12-1-2015

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Interaction of Alu Polymorphisms and Novel Measures of Discrimination in Association with Blood Pressure in African Americans Living in Tallahassee

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Key words: Alu polymorphisms, discrimination, blood pressure, racial health disparities, African Americans.

Short Title: Alu Polymorphisms, Discrimination, and Blood Pressure

Abstract African Americans are 40% more likely to be afflicted with hypertension in comparison to non-Hispanic, white Americans, resulting in a 30% higher instance of mortality due to cardiovascular disease. There is debate about the relative contributions of genetic and sociocultural risk factors to the racial disparity in hypertension.

We assayed three *Alu* insertion polymorphisms located in the angiotensin-1-converting enzyme (*ACE*)*, tissue plasminogen activator (PLAT), and* with no-lysine kinase 1 (*WNK1*) genes. We also estimated West African genetic ancestry and developed novel measures of perceived discrimination to create a biocultural model of blood pressure among African-American adults in Tallahassee, FL (n=158).

When tested separately, the *ACE Alu* non-insertion allele was significantly associated with higher systolic and diastolic blood pressure. In multiple regression analyses, West African genetic ancestry was not associated with blood pressure and reduced the strength of all blood pressure models tested. A gene x environment interaction was identified between the *ACE Alu* genotype and a new measure of unfair treatment that includes experiences by individuals close to the study participant. Inclusion of the *WNK1 Alu* genotype further improved this model of blood pressure variation.

Our results suggest an association of the *ACE* and *WNK1* genotypes with blood pressure that is consistent with their proposed gene functions. Perceived unfair treatment (to others) shows a threshold effect where an increase in blood pressure is demonstrated at higher values. The interaction between the *ACE* genotype and unfair treatment highlights the benefits of including both genetic and cultural data to investigate complex disease.

African Americans experience a disproportionate burden of the morbidity of hypertension in the United States and are 40% more likely than non-Hispanic, white Americans to be hypertensive (Office of Minority Health 2014). In order to improve our understanding of the etiology of racial disparities in hypertension, we present a biocultural approach, wherein both genetic and sociocultural risk factors are measured.

Alu insertion polymorphisms are transposable, repeated elements of ~300 nucleotides that often demonstrate linkage disequilibrium with causative alleles for common diseases and have the potential to causatively disrupt the function of genes through alternative splicing and modified expression and regulation (Deininger and Batzer 1999; Putku et al. 2011). *Alu* polymorphisms assayed in this study were chosen based on two criteria: 1) previous findings of association with hypertension in European populations and, where available, African American populations and 2) a viable physiological mechanism to explain an effect on blood pressure regulation.

The *ACE Alu* polymorphism is a 287 base pair insertion in intron 16 of the angiotensin-1-converting enzyme (*ACE*) gene (Asamoah et al. 1996). Inclusion of the *ACE Alu* accounts for 45-50% of the variation in *in vivo* levels of circulating angiotensin-I-converting enzyme (ACE) (Jern et al. 1999). ACE is the rate-limiting enzyme in the renin-angiotensin system (RAS), cleaving angiotensin I to angiotensin II (Ajala et al. 2012). Angiotensin II is the catalyzing agent of most of the action of the RAS, resulting in vasoconstriction via the inactivation of bradykinin. The *ACE Alu* non-insertion allele is generally associated with higher ACE plasma levels and, therefore, increased systolic and diastolic blood pressure (Ajala et al. 2012; Asamoah et al. 1996; Das et al. 2008; Hooper et al. 2002; Rotimi et al. 1996; Woo et al. 2012). However, a few studies have found the insertion allele to be associated with hypertension (Ismail et al. 2004; Panjaliya et al. 2013).

The *PLAT Alu* polymorphism is a 311 base pair insertion located on intron 8 of the *PLAT* gene (Tishkoff et al. 2000). The insertion allele is associated with increased tissue plasminogen activator (TPA) release *in vivo* (Jern et al. 1999). TPA is a serine protease that enzymatically cleaves plasminogen to activated plasmin (Jern et al. 1999), and plasmin is an enzyme that proteolyzes fibrin, the major structural component in blood clots (Ladenvall et al. 2003). Heightened plasma levels of TPA result in greater binding with plasminogen activator inhibitor-1 (PAI-1), which inhibits fibrinolysis (Valle-Garay et al. 2013). Therefore, the insertion allele has been associated with thrombosis, stroke, and adverse cardiovascular events (Valle-Garay et al. 2013).

The *AluYb8* insertion is a 300 base pair insertion within intron 10 of the with no-lysine kinase 1 (*WNK1*) gene. WNK1 is a serine/threonine kinase that regulates the co-transportation of sodium and potassium ions within the distal nephron convoluted tubule and collecting duct (Vidal-Petiot et al. 2013). *WNK1* has been identified as a locus of blood pressure regulation due to association with Familial hyperkalemic hypertension, or Gordon's Syndrome, an uncommon form of hypertension in humans. This condition is linked to up-regulation of *WNK1* gene activity, which increases ion activity and induces vasoconstriction (Vidal-Petiot et al. 2013). The insertion allele is associated with higher blood pressure and is found at significantly lower frequencies in sub-Saharan African populations than other ethnic groups (Putku et al. 2011).

Genetic ancestry is often included in a discussion of racial health disparities with an implicit assumption that genetic ancestry is associated in some manner with disease risk. Genetic ancestry is estimated using ancestry-informative markers (AIMs) that display large frequency differences between putative parental populations. Studies have found associations between West African genetic ancestry and disease phenotypes, including elevated serum creatinine (Peralta et al. 2010), increased risk of insulin resistance but not hypertension or body mass index (BMI) (Reiner et al. 2007), and BMI and higher bone mineral density (Fernández et al. 2003). However, these studies did not explain the

biological underpinning of such associations and did not include sociocultural variables that might be more relevant to an understanding of racial disparities in disease and related phenotypes.

Blood pressure has been associated with a range of sociocultural factors, including socioeconomic status (Cundiff et al. 2015) and psychosocial stressors (Dressler 1999). Some researchers have proposed that social stressors associated with systemic racism constitute unique stressors that contribute to excess hypertension among African Americans (Williams and Jackson 2005). However, most existing studies measure perceived discrimination only by reference to participants' personal exposure to unfair treatment. Dressler (1999) emphasizes that stress models should be informed by careful ethnographic work. In the first phase of our research, ethnographic interviews revealed that many study participants mentioned unfair treatment experienced by others – family, close friends, co-workers – when given open-ended questions querying their experiences with discrimination. Thus, in the second, survey phase of our research, we included standard measures of perceived discrimination, or unfair treatment (Williams and Jackson 2005; Williams et al. 1997), and developed novel measures to capture unfair treatment experienced by individuals close to the study participants.

Most research on the risk factors associated with high blood pressure seldom includes both genetic and sociocultural data, such that few studies are able to evaluate the relative importance of genetic versus sociocultural factors or to test for gene x environment interactions. In our study, three *Alu* polymorphisms were assayed as candidate genetic variants for hypertension in 158 individuals. Genetic ancestry was estimated from 1423 assayed AIMs. Sociocultural variables included novel measures of unfair treatment experienced by individuals close to the study participants, i.e. unfair treatment to others.

MATERIALS AND METHODS

Participant Selection. Data come from a multistage probability sample of African American adults in Tallahassee, FL (Table 1). We first stratified Census block groups using cluster analysis of neighborhood-level indicators of racial composition and material deprivation to maximize contrasts in relevant social stressors. Using data from the U.S. Census Bureau's American Community Survey, the cluster analysis was based on the following variables for Census Block Groups: percent of residents who identified as Black or African American, percent of residents with high school diploma or equivalent, percent of female-headed households, percent of vacant housing units, percent in poverty, median household income. Next, within each cluster, we randomly selected block groups and then residential addresses within sampled block groups. Last, we randomly selected one participant from among eligible adults (self-identified African American, age 25-65) in each household. The participant from each household was chosen beforehand so as not to bias the sample in favor of ambulatory individuals (those individuals more likely to come to the door). The final number of individuals with complete data for analyses reported here was 158. The research protocol was approved by the University of Florida IRB-01 (#364-2008) and IRB-02 (#2007-U-469). Informed consent was obtained from all participants prior to data collection.

Saliva Samples. Saliva samples were collected and stored using Oragene DNA Collection Kits (DNA Genotek, Ontario, Canada). DNA was extracted according to the manufacturer's protocol.

Genotyping the *Alu* Markers. Samples were individually genotyped for each of the three *Alu* polymorphisms: *ACE, PLAT, WNK1*. Primers for each *Alu* polymorphism insertion were designed based on previously published literature (Ajala et al. 2012; Hooper et al. 2002; Putku et al. 2011). PCR was performed with 2 µL DNA, 10 µL H_2O, 12.5 µL Apex™ RED Taq DNA Polymerase Master Mix 2.0X (Genesee, San Diego, CA), and 0.5 µL of the respective 10 µM primers/ reaction for 35 cycles: 94°C (5 min) and 94°C (30 sec), 58°C (30 sec), 72°C (30 sec). Amplicons were electrophoresed on 2% agarose gels. Insertion and non-insertion allele sizes were 490bps and 190bps, respectively for the *ACE Alu* insertion, 420bps/110bps for the *PLAT Alu* insertion, and 660bps/ 353bps for the *WNK1 Alu* insertion.

African Ancestry. Ancestry informative markers (AIMs) were identified from the Illumina African American Admixture panel (Illumina, San Diego, CA) and were genotyped using a custom Affymetrix Axiom Array that we designed (Affymetrix, Santa Clara, CA). Ultimately, 1423 AIMs had a genotyping success rate ≥ 95% and were used to estimate genetic ancestry. West African ancestry estimates for each of the African American study participants were generated using a Bayesian model with prior allele frequencies in the ADMIXMAP program (Hoggart et al. 2004). Prior allele frequencies were derived from the HapMap Yoruba (YRI) and CEPH European (CEU) populations in a two way model of admixture. YRI and CEU populations were selected to approximate the parental populations from which the admixture in our sample population was derived and while this selection will influence the relative proportion of ancestry estimated for admixed individuals, it is not expected to significantly influence the ancestry associations tested in our analyses.

We also used the AIMs to test if relatedness of the study participants influenced the results in our final model. To account for relatedness in our samples, we generated a matrix of pairwise relatedness estimates (IBD) for all possible pairs of individuals in our cohort using the genotypes of the ancestry informative markers (AIMs). We used this matrix to estimate the random effects of relatedness in a Q-K mixed model framework using the GLIMMIX procedure in SAS (Yu et al. 2006).

Sociocultural and Biological Data. Sociocultural and biological data were obtained during face-to-face interviews in participants' homes. Three resting blood pressure readings were collected using a standardized protocol in which respondents were seated for approximately 30 minutes and had not consumed alcohol or tobacco. Blood pressure was measured using an oscillometric monitor (UA-787, A&D Medical, Tokyo, Japan) that had been validated according to the European Society of Hypertension protocol (Longo et al. 2003). The first readings differed significantly from the latter two readings and, thus, an average of the latter two readings was used in the study analyses. Weight and height were measured using a digital scale and portable stadiometer and used to calculate BMI. Data on age, sex, antihypertensive medication use, years of education, and perceived discrimination were collected throughout the interview.

In the first phase of our research (in preparation), we conducted in-depth ethnographic interviews with 34 African Americans from varying socioeconomic backgrounds. Many respondents narrated their experiences of racism by citing the experiences of others, e.g. close friends, family, and coworkers. In the survey phase, on which this paper is based, we adapted a standard measure of perceived discrimination (Williams et al. 1997, Williams and Jackson 2005) that usually asks only about respondents' own experiences in nine social domains (e.g., police, courts, housing). We modified these questions to ask if "you or someone close to you" had been treated unfairly in each domain. If participants answered

"yes", we subsequently asked who had experienced the unfair treatment given the following options—yourself, your spouse, your parents, your child, your grandchild, a sibling, another relative, or a close friend. The discrimination scales used in our study included: any/major instances of unfair treatment experienced by the study participant during their lifetime (UT_Self) and any/major lifetime unfair treatment experienced by individuals close to the study participant (UT_Other). Unfair treatment was measured as an unweighted count of affirmative answers to the questions of unfair treatment in nine social domains with a maximum theoretical value of nine (actual range = 0-7; Table 1).

Statistical Analysis The presence of each *Alu* insertion was tested individually with systolic and diastolic blood pressure using association analyses. Two-way ANOVA analyses were used to test for a significant difference between the mean blood pressure values for each *Alu* insertion genotypic group. To determine a possible trend based on the number of insertion alleles, pairwise comparisons of the genotypic groups (Tukey's test) were performed.

Multiple linear regression models were constructed using R statistical software (R Core Team 2013) to test the association of combinations of the following variables with blood pressure levels: *Alu* polymorphisms, West African ancestry, years of education, household income, and measures of perceived unfair treatment as well as standard covariates of age, sex, BMI, and antihypertensive medication.

RESULTS

Genotype data for the *ACE, PLAT,* and *WNK1 Alu* polymorphisms and medical and sociocultural data were collected on 158 individuals (Table 1). We first tested for association of the individual *Alu* insertion alleles and genotypes with blood pressure. The only significant association was found with the *ACE Alu* insertion allele ($p=0.007134$ for systolic and $p=0.01098$ for diastolic blood pressure) and *ACE* genotypes ($p=0.0145$ for systolic, $p=0.0083$ for diastolic). A Tukey's test showed that one non-insertion allele increased systolic blood pressure by about ~0.1 mmHg, and a second non-insertion allele increased blood pressure by ~9.9 mmHg. For diastolic blood pressure one non-insertion allele led to an increase of ~2.2 mmHg per *ACE Alu* non-insertion allele, and a second non-insertion allele increased blood pressure by ~5.3 mmHg.

Multiple linear regression was next used to gain a more comprehensive picture of the variables that influence blood pressure. We first identified the optimal base model to explain blood pressure variation based on previously identified co-variates (e.g. age, sex, BMI, antihypertensive medication). The base model was then used to test the effect of the three *Alu* polymorphisms, West African genetic ancestry, two measures of unfair treatment and two measures of socioeconomic status (years of education and household income). Identification of the optimal base model and comparison of subsequent models was based on lower Akaike information criterion (AIC) and higher R^2 values.

The optimal base model for systolic blood pressure included age, sex, BMI, and antihypertensive medication use (Table 2). The optimal model for diastolic blood pressure included age, sex and BMI, but did not include antihypertensive medication use (Supplementary Table 1). Measures intended to capture socioeconomic status, such as years of education and household income, did not improve the model (Years education-AIC: 926.65, R^2: 0.2233, p-value: 0.65036; household income- AIC: 926.86, R^2: 0.2223, p-value: 0.93698). West African ancestry was found to reduce the strength of all models tested (e.g. base model: AIC: 926.82, R^2: 0.2186, p-value: 0.84773). The *ACE Alu* polymorphism was

the only genetic marker to improve the model on its own with a modest improvement of both AIC and R^2 values (Table 2).

Neither of the unfair treatment measures improved the model on their own. However, an interaction between the *ACE Alu* genotype and unfair treatment experienced by individuals close to the study participant (UT_Other) improved the model (Table 2). UT_Other was reported by 68.35 % of study participants, demonstrating its salience to participants' experiences of unfair treatment, with affirmative responses ranging from zero to seven (Figure 1). All categories of "Other" individuals had multiple responses, demonstrating that all categories were relevant to the measure of unfair treatment by others (Figure 2).

Although the interaction between the *ACE Alu* genotype and UT_Other did improve the model, the interaction effect was not significant. However, we noticed a possible threshold effect of unfair treatment on systolic blood pressure at UT_Other ≥ 3 (Supplementary Figure 1). Thus, we dichotomized the UT_Other variable into low (0-2) and high levels (3-7) of unfair treatment, which resulted in an improvement on the previous best model and the *ACE Alu* x UT_Other interaction became significantly associated with blood pressure (Table 2). Furthermore, the *ACE Alu* x UT_Other interaction showed a gene x environment effect such that only the non-insertion homozygous individuals demonstrated increased blood pressure with increased unfair treatment (Figure 3).

After refining the optimal model to include the interaction between *ACE Alu* and UT_Other, we tested to see if the other two *Alu* markers improved the model. We found that *WNK1 Alu* improved the model and was significantly associated with systolic blood pressure (Table 2).

We then tested to see if relatedness between participants influenced our results, specifically the significance of the *ACE Alu* x UT_Other interaction in the optimal model. We ran a Q-K mixed model that included age, sex, BMI, antihypertensive medication, *ACE Alu* genotype, *WNK1 Alu* genotype, UT_Other (high-low), and *ACE Alu* genotype x UT_Other (high-low) interaction and the relatedness matrix. This analysis identified that the interaction effect between *ACE Alu* genotype and UT_Other (high-low) remained significant even after accounting for relatedness (p-value of 0.0037).

It is important to note that the model improvements listed above reflect only modest improvements in the AIC and R^2 values. Nevertheless, the best model of variation for both systolic and diastolic blood pressure consisted of the base model plus the *ACE Alu* genotype, *WNK1 Alu* genotype, UT_Other, and *ACE Alu* genotype x UT_Other interaction. The statistical significance of the association of the *ACE Alu* genotype x UT_Other interaction is especially compelling given the relatively small sample size of our study.

DISCUSSION

Over the years, lengthy debate has surrounded the factors that influence blood pressure and, more specifically, the role of these factors in the racial disparity that exists in hypertension. Similarly, there has been debate on the role of the *ACE Alu* polymorphism in blood pressure variation. Seemingly contradictory articles have suggested the presence and absence of the insertion allele to be factors in cardiovascular disease morbidity. More recent studies have hypothesized that some of the conflict might signal varying degrees of linkage disequilibrium between the phenotypic trait locus and the *ACE Alu* polymorphism in different racial or ethnic groups (Reiner et al. 2007). When limiting the focus to African American populations, greater consensus exists for the association of increased blood

pressure with the non-insertion allele, although few evaluations of the *ACE Alu* polymorphism in African Americans have been conducted within the past decade (Reiner et al. 2007). Our study provides clarification of the *ACE Alu* association. Specifically, we find a significant and cumulative effect of the non-insertion allele with higher blood pressure, i.e. one non-insertion allele increased systolic blood pressure by ~0.1 mmHg, and a second non-insertion allele increased blood pressure by ~9.9 mmHg. As the non-insertion allele has been found to be associated with higher ACE activity and subsequently greater conversion to angiotensin-II and bradykinin inactivation, our results are consistent with the predicted physiological mechanism of *ACE* (Ajala et al. 2012).

To better understand the *ACE Alu* polymorphism's contribution to hypertension, we explored its effect in combination with two other *Alu* polymorphisms of interest, *PLAT* and *WNK1*. In this context, we found that the *WNK1 Alu* genotype significantly improved the model of blood pressure variation when added in combination with the *ACE Alu* x UT_Other interaction. This result suggests an association between *ACE* and *WNK1* gene functions. Studies of the *WNK* family of genes have determined their key role in vascular tonus (Zeniya et al. 2015). Angiotensin II, the product of ACE action, has been determined to regulate the *WNK3* and *WNK4* genes, which are involved in a phosphorylating cascade that leads to vasoconstriction and vascular tone (Zeniya et al. 2015). Although the role of the *WNK1* gene in this mechanism has not been well studied, a recent study implicated its function in the regulation of vascular tension in coordination with *WNK4* (Susa et al. 2012). Our results showing involvement of both *ACE* and *WNK1 Alu* polymorphisms provide support for a possible biological interaction of angiotensin II with the *WNK1* phosphorylation cascade that ultimately results in vasoconstriction. Furthermore, our study confirms results of previous studies of the *WNK* gene family, which elucidate the linkage between the renin-angiotensin and ion regulation systems in hypertension.

With the study of racial health disparities, incorporation of genetic ancestry seems an intuitive improvement from the previous system of racial self-identification. Several studies of African American populations have demonstrated associations of disease-related phenotypes with African ancestry (Fernández et al. 2003; Peralta et al. 2010; Reiner et al. 2007). In contrast, our study found no association of West African ancestry with either systolic or diastolic blood pressure and West African ancestry reduced the strength of all the models of blood pressure variation in this study. Our result undermines the validity of genetic ancestry as a risk factor for hypertension and its perceived role in contributing to the disparity of hypertension in African Americans. Instead, we suggest that sociocultural measures designed to capture the unique stressors for African Americans, such as experiences of unfair treatment, may better reflect the race-specific risk factors associated with hypertension.

The interaction between the *ACE Alu* genotype and unfair treatment to others (UT_Others) reflects the complex nature of the impact of discrimination on blood pressure in a population that demonstrates disparities in hypertension. Interestingly, an increase in blood pressure was only seen in the at-risk non-insertion homozygous individuals when UT_Others reached levels of three or greater. This result is consistent with the "weathering hypothesis," which proposes a threshold of experienced discrimination before effects on physical and biological health are observed (Geronimus et al. 2006). The accumulated, larger allostatic load, i.e. wear and tear on the body due to repeated or chronic stress, in

African Americans has been suggested to be a contributing factor in racial health disparities (Geronimus et al. 2006).

It is interesting to speculate on why unfair treatment to others is a salient measure of stress and why it appears to have a larger effect on blood pressure than unfair treatment to self. Stress exposures are typically measured by eliciting participant's perceptions of stressors. The focus on the individual is limited because it neglects social and cultural context and because people vary in their ability or willingness to report on their own stressful experiences (Young 1980; Dressler 2007). These limitations may be especially significant in trying to measure exposure to social stressors such as racial discrimination (as opposed to, say, stressful life events) because participants may be reluctant to report personal experiences of unfair treatment in order to avoid the stigma of discrimination. It may be less threatening to report on others' discrimination experiences, such that this measure yields less-biased measures of participant's exposures. Furthermore, denying that one has experienced discrimination could itself be a copying strategy. Finally, people may feel they have more control over things that they experience personally, whereas it may be more stressful to hear about bad experiences happening to people close to them over which they have little control. Regardless, the key consideration is that activation of physiological responses to stress does not require direct experience of a particular stressor. The same responses can be activated by vicarious exposure to the stressor. Furthermore, people who have higher exposure to vicarious racism may be more likely to suffer anticipatory stress responses or to perceive ambiguous situations as discriminatory when they enter social contexts where friends or family have been treated unfairly.

Our study highlights how a biocultural approach can provide a more complete understanding of complex diseases, such as hypertension. Our results would not have been detected without inclusion of both genetic and cultural data. Specifically, we identified an interaction between the *ACE Alu* polymorphism and unfair treatment to others and the *WNK1 Alu* polymorphism that improved our model of blood pressure variation. Our finding that only the measure of unfair treatment to others, i.e. not unfair treatment to self, improved the model demonstrates the value of novel measures of discrimination and suggests that the biological consequences of discrimination experiences are complex and not well understood. Further study is warranted to better understand the effects that genes and social stressors, like discrimination, have on disease outcomes, possibly leading to the manifestation of apparent genetic predispositions and contributing to racial disparities in health that persist today.

Acknowledgments This study was supported by NSF grants BCS 0820687 and BCS 0724032. We gratefully acknowledge the contributions of our Tallahassee participants as well as the knowledge and advice of our Tallahassee steering committee members. We thank Mitchell Schepps for initial statistical analyses and Franjo Ivankovic for creating Figure 2.

Received 28 July 2015; revision accepted for publication 6 October 2015.

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Table 1. Characteristics of study sample

Characteristics	**Total/Mean (SD)[1]**
N	158
Men:Women	51:107
Age (years)	40.96 (11.90)
Body mass index	32.47 (9.40)
Antihypertensive medication (yes:no)	48:110
Systolic BP	128.51 (20.91)
Diastolic BP	81.38 (13.26)
Education (years)	13.15 (2.40)
African ancestry	0.89 (0.072)
UT_Other	1.48 (1.57)
UT_Other (range)	0-7

[1]SD = standard deviation

Table 2: Optimal model choice using multiple linear regression analysis of systolic blood pressure

Model	Variable	β Coefficient	P-value	AIC	R^2_{adj}
Base	Age (Years)	0.418	**0.002**	924.86	0.227
	Sex (1=male, 2=female)	-14.769	**1.19E-05**		
	BMI	0.634	**0.00061**		
	BP Meds	5.366	0.167		
ACE	Age (Years)	0.422	**0.00168**	922.9	0.2415
	Sex (1=male, 2=female)	-14.424	**1.61E-05**		
	BMI	0.624	**0.00067**		
	BP Meds	4.422	0.254		
	ACE genotype	-4.184	0.0513		
ACE-UT_Other WNK1	Age (Years)	0.389	**0.0037**	920.95	0.2642
	Sex (1=male, 2=female)	-15.0211	**6.09E-06**		
	BMI	0.0597	**0.0007**		
	BP Meds	5.303	0.168		
	ACE genotype	-1.602	0.57243		
	WNK1 Genotype	9.04049	0.04517		
	UT_Other	0.09854	0.96601		
	I(ACE*UT_Other)	-2.04096	0.13869		
ACE-UT_Other WNK1[1]	Age (Years)	0.409	**0.00307**	916.03	0.2868
	Sex (1=male, 2=female)	-14.455	**1.10E-05**		
	BMI	0.574	**0.00086**		
	BP Meds	5.3185	0.21554		
	ACE genotype	-2.2318	0.39264		
	WNK1 Genotype	7.4704	0.08787		
	UT_Other	4.854	0.35144		
	I(ACE*UT_Other)	-13.5557	**0.0096**		

[1] Uses the dichotomized high/low UT_Other variable.

[2] A P value of 0.01 was considered significant after multiple comparison testing using a Bonferroni correction of 0.05/5 (5 = 3 Alu polymorphisms and 2 unfair treatment measures).

Supplementary Table 1: Multiple linear regression model of diastolic blood pressure

Model	Variable	β Coefficient	P-value	AIC	R^2_{adj}
Base	Age (Years)	0.17401	**0.0388**	800.21	0.122
	Sex (1=male, 2=female)	-5.11427	**0.0214**		
	BMI	0.47008	**3.29E-05**		
ACE	Age (Years)	0.16718	**0.0441**	796.67	0.146
	Sex (1=male, 2=female)	-4.88444	**0.0259**		
	BMI	0.44654	**6.56E-05**		
	ACE genotype	-3.32142	**0.0208**		
ACE-UT_Other WNK1	Age (Years)	0.1519	0.07	798.1	0.154
	Sex (1=male, 2=female)	-5.10741	**0.0198**		
	BMI	0.44858	**5.80E-05**		
	ACE genotype	-1.61949	0.3993		
	WNK1 Genotype	4.3832	0.1517		
	UT_Other	0.37208	0.6955		
	I(ACE*UT_Other)	-1.32503	0.1572		
ACE-UT_Other WNK1[1]	Age (Years)	0.15174	0.0681	796.51	0.163
	Sex (1=male, 2=female)	-4.82416	**0.0267**		
	BMI	0.43428	**9.24E-05**		
	ACE genotype	-2.19434	0.1622		
	WNK1 Genotype	3.52438	0.2402		
	UT_Other	2.90159	0.4148		
	I(ACE*UT_Other)	-6.99061	0.0589		

[1] Uses the dichotomized high/low UT_Other variable.

[2] A P value of 0.01 was considered significant after multiple comparison testing using a Bonferroni correction of 0.05/5 (5 = 3 Alu polymorphisms and 2 unfair treatment measures).

Figure Legends

Figure 1. Distribution of affirmative responses concerning unfair treatment experienced by individuals close to the study participant. The range of affirmative responses is shown on the X axis and the total number of individuals who reported 0-7 experiences is shown on the Y axis.

Figure 2. Distribution of individuals close to the study participant who experienced unfair treatment in the nine query areas (Unfair treatment on the job, by the police, by the courts, at school, obtaining housing, through the banking system, through the health care system, receiving service, e.g. from a mechanic, and from in-laws). Total number of affirmative answers is plotted on the Y axis.

Figure 3. Interaction of *ACE Alu* genotype and UT_Other (coded as high/low) with systolic blood pressure.

Supplementary Figure 1. Interaction of *ACE Alu* genotype and UT_Other (coded as 0-7) with systolic blood pressure. A threshold effect of unfair treatment on blood pressure is seen at UT_Other ≥ 3.