Protective effects of angiotensin-converting enzyme I/I and matrix metalloproteinase-3 6A/6A polymorphisms on dilatative pathology within the ascending thoracic aorta

Vaiva Lesauskaitea,*, Giedre Sinkunaite-Marsalkienea, Abdonas Tamosiunasb, Rimantas Benetisc

aLaboratory of Molecular Cardiology, Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania
bLaboratory of Population Research, Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania
cDepartment of Cardiac, Thoracic and Vascular Surgery, Kaunas University of Medicine, Kaunas, Lithuania

Received 21 June 2010; received in revised form 5 October 2010; accepted 7 October 2010; Available online 15 December 2010

Abstract

Objective: Activation of matrix metalloproteinases and the renin/angiotensin signaling pathways is under investigation with regard to their potential pathogenesis in dilatative pathology of the aorta. The purpose of this study was to explore matrix metalloproteinase-3 5A/6A and angiotensin-converting enzyme I/D polymorphisms as predisposing factors to dilatative pathology of the aorta.

Methods: We studied 107 patients who underwent aortic reconstruction surgery due to dilatative pathology of ascending thoracic aorta and a random sample of the population (n = 773), all from Lithuania. The insertion/deletion (−1171 5A/6A) polymorphism in the promoter region of matrix metalloproteinase-3 studied by real-time polymerase-chain-reaction amplification and the D and I alleles were identified on the basis of standard polymerase-chain-reaction amplification of the respective fragments from intron 16 of the angiotensin-converting enzyme gene.

Results: The frequency of the angiotensin-converting enzyme D allele was significantly higher in dilatative pathology of ascending thoracic aorta patients than in the reference group subjects (0.55 vs 0.48, respectively). The latter group had a significantly higher frequency of the angiotensin-converting enzyme I/I genotype than in dilatative pathology of ascending thoracic aorta patients (27.4% vs 16.5%, respectively). In the reference group, the frequency of combined angiotensin-converting enzyme I/I and matrix metalloproteinase-3 6A/6A genotypes was 7.5%, while in the dilatative pathology of ascending thoracic aorta patient group, there was no one carrying that combined genotype (p = 0.001).

Conclusions: The present study showing a role of angiotensin-converting enzyme and matrix metalloproteinase-3 in the development of dilatative pathology of ascending thoracic aorta permits us to entertain a possible protective mechanism for the combined effects of the angiotensin-converting enzyme I/I and the matrix metalloproteinase-3 6A/6A genotypes.

© 2010 European Association for Cardio-Thoracic Surgery. Published by Elsevier B.V. All rights reserved.

Keywords: Thoracic aorta; Aneurysm; Matrix metalloproteinase-3; Angiotensin-converting enzyme; Polymorphism

1. Introduction

Exhaustive research has been done on the basic etiologic and pathogenic mechanisms underlying thoracic aortic aneurysms. Studies have ascertained associations with particular genetic syndromes such as the mutations in filamin-A associated with the Loeys–Dietz syndrome, with the Ehlers–Danlos syndrome, or with fibrillin-1 producing the Marfan syndrome. Dilatative pathology within the ascending thoracic aorta (DPATA) can also be caused by inflammation as in giant cell arteritis or by atherogenesis. Some clinically important signal transduction pathways that can lead to disease progression, that is, activation of matrix metalloproteinases (MMPs) [1] or the renin/angiotensin signaling pathways [2], are under investigation with regard to their potential pathogenesis in dilatative pathology of the aorta. It was shown that MMPs are involved in vascular remodeling, and these appear to be active agents degrading extracellular matrix proteins with the appearance of phenotypic changes in smooth muscle cells (SMCs) [3]. MMPs are a family of zinc-dependent proteases capable of degrading extracellular matrices, basement membranes, and other proteins. MMP-3 (stroma-
lysin-1) degrades a broad substrate spectrum, including certain major constituents of the arterial wall (fibronectin; collagen types IV, V, IX, and X; gelatins; laminins; elastin; and proteoglycan proteins). Thus, MMP-3 may of be particular significance in arterial wall remodeling [4]. MMP-3 has a common polymorphism in the promoter region (−1171 bp), with one allele containing a run of six adenosines (6A) and the other allele having only five adenosines (5A). It was shown that an increased number of 5A alleles leads to increased expression of MMP-3 [5,6], and higher plasma MMP-3 activity [7].

Angiotensin-converting enzyme (ACE) is a key player in the renin–angiotensin system (RAS). Both experimental and clinical studies have shown the role of ACE and ACE-generated proteins in vascular remodeling during restenosis, hypertension, atherosclerosis, and aneurysm formation [8]. It was shown that angiotensin 2 modulates MMP expression in arterial and cardiac remodeling [9,10], and this has drawn attention to a link between the RAS pathway and MMP expression. Therefore, the present study explores MMP-3 5A/6A and ACE I/D polymorphisms as predisposing factors to DPATA.

2. Materials and methods

2.1. Study population

The study enrolled 107 patients (79 males, 28 females) who underwent aortic reconstruction due to DPATA at the Department of Cardiac, Thoracic, and Vascular Surgery, Kaunas University of Medicine Hospital, Lithuania in 2004—2008. The patients with aortitis (n = 2) and Marfan syndrome (n = 2) were excluded from further study. The mean age of patients was 60.9 ± 12.5 years. The patients were operated on due to dissection of the thoracic aorta (n = 32), poststenotic dilatation of the ascending aorta due to aortic stenosis (n = 26), or chronic aneurysm of the thoracic aorta (n = 45). There were 30 (29.1%) and 71 (68.9%) patients with bicuspid or tricuspid aortic valve, respectively. Two patients had aortic valve prostheses. History of hypertension and diabetes was assessed by review of each patient’s medical history. All patients underwent preoperative transthoracic echocardiography. Specimens of aortic tissue taken during aortic reconstruction surgery were fixed in 10% neutral buffered formalin for 24 h and then processed for routine paraffin embedding.

The reference group recruited 773 gender- and age-matched subjects (mean age 60.2 ± 7.6 years) from a random sample of the population of Kaunas screened within the international HAPIEE (Health, Alcohol and Psychosocial factors In Eastern Europe) study [11]. The random sample is representative of the Lithuanian urban population.

Information on (1) hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg); (2) whether the person had taken anti-hypertensive drugs within the prior 2 weeks); and (3) assessment of a history of diabetes (diabetes mellitus was determined either by self-reporting of the responders to the question: ‘Has a doctor ever told you that you have diabetes?” and/or fasting glucose level ≥7.8 mmol l−1) was taken from the epidemiological screening data. During screening, venous blood was taken in 3-ml K3 ethylene diamine tetraacetic acid (EDTA) vacutainers for genetic investigation. These samples were stored at −80 °C for subsequent DNA extraction.

Ethical approval was obtained from the Ethics Committee of Kaunas University of Medicine and Lithuanian Bioethical Committee (March 23, 2006, No. 11).

2.2. DNA extraction

The standard procedure [12] was used to extract genomic DNA from aortic wall sections taken during aortic reconstruction surgery. Genomic DNA from blood was isolated by using a Sorpoclean Genomic DNA Extraction Module kit (Sorpo Diagnostics, Vilnius, Lithuania), according to the manufacturer’s instructions. DNA quality and concentration were estimated by spectrophotometric analysis and by ethidium bromide-stained agarose gel under ultraviolet light. The latter method was used to evaluate the level of DNA degradation in the samples from paraffin-embedded aortic tissue. Photographs were documented through a video documentation system, the BioDocAnalyse 2.0 (Biometra, Göttingen, Germany).

2.3. Determination of genotypes

The D and I alleles were identified on the basis of polymerase-chain-reaction (PCR) amplification of the respective fragments from intron 16 of the ACE gene, according to the method of Rigat et al. [13].

The flanking oligonucleotide primer pairs used to characterize the I/D polymorphism and produce a 490-bp fragment, corresponding to the I allele, and a 190-bp fragment, corresponding to the D allele were as follows: 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′-GAT GTG GCC ATC ACA TTC GTC AGAT-3′ (TibMolBiol, Berlin, Germany). D/D homozygotes were amplified again with an insertion-specific primer pair. A 335-bp PCR product only in the presence of the I allele was amplified using 5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ as sense primer and 5′-TCG CCA GCC TTC CCA TGC CCATAA-3′ as antisense primer [14].

The insertion/deletion (−1171 5A/6A) polymorphism in the promoter region of MMP-3 was studied by real-time PCR as described in a previous publication [15].

2.4. Statistical analysis

Data on age were presented as mean ± SD. Differences between groups were analyzed with the unpaired Student’s t-test. Chi-square analyses were used to test deviations of genotype distribution from the Hardy–Weinberg equilibrium. Allele and genotype frequencies were determined between random samples from the urban population and DPATA patients. The two-tailed Fisher’s exact test was applied to analyze combined genotype distribution between the control and patient groups. Statistical analyses were done using the Statistical Package for Social Sciences (SPSS) statistical package (version 12.0; SPSS Inc., Chicago, IL, USA).
Table 1. Demographic and clinical characteristics of study participants.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DPATA patients (n = 103)</th>
<th>Reference group (n = 773)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.9 ± 12.5</td>
<td>60.2 ± 7.6</td>
<td>0.41</td>
</tr>
<tr>
<td>Males</td>
<td>78 (75.7%)</td>
<td>563 (72.8%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Hypertension</td>
<td>78 (75.7%)</td>
<td>582 (75.3%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (4.8%)</td>
<td>56 (7.2%)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Data are presented as number of patients (percentage) or mean ± SD; DPATA: dilatative pathology of ascending thoracic aorta.

3. Results

The demographic and clinical characteristics of the DPATA patients and reference group subjects are reported in Table 1. These data did not differ according to age, gender, hypertension, and diabetes.

Distribution of ACE I/D and MMP-3 5A/6A genotypes in the reference group was according to the Hardy–Weinberg equilibrium (Tables 2 and 3). The frequency of the ACE D allele was significantly higher in DPATA patients than in the reference group subjects (0.55 vs 0.48, respectively). The latter group had a significantly higher frequency of the ACE I/I genotype than DPATA patients (27.4% vs 16.5%, respectively).

There were no significant differences in the distribution of MMP-3 5A/6A genotypes and the frequency of 5A allele in DPATA and reference groups (Table 3).

The distribution of ACE and MMP-3 genotypes is presented together in Table 4. In the reference group, the frequency of combined ACE I/I and MMP-3 6A/6A genotypes was 7.5%, while in the DPATA patient group, there was no one carrying that combined genotype (p = 0.001). There were no other significant differences in the distribution of combined ACE I/D and MMP-3 5A/6A between reference and DPATA groups.

4. Discussion

Multiple studies have proven that MMP-mediated extracellular matrix degradation is implicated in the pathogenesis of thoracic aneurysm formation, and these MMPs are produced by medial SMCs [3]. In our study, we investigated MMP-3 5A/6A gene polymorphism to test the hypothesis that 5A alleles leading to increased expression of MMP-3 might be a predisposing condition for the development of DPATA. Our results show that the frequency of the MMP-3 promoter 5A/6A genotypes did not differ between the group of patients with DPATA and the reference group. There were also no significant differences in 5A allele frequency between both groups. We were not able to recover a publication documenting the prevalence of MMP-3 5A/6A polymorphism in patients with DPATA in available databases (those searched included Medline (PubMed), ScienceDirect, Wiley InterScience, Blackwell Synergy, and Oxford University Press (Oxford Journals)). Some studies of patients with abdominal aortic aneurysm indicate that the presence of the 5A allele predisposes to aneurysmal disease [5], while others did not prove such an association [16]. According to Deguara et al. [5], the frequency of MMP-3 5A/5A, 5A/6A, and 6A/6A genotypes in patients with abdominal aortic aneurysm was 30.4%, 51.4%, and 18.2%, respectively. Thus, the frequency of the 5A allele was significantly higher in patients with abdominal aortic aneurysm compared with the control population (0.56 vs 0.49, p = 0.0053). In our study, the frequency of MMP-3 5A/6A genotypes and the 5A allele frequency in patients with DPATA and the reference group (Table 3) did not differ significantly from those reported by Deguara et al. [5] in patients with abdominal aortic aneurysm and the control group, respectively. It could be argued that to prove a 5A allele association with DPATA may require a greater number of patients than we were able to include in the study.

Our study covered 107 patients with DPATA, while Deguara et al. [5] studied 405 patients with abdominal aortic aneurysm. The present study demonstrated that the D allele of ACE is much more frequent in patients with DPATA in comparison to the reference group. The D allele of the ACE gene is associated with increased serum levels of circulating ACE. The latter is decreased by insertion polymorphism [13]. There is some controversy about the ACE I/D genotype association with regard to acute aortic dissection. Some researchers claim an association of the D allele with aortic dissection [17], while others find no such association [18]. We did not analyze ACE I/D genotypes in DPATA because of a small number of patients than we were able to include in the study.

Table 2. ACE I/D genotype distribution and D allele frequency.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ACE genotypes n (%)</th>
<th>D allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I/I</td>
<td>I/D</td>
</tr>
<tr>
<td>DPATA group (n = 103)</td>
<td>17 (16.5%)*</td>
<td>58 (56.3%)</td>
</tr>
<tr>
<td>Reference group (n = 773)</td>
<td>212 (27.4%)</td>
<td>382 (49.4%)</td>
</tr>
<tr>
<td>p value</td>
<td>0.009</td>
<td>0.09</td>
</tr>
</tbody>
</table>

ACE: angiotensin-converting enzyme; and DPATA: dilatative pathology of ascending thoracic aorta.

* p < 0.05 versus reference group, accepted as statistically significant.

Table 3. MMP-3 5A/6A genotype distribution and 5A allele frequency.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MMP-3 genotypes n (%)</th>
<th>5A allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5A/5A</td>
<td>5A/6A</td>
</tr>
<tr>
<td>DPATA group (n = 103)</td>
<td>31 (30.1%)</td>
<td>49 (47.6%)</td>
</tr>
<tr>
<td>Reference group (n = 773)</td>
<td>190 (24.6%)</td>
<td>388 (50.2%)</td>
</tr>
<tr>
<td>p value</td>
<td>0.11</td>
<td>0.30</td>
</tr>
</tbody>
</table>

DPATA: dilatative pathology of ascending thoracic aorta; and MMP-3: matrix metalloproteinase-3.
genotypes separately in the group with thoracic aorta dissection, as it included a small number of patients (n = 32) and the frequency of ACE I/D was similar to that in the overall group bearing DPATA.

Thus, our data from patients with DPATA are concordant with the previous results, which identified the ACE D allele as a predisposing factor for abdominal aortic aneurysm development [19–21]. A role of ACE I/D polymorphism in the pathogenesis of abdominal aortic aneurysm is possibly due to the effect of angiotensin 2 [22]. It is necessary to stress that the morphology of dilative pathology of thoracic and abdominal aortas is different. The characteristic feature of abdominal aortic aneurysm is advanced, complicated atherosclerotic lesions in the intima, and infiltration by inflammatory cells in the media and adventitia, while specimens from thoracic aorta demonstrate a significantly smaller degree of inflammation. Thus, the main producers of MMPs in abdominal aortic aneurysm are inflammatory cells and medial SMCs [23]. It was demonstrated that infusion of angiotensin 2 leads to the development of abdominal aortic aneurysm in mice [24] due to its pro-inflammatory effects in attracting macrophages [8], which are the main producers of MMPs. By contrast, only the medial SMCs are the main MMP producers in the DPATA specimens [23]. A ‘trigger’ that induces a transition of medial SMCs from a contractile phenotype to a synthetic phenotype remains to be demonstrated.

Experimental studies provided evidence for a link between RAS and MMPs expression in arterial SMCs and cardiomyocytes [9,10]. Thus, we investigated whether the combined effect of ACE I/D and MMP-3 5A/6A genotypes can predispose to the development of DPATA. Our findings failed to demonstrate a combined ‘aggressive’ action of ACE D and MMP-3 5A alleles. The frequency of D/D and 5A/5A allele carriers was similar in both the DPATA and reference groups (7.8% and 5.8%, respectively). By contrast, a protective action of the ACE I and MMP-3 6A alleles was highlighted. There were no patients with I/I and 6A/6A alleles in the DPATA group, while in the reference group, the prevalence of such genotype carriers was 7.5%. Studies in vitro proved that endothelial cells with the ACE I/I genotype had lower levels of angiotensin 2 and high levels of angiotensin 1 [25]. Therefore, based on the above-mentioned experimental data [9,10,25], we suggest that the combined protective effect of ACE I/I and MMP-3 6A/6A might be explained by a lower expression of ACE to produce angiotensin 2 and a lower expression of MMP-3 in the aortic wall due to an angiotensin 2-stimulating effect.

The study has some limitations. Our reference group (n = 773) was constructed from a random sample of the population without prescreening by transthoracic echocardiography to exclude DPATA. Control subjects were matched only by age and gender to the DPATA group. This means that only clinically advanced patients with DPATA requiring surgery, and a random sample of the population were included in the study. Thus, our data reflect the impact of ACE I/D and MMP-3 5A/6A genotypes for clinically advanced DPATA development. Second, the study was limited only to an investigation of the ACE and MMP-3 genotypes. The functional significance of these genotypes was based on data from experimental studies reported by other researchers [6,13,25].

In conclusion, the present study showing a role of ACE and MMP-3 in the development of DPATA permits us to entertain a possible protective mechanism for the combined effects of the ACE I/I and the MMP-3 6A/6A genotypes. These findings offer the potential for further investigations of RAS and MMPs within the signaling pathways in the morphogenesis of DPATA.

Acknowledgment

We thank George Markelionis for editorial help.

References

and other non-communicable diseases in Central and Eastern Europe: rationale and design of the HAPIEE study. BMC Public Health 2006;18:255.


