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# Circulating transforming growth factor- $\beta$ 1 levels and the risk for kidney disease in African Americans

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Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is known to induce progression of experimental renal disease. Here we determined whether there is an association between serum levels of TGF- $\beta$ 1 and the risk factors for progression of clinically relevant renal disorders in 186 black and 147 white adults, none of whom had kidney disease or diabetes. Serum TGF- $\beta$ 1 protein levels were positively and significantly associated with plasma renin activity along with the systolic and diastolic blood pressure in blacks but not whites after controlling for age, gender, and body mass index. These TGF- $\beta$ 1 protein levels were also significantly associated with body mass index and metabolic syndrome and more predictive of microalbuminuria in blacks than in whites. The differential association between TGF- $\beta$ 1 and renal disease risk factors in blacks and whites suggests an explanation for the excess burden of end-stage renal disease in the black population, but this requires validation in an independent cohort. Whether these findings show that it is the circulating levels of TGF- $\beta$ 1 that contribute to renal disease progression or the findings reflect other unmeasured factors, further longitudinal studies are needed.

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Transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), a prominent member of a superfamily of proteins, regulates cell growth and differentiation, inflammation and immunity.<sup>1</sup> TGF- $\beta$ 1 is a fibrogenic cytokine that plays a key role in the pathogenesis of renal disease, and TGF- $\beta$ 1 blockade may prevent renal disease progression.<sup>2,3</sup>

Blockade of the renin angiotensin system (RAS) is an important therapeutic strategy for prevention of the progression of renal disease,<sup>4–6</sup> and experimental as well as clinical studies have shown that the benefits of RAS blockade may be in part related to the strong bidirectional relationship between RAS and TGF- $\beta$ .<sup>7–12</sup> Antonipillai *et al.*<sup>8</sup> reported that TGF- $\beta$ 1, at picomolar concentrations, promotes the secretion of renin by rat juxtaglomerular cells. Brezniceanu *et al.*<sup>9</sup> showed that active human TGF- $\beta$ 1 induces angiotensinogen gene expression in murine proximal tubular epithelial cells. It has also been reported that both renin and angiotensin II stimulate TGF- $\beta$ 1 expression. Huang *et al.*<sup>10</sup> demonstrated that human or rat recombinant renin, at physiological concentrations, induces TGF- $\beta$ 1 and other members of the fibrotic cascade in human/rat mesangial cells through a renin receptor dependent and enzymatic and angiotensin II independent, mechanism. Wolf *et al.*<sup>11</sup> reported that addition of angiotensin II to murine proximal tubular (MCT) cells induced TGF- $\beta$ 1 mRNA expression and synthesis and release of active TGF- $\beta$ 1 protein and that the growth promoting effects of angiotensin II on the cultured murine proximal tubular cells can be accomplished with the addition of TGF- $\beta$ 1 to the murine proximal tubular cells. In the clinical setting, drugs which block the RAS and prevent renal disease progression reduce serum levels of TGF- $\beta$ 1 protein.<sup>12</sup>

Hypertension affects over 1 billion individuals worldwide and is a leading risk factor for end-stage kidney disease (ESRD).<sup>13,14</sup> Hypertension is more prevalent in blacks, and renal disease progression and ESRD are more frequent in blacks compared to other ethnic/racial groups.<sup>15–17</sup>

We have reported that blacks with hypertension and ESRD have higher circulating levels of TGF- $\beta$ 1 protein compared to whites.<sup>18,19</sup> One potential explanation for the excess burden

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of ESRD in blacks is an increased level and/or biologic activity of TGF-β1 in blacks compared to whites.

In this study, we investigated the association between TGF-β1 protein levels and risk factors for renal disease progression in adult black or white subjects and report that peripheral blood TGF-β1 protein levels are positively associated with plasma renin activity (PRA), systolic blood pressure, diastolic blood pressure, BMI and metabolic syndrome (MBS)<sup>20</sup> in blacks, but not in whites. We also report that TGF-β1 protein levels are more predictive of microalbuminuria in blacks than in whites.

## RESULTS

### Study participants

The demographic and clinical characteristics of study participants, stratified by race and hypertension status, are shown in Table 1. The mean ( $\pm$  s.d.) age of the 333 study subjects was  $45.2 \pm 10.6$  years, and there were 186 blacks and 147 whites; the black participants were 4.7 years younger ( $P=0.0001$ ) and more frequently female ( $P=0.005$ ). The mean ( $\pm$  s.d.) BMI was high in both groups ( $29.3 \pm 6.1$  kg/m<sup>2</sup> for the entire cohort), but higher in blacks than whites ( $30.0$  vs  $28.4$  kg/m<sup>2</sup>,  $P=0.01$ ) and higher in female than male participants ( $29.8$  vs  $28.5$  kg/m<sup>2</sup>,  $P=0.05$ ). The differences between black and white subsamples with respect to the percentages of individuals classified as overweight ( $25$ – $30$  kg/m<sup>2</sup>) or obese were not significant ( $\geq 30$  kg/m<sup>2</sup>) ( $P=0.14$ ). In this group 37.6% of the blacks and 46.3% of the whites were hypertensive as defined by average clinic BP  $\geq 140/90$  mm Hg or being on antihypertensive medication ( $P=0.11$ ); 74% of

the hypertensives had stage 1 hypertension ( $140$ – $159/90$ – $100$  mm Hg), and 54% of hypertensives were on treatment for hypertension when they enrolled in the study; the latter were withdrawn from all antihypertensive medications for a minimum of two weeks prior to evaluation and collection of biologic specimens for laboratory studies including the serum collection for measure TGF-β1 concentration. The black and white participants had similar 24-h urine sodium excretion, urinary albumin to creatinine excretion ratio, and prevalence of metabolic syndrome.

**TGF-β1, PRA, and sodium excretion.** Serum total (active plus latent) TGF-β1 protein levels were measured in 333 adults (186 blacks and 147 whites) without kidney disease or diabetes, and were higher in blacks compared to whites, higher in hypertensives than in normotensives, and highest in black hypertensives (Table 1,  $P=0.01$ ), confirming and extending our earlier findings from an independent study comprised of 61 hypertensives and 90 normal controls.<sup>19</sup> In this and all subsequent analyses, TGF-β1 protein levels were square-root transformed to reduce the substantial positive skew and make the distribution approximately normal. The 10th and 90th percentiles of TGF-β1 protein concentration in the full sample were 26.52 and 55.12 ng/ml, respectively.

We also measured active TGF-β1 protein concentration in sera from 147 of the 333 subjects assayed for total TGF-β1 protein. The 147 specimens were selected on the basis of their availability. We found that 93% of the clinical specimens did not have measurable levels of active TGF-β1 protein, and the mean ( $\pm$  s.e.) level was  $12.45 \pm 2.32$  ng/ml in the 11 (7%)

**Table 1 | Characteristics of subjects, stratified by race and hypertension status<sup>a</sup>**

Variable	Black subjects		White subjects		P-value <sup>b</sup>
	Normotensive (n=116)	Hypertensive (n=70)	Normotensive (n=79)	Hypertensive (n=68)	
Sex (% male)	37.1	21.4	40.5	52.9	0.002
Age (year)	$40.7 \pm 9.1$	$47.1 \pm 10.7$	$46.1 \pm 10.9$	$49.9 \pm 9.9$	0.0001
BMI (kg/m <sup>2</sup> ) <sup>c</sup>	$29.4 \pm 6.6$	$31.2 \pm 6.1$	$27.4 \pm 5.5$	$29.4 \pm 5.5$	0.004
Systolic BP (mm Hg) <sup>d</sup>	$117 \pm 12$	$148 \pm 15$	$114 \pm 12$	$141 \pm 16$	0.0001
Diastolic BP (mm Hg) <sup>d</sup>	$75 \pm 8$	$91 \pm 9$	$75 \pm 8$	$90 \pm 9$	0.0001
Plasma glucose (mg 100 ml)	$80.9 \pm 23.6$	$81.4 \pm 15.2$	$80.1 \pm 29.7$	$80.8 \pm 20.3$	0.99
Serum creatinine (mg 100 ml)	$0.92 \pm 0.22$	$0.88 \pm 0.18$	$0.86 \pm 0.18$	$0.87 \pm 0.20$	0.18
PRA (ng/ml per h)	$1.3 \pm 1.4$	$0.8 \pm 1.1$	$2.1 \pm 1.5$	$1.7 \pm 1.6$	0.0001
Median (25th, 75th percentiles)	1.0 (0.3, 1.7)	0.3 (0.2, 1.1)	1.8 (1.0, 2.7)	1.2 (0.7, 2.1)	
Urinary Na Excretion (mEq/24 h)	$144 \pm 73$	$128 \pm 58$	$150 \pm 74$	$140 \pm 57$	0.32
Urinary albumin/creatinine ratio (mg/g)	$9.1 \pm 11.4$	$16.9 \pm 35.7$	$8.6 \pm 8.9$	$20.3 \pm 61.6$	0.001
Median (25th, 75th percentiles)	5.0 (3.7, 8.3)	7.9 (5.5, 15.0)	6.2 (4.5, 8.8)	7.5 (4.8, 12.5)	
Microalbuminuria (%)	3.9	9.2	2.7	7.8	0.27
Metabolic syndrome (%) <sup>e</sup>	4.6	17.2	6.4	22.1	0.0008
Number of metabolic syndrome criteria	$1.1 \pm 1.1$	$2.0 \pm 0.9$	$1.0 \pm 1.0$	$1.9 \pm 1.0$	0.001
TGF-β1 protein (ng/ml)	$42 \pm 15$	$46 \pm 19$	$38 \pm 14$	$42 \pm 14$	0.01
Median (25th, 75th percentiles)	41 (33, 49)	42 (36, 53)	39 (30, 45)	40 (34, 46)	

<sup>a</sup>Plus-minus values are mean  $\pm$  s.d.

<sup>b</sup>P-value based on *F*-test for 1-way analysis of variance for continuous variables (after log-transforming PRA and urinary albumin/creatinine ratio and square-root-transforming TGF-β1 protein) or  $\chi^2$  test of independence for binary variables.

<sup>c</sup>Height and weight were measured two times. Body mass index (BMI) was calculated as average weight (kilograms) divided by the square of average height (meters).

<sup>d</sup>Blood pressure was measured with a mercury sphygmomanometer using standard American Heart Association Guidelines, with subjects in the seated position and arm at heart level. Three readings were obtained and their average was treated as blood pressure measure. Hypertension status was defined as an average BP  $\geq 140/90$  mm Hg or being on antihypertensive medication. Individuals taking medication were withdrawn successfully from medication for at least 2 weeks before the study. Those whose BP exceeded 160/105 mm Hg, before or after being withdrawn from medication, were not eligible.

<sup>e</sup>Metabolic syndrome was defined according to the current recommended criteria of the National Cholesterol Education Program (Adult Treatment Panel (ATP) III).<sup>16</sup>

specimens that were positive. Among the sera tested for active TGF-β1 protein, measurable levels of active TGF-β1 were observed in two of 35 black normotensives (5.7%,  $11.89 \pm 1.64$  ng/ml), 4 of 56 black hypertensives (7.1%,  $6.71 \pm 3.57$ ), 2 of 30 white normotensives (6.7%,  $13.59 \pm 1.44$  ng/ml), and 3 of 37 white hypertensives (8.1%,  $19.75 \pm 4.75$  ng/ml).

PRA was lower in blacks compared with whites (Table 1,  $P=0.0001$ ), an observation that has also been made by others.<sup>21</sup> Among the four groups shown in Table 1, PRA was lowest in black hypertensives compared to the three other groups, consistent with previous reports of a low-renin profile in black hypertensives.<sup>21</sup>

TGF-β1 protein concentration was positively associated with (log-transformed) PRA in the entire cohort ( $P=0.005$ , adjusted for race, sex, age, and BMI); however, addition of a race-by-TGF-β1 protein interaction term revealed that this association was only present in blacks, with those at the 90th percentile predicted to have 3.24 [ $=\exp(1.17)$ ,  $P=0.0001$ ] times the level of PRA as those at the 10th percentile of protein; the projected ratio of PRA between whites at the two extreme deciles was only 1.09 [ $=\exp(0.09)$ ,  $P=0.71$ ] (Table 2A). The difference between blacks and whites in the relationship of PRA to TGF-β1 protein concentration was significant (Table 2A,  $P=0.0007$  for the race-by-TGF-β1 protein interaction term).

The bivariate relationship between PRA and TGF-β1 protein concentration, without adjustment for sex, age, or BMI, is shown in Figure 1a (blacks) and Figure 1b (whites). The difference in PRA between blacks and whites is large at low values of TGF-β1, and negligible at high values of TGF-β1 protein concentration.

As noted above, 24-h urinary sodium excretion rate was not different between blacks and whites ( $P=0.32$ ). However, although TGF-β1 protein concentration exhibited a non-significant negative association with sodium excretion in blacks and a non-significant positive association in whites (Table 2A), the difference in the association between blacks and whites, controlling for sex, age, and BMI, was nearly significant ( $P=0.06$ ).

### TGF-β1 and blood pressure

TGF-β1 protein concentration, adjusted for race, sex, age, and BMI, was weakly associated with systolic blood pressure in the group as a whole (6.4 mm Hg inter-decile difference (IDD = predicted difference between those at the 90th and 10th percentiles of TGF-β1 protein concentration),  $P=0.02$ ), but addition of the race interaction term again revealed that this association was primarily evident in black and not white subjects (IDD = 9.5 mm Hg;  $P=0.009$  for blacks; IDD = 2.3 mm Hg;  $P=0.58$  for whites; Table 2A and Figure 2a and b). This race-dependent association was also observed for diastolic blood pressure (IDD = 4.7 mm Hg;  $P=0.03$  for blacks; IDD = 1.5 mm Hg;  $P=0.56$  for whites; Figure 2c and d). For both systolic and diastolic measures, the covariate adjusted relationship of clinic blood pressure

**Table 2 | (A) Predicted difference (SE) in outcomes between subjects at the 90th percentile and subjects at the 10th percentile of TGF-β1 protein distribution<sup>a</sup> and (B) Predicted Odds Ratio (95 % CI) for microalbuminuria or metabolic syndrome between subjects at the 90th percentile and subjects at the 10th percentile of TGF-β1 protein distribution<sup>a</sup>**

Outcome	Black subjects	White subjects	Race-by-TGF-β1 interaction P-value
<b>(A)</b>			
PRA (ng/ml per h) <sup>b</sup>	1.17 (0.21)	0.09 (0.24)	0.0007
P-value	0.0001	0.71	
Urine Na excretion (mEq/24 h) <sup>b</sup>	-18.8 (14.0)	20.0 (15.7)	0.06
P-value	0.18	0.20	
Systolic BP (mm Hg) <sup>b,c</sup>	9.5 (3.6)	2.3 (4.2)	0.20
P-value	0.009	0.58	
Diastolic BP (mm Hg) <sup>b,c</sup>	4.7 (2.2)	1.5 (2.5)	0.34
P-value	0.03	0.56	
Pulse pressure (mm Hg) <sup>b,c</sup>	4.8 (2.4)	0.8 (2.8)	0.29
P-value	0.05	0.77	
BMI (kg/m <sup>2</sup> ) <sup>b,d</sup>	2.5 (1.1)	1.2 (1.3)	0.47
P-value	0.03	0.36	
Number of metabolic syndrome criteria (0-5) <sup>e,f</sup>	0.82 (0.21)	0.42 (0.23)	0.20
P-value	0.0001	0.07	
<b>(B)</b>			
Urine albumin: creatinine ratio $\geq 30$ mg/gm <sup>g</sup>	6.6 (1.4, 31.1)	2.4 (0.3, 19.0)	0.45
P-value	0.02	0.39	
Metabolic syndrome <sup>e,g</sup>	4.5 (1.0, 19.9)	3.0 (0.8, 11.6)	0.69
P-value	0.04	0.11	

<sup>a</sup>Each outcome was analyzed in a regression analysis performed on the full sample that included race, log-transformed TGF-β1 protein, and a race-by-TGF-β1 interaction term while controlling for sex and age; analyses, except those predicting BMI and metabolic syndrome, also controlled for BMI.

<sup>b</sup>Ordinary linear regression analysis.

<sup>c</sup>Blood pressure was measured with a mercury column using standard American Heart Association Guidelines, with subjects in the seated position and arm at heart level. Three readings were obtained and their average was treated as the blood pressure measure.

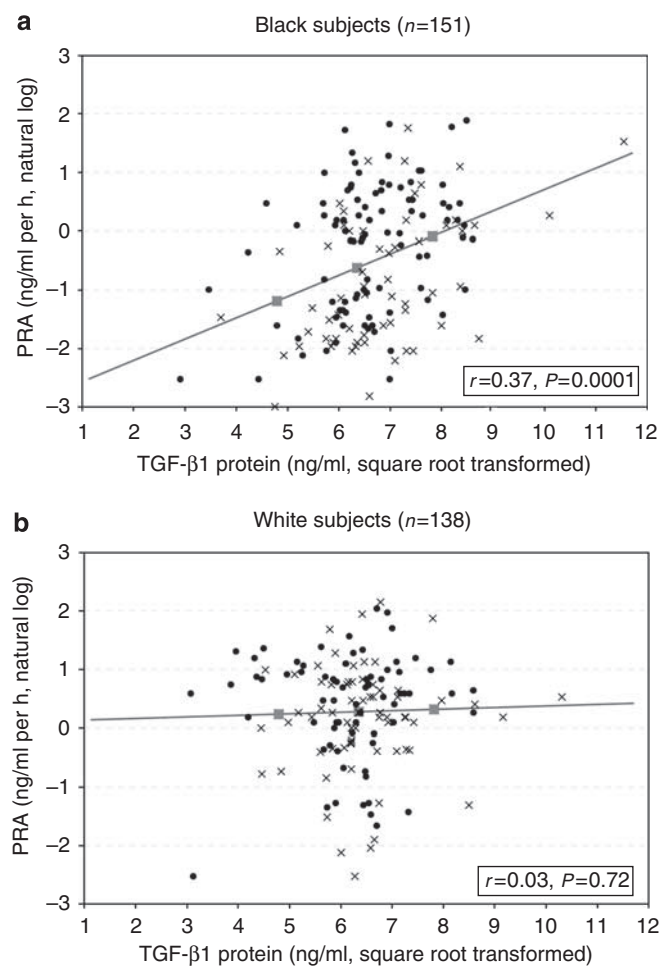
<sup>d</sup>Height and weight were measured two times. Body mass index (BMI) was calculated as average weight (kilograms) divided by the square of average height (meters).

<sup>e</sup>Metabolic syndrome was defined according to the current recommended criteria of the National Cholesterol Education Program (Adult Treatment Panel III).<sup>20</sup>

<sup>f</sup>Ordered probit regression analysis.

<sup>g</sup>Logistic regression analysis.

with TGF-β1 protein was more than three times greater in blacks compared to whites, although the difference between racial groups (interaction term) was not statistically significant (Table 2A). Mean arterial pressure exhibited the same pattern as systolic and diastolic blood pressure (not shown). Pulse pressure was also related to TGF-β1 protein in blacks ( $P=0.05$ ), but not in whites ( $P=0.77$ ).



**Figure 1 | Bivariate relationship of peripheral blood transforming growth factor  $\beta$ 1 protein concentration to plasma renin activity in black and white subjects.** Peripheral blood total transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1, ng/ml) level was measured after acid activation and neutralization of serum and using an isoform-specific TGF- $\beta$ 1 ELISA. Plasma renin activity (PRA, ng/ml per h) was measured using an enzyme-based method and by quantification of generated Ang I by radioimmunoassay.<sup>22</sup> Scatterplots show the bivariate relationship of TGF- $\beta$ 1 protein concentrations to PRA in black subjects (a) and white subjects (b). TGF- $\beta$ 1 protein measure was transformed using the square root transformation to reduce the positive skewness and make the resulting distribution approximately symmetric. PRA was transformed to natural logarithms (ln) to reduce skewness and heteroskedasticity. Pearson correlations ( $r$ ) and their associated  $P$ -values are presented. The three boxes on the regression line show the predicted value of the outcome variable for subjects at the 10th, 50th, and 90th percentiles of the overall distribution of TGF- $\beta$ 1 protein. Numbers in parenthesis are the number of subjects studied. The symbols 'o' and 'x' are used to identify normotensive and hypertensive subjects.

### TGF- $\beta$ 1 and microalbuminuria

Six percent of blacks and 5.1% of whites had microalbuminuria. As expected, the proportion with microalbuminuria was higher in hypertensives compared with normotensives (Table 1), although the majority of these mild hypertensives did not have microalbuminuria. Controlling for sex, race, age, and BMI in a logistic regression analysis, TGF- $\beta$ 1 was

significantly associated with microalbuminuria overall in blacks, but not in whites. The predicted odds ratio for the presence of microalbuminuria between a black subject at the 90th percentile compared to someone at the 10th percentile of TGF- $\beta$ 1 protein levels was 6.6 (95% CI: 1.4, 31.1;  $P=0.02$ ), whereas for whites it was 2.4 (95% CI: 0.3, 19.0;  $P=0.39$ ) (Table 2B). Despite the association being substantially stronger in blacks than in whites, the interaction was not statistically significant (Table 2B,  $P=0.45$ ).

### TGF- $\beta$ 1 and BMI

TGF- $\beta$ 1 was positively associated with BMI, controlling for race, sex, and age, overall ( $B=2.0$  kg/m<sup>2</sup> IDD,  $P=0.02$ ), and for blacks ( $B=2.5$  kg/m<sup>2</sup> IDD,  $P=0.03$ ) but not whites ( $B=1.2$  kg/m<sup>2</sup> IDD,  $P=0.36$ ) (Table 2A and Figure 3a and b). The relationship of TGF- $\beta$ 1 to BMI was two times as strong for blacks as for whites, but the difference was not statistically significant (Table 2A,  $P=0.47$  for the race interaction term).

### TGF- $\beta$ 1 and metabolic syndrome

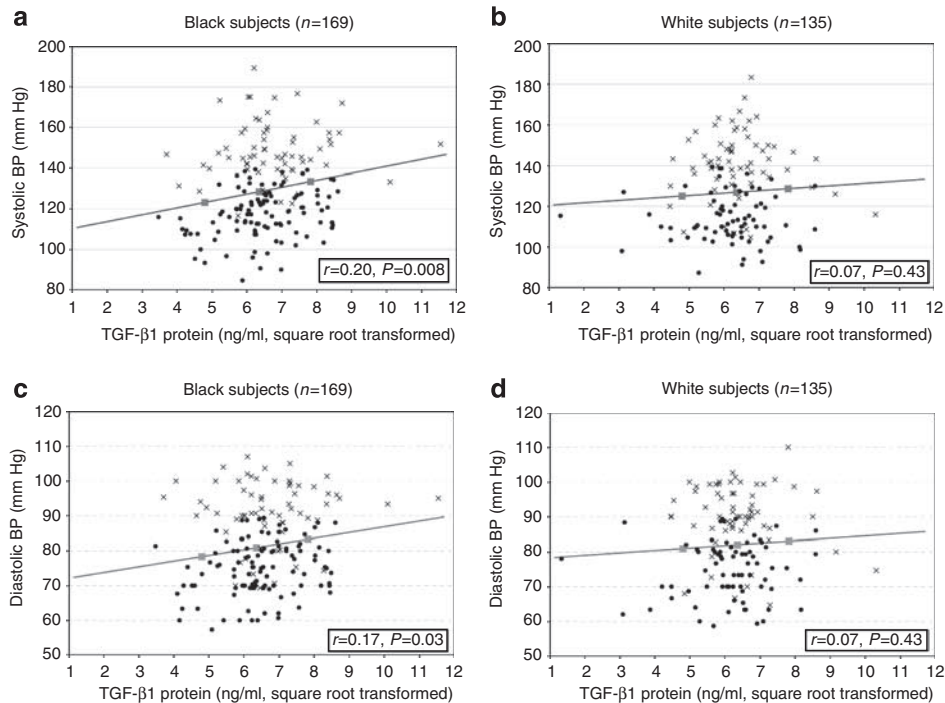
Subjects were characterized based on the number of features (0–5) of MBS (elevated BP, increased waist circumference, elevated triglycerides, elevated fasting glucose, and low HDL cholesterol) present, and also whether they had three or more criteria for MBS. There was little difference between the blacks and whites in either the number of features of MBS or the proportion of participants with three or more criteria for MBS. TGF- $\beta$ 1 protein levels were significantly associated with number of features of MBS (in an ordered probit analysis) as well as the presence of MBS (logistic analysis), controlling for sex, age, and race. Inclusion of the race interaction term again revealed that the association with number of MBS features was strong in blacks ( $P=0.0001$ ), but only marginal in whites ( $P=0.07$ , Table 2A). Similarly, the predicted odds of meeting criteria for MBS (having three or more criteria) was 4.5 (95% CI: 1.0, 19.9;  $P=0.04$ ; Table 2B) for blacks at the 90th percentile of TGF- $\beta$ 1 concentration compared to those at the 10th percentile, and 3.0 (0.8, 11.6;  $P=0.11$ ) for whites. The associations in blacks were stronger, but not significantly stronger, than those in whites (Table 2A and B).

### DISCUSSION

Hypertension and ESRD are increasing in prevalence worldwide, and both conditions are more common in blacks compared to whites.<sup>24</sup> We report a positive association, primarily in blacks, between circulating TGF- $\beta$ 1 protein concentration and plasma renin activity, blood pressure, microalbuminuria, BMI, and MBS. Our findings suggest that TGF- $\beta$ 1, a known mediator of renal disease, may modulate multiple renal risk factors, and may be biologically more active in blacks compared to whites.

An earlier study has shown that TGF- $\beta$ 1, at picomolar concentrations, induces the secretion of renin by rat renal juxtaglomerular cells.<sup>8</sup> Our study showing that TGF- $\beta$ 1 levels are positively associated with PRA, to the best of our





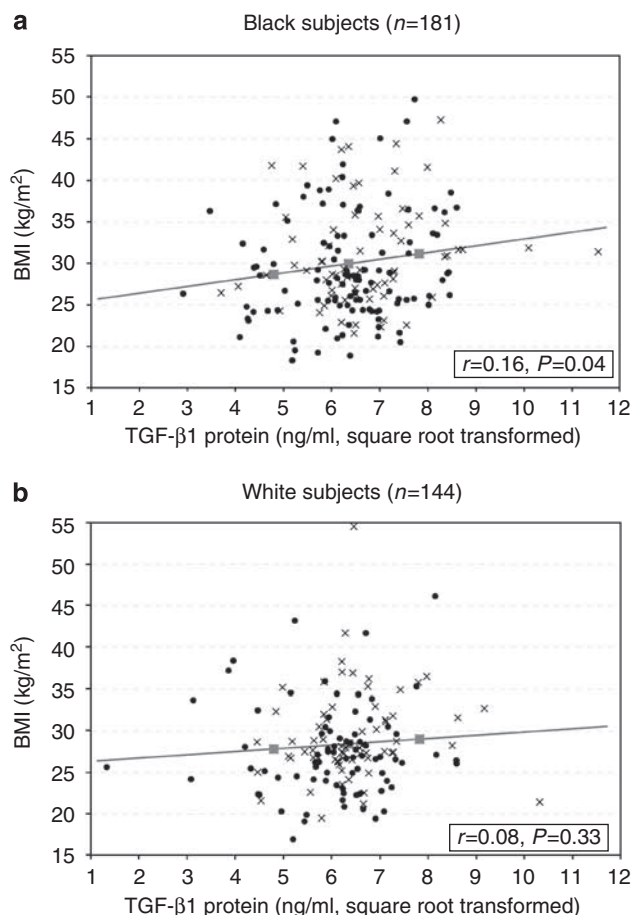
**Figure 2 | Bivariate relationship of clinic systolic and diastolic blood pressure to peripheral blood TGF- $\beta$ 1 protein concentration in black and white subjects.** Peripheral blood total transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1, ng/ml) level was measured after acid activation and neutralization of serum and using an isoform-specific TGF- $\beta$ 1 ELISA, and transformed using the square root transformation to reduce the positive skewness and make the resulting distribution approximately symmetric. Blood pressure measurements were made with a mercury column according to American Heart Association Guidelines, with patients in the seated position and arm at heart level.<sup>23</sup> Three readings were obtained and their average was treated as the blood pressure measure. Scatterplots show the bivariate relationship of TGF- $\beta$ 1 protein concentrations to systolic (a and b) or diastolic (c and d) blood pressure. Pearson correlations ( $r$ ) and their associated  $P$ -values are presented. The three boxes on the regression line show the predicted value of the outcome variable for subjects at the 10th, 50th, and 90th percentiles of the overall distribution of TGF- $\beta$ 1 protein. Numbers in parenthesis are the number of subjects studied. The symbols 'o' and 'x' are used to identify normotensive and hypertensive subjects.

knowledge, is the first clinical study reporting a positive relationship between TGF- $\beta$ 1 and the RAS in humans. The association of TGF- $\beta$ 1 and renin may be mechanistically relevant for fibrosis, vascular pathology, and the progression of renal disease.

The positive association between TGF- $\beta$ 1 and PRA in blacks is intriguing, and may even appear paradoxical, as PRA levels are lower in blacks compared to whites, and lowest in the black hypertensives, and serum TGF- $\beta$ 1 levels are higher in blacks compared to whites and highest in the black hypertensives. A linear and close looped relationship between TGF- $\beta$ 1 and PRA in blacks would predict that blacks would have a higher level of PRA compared to whites. One potential explanation that can account for the lower PRA in blacks despite a higher TGF- $\beta$ 1 is that TGF- $\beta$ 1 not only stimulates renin secretion but also has an additional and stimulatory effect on sodium reabsorption by the kidney, and the increased sodium (and water) reabsorption results in renin suppression and low-renin hypertension (a well documented form of hypertension in the black population). Partial support for this concept is provided by our findings that 24 h urine sodium excretion is lowest in the black

hypertensives (Table 1), and the association between 24 h urine sodium excretion and TGF- $\beta$ 1 protein levels is negative in blacks and positive in whites (Table 2). Additional measurements of the response of blood pressure, PRA and TGF- $\beta$ 1 to salt loading should help clarify our observations. It is note worthy that TGF- $\beta$ 1 has been reported to increase the activity of serine/threonine kinase, SGK,<sup>25</sup> a kinase expressed in several tissues<sup>26</sup> including the sodium reabsorption sites in the human kidney.<sup>27</sup> Importantly, SGK from *Xenopus* as well as from humans has been shown to increase the activities of the two sodium channels located in the distal tubule and ascending thick limb of loop of Henle, the epithelial sodium channel activity and the sodium, potassium, and chloride co-transporter.<sup>28,29</sup>

The positive association between TGF- $\beta$ 1 and PRA in blacks may also result from renin or angiotensin II functioning as the stimulus for TGF- $\beta$ 1 synthesis and secretion. Huang *et al.*<sup>10</sup> observed that human or rat recombinant renin, at physiological concentrations, induces TGF- $\beta$ 1 through a renin receptor-dependent mechanism. Our study does not resolve whether the observed and positive association results from TGF- $\beta$ 1 stimulating renin release,



**Figure 3 | Bivariate relationship of body mass index to peripheral blood TGF-β1 protein concentration in black and white subjects.** Peripheral blood total transforming growth factor β1 (TGF-β1, ng/ml) level was measured after acid activation and neutralization of serum and using an isoform-specific TGF-β1 ELISA, and transformed using the square root transformation to reduce the positive skewness and make the resulting distribution approximately symmetric. Height and weight were measured two times. Body mass index (BMI) was calculated as average weight (kilograms) divided by the square of average height (meters). Scatterplots show the bivariate relationship of TGF-β1 protein concentrations to BMI in black subjects (a) and white subjects (b). Pearson correlations ( $r$ ) and their associated  $P$ -values are presented. The three boxes on the regression line show the predicted BMI for subjects at the 10th, 50th, and 90th percentiles of the overall distribution of TGF-β1 protein. Numbers in parenthesis are the number of subjects studied. The symbols 'o' and 'x' are used to identify normotensive and hypertensive subjects.

renin or Ang II inducing TGF-β1 secretion, or due to mutual stimulation as documented in pre-clinical models.<sup>7–11</sup> Irrespective of the directionality, it is worth emphasizing that the positive association between TGF-β1 and renin was observed only in blacks and not in whites.

Experimental studies suggest a causative role for TGF-β in vascular remodeling, blood pressure regulation, and renal disease progression.<sup>30</sup> Anti-TGF-β antibodies lower blood pressure, reduce proteinuria and prevent renal function decline in rats.<sup>31</sup> Deletion of the gene for Emilin-1, a negative

regulator of TGF-β1, results in greater availability of TGF-β1, increased peripheral vascular resistance, and increased blood pressure in mice.<sup>32</sup> Moreover, inactivation of a single TGF-β1 allele normalizes blood pressure in the Emilin-deficient mice.<sup>32</sup> Our new observations, in addition to confirming and extending a quantitative difference in TGF-β1 protein levels between normotensives and hypertensives, advances the hypothesis that a qualitative difference may exist between blacks and whites in the association between TGF-β1 levels and blood pressure.

Body mass and blood pressure are positively associated in both blacks and whites; however, the age-adjusted prevalence of hypertension is greater in blacks compared to whites for BMI ranging from 18.5 to greater than 31.<sup>33</sup> The association of TGF-β1 with both blood pressure and BMI in blacks may partially explain the increased risk of hypertension for a given level of BMI in blacks, as well as previously reported associations between BMI and ESRD.<sup>34,35</sup>

MBS, a proinflammatory and prothrombotic disorder, increases the risk for cardiovascular disease as well as kidney failure.<sup>23</sup> Neither the average number of criteria for MBS nor the prevalence of metabolic syndrome differed between blacks and whites in our study, but the positive association of TGF-β1 concentration with both prevalence of MBS and the number of features of MBS was observed only in blacks. TGF-β1 increases plasminogen activator inhibitor (PAI)1 production,<sup>22</sup> a causal factor in the development of insulin resistance, type II diabetes, and associated cardiovascular complications. On the other hand, adipose tissue is a source of increased TGF-β1 production<sup>36</sup> and TGF-β1 concentration is greater in obese compared with non-obese hypertensives.<sup>37,38</sup> Regardless of the direction of the relationship between TGF-β1 and MBS, TGF-β1 may be a potentially modifiable target, as well as a novel candidate genetic locus and biomarker for these important conditions, and may contribute to increased complications associated with diabetes in blacks compared to other groups.

Microalbuminuria may reflect generalized endothelial cell dysfunction as well as early renal injury. TGF-β1 concentration was positively associated with microalbuminuria only in blacks and not in whites. Our finding suggests a mechanism for the greater prevalence of hypertension associated renal damage in blacks compared to whites as well as an additional pharmacologic target for reducing albuminuria. Previous studies have indeed suggested that beneficial effects of RAS inhibition on preventing renal disease progression may be mechanistically linked both to lowering TGF-β1 levels and reducing proteinuria.<sup>12,39</sup>

Our study has a number of limitations. Both the utility of characterizing populations based on racial/ethnic categories as well as the validity of self-identified race/ethnicity for stratifying populations in clinical studies have been challenged. In this regard, we note that racial and ethnic differences exist in the development of ESRD in the USA.<sup>15,16</sup> With respect to the validity of self-identified race/ethnicity, Tang *et al.*<sup>40</sup> have recently analyzed genetic data for

326 microsatellite markers that were typed uniformly in a large multiethnic population ( $n = 3636$ ) and found a near-perfect correspondence of genetic cluster analysis of microsatellite markers with the four self-reported race/ethnicity categories, white, African American, East Asian, and Hispanic.

Our investigation can also be criticized for not ascertaining TGF- $\beta$ 1 availability at the tissue/cellular level in view of the substantial post-translational regulation of TGF- $\beta$ 1 bioactivity,<sup>41</sup> and differential tissue availability remains a plausible mechanism for our observations. In this regard, Huang *et al.*<sup>42,43</sup> have reported renal protection by latent TGF- $\beta$  in experimental mouse models of ureteral obstruction and crescentic glomerulonephritis. The intriguing hypothesis that the active version is pathogenetic whereas an excess of latent form is protective merits investigation in the clinic. We measured active as well as latent TGF- $\beta$ 1 in sera from a subset of subjects assayed for total TGF- $\beta$ 1 (active plus latent). Our observations that the active version is found in only 7% of peripheral blood specimens, and with similar frequencies in normotensives and hypertensives suggest that the presence or absence of the latent form is unlikely to explain the associations observed in our study.

In summary, we find positive associations of TGF- $\beta$ 1 with PRA, blood pressure, BMI, metabolic syndrome, and microalbuminuria, primarily in black subjects with either normal blood pressure or mild hypertension, and not in white subjects with similar clinical characteristics. Our findings suggest that the biologic activity of TGF- $\beta$ 1 is higher in blacks compared to whites. Should the significant association between TGF- $\beta$ 1 and mediators of renal disease in blacks be validated in an independent cohort, a new and targetable mechanism for the rapid progression and the excess burden of ESRD in the black population would emerge.

## METHODS

### Study participants

We studied 333 black and white subjects with or without mild hypertension, and without overt kidney disease or cardiovascular disease. The study participants are from a cross-sectional study of race/ethnicity and blood pressure, The Neighborhood Study of Blood Pressure and Sleep<sup>44</sup> was conducted from 1999 through 2003 at four New York City institutions – Weill Cornell Medical College, Mount Sinai School of Medicine, Harlem Hospital, and the North General Hospital – using a common protocol. The study protocol and consent form were approved by the institutional review committees for human subject research at each institution.

Participants were 18–65 years of age, had no known cardiovascular disease or diabetes, and had no medical problems other than hypertension. Participants were asked to self-report their ethnic group/race. The mutually exclusive categories were ‘Black’, ‘White’, ‘Asian’ or ‘Other ... please specify’. The participants were also asked whether they considered themselves to be Spanish/Hispanic/Latino. Those who identified themselves as primarily ‘White’ or ‘Black’ were eligible to participate. The study participants included 186 blacks, three of whom considered themselves to also be Hispanics, and the

147 whites including 8 who considered themselves to also be Hispanic.

Subjects could be either normotensive or mildly hypertensive. Hypertension status was defined as an average clinic BP  $\geq 140/90$  mm Hg at recruitment or being on antihypertensive medication. Individuals taking medication were withdrawn successfully from medication for at least 2 weeks prior to the study. Those whose BP exceeded 160/105 mm Hg, before or after being withdrawn from medication, were not eligible.

### Blood pressure measurements

Blood pressure measurements were made by trained research personnel using a mercury sphygmomanometer according to the American Heart Association Guidelines, with patients seated and arm at heart level.<sup>45</sup> Three readings were obtained and their average was recorded as the clinic blood pressure measure.

### Laboratory tests

All subjects were on *ad libitum* diet. All laboratory tests were performed with the biologic specimens collected at the same time, and at the time clinical blood pressure measurements were made. The specimens were collected by a minimum of two-weeks following the withdrawal from antihypertensive medications. Prior to withdrawal, 10 of the 70 black hypertensives and 11 of the 68 white hypertensives were on either angiotensin-converting inhibitor or angiotensin II receptor blocker.

PRA was measured using an enzyme-based method and by quantification of generated Ang I by radioimmunoassay.<sup>46</sup> Twenty-four-hour urine collections were analyzed by the Weill Cornell Medical College General Clinical Research Center for sodium, creatinine, and albumin. Urinary albumin and creatinine concentrations were calculated using Bayer’s DCA 2000 micro-albumin/creatinine low and High Control Kit with the company’s DCA 2000 + Analyzer. If the albumin result was  $< 5$  mg/l, the lowest limit of detection, the concentration calculated from Diagnostic Product Corporation’s radioimmunoassay kit was used. Microalbuminuria was defined as an albumin/creatinine ratio  $> 30$  mg/gm.

### TGF- $\beta$ 1 protein measurement

Peripheral venous blood was obtained from the study participants and serum was isolated and stored at  $-70^{\circ}\text{C}$  until assayed for TGF- $\beta$ 1 protein. Serum TGF- $\beta$ 1 protein concentration was measured using an isoform-specific TGF- $\beta$ 1 ELISA according to the manufacturer’s protocol (Quantikine Human TGF- $\beta$ 1 Immunoassay, R&D Systems Inc. Minneapolis, MN, USA). Total TGF- $\beta$ 1 levels were measured after acid activation (acetic acid/urea) and neutralization (NaOH/HEPES) of serum from all 333 subjects. Active TGF- $\beta$ 1 (serum assayed without the activation step) was measured in all 147 available sera from the 333 subjects. A TGF- $\beta$ 1 standard curve was constructed by using 2000, 1000, 500, 250, 125, 62.50, 31.25 pg/ml recombinant TGF- $\beta$ 1 protein, and a curve-fitting software program was used to quantify TGF- $\beta$ 1 protein concentration in the sera. The minimum detectable level is 7.0 pg/ml and the intra-assay and inter-assay median coefficients of variation are reported to be 5.3 and 10.9%, respectively.

### Definition of metabolic syndrome

Metabolic syndrome was defined according to the current recommended criteria of the National Cholesterol Education Program (Adult Treatment Panel (ATP) III).<sup>20</sup>

### Statistical analysis

Descriptive statistics are reported as percentages for binary/categorical measures or means and s.d. for continuous measures; the median and quartiles are also reported for severely skewed measures. Regression analyses were used to estimate and test the relationship of serum TGF-β1 protein concentrations to PRA, blood pressure, urinary sodium excretion, BMI, microalbuminuria, and MBS; linear regression for the continuous outcome measures, logistic regression for the binary measures, and ordered probit regression for the number of metabolic syndrome criteria measure (an ordinal categorical measure ranging from 0 to 5). PRA was transformed to natural logarithms to reduce skewness and heteroskedasticity. For all regression analyses, the TGF-β1 protein measure was transformed using the square root transformation to reduce the positive skewness and make the resulting distribution approximately symmetric and normal. Regression estimates are reported as the predicted difference in the outcome between someone at the 90th percentile and someone at the 10th percentile of the TGF-β1 distribution. All analyses were performed on the full sample, with and without the inclusion of a multiplicative race-by-TGF-β1 interaction term. The inclusion of this interaction term yielded separate estimates for blacks and whites of the effect of TGF-β1, and a test of the black-white difference. Sex and age were statistically controlled for in all analyses, and BMI was controlled when predicting all outcomes except BMI and metabolic syndrome.

For the continuous outcome measures, we also estimated LOESS models for blacks and whites, to detect any non-linear relationships. None were found and therefore the results of the LOESS models are not presented.

### DISCLOSURE

All the authors declared no competing interests.

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