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A New Investigative Approach: How the Angiotensin-Converting Enzyme Gene Interacts with Coronary Risk Factors

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Abstract

How each Angiotensin-Converting Enzyme (ACE) genotype modulates the magnitude of the effect of each coronary risk factor.

Background: Coronary Artery Disease (CAD) is based on atherosclerosis, which is a multifactorial-multigenic disease. This study investigated when interacting with them in the genesis of this pathology.

Methods: The study included 630 subjects, including one group with 396 CAD-confirmed patients and another with 234 controls without angiographic lesions. Classical risk factors of CAD and ACE gene polymorphisms were evaluated using nested PCR. The association between the DD, ID and II genotypes with classical risk factors was analysed statistically by comparing the patient and control groups using the binomial frequency comparison test and the power test.

Results: Of the 630 individuals, 286 presented the DD genotype, and there were 251 ID genotypes and 93 II genotypes. In the comparative analysis of the genotype frequencies between the patient and control groups, the DD genotype showed the most significant difference and the highest power (p=0.0020; power=0.8454). In the analysis of the interaction between risk factors and the ACE genotype in the DD genotype, a significant difference and a high power in the family history of CAD (p=0.0043; power=0.08355); (p=0.0010; power=0.9155), high total cholesterol levels (p<0.0001; power=0.9983) and high LDL cholesterol levels (p=0.0004; power=0.9702) were found.

Conclusion: The results indicate that ACE genotypes interact differently with the same risk factors in each individual, influencing the development of CAD.

Keywords: Coronary artery disease; Atherosclerosis; Traditional coronary risk factor; Angiotensin converting enzyme gene; Genetic polymorphism

Introduction

The clinical identification of coronary artery disease (CAD) presents a broad spectrum of characteristics, ranging from asymptomatic individuals to those diagnosed with coronary syndromes, such as stable angina pectoris, silent myocardial ischaemia, unstable angina, myocardial infarction, ischaemic cardiomyopathy, and sudden cardiac death [1,2]. It is a multifactorial and multigenic disease for which classic coronary risk factors, such as dyslipidaemia, high blood pressure (HBP), diabetes mellitus (DM), obesity, smoking, and a first-degree family history of early coronary disease, are important and should be taken into account when identifying prospective patients. These factors, however, do not fully explain the genesis or the natural history of the disease, and possible new risk factors associated with CAD have been identified, including genes related to the renin-angiotensin system (RAS) [3].

Angiotensin converting enzyme (ACE) is a key component of RAS and thekinin-kallikrein system. ACE is a zinc metallopeptidase that cleaves the C-terminal (His-Leu) dipeptide from angiotensin I and generates a physiologically active peptide, angiotensin II, performing activities in the endothelium promoting cardiovascular homeostasis. ACE and angiotensin II are highly present in cardiac myocytes and are related to cell growth and the formation of the extracellular matrix, which is also important due to the release of cytokines, such as IL-6 and tumour necrosis factor. Thus, in pathological conditions, the level of angiotensin II, which is regulated by ACE, may contribute to the cardiac morphological changes involved in the development of atherosclerotic processes [4-6].

The ACE gene is located on chromosome 17q23.3. The polymorphism of this gene has a locus of quantitative trait that is in linkage disequilibrium with an Alu of a 287 bp insertion/deletion (I/D) in intron 16 that results in three genotypes as follows: a homozygous deletion (D/D); a heterozygous insertion/deletion (I/D) and a homozygous insertion (I/I). These three genotypes represent one of the main determinants of ACE plasma activity, with the highest levels being in the homozygotes for the D allele and the lowest in the homozygotes for the I allele. The heterozygotes (ID) show intermediate levels [7-11].

Association studies between the ACE gene and CAD suggest several hypotheses to explain the possible mechanisms by which

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this gene influences the development of CAD. Some studies provide conflicting results and fail to establish a clear relationship between the ACE gene and traditional risk factors [12-14]. In a case-control study [15], researchers reported that genotypes of the ACE gene showed significant associations in the development of CAD, in both DD [OR: 2.16; 95% CI: (60.60-67.40)] and the ID [OR: 1.48; 95% CI: (93.28-97.72)] and that the coexistence of DM and HBP may be a risk modifier of the disease. In another study (16), researchers investigated whether the insertion/deletion (I/D) polymorphism in the ACE gene and serum ACE levels were associated with the traditional risk factors for CAD. They found a significant difference between the control and the coronary patient groups regarding the levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol ($p<0.05$) [16].

Considering that the genotypes DD, ID and II of ACE constitute distinct populations regarding the reflexes of the interaction between risk factors and the ACE genotype and treating each genotype as a subpopulation with specific characteristics, this study aimed to investigate the way in which each genotype of ACE influences the magnitude of the effect on CAD, as well as to identify the interactions of these genotypes with the traditional risk factors in the genesis of the disease.

Material and Methods

Study object

This study was performed in the cardiology department of the Clinical Hospital from the Federal University of Uberlandia and the Institute of Cardiology from the same municipality, state of Minas Gerais, Brazil. The data were collected from February 2015 to June 2016. A total of 630 subjects were distributed into two groups. The target group, consisting of 396 patients, had symptoms characteristic of coronary syndrome. All the subjects were subjected to a coronary angiography in order to identify a significant obstructive atherosclerotic lesion in the coronary arteries. The control group, consisting of 234 control subjects, were also subjected to a coronary angiography for other indications. All the subjects from the control group did not present any evidence of atherosclerotic coronary lesion. In both groups the presence or absence of classic coronary risk factors, such as serum total cholesterol (>239 mg/dL), HDL-cholesterol (HDL-c<30 mg/dL), LDL- (VLDL-c>30 mg/dL), triglycerides (>150 mg/dL), HBP, DM, smoking, obesity (Body Mass Index- BMI>30) and a history of coronary artery disease with close relatives, were recorded.

Ethical considerations

The present study adheres to the 1975 Declaration of Helsinki and its updates and was approved by the Brazil's Platform of ethics and research council, which is a unified national database of the research recordings involving human beings (CAAE 01736412.9.0000.5152). Through a written consent form, all the subjects included in the study were informed of the procedures, risks, benefits and the right to have their data erased at any time.

Genotype identification

Genotyping of the ACE gene (HUGO Gene Nomenclature Committee: 2707) was performed according to the following protocol: peripheral venous blood samples were collected from each individual in a 3-ml vacutainer tube with EDTA. The samples were stored and frozen at -20°C until extraction of the leukocyte DNA and the subsequent polymerase chain reaction.

The DNA extraction was performed with brazol reagent (LGC Biotechnology Ltd.). Each reaction contained 100 ng of genomic DNA, 25 pmol of primer, 1.5 mmol/L of $MgCl_2$, 0.5 mmol/ L of each dNTP, a Taq DNA polymerase unit and 1X Taq DNA polymerase buffer in a volume of 30 μL reaction. The PCR was performed using 30 cycles at 93°C for 1.5 min, 58°C for 2 min and 72°C for 2 min. The fragment corresponding to intron 16 was amplified with the following primers: Sense: 5'-CTG CAG ACC ACT CCC ATC CTT TCT-3 ' and Anti-sense: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T- [13]. The primers for this reaction were specific for detecting the I/D polymorphism. After the amplification, the products were visualized on a 2% agarose gel that was stained with ethidium bromide, and the ACE genotypes were then characterized. Homozygous individuals for the deletion (D/D) had a band of 190 bp in the gel, the heterozygotes (I/D) presented two bands, with one at 490 bp and the other at 190 bp and the homozygotes for the insertion (I/I) presented a band of 490 bp [17,18]. To increase specificity to the D allele, a Nested-PCR reaction was performed using the products of the above amplification with the following primers: sense 5'-TGG GAC CAC AGC GCC CGC CAC TAC -3 'and nonsense 5'- TCG CCA GCC CTC CA TGC CCA TAA -3' [19].

Statistical analysis

The sample size was calculated to obtain a confidence level of 0.95 and a type I error of 0.05. It was considered that the positivity of CAD in the studied population was 5% in adults over 40 years [20]. The data analysis was performed using the binomial test to compare the percentage frequencies of the ACE genotypes and the traditional risk factors in the group of patients and controls [21]. The p-value used for decision-making regarding the rejection or non-rejection of the null hypothesis was obtained by calculating the proportion and combined samples of the patient groups and controls and the subsequent calculation of the Z test [22]. An analysis of statistical power for the data was also performed. The statistical power analysis used statistical tools applied for a more effective analysis that covers a power analysis of the correlation of multivariate sets and methods considering the size of the effect, the efficacy of the qualified dependent variables, and the expanded power for multiple regression/correlation.

The power of the test was determined according to the level of statistical significance, sample size and the magnitude of the effect. After assessing the various statistical inferences, the objective to be reached was the level of significance $p \leq 0.05$ and power ≥ 0.80, which was adopted in the present study [22]. All the tests were processed using Bioestat 5.3 software.

Results

In the sampled subjects of 630 individuals, the mean age was 60.78 years old (± 10.70), and 49% were women and 286 had the DD genotype, 251 were genotype ID and 93 were genotype II. The frequency of the DD, ID and II genotypes analysed by the binomial test between both the patient and control groups showed a significant difference in all the analyses. The DD genotype group was significantly different ($p=0.0020$; power=0.8454), while for both II ($p=0.0600$; power=0.4409) and ID ($p=0.0785$; power=0.4081) the differences were not significant (Table 1).

The DD, ID and II genotypes showed that the less frequent genotype was the II, which was 13.89% of the patients ($p<0.0001$) and that the DD and ID genotypes had equivalent frequencies of 44.19% and 41.92%, respectively. Of the 234 individuals in the control group, 111 (47.34%) presented the DD genotype, 85 (36.32%) the genotype ID and 38 (16.24%) the genotype II. The mean age was 58.20 years

Variables	Patients	Controls	p-value	power
	n=396	n=234		
ACE DD	175	111	**0.002**	**0.8454**
ACE ID	166	85	0.0785	0.4081
ACE II	55	38	0.06	0.4409
The significant probability (p-value<0.05) and high-power test (>0.80) are in bold.				

Table 1: Results of the Binomial test to compare the frequency differences of the DD, ID and II genotypes of the ACE gene between the patient and control groups.

Variables	Control group				Patient group			
	DD+ID+II	DD	ID	II	DD+ID+II	DD	ID	II
	n=234	n=111	n=85	n=38	n=396	n=175	n=166	n=55
	p (%)	p (%)	p (%)	p (%)	p (%)	p (%)	p (%)	p (%)
Age (years)	58.20 (± 10.95)	58.48 (± 10.26)	57.62 (± 11.75)	58.97 (± 11.31)	62.31 (± 10.27)	63.05 (± 10.03)	61.25 (± 10.28)	63.12 (± 10.90)
Females	0.58	0.65	0.52	0.53	0.43	0.4	0.45	0.47
Hypertension	0.42	0.42	0.38	0.53	0.58	0.54	0.6	0.64
Diabetes mellitus	0.44	0.45	0.48	0.32	0.45	0.5	0.42	0.4
Total Cholesterol*	0.24	0.29	0.18	0.24	0.41	0.35	0.46	0.45
HDL-c**	0.21	0.17	0.25	0.21	0.19	0.19	0.19	0.22
LDL-c***	0.15	0.18	0.13	0.11	0.3	0.26	0.34	0.29
VLDL§	0.32	0.37	0.27	0.32	0.39	0.33	0.42	0.45
Triglycerides§§	0.31	0.32	0.32	0.26	0.38	0.35	0.42	0.38
Smoking	0.41	0.37	0.49	0.37	0.49	0.49	0.47	0.53
Obesity§§§	0.24	0.25	0.24	0.21	0.23	0.25	0.21	0.22
Sedentary lifestyle	0.47	0.45	0.51	0.42	0.51	0.53	0.48	0.56
CAD family history	0.22	0.22	0.21	0.26	0.34	0.38	0.31	0.29
*Levels>239 mg/dL; ** Levels Lower<40 mg/dL; *** Levels>159 mg/dL; §Levels>30 mg/dL; §§Levels>150 mg/dL; §§§ IMC>30; C Indicates Control Group and P Indicates Patient Group.								

Table 2: Clinical and laboratory characteristics within the control and patient groups in the ACE genotypes.

Variables	DD		ID		II	
	Patients (n=175)		Patients (n=166)		Patients (n=55)	
	Controls (n=111)		Controls (n=85)		Controls (n=38)	
	p-value	power	p-value	power	p-value	power
Smoking	0.0429	0.5289	0.716	0.0448	0.1369	0.3253
Hypertension	0.0609	0.4662	**0.001**	**0.9155**	0.2886	0.1821
Diabetes mellitus	0.3874	0.1348	0.3136	0.1691	0.4071	0.126
Total Cholesterol*	0.2889	0.1833	**<0.0001**	**0.9983**	0.0321	0.5866
HDL-c**	0.71	0.0451	0.2645	0.1968	0.9296	0.0046
LDL-c***	0.1301	0.3323	**0.0004**	**0.9702**	0.0322	0.6042
VLDL§	0.511	0.093	0.019	0.6679	0.179	0.268
Triglycerides§§	0.6029	0.0685	0.1305	0.3295	0.2328	0.2205
Obesity§§§	0.9875	0.8942	0.6576	0.0577	0.9296	0.0046
Sedentary Lifestyle	0.2148	0.2351	0.6529	0.0577	0.1764	0.2693
CAD Family History	**0.0043**	**0.8355**	0.1089	0.3685	0.7694	0.0338
*Levels>239 mg/dL; ** Levels<40 mg/dL; *** Levels>159 mg/dL; § Levels<30 mg/dL; §§ Levels>150 mg/dL; §§§IMC >30. The Significant Probability (p-value<0.05) and Power Test (>0.80) are in bold.						

Table 3: Binomial test comparing the frequency differences of the coronary risk factors between the patient and control groups for the ACE genotypes.

old (± 10.95) with no significant difference between the genotypes. In the control group, 58% were women, and the DD genotype (65%) predominated. There was no significant association of the genotypes with any of the classic coronary risk factors (Table 2).

Of the 396 individuals in the patient group, 175 (44%) presented the DD genotype, 166 (41%) were ID and 55 (13.88%) were II. The mean age was 62.31 years old (± 10.27), with no significant difference among the genotypes, and 57.07% were men, with the DD genotype (60%) predominating (Table 2).

In the analysis shown in Table 3, when comparing the patient groups versus the control group, in patients with the DD genotypes, a family history of CAD showed a high significance and high power (p=0.0043; power=0.8355). In the same comparison, no associations between the DD genotype with smoking, HBP, DM, total high cholesterol, low HDL-c, high LDL-c, high triglycerides, obesity and sedentary lifestyle were identified. Among the patients with the ID genotype, an association with a high significance and high power was observed (p=0.0010; power=0.9155) with level of total cholesterol (p<0.0001; power=0.9983) as well as LDL-c (p=0.0004; power=0.9702). Among the patients with genotype II, there was no significant relationship with any of the coronary risk factors in the comparison of either the group of patients or the controls. An obesity and sedentary lifestyle showed no significant association with any specific genotype in either the patient group or in the controls (Table 3).

In the comparison between the patients and the controls, diabetes

mellitus showed a low significance and low power in all the genotypes (DD genotypes, p=0.3874; power=0.1348; ID genotypes, p=0.3136; power=0.3136; II genotype, p=0.4071; power=0.1260) (Table 3). Evaluation of the smoking frequency among the patients compared to the controls was performed. In the patients with the DD genotype, it was significant (p=0.0429) but had a low power (power=0.5289). For both the ID and II genotypes, the significance was low as was the power of the test, when making the same comparison (Table 3).

Discussion

The analysis of the association of the ACE genotypes with each risk factor provides a better understanding of the multiple molecular mechanisms possibly related to the genesis of this pathology. This study investigated how each ACE genotype altered the magnitude of the effect in CAD and measured how the interaction between these genotypes, with traditional risk factors, influenced the genesis of this disease.

Risk algorithms, with a separate multivariate analysis, are commonly used to assess the risk of specific events of atherosclerotic cardiovascular disease. D'Agostino et al. studied a single multivariable risk function that predicts the risk of developing the disease in a large-sized population sample and determined that the classical coronary risk factors participate in the development of the CAD [23].

The classical risk factors are associated with a portion of patients with CAD and may affect the clinical identification of the disease. The ACE gene also seems to variably influence the phenotypic expression of CAD. The relationship between the ACE gene and the genesis of CAD is controversial [24-29]. It and Shen demonstrated, in a meta-analysis of 118 studies, a positive association between the DD and ID genotypes and CAD, identifying the significant heterogeneity of these results [30]. Such incongruities in scientific research are related to studies that treat different populations that have their own characteristics as a single population. Considering the overlapping of different populations with unique characteristics may lead to conflicting results that will almost always point to the particularities of the subpopulation in a larger number in the sampled population.

The present study evaluated this hypothesis and treated each individual genotype (DD, ID and II) of the ACE as a subpopulation with its own characteristics, making it possible to evaluate the importance of each coronary risk factor for the individuals from each genotype. All the genotypes showed significant differences when comparing the patients and controls, showing that the DD genotype had the greatest significant difference (p=0.0020) and highest power (power=0.8454) (Table 1). These results are also demonstrated in other studies [31-33]. CAD is a multigenic condition, with several genes related to its occurrence, in addition to environmental factors. By treating the genotypes as subsamples, it was possible to infer the association with CAD, which would not have been possible if the genotypes had been analysed as constituents of the same sample.

Smokers with the DD genotype were more frequent among the patients (49%) than among the controls (37%). This difference was statistically significant (p=0.0429) but with low power (power=0.5289). Therefore, smoking, by comparing the patients and controls, does not seem to favour the development of CAD for the DD genotype, which was also not relevant in the comparison with the other genotypes. Family history was significantly important for the DD genotype (p=0.0043; power=0.84) and did not show significant differences when interacting with the genotypes ID and II. The arterial hypertension showed no association with the DD genotype in the development of CAD (p=0.0609; power=0.4662). However, this same risk factor presented an important correlation with the genotype ID (p=0.0010; power=0.9155). For genotype II, in the comparison between the patients and controls, no statistical relevance was identified with a high power in any coronary risk factor. This demonstrates that the participation of these genotypes in the genesis of CAD is determined by more complex associations than other studies have indicated when analysing the genotypes as part of a single sample [34-38].

The present study found that low levels of HDL-c are not associated with CAD in any of the ACE genotypes, either in the patient group or in the controls. Roberts also demonstrated, through the genetic risk score, that high plasma HDL-c does not protect against CAD [39]. Obesity was identified as an important risk factor for the development of CAD [40]. However, in this study, there was no association of obesity with any of the ACE genotypes, in either the patients and or in the controls. In a review of several prospective studies, Ford and Caspersen demonstrated the importance of a sedentary lifestyle as a risk factor for CAD [41]. In contrast, the present research revealed that none of the ACE genotypes were associated with a sedentary lifestyle.

The relationship of diabetes mellitus with CAD and the I/D polymorphism of the ACE gene revealed a significant relationship with the DD and ID genotypes [42]. In the present study, there was no association between diabetes mellitus and CAD in the genotypes DD (p=0.3874; power=0.1348), ID (p=0.3136; power=0.3136) and II (p=0.4071; power=0.1260). This divergence of results may be related to the methodology used in the statistical analysis, in which the power of the test can be an important result to determine the real participation of this variable in the association with the ACE gene in CAD.

The ACE gene insertion/deletion polymorphism is recognized as a low-penetrance mutation and may be in linkage disequilibrium with other functional variants. The analysis of multiple genetic markers in the context of environmental factors is necessary to identify whether common risk factors are relevant. A meta-analysis of four studies, involving 55, 685 participants, studied polymorphisms associated with coronary risk and demonstrated that genetic and lifestyle factors were independently decisive for the onset of CAD [43]. In another meta-analysis of 40 case-control studies with 34,993 participants, the D allele of the ACE I/D polymorphism was significantly associated with an increased risk of myocardial infarction in genetic comparison models [44].

In the present study, the analysis done with the ACE genotypes allowed us to infer that there was an association between the DD genotype and an increased risk of CAD. However, as each genotype interacts with modulating external variables, its effect would not be well defined in such a way. Thus, it was identified a form of the analysis considering each genotype DD, ID and II as three distinct genotype populations whose behaviour, in relation to the different risk factors, is variable. This form of statistical analysis, in association with the p-value and power test, opens the door for establishing the participation of the ACE gene polymorphism and the manner in which it modulates the expression of classical risk factors in the development of CAD.

Conclusion

Our results indicated that different genotypes of the ACE gene are associated, in different ways, with the same coronary risk factors influencing the development of CAD. This information may help identify individuals with better predictability and greater susceptibility to CAD, aiding the interpretation of many aspects of these interfaces in clinical practice.

Disclosures

The authors report no relationships that could be construed as a conflict of interest.

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