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Urinary angiotensinogen is correlated with blood pressure in men (Bogalusa Heart Study)

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Abstract

Background—The Bogalusa Heart Study is a long-term study on cardiovascular disease and has followed a biracial (black/white) population from childhood. Risk factor data pertaining to many patients have been collected over 35 years, and the time course of hypertension has been documented by repeated examinations and measurements. Considerable sex and racial differences have been found to be related to cardiovascular disease. Urinary angiotensinogen (UAGT) is a novel biomarker for the intrarenal activity of the renin–angiotensin system in hypertension and kidney disease. We aimed to determine the relationship of UAGT with traditional cardiovascular disease risk factors in asymptomatic young adults in this biracial population.

Method—We recruited 251 individuals and collected a single random spot urine sample from each one. Because UAGT is significantly increased in diabetic patients and the use of antihypertensive drugs affects UAGT levels, we excluded patients who had diabetes, who were receiving antihypertensive treatment, or both. Consequently, 190 participants were included for this analysis.

Results—UAGT levels did not differ with race or sex, but were significantly correlated with SBP (r = +0.23, P = 0.0015) and DBP (r = +0.24, P = 0.0012). Moreover, high correlations were shown in men, especially in black men (SBP, r = +0.85, P = 0.0005 and DBP, r = +0.72, P = 0.0079). Thus, UAGT is correlated with blood pressure in men, even when they do not show overt proteinuria or albuminuria.

Conclusion—The biomarker, UAGT, may facilitate the identification of individuals that are at increased risk for the development of hypertension and early asymptomatic renal disease.

Keywords

angiotensinogen; clinical study; ELISA; hypertension; renin-angiotensin system; urine

Introduction

Uncontrolled hypertension induces structural and functional alterations in the kidney, which can eventually lead to end-stage renal disease [1]. Effective control of blood pressure (BP)

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retards the progression of renal failure and reduces the morbidity and mortality rates associated with hypertensive vascular disease [2–5]. Identification of individuals at risk of developing hypertension and renal disease is of cardinal importance.

The Bogalusa Heart Study [6–9] is a long-term study of cardiovascular disease (CVD) and has followed a biracial (black/white) population from childhood. Risk factor data on many patients have been collected over 35 years, and the time course of hypertension has been documented by repeated examinations and measurements. Considerable sex and racial contrasts have been noted in relation to CVD [6–9].

Recent findings related to the renin–angiotensin system (RAS), one of the most important mechanisms of BP regulation and water–electrolyte homeostasis, have provided an improved understanding of the pathophysiology of hypertension [10,11]. In recent years, the focus of interest on the RAS has shifted to the role of the local/tissue RAS in specific tissues [12]. Many studies have reported the importance of the tissue RAS in the brain [13], heart [14], adrenal glands [15], vasculature [16,17], and kidneys [10,11]. Further, there is substantial evidence that the major fraction of angiotensin II present in renal tissues is locally generated from angiotensinogen (AGT) delivered to the kidney as well as from AGT locally produced by the proximal tubule cells [18]. Renin secreted by the juxtaglomerular apparatus cells into the renal interstitium and vascular compartment also results in the local generation of angiotensin I [19]. Angiotensin-converting enzyme is abundant in the kidney and is present in the proximal tubules, distal tubules, and collecting ducts [20]. Angiotensin I delivered to the kidney can also be converted to angiotensin II [21]. Therefore, all of the components necessary to generate intrarenal angiotensin II are present along the nephron [10,11].

AGT is the only known substrate for renin, the rate-limiting enzyme of the RAS. Because the level of AGT is close to the Michaelis–Menten constant for renin, changes in AGT levels as well as renin levels can control the activity of the RAS, and upregulation of AGT levels may lead to increases in the angiotensin peptide levels and in BP [22,23]. Recent studies [24–32] on experimental animal models and transgenic mice have documented the involvement of AGT in the activation of the RAS and development of hypertension. Genetic manipulations that lead to overexpression of AGT have consistently been shown to cause hypertension [33,34]. In human genetic studies, a linkage has been established between the *AGT* gene and hypertension [35–38]. Thus, AGT plays an important role in BP regulation.

Recently, we reported that urinary excretion rates of AGT provide a specific index of the intrarenal RAS status in angiotensin II-dependent hypertensive rats [39–43]. Further, we recently developed a direct quantitative method to measure urinary AGT (UAGT) using human AGT ELISA [44]. This method has recently been used to determine the urinary excretion rates of AGT, and these rates have been found to be a novel biomarker for the intrarenal activity of the RAS in chronic kidney disease [45,46], hypertension [47], and diabetic nephropathy [48, 49]. The current study was performed to determine the relationships of UAGT with traditional CVD risk factors in asymptomatic young adults in the biracial populations of the Bogalusa Heart Study.

Methods

Study design and participant recruitment

The protocol of this observational study was approved by the Institutional Review Board and by the Clinical and Translational Research Center at Tulane University. Two hundred and fifty-one individuals were recruited in Tulane University Health Sciences Center and associated clinics, and samples were obtained from them after acquiring written informed consent. It has been reported that UAGT is significantly increased in diabetic patients [46], and the use of

antihypertensive drugs also affects UAGT levels [47]. Therefore, we excluded patients who had diabetes, those who were receiving antihypertensive therapy, or both, from further analyses. Consequently, 190 samples were analyzed. A random spot urine sample and a blood sample were obtained from the participants when they visited our clinic. Height and body weight were also recorded on the same day. BP was measured as previously described [46, 50]. Briefly, participants sat quietly for at least 5 min prior to BP measurements in their right arms. We used an automated manometer (Omron Corporation, Kyoto, Japan) to make at least three BP measurements, 2 min apart. The mean BP values were calculated from the last two SBP and DBP readings that fell within 5 mmHg of each other.

Measurements

Serum concentrations of sodium, creatinine, and cystatin C, and urinary concentrations of sodium and protein were measured in the clinical laboratory in the Tulane University Health Sciences Center with an AU 400 Auto-analyzer (Olympus, Melville, New York, USA). Urinary concentrations of albumin and creatinine were measured with a DCA 2000 Analyzer (Bayer AG, Leverkusen, Germany). Urinary concentrations of AGT were measured with human AGT ELISA kits as previously described [44]. The coefficients of variation of the intraassay and of the interassay of this AGT ELISA were 4.4 and 4.3%, respectively [44].

Statistical analyses

Pearson correlation coefficients and Spearman correlation coefficients were used for parametric data and nonparametric data, respectively. The standard least squares method was used for multiple regression analysis. Comparisons of continuous variables between groups were performed using one-way analysis of variance and Dunnett's test. All data are presented as means ± SEM. A P value of less than 0.05 was considered significant. All computations, including data management and statistical analyses, were performed with the JMP software (SAS Institute, Cary, North Carolina, USA).

Results

Participants' profiles and laboratory data

The demographics and baseline laboratory data of the study participants are summarized in Tables 1 and 2, respectively. The important finding is that serum creatinine levels, urinary protein–creatinine ratio, fractional excretion of sodium, serum cystatin C levels, or urinary albumin–creatinine ratio are not different and thus renal functions were similar among the groups in this study.

Single regression and correlation analyses

We performed single regression and correlation analyses of UAGT–creatinine ratio (UAGT/UCre) with clinical parameters. The UAGT/UCre levels were not correlated with age, height ($r = -0.03$, $P = 0.6481$), body weight ($r = +0.00$, $P = 0.9745$), BMI, serum sodium levels, serum creatinine levels ($r = -0.11$, $P = 0.131$), serum cystatin C levels, or fractional excretion of sodium. However, the UAGT/UCre levels were significantly and positively correlated with the SBP (Fig. 1a, $r = +0.23$, $P = 0.0015$), DBP (Fig. 1b, $r = +0.24$, $P = 0.0012$), urinary sodium–creatinine ratio ($r = +0.16$, $P = 0.0342$), urinary albumin–creatinine ratio ($r = +0.39$, $P < 0.0001$), and urinary protein–creatinine ratio ($r = +0.47$, $P < 0.0001$).

Multiple regression analysis

Factors that were found to have a significant correlation with the UAGT/UCre levels in the single regression analyses (SBP, DBP, urinary sodium–creatinine ratio, urinary albumin–creatinine ratio, and urinary protein–creatinine ratio) were used as explanatory variables in the

multiple regression analysis. To reduce the impact of multicollinearity, we selected explanatory variables such that the mean sum of the squares of the residual would be minimal in the multiple regression analysis. As a result, SBP and urinary albumin–creatinine ratio were excluded from the multiple regression analysis (Table 3). The remaining three parameters were subjected to multiple regression analysis by stepwise method for UAGT/UCre. These three parameters accounted for almost 30% of the variations in UAGT/UCre levels (Fig. 1c; $r = 0.54$, $R^2 = 0.29$, $P < 0.0001$).

Relationship of urinary angiotensinogen–creatinine ratio levels with blood pressure in the study populations

The average UAGT/UCre levels did not differ with race or sex (Fig. 1d). However, as described above, the UAGT/UCre levels were significantly correlated with the SBP and DBP. Moreover, these levels were strongly correlated in men, especially in black men with the SBP (Fig. 1e; $r = +0.85$, $P = 0.0005$) and DBP (Fig. 1f; $r = +0.72$, $P = 0.0079$).

Discussion

In this study, we recruited men and women without any bias in the selection process. Accordingly, there were some differences among the participant groups in terms of height, body weight, and serum creatinine levels (Tables 1 and 2). However, most of these differences may have stemmed from well recognized sex-related differences, and we emphasize here that none of these parameters (height, body weight, and serum creatinine levels) were correlated with the UAGT/UCre levels. Therefore, it seems unlikely that the above differences affected the final results reported herein.

Although most of the circulating AGT is produced and secreted by the liver, the kidneys also produce AGT [11]. Intrarenal AGT mRNA and protein have been localized in the proximal tubule cells, indicating that intratubular angiotensin II may be derived from locally formed and secreted AGT [51,52]. In the proximal tubule cells, AGT appears to be directly secreted into the tubular lumen; in addition, some AGT is broken down intracellularly to produce metabolites, which are then secreted into the tubular lumen [53]. Proximal tubular AGT concentrations in anesthetized rats have been reported to be in the range of 300–400 nmol/l and greatly exceed the free angiotensin I and II tubular fluid concentrations [10]. Because of its molecular size (50–60 kDa), little plasma AGT is expected to filter across the glomerular membrane, which further supports the concept that proximal tubular cells secrete AGT directly into the tubules [54].

To determine whether circulating AGT is a source of UAGT, human AGT was infused into hypertensive and normotensive rats, and it was found that circulating human AGT was not detectable in the urine [42]. The failure to detect human AGT in the urine indicates limited glomerular permeability, tubular degradation, or both. These findings support the hypothesis that UAGT is derived from the AGT that is formed and secreted by the proximal tubules and not from plasma AGT.

As described previously, we reported that UAGT excretion rates provide a specific index of the intrarenal RAS status in angiotensin II-dependent hypertensive rats [39–43]. To determine whether the increase in UAGT excretion was simply a nonspecific consequence of the proteinuria and hypertension, further studies were performed in a rat model of hypertension established by administering a deoxycorticosterone acetate salt and a high-salt diet to the rats [42]. Although the urinary protein excretion rates in the deoxycorticosterone acetate salt-induced volume-dependent hypertensive rats were increased to the same or greater extent, the UAGT levels were significantly lower in the volume-dependent hypertensive rats than in

angiotensin II-dependent hypertensive rats and were not greater than the levels in control rats [42].

Similar observations were made in patients with chronic kidney disease [46]. The UAGT/UCre levels in patients with type 2 diabetic nephropathy (795.67 ± 296.03 μg/g) or with membranous nephropathy (503.53 ± 297.68 μg/g) were much higher than the overall average levels in patients with chronic kidney disease (273.17 ± 62.22 μg/g) [46]. Importantly, activation of the intrarenal RAS has been reported to be involved in the progression of renal injury in human patients with diabetic nephropathy [55,56] or membranous nephropathy [57]. In contrast, the UAGT/UCre levels in patients with minimal change disease (8.28 ± 3.70 μg/g) were similar to those in control individuals (10.78 ± 3.42 μg/g), even though the patients with minimal change had severe proteinuria [46]. The findings in hypertensive animals as well as those in patients with chronic kidney disease support the hypothesis that the enhanced UAGT observed in this study was not a nonspecific consequence of proteinuria.

Further, important evidence was recently reported in relation to juvenile patients with type 1 diabetes [49]. The UAGT/UCre levels were compared between patients with type 1 diabetes and the age and sex-matched control individuals. Neither the urinary albumin–creatinine ratio nor the urinary protein–creatinine ratio was found to be increased in patients with type 1 diabetes compared with the control individuals, suggesting that these patients were in the premicroalbuminuric phase of diabetic nephropathy. However, the UAGT/UCre levels were significantly higher in the patients with type 1 diabetes than in the control individuals. These data indicate that in patients with type 1 diabetes, the increase in UAGT levels precedes the increase in urinary albumin levels. Taken together, these data indicate that augmented UAGT excretion is not simply a nonspecific consequence of proteinuria.

Hypertension is a multi-etiological disease and multiple factors are involved [1,58]. Our data suggest that in men, especially in black men, the pathway described in Fig. 2 is a major contributor to the pathogenesis of hypertension. In human genetic studies, a linkage has been established between the *AGT* gene and hypertension [35–38,59]. Also, oxidative stress-induced intrarenal AGT enhancement is reported to play an important role in the development of hypertension in animal models [60–62] and in clinical studies [48,55]. In addition, it is also reported that metabolic disorders augment AGT expression [63–66]. These factors may lead to increases in kidney/UAGT levels. Renin is secreted by the juxtaglomerular apparatus [67, 68] and by principal cells of collecting ducts [54,69–72]. Angiotensin-converting enzyme is also abundant in the kidney and is present in the proximal tubules, distal tubules, and collecting ducts [20]. Because the level of AGT is close to the Michaelis–Menten constant for renin [22,23], increases in kidney/UAGT levels may lead to the increases in kidney/urinary angiotensin I and thus angiotensin II levels. The augmented kidney/urinary angiotensin II levels are reported to enhance salt sensitivity and lead to BP increases via salt retention [73–75]. Although it is not possible to address these mechanistic issues in this observational study, our results are consistent with this perspective. Obviously, further studies, including interventional studies and a randomized clinical trial, are required to confirm its applicability.

The limitation of this study comes from the nature of the observational study using one-point sample collection. Although these data would establish a foundation of the normal range allowing comparison with data from patients with various diseases, we believe that a larger multicenter, randomized, control study is required to extend these observations. A prospective study to determine the relationship between the effect of RAS blockade on UAGT and CVD events will be helpful in assessing the clinical significance of the decrease in UAGT with RAS blockade. On the basis of these findings, a randomized clinical trial has been projected to establish the clinical significance of the decrease in UAGT with RAS blockade.

In summary, in asymptomatic young adults taken from a biracial population of the Bogalusa Heart Study, UAGT/UCre levels were significantly and positively correlated with the SBP, DBP, urinary sodium–creatinine ratio, urinary albumin–creatinine ratio, and urinary protein–creatinine ratio. They were not correlated with age, height, body weight, BMI, serum sodium levels, serum creatinine levels, serum cystatin C levels, or fractional excretion of sodium, and did not vary with race or sex.

However, these levels were strongly correlated with SBP and DBP, especially in black men. Because UAGT originates from the AGT that is formed and secreted by the proximal tubules and not from plasma AGT, the enhanced UAGT may not be just a nonspecific consequence of proteinuria, but rather indicate activation of the intrarenal RAS. These data suggest that UAGT excretion is correlated with the BP in men, even when they do not show overt proteinuria or albuminuria. Although a longer observation period is required to study long-term clinical outcomes such as cardiac events for which UAGT acts as a biomarker, UAGT may facilitate the identification of individuals at increased risk for the development of hypertension, and early asymptomatic renal disease.

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Abbreviations

AGT	angiotensinogen
CVD	cardiovascular disease
RAS	renin–angiotensin system
UAGT	urinary angiotensinogen
UAGT/UCre	urinary angiotensinogen–creatinine ratio

References

1. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension 2003;42:1206–1252. [PubMed: 14656957]
2. Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, et al. Blood pressure, stroke, and coronary heart disease. Part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. Lancet 1990;335:827–838. [PubMed: 1969567]
3. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). JAMA 2002;288:2981–2997. [PubMed: 12479763]
4. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT-llt). JAMA 2002;288:2998–3007. [PubMed: 12479764]

5. Heerspink HJ, Ninomiya T, Zoungas S, de Zeeuw D, Grobbee DE, Jardine MJ, et al. Effect of lowering blood pressure on cardiovascular events and mortality in patients on dialysis: a systematic review and meta-analysis of randomised controlled trials. Lancet 2009;373:1009–1015. [PubMed: 19249092]
6. Voors AW, Foster TA, Frerichs RR, Webber LS, Berenson GS. Studies of blood pressures in children, ages 5–14 years, in a total biracial community: the Bogalusa Heart Study. Circulation 1976;54:319–327. [PubMed: 939029]
7. Bao W, Srinivasan SR, Valdez R, Greenlund KJ, Wattigney WA, Berenson GS. Longitudinal changes in cardiovascular risk from childhood to young adulthood in offspring of parents with coronary artery disease: the Bogalusa Heart Study. JAMA 1997;278:1749–1754. [PubMed: 9388151]
8. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650–1656. [PubMed: 9614255]
9. Li S, Chen W, Srinivasan SR, Bond MG, Tang R, Urbina EM, Berenson GS. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. JAMA 2003;290:2271–2276. [PubMed: 14600185]
10. Navar LG, Harrison-Bernard LM, Nishiyama A, Kobori H. Regulation of intrarenal angiotensin II in hypertension. Hypertension 2002;39:316–322. [PubMed: 11882566]
11. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin–angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacol Rev 2007;59:251–287. [PubMed: 17878513]
12. Dzau VJ, Re R. Tissue angiotensin system in cardiovascular medicine. A paradigm shift? Circulation 1994;89:493–498. [PubMed: 8281685]
13. Baltatu O, Silva JA Jr, Ganten D, Bader M. The brain renin–angiotensin system modulates angiotensin II-induced hypertension and cardiac hypertrophy. Hypertension 2000;35:409–412. [PubMed: 10642333]
14. Dell'Italia LJ, Meng QC, Balcells E, Wei CC, Palmer R, Hageman GR, et al. Compartmentalization of angiotensin II generation in the dog heart evidence for independent mechanisms in intravascular and interstitial spaces. J Clin Invest 1997;100:253–258. [PubMed: 9218500]
15. Mazzocchi G, Malendowicz LK, Markowska A, Albertin G, Nussdorfer GG. Role of adrenal renin-angiotensin system in the control of aldosterone secretion in sodium-restricted rats. Am J Physiol Endocrinol Metab 2000;278:E1027–E1030. [PubMed: 10827004]
16. Danser AH, Admiraal PJ, Derkx FH, Schalekamp MA. Angiotensin I-to-II conversion in the human renal vascular bed. J Hypertens 1998;16:2051–2056. [PubMed: 9886896]
17. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994;74:1141–1148. [PubMed: 8187280]
18. Ingelfinger JR, Pratt RE, Ellison K, Dzau VJ. Sodium regulation of angiotensinogen mRNA expression in rat kidney cortex and medulla. J Clin Invest 1986;78:1311–1315. [PubMed: 3533999]
19. Moe OW, Ujiie K, Star RA, Miller RT, Widell J, Alpern RJ, Henrich WL. Renin expression in renal proximal tubule. J Clin Invest 1993;91:774–779. [PubMed: 7680667]
20. Casarini DE, Boim MA, Stella RC, Krieger-Azzolini MH, Krieger JE, Schor N. Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. Am J Physiol 1997;272:F405–F409. [PubMed: 9087685]
21. Komlosi P, Fuson AL, Fintha A, Peti-Peterdi J, Rosivall L, Warnock DG, Bell PD. Angiotensin I conversion to angiotensin II stimulates cortical collecting duct sodium transport. Hypertension 2003;42:195–199. [PubMed: 12835330]
22. Gould AB, Green D. Kinetics of the human renin and human substrate reaction. Cardiovasc Res 1971;5:86–89. [PubMed: 4322712]
23. Brasier AR, Li J. Mechanisms for inducible control of angiotensinogen gene transcription. Hypertension 1996;27:465–475. [PubMed: 8613188]
24. Ding Y, Davisson RL, Hardy DO, Zhu LJ, Merrill DC, Catterall JF, Sigmund CD. The kidney androgen-regulated protein promoter confers renal proximal tubule cell-specific and highly androgen-responsive expression on the human angiotensinogen gene in transgenic mice. J Biol Chem 1997;272:28142–28148. [PubMed: 9346970]

25. Kimura S, Mullins JJ, Bunnemann B, Metzger R, Hilgenfeldt U, Zimmermann F, et al. High blood pressure in transgenic mice carrying the rat angiotensinogen gene. EMBO J 1992;11:821–827. [PubMed: 1547785]
26. Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, et al. Chimeric renin–angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. J Biol Chem 1993;268:11617–11621. [PubMed: 8505294]
27. Bohlender J, Menard J, Ganten D, Luft FC. Angiotensinogen concentrations and renin clearance: implications for blood pressure regulation. Hypertension 2000;35:780–786. [PubMed: 10720595]
28. Smithies O. Theodore cooper memorial lecture. A mouse view of hypertension. Hypertension 1997;30:1318–1324. [PubMed: 9403547]
29. Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE, Sigmund CD. Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. J Clin Invest 1996;97:1047–1055. [PubMed: 8613528]
30. Kobori H, Ozawa Y, Satou R, Katsurada A, Miyata K, Ohashi N, et al. Kidney-specific enhancement of ANG II stimulates endogenous intrarenal angiotensinogen in gene-targeted mice. Am J Physiol Renal Physiol 2007;293:F938–F945. [PubMed: 17634399]
31. Sachetelli S, Liu Q, Zhang SL, Liu F, Hsieh TJ, Brezniceanu ML, et al. RAS blockade decreases blood pressure and proteinuria in transgenic mice overexpressing rat angiotensinogen gene in the kidney. Kidney Int 2006;69:1016–1023. [PubMed: 16528251]
32. Lavoie JL, Lake-Bruse KD, Sigmund CD. Increased blood pressure in transgenic mice expressing both human renin and angiotensinogen in the renal proximal tubule. Am J Physiol Renal Physiol 2004;286:F965–F971. [PubMed: 15075192]
33. Smithies O, Kim HS. Targeted gene duplication and disruption for analyzing quantitative genetic traits in mice. Proc Natl Acad Sci U S A 1994;91:3612–3615. [PubMed: 8170956]
34. Kim HS, Krege JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, et al. Genetic control of blood pressure and the angiotensinogen locus. Proc Natl Acad Sci USA 1995;92:2735–2739. [PubMed: 7708716]
35. Inoue I, Nakajima T, Williams CS, Quackenbush J, Puryear R, Powers M, et al. A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. J Clin Invest 1997;99:1786–1797. [PubMed: 9120024]
36. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al. Molecular basis of human hypertension: role of angiotensinogen. Cell 1992;71:169–180. [PubMed: 1394429]
37. Zhao YY, Zhou J, Narayanan CS, Cui Y, Kumar A. Role of C/A polymorphism at −20 on the expression of human angiotensinogen gene. Hypertension 1999;33:108–115. [PubMed: 9931090]
38. Ishigami T, Umemura S, Tamura K, Hibi K, Nyui N, Kihara M, et al. Essential hypertension and 5′ upstream core promoter region of human angiotensinogen gene. Hypertension 1997;30:1325–1330. [PubMed: 9403548]
39. Kobori H, Harrison-Bernard LM, Navar LG. Expression of angiotensinogen mRNA and protein in angiotensin II-dependent hypertension. J Am Soc Nephrol 2001;12:431–439. [PubMed: 11181790]
40. Kobori H, Harrison-Bernard LM, Navar LG. Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. Hypertension 2001;37:1329–1335. [PubMed: 11358949]
41. Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. Kidney Int 2002;61:579–585. [PubMed: 11849399]
42. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG. Urinary angiotensinogen as an indicator of intrarenal angiotensin status in hypertension. Hypertension 2003;41:42–49. [PubMed: 12511528]
43. Kobori H, Prieto-Carrasquero MC, Ozawa Y, Navar LG. AT1 receptor mediated augmentation of intrarenal angiotensinogen in angiotensin II-dependent hypertension. Hypertension 2004;43:1126–1132. [PubMed: 15037565]
44. Katsurada A, Hagiwara Y, Miyashita K, Satou R, Miyata K, Ohashi N, et al. Novel sandwich ELISA for human angiotensinogen. Am J Physiol Renal Physiol 2007;293:F956–F960. [PubMed: 17553939]
45. Yamamoto T, Nakagawa T, Suzuki H, Ohashi N, Fukasawa H, Fujigaki Y, et al. Urinary angiotensinogen as a marker of intrarenal angiotensin II activity associated with deterioration of renal

function in patients with chronic kidney disease. J Am Soc Nephrol 2007;18:1558–1565. [PubMed: 17409316]

46. Kobori H, Ohashi N, Katsurada A, Miyata K, Satou R, Saito T, Yamamoto T. Urinary angiotensinogen as a potential biomarker of severity of chronic kidney diseases. J Am Soc Hypertens 2008;2:349–354. [PubMed: 18958182]
47. Kobori H, Alper AB, Shenava R, Katsurada A, Saito T, Ohashi N, et al. Urinary angiotensinogen as a novel biomarker of the intrarenal renin–angiotensin system status in hypertensive patients. Hypertension 2009;53:344–350. [PubMed: 19075095]
48. Ogawa S, Kobori H, Ohashi N, Urushihara M, Nishiyama A, Mori T, et al. Angiotensin II type 1 receptor blockers reduce urinary angiotensinogen excretion and the levels of urinary markers of oxidative stress and inflammation in patients with type 2 diabetic nephropathy. Biomark Insights 2009;4:97–102. [PubMed: 19652765]
49. Saito T, Urushihara M, Kotani Y, Kagami S, Kobori H. Increased urinary angiotensinogen is precedent to increased urinary albumin in patients with type 1 diabetes. Am J Med Sci 2009;338:478–480. [PubMed: 19884815]
50. Kobori H, Katsurada A, Ozawa Y, Satou R, Miyata K, Hase N, et al. Enhanced intrarenal oxidative stress and angiotensinogen in IgA nephropathy patients. Biochem Biophys Res Commun 2007;358:156–163. [PubMed: 17482564]
51. Ingelfinger JR, Zuo WM, Fon EA, Ellison KE, Dzau VJ. In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. An hypothesis for the intrarenal renin angiotensin system. J Clin Invest 1990;85:417–423. [PubMed: 2298917]
52. Darby IA, Sernia C. In situ hybridization and immunohistochemistry of renal angiotensinogen in neonatal and adult rat kidneys. Cell Tissue Res 1995;281:197–206. [PubMed: 7648615]
53. Lantelme P, Rohrwasser A, Gociman B, Hillas E, Cheng T, Petty G, et al. Effects of dietary sodium and genetic background on angiotensinogen and renin in mouse. Hypertension 2002;39:1007–1014. [PubMed: 12019284]
54. Rohrwasser A, Morgan T, Dillon HF, Zhao L, Callaway CW, Hillas E, et al. Elements of a paracrine tubular renin-angiotensin system along the entire nephron. Hypertension 1999;34:1265–1274. [PubMed: 10601129]
55. Ogawa S, Mori T, Nako K, Kato T, Takeuchi K, Ito S. Angiotensin II type 1 receptor blockers reduce urinary oxidative stress markers in hypertensive diabetic nephropathy. Hypertension 2006;47:699–705. [PubMed: 16505207]
56. Mezzano S, Droguett A, Burgos ME, Ardiles LG, Flores CA, Aros CA, et al. Renin–angiotensin system activation and interstitial inflammation in human diabetic nephropathy. Kidney Int 2003;64 (Suppl 86):S64–S70.
57. Mezzano SA, Aros CA, Droguett A, Burgos ME, Ardiles LG, Flores CA, et al. Renal angiotensin II up-regulation and myofibroblast activation in human membranous nephropathy. Kidney Int 2003;64 (Suppl 86):S39–S45.
58. Cowley AWJ. Long-term control of arterial blood pressure. Physiol Rev 1992;72:231–300. [PubMed: 1731371]
59. Watkins WS, Hunt SC, Williams GH, Tolpinrud W, Jeunemaitre X, Lalouel JM, Jorde LB. Genotype-phenotype analysis of angiotensinogen polymorphisms and essential hypertension: the importance of haplotypes. J Hypertens 2010;28:65–75. [PubMed: 19770777]
60. Kobori H, Nishiyama A, Abe Y, Navar LG. Enhancement of intrarenal angiotensinogen in Dahl salt-sensitive rats on high salt diet. Hypertension 2003;41:592–597. [PubMed: 12623964]
61. Kobori H, Nishiyama A. Effects of tempol on renal angiotensinogen production in Dahl salt-sensitive rats. Biochem Biophys Res Commun 2004;315:746–750. [PubMed: 14975764]
62. Yanes LL, Sartori-Valinotti JC, Iliescu R, Romero DG, Racusen LC, Zhang H, Reckelhoff JF. Testosterone-dependent hypertension and upregulation of intrarenal angiotensinogen in Dahl salt-sensitive rats. Am J Physiol Renal Physiol 2009;296:F771–F779. [PubMed: 19211690]
63. Leehey DJ, Singh AK, Alavi N, Singh R. Role of angiotensin II in diabetic nephropathy. Kidney Int 2000;58:S93–F98.
64. Strazzullo P, Galletti F. Impact of the renin–angiotensin system on lipid and carbohydrate metabolism. Curr Opin Nephrol Hypertens 2004;13:325–332. [PubMed: 15073492]

65. Yasue S, Masuzaki H, Okada S, Ishii T, Kozuka C, Tanaka T, et al. Adipose tissue-specific regulation of angiotensinogen in obese humans and mice: Impact of nutritional status and adipocyte hypertrophy. Am J Hypertens. 2010 [Epub ahead of print]. 10.1038/ajh.2009.263

66. Okada S, Kozukaa C, Masuzaki H, Yasue S, Ishii-Yonemoto T, Tanaka T, et al. Adipose tissue-specific dysregulation of angiotensinogen by oxidative stress in obesity. Metabolism. 2009 [Epub ahead of print]. 10.1016/j.metabol.2009.11.016

67. Davis JO, Freeman RH. Mechanisms regulating renin release. Physiol Rev 1976;56:1–56. [PubMed: 1108062]

68. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology, and molecular biology of renin secretion. Physiol Rev 1990;70:1067–1116. [PubMed: 2217555]

69. Rohrwasser A, Ishigami T, Gociman B, Lantelme P, Morgan T, Cheng T, et al. Renin and kallikrein in connecting tubule of mouse. Kidney Int 2003;64:2155–2162. [PubMed: 14633138]

70. Prieto-Carrasquero MC, Harrison-Bernard LM, Kobori H, Ozawa Y, Hering-Smith KS, Hamm LL, Navar LG. Enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. Hypertension 2004;44:223–229. [PubMed: 15226276]

71. Prieto-Carrasquero MC, Kobori H, Ozawa Y, Gutierrez A, Seth D, Navar LG. AT1 receptor-mediated enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. Am J Physiol Renal Physiol 2005;289:F632–F637. [PubMed: 15870381]

72. Prieto-Carrasquero MC, Botros FT, Pagan J, Kobori H, Seth DM, Casarini DE, Navar LG. Collecting duct renin is upregulated in both kidneys of 2-kidney, 1-clip Goldblatt hypertensive rats. Hypertension 2008;51:1590–1596. [PubMed: 18426992]

73. Franco M, Tapia E, Santamaria J, Zafra I, Garcia-Torres R, Gordon KL, et al. Renal cortical vasoconstriction contributes to development of salt-sensitive hypertension after angiotensin II exposure. J Am Soc Nephrol 2001;12:2263–2271. [PubMed: 11675402]

74. Rodriguez-Iturbe B, Pons H, Quiroz Y, Gordon K, Rincon J, Chavez M, et al. Mycophenolate mofetil prevents salt-sensitive hypertension resulting from angiotensin II exposure. Kidney Int 2001;59:2222–2232. [PubMed: 11380825]

75. Rodriguez-Iturbe B, Vaziri ND, Herrera-Acosta J, Johnson RJ. Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: all for one and one for all. Am J Physiol Renal Physiol 2004;286:F606–F616. [PubMed: 15001451]

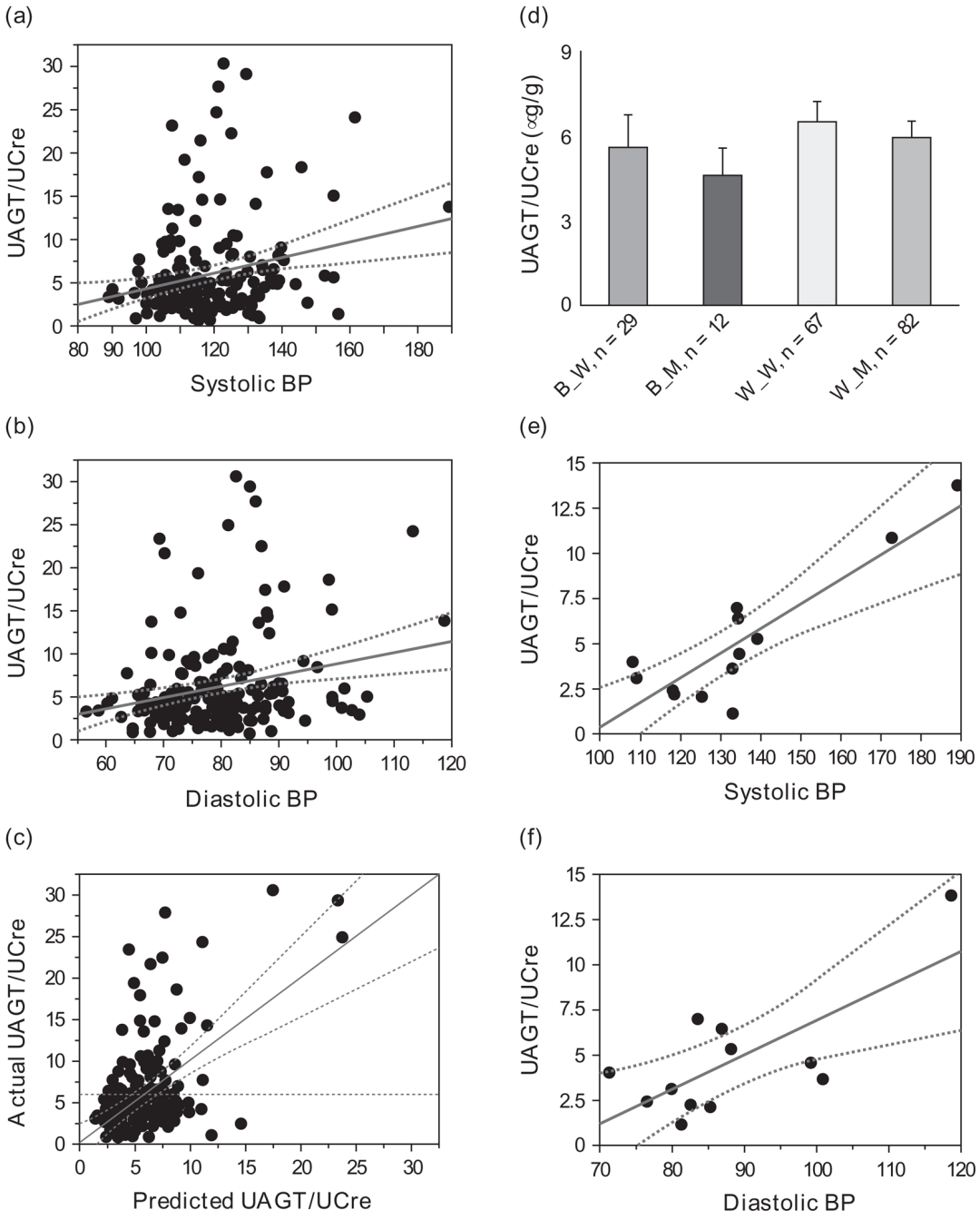

Fig. 1.

(a and b) Single regression analyses for urinary angiotensinogen–creatinine ratio levels. UAGT/UCre levels are not correlated with age, height ($r = -0.03$, $P = 0.6481$), body weight ($r = +0.00$, $P = 0.9745$), BMI, serum sodium levels, serum creatinine levels ($r = -0.11$, $P = 0.131$), serum cystatin C levels, or fractional excretion of sodium. However, UAGT/UCre levels are significantly and positively correlated with SBP (a; $r = +0.23$, $P = 0.0015$), DBP (b; $r = +0.24$, $P = 0.0012$), urinary sodium–creatinine ratio ($r = +0.16$, $P = 0.0342$), urinary albumin–creatinine ratio ($r = +0.39$, $P < 0.0001$), and urinary protein–creatinine ratio ($r = +0.47$, $P < 0.0001$). (c) Multiple regression analyses for UAGT/UCre levels. Only three parameters (DBP, urinary sodium–creatinine ratio, and urinary protein–creatinine ratio) can account for

almost 30% of variation of UAGT/UCre levels ($r = 0.54$, $R^2 = 0.29$, $P < 0.0001$). (d–f) Relationship of UAGT/UCre levels with blood pressure in populations. UAGT/UCre levels were not different among race or sex (d). However, as described in this figure, UAGT/UCre levels were significantly correlated with SBP and DBP. Moreover, high correlations were shown in men, especially in black men in SBP (e; $r = +0.85$, $P = 0.0005$) and DBP (f; $r = +0.72$, $P = 0.0079$). UAGT/UCre; urinary angiotensinogen–creatinine ratio (in μg/g).

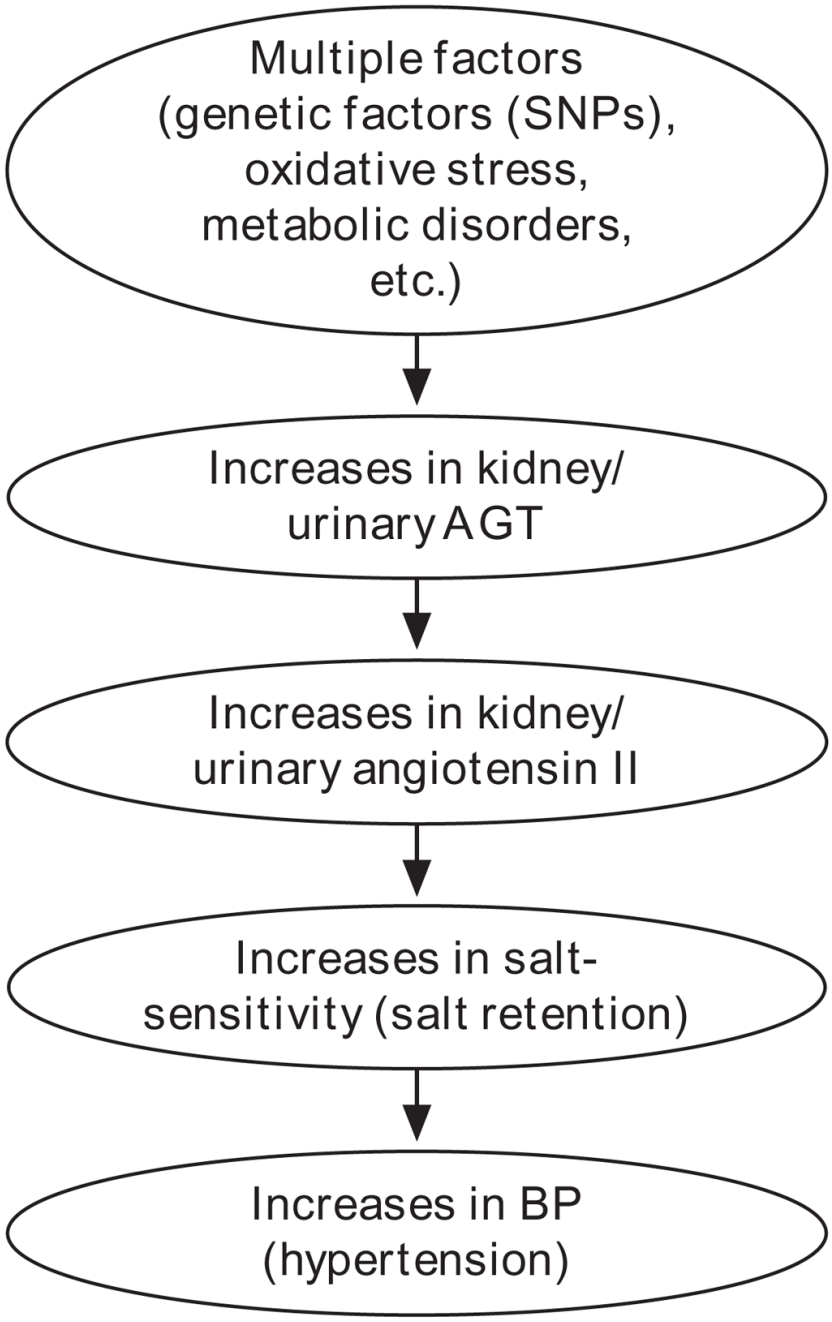

Fig. 2.
Perspectives based on the findings in this observational study. SNP, single-nucleotide polymorphisms

Table 1

Participants' profiles

Parameters	Black women *N* = 29	Black men *N* = 12	White women *N* = 67	White men *N* = 82	*P*
Age (year)	38.62 ± 1.06	40.25 ± 1.37	40.84 ± 0.57	40.52 ± 0.44	0.1769
Height (cm)	164.21 ± 1.31	178.33 ± 2.60	163.89 ± 0.76	178.13 ± 0.67	<0.0001*
Body weight (kg)	83.16 ± 4.08	100.13 ± 8.59	82.11 ± 2.61	93.80 ± 1.81	0.0005*
BMI	30.56 ± 1.24	31.17 ± 2.17	30.52 ± 0.93	29.51 ± 0.52	0.6864
SBP (mmHg)	113.91 ± 1.73	131.64 ± 6.06	115.38 ± 1.63	118.84 ± 1.55	0.0009*
DBP (mmHg)	75.21 ± 1.48	87.94 ± 3.70	77.36 ± 1.00	81.05 ± 1.16	0.0002*

*P <0.05.

Table 2

Laboratory data

Parameters	Black women *N* = 29	Black men *N* = 12	White women *N* = 67	White men *N* = 82	*P*
Serum Na (mEq/l)	138.59 ± 0.35	139.08 ± 0.56	138.56 ± 0.23	138.59 ± 0.26	0.8835
Serum Creatinine (mg/dl)	0.78 ± 0.03	1.09 ± 0.05	0.78 ± 0.01	1.02 ± 0.02	<0.0001*
UNa/UCre (mEq/g)	106.55 ± 14.24	69.91 ± 10.16	111.46 ± 7.08	93.47 ± 7.29	0.1134
UPro/UCre (g/g)	0.06 ± 0.01	0.07 ± 0.03	0.08 ± 0.01	0.06 ± 0.01	0.5314
FENa (%)	0.59 ± 0.07	0.54 ± 0.08	0.61 ± 0.04	0.67 ± 0.05	0.6314
Serum cystatin C (mg/l)	1.21 ± 0.09	1.27 ± 0.14	1.10 ± 0.05	1.27 ± 0.05	0.1617
UAlb/UCre (mg/g)	11.92 ± 4.08	7.62 ± 2.52	9.02 ± 1.24	8.30 ± 2.01	0.7519

FENa, fractional excretion of sodium; UAlb/UCre, urinary albumin–creatinine ratio; UNa/UCre, urinary sodium–creatinine ratio; UPro/UCre, urinary protein–creatinine ratio.

*P <0.05.

Table 3

Multiple regression analysis by stepwise method for urinary angiotensinogen–creatinine ratio

Parameters	Estimate	*F* ratio	*P*
Intercept	−6.41	0.00	1.0000
UPro/UCre (g/g)	29.41	51.66	0.0000*
DBP (mmHg)	0.11	10.92	0.0012*
UNa/UCre (mEq/g)	0.01	4.71	0.0314*
UAlb/UCre (mg/g)	0.00	2.41	0.1221
SBP (mmHg)	0.00	1.18	0.2798

UAlb/UCre, urinary albumin–creatinine ratio; UNa/UCre, urinary sodium–creatinine ratio; UPro/UCre, urinary protein–creatinine ratio.

*$P < 0.05$.