ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISMS IN CORONARY ARTERY DISEASE- A CONTROVERSIAL ROLE

Maria-Cristina Apăvăloaie¹,², Iris Bararu¹,³, Manuela Ciocoiu², Magda Bădescu²*, Cătălina Arsenescu-Georgescu¹,³
University of Medicine and Pharmacy "Grigore T. Popa"-Iaşi
Faculty of Medicine
1. Department of Medical Specialties (I)
2. Department of Morpho-functional Sciences
3. Institute for Cardiovascular Diseases "Prof. Dr. George I. M. Georgescu" Iaşi
*Corresponding author. E-mail: magda.badescu@gmail.com

ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISMS IN CORONARY ARTERY DISEASE-A CONTROVERSIAL ROLE (Abstract): Coronary artery disease (CAD) is the most prevalent cause of morbidity and mortality worldwide. It is a multifactorial disease, which is influenced by genetic and environmental factors. The major risk factors of CAD are age, male gender, hypertension, diabetes, hyperlipidemia, smoking, family history of CAD and obesity. Genetic factors add further information in predicting CAD. The relationship between genetic risk factors and the development of CAD is not well understood, likely due to the complex interrelation of genetic and environmental risk factors. One of the currently explored genetic factors is angiotensin converting enzyme (ACE) polymorphisms, which may have an influence on the progression of coronary artery disease. We present the results of several recently published studies (2012, 2013) on the association between traditional cardiovascular risk factors and novel genetic risk factors (polymorphisms of ACE gene: insertions/deletions). The results of these studies are controversial. Most of them show an association between DD polymorphisms and D allele and the severity of stenotic coronary lesions, evaluated using the Gensini score (Gensini>6). However, others found no association. These differences may be due to geographical discrepancies, ethnical characteristics, small number of subjects included and variability of used techniques. We can therefore draw the conclusion that an international study on a large number of patients, using the same technique for genetic determination and the same statistical software should be performed. Key words: ANGIOTENSIN CONVERTING ENZYME, GENETIC POLYMORPHISMS, CORONARY ARTERY DISEASE, STENOSIS.

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in Westernized societies (1). It is well known that the etiology of this devastating disorder involves both genetic and environmental factors. Sequence variants of the components of the renin-angiotensin-aldosterone system are suggested to have significant influences on cardiovascular homeostasis. Both gene targeting and transgenic studies in mice have clearly suggested a critical role of the angiotensin converting enzyme (ACE) gene in blood pressure regulation. Thus, the ACE gene has been
recognized as a top candidate gene for cardiovascular research. The relationship between genetic risk factors and the development of coronary artery disease (CAD) is not well understood, likely due to the complex interrelation of genetic and environmental risk factors (3).

CARDIOVASCULAR RISK FACTORS
Risk factors for CAD were officially described after the discoveries of the Framingham study:

1. Conventional risk factors: age (over 45 years for men, over 55 years for women), family history of heart disease, race (higher incidence in African-Americans);
2. Modifiable risk factors: smoking, high total cholesterol level (especially LDL-cholesterol), hypertension, lack of physical activity, obesity, diabetes, metabolic syndrome, stress or depression;
3. Nontraditional risk factors (novel risk factors): inflammatory markers such as C-reactive protein, coronary calcium, lipoprotein (a), interleukin-6, fibrinogen, homocysteine, small-dense low-density lipoprotein, insulin resistance;
4. Genetic factors, which represent the latest discoveries in the field of cardiology: ACE gene polymorphisms (4).

ANGIOTENSIN CONVERTING ENZYME POLYMORPHISMS
ACE is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. Several different names refer to this enzyme in the scientific literature. ACE converts the inactive decapeptide angiotensin I (Ang I or Ang 1-10) to the active octapeptide and potent vasoconstrictor angiotensin II (Ang II or Ang 1-8), which is the main active product of the renin–angiotensin system (RAS). Long-term regulation of blood pressure and blood volume in the body is controlled by RAS.

Angiotensin II is a potent vasoconstrictor. It also acts on the adrenal cortex, causing the release of aldosterone, which stimulates tubules in the kidneys, allowing them to reabsorb more sodium and water from the urine. These effects directly act to increase the amount of fluid in the blood, making up for a loss in volume, and to increase blood pressure. Angiotensin II also mediates cell growth and proliferation by stimulating various cytokines and growth factors (5). Furthermore, angiotensin II may induce endothelial dysfunction by reducing nitric oxide bioavailability (6). These findings emphasize the importance of angiotensin II in cardiovascular pathophysiology and motivate exploration of the role of RAS in atherosclerosis and other cardiovascular outcomes. ACE also plays an important role in another hormonal system, the kallikrein kinin–cascade. ACE metabolizes bradykinin, which is a strong vasodilator, forming the inactive metabolite bradykinin 1-5. Therefore, ACE plays a prominent role in blood pressure regulation through this pathway as well (7, 23).

The ACE gene is located on chromosome 17q23. It spans 21 kb, and comprises 26 exons and 25 introns (8). It contains a polymorphism due to an insertion (I) or a deletion (D) of a 287 base pair (bp) alu sequence in intron 16, resulting in the 3 genotypes of insertion/insertion (II), insertion/deletion (ID), and deletion/deletion (DD). (8, 9, 10). The I/D polymorphism is associated with circulating and tissue ACE levels. Individuals homozygous for the D allele had higher tissue and plasma ACE concentrations than heterozygotes and II homozygotes (11). ACE polymorphism has
attracted a great deal of information as a potential cause of genetic variation in cardiovascular physiological function (12). Consequently, the ACE I/D polymorphism have been associated with various cardiovascular diseases, such as atherosclerosis (13), hypertension (14), and coronary artery disease (CAD) (15). ACE, as a candidate gene, can associate with traditional risk factors in predicting the severity of CAD (3, 24).

**GENETIC STUDIES**

The relationship between ACE polymorphisms and other cardiovascular risk factors (CRF) is not well understood in CAD, likely due to the complex interrelation of genetic and environmental risk factors. Several studies were aimed to investigate the associations of CAD risk factors and ACE polymorphisms in patients with CAD.

Patients included in the studies were referred for coronary angiography as a result of various clinical indications.

The experimental protocol was approved by the Ethics Committees, the study procedures being in accordance with the principles of the *Declaration of Helsinki II*, and all subjects provided written informed consent prior to enrollment.

Physical examination was performed on and a detailed family history was obtained for all subjects. The severity of CAD was evaluated according to the number of stenotic vessels on coronary angiography. The coronary angiography findings were reviewed by independent operators. The angiography results were reported as Gensini scores, which were computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its importance based on location. Reduction in the lumen diameter and the angiographic appearance of concentric lesions and eccentric plaques were quantitatively evaluated. Gensini scores higher than 6 were considered a criterion for having CAD in the patients.

Informations about the conventional risk factors for CAD (1. hyperlipidemia, 2. hypertension, 3. diabetes mellitus, 4. family history of CAD, 5. smoking, 6. obesity) were obtained from all subjects. All studies used well defined inclusion criteria, according to strict definitions of the risk factors.

Hyperlipidemia was defined based on low-density lipoprotein cholesterol (>100 mg/dL for subjects with CAD) and/or high-density lipoprotein cholesterol (<40 mg/dL for men and <50 mg/dL for women) and triglyceride levels (>150 mg/dL), or prior requirement of lipid lowering medications. Hypertension was defined as blood pressure level above 140/90 on 3 different occasions or requirement of pharmacological intervention to lower blood pressure. Diabetes mellitus was defined as fasting venous blood glucose ≥126 mg/dL on 3 separate occasions or requirement of pharmacological intervention for diabetes mellitus. Family history of CAD was defined as having a first degree relative with established CAD before age 50, or before menopause. Cigarette smoking was assessed by asking participants about prior and current history of smoking habits. Body mass index (BMI) was calculated as weight (kg)/height (m²). Overweight or obesity were defined based on the BMI levels (>25-29.9 and ≥30 kg/m² respectively).

CAD was defined as a documented coronary stenosis of at least 70% by quantitative coronary angiography in one or more major (more than 2 mm in diameter by
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quantitative coronary angiography) coronary arteries. The patients included were divided into groups according to the number of diseased vessels (coronary obstruction of at least 70%) as determined by the number of diseased major coronary arteries (left anterior descending, left circumflex, and right coronary artery). Patients were grouped into 0, 1, 2, 3, or 4 vessel disease groups. Some studies selected patients without significant coronary obstruction by coronary angiography (less than 30%) as controls.

All studies extracted the DNA, used polymerase chain reaction (PCR) amplification and different SPSS programs for statistical analysis.

ANALYSIS OF THE STUDIES
Zhou Y.F. et al (16), enrolled 603 patients in three groups: 260 subjects were enrolled in the elderly control group (Male, n = 150, Female, n = 110), whose age were from 75 to 85 years (81.6 ± 4.55 years). They were admitted to the hospital for medical examination and routine treatment from the October 2006 to March 2013. They did not have hypertension, diabetes, coronary heart disease (CHD) or any other CVD. Two hundred and five patients aged 74 to 86 years (79.8 ± 6.28 years) were enrolled in elderly hypertensive group (male, n = 103, female, n = 102). One hundred and thirty eight patients aged 75 to 83 years (79.12 ± 5.21 years) were enrolled in elderly diabetic hypertension group (male, n = 70, female, n = 68). The result was that in Chinese elderly population DD genotype and D allele in ACE gene were associated with hypertension and lipid levels. However, they were not risk factors for type 2 diabetes.

Guney A.I. et al. (2), enrolled 343 patients with CRF and angiographically proven CAD. Patients were grouped into 0, 1, 2, 3, or 4 vessel disease groups. Patients without significant coronary obstruction by coronary angiography (less than 30%) were selected as controls (140 patients). Their results showed that the frequency of the DD genotype was significantly higher in patients. D allele frequency was higher among CAD subjects when compared to the control group. The number of stenotic vessels were found to be statistically associated with a high frequency of DD polymorphism and D allele and a low frequency of I allele in patients, especially in male patients. The control group displayed II and ID genotypes more frequently than did the patients. The ACE I/D genotype was associated with hyperlipidemia and smoking history. They considered that the DD polymorphism and D allele may affect the severity of CAD, while I allele may have a protective effect. In conclusion, the ACE I/D genotype may interact with conventional risks criteria in determining the risk of CAD.

Dhar S. et al. (17), included 217 clinically diagnosed cases with CAD from the outdoor of Cardiology Department of Ramakrishna Mission Seva Pratishthan. 255 age and sex matched medically diagnosed healthy controls were taken for the study which were negative for Treadmill test (TMT _ve). All the samples were diagnosed by biochemical tests and other medical tests like electrocardiogram, echocardiography, or treadmill test.

The results of this study outlined that both DD [OR: 2.16; 95%CI: (60.60e67.40)] and ID [OR: 1.48; 95%CI: (93.28e97.72)] genotypes of the ACE gene showed significant associations in the development of CAD. Coexistence of diabetes and hyper-
tension was found to be a risk modifier of the disease. Tobacco intake in various forms elevates the risk of the disease among the cases with risk genotypes. Therefore, ID and DD genotypes of ACE gene came out to be predisposing factors for the CAD cases in our study population.

Poorgholi L. et al. (18) performed a cross-sectional study on a total of 1050 individuals referred to hospital for coronary angiography. 676 CAD-positive patients (documented by coronary angiography and Gensini scores higher than 6) and 374 CAD-negative patients were evaluated for ACE gene I/D polymorphism via the Polymerase Chain Reaction Amplification method. Patients’ age, sex, smoking status and its duration as well as family history of CAD, hypertension, and diabetes mellitus were recorded.

The results showed that 504 (74.6%) of the CAD-positive patients were male, and the mean age of this group was 60 (60 ± 10). In the CAD-negative individuals, the mean age was 56 (56 ± 10) and 196 of them were male (52.4%). After the analysis of all groups and gender subgroups, neither genotype nor allele frequency was significantly different between the CAD-positive nor CAD-negative groups (p values for genotypes and allele frequencies were 0.494 and 0.397, respectively). Therefore, ACE gene I/D polymorphism were not associated with an increased risk of CAD in an Iranian population.

Sahin S. et al. (19), investigated whether the insertion/deletion (I/D) polymorphism in the ACE gene and serum ACE levels are associated with traditional risk factors of CAD. They enrolled 250 individuals without CAD and 750 individuals suffering from CAD who were angiographically diagnosed. Biochemical risk factors, the ACE (I/D) gene polymorphism, and ACE serum levels were compared. Compared to the control group, the CAD group showed significantly higher serum ACE levels (P < 0.001). The highest ACE levels were found in those with the DD genotype. Other genotypes also presented statistically significant differences. They observed a significant difference between the control and coronary patient groups regarding the levels of total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol (P < 0.05). ACE (I/D) genotypes and serum ACE levels may be associated with risk factors and the development of CAD.

**DISCUSSION**

We presented the results of several recently published studies (2012, 2013) on the association between traditional CRF (hypertension, diabetes, hyperlipidemia, obesity, smoking habit, family history) and novel genetic risk factors (ACE deletion/insertion polymorphisms).

In all studies, DNA was extracted from peripheral leukocytes and amplified by polymerase chain reaction (PCR). Data were analyzed with different statistical softwares, such as SPSS 11.5, SPSS 13.0, SPSS 14.0, SPSS 18.0, SAS 9.2 packages.

Inclusion criteria were similar between studies, but some studies chose healthy subjects for comparison (16) while others used patients without significant stenosis (less than 30%) for the second group (2, 18).

The results of those studies were controversial. Most of them showed an association between genetic risk factors (DD genotype, D allele) and the severity of stenotic coronary lesions, evaluated using the Gensini score (Gensini>6). Gensini
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score is based on the number of stenotic coronary artery segments, degree of luminal narrowing and the regional importance of lesion location in the coronary tree. However, Poorgholi L. et al. (18), found no association between ACE I/D polymorphism and the progression of coronary artery disease in an Iranian population.

There are multiple implications of ACE gene in CAD. The presented studies focused on the development and severity of stenosis. Other directions scientists are working on are left ventricular dysfunction and in-stent restenosis, in the context of ACE gene polymorphisms. In-stent restenosis occurs after treatment of coronary artery stenosis in 12% to 32% of coronary interventions with stents. Experimental and clinical studies have suggested that the deletion/insertion (D/I) polymorphism of the ACE gene plays a role in this. Jorgensen E. et al. (20), performed a study on 369 patients after stent implant and concluded that the D/I polymorphism is not an independent predictor of coronary in-stent restenosis in general, but it may be of clinical importance in patients treated with ACE inhibitors or angiotensin receptor antagonists. Martinez R.A. et al. (21) also stated genetic variations of the ACE gene could be a genetic factor related to coronary artery disease in the Mexican mixed racial ancestry individuals, but do not support its role as a risk factor for developing restenosis after coronary stenting. However, the meta-analysis by Wang S et al. (22), showed that the DD genotype of ACE I/D polymorphism was significantly associated with increased risk of restenosis, particularly for PTCA stent.

CONCLUSIONS
We believe that these differences may be due to geographical discrepancies, ethical characteristics, rather small number of study subjects and variability of the used techniques. We can therefore draw the conclusion that an international study on a large number of patients, using the same technique for genetic determination and the same statistical software should be performed.

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