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Genetic variants in the Renin-Angiotensin System genes are associated with Cardiovascular-Renal-related risk factors in Mexican Americans

Farook Thameem^{1,*}, V. Saroja. Voruganti², Xin He¹, Subrata D. Nath¹, John Blangero², Jean W. MacCluer², Anthony G. Comuzzie², Hanna E. Abboud¹, and Nedat H. Arar¹

¹Division of Nephrology, Department of Medicine, University of Texas Health Science Center, San Antonio, TX 78229, USA

²Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX 78227, USA

Abstract

The aim of this study was to examine whether the *ACE-I/D*, *AGT-M235T*, and *AT1R-A1166C* polymorphisms of the renin-angiotensin system (RAS) genes are associated with cardiovascular and renal-related risk factors in Mexican Americans. Study participants (N=848) were genotyped by Taqman assays. Association analyses were performed by measured genotype approach. Of the phenotypes examined, the *ACE-I/D*, *AGT-M235T*, and *AT1R-A1166C* polymorphisms exhibited significant association with systolic blood pressure, glomerular filtration rate and body mass index, respectively. The data suggest that the polymorphisms examined in the RAS may modulate the risk factors associated with cardiovascular-renal disease.

Keywords

ACE; *AGT*; *AT1R*; polymorphisms; association analysis; SBP; GFR; BMI; Mexican Americans

The renin-angiotensin system (RAS) plays a central role in the regulation of sodium metabolism, vascular tone, blood pressure, renal haemodynamics, and vascular modelling. Genetic variants of its major components such as the insertion/deletion polymorphisms of angiotensin converting enzyme I (*ACE-I/D*), the M235T variant of angiotensinogen (*AGT-M235T*), and the A1166C polymorphism of angiotensin II type 1 receptor (*AT1R-A1166C*) genes have been implicated in the pathogenesis of hypertension, cardiovascular and renal diseases (Miller and Scholey, 2004). A large number of association studies of cardiovascular-renal-related traits and the above mentioned polymorphisms in the RAS have been mostly conducted in Caucasians and Asians. Furthermore, the association studies have yielded conflicting results. Therefore, the objective of this study is to investigate whether these three polymorphisms are associated with sub clinical cardiovascular and renal-related risk factors in a cohort representing the Mexican American population.

The San Antonio Family Heart Study (SAFHS) family member recruitment and a variety of metabolic, hemodynamic, anthropometric, and demographic data collection procedures from

* Corresponding author: Farook Thameem, Ph.D. Division of Nephrology, Department of Medicine The University of Texas Health Science Center at San Antonio 7703 Floyd Curl Drive San Antonio, Texas 78229, USA Phone: 210-567-4787 ; FAX: 210-567-4712 thameem@uthscsa.edu.

more than 40 extended families were described previously (MacCluer et al., 1999). Proband for the SAFHS were selected randomly from a census tract in San Antonio of low-income Mexican Americans regardless of preexisting medical conditions. Estimation of glomerular filtration rate (eGFR) and albumin to creatinine ratio (ACR) have already been described (Arar et al., 2008). The quantitative trait values of ACR (*ln* ACR) and triglycerides (*ln* TGL) were log transformed and used in the association analyses since their raw data were non-normally distributed. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved all procedures, and all subjects gave informed consent.

In this study of 848 participants in 21 families, 52%, 22% and 14% of the subjects had hypertension, type 2 diabetes, and albuminuria, respectively. Genotyping of *AGT-M235T* (rs699), and *AT1R-A1166C* (rs5186) polymorphisms was performed by TaqMan assays (Applied Biosystems, USA). Genotyping of *ACE-I/D* (rs1799752) polymorphism was performed by PCR as described (Lovita et al., 2001). The allele and genotype frequencies were checked for mendelian inconsistencies using INFER, the subroutines of the program PEDSYS and are presented in Table 1. Genotypic data were consistent with the Hardy-Weinberg Equilibrium expectations. Association analysis in our family data was carried out using the measured genotype approach within the variance components analytical framework implemented in SOLAR (Almasy and Blangero, 2004). To guard against potential effects of hidden population stratification, we also employed the quantitative trait disequilibrium test (QTDT) approach (Abecasis et al., 2000). This approach permits a formal test of likely population heterogeneity on a marker-specific basis (Havill et al., 2005). Using this test, we found no evidence for such stratification. Additionally, the results of the QTDT analyses were consistent with the more powerful measured genotype analyses.

Of the phenotypes examined for association, *ACE-I/D* polymorphism exhibited significant association ($P = 0.0031$) with systolic blood pressure (SBP) after accounting for trait-specific covariate effects (Table 2). The carriers of the **D** allele had significantly increased SBP values [$I = 123.3 \pm 1.4$; $ID = 125.8 \pm 1.2$; and $D = 128.3 \pm 1.5$; $P = 0.003$]. Our association results are in agreement with several studies, including a Hispanic cohort demonstrating that the **D** allele is consistently associated with increased SBP values (Bautista et al., 2008). As can be seen from Table 2, association analyses also revealed the presence of significant association between the *AGT-M235T* variant and eGFR ($P = 0.025$) after accounting for trait specific covariates (Table 2). The carriers of the **T** allele had significantly decreased eGFR values [$M = 104.5 \pm 1.5$; $MT = 101.4 \pm 1.3$; and $T = 98.3 \pm 2.3$; $P = 0.025$]. Our finding confirms earlier report that the **T** allele is associated with decline in renal function (Fabris et al., 2005). Of the phenotypes examined, the *AT1R-A1166C* polymorphism demonstrated significant association with BMI ($P = 0.007$) after accounting for age, sex, and diabetes.

Although the elucidation of the pathophysiological mechanisms underlying the risk associated with the examined RAS gene polymorphisms is beyond the scope of this study, it is reasonable to assume that genetic variants may influence, through different pathways, the activation of the RAS at the systemic levels. Many vascular cells as well as adipocytes express all the components of RAS. Angiotensin II, the major effector of RAS acts primarily through AT1R. The variants examined in this study including AT1R-A1166C have been associated with increased RAS activity (van Geel et al., 2000). Activation of the RAS in adipose tissue has been implicated in the regulation of adiposity and overt obesity through its ability to increase fat cell growth and differentiation, increase synthesis, uptake and storage of fatty acids and triglycerides (Jones et al., 1997). Moreover, activation of the RAS in adipose tissue may represent an important link between obesity and hypertension. Consistent with this notion, it is reasonable to predict from our data that the variants of the RAS examined may be associated with activation of the RAS in vascular and adipose tissue and thereby contributed to the altered blood pressure and BMI.

The mechanism by which the AGT-M235T polymorphism affects renal function estimated by GFR remains to be elucidated. The AGT-M235T polymorphism has been associated with increased production of angiotensin II. Angiotensin II regulates renal hemodynamics and tubular transport, stimulates cytokine formation and release (Ibrahim et al., 1997). Thus, increased production of angiotensin II due to AGT-M235T within nephrons and their vasculature may participate in local renal injury through altering both hemodynamic and non-hemodynamic/trophic actions of angiotensin II and thereby contribute to renal dysfunction.

This study has limitation in that it focused on three variants of the RAS components examined previously and has not attempted comprehensive tagging of all common variation within the RAS. In addition, the GFR estimated by the **Modification of Diet in Renal Disease** (MDRD) formula has not been validated in Mexican-Americans. However, the MDRD equation is commonly used to estimate GFR and have been employed in several genetic studies (Puppala et al., 2007, Schelling et al., 2008; Freedman et al., 2008). It is to be noted that while allowing for multiple testing of SNPs preserves the significance of our three best associations, we, however, did not consider corrections for tests across multiple phenotypes since each phenotype can be considered to reflect an unique hypothesis.

In conclusion, the present study adds evidence for the role of RAS gene polymorphisms in modulating the risk factors related to cardiovascular-renal disease in Mexican Americans.

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Table 1Allele and Genotype frequencies of the selected variants from the *RAS* Genes

Gene (polymorphism)	Major/minor allele (%)	Genotypes (%)		
Angiotensin converting enzyme I (<i>ACE-I/D</i>)	I (57) / D (43)	II (32)	ID(48)	DD (20)
Angiotensinogen (<i>AGT-M235T</i>)	T (69) / M (31)	MM (7)	MT (47)	TT (46)
Angiotensin II type 1 receptor (<i>AT1R-A1166C</i>)	A (69) / C (31)	AA (48.5)	AC (42)	CC (9.5)

Table 2

Association analysis between selected polymorphisms from the *RAS* genes and Cardiovascular-renal related traits in SAFHS data

Phenotypes	<i>ACE-I/D</i> <i>P</i> value	<i>AGT-M235T</i> <i>P</i> value	<i>AT1R-A1166C</i> <i>P</i> value
Type 2 diabetes ^a	0.539	0.222	0.950
Body mass index ^b	0.868	0.353	0.007
Total Cholesterol ^c	0.982	0.347	0.198
High density lipoprotein cholesterol ^d	0.793	0.332	0.670
Triglycerides (log transformed) ^c	0.499	0.738	0.553
Systolic blood pressure ^d	0.003	0.680	0.311
Diastolic blood pressure ^d	0.120	0.933	0.885
Albumin to creatinine ratio (log transformed) ^e	0.718	0.161	0.505
Glomerular filtration rate ^e	0.402	0.025	0.885

^aData adjusted for age, sex and BMI;

^bData adjusted for age, sex and diabetes;

^cData adjusted for age, sex, diabetes duration, lipid lowering medication and BMI;

^dData adjusted for age, sex, diabetes, duration of diabetes and antihypertensive treatment;

^eData adjusted for diabetes, diabetes duration, systolic blood pressure, and antihypertensive treatment. GFR was estimated by MDRD equations.