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The association of hypertension with renin–angiotensin system gene polymorphisms in the Lebanese population

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Abstract

Aim: The study objective was to examine the association of hypertension in the Lebanese population with three renin–angiotensin system gene polymorphisms (RAS): angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and angiotensin-receptor type 1 (AT1R).

Methods: A total of 270 subjects (124 hypertensive vs 146 normotensive) were genotyped for ACE insertion (I)/deletion (D), AGT (M235T), and AT_1R (A1166C) gene polymorphisms by polymerase chain reaction and restriction fragment length polymorphism.

Results: The studied genes showed no deviation from Hardy–Weinberg equilibrium. No association could be reported with the ACE I/D polymorphism, although the D allele frequency was high (77%) in patients. AGT TT genotype prevalence was found to be lower in hypertensive versus normotensive subjects ($p < 0.0001$). AT_1R CC and AC genotypes were significantly more frequent in hypertensive than normotensive subjects ($p < 0.0001$).

Conclusion: The first conducted study on the RAS gene polymorphisms in Lebanese hypertensive patients demonstrated a possible association of the AGT T and AT_1R C alleles with hypertension.

Keywords

Angiotensin converting enzyme, angiotensin receptor type 1, angiotensinogen, gene, hypertension, polymorphism

Introduction

Hypertension (HTN), or high blood pressure (BP), is the most common risk factor for myocardial infarction,[1] stroke, end-stage renal disease and peripheral vascular diseases.[2] HTN is present in approximately two-thirds of all persons over the age 65 years. Although ageing, obesity and environmental factors such as alcohol consumption[3] contribute to the onset of HTN, genetic factors also determine a substantial proportion of the variance of BP in the general population.[1] The renin–angiotensin system (RAS) is an important system of BP control through its effect on vascular tone, renal haemodynamics and sodium and volume homeostasis. Several studies have established that the RAS, including the angiotensin-converting enzyme (ACE) insertion (*I*)/deletion (*D*), the angiotensinogen (AGT) M235T and the angiotensin-receptor type 1 (AT_1R) A1166C genetic polymorphisms have all been implicated in the pathogenesis of essential HTN.[4,5]

RAS gene associations with high BP have been reported across various populations and ethnic groups. A high prevalence of the *D* allele and/or *DD* genotype has been shown in hypertensive subjects throughout the world.[5–15] The AGT *TT* genotype has been associated with HTN similarly across a wide range of populations. Numerous studies have also demonstrated an association between the AT_1R A1166C SNP polymorphism and essential HTN.[16–22] However, some of the evidence identifying associations between HTN and the RAS gene polymorphisms has been conflicting. For example, several studies have reported no association with the ACE *I*/*D*,[23–27] the AGT M235T[28–30] or the AT_1R A1166C polymorphisms.[31–33] It has been suggested that this inconsistency could be due to variation in genetic and environmental backgrounds across the numerous populations.[34]

Previous studies identified some populations of the Middle East as having lower frequencies of the ACE

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insertion, this being indicative of ancient populations; the ancestral state of the *Alu* repeat being the *D* allele.[35–39] Lebanon is a small country of 10,452 km^2 on the eastern shores of the Mediterranean Sea, with a population of approximately 4 million people, with a worldwide diaspora estimated at 15 million. The fact that the Lebanese population had such a low frequency of the *I* allele, then, is also considered to be indicative of this population being ancestral, with regards to human demographic history. The prevalence of HTN among the Lebanese is estimated at 23.1%, and up to the date of the present study there has been no information on RAS gene polymorphisms among Lebanese hypertensive patients. Given the nature of ACE *I*/*D* polymorphism in the Middle Eastern populations, it seems appropriate to question the health consequences of a polymorphism closely associated with the RAS. Specifically, does the high frequency of the *D* allele correspond with hypertension in a population where the *DD* genotype is predominant? Less information is available on the global trends of the AGT and A1166C polymorphisms, which are also implicated in hypertension, so predictions are difficult to make with regards to these two markers. Thus, this study aimed at investigating the association between genetic polymorphisms at three RAS genes (ACE, AGT, and AT_1R) and BP in a population-based sample from Lebanon.

Materials and methods

Study sample

Hypertensive patients. The patient sample (age 29–85; onset HTN < 60 years; otherwise healthy) collection took place in 10 different community pharmacies located in different areas of Lebanon. All records of adult male and female patients taking antihypertensive drugs and/or patients with systolic BP ≥ 140 and/or diastolic BP ≥ 90 were selected. One-hundred and twenty-four samples were collected (61:63 male:female), mean age of sample = 63 years (SD = 14 years).

Normotensive individuals. The control sample was chosen as for the patient sample except that the individuals had never been treated with medication for HTN and their systolic and diastolic BPs were < 140 and < 90 mmHg respectively. One-hundred and forty-six samples were collected (69:77 male:female), mean age of sample = 27 years (SD = 9 years). All subjects agreed to participate in the study after full explanation of what was involved, and the project was approved by the School of Pharmacy Research Committee.

Genomic protocols

Sample collection and DNA extraction. Each subject provided a cheek swab for DNA extraction. The presence of the insertion/deletion allele in intron 16 of the ACE gene was detected using the method of Rigat *et al*[40] with some modifications.[41] The *I* and *D* alleles were manifest as 490 bp and 190 bp bands respectively. Because of the possibility of preferential amplification of the *D* fragment in relation to the *I* fragment, resulting in mistyping of *I*/*D* as the *DD* genotype, all *DD* genotypes were reconfirmed[42] in additional gels.

AGT (M235T) and AT_1R (A1166C) polymorphism genotyping. Polymerase chain reaction and restriction fragment length polymorphism (RFLP) based protocols were used to identify the AGT[43] and AT_1R[44] gene polymorphisms. The homozygous M235T was represented as 164 bp. Both 164 bp and 140 bp fragments were apparent for heterozygotes. *TT* homozygotes were identified as a single band of 140 bp. The AT_1R *CC* genotype gives two bands of 224 bp and 143 bp, and the heterozygous *AC* genotype as three bands of 367 bp, 224 bp and 143 bp. The *AA* genotype presents a single band of 367 bp.

Statistical analysis

Age, sex, weight, height, body mass index (BMI) and ACE, AGT and AT1R genotypes were tabulated for hypotensive and normotensive individuals. The association between ACE, AGT and AT1R genotypes with the risk of hypertension was estimated by computing odds ratios (OR). We also adopted a generalised linear modelling (GLM) approach for hypertension with fixed effects, implemented in R (R: A Language and Environment for Statistical Computing, R Development Core Team, www.R-project.org). Binomial error distributions were assumed and logistic regression used to select the best-fitting minimal adequate model.[45,46] This regression approach also allows control of confounding variables.[47] The significance level of all tests was set at 0.05.

Results

The genotype frequencies of each of the three genes were in Hardy–Weinberg equilibrium, both in the total sample group and segregated into hypertensive and normotensive groups. Genotype and allele frequency comparisons between hypertensive and normotensive groups are presented in Table 1. Odds ratios for each of the three markers for both full and reduced datasets are presented in Table 2.

Using the full dataset, the minimum adequate model for hypertension explained 52.13% of the total variation, with an Akaike Information Criterion (AIC) score of 73.33 (Table 3(a)). Importantly, 69% of the total deviance is explained by age. Weight and height are weakly correlated with age (R^2 = 0.035 and 0.126 respectively) and are significant effects. The AT1 genotype also has a significant effect on

Table 1. Genotype/allele frequency comparison of the ACE I/D, AGT M235T and AT_1R A1166C genes among hypertensive and normotensive subjects

Gene	ACE (I/D)			AGT (M235T)			AT_1R (A1166C)		
Genotype	II	ID	DD	MM	MT	TT	AA	AC	CC
Hypertensive									
N = 124	9	37	78	34	72	18	31	64	29
(%)	(7)	(30)	(63)	(27)	(58)	(15)	(25)	(52)	(23)
Normotensive									
N = 146	12	58	76	38	59	49	83	52	11
(%)	(8)	(40)	(52)	(26)	(40)	(34)	(57)	(36)	(7)
p		0.015			< 0.0001			< 0.0001	
Allele	I		D	M		T	A		C
Hypertensive	0.22		0.78	0.56		0.44	0.51		0.49
Normotensive	0.28		0.72	0.46		0.54	0.75		0.25
p		< 0.01			< 0.0001			< 0.0001	

p values derived from χ^2 analyses.
A: adenine, ACE: angiotensin converting enzyme, AGT: angiotensinogen, AT_1R: angiotensin receptor subtype 1, C: cytosine, D: deletion, I: insertion, M: methionine, N: number, T: threonine.

Table 2. Table of odds ratios (*N* = 270)

Gene	Odds ratio	*p*	95% CI	*N*
AT1	3.952	< 0.001	2.345 to 6.661	270
AGT	2.975	< 0.001	1.622 to 5.455	270
ACE	1.562	< 0.1	0.959 to 2.544	270

Table 3(a). Summary of minimum adequate model of hypertension (binomial response, *N* = 270)

Fixed effects	d. f.	Parameter estimate (SE)	% deviance explained	*p*
Age	1	0.202 (0.038)	121.79	< 0.0001
Weight	1	–1.118 (0.623)	33.026	< 0.0001
Height	1	–0.955 (0.315)	26.187	< 0.0001
AT1	2		7.064	0.029
Weight: Height	1	0.008 (0.004)	6.106	0.013

Total deviance explained by the main effects and first order interactions = 50.49%. For each term, the deviance explained refers to the change in deviance attributed to the term in question when fitted last, as a proportion of total deviance explained by the main effects in the minimum adequate model. *p* values were estimated by comparison with the reduced model not containing the term in question.

Table 3(b). Summary of minimum adequate model of hypertension (binomial response, *N* = 270) with age removed from the model

Fixed effects	d.f.	Parameter estimate (SE)	% deviance explained	*p*
Height	1	–0.358 (0.047)	126.53	< 0.0001
Weight	1	0.212 (0.03)	116.22	< 0.0001
AT1	2		15.835	0.0004
AGT	2		8.823	0.012
ACE	2		7.883	0.019

Total deviance explained by the main effects = 73.9%.

hypertension. However, it is fully expected that hypertension should correlate with age and the mean ages of the hypertensive and normotensive groups are significantly different ($t_{205} = 24.7$, $p < 0.001$). When age is removed from the model the minimum adequate model for hypertension explains 73.9% of the deviance, but with an AIC score of 188.45 (Table 3(b)). Interestingly, AT1, AGT and ACE now all have significant effects.

Discussion

Conflicting results are found in the literature on the relationship between HTN and the RAS gene polymorphisms, i.e. ACE I/D, AGT M235T and AT_1R A1166C. This study aimed at determining the polymorphism of the RAS genes based on a population study in order to contribute to the current debate by examining a specific population at the extreme end of the frequency range for the ACE polymorphisms. The initial study hypothesis was that polymorphisms associated with increased RAS activity may predispose to HTN, whereas those that decrease RAS activity might offer some protection. For example, it might be expected that the AGT T allele, which is associated with markedly higher plasma AGT,[48] the ACE D allele, which is associated with greater ACE activity,[40] and the AT_1R C allele, which is associated with greater responses to angiotensin II at lower concentrations,[49] might all be more prevalent within a hypertensive population.

Generally, ACE D allele was at a higher prevalence in the Lebanese (72%) relative to Caucasian populations (typically ~56%), African (~60%) and Asian populations (~39%).[34] Specifically, for the ACE I/D polymorphism, our results showed a significant difference between hypertensive and normotensive groups across genotypes ($p < 0.05$), although the OR for the DD genotype was not significant ($p = 0.08$). These results are not quite in agreement with previously published ACE–HTN linkage and association studies.[26] The D allele frequency was significantly different between the hypertensive and normotensive groups (χ^2, $p < 0.01$), but again the OR was not significant (OR 1.370, $p = 0.136$).

AGT 235T allele was found to have a frequency of 49%, which is higher than the published frequency of 42% in Caucasian but lower than the 77% in African and Asian populations.[34] The genotype frequencies differed significantly between the two groups and the OR 2.97 was significant ($p < 0.001$). Jeunemaitre and associates[4] were the first to report the linkage of the molecular variants M235T with HTN in Caucasian populations. Again, there has been some inconsistency, with some studies in support of this finding,[18] whereas others were not.[50] Positive findings from previous studies have reported the involvement of the T allele, whereas, here, we found that the MM/MT genotypes increased the risk of hypertension. Thus, this study provides no evidence in support of the initial hypothesis that the T allele is associated with higher risk of HTN.

As for AT_1R polymorphisms, the frequency of the C allele in the Lebanese is 36%, which compares well with general Caucasian populations being at around 35%, but differs markedly from Asian populations, where it is around 5% (NCBI database). The genotypes differ significantly between hypertensive and normotensive subjects (χ^2, $p < 0.001$) and the CC/AC genotypes give a significant OR 3.95 ($p < 0.001$). Since its first description, little progress has been made in unravelling the functional significance of AT_1R polymorphism with respect to diseases, although the AT1 receptor mediates most of the known physiological effects of angiotensin II. In agreement with this study, AT_1R CC and/or AC genotypes have shown a significant interaction with the disease phenotype in previous studies. Such a finding supports the initial hypothesis that increased RAS activity predisposes to hypertension in that the CC variant of the AT_1R (A1166C) polymorphism has previously been seen to be associated with increased responsiveness to angiotensin II.[49]

With a sample size of $N = 270$, a simple χ^2 goodness-of-fit test for association has sufficient power ($1\text{-}\beta = 0.8$; $\alpha = 0.05$),[51] should the effect size be greater than $w = 0.17$, which is considered to be somewhere between a small to medium effect size. Given this is the case, we would have to conclude that there is *not* a small to medium effect size for the ACE polymorphism. Hence, in order to determine whether the ACE polymorphism plays any role in influencing hypertension, a larger study is required.

The opportunistic design of this study resulted in the hypertensive and normotensive groups being age-stratified, which generated an obvious flaw in that a proportion of the younger, currently normotensive individuals may go on to develop HTN. Regression analyses identified that age alone explains 69.4% of the deviance. Weight and height are weakly correlated with age in these datasets ($R^2 = 0.036$ and 0.126 respectively). A more involved model including all the other significant terms is given in Table 3(a). Although this model shows weight, height and AT1 to also be significant, this model does in fact explain less of the total deviance (50.49%) than the age-alone model. When age is removed as a main effect, weight, height and the three markers generate a model that explains 73.9% of the total deviance (Table 3(b)). Weight and height are relatively weakly correlated ($R^2 = 0.27$), but models with one of these two terms removed are inferior, explaining around 21% of the total deviance. Interestingly, BMI did not remain as a significant factor in any of the models. It is difficult to explain why height and weight remain as influential factors. Each marker appears to explain small, but significant proportions of the deviance. In some respects, this is to be expected as very few association studies of complex traits identify markers of large effect size. The genotypes explain around 2% of the deviance, which is significant but probably not biologically meaningful. Associations are typically reported giving OR. However, Table 2 shows that no significant OR is identified for ACE. The underlying rationale for each of these two approaches to identifying the influence of genotypes on HTN is quite different, hence the results are not directly comparable; indeed the interpretation of OR is not always straightforward.[52] If anything, this study has illustrated how markers that appear to have a large influence using OR may actually be responsible for very little of the total variation in a given phenotype. Nevertheless, it does appear that each of these three markers warrant further investigation as regards their influence of HTN.

This RAS gene polymorphisms study has shown inconsistencies with respect to other previous population studies on HTN. Whether these differences represent the historic multi-ethnic origin of the Lebanese population remains to be resolved; certainly the variation in allele frequencies between Lebanese and other broad ethnic groups (e.g. Caucasian, Asian and African) suggests that this could be the case.[39]

Conclusion

Of the demographic factors studied, weight and height of the individual contributed to the greatest proportion of variation in HTN in the study populations, despite these being only weakly correlated with the age of the subjects. Logistic regression analysis identified a small, but significant influence of AT1, AGT and ACE on HTN. When age is controlled for, AGT has the greatest influence. OR analysis also suggests that AT1 plays a significant role.

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Conflict of interest

None declared.

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