

Genotypes of the renin-angiotensin-aldosterone system: on the search of cardiovascular diseases

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Associations between cardiovascular diseases and diverse polymorphisms have been studied with interest in recent years. A polymorphism is the presence within a genetic *locus* of allelic variants (or alleles) that can be explored by analysis of the DNA (genetic polymorphism) or protein product (phenotypic polymorphism). An allele is an alternative version of a DNA nucleotide sequence in a specific *locus*.

Generally speaking, associations between a polymorphism and a specific disease have been reported in the bibliography more frequently than studies evaluating the specific mechanisms that relate such a genotype to a disease (or phenotype). In investigative and epidemiological terms, this is logical method for establishing associations and new pathogenic hypotheses. However, it raises several questions regarding topics such as publication bias (studies that yield positive results are more likely to be published than studies with negative results), the biological meaning of the association (causality), and the conditions in which the observation is made (and its validity).

The problem of the molecular or cellular mechanisms underlying a given association between a genotype and a phenotype is being investigated –both clinically and experimentally– and is relevant in terms of causality and possible preventive or therapeutic interventions in cardiovascular diseases (e.g., conductual, pharmacological, or genetic).

Among the criteria that ideally should be met by studies of genotype-disease associations in order to establish causality, large sample size and small *p* values are important. In addition, biologically

reasonable associations should be found in alleles that affect a certain gene product in a way that makes physiological sense. On the other hand, there should be an initial study and an independent study reproducing the results of the initial study. The association should be observed in familial and population studies. Finally, the odds ratio and/or attributable risks should be high.¹

Among the polymorphisms that have been most studied in relation to the cardiovascular diseases, on which the work of Hernández et al² has focused, are polymorphisms of the renin-angiotensin-aldosterone system (RAAS), particularly the insertion/deletion (I/D) polymorphism of the angiotensin I converting enzyme (ACE) gene, M235T polymorphism of the angiotensinogen gene, and A1166C polymorphism of the type I receptor of angiotensin II (R-AT₁).

ACE (or kininase II) hydrolyzes the decapeptide angiotensin I to the vasoactive octapeptide angiotensin II. In addition, it metabolizes bradykinin (1-9) to its inactive form (1-7) and angiotensin (1-7) to its inactive form (1-5). Consequently, ACE activity may somehow determine circulating and tissue concentrations of angiotensin II and contribute to regulating vascular tone, blood pressure, and possibly cardiovascular remodeling processes. In different populations, I/D polymorphism of the ACE gene determines almost 50% of circulating ACE titers. Higher circulating ACE concentrations are associated with the presence of the D allele and may be related to an increase in cardiovascular and renal morbidity. In humans, this polymorphism could be a marker of a nearby variant sequence, not yet identified, that modulates expression of the ACE gene so that the D allele is associated with more ACE activity in plasma, lymphocytes, and cardiac tissue. A similar genetic polymorphism of the activity of this enzyme has been observed in rats, thus providing an extremely useful experimental model for studying some aspects of this polymorphism.^{3,4}

Angiotensinogen is an extracellular glycoprotein that is synthesized and released mainly by

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hepatocytes (as well as adipocytes and astrocytes) in constitutive form (non-induced). The production of plasma angiotensin II is very sensitive to small changes in angiotensinogen concentration (through the action of renin). Circulating and tissue angiotensinogen levels correlate inversely with renin levels. A polymorphism in exon 2 of this gene (substitution of methionine by treonine in position 235 of the coded protein [M235T]) would result in higher angiotensinogen titers.

On the other hand, most of the deleterious cardiovascular effects of angiotensin II take place through R-AT₁ (such as vasoconstriction, aldosterone synthesis, induction of cell growth and hypertrophy, and Nf-KB activation). These receptors are expressed in a variety of cells in different organs (like vascular smooth muscle cells, myocytes, renal mesangial and proximal tubule cells, and the glomerulosa zone of the adrenal glands). The R-AT₁ polymorphism (A1166C) is located in the untranslated 3' region of mRNA and corresponds to a substitution of adenine by cytosine. The functional effects of this polymorphism are little known.

With respect to these three RAAS polymorphisms, the following observations are consistently made in humans:

- ACE concentrations are higher in carriers of the D allele, with no changes in renin, angiotensin II, and aldosterone, or differences in the conversion of angiotensin I to angiotensin II. Circulating bradykinin has been found to have a more prolonged half-life in subjects with genotype II. In recent experimental studies in rats with a similar ACE polymorphism, lower titers of circulating and tissue neutral endopeptidase (another ectoenzyme that metabolizes many peptides with cardiovascular effects) have been observed in the presence of high ACE values.⁴

- Increased angiotensinogen values in carriers of the T235-TT angiotensinogen variant (with lower renin and prorenin concentrations due to negative feedback, but no effect on the titers of ACE, angiotensin II, aldosterone, or R-AT₁ density or affinity), the variant associated with a greater coronary risk in the recent study of Hernández et al.²

- There is no convincing evidence of a relation between R-AT₁ density and/or functionality and the presence of the C allele of this receptor. Genotype CC is associated with greater vasoconstriction through a mechanism that has not yet been clarified.

As regards the three polymorphisms under discussion and cardiovascular diseases, Danser and Schunkert affirm that the best available evidence in favor of a clinically relevant relation is that suggesting a relation between I/D ACE polymorphism and arterial hypertension (AHT) in men.⁵⁻⁷ The risk of presenting AHT is 1.6 times greater in men with the genotype

ACE DD.⁶ This clinical-epidemiological observation also has a recent experimental correlate (Ocaranza et al, *Journal of Hypertension*, in press). According to the clinical observation mentioned, the risk of individuals with the ACE DD genotype developing acute myocardial infarction (AMI) attributable to AHT phenotype is calculated as close to 3.4%, a minimal figure⁵ that concurs with the absence of a greater risk of a first coronary event associated with this genotype found by the authors of the Spanish study.

In this population study of cases and controls carried out in the Canary Islands (74% of the sample were men), TT homozygosis of the angiotensinogen gene was found to predispose independently to the appearance of a first coronary event, with an odds ratio (OR) of 1.9 (1.1-3.4). Compared to the risk associated with this genotype, the estimated risks (OR) associated with other coronary risk factors were 4.4 for diabetes; 4 for total cholesterol/HDL ratio; 2.7 for smoking; 2.2 for alcohol consumption; and 2.1 for AHT. No association was found between the risk of a coronary event and I/D ACE polymorphisms or R-AT₁ (A1166C). The results of this study are similar to those found in other populations,^{8,9} including one made in another region of Spain.¹⁰ It should be noted that there are studies of similar design that have obtained negative results (particularly when the number of women in the study increases).¹¹ Therefore, the present study clearly indicates the need for more similar data in different human groups, hopefully simultaneous, with additional parameters that allow the causal mechanisms of these associations to be explained.

At the moment, and although this is a very attractive area of investigation from which we should learn much in the future, no clear conclusions can be drawn regarding the relation between RAAS polymorphisms and the effect produced when the system is blocked pharmacologically. The results so far obtained (mainly in AHT) vary in accordance with the study populations (healthy/ill), the duration of treatment, doses used, sodium intake, and the use of other drugs. Pharmacogenomic studies could explain the variability of the effect of drugs that inhibit the RAAS. Individual pharmacological effects could depend on interactions between these polymorphisms that result in different circulating and tissue levels of the different peptides and enzymes in the RAAS.

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