Combined Analysis of Genetic and Environmental Factors on Essential Hypertension in a Brazilian Rural Population in the Amazon Region

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Summary

Objective: In the present study, we evaluated the contribution of six genetic polymorphisms of the Renin-Angiotensis-Aldosterone system (RAAS) and clinical risk factors in the development of essential hypertension in a Brazilian rural population in the Amazon region.

Methods: Eighty-two hypertensive patients and seventy-eight normotensive individuals were evaluated. Genotyping for renin (REN G1051A), angiotensinogen (AGT) M235T, insertion/deletion of angiotensin-converting enzyme (ACE I/D), angiotensin II type 1 receptor (AGTR1) A1166C and aldosterone synthase (CYP11B2) C344T polymorphisms were performed using polymerase chain reaction, with further restriction analysis when required. The influence of genetic polymorphisms and clinical risk factors on blood pressure variation was assessed by stepwise linear regression.

Results: We report the co-occurrence of clinical risk factors and angiotensin-converting enzyme (ACE) gene polymorphism in a Brazilian rural population in the Amazon region. Our results indicate that increase of systolic blood pressure (SBP) is favored by ACE I/D- D allele and advanced age, while alcohol consumption and aging are associated with high diastolic blood pressure (DBP).

Conclusion: These findings suggest that in the Santa Isabel do Rio Negro population, the residents that carry ACE-D allele or have an alcohol consumption habit present higher values of SBP and DBP, respectively, with the passing of years.

Key words: Polymorphisms, essential hypertension, environmental risks, genetic.

Introduction

Essential hypertension (EH) is a multifactorial disease triggered by several genetic and multiple environmental factors in conjunct. Epidemiological studies have suggested that genetic variants, including those of the genes for angiotensinogen (AGT)[1], renin (REN)[2], angiotensin-converting enzyme (ACE)[3], angiotensin II receptor type 1 (AGTR1)[4,5], and aldosterone synthase (CYP11B2)[6] increase the risk for EH. However, the influence of polymorphic forms of these genes has shown conflicting results in different populations[7,8]. This scenario might be reflecting the variable impact of the genetic background of populations and the interaction of environmental factors, which, in turn, might be modulating this molecular background. Recent literature data have shown that unfavorable genotype/allele alone might indicate a minor or nonsignificant association with EH. However, the co-occurrence of different unfavorable genotypes and clinical risk factors can increase the risk of hypertension. Data indicate that the most important environmental risk factors are advanced age, alcohol intake, smoking and body size. The relationship of those factors with unfavorable genotype such as ACE-DD and Methylenetetrahydrofolate reductase (MTHFR) 677-TT can greatly increase the EH risk, when compared with patients who do not present the clinical risks[9].

In this work, we aimed at investigating the association among AGT-M235T, REN-G1051A, ACE-I/D, AGTR1-A1166C, CYP11B2-C344T genetic markers and common anthropometrical factors with essential hypertension, in an isolated community in the Amazon region in Brazil.

Methods

The municipality of Santa Isabel do Rio Negro lies in the northwest of Amazonas State (0º 28' S and 65º 32' W), in the region of the Alto Rio Negro. Santa Isabel do Rio Negro occupies an area of 62, 846 Km2 (IBGE, 2003), 90% of which is covered by the Amazonian Rain Forest. The majority of the population of 10, 561 inhabitants is of indigenous ancestry; 4220 of them reside in the municipality's seat and 6341 in riverine communities in the municipality's territory, including the Yanomami Indigenous Territory[10].

Subjects chosen for this study were obtained using the Conglomerate Systematic Sampling Technique, in which each residence was considered a unit sample. One hundred and fifteen residences were visited, and one adult from each residence was chosen. A total of eighty-two individuals were classified as hypertensive according to the following criteria: 1)

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blood pressure (BP) ≥ 140/90 mmHg (derived from repeated measurements performed in accordance with international guidelines)[11]; 2) absence of antihypertensive, and 3) exclusion of secondary forms of hypertension. The normotensive controls consisted of 78 individuals, characterized by no family history of EH or other cardiovascular disorder and BP < 140/90 mmHg.

All participants were submitted to a complete physical and routine biochemical analyses and provided written informed consent.

Participants were classified as Caucasian, Negroid, Indigenous or racially mixed (Mestizo) according to a set of phenotypic characteristics[12]. Individuals who had ever smoked more than five cigarettes per day for at least a year were classified as smokers and the ones who drank 30-40 ml of alcohol or more per day were considered drinkers.

Genotyping ACE I/D[3], AGT M235T[13], REN G1051A[2], AGTR1 A1166C[4], and C344T[6] were assessed by the PCR-restriction fragment length polymorphisms method. Quality control for these assays was assessed by randomly selecting samples from hypertensive and normotensive groups to be genotyped again.

Anthropometrical and clinical data were compared by the t-Student for quantitative data, and by χ^2 test for qualitative data. The genotypic frequencies were computed using the SPSS statistical program (version 10.0), and allelic frequencies were calculated by direct gene counting. The Hardy-Weinberg equilibrium (HWE) for distribution of the genotypes was tested by χ^2 test, using the GENIOC program[14]. We also performed a stepwise linear regression (SPSS program, version 12.0) to explore the effects of genotypes and anthropometrical factors on hypertension. In this analysis, the dependent variables were systolic blood pressure (SBP) and diastolic blood pressure (DBP), while independent variables included age, body mass index (BMI), gender (0 = female, 1 = male), smoking status (0 = non-smoker, 1 = smoker), alcohol consumption (0 = non- drinker, 1 = drinker), Diabetes mellitus (0 if glucose <100mg/dl, 1 to else), ethnicity (ethny_1 [African descendant], ethny_2 [Caucasian], ethny_3 [Indigenous]; ethny_4 [Mestizo]). Values 0 and 1 were attributed if ethnicity was absent or present, respectively.

Results

Age, alcohol consumption, and smoking weighed more frequently among hypertensives than in normotensives (Tab.1). These results are supported by previous studies that demonstrated that alcohol consumption contributes to EH development by stimulation of the sympathetic nervous system and increased production of adrenocorticoid hormones[15], while age and smoking act as co-factors that, in association with salt sensitivity, alcohol consumption or obesity, collaborate to the development of this disease[16]. Diabetes mellitus and obesity are also important clinical risk factors for hypertension, but their frequencies were not significantly different between the groups, even among patients that exhibit a slightly high BMI. Moreover, the ethnicity profile observed in this Amazon population (where a great number of individuals from both groups are Indigenous descendents or Mestizo, and a lesser number are Negroid or Caucasoid) was quite similar between normotensives and hypertensives.

Genotypic distribution of each polymorphism was in agreement with HWE expectations (p >0.05) in both groups. Allelic frequencies of all polymorphisms did not show any difference between normotensives and hypertensives (Tab.2). However, the genotypic distributions of ACE I/D differed between the groups: 9.76% hypertensives versus 5.1% normotensives had ACE-DD genotype; and 57.32% hypertensive versus 78.2% normotensives had ACE-II genotypes. It was observed that postulated "worse" ACE (DD) genotype was slightly more frequent in hypertensives than in normotensives. These unfavorable alleles/genotypes are associated with physiological changes that can lead to hypertension development. Yet, worldwide results have been

Table 1 - Anthropometrical and clinical characteristics

		Normotensives (n = 78)	Hypertensives (n = 82)	p*
Gender	Female/Male	47/31	49/33	Nonsignificant
Age (year)		36.26 ± 12.46	49.27 ± 18.58	< 0.05
Ethnicity (%)	Negroid	1.3	0.0	Nonsignificant
	Caucasoid	5.1	9.1	
	Indigenous	32.1	40.9	
	Mestizo	61.5	50.0	
Smoking (%)		26.9	50.0	< 0.05
Alcohol Consumption (%)		33.3	45.5	< 0.05
Diabetes mellitus (%)		9.0	9.1	Nonsignificant
BMI (kg/m2)		24.62 ± 3.69	26.07 ± 3.43	Nonsignificant
SBP (mmHg)		111.73 ± 12.05	149.37 ± 11.81	< 0.05
DBP (mmHg)		70.76 ± 8.64	92.31 ± 4.38	< 0.05

*Data presented as mean ± standard error of the mean. * = T-test.*

Table 2 - Genotypic and allelic frequencies

RAAS polymorphisms	Normotensives	Hypertensives	p*
AGT M235T genotypes			
MM	08 (10%)	07 (8.54%)	
MT	56 (72.5%)	50 (60.97%)	0.154
TT	14 (17.5%)	25 (30.49%)	
AGT alleles			
M	36 (45.15%)	35.5 (43.3%)	
T	42 (53.85%)	46.5 (56.7%)	0.775
REN G1051A genotypes			
AA	40 (51.3%)	44 (53.66%)	
GA	22 (28.2%)	30 (36.59%)	0.136
GG	16 (20.5%)	08 (9.76%)	
REN alleles			
A	51 (65%)	59 (71.95%)	
G	27 (35%)	23 (23.05%)	0.370
ACE I/D genotypes			
II	61 (78.21%)	47 (57.32%)	
ID	13 (16.67%)	27 (32.93%)	0.019
DD	04 (5.13%)	08 (9.76%)	
ACE alleles			
I	67 (86%)	60 (73.17%)	
D	11 (14%)	22 (26.86%)	0.199
AGTR1 A1166C genotypes			
AA	55 (70.5%)	46 (56.10%)	
AC	20 (25.6%)	30 (36.59%)	0.157
CC	03 (3.8%)	06 (7.32%)	
AGTR1 alleles			
A	65 (83%)	61 (74.39%)	
C	13 (17%)	21 (25.61%)	0.169
CYP11B2 C344T genotypes			
CC	14 (17.9%)	12 (14.63%)	
TC	36 (46.2%)	48 (58.54%)	0.288
TT	28 (35.9%)	22 (26.83%)	
CYP11B2 alleles			
C	32 (41%)	36 (43.90%)	
T	46 (59%)	46 (56.10%)	0.713

** p value from ±2 test for genotype and allele distribution comparison.*

controversial and, consequently, the frequencies of ACE genotypes vary widely[7,17,18]. Genotypic frequencies of M235T, G1051A, A1166C and C344T failed to show any difference in either group ($p > 0.05$).

To investigate which genetic polymorphism and/or clinical variant is associated with high BP variation, we analyzed the genetic and clinical profile of normotensives and hypertensives simultaneously. Stepwise linear regression showed that ACE I/D polymorphism (D allele) and age contributed to SBP variation ($F = 12.958$, $p = 0.000$). The D allele supports an increase of 7.8 mmHg in SBP ($b = 7.829$, $p = 0.006$), while advanced age contributes to a 0.471 mmHg increase in SBP, with each additional year ($b = 0.471$, $p = 0.000$). This polymorphism is responsible for half the variance in plasma

ACE levels, which results in a large / in wide inter-individual variability[19]. It was observed that homozygotes for the D allele display serum ACE levels were almost twice as high as I allele homozygotes. However, specific haplotypes constituted by I/D and single nucleotide polymorphisms within the ACE gene were more accurate in determining ACE plasma levels. According to Keavney et al[19], the use of ACE haplotype analysis was able to determine 36% of the phenotype variance of ACE levels. Despite the physiological damage caused by ACE I/D polymorphism, recent studies have demonstrated that clinical risk factors can raise ACE gene susceptibility. Several studies have shown that advanced age, drinking, smoking and body size are important clinical risk factors for EH, especially when they occur simultaneously with the ACE-DD genotype[7]. In accordance with literature reports, our findings also suggest that ACE I/D polymorphism and a specific clinical risk factor collaborate to EH. Stepwise linear regression indicated that individuals who carry D allele have an increase of 7.8 mmHg in SBP when compared with those who have only I allele. In addition to I/D polymorphism, aging also contributes to the unfavorable effects of D allele, causing a progressive increase of SBP. In light of our findings, we believe that high variation of SBP may be, in part, determined by a genetic (ACE-D allele) predisposition and advanced age. Therefore, an unfavorable genotype associated with a clinical risk factor might be acting in hypertension development. We also observed that DBP variation is dependent on alcohol consumption and advanced age ($F = 4.305$, $p = 0.016$). Statistical analysis suggested that consumption of alcohol (more than 3 drinks per day) could contribute to an increase of 4781 mmHg in DBP. Advanced age also supports DBP increase by 0.140 mmHg per year. Our observation corroborates previous reports whose authors showed that heavy drinkers have a reduction in calcium absorption that could lead to the development of EH[19]. Moreover, presence of alcohol in an organism disturbs the renin-angiotensin system function, stimulates the sympathetic nervous system (possibly due to fluctuating blood alcohol levels), and increases production of adrenocorticoid hormones[20]. Literature reports also relate that consumption of about 40 ml of alcohol, per day, results in BP increase and, consequently, in a doubling of hypertension (SBP > 160mm Hg or DBP > 95mmHg)[19]. In this Amazon population, continuous alcohol consumption down the years contributed to the increase of DBP and, consequently, raised the number of hypertension cases.

Conclusions

Multifactorial analysis of genetic polymorphisms and clinical-antropometrical factors suggested that combinations of ACE D allele with advanced age and alcohol consumption, along with aging contributes to SBP and DBP high variation, respectively, in the Santa Isabel do Rio Negro population.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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