Angiotensin-converting enzyme and angiotensin II receptor 1 polymorphisms: association with early coronary disease

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Abstract

Objective: to examine the association between coronary artery disease and polymorphisms at the angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R) genes. Methods: A total of 181 patients younger than 50 years and 240 controls from the same homogeneous Caucasian population (Asturias, Northern Spain) were genotyped (using polymerase chain reaction) for the ACE insertion/deletion (ACE-I/D) and the AT1R A/C transversion (AT1R-A/C) (3'-untranslated region) polymorphisms. Results: The DD-genotype was at a non-significant higher frequency among patients (50%) than in controls (41%). No difference between the two groups was found for the AT1R-genotypes. Distribution of ACE-genotypes according to AT1R-genotypes showed a significant association between ACE-DD and AT1R-CC and early coronary disease. Among the CC patients 58% were DD, compared to 21% among the controls (p = 0.02; OR = 5.32, 95%CI = 1.45, 19.51). We determined the distribution of these genotypes among the hypertensive and non-hypertensive patients. Frequencies of ACE- or AT1R-genotypes did not differ between the two groups. However, we found an interaction between the DD- and CC-genotypes in the group of normotensives. Among the CC patients, 13% of the hypertensives and 75% of the normotensives were DD (p = 0.014). Conclusions: Our results indicate a synergistic contribution of ACE and AT1R polymorphisms to the risk of coronary artery disease. This gene–gene interaction could have clinical implications. Approximately 2% of individuals in our population are CC1DD, and the genotyping of both polymorphisms could identify those with a high relative risk for coronary artery disease. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Coronary artery disease; Hypertension; Angiotensin-converting enzyme; Angiotensin receptor; DNA-polymorphism

1. Introduction

Coronary artery disease (CAD) is one of the most frequent causes of morbidity and mortality in industrialized countries. Both environmental and genetic factors contribute to the development of myocardial infarction and unstable angina.

Several studies have suggested that the renin–angiotensin system, which regulates blood pressure, plays an important role in the pathogenesis of CAD [1,2]. Angiotensin II receptors and the angiotensin-converting enzyme (ACE) are two major components of the renin–angiotensin system.

Angiotensin II is a potent regulator of cardiovascular homeostasis and binds to two different G-protein-coupled receptors. The type 1 receptor (AT1R) mediates the cardiovascular actions of angiotensin II and has been the most extensively studied [3–6]. AT1R is expressed in vascular smooth muscle cells and in the myocardium, and for this reason a possible association between the AT1R gene and myocardial infarction has been investigated [7]. The AT1R gene is polymorphic, and an A/C transversion at the 3’-untranslated region has been linked to essential hypertension [8].

The angiotensin II type 2 receptor (AT2R) inhibits cell proliferation and mediates programmed cell death. In contrast with the AT1R, little is known about AT2R.

ACE catalyses the conversion of angiotensin I to
angiotensin II. A polymorphism consisting in an insertion/deletion (I/D) of a 287 base pair (bp)-sequence at intron 16 of the ACE gene is associated with increased levels of the enzyme and has been described as a risk factor for myocardial infarction and left ventricular hypertrophy, especially among subjects considered to be at low risk according to common criteria [9–14]. However, other authors failed to find such an association [15–17].

Recently, one study has shown that the AT1R-A/C and ACE-I/D polymorphisms interact synergistically to increase the risk of myocardial infarction. Thus, individuals with the AT1R-CC- and the ACE-DD-genotypes would be at an increased risk of myocardial infarction [18].

In this study, patients and controls were genotyped for the AT1R and ACE polymorphisms in an attempt to define the role of both polymorphisms in the development of CAD.

2. Methods

2.1. Patients and controls

A total of 181 male patients were selected from people who had suffered an episode of myocardial infarction or unstable angina, defined according to the WHO criteria. All patients were Caucasian from the same region (Asturias, Northern Spain), and aged 50 years or less (average age 43 years, range 26–50).

Patients with a documented history of hypertension or having systolic blood pressure values equal to or greater than 140 mmHg in more than one determination were defined as hypertensives. Those with a history of hypercholesterolaemia or showing total cholesterol concentrations equal to or greater than 200 ml/dl were diagnosed as having hypercholesterolaemia. Patients with a history of diabetes or basal glucose greater than 120 mg/dl were defined as diabetics. A smoking history from all subjects was collected by means of a structured questionnaire. For methodological purposes, ex-smokers, defined as those maintaining 1 year of abstinence, were grouped with smokers. Table 1 summarizes data from patients.

We also analysed 240 healthy controls (150 males, 90 females) from the same population, matched with patients for age and ethnicity. These controls (Hospital staff, blood bank donors) were recruited through the Cardiology and Molecular Genetics Departments of the Hospital Central de Asturias, as part of an analysis of genetic factors involved in the risk of cardiovascular diseases (CAD and venous thromboembolism) [19]. Consent was obtained from patients and controls before participation, following the recommendations of the World Medical Association Declaration of Helsinki [20].

2.2. ACE-genotyping

For the analysis of the ACE-I/D polymorphism genomic

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<th>Anthropometric characteristics and average lipoprotein values in the 181 patients with early CAD</th>
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<td>Average age (years)</td>
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<td>Diabetics</td>
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<td>Hypertensives</td>
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DNA was polymerase chain reaction (PCR) amplified with primers 5’-CTGGAGACCTCCCACATTTCTTCT-3’ and 5’-GATGTGCCCACATTCGTCAGAT-3’. PCR consisted of 30 pmol of each primer, 2 mM each deoxynucleotide triphosphates, 2 mM MgCl₂, 1×buffer and 0.5 units of Taq DNA polymerase (Promega, Madison, WI), in a total volume of 30 μl. After an initial denaturation at 98°C, followed by 31 cycles of 15 s at 98°C, 1 min at 58°C and 30 s at 75°C, 10 μl of each reaction were electrophoresed on a 3% agarose gel. Allele sizes were 490 bp (I), and 190 bp (D).

By using this conventional method (PCR with primers flanking the insertion allele), mistyping of the DD-genotype may occur. Therefore, we confirmed each DD-genotype by using an insertion-specific primer, following a previously described protocol [22].

2.3. AT1R-genotyping

For the analysis of the A/C-polymorphism at the 3’-untranslated region of the AT1R gene we developed a PCR protocol that used a primer containing a mismatch, introducing a site for the BclI restriction enzyme. A 176 bp fragment was amplified under the same conditions described for the ACE-genotyping, with primers 5’-GCAGCACCTACTACCAATGTAGAT-3’ and 5’-TGTTCTTCGAGCGCCGT-3’ (annealing temperature at 58°C). Ten μl of each PCR were digested with BclI (Boehringer Mannheim, Germany) and electrophoresed on a 3% agarose gel. The AT1R alleles were visualized as fragments of 176 bp (C) and 156 bp (A).

The accuracy of this method was assayed by direct sequencing of five of each of the three genotypes.

2.4. Statistical analysis

Differences between frequencies were assessed using the chi-square test, and a p-value of 0.05 or less was considered as significant. We also calculated the odds ratio (OR)
and their 95% confidence interval (CI), using the computer program BMDP—New Systems (BMDP Statistical Software, Cork, Ireland).

3. Results

We genotyped 181 patients with early coronary heart disease (patients that were younger than 50 years when they suffered the first episode) and 240 controls from the same population, for both ACE and AT1R polymorphisms. Data from patients are shown in Table 1. All patients had at least one recognized risk factor for CAD. For the genotyping of the polymorphism at the 3'-untranslated region of the AT1R gene (nucleotide 1166, adenine or cytosine) we developed a method that uses a mismatched primer, creating a BcII-site (Fig. 1). Genotypes at both genes were in the Hardy–Weinberg equilibrium.

The group of patients showed an increased frequency of the ACE-DD-genotype compared to controls (50% vs. 41%). However, this difference was non-significant ($p = 0.07$) (Table 2). Among the 181 patients, no significant difference was found for the DD-frequency among hypertensives ($n = 61$; DD = 52%) and normotensives ($n = 120$; DD = 48%).

Genotype frequencies for the AT1R polymorphism did not differ between patients and controls (Table 2). In addition, no significant difference between hypertensive and normotensive patients was found for this polymorphism. Genotype frequencies for both ACE and AT1R polymorphisms did not differ between patients with and without hypercholesterolaemia or diabetes, or between smokers and non-smokers.

We found a significant association between the ACE-DD+AT1R-CC-genotypes and early CAD. Thus, among the CC individuals, 58% of the patients (14 / 24) and 21% of the controls (5 / 24) were DD ($p = 0.018$; OR = 5.32, 95% CI = 1.45; 19.51) (Table 3). Among the CC patients, eight and 16 were hypertensive and normotensive, respectively. A significant difference for the DD+CC-genotypes was found between both groups: 13% (1 / 8) of the CC-hypertensives and 75% (12 / 16) of the CC-normotensives were also DD ($p = 0.014$) (Table 4).

4. Discussion

Both environmental and genetic factors contribute to the risk of CAD, and common polymorphisms at several genes have been described as genetic risk factors for CAD. Thus, the AT1R has been linked to essential hypertension in several families, and polymorphisms at this gene have been associated with an increased risk of hypertension [8]. A characteristic of polymorphisms that are genetic risk factors is the early onset of the disease among carriers of the genotype that confers susceptibility.

We genotyped 181 CAD patients aged 50 years or less, that are considered as having early CAD by most authors. Compared to other studies, our work was based on a smaller number of patients. However, these patients were...
from the same region, and we excluded the effect of admixture of individuals from different geographical origin, a feature frequently found while working with people from larger heterogeneous populations.

In our population, ACE and AT1R allele frequencies were similar to those described for other Caucasian populations [18,21].

The ACE-DD-genotype was at a non-significant increased frequency among patients. This is in agreement with previous reports that described an association between early CAD and the DD-genotype [10–14].

The A/C polymorphism at the AT1R gene failed to show an association with the disease in our population. However, a significant interaction between the ACE-DD- and AT1R-CC-genotypes was found. This is in agreement with a previous work that described an increased risk for CAD among individuals with the DD+CC-genotypes [18]. In their multicentric study, Tiret et al. found an interaction between the ACE-DD- and AT1R-CC-genotypes for both low-risk and high-risk patients. Compared to controls, ORs for the CC+DD-genotypes were 13.3 and 5.59 for low-risk and high-risk patients, respectively [18]. Our patients had at least one recognized risk factor for CAD and thus can be regarded as a high-risk group. We found an OR for the DD+CC-genotypes of 5.32, close to that described by Tiret et al. for their high-risk group. In their analysis of Norwegian patients with early CAD, Berge et al. failed to confirm the ACE-DD+AT1R-CC interaction. This could be partly attributed to the small number of controls with the CC-genotype (only six individuals) included in their study [21].

Hypertension is a recognized risk factor for CAD. Because both ACE-DD- and AT1R-CC-genotypes have been linked to hypertension, it seems plausible that the increased frequency of the DD+CC-genotypes in our patients should be a consequence of an increased frequency of DD+CC carriers among the CAD patients who were hypertensive. We found exactly the opposite, with a lower frequency of DD+CC among hypertensive than among normotensive patients, suggesting that the association between these genotypes and CAD is independent of hypertension [23]. Although only eight and 16 of the CC patients were hypertensives and normotensives, respectively, frequencies according to ACE-genotypes differed significantly between both groups (Table 4).

The ACE–AT1R interaction could have clinical implications. Approximately 2% (5/240) of individuals in our population are DD+CC. Genotyping of both polymorphisms could identify individuals with a high relative risk for CAD. This could be of special interest among patients who have other risk factors, thus being candidates for clinical trials with the ACE inhibitors and AT1R antagonists.

Finally, the method introduced in our study for the analysis of the AT1R-polymorphism avoids the use of allele-specific radiolabelled oligonucleotides, permitting an accurate, rapid and cheaper genotyping of large number of individuals.

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References


