Angiotensinogen Gene in Human Hypertension
Lack of an Association of the 235T Allele Among African Americans

Charles Rotimi, Linda Morrison, Richard Cooper, Catherine Oyejide, Eme Effiong, Modupe Ladipo, Babatunde Osotemihen, Ryk Ward

Abstract The frequency of the 235T and 174M alleles of the angiotensinogen gene, previously reported to be associated with hypertension in Caucasians and Japanese, was compared between 57 hypertensive African Americans and 130 normotensive African Americans sampled as part of a community survey of hypertension in the Chicago area. The frequency of the 235T allele was unrelated to hypertension status (cases, 83%, control subjects, 82%), as was true for the 174M allele. Compared with Caucasians, the frequency of the 235T allele was twice as high in this African American population, while the frequency of the 174M allele was similar. Even higher frequencies of the 235T allele (93%) were noted in a sample of 122 Nigerians. It appears that the 235T allele is very common in populations of West African origin, although we found no evidence that it confers risk of hypertension. (Hypertension. 1994;24:591-594.)

Key Words • hypertension, essential • angiotensinogen • genes • blacks

As an essential substrate for the renin-angiotensin system, angiotensinogen (AGT) plays an important role in hydromineral balance and the control of blood pressure.1-4 Walker et al1 observed a highly significant correlation between plasma AGT concentration and blood pressure in a large cross-sectional epidemiological study. Within the context of a family study, Watt and coinvestigators6 reported higher plasma AGT levels in young adults with an elevated blood pressure whose parents also had high blood pressure compared with young adults with low blood pressure whose parents also had low blood pressure. Plasma AGT is also reported to be higher in hypertensive subjects and in the offspring of hypertensive parents.7 In addition, overexpression of the AGT gene causes elevated blood pressure in transgenic mice carrying the rat AGT gene.8

A recent collaborative investigation of the AGT gene in siblings from Utah and Paris sharpened these findings by reporting both linkage and association of AGT molecular variants (235T and 174M) with hypertension, suggesting that these AGT polymorphisms may represent markers of an inherited predisposition to essential hypertension in humans.9 These findings have recently been extended to a Japanese population, where a significant association was also noted between hypertension and the 235T allele, along with a substantial increase in the population frequency of the 235T allele.10 Most recently, despite the failure to identify an association with either the 235T allele or the 174M allele, linkage between essential hypertension and the AGT gene has been demonstrated in a set of British families.11 The probable involvement of the AGT genomic region with blood pressure regulation is strengthened by two reports of an association between proteinuric preeclampsia and AGT polymorphisms: one with the 235T allele,12 the other with a microsatellite polymorphism.13

We report the findings of an investigation of the population frequency of the molecular variants 235T and 174M of the AGT gene and their association with blood pressure in samples from black populations of Chicago, Ill., and Ibadan, Nigeria.

Methods

Participants

African Americans were recruited from among the participants of an ongoing population survey of hypertension in the village of Maywood, a western suburb of Chicago. Of the 27 139 residents of Maywood, 84% are African Americans. A total of 1525 people 25 to 74 years of age (711 men, 814 women) participated in the hypertension survey, which was initiated in 1991. To study people with maximal contrasts with respect to a genetic predisposition to hypertension, participants were selected from the extremes of the bivariate age–blood pressure distribution. Cases (hypertensive subjects) were younger individuals with high blood pressure (systolic blood pressure $\geq$140 or diastolic blood pressure $\geq$90 mm Hg) or who were taking antihypertensive medication. A total of 57 cases and 130 control subjects who participated in this study were typed at both loci and had complete data for all variables.

Blood pressure, height, and weight were measured with a standardized protocol. All blood pressures were measured on the right arm with subjects in the sitting position using an appropriately sized cuff. Systolic and diastolic blood pressures were recorded to the nearest 2 mm Hg as the first and the fifth Korotkoff phases by using a standard mercury manometer. Body mass index (BMI) was calculated as the measured weight in kilograms divided by height in meters squared.
The 122 Nigerian participants included men and women recruited from a traditional Yoruba community (Idikan) in Ibadan, plus patients attending the general outpatient clinic of the University College Hospital, Ibadan. Because information regarding blood pressure and hypertension history was limited, only population allele frequencies are displayed for the Nigerian participants. Both phases of the study were reviewed and approved by the Loyola University Medical Center IRB Committee.

Blood Collection and DNA Extraction
A 10-ml sample of venous blood was collected in an EDTA vacutainer from consenting participants. Within an hour of drawing, the Buffy coat was separated from the blood by centrifugation at 800 to 900g for 10 minutes and stored at −70°C until shipment to the University of Utah. Genomic DNA was isolated from the Buffy coat according to standard methods.14 Phenol extraction was followed by ethanol precipitation of DNA.

Identification of AGT Variants
Genomic DNA extracted from the Buffy coat was adjusted to a concentration of 100 g/mL and used as a template for amplifying the second exon of the AGT gene by the polymerase chain reaction (PCR). We used the first set of second exon primers described by Jeunemaitre et al14 and followed their PCR protocol. Following amplification, the DNA was denatured and 100 μL of the denatured DNA was spotted onto each of two nylon membranes. The blots were neutralized, the DNA was cross-linked to the membrane, and each membrane was hybridized overnight with one of the following radiolabeled probes: 5'-GGCTCCCATCAGGGAGC-3' (detects the 235M allele by hybridization to the sense strand) and 5'-GCTCCCTGACGGGAGGCC-3' (detects the 235T allele by hybridization to the antisense strand). Autoradiographs were obtained, and the presence or absence of the 235M allele and of the 235T allele was scored from each blot. Homozygotes were defined by samples that exhibited a spot only on a single membrane, while heterozygotes had spots on both membranes. Each membrane was then denatured to strip the 235T probes and rehybridized with the following probes to detect alleles at position 174: 5'-CTGTCCACGGTGATG-3' (detects allele 174T by hybridization to the antisense strand) and 5'-CACCACCATGGAGCAG-3' (detects allele 174M by hybridization to the sense strand). Autoradiographs were then obtained, and the membranes were scored to identify genotypes as above.

Statistical Analyses
Differences in the distribution of genotypes between groups (blacks versus whites; African Americans versus Nigerians; men versus women; and hypertensive versus control subjects) were determined by the χ2 procedure, as was the association between allele frequencies and hypertension status. The potential confounding influences of age and BMI were assessed in a multiple logistic regression model. Statistical significance occurred if a computed two-tailed probability value was less than 5% (P<.05).

Results
The frequency of the AGT 235 TT genotype (homozygote) among African Americans and Nigerians is displayed in the Figure and compared with a sample of whites from the Salt Lake City area. The frequency of the 235T allele (Table 1) in the African American sample was 83% and increased to 93% in Nigerians, a population with little or no European admixture. Among whites, the 235T allele had a frequency of only 41% in a random sample from Utah (n=611; R. Ward et al, unpublished data, 1993). The population difference between whites and either group of blacks for this allele was highly significant (P<.001). By contrast, the frequency of the 174M allele among both African American and Nigerians (Table 1) was approximately only one half the reported value for whites, though the difference was significant only for the African American sample (P<.01).

Table 2 displays the characteristics of the African American participants according to hypertension status. As a result of the sampling strategy, control subjects were older and had lower systolic and diastolic blood pressures. Seventy-six percent of cases were being treated by antihypertensive medication. There was no significant difference in the frequency of either the 235T or 174M allele between the hypertensive and control populations.
TABLE 3. Comparison of Allele Frequencies in Hypertensive and Control Subjects

<table>
<thead>
<tr>
<th>Allele M235T</th>
<th>Allele T174M</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>P, %</td>
</tr>
<tr>
<td>Control subjects</td>
<td>260</td>
</tr>
<tr>
<td>Hypertensive subjects</td>
<td>114</td>
</tr>
</tbody>
</table>

M indicates methionine; T, threonine; n, number of alleles analyzed; P, allele frequency; and x², test of homogeneity.

subjects (Table 3). The observed lack of association was independent of age and body weight. Although the 23ST allele occurred somewhat more frequently among the 16 hypertensive men compared with the 41 hypertensive women (88% versus 80%), this difference was not significant.

Discussion

The frequency of the 23ST molecular variant of the AGT gene appears to be twice as high among blacks as whites—a striking ethnic difference for a potential matter of predisposition to hypertension. Furthermore, the gene frequency is significantly higher in the Nigerian sample, suggesting some degree of variation between blacks in the United States and in West Africa. Even though Nigerian participants were not recruited through a defined sampling protocol and thus may not be entirely representative of the general population, the frequency of the 23ST allele is so high that it is unlikely that this would result in significant bias. Since US blacks are primarily of West African descent and share a common genetic ancestry, the lower frequency of the 23ST allele in US blacks relative to Nigerians probably reflects the consequences of European admixture. Hence, it is likely that the 23ST allele is close to fixation in populations of African ancestry. This observation, coupled with the reported high frequency of 75% in Japanese populations, suggests that the 23ST variant may be the ancestral allele for the AGT gene. Conversely, the evolutionary younger 235M allele may exhibit high frequencies only in Caucasian populations.

While the initial study on Caucasian subjects resulted in an odds ratio (OR) of 1.87 for the combined set of most severe index cases ($\chi^2 = 11.1$, $P < .001$), a subsequent study of Caucasians with severe hypertension failed to find any significant association ($\chi^2 = 0.24$, $P = .63$). Thus, the overall association of the 23ST allele with hypertension in Caucasians may be weaker than initially suggested by the Salt Lake City and Paris findings. However, despite the high frequency of the 23ST allele in Japanese, the comparison between 105 hypertensive and 81 normotensive subjects found an OR of 2.67 (allele frequencies of 89% versus 75%), suggesting a stronger association in Japanese. In contrast to the initial results from Caucasian and Japanese subjects, our data provide no support for the existence of an association between hypertension and the 23ST allele in the African American population of Chicago.

Although our negative result could be taken to imply that there is essentially no relation between the AGT gene and risk of hypertension within the African American population, some limitations to the present study must be taken into account. First, like the other studies, our study is retrospective and thus provides only an imperfect insight into causal processes. Second, given the high frequency of the 23ST allele in the African American population (80% to 90%), attempts to find an association between this putative risk factor and hypertension are inherently constrained by low statistical power. Even if the OR in African Americans was as high as in the Japanese study (OR = 2.67), a sample of 200 individuals in each group would be required to give an 80% statistical power of detecting a significant effect. If the OR in African Americans were more akin to that observed in the Caucasian sample from Salt Lake City and Paris ($OR = 1.87$), even larger sample sizes (in excess of 500 individuals per group) would be required. Clearly, our failure to identify a statistically significant association in a sample of 57 hypertensive and 130 control subjects cannot be taken as conclusive proof that no such association exists in the African American population. Against these caveats must be set the fact that in our data the frequency of the 23ST allele is so similar in the two groups that, were an association with hypertension found within the African American population, the attributable risk is likely to be substantially lower than that for the Caucasian population.

Overall, the results of this study, in conjunction with previous reports, reinforce the notion that a full understanding of the genetic contribution to the pathophysiology of essential hypertension requires the evaluation of several different ethnic groups. As exemplified by the comparison of results among Caucasians, Japanese, and African Americans, the same molecular variant may exhibit very different risk associations in genetically different populations. In addition, as a recent British study demonstrated, the apparent negative findings with respect to the 23ST allele cannot be taken to imply that segregation of genetic variants in the AGT genomic region has no influence on the familial distribution of hypertension. The literature to date suggests that mendelian segregation at the AGT 235T/M polymorphism may have etiologic relevance for the distribution of hypertension within the Japanese population, variable relevance for the Caucasian population, but essentially no relevance for assessment of the distribution of hypertension risk within the African American population. However, none of these studies address the issue of whether the methionine to threonine substitution at position 235 has direct relevance for the physiological role of the AGT gene. Similarly, these studies do not address the issue of whether population differences in the frequency of the 23ST allele are in any way related to differences in the prevalence of hypertension. This latter issue would be best addressed by using comparable methods to measure AGT plasma levels in the different ethnic groups. If the 23ST allele is consistently related to AGT levels, then the population means for plasma AGT concentration should follow the rankings of the allele frequency: African American > Japanese > Caucasian. Such comparative data appear not to exist.
system and pathophysiological processes. It also indicates the importance of defining genotypes at the DNA level and suggests that haplotype analyses will be required to determine whether causation is likely to be directly related to a specific DNA substitution or to a larger genomic segment. Our data indicate that compared with Caucasians, populations of African ancestry have appreciably higher frequencies of the 235T allele but lower frequencies of the 174M allele, suggesting a more negative linkage disequilibrium. The different haplotype distribution in populations of African ancestry is confirmed by the different distribution of microsatellite alleles in both the Chicago and Nigerian populations compared with those found in Caucasians from Utah (data not shown). Hence, disease risk will have to be evaluated in terms of specific haplotypes, and haplotypic associations are likely to be markedly different in different ethnic groups. Similar considerations apply to the evaluation of the pathophysiological consequences associated with other components of the renin-angiotensin system, such as the relation between polymorphisms at the angiotensin-converting enzyme gene and hypertension and its determinants,19,20 myocardial infarction,21,22 and idiopathic cardiomyopathy.23 In each instance, the recent conclusion of an expert National Institutes of Health panel has considerable significance for designing and interpreting cross-cultural studies: “The most important genetic determinants of disease in a minority group may not be the same as in the population at large.”24 In particular, understanding the potential importance of genetic variants of the renin-angiotensin system as factors influencing the high prevalence of hypertension in African American populations will require a combined attack at the molecular, physiological, and epidemiological levels.

Acknowledgments
This work was supported in part by grants from the National Institutes of Health (grants HL-45508 and HL-47910). We thank Drs A. Asekun-olarimよい, T. Adeniran, and B. Ogunsmi of the University of Ibadan for their assistance with the collection and initial laboratory processing of the Nigerian specimens. We also wish to express our gratitude to Dr Jean-Marc Lalouel not only for sharing the preliminary data with us but also for advice and encouragement during this study.

References
C Rotimi, L Morrison, R Cooper, C Oyejide, E Effiong, M Ladipo, B Osotemihen and R Ward

Hypertension. 1994;24:591-594
doi: 10.1161/01.HYP.24.5.591

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/24/5/591

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/