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Impact of Renin-Angiotensin-Aldosterone System Gene Variants on the Severity of Hypertension in Patients With Newly Diagnosed Hypertension

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Background: The severity of hypertension has prognostic significance. Previous studies have assessed the relationship between renin-angiotensin-aldosterone system (RAAS) genotype and the severity of hypertension in either treated patients or those who have only recently discontinued treatment.

Methods: We assessed the impact of RAAS genotype on ambulatory and office blood pressure (BP) in 231 newly diagnosed hypertensive patients of African ancestry who had never received therapy. Subjects were genotyped for variants of the angiotensin-converting enzyme (insertion/deletion), angiotensinogen (M235T, −20A→C), and aldosterone synthase (*CYP11B2*)(−344C→T) genes.

Results: The *CYP11B2* gene polymorphism was associated with systolic BP (SBP). In comparison to subjects with at least one copy of the −344C allele (n = 75), patients who were homozygous for the −344T allele (n = 156) had both higher ambulatory SBP (150 ± 1 *v* 144 ± 1 mm Hg, $P = .002$ before and $P = .01$ after adjusting for multiple genotyping) and office SBP (163 ± 2 *v* 156 ± 2 mm Hg, $P = .01$ before and $P = .05$ after adjusting for multiple genotyping). Neither the angiotensin-converting enzyme insertion/deletion nor the angiotensinogen gene polymorphisms were associated with ambulatory or office SBP or diastolic BP (DBP). The *CYP11B2* gene variant also did not affect DBP.

Conclusion: A variant within the *CYP11B2* locus has a clinically important impact on the severity of SBP changes in individuals with newly diagnosed hypertension who are of African ethnicity. Am J Hypertens 2003;16: 1006–1010 © 2003 American Journal of Hypertension, Ltd.

Key Words: Genes, angiotensinogen, angiotensin-converting enzyme, aldosterone, hypertension.

A number of studies have provided data to implicate genes that influence the activity of the renin-angiotensin-aldosterone system (RAAS) in the control of blood pressure (BP) and the development of hypertension. Angiotensinogen (AGT),[1–5] angiotensin-converting enzyme (ACE)[6,7] and aldosterone synthase (*CYP11B2*)[8–12] gene variants have all been shown to produce effects on BP in humans in some studies. Because the severity of hypertension has prognostic significance,[13,14] knowledge of the impact of candidate genes on BP in hypertension is of considerable importance. Studies conducted in previously treated patients that examined the relationship between RAAS genotype and the severity of hypertension have provided contradictory data.[15,16] Preclinical studies indicate that prior therapy modifies the natural history of hypertension, probably through an impact on vascular remodeling.[17] Therefore, in the present study we evaluated the impact of RAAS gene variants on

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BP in newly diagnosed hypertensive patients who had never received previous therapy. Genotype effects were studied not only on office BP measurements but also on ambulatory BP, which has more prognostic relevance[18] and allows the exclusion of isolated office hypertension.[19]

Methods

This study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand (approval numbers M951122 and M010111).

Study Groups, BP Measurements, and Hypertension Grading

To avoid population stratification through ethnic diversity, only hypertensive patients of African ethnicity who were historically derived from the same gene pool (Nguni, Sotho and Venda chiefdoms) of South Africa living in urban and peri-urban areas of Johannesburg were selected. Consecutive hypertensive patients from district clinics in suburban areas of Johannesburg were recruited if they had provided consent, they had both conventional and daytime ambulatory BP ≥140 or ≥90 mm Hg, and were newly diagnosed with hypertension. Patients who had received prior antihypertensive therapy or who had secondary forms of hypertension, type 1 diabetes mellitus, uncontrolled type 2 diabetes mellitus (defined as an $HbA_{1C} >$ 10%), renal or endocrine disease, or clinically important cardiac pathology were excluded. All patients had ambulatory BP (ABP) measurements (SpaceLabs model 90207; SpaceLabs, Redmond, WA) performed at least half hourly during the day and hourly during the night from 10 PM to 6 AM, and ambulatory monitors were calibrated using standard techniques. All patients were advised not to smoke or to imbibe alcohol or ingest caffeine during this period. Daytime hours were predefined as 6 AM to approximately 8 PM and night hours from 8 PM to approximately 6 AM.

Genotyping

Deoxyribonucleic acid was extracted as previously described.[20] Polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism-based techniques were performed to genotype patients for the insertion/deletion (I/D) polymorphism of the ACE gene,[20] the M235T and −20A→C polymorphisms of the AGT gene,[5] and the −344C→T polymorphism of the *CYP11B2* gene,[21] as previously described. Because strong concordance between the M235T polymorphism and the −6G→A, and −532C→T variants has previously been described by our group in the population sampled,[5] the M235T polymorphism was used as a surrogate marker for the −6G→A, and −532C→T polymorphisms. We also considered genotyping patients for a previously described polymorphism of the angiotensin II type 1 receptor (AIIR) polymorphism. However, as the AIIR gene polymorphism occurs infrequently in subjects of African ancestry,[22] this variant was not studied.

Table 1. Demographic and clinical characteristics of newly diagnosed hypertensive patients

n	231
Age (y)	50.6 ± 0.7
% Nguni/Sotho/Venda	58/40/2
% Female	70
Body mass index ($kg \cdot m^{-2}$)	30 ± 0.5
Type 2 diabetes mellitus (%)	6.0
Office SBP/DBP (mm Hg)	161 ± 1/99 ± 1
24-h SBP/DBP (mm Hg)	148 ± 1/95 ± 1
Daytime SBP/DBP (mm Hg)	153 ± 1/101 ± 1
Nighttime SBP/DBP (mm Hg)	142 ± 1/89 ± 1
Grade of hypertension (% I/II/III)	43/41/15

DBP = diastolic blood pressure; SBP = systolic blood pressure.

Data Analysis

Analysis to detect genotype effects on BP was performed by using MANCOVA techniques with age, gender, and body mass index (BMI) included in the analysis. Because there is no consensus as to when probability values should be reported as unadjusted versus adjusted values,[23,24] and because all genes assessed were based on a priori hypotheses, both unadjusted and adjusted probability values are reported. αValues were adjusted using Bonferroni's method.[25] Continuous data are expressed as mean ± SEM.

Results

Demographic and Clinical Data

The study group had a preponderance of women and individuals with an increased BMI (Table 1). Both mean office and ABP values were considerably elevated compared to conventional criteria for the upper limit of normal. Except for the M235T polymorphism, in which case more women were noted in the TT genotype group, genotype did not predict demographic or other clinical characteristics (data not shown).

Blood Pressure

The ACE and angiotensinogen polymorphisms were not associated with ambulatory or office BP, systolic BP (SBP) (Figs. 1, 2), or diastolic BP (DBP) (Figs. 3, 4). The *CYP11B2* gene polymorphism was also not associated with DBP (Figs. 3, 4). However, the *CYP11B2* gene polymorphism was associated with both ambulatory and office SBP (Fig. 1). The association between *CYP11B2* genotype and ambulatory SBP was for 24-h values (Fig. 1) as well as for day and night values (Fig. 2). Although the M235T variant of the AGT showed a trend for an association with nocturnal DBP, this failed to reach significance after adjusting for multiple genotyping (Fig. 4).

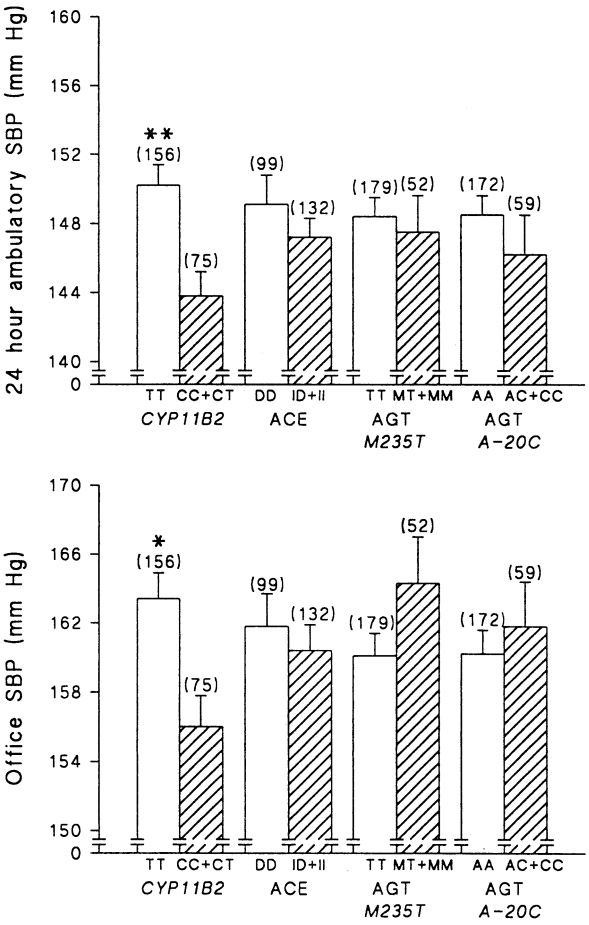

FIG. 1. Effects of renin-angiotensin-aldosterone gene polymorphisms on mean 24-h ambulatory and office systolic blood pressures (SBP) in patients with newly diagnosed, never previously treated hypertension. ACE = angiotensin-converting enzyme; AGT = angiotensinogen; *CYP11B2* = aldosterone synthase. *$P < .01$, **$P < .002$ before and *$P < .05$, ** $P < .01$ after adjusting for multiple genotyping when compared with the CC+CT genotype group.

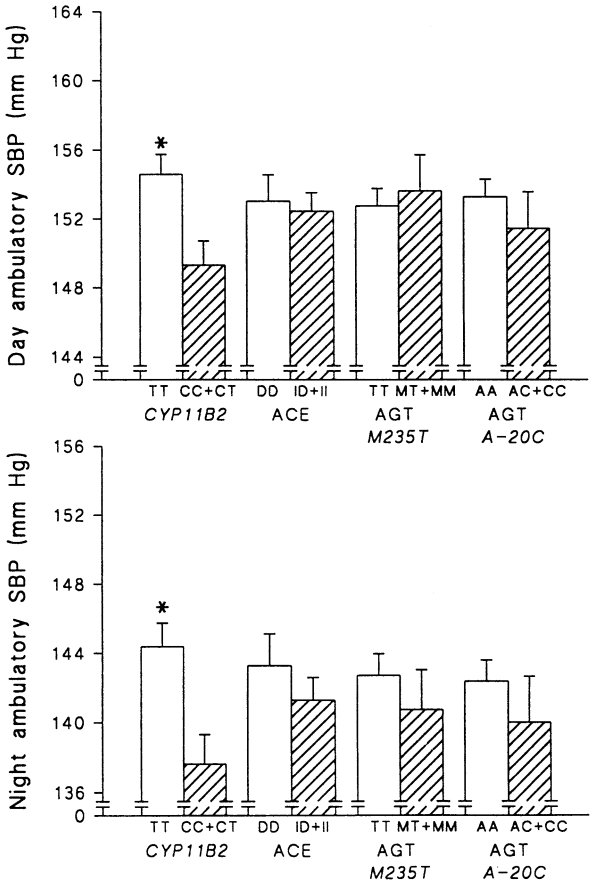

FIG. 2. Effects of renin-angiotensin-aldosterone gene polymorphisms on mean day and night ambulatory systolic blood pressures (SBP) in patients with newly diagnosed, never previously treated hypertension. Abbreviations and sample sizes as in Fig. 1. *$P = .01$ before and $P = .05$ after adjusting for multiple genotyping when compared with the CC+CT genotype group.

Discussion

The main findings of the present study are that the −344C→T polymorphism of the *CYP11B2* gene is associated with both ambulatory and office SBP in newly diagnosed hypertensive patients who have never received antihypertensive therapy. The considerable difference in SBP between *CYP11B2* genotype groups (6 mm Hg on ambulatory monitoring) is indicative of a clinically significant effect. It should be noted that statistically significant differences in BP were found between *CYP11B2* genotype groups irrespective of whether the stringent Bonferroni method was applied to account for multiple genotyping.

The finding of an association between the −344T allele of the *CYP11B2* gene and an increased BP in hypertensive individuals is in contrast to previously published data in a small sample (n = 65) of hypertensive subjects (with previously treated patients included in the analysis); in that study, the investigators found that a greater frequency of patients who had at least one copy of the −344C allele had inappropriate nocturnal decreases in BP.[10] However, the present data are in agreement with findings from a sample of the general population, in which subjects with the −344T allele were noted to have a reduced nocturnal decrease in BP as compared to other genotype groups.[26] Importantly, in the present study the differences in BP noted between *CYP11B2* genotype groups was for 24-h, day and night values and not for differences between day and night values (nocturnal decreases in BP).

A possible explanation for the association between the −344C→T variant of the *CYP11B2* gene and systolic BP in the present study is that the −344C→T variant of this gene influences aldosterone production.[9,12,27–29] The −344C→T polymorphism occurs at a putative binding site for steroidogenic transcription factor–1 (SF-1), and is associated with a fourfold increase in SF-1 binding to the −344C allele.[30] Because SF-1 binding to this region of the *CYP11B2* gene is nonfunctional,[31] the presence of the −344C allele is viewed as promoting competition between functional and nonfunctional binding of SF-1 to the promoter region of the gene. Consequently, the −344C allele may lead to a reduced transcription and, by inference, the

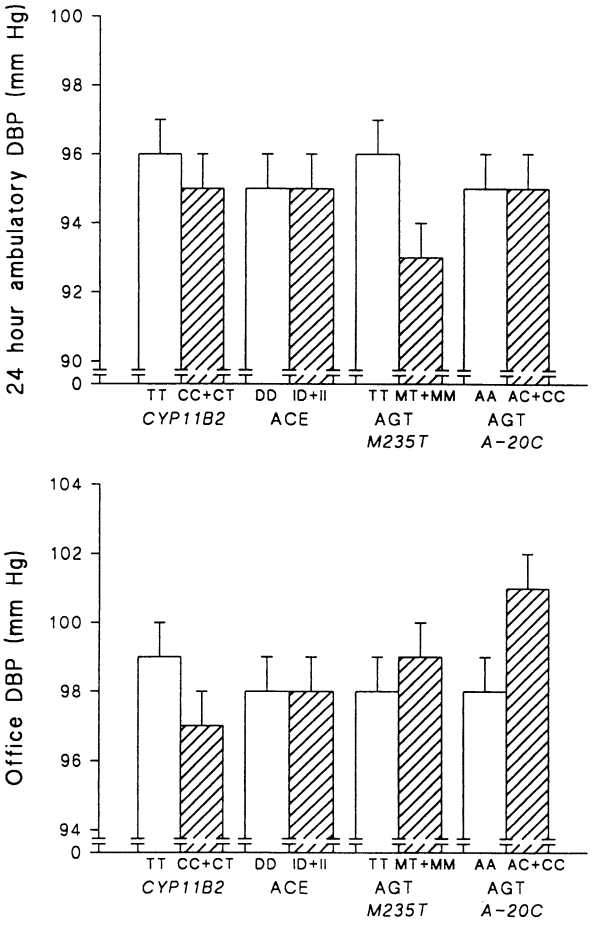

FIG. 3. Effects of renin-angiotensin-aldosterone gene polymorphisms on mean 24-h ambulatory and office diastolic blood pressures (DBP) in patients with newly diagnosed, never previously treated hypertension. abbreviations and sample sizes as in Fig. 1. No differences were noted between genotype groups.

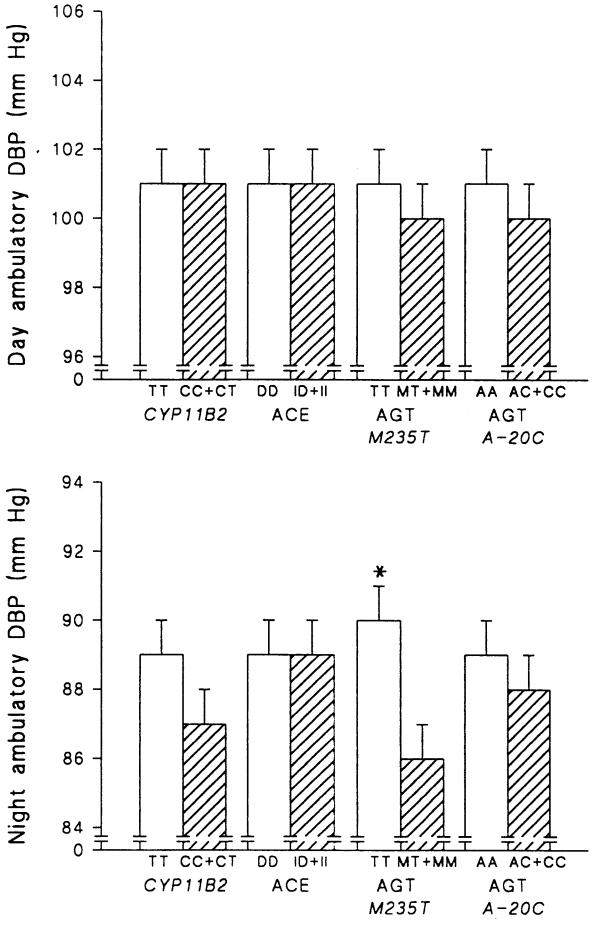

FIG. 4. Effects of renin-angiotensin-aldosterone gene polymorphisms on mean day and night ambulatory diastolic blood pressures (DBP) in patients with newly diagnosed, never previously treated hypertension. Abbreviations and sample sizes as in Fig. 1. * $P = .03$ before and $P = .08$ after adjusting for multiple genotyping.

presence of the −344T allele may lead to an increased transcription. Alternatively, the −344C→T polymorphism could be in linkage disequilibrium with an alternative and as yet unidentified functional variant either in or near the *CYP11B2* gene. Discrepant findings regarding the allele of importance[8–12,26] supports a notion that an alternative variant is involved and that the −344C→T polymorphism is a marker of the effects of an unidentified functional variant. Irrespective of the molecular mechanisms responsible, an increase in aldosterone production could influence BP through changes in vascular structure and tone as well as blood volume.[32]

The findings have potentially important clinical significance. The quantitative effects of *CYP11B2* genotype on SBP are substantial and equate to a >25% greater risk of stroke,[33] a frequently noted complication of hypertension in subjects of African ancestry. Also, genotyping at the *CYP11B2* locus may identify a subgroup that may respond more favorably to antihypertensive substances that modify either aldosterone synthesis or its receptors.

In conclusion, data obtained in the present study suggests that variation in the *CYP11B2* gene predicts the severity of BP in newly diagnosed hypertensive individuals. Because the severity of hypertension determines cardiovascular risk, and because aldosterone is an important target in hypertension, genotyping for the −344C→T polymorphism of the *CYP11B2* gene may have prognostic as well as therapeutic potential in a selected group of patients. These hypotheses need to be evaluated in prospective studies.

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