Genetic Variation of the Renin-Angiotensin System and Chronic Kidney Disease Progression in Black Individuals in the Atherosclerosis Risk in Communities Study

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The renin-angiotensin system (RAS) regulates BP and may affect chronic kidney disease (CKD) through induction of tissue growth and fibrosis. The angiotensinogen (AGT) promoter G(−6) allele lowers transcription and is inversely associated with hypertension. In white individuals, the A1166C 3′-UTR variant of angiotensin II type 1 receptor (AT1R) has been associated with CKD. CKD associations with these RAS genes are uncertain in high-risk black populations. A prospective population-based study of CKD risk was conducted among 3706 black individuals without severe renal dysfunction at baseline (serum creatinine ≥177 μmol/L [2.0 mg/dl] for men, ≥159 μmol/L [1.8 mg/dl] for women) to examine associations with AGT and AT1R. Incident CKD progression was defined as kidney disease hospitalization or increase in serum creatinine level ≥35 μmol/L (0.4 mg/dl) above baseline. During mean follow-up of 10.2 yr, CKD progression incidence rate (per 1000 person-years) was 8.2 (log-rank P = 0.03) and nonsignificantly higher among AT1R C1166 carriers. Adjusting for hypertension and major CKD risk factors, AGT G(−6) decreased risk (relative risk 0.75; 95% confidence interval 0.57 to 0.98). AT1R C1166 increased risk only among those with hypertension (relative risk 1.65; 95% confidence interval 1.14 to 2.39). The AGT G(−6)A polymorphism may play a role in CKD progression in black individuals, consistent with in vitro effects on AGT levels and renal remodeling but independent of BP. The AT1R C1166 allele may increase susceptibility but only in the presence of hypertension.


Chronic kidney disease (CKD) is a multifactorial disorder, with inherited components playing a role (1) in addition to the major risk factors of diabetes (2) and hypertension (3). The black community is particularly susceptible to CKD, in whom disease risk is two to three times that of white individuals (4,5).

The renin-angiotensin system (RAS) regulates BP and may have nonhemodynamic effects on renal structures by inducing cellular proliferation and fibrosis, affecting CKD (6,7). Of particular interest have been the angiotensinogen (AGT) G-to-A functional variant located at nucleotide −6 of the promoter [G(−6)A] (8) and the angiotensin II type 1 receptor (AT1R) gene A-to-C variant located in the 3′-untranslated region at nucleotide 1166 (A1166C), which has been associated with essential hypertension (9).

The AGT A(−6) promoter variant is the ancestral allele (10,11) and has a higher frequency in black (0.84) than white individuals (0.48) (12). The A(−6) ancestral allele increases basal transcription of the AGT gene (8), resulting in increases of AGT, the precursor for angiotensin II (Ang II). Ang II regulates both BP and fibrosis (6), and in animal models, increased levels contribute to glomerulosclerosis and extracellular matrix (ECM) synthesis through induction of TGF-β (7,13). Compared with the A(−6) allele, the G(−6) variant of AGT has been shown to be protective for coronary heart disease (CHD) (14). The AGT M235T polymorphism has been associated with ESRD (15), but there has been no study of the tightly linked G(−6)A functional polymorphism and CKD.

The major cardiovascular effects of Ang II are mediated through its receptor, AT1R. AT1R may also regulate cell proliferation and vascular ECM protein synthesis with effects on renal vascular and glomerular fibrosis (6,16). The C1166 variant has been associated with hypertension (9), myocardial infarction (17), and increased response to Ang II in human arteries (18–20). In white individuals, the AT1R C1166 variant has been associated with renal disease (21,22).

There has been no investigation of RAS genetic variation and CKD in a large U.S. population-based study of black individuals. Few studies have examined the mediating effects of BP on the association of RAS genetic variation and CKD. With these considerations in mind, we conducted a prospective study of a
community-based middle-aged cohort of 3706 black individuals to determine risk for CKD progression associated with both AGT G(−6)A and AT1R A1166C genetic variants and to investigate whether risk is independent of BP. Compared with A(−6) of AGT, we hypothesized that G(−6) would decrease CKD progression risk. On the basis of associations with essential hypertension and kidney disease in white individuals, we hypothesized that AT1R C1166 carriers would be at increased risk. On the basis of the increased Ang II response associated with the AT1R C1166 allele, we postulated that the AT1R C1166 allele would modify the risk for CKD progression with AGT.

Materials and Methods

Study Population

The Atherosclerosis Risk in Communities (ARIC) Study recruited 15,792 adults aged 45 to 64 yr at baseline in 1987 through 1989 from four US communities: Forsyth County, NC; Jackson, MS; Minneapolis, MN; and Washington County, MD. Participants underwent four standardized examinations in field center clinics, scheduled approximately every 3 yr, with the last visit ending in 1999 (23). In addition to yearly telephone interviews, hospitalizations and deaths were ascertained and records were abstracted as described previously (22,24). The institutional review boards of each participating institution approved the study protocol. Written informed consent was obtained from all participants at each examination.

Of the 4266 black individuals, 560 were excluded from this analysis: 167 had missing serum creatinine (Scr), 36 had severe hypercreatinemia (Scr ≥177 μmol/L [2.0 mg/dl] for men, Scr ≥159 μmol/L [1.8 mg/dl] for women) (25), eight individuals were missing covariates (diabetes, BP, hypertension medications, CHD history, or BMI), and 349 mg/dl] for women) (25), eight individuals were missing covariates defined by history of CHD revascularization procedures or electrocardiogram evidence of myocardial infarction (MI). Baseline GFR was estimated from calibrated Scr using the simplified equation developed using data from the Modification of Diet in Renal Disease Study (28,29) as follows: GFR (ml/min per 1.73 m^2) = 186.3 × (Scr)−1.154 × (age)−0.203 × (0.742 if female) × (1.21 if black).

AGT and AT1R Genotyping

Genotyping of the −6 G-to-A promoter polymorphism of AGT and the 3′ AT1R untranslated region 1166 A-to-C polymorphism was performed only among black individuals and was detected using the TaqMan assay (Applied Biosystems, Foster City, CA). Oligonucleotide sequences for PCR primers and TaqMan probes are available upon request from the authors. Allele detection and genotype calling were performed using the ABI 7700 and the Sequence Detection System software (Applied Biosystems).

Ascertainment of CKD Progression

CKD progression was defined as either (1) an increase in Scr ≥35 μmol (0.4 mg/dl) above baseline or (2) a hospitalization discharge or death coded for chronic renal disease (International Classification of Diseases, Ninth Revision [ICD-9] codes 581 to 583 or 585 to 588); hypertensive renal disease (ICD-9 code 403); hypertensive heart and renal disease (ICD-9 code 404); unspecified disorder of kidney and ureter (ICD-9 code 593.9); diabetes with renal manifestations (ICD-9 code 250.4); kidney transplant, renal dialysis, or adjustment/fitting of catheter (ICD-9 codes V42.0, V45.1 or V56); or either hemodialysis (ICD-9-CM procedure code 39.95) or peritoneal dialysis (ICD-9-CM procedure code 54.98) without acute renal failure (ICD-9 codes 584, 586, 788.9, and 958.5), as the primary or secondary hospitalization code. Scr was measured at baseline and at the 3-yr (University of Minnesota) and 9-yr (ARIC central laboratory, Houston, TX) follow-up visits using a modified kinetic Jaffe method (26,30). Lack of standardization of Scr values can lead to biased estimates of GFR, particularly at higher levels of GFR (51). Accordingly, Scr measurements were corrected for interlaboratory differences and calibrated to the Cleveland Clinic measurement standards by subtraction of 0.24 mg/dl from baseline and year 3 measurements and by addition of 0.18 mg/dl to year 9 measurements, after repeat analysis of 29 frozen samples to assess interlaboratory variation (32). Assessment of variability within ARIC participants revealed that 0.18 mg/dl was the minimal change in creatinine at which 95% confidence existed that a true change had occurred (methodologic variability, SD = 0.05, and within-person variability, SD = 0.04) (30). A rise in Scr was defined as a change of at least twice this amount (35 μmol or 0.4 mg/dl) as in previous analyses (5,33–35). Ancillary analyses compared association with CKD hospitalization and creatinine rise as separate outcomes as well as alternative definitions of CKD. In addition, we defined the incidence of moderately decreased kidney function as estimated GFR <60 ml/min per 1.73 m^2 during follow-up. In our subsidiary analyses, among those without moderately decreased kidney function, we used this case definition (and corresponding follow-up time) in lieu of Scr rise for our alternative combined definition of CKD progression. Albuminuria was also assessed from an untimed urine sample collected at the year 9 visit. Albumin and creatinine levels were measured in the University of Minnesota Physicians Outreach Laboratories (Minneapolis, MN), albumin by a fluorescence immunoassay (assay sensitivity, 2.0 mg/L), and creatinine using the Jaffe method. Macroalbuminuria was defined as urinary albumin-to-creatinine ratio ≥300 μg/mg.

Statistical Analyses

Tests of differences in baseline characteristics by AGT G(−6) allele carrier status and by AT1R C1166 carrier status were performed using
t test and \( \chi^2 \) tests. The primary analysis for the study evaluated time to CKD progression with (1) AGT genetic variation and (2) AT1R genetic variation. For cases, follow-up time was assessed from baseline until the visit date at which the Scr rise occurred (Scr-based CKD cases), the date of hospitalization discharge or death (hospitalization-based cases), or the earlier of the two dates for participants who met both case definitions. Noncases were censored at the earlier of the date of last contact (or date of non-CKD death) or January 1, 2000. The cumulative incidence curves for time to CKD progression as a function of age during follow-up were estimated by the Kaplan-Meier method (36), and differences in cumulative incidence by genetic variation were compared with the use of the log-rank test. Proportional hazards models (37) were constructed to examine separately AGT and AT1R variation as independent predictors of CKD progression. To determine whether AGT affects CKD progression differently by AT1R alleles, we performed analyses stratified by AT1R C1166 carrier status and tests of interaction. Genetic variation was modeled for dominant genetic effects of the more recently derived evolutionary alleles AGT G(−6) and AT1R C1166 (10,11). Genotypic effects were examined with similar results. Baseline variables that were thought to influence renal function were chosen as covariates: Gender, age, body mass index (BMI), diabetes, JNC6 BP categories, hypertension medication use, CHD history, and GFR. To examine further the possible mediating effects of BP, in lieu of JNC6 BP categories, we also adjusted for SBP and DBP (singly or in combination) with hypertension medication use. To verify consistency across high-risk subgroups, we stratified by diabetic and hypertensive status. In cross-sectional analyses of RAS genetic variation and macroalbuminuria at visit 4, logistic regression methods were used with visit 4 covariates.

Results
Genotypic Frequencies

There were 3449 black participants with AGT data and 3638 individuals with AT1R genotypes. Distribution of AGT −6 AA, GA, and GG genotypes was 70.6, 26.8, and 2.6%, respectively. For the AT1R 1166 3′ UTR polymorphism, distribution of AA, CA, and CC genotypes was 88.2, 11.5, and 0.3%, respectively. Genotype frequencies agreed with Hardy-Weinberg equilibrium expectations. Frequencies of less common alleles were 16.0% for AGT G(−6) and 6.1% for AT1R C1166, comparable to previously published estimates in black individuals (11,12,38).

CKD Progression Risk Factors at Baseline

Table 1 summarizes characteristics for all black individuals without severe baseline renal dysfunction and by AGT G(−6) and AT1R C1166 carrier status. Risk factor profile was predominantly similar by genotype. Baseline diabetes status, GFR, and SBP did not differ by RAS genotype. However, mean DBP was higher among those who were homozygous for the AGT ancestral A(−6) allele compared with G(−6) carriers (respectively, mean DBP = 79.9 versus 78.4 mmHg; \( P < 0.001 \)). There was no difference in BP by AT1R variation.

Incident CKD Progression

During mean follow-up of 10.2 yr, 312 CKD progression cases developed in our total sample (incidence 8.2 per 1000 person-years). Of cases, 38.8% had been hospitalized for CKD (\( n = 121 \)), 42.0% developed compromised renal function according to the SCR-based outcome (\( n = 131 \)), and 19.2% were identified with both definitions (\( n = 60 \)). Carriers of the AGT G(−6) allele had a lower incidence rate of CKD (72 cases, incidence 6.9 per 1000 person-years) compared with those who were homozygous for the ancestral A(−6) allele (221 cases, incidence 9.0 per 1000 person-years). Carriers of the AT1R C1166 allele had a higher incidence rate of CKD (42 cases, incidence 16.0%) for AGT G(−6) and 6.1% for AT1R C1166, comparable to previously published estimates in black individuals (11,12,38).

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Table 1. Selected baseline characteristics of black adults aged 45 to 64 yr by AGT G(−6) and AT1R C1166 carrier status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Black Individuals, All Genotypes (( n = 3706 ))</th>
<th>AGT (( n = 3449 ))</th>
<th>ATIR (( n = 3638 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( A/A ) ( (n = 2435) )</td>
<td>( G(−6) ) Carriers (( n = 1014 ))</td>
</tr>
<tr>
<td>Age (yr; mean [SD])</td>
<td>53.5 (5.8)</td>
<td>53.5 (5.8)</td>
<td>53.7 (5.9)</td>
</tr>
<tr>
<td>Female (( n ) [%])</td>
<td>2283 (61.6)</td>
<td>1490 (61.2)</td>
<td>636 (62.7)</td>
</tr>
<tr>
<td>Diabetes (( n ) [%])</td>
<td>702 (18.9)</td>
<td>457 (18.8)</td>
<td>196 (19.3)</td>
</tr>
<tr>
<td>SBP (mmHg; mean [SD])</td>
<td>128.5 (21.0)</td>
<td>128.9 (21.4)</td>
<td>127.6 (20.7)</td>
</tr>
<tr>
<td>DBP (mmHg; mean [SD])</td>
<td>79.5 (12.1)</td>
<td>79.9 (12.3)</td>
<td>78.4 (11.7)</td>
</tr>
<tr>
<td>Hypertension medication use (( n ) [%])</td>
<td>1548 (41.8)</td>
<td>1044 (42.9)</td>
<td>408 (40.2)</td>
</tr>
<tr>
<td>Prevalent hypertension (( n ) [%])</td>
<td>2045 (55.2)</td>
<td>1368 (56.2)</td>
<td>544 (53.7)</td>
</tr>
<tr>
<td>Prevalent CHD (( n ) [%])</td>
<td>165 (4.5)</td>
<td>99 (4.1)</td>
<td>55 (5.4)</td>
</tr>
<tr>
<td>SCR (mg/dl; mean [SD])</td>
<td>0.9 (0.2)</td>
<td>0.9 (0.2)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²; mean [SD])</td>
<td>103.3 (23.8)</td>
<td>102.8 (23.8)</td>
<td>104.2 (23.6)</td>
</tr>
<tr>
<td>GFR &lt; 90 ml/min per 1.73 m² (( n ) [%])</td>
<td>1131 (30.5)</td>
<td>759 (31.2)</td>
<td>294 (29.0)</td>
</tr>
<tr>
<td>BMI (kg/m²; mean [SD])</td>
<td>29.6 (6.1)</td>
<td>29.7 (6.2)</td>
<td>29.4 (6.0)</td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30 kg/m²; ( n ) [%])</td>
<td>1512 (40.8)</td>
<td>999 (41.0)</td>
<td>398 (39.3)</td>
</tr>
</tbody>
</table>

\[ ^\text{a} \text{AGT, angiotensinogen; ATIR, angiotensin II type 1 receptor; BMI, body mass index; CHD, coronary heart disease; DBP, diastolic BP; SBP, systolic BP; SCR, serum creatinine.} \]

\[ ^b p < 0.001, \text{AGT G(−6) carriers versus noncarriers.} \]
incidence 9.6 per 1000 person-years) compared with noncarriers (265 cases, incidence 8.1 per 1000 person-years). Figure 1 illustrates the cumulative incidence of CKD progression by carrier status for the examined RAS alleles. Incident CKD progression as a function of age during follow-up was significantly lower among carriers of the G(−6) promoter polymorphism of AGT when compared with noncarriers (log-rank test, \( P = 0.026 \)). However, there was no significant difference by AT1R C1166 carrier status (log-rank test, \( P = 0.31 \)).

Unadjusted and Multivariate Analyses
To determine effects of AGT and AT1R on CKD progression, we constructed a series of proportional hazards models that adjusted for known risk factors (Table 2). The risk effects of RAS gene variation on CKD progression were comparable when stratified by gender, diabetic status, and baseline kidney function of GFR 90 ml/min per 1.73 m² (results not shown) with formal tests of interaction NS (\( P > 0.53 \) for interaction). The AGT G(−6) allele was associated inversely with CKD with an unadjusted relative risk (RR) of 0.77 (95% confidence interval [CI] 0.59 to 1.00). Using SCr or estimated GFR as a covariate for baseline renal function in our multivariate models did not significantly alter our results (models 2 and 3). Without adjustment for hypertension, the AGT G(−6) allele was protective for CKD progression independent of other major CKD risk factors, including diabetes (model 4: RR 0.74; 95% CI 0.56 to 0.96). By contrast, carrying the AT1R C1166 allele was not a significant risk factor for CKD progression with an unadjusted RR of 1.18 (95% CI 0.85 to 1.64). Adjustment for several major CKD risk factors (model 4) increased the RR of the AT1R C1166 allele to 1.31 (95% CI 0.94 to 1.81).

When we examined the 3381 individuals (288 cases) with both AGT and AT1R genotypes, adjusting for the covariates of model 3, compared with individuals with neither the AGT G(−6) allele nor the AT1R C1166 variant, the RR of CKD was 1.21 (95% CI 0.80 to 1.84) for those who carried the AT1R C1166 allele only, 0.74 (95% CI 0.55 to 0.99) for those who carried the AGT G(−6) allele only, and 1.02 (95% CI 0.57 to 1.82) for those who carried both. A formal test of interaction was NS (\( P = 0.73 \) for interaction).

To tease apart the complex relationship among RAS genetic variation, BP, and kidney disease, we performed multivariate analyses stratified by hypertensive status. Adjusting for age, gender, diabetes, BMI, CHD, and GFR (Figure 2), the RR for CKD progression of the AGT G(−6) allele was comparable between those without (RR 0.68; 95% CI 0.42 to 1.12) and with hypertension (RR 0.78; 95% CI 0.57 to 1.07) with no interaction (\( P = 0.88 \)), demonstrating that it was appropriate to include hypertensive status as a covariate in our model assessing risk effects of AGT. Adjusting for age, gender, diabetes, BMI, CHD, GFR, hypertension medication use, and JNC6 BP categories demonstrated that RR of CKD progression for AGT G(−6) was 0.75 (95% CI 0.57 to 0.98) and was independent of major CKD risk factors. Because of the association of AGT variation and DBP, we examined a model adjusting only for DBP (RR 0.78; 95% CI 0.60 to 1.02) and a separate model adjusting for age, gender, diabetes, BMI, CHD, GFR, hypertension medication use, and DBP (RR 0.74; 95% CI 0.57 to 0.97) with no significant changes in the risk association.

When we stratified by hypertensive status and examined the risk association of the AT1R C1166 allele on CKD progression (Figure 2), the RR among the 1631 individuals without hypertension (86 cases) was 0.74 (95% CI 0.36 to 1.54) and among the 2007 individuals with hypertension (221 cases) was 1.65 (95% CI 1.14 to 2.39). There was a significant interaction on risk for CKD progression between AT1R C1166 carrier status and hypertension (\( P = 0.042 \), adjusting for age, gender, diabetes, BMI, CHD, and GFR. Among individuals with hypertension, AT1R C1166 predicted CKD progression (RR 1.55; 95% CI 1.04 to 2.31) independent of AGT G(−6) carrier status, after adjustment for major CKD risk factors and AGT carrier status. Therefore, the

![Figure 1. Cumulative incidence of chronic kidney disease (CKD) progression by angiotensinogen (AGT) and angiotensin II type 1 receptor (AT1R) genetic variation.](image-url)
Table 2. Unadjusted and adjusted RR for CKD progression for AGT and AT1R polymorphismsa

<table>
<thead>
<tr>
<th>Modelb</th>
<th>AGT G(−6) Carriers versus Noncarriers (n = 3449)</th>
<th>AT1R C1166 Carriers versus Noncarriers (n = 3638)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>RR (95% CI) for CKD Progression</td>
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</tr>
<tr>
<td>Model 1</td>
<td>0.77 (0.59 to 1.00)</td>
<td>1.18 (0.85 to 1.64)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.73 (0.56 to 0.95)c</td>
<td>1.21 (0.87 to 1.67)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.75 (0.58 to 0.98)c</td>
<td>1.30 (0.94 to 1.81)</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.74 (0.57 to 0.97)c</td>
<td>1.27 (0.91 to 1.76)</td>
</tr>
<tr>
<td></td>
<td>0.74 (0.56 to 0.96)c</td>
<td>1.31 (0.94 to 1.81)</td>
</tr>
</tbody>
</table>

aCI, confidence interval; CKD, chronic kidney disease; RR, relative risk.
bModel 1 adjusts for age, gender, and diabetes; model 2 = model 1 + SCr; model 3 = model 1 + GFR; model 4 = model 1 + GFR, BMI, and CHD.

Results were similar to our combined definition. For a hospitalization or death with CKD, in the fully adjusted model (covariates age, gender, diabetes, BMI, CHD, JNC6 BP categories, hypertension medication use, and GFR), the AGT G(−6) allele decreased risk (RR 0.75; 95% CI 0.52 to 1.06) whereas the AT1R C1166 allele increased risk by 1.39 (95% CI 0.90 to 2.15). AT1R C1166 increased risk for a renal disease hospitalization only among those with hypertension (RR 1.73; 95% CI 1.06 to 2.81) but not among those without hypertension (RR 0.55; 95% CI 0.19 to 1.61). For a rise in serum creatinine ≥35 μmol/L (0.4 mg/dL), the RR associations were attenuated but in the same direction, with AGT G(−6) at 0.84 (95% CI 0.60 to 1.17) and AT1R C1166 at 1.05 (95% CI 0.68 to 1.64) in the fully adjusted model.

Results were similar but NS when we alternatively defined CKD progression as a combination of either (1) a renal disease hospitalization or (2) incidence of moderately decreased kidney function (GFR <60 ml/min per 1.73 m2). AGT G(−6) carriers were at decreased risk (RR 0.82; 95% CI 0.62 to 1.07), after adjustment for all covariates. Similar to our main results, the AT1R C1166 increased risk among those with hypertension (RR 1.27; 95% CI 0.86 to 1.88) but not among those without hypertension (RR 0.78; 95% CI 0.35 to 1.70).

When we examined risk for a hospitalization with an ESRD code, in the fully adjusted model, AGT G(−6) decreased risk among those without hypertension (RR 0.37; 95% CI 0.10 to 1.43) but not among those with hypertension (RR 1.13; 95% CI 0.61 to 2.07). AT1R C1166 increased risk (RR 1.48; 95% CI 0.70 to 3.15) during follow-up. Again, AT1R C1166 increased risk only in the presence of hypertension (RR 1.82; 95% CI 0.75 to 4.43) but not among those without hypertension (RR 0.94; 95% CI 0.20 to 4.50). However, our analysis of ESRD was severely limited by the number of incident cases (n = 77).

Quantitative data on albuminuria was only available for study participants who attended the last visit (n = 1941). Using logistic regression adjusting for all covariates, AGT G(−6) was not associated with prevalence of macroalbuminuria (odds ratio [OR] 1.10; 95% CI 0.61 to 1.99). Similarly, AT1R C1166 did not significantly increase risk among those with (OR 1.00; 95% CI 0.40 to 2.48) and without hypertension (OR 6.68; 95% CI 0.18

ATIR C1166 allele was associated significantly with risk for CKD progression in the presence of hypertension.

Subsidiary Analyses
The association of RAS genetic variation and CKD was robust across several definitions of CKD progression (including those defined by GFR, SCr, and urine albumin). When the two components of CKD progression were analyzed separately, results were similar to our combined definition. For a hospitalization or death with CKD, in the fully adjusted model (covariates age, gender, diabetes, BMI, CHD, JNC6 BP categories, hypertension medication use, and GFR), the AGT G(−6) allele decreased risk (RR 0.75; 95% CI 0.52 to 1.06) whereas the AT1R C1166 allele increased risk by 1.39 (95% CI 0.90 to 2.15). AT1R C1166 increased risk for a renal disease hospitalization only among those with hypertension (RR 1.73; 95% CI 1.06 to 2.81) but not among those without hypertension (RR 0.55; 95% CI 0.19 to 1.61). For a rise in serum creatinine ≥35 μmol/L (0.4 mg/dL), the RR associations were attenuated but in the same direction, with AGT G(−6) at 0.84 (95% CI 0.60 to 1.17) and AT1R C1166 at 1.05 (95% CI 0.68 to 1.64) in the fully adjusted model.

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to 244.12) in the fully adjusted model. However, our analysis of macroalbuminuria was also limited by the number of cases (n = 65).

To assess whether the associations of RAS genetic variation and CKD progression could be an artifact as a result of a survival effect, we examined whether RAS genetic variation was associated with loss to follow-up. In our cohort, loss to follow-up did not differ by either AGT G(−6) carrier status (P = 0.68) or AT1R C1166 carrier status (P = 0.45). In addition, in our cohort, there were 520 deaths during follow-up. When we examined the effect of RAS genetic variation on CKD only among the 520 mortality events, the risk factor adjusted effects on CKD (n = 99 cases) were attenuated but similar to that seen in the entire cohort for AGT G(−6) carrier status (RR 0.86; 95% CI 0.52 to 1.42) and AT1R C1166 carrier status (RR 1.12; 95% CI 0.59 to 2.14).

Discussion

AGT variation is associated with CKD progression in black individuals, independent of the effects of diabetes and BP. Compared with the ancestral A(−6) allele, carriers of G(−6) may have lower risk, consistent with effects of this promoter polymorphism on a lower rate of transcription in vitro and of decreased cardiovascular disease risk (8,14). Consistent with its effects on modulating AGT transcription and renal remodeling (6,7,13), it may play a BP-independent role in CKD progression, with G(−6) carriers having a RR of CKD progression of 0.75 (95% CI 0.57 to 0.98). When we examined AT1R, the C1166 allele was positively associated with CKD progression in black individuals independent of major CKD risk factors but only among those with hypertension. Our findings are comparable to risk effects of AT1R C1166 on chronic renal insufficiency (22) and progression of interstitial nephritis (39). There was no interaction between AGT and AT1R genetic variation on CKD progression.

Although there have been studies of AT1R A1166C on CKD, the AGT G(−6)A polymorphism has never been studied before in a prospective, population-based study of black individuals. However, the AGT M235T allele has been associated with diabetic kidney disease and IgA nephropathy (15,40–42), although several studies also found no association (43,44). The T235 allele and the A(−6) allele both are in tight linkage disequilibrium, are thought to be ancestral alleles (45), and have been associated with essential hypertension (46). However, there is more evidence that the G(−6)A polymorphism (rather than M235T) may be the functional variant (8,47).

The prevailing explanation for the association between AGT and kidney disease has focused on BP control, with subsequent effects on renal function. The G(−6) allele is associated with decreased transcriptional activity and less risk for hypertension (46). Better BP regulation would result in less subsequent renal damage (48). However, the association of AGT and CKD progression was independent of BP in our study. Risk associations of AGT G(−6)A and CHD were similar in magnitude and also independent of hypertension (14).

We propose that RAS genetic variation may affect CKD progression through two pathways: Modulation of BP and separately through kidney remodeling. The AGT G(−6) variant is less transcriptionally active with lower levels of AGT mRNA. AGT is the precursor of Ang II, which has effects beyond BP control. Ang II can affect mesangial cell proliferation (7) and renal ECM protein synthesis through induction of TGF-β (6,13). AGT variants have been associated with worse kidney disease progression across several modalities, including IgA nephropathy and diabetic ESRD (15,40–42). Animal models demonstrate that in the absence of AGT synthesis, there is reduced hypertension-induced end-organ damage (49). Perhaps the role of the AGT G(−6) allele on renal remodeling may explain its decreased risk effect on CKD progression.

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Previous studies of AT1R demonstrated increased risk of the C1166 allele for progression of interstitial nephritis (39) and for chronic renal insufficiency (22). However, several previous studies also showed no associations (15,41). The AT1R C1166 allele was associated with increased risk for essential hypertension (9). However, in our study, it was associated with neither BP nor hypertension. In our prospective analysis, the AT1R C1166 allele predicted CKD progression only among those with hypertension. AT1R is the predominant receptor for the RAS, and the C1166 allele is associated with increased vascular responsiveness to RAS ligands (18–20). Among individuals with hypertension, the C1166 allele significantly increased risk for CKD progression. Perhaps AT1R C1166 carriers are genetically susceptible to CKD progression but only if exposed to the physiologic milieu concurrent with hypertension. Hypertension is one of the major causes of CKD (3), and its vascular and renal actions cause increased fibrosis, renal scarring, and subsequent deterioration of kidney function (48). Perhaps C1166 carriers, with increased vascular responsiveness in RAS, may be more susceptible to vascular damage and ECM deposition in the context of hypertension exposure. C1166 carriers are genetically susceptible to CKD, but the effects of hypertension were necessary to further damage of renal function.

We provide evidence that genetic variation of AT1R may affect CKD risk independent of diabetes among hypertensive black individuals, a population at particularly high risk for kidney disease. In addition, we examined the association of kidney disease with the AGT G(−6)A variant, which has never been examined before in this context. The AGT G(−6)A variant may predict risk independent of both diabetes and hypertension. However, there are several limitations to our study. Because CKD is heterogeneous, the influence of genetic variation of RAS on specific causes of kidney disease may not be inferred directly. However, the degree of sensitivity afforded by our definition provides sufficient power to examine a severe disease in a longitudinal, community-based setting (35), and our results demonstrate that genetic variation of RAS affects overall CKD risk. Although creatinine-based definitions of CKD progression can be insensitive, they should be specific and show the expected association with traditional risk factors (50). Furthermore, our examination of RAS genetic variation and alternative measures of renal disease demonstrate the consistency of our results. Hospitalization or death with a CKD diagnosis code does not allow for quantification of the amount of kidney disease progression. However, among individuals without hy-
percreatinemia at baseline, such a diagnosis likely denotes a substantial rise in SCr. Another limitation is that we cannot determine to what extent RAS genetic variation may explain increased risk for black individuals compared with white individuals, as RAS genotyping was performed only in black individuals. This question is particularly provocative in the context of the significantly different frequencies of the ancestral A(−6) risk allele in black and white individuals (0.84 versus 0.48, respectively) (12). However, it is possible the G(−6) allele could serve as a proxy for background genetic variation of white individuals (and their lower risk), rather than possessing a causative association with kidney disease. This study of RAS variation and kidney disease has the largest sample to date, which afforded detailed analyses not possible in smaller studies.

Although our study focused on black individuals because of high rates of kidney disease within the population, the use of self-described race and ethnicity has its limitations in the context of genetic studies. Race and ethnicity are socially constructed categories, largely based on skin color (51). Categorization by race suggests genetic homogeneity in frequently heterogeneous populations, such as black individuals, an ethically admixed group that is composed of individuals of African, European, Latino, and Native American ancestry (51,52). In addition, in disease models, race is often used as a flawed surrogate for multiple environmental and genetic factors that differentiate high- and low-risk populations (53). The use of race in clinical studies has its limitations; however, we do not believe that these aspects detract from the association of RAS genetic variation with CKD progression in our study population for several reasons. First, the AGT G(−6)A single nucleotide polymorphism is a functional variant, although it could still be in linkage disequilibrium with another functional single nucleotide polymorphism associated with CKD risk. Second, a recent study using genetic cluster analysis suggested a degree of genetic homogeneity within self-identified race/ethnicity categories, with sufficient variability between groups to distinguish between black, white, Hispanic, and East Asian race (54). In addition, several studies have suggested that the potential impact of population stratification is minimal in association studies of black populations (52,55,56).

Conclusion

RAS genetic variation predicts moderately elevated risk for CKD progression in black individuals. The association of AGT is independent of major CKD risk factors and of AT1R. Similarly, AT1R predicts risk among those with hypertension, independent of AGT. Studying the pathways that mediate this association may shed light on the pathophysiology of CKD and help to delineate mechanisms that may explain the excess risk among black individuals. The modest sizes of the risk associations limit utility for screening and individualized therapy. However, if multiple genes of small and moderate effect on CKD are identified, then they may compose panels for risk assessment (57). In addition, these observations support mounting data that inhibition of the RAS decreases CKD progression (16,58). Consistency of the findings supports the hypothesis that much of CKD is multifactorial with significant inherited components in black individuals.

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