Original

El polimorfismo inserción/deleción del gen de la enzima convertidora de angiotensina y su asociación con algunas complicaciones en pacientes con diabetes mellitus tipo 2

(Angiotensin Converting Enzyme Insertion/Deletion Polymorphism and its Association with Complications in Patients with type 2 Diabetes Mellitus)

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Abstract

Background: Diabetes mellitus is a disease with serious repercussions on the Costa Rican population and it predisposes patients to diverse complications. The exact origin of these complications is not known; it has been suggested that certain polymorphisms are strongly associated with the appearance of some of them.

Aim: To determine if there is a relation between complications from diabetes and the insertion/deletion polymorphism of the angiotensin converting enzyme.

Methods: This investigation included 225 subjects. 109 were cases and 116 control subjects. A blood sample was taken from each participant and fibrinogen levels were measured; also, using a polymerase chain reaction of the intron 16 of the angiotensin converting enzyme gene, the insertion/deletion mutation of the angiotensin converting enzyme was studied. Then, the results were compared to the presence of diabetes or its complications.

Results: A greater number of controls were found to have insertion/deletion polymorphism, however this was not considered statistically significant. In the case group, none of the polymorphisms showed a dominant behavior. D/D was associated with high levels of fibrinogen.

Conclusion: No strong association was found between the polymorphisms of the Angiotensin converting enzyme and suffering from Diabetes mellitus type 2 in the studied population. Using a logistic regression analysis, it was determined that the polymorphism D/D is significant regarding the predisposition to develop diabetes, but it explains only 3,58% of this variable, therefore there are variables that have a greater significance. Yet, elevated levels of fibrinogen are related with the polymorphism D/D. It can be established that in the studied population there is no relation between the polymorphisms of the angiotensin converting enzyme and the emergence of complications from diabetes.

Keywords: Diabetes mellitus, angiotensin converting enzyme, polymorphism, PCR

Diabetes mellitus (DM) is a set of metabolic disorders that share hyperglycemia as a common phenotype. [1] Different types of diabetes exist, and they are related to a complex interaction between genes, environment and lifestyles. Factors contributing to hyperglycemia may be due to decreased or absent secretion of insulin, decreased glucose consumption or increased glucose production. [2,3] The lack of glucose metabolism regulation causes several pathophysiological changes in multiple organs; most common complications include dyslipidemia, nephropathy and vascular abnormalities. [4]

Nowadays, there are more than 143 million people with DM worldwide [5], representing one of the major health problems worldwide as it implies high costs for any country. In Costa Rica it is estimated that by 1998 the self-reported prevalence of DM was 4.8% for people over 20 years-old, by 2006 it was estimated that the percentage of diabetic patients went up to 5.3% in this population, [1] showing the importance that this disease has claimed along time for health authorities in Costa Rica. [6]

According to the CARMEN survey [7] for the diabetic population, gender percentages indicate that the prevalence is higher in women (3.4%) than men (2.2%), the average age at death is 65, and also it is known that the prevalence of DM is lower in rural areas, both inside and outside the Costarican Central Valley. [8] The urban area showed a 10,2% prevalence both in and out from the Central Valley. [8]

Angiotensin converting enzyme (ACE)

ACE is a protein that has evidence of possibly having pleiotropic effects and a role in several diseases, not only in hypertension. [9] ACE is a regulating enzyme of the renin-angiotensin-aldosterone system (RAAS); when ACE activates, angiotensin I is converted to angiotensin II, a vasoconstrictor. ACE is also known to inactivate bradykinin and kallikrein, which are vasodilator molecules (Figure 1). Because of these effects, ACE increases blood pressure [10].

The ACE gene is located in chromosome 17 q23 and contains a polymorphism that can be an insertion (I) or a deletion (D) of 287 base pairs in the 16th intron. The genotype D/D has been associated with higher ACE levels and a 4 times higher activity than genotype I/I, 11 as well as higher blood pressure levels, obesity and cardiovascular risk [10]. Other studies suggest that the ACE I/D polymorphism is a risk factor for developing kidney disease in type I diabetes. [12]

Materials and Methods

A population of 225 individuals was studied, of whom there were 109 cases and 116 controls, the case group were those diabetic patients who were treated in areas covered by the Basic Teams for Comprehensive Health Care (Equipo Básico de Atención Integral de Salud, EBAIS), within the comprehensive health care program of the Costarican Social Security Fund (Caja Costarricense del Seguro Social, CCSS)-University of Costa Rica agreement, from March 2007 to July 2008. The control group were those non-diabetic individuals willing to participate freely in the study.

Each individual had to sign an informed consent form (project number 807-A5-311, approved by the document VI-8163-2008) to be included in the investigation as provided by the Bioethics Committee of the Universidad de Costa Rica´s Research Vice-Rectory.

Because of a coincidence in terms of patients studied, in both case and control groups there was a clear majority of women; 86 (74%) of control individuals, and 73 (67%) of the case individuals were women.

Studies performed

• **Sample gathering and stability**: Two peripheral blood samples were obtained from each patient, one with EDTA as an anticoagulant and the other one with citrate. This was kept refrigerated since it was drawn, and plasma was separated from blood cells as soon as possible.

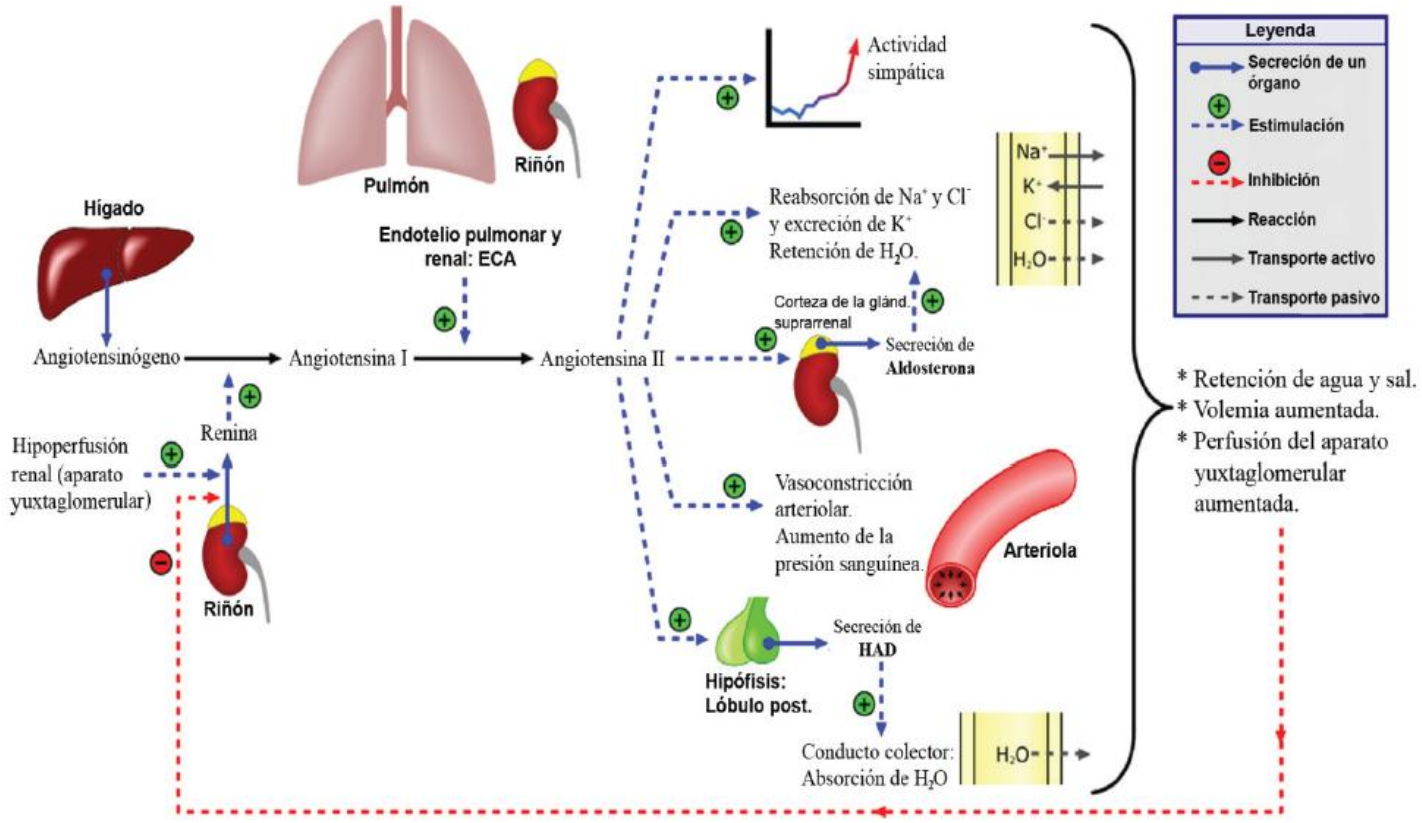

Figure 1. Summary of ACE ´s physiology and action in the rennin-angiotensin-aldosterone system, adapted from reference 23

• **Molecular-genetic analysis**: DNA isolation was made by the standard precipitation method with NaCl, as described by Miller et al. [13] The I/D mutation study for the ACE gene was performed through the polymerase chain reaction (PCR) of intron 16, in ACE gene, according to the protocol described by Rigat, [14] to obtain a fragment of 190 base pairs (bp) in the absence of insertion (D allele) and a fragment of 490 bp in the presence of the insertion (I allele). The bands obtained were analyzed on polyacrylamide gels by electrophoresis (Fig. 2).

• **Coagulation analysis**: plasma was frozen to -70° C to be analyzed in a two month-maximum time lapse, values of plasma fibrinogen (FGO) were obtained by the method described by Clauss in 1957 [15].

• **Data analysis:** The information obtained by interviews and laboratory tests was digitized and data analysis was made using the Epi-Info6d software; a binomial logistic model was also used, using the version 13.0 SPSS software (SPSS Inc, Chicago, USA) for Windows. Finally, we determined the allele frequencies with the balance proposed by the Hardy-Weinberg law using the χ2 test.

Results

We compared the number of individuals in the study who suffered strokes, dyslipidemia, nephropathy and ischemic heart disease in both groups without obtaining a significant difference between the number of cases and number of controls who suffered such pathologies. In the case of high blood pressure (hypertension), there was a significant difference, according to what was expected. (Table 1)

As for the ACE I/D gene polymorphism, there was a greater number of controls with the ACE I/D

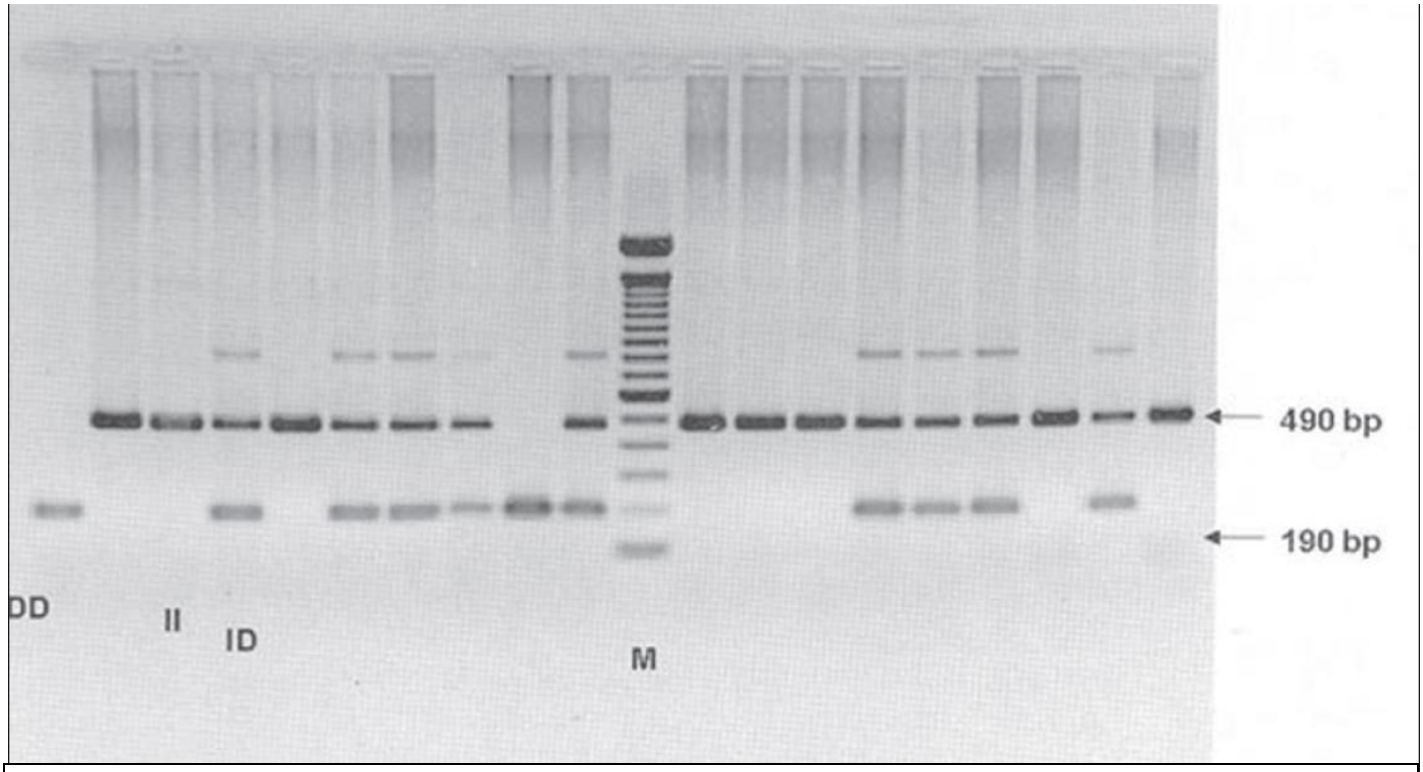

Figure 2. The ACE polimorphism for intron 16 is shown after PCR.

polymorphism, without being significant. In the case group, no polymorphism showed a dominant behavior, with almost the same number of individuals for each polymorphism.

We looked for the relationship between having or not a stroke and the ACE polymorphism for each individual, without a significant difference between any of the 3 polymorphisms for this disease. A similar behavior was observed when looking for a relationship between ACE polymorphism and the presence of ischemic heart disease, dyslipidemia, hypertension and nephropathy.

An association between ACE polymorphism and having or not DM was also looked for, obtaining a p= 0.334, which indicates that there was no significant difference for any of the polymorphisms studied.

When performing a $\chi 2$ comparing the variable FGO with D/D polymorphism, a p= 0.026 was obtained, which indicates that the D/D polymorphism is associated with abnormal levels of FGO, so that the D/D polymorphism is apparently associated with some complications of diabetes (Table 2), as it is a confusing variable.

The polymorphism allelic frequencies for the controls met the Hardy-Weinberg equilibrium. The ACE I/D does not meet the Hardy-Weinberg equilibrium ($\chi 2 = 10,36$; df= 2; p = 0.005).

Finally, it was seen that among the 116 individuals of the control group, 68% had normal plasma FGO levels while 29% of this group had altered plasma FGO levels. For the case group 72% out of 109 individuals had altered plasma FGO levels, while 22% had normal levels.

Discussion

As for the ACE polymorphism and its association with cerebrovascular disease, this research shows no association with each other and data indicate that the endothelial damage and prothrombotic state present in diabetes must have other factors besides this gene. Similar results can be found in the literature, giving much greater weight to this finding [16].

The literature indicates that the sequence of the I allele in the ACE I/D, is similar to that of a "silencer", this could explain the association of the D allele with high plasma levels of ACE, producing hypertension (HTN); [17 -19] the

study population slightly showed this behavior without being statistically significant.

The presence or absence of hypertension was not significantly different when compared with the 3 studied polymorphisms; however, by the applied logistic regression model, the D/D polymorphism is related to a predisposition to develop diabetes, but this variable can barely explain 3,58% of the variability, so that the important etiology of DM depends on other factors. One idea that emerges is that perhaps the D/D polymorphism is a confounding variable when studying the I/D ACE polymorphism, this means that possibly there is another variable not taken into account, besides I/D ACE polymorphism that really influences suffering or not from DM and its complications; then the fact of having or not DM and its complications is associated with this factor, which was not considered in this study. This finding may also be due to sample size, which in this case is small for genetic research purposes. Also, it could be because the study population does not have a true relationship between hypertension and the ACE polymorphism, which is shown in the results; Mondry et al [20] obtained similar results with a larger sample size, so that this supports the idea that there is no relationship between hypertension and ACE in the study population. Both nephropathy, dyslipidemia, as in ischemic heart disease showed no tendencies towards any of the studied polymorphisms. In Costa Rica, specifically in the Central Valley, a study has been made, revealing the genetic origins of the population [21] and another one that investigates conditions according to gene origins, showing that the more the Western-African heritage a person has, the greater the risk of myocardial infarction and hypertension, while the more the indigenous heritage, the greater the risk of metabolic syndrome. [22] However, there were no Costa Rican reports found about ACE I/D polymorphisms and their relationship with the complications studied in this research. Reports were found from Asian, European and U.S. populations [10, 12], with very different origins compared to our country.

The allelic frequencies of the ACE I/D polymorphism for the cases group do not meet the balance proposed by the Hardy-Weinberg equilibrium. This is probably due to sample size which is not suitable for this type of analysis (n = 225), coupled with the fact that ideally the analysis is applied to samples in which different generations are included, so that the mutation´s behavior could be compared throughout generations. Further research is suggested to increase the sample size and achieve statistically significant results.

For an inference about the ACE gene polymorphism on the Costa Rican population, it is necessary a bigger study in which an adequate sampling is done, taking into account epidemiological parameters; once an adequate number is obtained and an appropriate selection of each individual´s original location is made, the variability of the polymorphism in the Costa Rican population should be established, and then an inference could be made about the relationship of the ACE gene in certain pathologies in the Costa Rican population.

Levels of FGO in the diabetic population are known to be generally changed, [17] this was important when performing data analysis and obtaining an apparent association of the D/D polymorphism with some of the studied complications. When analyzing this background detail it was concluded that individuals with the D/D polymorphism are linked to altered FGO values, this is why FGO is identified as the cause of the associated complications, subtracting the effect of the FGO variable, the real effect of the polymorphism meant no significant value.

Conflicts of interest: none of the authors reported conflicts of interest performing this research.

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