PAH. Familial PAH accounts for at least 6% of cases of IPAH and mutations in the bone morphogenetic protein receptor 2 (BMPR2) have been identified in the majority of cases of FPAH. PAH can also be associated with a number of conditions (Associated Pulmonary Arterial Hypertension; APAH), which together account for most other cases of PAH. Assessment of PAH usually includes a functional class rating initially devised by the New York Heart Association (NYHA) for Chronic Heart Failure and then adapted for PAH by the World Health Organisation (WHO). WHO Functional class (WHO FC) measures the severity of PAH and reflects the impact on a patient's life in terms of physical activity and symptoms. There are four classes, with WHO FC I being the least severe and WHO FC IV being the most advanced. PAH is a rare disease, with an estimated prevalence of 30–50 cases per million. The prevalence of PAH in certain at-risk groups is substantially higher. For example, in HIV-infected patients the prevalence is 0.46%; in patients with sickle cell disease the prevalence is 20–40%; and in patients with systemic sclerosis the prevalence has been reported to be up to 16%. Idiopathic PAH is more common in young women with a mean age of diagnosis of 36 years. The exact cause behind the development of PAH remains unknown. However, research has lead to a better understanding of the underlying mechanisms. PAH is recognised as a complex, multi-factorial condition involving numerous biochemical pathways and different cell types. Endothelial dysfunction is believed to occur early on in disease pathogenesis, leading to endothelial and smooth muscle cell proliferation and structural changes or 'remodelling' of the pulmonary vascular bed resulting in an increase in PVR. Vascular remodelling itself involves all layers of the vessel wall and is characterised by proliferative and obstructive changes involving many cell types, including endothelial, smooth muscle and fibroblasts. Inflammatory cells and platelets may also play a significant role in PAH. Endothelial cell dysfunction results in reduced production of vasodilators, such as nitric oxide (NO) and prostacyclin, and over production of vasoconstrictors, such as thromboxane A2 and endothelin-1 (ET). As presented in the previous slide one aspect of PAH pathogenesis is the reduced production of the naturally occurring vasodilators, prostacyclin and nitric oxide. Prostacyclin is a potent vasodilator as well as an inhibitor of platelet activation. It is believed that patients with PAH have low levels of prostacyclin, which could
Pulmonary Arterial Hypertension (PAH) and PAH in Systemic Sclerosis (SSc)

Comments

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Pulmonary arterial hypertension (PAH) is a debilitating chronic disease of the small pulmonary arteries that is characterized by vasoconstriction and vascular remodeling (1). Endothelial dysfunction is believed to occur early on 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. PAH is either idiopathic or occurs in association with various conditions such as connective tissue diseases, HIV infection, portal hypertension, chronic hypoxic pulmonary disease, left heart disease, and left-to-right congenital shunts. Although some pathophysiological properties of PAH related to these diseases are similar especially in the terminal phase, mechanisms of increase in pulmonary arterial pressure (PAP) and thereby appropriate treatment are different according to etiology, especially in early phase of the diseases.

It is important to consider that most of studies regarding histopathological properties and proper treatment in PAH have been done in idiopathic PAH. The results of these studies may not be valid for PAH that related to other etiologies. Especially, mechanism of PAH in left heart disease, i.e. systolic/diastolic dysfunction or valvular heart disease, is different from idiopathic PAH. Early increase in PAP is related to increase in pulmonary capillary wedge pressure, and reactive pulmonary arterial vasoconstrictive state without vascular structural changes. This could trigger some mechanisms within the lungs that result in a disproportionate permanent increase in the PAP for later time. Pulmonary arterial structural changes occur in very late phase of the disease. The mechanism of PAH in hypoxemic pulmonary diseases, i.e. chronic obstructive pulmonary disease, is also different.

Among the mechanisms of increase in PAP, the expression of angiotensin converting enzyme has been reported to be increased in pulmonary arteries of PAH patients (2, 3) with a functional predominance at the site of arteriolar remodeling (4). Angiotensin II stimulates hypertrophy of human pulmonary artery smooth muscle cells in culture (4). It is thus possible that angiotensin II contributes to abnormal pulmonary vascular tone and remodeling in PAH.

In humans, there have been but a few studies on the effects of renin-angiotensin system blockers on PAH; almost all have involved acute administration of angiotensin converting enzyme inhibitors, and different findings have been reported (5-7). A study using losartan in PAH secondary to chronic obstructive pulmonary disease was also in the acute setting and oral dosing with losartan (50 mg) produced a significant reduction in mean PAP and total pulmonary vascular resistance (8). On the contrary, a pilot study to evaluate the effects of losartan on PAP, exercise capacity, quality of life, arterial blood gases and safety did not demonstrate any benefit in patients with cor pulmonale secondary to severe chronic obstructive pulmonary disease (9).

In this issue of Anatolian Journal of Cardiology, Bozbaş et al. presented a study results implying that losartan is non-inferior to nifedipine for reducing PAP and improving exercise capacity in patients with secondary PAH (10). The patients with PAH enrolled in the study were in two very different conditions that lead to increase in PAP by different mechanisms, namely hypoxemic lung disease and left heart diseases. Therefore, it is expected that the effects of losartan and/or nifedipine on PAP may be different between the groups. But, it is not clear that the effects of the drugs were similar between patients with left heart disease and those with hypoxemic lung disease. Beyond the total number of the study group, the number of the patients with hypoxemic lung disease was too low to separately analyze the effect of both drugs on PAP and the other variables in this group. To obtain a clinical implication, the effect of the drugs would be compared separately in each patients group. According to the findings of the study, it is reasonable to say that the effects of losartan and/ or nifedipine on PAP and exercise capacity are similar in a patient group of secondary PAH, majority of which was due to left heart disease. This result may not be valid for other conditions that cause PAH.
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BMPR2 mutations in pulmonary arterial hypertension with congenital heart disease


ABSTRACT: The aim of the present study was to determine if patients with both pulmonary arterial hypertension (PAH), due to pulmonary vascular obstructive disease, and congenital heart defects (CHD), have mutations in the gene encoding bone morphogenetic protein receptor (BMPR)-2.

The BMPR2 gene was screened in two cohorts: 40 adults and 66 children with PAH/CHD. CHDs were patent ductus arteriosus, atrial and ventricular septal defects, partial anomalous pulmonary venous return, transposition of the great arteries, atrioventricular canal, and rare lesions with systemic-to-pulmonary shunts.

Six novel missense BMPR2 mutations were found in three out of four adults with complete type C atrioventricular canals and in three children. One child had an atrial septal defect and patent ductus arteriosus; one had an atrial septal defect, patent ductus arteriosus and partial anomalous pulmonary venous return; and one had an aortopulmonary window and a ventricular septal defect.

Bone morphogenetic protein receptor 2 mutations were found in 6% of a mixed cohort of adults and children with pulmonary arterial hypertension/congenital heart defects. The current findings compliment recent reports in mouse models implicating members of the bone morphogenetic protein transforming growth factor-β pathway inducing cardiac anomalies analogous to human atrioventricular canals, septal defects and conotruncal congenital heart defects. The small number of patients studied and the ascertainment bias inherent in selecting for pulmonary arterial hypertension require further investigation.


Pulmonary arterial hypertension (PAH) consists of a group of vascular abnormalities with elevated pulmonary arterial pressure and pulmonary vascular resistance. The clinical spectrum includes familial and sporadic idiopathic PAH (IPAH) (previously referred to as primary pulmonary hypertension), as well as PAH related to congenital heart disease (CHD), portal hypertension, connective tissue diseases, HIV-infection and appetite suppressant exposure [1]. Germline mutations of bone morphogenetic protein receptor (BMPR)-2, a member of the transforming growth factor (TGF)-β superfamily, have been found in familial and sporadic forms of IPAH [2–5], and in appetite-suppressant PAH [6], but not in PAH with HIV infection or PAH with connective tissue diseases [7, 8].

BMPR2 mutations have not been previously reported in PAH with CHD (PAH/CHD) in whom the PAH is due to pulmonary vascular obstructive disease. The natural history of CHD associated with large systemic-to-pulmonary shunts (e.g. atrial and ventricular septal defects, patent ductus arteriosus) results in pulmonary vascular obstructive disease, i.e. the Eisenmenger syndrome (ES) [9]. Approximately one third of all patients with CHD who do not undergo early “corrective” surgery, or who die from other causes, will die from pulmonary vascular disease [10]. Although the pathophysiological mechanisms, which lead to the histopathological changes seen in ES, are not completely understood, CHD repaired within the first 2 yrs of life is unlikely to lead to pulmonary vascular disease [10]. It is unclear in certain patients with CHD whether the PAH results from increased flow, a primary pulmonary vascular abnormality or both.

Members of the TGF-β/BMP signaling pathway are particularly important in vasculogenesis and embryonic heart development [11–16]. Heterodimers of BMPR2 form a heterotetramer with type-1 receptors, BMPR1a (activin receptor-like kinase (ALK)-3) and BMPR1b (ALK-6), in the presence of a BMP ligand such as BMP2 or BMP4. Mice with tissue-specific inactivation of ALK3 (BMPR1a) have abnormal endocardial cushion morphogenesis [11, 13]. BMPR2 has been implicated in abnormal septation in the mouse, resulting in a conotruncal abnormality, i.e. truncus arteriosus [15]. Jiao et al. [16] has reported that cardiac muscle conditional knockout of BMP4 results in reduced atrioventricular septation.

To ascertain whether genetic mutations either predispose to PAH/CHD in general, or to a specific cardiac abnormality, the DNA sequence of the BMPR2 gene was determined in two patient cohorts; 40 adults and 66 children with PAH/CHD.

Material and methods

Study subjects

All studies and procedures were approved by the Columbia Presbyterian Medical Center Institutional Review Board (Columbia University, New York, NY, USA) and comply
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Association Study of Angiotensin-Converting Enzyme Ins/Del Polymorphism with Hypertension in Punjabi Population

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KEYWORDS ACE; Hypertension; I/D polymorphism

ABSTRACT Angiotensin-converting enzyme (ACE) is the key enzyme of the Renin-angiotensin system (RAS) which maintains the blood pressure homeostasis in our body. The association of the ACE insertion (I) or deletion (D) with essential hypertension has been demonstrated by many studies. The present study is aimed to determine the association, if any, of ACE I/D polymorphism with essential hypertension in Punjabi population. The ACE I/D polymorphism genotype frequencies were calculated by comparing essential hypertensive patients with ethnically similar normotensive controls. The samples were collected from the outpatient departments of various hospitals of Punjab. The subjects who had systolic blood pressure (SBP) of 140 mmHg or greater, and diastolic blood pressure (DBP) of 90 mmHg or greater, or were using any antihypertensive medication were considered as hypertensive. The DNA samples from the patients (100) and controls (100) were isolated, amplified by PCR and analyzed on agarose gel. When all the genotypes were compared in patients and controls, the chi square value was 0.444, which was not significant at 5% level. The age, height and weight were analyzed in the three different categories DD, ID, II which did not show any significant relationship with the disease. A consistent increase was seen in the SBP and DBP in all the three genotypes from DD, ID to II respectively. This increase was statistically significant for DBP especially in case of DD vs II at 5% level (t=2.34, p<0.05).

INTRODUCTION

Hypertension is a major risk factor for cardiovascular disease both in the developed and developing countries. It is estimated that by 2020 the burden of cardiovascular disease in India would surpass any other country in world (Gupta et al. 2004). There are various environmental, genetic and physiological factors that affect hypertension.

Renin-angiotensin system (RAS) in the body is a hormone pathway to regulate the blood pressure and blood volume in the body (Inagami 1994). Angiotensin-I converting enzyme (ACE) is a key enzyme of the system which activates the angiotensin-I (decapeptide) by cleaving it into angiotensin II (octapeptide) which is a potent vasoconstrictor and inactivates the vasodilator peptide bradikinin (Richard et al. 2004; Turner et al. 2000).

Most of the association studies are based on the insertion or deletion of the 287 bp Alu repeat in the intron 16 of the gene, in which D allele is found to be associated with an increase in the plasma ACE levels in a codominant fashion (Rigat et al 1990). Several studies have reported the association of the ACE I/D polymorphism and its susceptibility to hypertension (Zee et al. 1990; Morise et al. 1994). However, it is noticed that these results have not been replicated in certain other studies (Chiang et al. 1997; Kiema et al.1997). It has also been reported that the increase in the blood pressure is gender specific and association is stronger in men than in women (O’Donnell et al. 1998). It is true that blood pressure is also influenced by the interaction of genetic and some environmental factors, but the detection of statistically significant relationship between the ACE I/D polymorphism and various other environmental factors has received less than required attention. Such interactions can give rise to differences in the genotypic and phenotypic variations in different environments. Keeping all the above factors in mind, the present case control study was designed to see the association, if any, of the ACE I/D polymorphism with hypertension and to evaluate the effect of various other parameters like age, height and weight on essential hypertension in the population of Punjab.

MATERIAL AND METHODS

Study sample: In the present investigation
blood samples of 100 hypertensive patients and 100 samples of age, sex and caste matched normal, healthy individuals as control group were collected in tubes containing 0.5M EDTA as an anticoagulant. The samples were transported on ice to the laboratory and stored at -20°C till further analysis. All the patient samples were collected from the outpatient departments of various hospitals of Punjab and control samples were collected from normal healthy population after obtaining the informed consent. Various parameters like age, height, weight, smoking habits and dietary patterns were recorded in a questionnaire.

The samples, of only those patients were included in the study who were classified as hypertensive, according to the criterion of JNC VII i.e. who had systolic blood pressure (SBP) of 140mmHg or greater, and diastolic blood pressure (DBP) of 90mmHg or greater, or were using any anti-hypertensive medication.

**ACE Insertion/deletion Genotyping:** The genomic DNA was isolated using standard protocol (Gill et al. 1987). The quality of DNA was checked by 1% agarose gel electrophoresis and the quantity was determined by spectrophotometric analysis. Amplification of the isolated DNA was achieved using standard polymerase chain reaction (PCR) with the following primers, 5’-CTG GAG AGC CAC TCC CAT CCT TTCT T-3’ and 5’-GAC GTG GCC ATC ACAA TCC T-3’ (Tiret et al. 1992). In a total reaction volume of 10 µl, 100ng of genomic DNA was used along with 50 µM of each of dNTPs, 10µM Tris-HCl (pH 9.0), 1.5 µM MgCl₂, 50µM KCl, 0.01% gelatin, 0.13 µM of each primer and 0.25 units of Taq polymerase (Bangalore Genei, Bangalore). The initial denaturation at 95°C for 5 minutes was followed by thirty-five cycles of denaturation at 95°C for 45 sec, annealing at 56°C for 45 sec and extension at 72°C for 45 sec. The final extension was carried out at 72°C for 10 minutes. The products, thus obtained, were then electrophoresed on 2% agarose gel and the results were seen by ethidium bromide staining on UV transilluminator.

**Statistical Analysis:** Data analysis was done with the help of an SPSS version 7.5. The genotypes were analyzed using chi-square analysis using 3X2 tables. Means were compared by using one-way ANOVA and the SBP and DBP in the patients were compared by t-test.

**RESULTS**

As no reports are available regarding the association between ACE I/D polymorphism and hypertension in the population of Punjab, so the present case control study examined the possible association of ACE I/D polymorphism in the hypertensive Punjabi population.

The patient group comprised of 40 males and 60 females. The mean age of the patients was 52.85 ± 11.64 and the mean height and weight of this group was 160.45 ± 17.75 (mean ± SD) and 69.6 ± 11.83 respectively. 14% of patients were smokers and 35% of the patients were taking non-vegetarian diet (Table 1).

In the control group there were 60 females and 40 males. The mean age of the group was 50.7 ± 10.07 whereas the mean height and weight were 162.27±7.74 and 64.39 ± 10.78 respectively. 10% of these were smokers and 45% were taking non-vegetarian diet (Table 1).

Table 2 shows the data pertaining to all the genotypes and the allele distribution in hypertensive patients and the normal healthy control group. The frequency of D/D heterozygotes as compared to homozygotes was higher both in the patient and control group. The results were however, statistically insignificant.

We have also analyzed the association of all the three genotypes with the phenotypic variables such as age, height and weight. Means and standard deviations for all the three genotypes were calculated and these values were compared using one-way ANOVA to see if the difference in the values was significant or not. On analysis it was seen that there was no significant difference between the age, height

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**Table 1: Demographic characteristics of subjects.**

<table>
<thead>
<tr>
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<th>Patients</th>
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<td>Females</td>
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<td>Age</td>
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* (Mean±SD)
Increased frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes in Taiwan

Ming-Chia Hsieh¹, Shiu-Ru Lin², Tusty-Jiuang Hsieh², Chin-Hsun Hsu², Hung-Chun Chen¹, Shyi-Jang Shin¹ and Juei-Hsiung Tsai¹

Departments of ¹Internal Medicine and ²Clinical Pathology, Kaohsiung Medical University, Kaohsiung, Taiwan

Abstract

Background. Diabetes is one of the major causes of end-stage renal failure in the Taiwanese population. Previous studies have shown that angiotensin-converting enzyme (ACE) inhibitor can improve glucose utilization and suppress hepatic glucose production and the renin–angiotensin system may play an important role in the initiation and progression of diabetic nephropathy. Thus, ACE gene polymorphism may be associated with type 2 diabetes and diabetic nephropathy.

Methods. To investigate the distribution of ACE-I/D genotype in type 2 diabetes and diabetic nephropathy, we examined 336 patients with type 2 diabetes (157 without nephropathy and 179 with nephropathy) and 263 age-matched normal controls. The diagnosis of nephropathy was made when daily protein loss exceeded 500 mg. ACE gene polymorphism was analysed by use of polymerase chain reaction.

Results. Our study revealed that the frequency of the D allele of the ACE gene was 29.3% in normal controls. The frequency of ACE DD genotype was significantly higher in type 2 diabetics compared with normal controls (18.2 vs 9.1%, P<0.01). The frequency of ACE DD genotype in patients with diabetic nephropathy was significantly higher than in patients without nephropathy (22.3 vs 13.4%, P<0.05). To determine whether ACE gene polymorphism was associated with the severity of diabetic nephropathy, we divided patients with diabetic nephropathy into dialysis and non-dialysis groups. The frequency of ACE DD genotype in the dialysis group was significantly higher than in non-dialysis group (28.7 vs 15.3%, P<0.05).

Conclusion. Our results indicate that the frequency of ACE DD genotype is markedly higher in patients with type 2 diabetes, and the ACE DD genotype is significantly associated with diabetic nephropathy.

Keywords: ACE genotype; diabetic nephropathy; type 2 diabetes

Introduction

The prevalence of diabetes mellitus is 3.2% in mainland China [1] and 12.4% in Taiwan [2], and more than 90% of patients suffer from type 2 diabetes. The susceptibility to type 2 diabetes is strongly inherited, as evidenced by a high concordance in twins and the strong familial aggregation. A history of type 2 diabetes in a first-degree relative doubles an individual’s risk of acquiring diabetes, and offspring of two diabetic parents have an 80% lifetime incidence of acquiring the disease [3,4]. Additional evidence for a genetic role is suggested by the wide variation in incidence of prevalence among different ethnic groups.

Diabetic nephropathy is an important cause of end-stage renal disease, accounting for 26.6% of patients on dialysis in Taiwan [5] and 4.7% in mainland China [6]. The pathogenesis of this complication is not clearly understood, but available data suggests that multiple factors such as haemodynamic alterations, metabolic abnormalities, various growth factors, and genetic factors contribute to this complication [7]. Genetic predisposition to diabetic nephropathy in patients with non-insulin-dependent diabetes mellitus (NIDDM) has been reported [8,9]. Prior studies have shown that angiotensin-converting enzyme (ACE) inhibitors can improve glucose utilization and suppress hepatic glucose production in patients with type 2 diabetes [10,11]. Also the renin–angiotensin system may play a critical role in the initiation and progression of diabetic nephropathy [12]. ACE gene polymorphism is correlated to serum and tissue ACE activity [13–17], thus, ACE polymorphism may be associated with type 2 diabetes and diabetic nephropathy.

The ACE gene consists of 26 exons and spans 21 kb on chromosome 17. The polymorphism consists of the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence within intron 16, and the D allele is associated with higher serum ACE activity [13–17]. According to previous studies, there are differences in the frequencies of I/D polymorphism in different ethnic groups [18–23]: the frequency of the D allele is considerably lower in Asians than in Caucasians. In NIDDM...
patients there are conflicting results regarding the relationship between the ACE genotype and diabetic nephropathy [24–29].

Although several studies on ACE gene polymorphism in type 2 diabetes have been performed in white and Japanese populations, the relationship between them has not been clarified. Moreover, no large-scale study of ACE gene polymorphism in type 2 diabetes has been performed in Taiwan. Therefore, our study was designed to determine whether ACE polymorphism is associated with type 2 diabetes and diabetic nephropathy in the Taiwanese population.

**Subjects and methods**

**Patient population**

The study included 263 age-matched normal controls and 336 patients with type 2 diabetes for more than 5 years. The normal controls were recruited from individuals who had a general health evaluation at the Kaohsiung Medical University Hospital. Diabetic patients were recruited from the diabetic clinic of the Metabolism Division of Kaohsiung Medical University Hospital and diabetic patients with dialysis-dependent diabetic nephropathy were recruited from the dialysis centre of Kaohsiung Medical University Hospital. All these patients received a baseline examination, blood biochemistry, urinalysis, renal sonography, chest X-ray, and retinal funduscopy. Diabetic nephropathy was defined as overt proteinuria (>500 mg/day) with or without elevated serum creatinine and hypertension. The patients with diabetic nephropathy were divided into dialysis and non-dialysis groups. Patients with renal disease other than diabetic nephropathy were excluded. All subjects were divided into four groups: group 1, normal controls; group 2, diabetes without nephropathy; group 3, diabetes with nephropathy—non-dialysis group; and group 4, diabetes with nephropathy—dialysis group.

**Determination of genotypes**

In accordance with standard methodology, genomic DNA was extracted from peripheral blood with a blood DNA kit (Puregene Genta System, Minneapolis, USA). Genomic DNA was suspended in 10 mmol Tris–HCl, 1 mmol/EDTA pH 8.0, and concentrations of DNA were measured by spectrophotometry.

To determine the ACE genotype of the patients, a genomic DNA fragment on intron 16 of the ACE gene was amplified by polymerase chain reaction (PCR) using a flanking primer pair and a primer pair that recognizes insertion-specific sequence. The sequence of flanking primer pair used was 5′-CTGGAGACCACCTCCCATCCCTTCG-3′ and 5′-GATGTGGCCATCACATTGGTG-3′) and 5′-GATGTGGCCATCACATTGGTG-3′) PCR amplification products were obtained using 50 μl reactions (1 μg genomic DNA, 500 pmol of primers, 0.5 mmol/l each of deoxy-ATP, GTP, CTP, and TTP, and 3 mmol/l MgCl₂; 1 U Taq DNA polymerase (Boehringer Mannheim); 50 mmol/l KCl; 0.001% gelatin; and 10 mmol/l Tris–HCl pH 8.3) with 4 min of denaturation at 94°C, followed by 35 cycles of 15 s at 94°C, 5 s at 67°C (annealing), and 30 s at 74°C (extension) in a thermal cycler (PTC-100, MJ Research, Watertown, MA, USA). Reaction was terminated at 72°C for 2 min. Fragments without insertion (D allele) and with insertion (I allele) of

199 and 479 bp were separately detected on a 3% agarose gel containing ethidium bromide. To increase the specificity of DD genotyping, PCR amplifications were performed with an insertion-specific primer pair (5′-TCTCGGCTC-3′ and 5′-GATGTGGCCATCACATTGGTG-3′) mixed in 25 μl reaction (0.5 μg genomic DNA, 500 pmol of primers; 0.5 mmol/l each of deoxy-ATP, GTP, CTP, TTP, and 1.5 mmol/l MgCl₂; 0.5 U Taq DNA polymerase (Boehringer Mannheim); 50 mmol/l KCl; 0.001% gelatin; and 10 mmol/l Tris–HCl pH 8.3) with 4 min of denaturation at 94°C, followed by 35 cycles of 15 s at 94°C, 5 s at 62°C (annealing), and 30 s at 74°C (extension). Only the I allele produces a 335 bp amplicon. The 335 bp fragment was identified on a 3.0% agarose gel containing ethidium bromide. The reaction yields no product in the sample of DD genotype.

**Statistical analysis**

All values are reported as means ± SEM. The levels of the variables between each group were compared by analysis of variance (ANOVA). Genotype distribution and allele frequencies between groups were analysed by a χ² test. A P value <0.05 was considered statistically significant.

**Results**

At baseline the four groups were similar regard to sex, age, and body mass index (Table 1). Diabetic patients had higher blood pressures, and diabetic patients with nephropathy had significantly higher systolic pressures than patients without nephropathy. The serum triglyceride level was higher in diabetic patients (groups 2–4) than in normal controls (P<0.001). The serum cholesterol concentration was significantly higher in groups 2 and 3 than in group 1 (P<0.001). The HbA₁c and duration of diabetes were not different between diabetic groups (groups 2–4).

Table 2 shows the ACE genotype distributions of normal controls and patients with type 2 diabetes. The frequency of the ACE D allele was 29.3% in normal controls, however, the ACE DD genotype was more frequent in diabetic patients (18.2% vs 15.3%, P<0.05) and dialysis (44.3% vs 34.7%, P<0.05) groups. The diabetic clinic of the Metabolism Division of Kaohsiung Medical University Hospital. The study included 263 age-matched normal controls and 336 patients with type 2 diabetes. The characteristics of the patients with type 2 diabetes according to ACE genotype are shown in Table 5. There were no significant differences with respect to age, body mass index, blood pressures, blood levels of cholesterol and triglyceride, HbA₁c, and duration of diabetes. The percentages of nephropathy (65.6% vs 48.2%, P<0.05) and dialysis (44.3% vs 21.1%, P<0.01) were higher in patients with ACE DD genotype than in patients with ACE II genotype.
The echocardiographic assessment of the right ventricle: what to do in 2010?

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For many years, the echocardiographic quantitative assessment of right ventricular (RV) function has been difficult owing to the complex RV anatomy. Identifying an accurate and reliable echocardiographic parameter for the functional assessment of the RV still remains a challenge. The review presents a summary of the most studied and presently used parameters of RV function, with their reported normal values, as well as advantages and limitations of use. Combinations of these parameters are used in daily clinical practice, each one offering only partial information about the status of the RV. Myocardial velocity and strain rate imaging have promising results in the assessment of RV function. There is hope that novel myocardial deformation parameters and three-dimensional echocardiography-derived parameters may add value to the examination of the RV, but validation studies are still needed.

Keywords Right ventricle • Echocardiography • Tissue Doppler • Strain rate imaging • 3D echocardiography

Introduction

For decades, the right ventricle (RV) has been considered ‘dispensable’ for cardiac function and consequently ignored. The introduction of the Fontan procedure for complex congenital heart disease in 1968, a technique that directly connects the right atrium to the pulmonary artery, thus ‘bypassing’ the RV, cemented this belief. Only in the second half of the past century, after recognizing its key role in various physiological and pathological conditions, the RV regained attention. The RV performance defines prognosis in patients with congenital heart disease. In this population group, the RV may be subjected to either volume (atrial septal defect, pulmonary, and/or tricuspid regurgitation) or pressure overload (pulmonary stenosis, atrial switch operations, congenitally corrected transpositions). Assessing RV morphology and function is of paramount importance in acquired diseases as well. The RV has a great impact on the prognosis of patients with pulmonary hypertension, myocardial infarction involving the RV, and left ventricular (LV) dysfunction.

Echocardiography, being non-invasive, widely available, relatively inexpensive, and having no side effects, is the modality of choice for the assessment of morphology and function of the RV in clinical practice. Recent developments have provided several new methods for analysing the RV, each having advantages and disadvantages. Doppler myocardial imaging (DMI), speckle tracking, or 3D echocardiography (3D Echo) are some of the techniques that may now add to a better understanding of RV function.

In this article, we review the currently available echocardiographic techniques and parameters for RV assessment in clinical practice and for research purposes, with a focus on acquired heart diseases.

Anatomy and physiology of the right ventricle

The RV is positioned directly behind the sternum, anterior to the left ventricle (LV). It has a complex geometry, appearing triangular when viewed from the front, and crescentic when viewed in a transverse section of the heart, with the septum being the most important determinant of shape. Under normal loading conditions, the septum arches into the RV both in systole and diastole. This complex geometry cannot be fitted to simple geometric models, which presents important limitations for the estimation of RV volume and function based on two-dimensional (2D) tomographic views.

In a normally developed RV with atroventricular and ventriculo-arterial concordance and normal tricuspid and pulmonary valves, three anatomical parts of the RV can be distinguished: the inlet part which accommodates the tricuspid valve, the trabeculated apical...
areas can be determined.\textsuperscript{37} In normal individuals, RV area and mid-cavity diameter should be smaller than those of the LV, thus allowing a simple visual assessment of RV area.

Assessment of the structure and architecture of the RV walls can identify features which suggest a particular aetiology, such as RV infarct or ARVC. For instance, the presence of localized RV free wall aneurysms is a major diagnostic criteria for ARVC, while a high degree of trabeculation, increased thickness of the moderator band with a hyperechogenic appearance, and RVOT dilatation can also support this diagnosis.\textsuperscript{38} However, on the basis of visual echocardiographic assessment solely, identification of functional abnormalities is inaccurate, frequently resulting in false-positive findings,\textsuperscript{39,40} and newer echocardiographic techniques help in a more accurate evaluation.

The maximum limit for normal thickness in the RV free wall is 5 mm,\textsuperscript{31} above which the ventricle is considered to be hypertrophied. Right ventricular hypertrophy can be seen in various pathological states: RV pressure overload, biventricular hypertrophic cardiomyopathies, and deposit diseases. For RV mass quantification, real-time 3D echocardiography (RT3DE) is superior to 2D Echo,\textsuperscript{46} and post-processing of full-volume datasets can lead to LV mass and volume calculation.\textsuperscript{41} The excellent accuracy and reproducibility of cardiac magnetic resonance imaging (MRI) is well established, making MRI a gold standard technique in quantifying the RV chamber.\textsuperscript{16,42,43} However, it remains confined to experienced centres and involves high costs. Several studies showed good correlations between RT3DE and MRI-measured RV volume, ejection fraction, and mass.\textsuperscript{40}

**Assessment of right ventricular function**

**Global right ventricular function**

**Standard parameters**

Unlike the LV, where biplane methods are accepted and widely used for a global assessment of systolic function, a quantitative approach towards evaluating RV global function is more difficult to achieve owing to its more complex shape.\textsuperscript{44} Therefore, surrogate parameters were developed and were further validated against ejection fraction derived using isotopic methods or MRI.

Right ventricular outflow tract shortening fraction (RVOT-SF) is obtained from a parasternal short-axis view at the base of the heart where the end-diastolic RV outflow tract diameter (EDRVOTD) and end-systolic RVOT diameter (ESRVOTD) can be measured and the shortening fraction is calculated using the formula: RVOTSF (%) = (EDRVOTD − ESRVOTD)/EDRVOTD (Figure 4A). Lindqvist et al.\textsuperscript{45} found that RVOT fractional shortening correlates well with longitudinal function, pulmonary pressure gradient, and RV-right atrial (RA) pressure gradient. Care must be taken when measuring this parameter, as there are no defined landmarks for orientating the image with precision, and thus significant inaccuracies may result from oblique plane acquisitions.

Right ventricular fractional area change (RVFAC) expresses the percentage change in RV area between end-diastole and end-systole. It is obtained from a four-chamber view where the RV end-diastolic (RVEDA) and end-systolic areas (RVESA) are measured, and the RVFAC is calculated as follows: RVFAC (%) = (RVEDA − RVESA)/RVEDA (Figure 4B). It has a good correlation with MRI-derived RVEF and was shown to have prognostic significance in patients with myocardial infarction and pulmonary hypertension.\textsuperscript{46–48} Its main limitation is related to the need of good endocardial border delineation, which can be difficult to achieve in the highly trabeculated RV.

**Tricuspid annular plane systolic excursion (TAPSE)** has proved a useful index for evaluating RV longitudinal function. It is especially attractive in clinical practice given the ease with which it is measured using an M-mode cursor passed through the tricuspid lateral annulus in a four-chamber view (Figure 5A and 8). This parameter measures the extent of systolic motion of the lateral portion of the tricuspid ring towards the apex. It has been shown to have a good correlation with isotopic derived RVEF,\textsuperscript{57} although Anavekar et al.\textsuperscript{49} failed to find any correlation between TAPSE and MRI-derived ejection fraction. Normal values for TAPSE are 15 – 20 mm. The prognostic value of TAPSE was emphasized in cardiac failure and myocardial infarction.\textsuperscript{50,51} Samad et al.\textsuperscript{52} assessed TAPSE in patients after a first acute myocardial infarction, and showed that TAPSE ≤15 mm was associated with increased mortality (45% at 2 years) compared with patients having TAPSE >20 mm (4%). Although simple to use, TAPSE has some inherent limitations mostly because assessment is restricted to the longitudinal function of the RV free wall, disregarding the contribution of the interventricular septum and the RVOT.\textsuperscript{53} As TAPSE is measured relative to transducer position and was shown to be influenced by the functional status of the LV,\textsuperscript{54} care must be taken when interpreting this parameter in longitudinal studies of patients undergoing procedures that affect the overall heart motion (cardiac surgery).\textsuperscript{55}

The myocardial performance index (MPI, Tei index) differs from the previously described parameters in that it is derived from physiological rather than structural features. It is calculated as the ratio between the sum of the times of the isovolumic periods and the ejection time for the RV.\textsuperscript{56} The MPI is a parameter of global function, combining information on both systole and diastole. Unlike the left heart, where these time intervals can be determined during the same cardiac cycle (owing to the possibility of aligning the mitral and the aortic valves in the same view), measuring MPI for the right heart using conventional Doppler techniques is less accurate, as it needs at least two different cardiac beats for determining the time periods. The ejection time can be determined from the parasternal short-axis view at the pulmonary valve, while isovolumic intervals are derived based on the tricuspid flow. Myocardial performance index was shown to correlate with radionuclide-derived RVEF.\textsuperscript{57} Normal values for MPI are 0.28 ± 0.04, and it usually increases in diseases associated with RV dysfunction.\textsuperscript{58} Furthermore, it was shown to be useful in the longitudinal follow-up of patients with chronic thrombo-embolic pulmonary hypertension who undergo pulmonary thrombendarterectomy, in whom RV MPI decreases after treatment.\textsuperscript{59,60} However, the use of this index is limited by the absence of the isovolumic periods in the normal RV as well as the pseudonormalization of the index when RA pressure is increased as shown by Yoshifuku et al.\textsuperscript{61} (Figure 6). The increased RA pressure determines a shortening of the IVRT that will result in a decreased value of the MPI index.

Another parameter of contractility is RV dP/dt measured on the tricuspid regurgitation envelope. Although it was shown to be highly load-dependent and not reflecting the contractile status of
areas can be determined. In normal individuals, RV area and mid-cavity diameter should be smaller than those of the LV, thus allowing a simple visual assessment of RV area.

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Whether myocardial deformation parameters derived from the RV have added value in daily clinical routine remains a question to be answered, especially given that only small populations have been investigated and normal values are yet to be established.

Visual assessment of RV regional function has proved useful in the diagnosis of patients with PE. McConnell et al.\(^9\) described a specific pattern of RV dysfunction in patients with PE, with severe hypokinesia of the mid and basal segments of the RV free wall and hyperkinesia of the apical portion of the same wall. The specificity and sensitivity of this sign for diagnosing PE were 77 and 94%, respectively. However, when validated against helical CT diagnosis of PE, the sensitivity of the McConnell sign was very poor (16%) even if with a high specificity (96%).\(^9\) Moreover, Casazza et al. showed that regional free RV wall dysfunction was similar in acute PE and RV infarction.\(^9\)\(^,\)\(^9\)

**Right ventricular diastolic function**

Several echocardiographic parameters can be determined for evaluating RV diastolic function, but less data exist regarding their accuracy.

*The tricuspid inflow pattern is obtained in a four-chamber view by placing a cursor at the tips of the tricuspid valve. It is similar to the mitral pattern, although velocities are smaller and there are marked inspiratory variations (Figure 12).*\(^9\)

The transhepatic flow in a normal individual is characterized by the presence of the three waves: S, D, and A, the latter corresponding to atrial contraction. The fraction of hepatic flow measured as time-velocity integrals (TVIs) of the S and D waves: TVI S/(TVI S + TVI D) was shown to correlate with RA pressures. Furthermore, values below 55% predict an RA pressure of above 8 mmHg with good sensitivity and specificity. Nagueh et al.\(^\)\(^6\) showed that hepatic venous flow dynamics relate best among several parameters (e.g. tricuspid flow, hepatic flow, and inferior vena caval diameters) to mean RA pressure and can be used clinically to estimate mean RA pressure.

**Estimation of RA pressure is most often done by assessing the IVC diameter and its degree of collapse with inspiration.\(^1\)**

TDI was also employed in analysing diastolic function in the RV. Using the ratio between the tricuspid flow early diastolic velocity (E) and peak early diastolic velocity of the lateral tricuspid annulus (E'), the E/E' ratio was found to have a good correlation with mean invasively measured RA pressure (r = 0.75; P<0.001). An E/E' > 6 had a sensitivity of 79% and a specificity of 73% to detect an RA pressure >10 mmHg.\(^1\)

**Conclusions**

For many years, the echocardiographic quantitative assessment of RV function has been difficult owing to the complex RV anatomy. Identifying an accurate and reliable echocardiographic parameter for the functional assessment of the RV still remains a challenge. Myocardial velocity and strain rate imaging have promising results in the assessment of RV function. In Table 2, we present a summary of the most studied and presently used parameters of RV function, with their reported normal values, as well as advantages and limitations of use. Combinations of these parameters are used in daily clinical practice, each one offering only partial information about the status of the RV. There is hope that novel myocardial deformation parameters and 3D Echo-derived parameters may add value to the examination of the RV, but validation studies are still needed.
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Estimation of RA pressure is most often done by assessing the IVC diameter and its degree of collapse with inspiration.\textsuperscript{13}

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Genetic polymorphisms of the renin–angiotensin system and complications of insulin-dependent diabetes mellitus

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Abstract

Objective. Patients with insulin-dependent diabetes mellitus (IDDM) have a high risk of developing diabetic nephropathy, retinopathy and cardiovascular diseases. The contribution of gene polymorphisms of the renin angiotensin system to these complications is controversial and may differ among populations.

Methods. In 257 Dutch IDDM patients (188 with urinary albumin excretion (UAE) <30 mg/24 h), logistic regression analysis was used to study the relationships among, on the one hand, the insertion/deletion gene polymorphism of the angiotensin-converting enzyme gene (ACE-ID), the M235T gene polymorphism of the angiotensinogen gene (AGT-M235T), and the A1166C gene polymorphism of the angiotensin type 1 receptor gene (AT1-A1166C), and, on the other hand, UAE, retinopathy, hypertension, and coronary heart disease.

Results. The T-allele of the AGT-M235T polymorphism was associated with an increased risk of an elevated UAE (odds ratio (OR) 3.03; 95% confidence interval (CI) 1.06–8.61), but only when interaction with the D-allele of the ACE-ID polymorphism was considered. A previously described positive interaction between the T-allele of the AGT-M235T polymorphism and the D-allele of the ACE-ID polymorphism could not be confirmed. The T-allele was also associated with an increased risk of retinopathy (OR 3.89, 95% CI 1.79–8.47). The CC-genotype of the AT1-A1166C polymorphism was associated with hypertension (OR 3.58; 95% CI 1.23–10.37).

Conclusions. In a Dutch IDDM population, including 69 patients with (incipient) diabetic nephropathy, the T-allele of the AGT-M235T polymorphism is associated with an elevated UAE and diabetic retinopathy and the CC-genotype of the AT1-A1166C polymorphism is associated with hypertension. A previously described interaction between the AGT-M235T and the ACE-ID polymorphisms could not be confirmed. Since the number of nephropathic patients in this study is small, these conclusions must be interpreted with caution.

Keywords: insulin-dependent diabetes mellitus; complications; angiotensin-converting enzyme gene polymorphism; angiotensinogen gene polymorphism; angiotensin II type 1 receptor gene polymorphism

Introduction

Patients with insulin-dependent diabetes mellitus (IDDM) have a high risk of developing severe complications, such as diabetic nephropathy, retinopathy and cardiovascular disease. Studies demonstrating familial clustering of diabetic nephropathy, cardiovascular disease and hypertension [1–3] suggest that, in addition to poor glycaemic control, genetic factors may affect susceptibility to the development of diabetic micro- and macroangiopathy. In this context, genetic polymorphisms of the renin–angiotensin system (RAS) are attractive candidates to be studied, since inhibition of the activity of this system has shown to retard the development of diabetic complications, such as nephropathy and retinopathy [4,5].

Three genetic polymorphisms of the RAS have been studied. One meta-analysis of the insertion/deletion gene polymorphism of the angiotensin-converting enzyme gene (ACE-ID) has shown that, in IDDM patients, the D-allele is associated with diabetic nephropathy in a dominant model [6], although two other meta-analyses showed only a tendency towards this relationship [7] or severe heterogeneity impairing pooling of the data available [8]. The DD-genotype has been shown to be associated with an increased risk of coronary heart disease in IDDM patients, and an
increased risk of myocardial infarction in NIDDM patients [9], whereas the excess risk for myocardial infarction due to this genotype in a large general population was at most 10% [10]. Another gene polymorphism of the RAS, the angiotensinogen gene M235T polymorphism (AGT-M235T), has not been associated with an increased risk of diabetic nephropathy in a recent meta-analysis [11]. However, for this meta-analysis, the results of just six studies in IDDM patients and six studies in NIDDM patients were available and the association between nephropathy and the AGT-M235T polymorphism was not analysed in IDDM patients separately. The available studies of this polymorphism in IDDM patients showed an increased risk of diabetic nephropathy in some studies [12,13], but not in all [14–16]. In addition, a third gene polymorphism, the A1166C polymorphism of the angiotensin-II-type 1 receptor gene (AT1-A1166C), was not associated with diabetic nephropathy or retinopathy in Caucasian IDDM patients [17]. However, this genotype was associated with coronary artery stenosis in another study of diabetic patients (probably NIDDM) [18]. Interactions between the D-allele of the ACE-ID polymorphism and the T-allele of the AGT-M235T polymorphism have been found to be associated with nephropathy in IDDM patients with proliferative retinopathy [12], but have not been studied extensively.

Thus, with regard to the major complications of IDDM, the contribution of and the interactions among several gene polymorphisms of the RAS have not been fully elucidated, and may in fact differ among populations. Therefore, we analysed the associations of three gene polymorphisms and diabetic complications in a group of Caucasian IDDM patients in The Netherlands.

Methods

All Caucasian IDDM patients older than 17 years who visited the outpatient clinic of the Department of Medicine of the Isala Clinics, Wezenlanden Location in Zwolle between September 1993 and March 1994 were asked to participate in the study. A total of 268 patients gave informed consent. Clinical characteristics were gathered from the records and from interviewing the patients. IDDM was defined as onset of diabetes before the age of 30 years and necessity of insulin therapy within 6 months after the onset of the disease. Patients were classified according to the amount of albuminuria using the median result of three 24-h urine collections. Urinary albumin excretion (UAE) was determined with a nephelometric technique (Beckmann Instrument Inc., Brea, CA, USA). Normoalbuminuria was defined as UAE < 30 mg/24 h; microalbuminuria as UAE 30–300 mg/24 h, and macroalbuminuria as UAE ≥ 300 mg/24 h. Smoking was defined as more than one cigarette, cigar or pipe per day. Retinopathy was assessed by an experienced ophthalmologist and graded as: none, background, non-proliferative or proliferative. Coronary heart disease was defined as history of treatment or admission for myocardial infarction, unstable angina pectoris (diagnosis made by an experienced cardiologist), percutaneous transluminal coronary angioplasty or coronary artery bypass graft, or stable angina pectoris with angiographic evidence of significant (> 50%) coronary artery stenosis or positive exercise ECG-test result. Data on stroke and transient ischaemic attacks were not collected. The study was approved by the medical ethics committee of the Wezenlanden Ziekenhuis and all patients gave informed consent.

On the day of the study visit, patients took their regular diet and medication. In the hospital height and weight were determined to calculate the body mass index (BMI). Blood pressure was taken at the right arm in the sitting position after 5 min of rest using a sphygmomanometer with appropriate cuff size. The first Korotkoff sound was taken as the systolic and the fifth Korotkoff sound as the diastolic blood pressure. Patients were considered hypertensive if blood pressure exceeded the limit of 140/90 mmHg at two or more occasions and/or if they used antihypertensive medication. Blood samples were drawn for measurement of serum creatinine, serum cholesterol, HbA1c and plasma ACE level, and for the determination of the genotypes.

Serum creatinine was determined with the Jaffé reaction with a Hitachi 1717 automatic analyser; HbA1c with high-pressure liquid chromatography (normal range 3.4–6.5%). Serum ACE levels were measured spectrophotometrically based on the decrease of extinction at a wavelength of 340 nm [19].

Determination of genotypes

Genomic DNA was obtained from the white cell pellets using an isoamylalcohol–chloroform extraction. To determine the ACE-ID genotype, DNA (100 ng) was amplified using the polymerase chain reaction (PCR). Thirty-two cycles of amplification were performed with a High Bas DNA Thermal Cycler with denaturation for 1 min at 94°C, annealing for 1.5 min at 58°C and extension for 2 min at 72°C. Each 50 μl reaction mixture contained 1.0 μl 50 mM magnesium chloride, 5.0 μl 1% W-1, 2.5 μl DMSO, 5.0 μl 2 mM dNTP polymerization mix, 0.2 μl 10× Taq DNA polymerase and 100 ng unlabelled primers. The PCR primers with the sequences reported by Tiret [20] were used: ACE-1 24-mer Isogen Bioscience (5'-CTGAGACCATCCATCTCTTTC-3') and ACE-2 25-mer Isogen Bioscience (5'-GATGTCGAACATCATGCATGCAGAT-3'). The reaction products were electrophoresed on 2% agarose gels and stained with ethidium bromide. Under ultraviolet light two bands, insertion (I; 490 bp) and deletion (D; 190 bp) were visible. Subjects were classified according to the presence or absence of a 287 bp base pair insertion in intron 16 of the ACE gene as II, ID or DD. Preferential amplification of the smaller 190 bp deletion allele (D) in ID heterozygotes has led to their mistyping as DD homozygotes [21]. To exclude this possibility, all ID homozygotes were retyped using an I-specific sense primer (5'-TTTAGAGACGGAGTCTCGCTC-3') with modification in reaction conditions such that denaturation took place at 93°C for 1 min, annealing at 68°C for 1.5 min, and extension at 72°C for 2 min. When a ‘DD’ sample amplified using the I-specific primer, it was recoded ‘ID’.

Determination of the AGT-M235T genotype was performed by enzymatic amplification of DNA with the PCR described above using the unlabelled primers ANGOL 1C Isogen Bioscience (5'-GCTGTCCACACTGGACCACCC-3') and ANGOL 2D Isogen Bioscience (5'-GTCGCAGGGCTTGCTCTTCT-3'). The PCR products were digested with the restriction enzyme Aspl at 37°C for 2 h. DNA fragments were separated by electrophoresis on a 2%/agarose gel stained with ethidium bromide and were visualized using