Angiotensinogen Gene Promoter Region Variant Modifies Body Size–Ambulatory Blood Pressure Relations in Hypertension

Armindo D. Tiago, MD; Nilesh J. Samani, MD; Geoffrey P. Candy, PhD; Richard Brooksbank, MS; Elena N. Libhaber, MS; Pinhas Sareli, MD; Angela J. Woodiwiss, PhD; Gavin R. Norton, MD, PhD

Background—The extent to which genes modify the relationship between risk factors for hypertension and blood pressure (BP) is unclear. As angiotensinogen is expressed in adipose tissue and angiotensinogen (AGT) gene promoter variants influence the production of angiotensinogen, we evaluated the role of AGT gene variants as potential modifiers of body size–BP relations.

Methods and Results—Five hundred twenty-one hypertensives of African origin sampled from a group with a high mean body mass index (BMI) had 24-hour ambulatory BP (ABP) measurements determined off therapy and were genotyped for the AGT –6G→A, –532C→T, –20A→C, and 704T→C (M235T) gene variants. Genotypes were also determined in 547 control subjects of African origin who had a normal clinic BP. The –6A and –532C alleles were concordant with the M235T variant. Although AGT gene variants had no independent effects on either the presence of hypertension or ABP values in hypertensives, the –20A→C polymorphism had a marked influence on the relation between ambulatory systolic BP and BMI. This relation was present in patients homozygous for the –20A allele (n=399, r=0.23, P=0.0001), but absent in those with at least one copy of the –20C allele (n=122, r=0.01, P=0.89). The M235T polymorphism did not impact on the BMI-BP relation. Specificity of the –20A→C polymorphism effect on the BMI-BP relation is further indicated by the lack of effect on the systolic BP-age relation.

Conclusion—An AGT gene promoter region variant is an important modifier of the relation between body size and BP. Hence, these data corroborate the notion that genetic modifiers can produce a profound impact on BP-phenotypic relations. (Circulation. 2002;106:1483-1487.)

Key Words: genetics ◼ hypertension ◼ obesity

Body size is associated with blood pressure (BP). However, not all obese persons have an increased BP and even in severe obesity 40% of subjects may have a normal BP. The reasons for these diverse effects of body size on BP have not been identified. Factors that influence the relationship between body size and BP in hypertension require elucidation as they may predict the impact of weight reduction on BP. The BP response to obesity may be influenced by genetic factors, which in turn may depend on modifying alleles in the genetic background. Hence, modifier genes have been suggested to have a substantial effect on the impact of body size on BP. However, as yet there are no data to show a profound influence of genetic modifiers on body size–BP relations in human studies. Production of angiotensinogen, which is in part derived from adipose tissue, is influenced by polymorphisms in the angiotensinogen (AGT) gene. These polymorphisms, notably an exon 2 variant (704T→C [M235T]), which is in linkage disequilibrium with potential functional promoter region polymorphisms, and a promoter region variant (–20A→C) have been variably linked with the risk of hypertension. Therefore, the aim of the present study was to assess whether these AGT gene polymorphisms substantially influence the impact of body size on BP in human hypertension.

Methods

This study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand (approval No. M951122).

Study Groups, BP Measurements, and Hypertension Grading

Five hundred fifty-six consecutive hypertensive patients of African ancestry initially screened at district clinics in suburban areas of Johannesburg and referred to tertiary centers for more thorough...
clinical assessments were recruited if they had mean daytime ambulatory diastolic BPs (DBPs) >90 mm Hg (Spacelabs model 90207) off medication. Patients with auscultatory BPs <200/115 mm Hg had ambulatory measurements performed after at least 2 weeks off medication. A minority of patients (6%) with either first visit auscultatory BPs ≥200/115 mm Hg and with either target organ damage or two or more additional risk factors for cardiovascular disease had 24-hour BP monitoring performed within a shorter period off medication. To avoid population stratification, only patients of the Nguni, Sotho, and Venda chiefdoms of South Africa were selected. In addition, patients with type I diabetes mellitus, uncontrolled type II diabetes mellitus (defined as hemoglobin A1C of >10%), renal and endocrine disease, and clinically important cardiac pathology (clinically significant arrhythmias, heart failure, valvular disease, ischemic heart disease, previous myocardial infarction, and unstable angina) were excluded. Ambulatory BP (ABP) measurements were performed at least every half hour during the day (6 AM to 10 PM) and hourly during the night (10 PM to 6 AM) and ambulatory monitors were calibrated using the standard techniques. All patients were advised not to smoke, imbibe alcohol, or ingest caffeine during this period. Grading of the severity of hypertension was determined from the mean of daytime ABP measurements on the basis of standard criteria (grade I, 140 to 159/90 to 99; grade II, 160 to 179/100 to 109; grade III, ≥180/100 mm Hg).12 Five hundred twenty-one patients had >90% and the rest >85% of intended ABP recordings obtained.

To evaluate whether the genetic variants examined were associated with the development of BP, a case-control study was also performed. Five hundred forty-seven control subjects of African origin without a family history of hypertension were recruited from suburban areas of Johannesburg and were considered normotensive if the subjects had been residents of an urban area for at least 2 years. Additional statistical analysis was performed assessing the relationship between allele/genotype and the presence of hypertension, in which all 556 patients were included, regression analysis was only performed in those patients for whom >90% of intended ABP recordings were obtained. The impact of genotype on ABP values was evaluated by using ANCOVA techniques, with age, gender, and BMI used as covariates, and also by assessing the effect of genotype on the regression relations between ABP and other phenotypic parameters. Continuous data are expressed as mean±SEM.

Results

Demographic and Clinical Data

Demographic and clinical data are shown in the Table. Both the case and the control groups had a preponderance of females and individuals with an increased BMI. The preponderance of females reflects the gender distribution of patients attending district clinics rather than a profoundly greater incidence of hypertension in African women compared with men. Except for a higher mean BMI in the case group (Table), the case and control groups were matched according to all other demographic features including the frequency of subjects from different chiefdoms. Although the majority of patients had grades I to II hypertension as determined from mean daytime ABPs, a high percentage of patients with grade III hypertension were also recruited (Table).

Genotype Effects on Risk of Hypertension and ABP

The genotype frequencies of both the –20A→C and M235T polymorphisms were in Hardy-Weinberg equilibrium. Neither variant was independently associated with the presence of hypertension (Figure 1). Further, neither polymorphism showed a quantitative association with 24-hour (Figure 2), day, or night ABP. Similarly, no association between genotype and the grade of hypertension was evident.

Genotype Effects on BP-Phenotypic Relations

Both age and BMI showed significant correlations with systolic BP (SBP) (Figure 3), but not diastolic ABP, in the
The main finding of the present study is that a potentially functional promoter region variant of the AGT gene has a marked influence on the relation between body size and systolic BP in hypertensives. Because the –20A→C polymorphism did not produce an independent effect on either BP in hypertensives or the risk of development of hypertension when accounting for body size differences between subjects, the effect of the variant reflects a moderating influence of the genotype on the BMI-BP relationship rather than an effect of body size on the relationship between genotype and BP. This distinction is important as it implies that the presence of the risk genotype is insufficient to account for a BP effect alone. Rather, the risk genotype in part determines the overall effect of body size on BP. The presence of the –20C allele abolished the impact of body size on BP, whereas in those patients homozygous for the –20A allele a significant relationship between body size and SBP was evident. Although an angiotensin-converting enzyme gene insertion/deletion polymorphism has previously been shown to influence the slope of the relationship between body size and BP in humans, the novelty of the present data is that they show a gene modifier effect that abolishes this relation. This profound context-dependent effect of a genetic polymorphism is in keeping with the complex nature of polygenic traits and could assist in predicting the effect of body size on BP in humans.

A large number of studies attempting to associate AGT gene variants with risk of hypertension have produced highly variable results. Sample sizes inadequate to limit the risk of false-positive or false-negative results, poor phenotypic characterization, and population admixture may limit the outcome of case-control studies. With respect to these limitations, our study has a sufficiently large sample size (556 cases and 547 controls) to avoid false-negative results (for the –20A→C and the M235T gene variants), and the presence of hypertension has been confirmed in all patients using 24-hour ABP monitoring with patients off medical therapy. Moreover, patient and control groups were not only matched for their usual demographic characteristics, including racial background, but also for more precise ethnic backgrounds (chief-doms that are historically derived from the same gene pool). Although our present data do not support an independent role for the AGT gene in contributing to either the development or the severity of hypertension in this population, the possibility of epistatic interactions with other genes, moderating the impact of the AGT gene, cannot be excluded.

In the present study, we evaluated genetic effects on BP considered as a continuous trait in a hypertensive group rather than in a cross section of the population. Our reasons for the choice of study sample were 3-fold. First, to improve on the statistical power of detecting genotype-phenotype interac-

**Figure 1.** Effects of AGT gene –20A→C and M235T polymorphisms on the risk of developing hypertension. Genotype odds ratios (OR) and 95% confidence intervals (C.I.) were determined assuming that the –20A and 235T alleles produced dominant effects. No gene variants were a significant risk factor for hypertension.

**Figure 2.** Effects of AGT gene –20A→C and M235T polymorphisms on mean 24-hour ABP in hypertensives.
tions using ABP measurements, a group sampled with a relatively wide distribution of BPs as opposed to a group sampled with BPs clustered around the median (general population) was necessary. Second, effects of body size on BP are more relevant to hypertensives, as it is to this group that advice regarding weight reduction would be given to assist with BP control. Third, in patients of African origin there is a higher prevalence of more severe hypertension. Therefore, equally as important as assessing the contribution of genes to BP in the general population is an approach that assesses the role of genes in contributing to the severity of hypertension in groups with a higher prevalence of grades II and III hypertension. A major strength of our study is the informative distribution of the M235T variant did not influence body size—BP relations, the M235T variant was used as a marker of the effects of the −6G→A and −532C→T variants, both of which have previously been shown to influence angiotensinogen concentrations. As the M235T variant did not influence body size—BP relations, these data would suggest that the −6G→A and −532C→T polymorphisms are not significant modifiers of body size—BP relations. However, further studies in populations with an informative distribution of −6G→A and −532C→T alleles will be required before this conclusion can be drawn with certainty.

At this stage the clinical implications of our findings are speculative. However, if the results are confirmed in other studies and populations, then it is conceivable that the effect of the AGT gene could, for example, influence therapeutic choices. If the AGT −20A→C variant influences the effect of BMI on BP, then pharmacological agents targeting the RAS may be particularly efficacious in obese hypertensive subjects with the deleterious genotype. Moreover, if body size effects on BP are genotype dependent, then one would expect that...
weight reduction would be beneficial with respect to BP effects in patients with the risk genotype, but not the nonrisk genotype. These hypotheses have yet to be tested.

In summary, the results of the present study indicate that in the absence of an independent effect on BP, an AGT gene promoter region polymorphism markedly influences the body size–BP relationship, at least in hypertensives. These context-dependent effects of the AGT gene on BP could be useful in predicting the effect of changes in body size on BP. These data show that genetic factors impact profoundly on the relation between body size and BP, an effect previously purported to significantly influence BP in human hypertension.

Acknowledgments

This work was supported by the University Research Council of the University of the Witwatersrand, The Medical Faculty Research Endowment Fund of the University of the Witwatersrand, the C.V. Hodges Charitable Trust, the H.E. Griffin Charitable Trust, the Southern African Hypertension Society supported by an open grant from Astra-Zeneca, and the Medical Research Council of South Africa. A.D.T. was supported by a grant from the Netherlands Organization for International Cooperation in Higher Education.

References

Angiotensinogen Gene Promoter Region Variant Modifies Body Size—Ambulatory Blood Pressure Relations in Hypertension

Armando D. Tiago, Nilesh J. Samani, Geoffrey P. Candy, Richard Brooksbank, Elena N. Libhaber, Pinhas Sareli, Angela J. Woodiwiss and Gavin R. Norton

_Circulation_. 2002;106:1483-1487; originally published online August 26, 2002;
doi: 10.1161/01.CIR.0000029093.93362.FC
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/106/12/1483

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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 _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/