

Research paper

Prevalence and cardiometabolic associations of the glucocorticoid receptor gene polymorphisms N363S and *BclI* in obese and non-obese black and white Mississippians

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ABSTRACT

OBJECTIVE: Polymorphisms (SNP) in the glucocorticoid receptor (GR) gene can alter sensitivity to glucocorticoids. Previous studies of the N363S and *BclI* SNP in the GR gene have shown a metabolic syndrome phenotype in mostly non-African populations. The obesity phenotype of African Americans (AA) seems to be more severe than that of Caucasians. **DESIGN:** We aimed to assess the prevalence of N363S and *BclI* in obese and non-obese Caucasian (n=26) and African (n=23) Mississippians (age: 23-63 years) to investigate associations with body composition (body mass index/BMI, waist-to-hip ratio), metabolic parameters (salivary cortisol, fasting glucose and insulin, hemoglobin A1C, fructosamine, HOMA-IR index), and psychological stress perception (blood pressure/BP, perceived stress scale/PSS). **RESULTS:** All subjects were homozygous for wildtype N363N. *BclI* polymorphism genotype frequencies among the 23 AA were: homozygous CC (57%), GG (4%), and heterozygous CG (39%), and among the 26 white women: homozygous CC (35%), GG (19%), and heterozygous CG (46%). Linear and logistic regression analyses including a parsimonious model identified BMI as a statistically significant parameter between the two ethnic groups (BMI was 3.13 kg/m² higher in AA). Within the AA group, BMI, waist-to-hip ratio, log (HOMA-IR), PSS scores, BP, and hyperlipidemia showed no statistically significant relationships for the *BclI* polymorphism. PSS scores were 15.2 for AA vs. 14.7 for white women (normal mean: 14.7 vs. 12.8). **CONCLUSION:** Black Mississippians

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have a higher BMI than whites, which may be related to the presence of the *BclI* polymorphism and increased glucocorticoid sensitivity. Although more blacks (52%) than whites (38%) had elevated BP, PSS scores in both groups suggest that a high BMI is not regarded as abnormal or stressful. This might negatively impact behavior change regarding lifestyle modifications with increased physical activity and healthier food choices. Larger studies, particularly in African populations, are needed to better define metabolic and psychological characteristics in relation to the N363S and *BclI* GR gene polymorphisms.

Key words: Glucocorticoid receptor, Polymorphism, *BclI*, Diabetes, Stress, Body mass index, Waist circumference, Obesity

INTRODUCTION

Obesity, which occurs when more calories are consumed than burned over time, has become an epidemic in the United States. According to recent data (the 2007-2008 National Health and Nutrition Examination Survey which actually collects measured and not self-reported heights and weights) from the Centers for Disease Control (CDC) and Prevention, approximately 36% of women are obese and an estimated 17% of American children aged 2-19 years are overweight or obese. In Mississippi, approx. 34% of the population is overweight and another 34% are obese, leaving 32% of Mississippians having normal body weight, as defined by the body mass index (BMI).^{1,2} Obesity is a risk factor for developing type 2 diabetes and nearly 26 million children and adults in the U.S. have diabetes with another estimated 79 million Americans having prediabetes and therefore being at risk for developing type 2 diabetes. National healthcare spending, including medical bills of an obese individual, is 42% higher than that of someone who is not obese according to CDC data. In 2011, Gallup's annual health and healthcare survey found American men were on average 15 lbs over their ideal weight and American women were 22 lbs over their ideal weight. The cost of caring for someone with diabetes is \$1 out of every \$5 in total healthcare costs according to the American Diabetes Association. Obesity is not only very prevalent in the U.S. but also worldwide, with variable rates in European countries and sociodemographic, environmental, ethnic, and dietary factors playing a major role.³⁻¹²

About 40-80% of the variation in obesity has been linked to genetic factors. Analyzing the human

genome for possible gene variants associated with obesity led to the discovery of major obesity loci on chromosomes 2, 5, 10, 11, 16, and 20.¹³⁻¹⁸ Differential polygenic predispositions across ethnicities could therefore explain differential development of obesity and other disease states.

The frequency of such SNPs varies greatly among ethnic populations and the effects of these polymorphisms may differ among races related to different combinations of polymorphisms of several genes and their protein interactions and differences in environmental and socioeconomic factors.^{19,20} The obesity phenotype of African Americans seems to be more severe than that of Caucasians. We here examined whether glucocorticoid receptor polymorphisms (SNP) mediated this ethnicity-obesity relationship. SNPs of the GR may play a role in aggravating the course of certain diseases. Some of these conditions are listed in Table 1.

Cortisol is a stress hormone associated with various disease states including obesity.^{3,4,28} Glucocorticoid sensitivities vary greatly between individuals and may help explain why individuals respond differently to the

Table 1. Role of GR gene polymorphisms in various diseases

- progression of lung disease in young patients with cystic fibrosis²¹
- increased disease activity in inflammatory bowel diseases²²
- reduced susceptibility to develop rheumatoid arthritis²³
- avascular necrosis of the femoral head²⁴
- decreased bone mineral density in patients with endogenous hypercortisolism²⁵
- susceptibility to develop major depression²⁶
- increased risk for impaired endothelial function²⁷

same amount of glucocorticoid.^{29,30} The best known polymorphisms in the GR gene related to an altered GC sensitivity are: ER22/23EK, N363S, and *BclII* and *TthIII* restriction fragment length polymorphisms. At present, data regarding their effect on cardiovascular risk factors, body composition, metabolic parameters, and psychological stress are mixed.³¹⁻³⁵ This may in part be related to the respective ethnic group studied, including mostly Caucasians (French, Swedish, Dutch, Anglo-Celtics, Northern Europeans, Hungarians) but also Indians, Middle Eastern, Asians, Vietnamese, and Koreans. Recently, a heterozygous nucleotide substitution in exon 2 of the GR, the D401H mutation, has been found to potentially predispose Colombians to obesity, hypertension, and other manifestations of the metabolic syndrome.²⁹ Our study population consisting of black and white Mississippians was designed to examine whether possible associations between N363S and *BclII* polymorphisms and various obesity-related clinical parameters help explain the associations previously observed between ethnicity and clinical parameters.

SUBJECTS AND METHODS

Study design and data collection

This study was conducted in accordance with the declaration of Helsinki and approved by the Institutional Review Board of the University of Mississippi Medical Center. All study subjects signed the informed consent. Our main objectives were to assess the prevalence of polymorphisms N363S and *BclII* in the glucocorticoid receptor gene in obese and non-obese Caucasian and African American (AA) individuals from Jackson, Mississippi, and to investigate associations with body composition, metabolic parameters, and psychological stress measures. All of our study participants were women. All participants perceived themselves as healthy. The age range was 24-63 years overall (32-58 yrs for AA, 24-63 yrs for white women).

We analyzed two distinct ethnic groups: (1) obese and non-obese Caucasian females (N=26) and (2) obese and non-obese AA females (N=23). After informed consent, venous blood was drawn to obtain 20 cc EDTA blood to extract genomic DNA for SNP analyses. Obesity was defined as a BMI >30.

Glycemic control was assessed by measuring fast-

ing glucose (in mg/dl) and insulin (in microIU/ml), hemoglobin A1C, and fructosamine (micromol/L) levels. HOMA-IR index was computed adjusting for glucose values in mg%. We additionally collected BMI, waist-to-hip ratio, systolic and diastolic blood pressure. Morning cortisol levels were obtained by collecting saliva at 8 AM measured in our laboratory in mcg/dl and converted to ng/dl (normal, 100-750 ng/dl, 7AM-9AM, Mayo Clinic Laboratories). To measure the perception of stress, we used the perceived stress scale (PSS).³⁷

We compared the homozygous and heterozygous genotypes between Caucasian and African American female differences in relation to the aforementioned clinical parameters. In addition to this analysis, we performed a separate evaluation of the impact of CC (homozygous) and CG (heterozygous) genotypes on our study parameters within the AA group (group 2).

Genetic analysis

Allelic Discrimination Assays for the GR BclII and N363S Polymorphism

DNA was extracted using the commercial Pure-gene DNA Isolation Kit (Centra Systems) and 15 ng of DNA was used in each genotyping assay. The *BclII* (rs41423247) and N363S (rs6195) polymorphisms of the GR gene were detected by using the Applied Biosystems 7300 Real-Time PCR System with TaqMan SNP assay reagents and genotyping analyzed by SDS 2.1 package (Applied Biosystems). The sequences of probes were 5'-FAM-TCTGCTGATCAATCT-3' and 5'-VIC-TCTGCTGATGAATCT-3' for *BclII* and 5'-FAM-CTATTCCAATTTTCGGAACCA-3' and 5'-VIC-CTATTCCAATTTTCGGAACCA-3' for N363S. Reaction components and amplification parameters were based on the manufacturer's instructions using an annealing temperature of 60°C and optimized concentrations for primers and probes of 400 nmol/l and 50 nmol/l, respectively. The results from the TaqMan SNP assay were confirmed by PCR-RFLP analysis using the *BclII* and N363S restriction enzymes and digestion for 1 h at 37°C.

Statistical analysis

Analyses were conducted using linear regression for continuous response variables and logistic regression for binary response variables (elevated

blood pressure and hyperlipidemia). For the binary responses the results are presented as odds ratios (OR), i.e. the odds of the response being present are increased by the reported ratio for every one unit increase in the predictor.

A series of regression models were constructed focusing on ethnicity as a predictor. First, an unadjusted model was constructed. The second model was adjusted for age. The third was adjusted for age and genotype. Fourth, a full model containing every covariate was fashioned. Finally, a parsimonious model was built using a manual backward elimination technique while providing that ethnicity remained in the model. The AA group (group 2) was further examined with the same approach, while genotype served as the primary predictor. Statistical significance was defined as $\alpha < 0.05$.

RESULTS

Blood samples of the two groups of individuals (26 Caucasian and 23 AA) were analyzed for N363S and *BclI* polymorphisms. All of the participants in our study were found to carry a homozygous genotype (N363N). Genotype frequencies of the *BclI* polymorphism in the GR gene for our two groups were: group 1 (CC=35%, CG=46% and GG=19%) and group 2 (CC=57% and CG 39%). Demographic information for the participants and results are shown in Table 2.

White participants were on average 49 years old with BMIs of 30.7 kg/m², while AA participants were on average 46 years old with BMIs of 37.3 kg/m². BMI ($p=0.012$), HbA1c ($p=0.030$), fasting glucose, ($p=0.071$) and fasting insulin ($p=0.092$) showed potential differences between ethnicities. A lower percentage of our white participants (38%) had elevated blood pressure compared with AA (52%).

Waist circumference ranged from 87 cm to 143 cm in black women, and from 65 to 139 cm in white women. Hip circumference ranged from 101 cm to 150 cm in black women, and from 57 cm to 145 cm in white women.

Age and HbA1c differences were supported between AA and white participants, primarily occurring for the CC genotype, while only BMI showed a statistically significant difference for the CG geno-

type. BMI, HbA1c, and fasting insulin also showed potential differences between our two groups and were followed up in adjusted models.

Regression analyses were used to examine the effect of the ethnicity on metabolic/CVD outcomes. Unadjusted, adjusted for age, *BclI*, and fully and parsimonious models were included in our statistical analyses to observe any increasing statistical significance along our regression models. Logistic regression odds ratios were reported for binary variables (elevated BP and hyperlipidemia), and where we had problems with the collinearity of our variables we excluded them from the regression model.

We started the analysis of our metabolic/CVD outcomes with an unadjusted regression model. This initial model suggested a possible effect of genotype on the relationship between ethnicity and BMI (6.62, $p=0.010$), waist-to-hip ratio (0.04, $p=0.081$), log (HOMA-IR)(0.37, $p=0.081$), and HbA1c (0.28, $p=0.059$).

To examine further our primary question of the effect of genotype on the relationship between ethnicity and metabolic/CVD outcomes, we added subsequently age and *BclI* polymorphism to the initial unadjusted model.

The adjusted relationships between ethnicity and the outcomes remained similar when age was added to the initial model. We observed a stronger statistical difference in this model 1 for BMI (7.89, $p=0.001$), waist-to-hip ratio (0.053, $p=0.035$), log (HOMA-IR) (0.42, $p=0.05$), and HbA1c (0.34, $p=0.021$).

For the relationships between ethnicity and the metabolic/CVD outcomes (Table 3), African American ethnicity was associated with a 7.89 kg/m² higher BMI in age adjusted models (95% CI: [3.27, 12.51]), $p=0.001$. AA also showed higher waist-to-hip ratios, 0.053 (95% CI: [0.003, 0.10]), $p=0.035$, log (HOMA-IR), 0.42 (95% CI: [-0.007, 0.84]) $p=0.05$ and HbA1c 0.34 (95% CI: [0.057, 0.64]) $p=0.021$. The other response variables did not reach statistical power to conclude that ethnicity was a significant predictor.

The results obtained after the addition of the *BclI* polymorphism to the regression model have indicated that the *BclI* polymorphism is unlikely to be the driver of observed ethnicity differences in the

Table 2. Mean values and standard deviation (SD) for clinical, biochemical, and psychological parameters for the Bc/I polymorphism (C and G alleles) in Caucasian and African American Mississippians

Characteristics	Caucasian (white)				African American (black)		
	CC	CG	GG	Total	CC	CG	Total
	N=9 (35%)	N=12 (46%)	N=5 (19%)	N=26	N=13 (57%)	N=9 (39%)	N=23
Age (y)	52.8 (11.1)	47.3 (12.6)	46.8 (14.5)	49.1 (12.3)	45.2 (7.8)	45 (7.68)	45.5 (7.6)
BMI	33.8 (9.6)	28.8 (9.4)	29.8 (9.7)	30.7 (9.4)	39.55 (8.69)	35.03 (4.93)	37.3 (7.6)
Waist-to-hip ratio	0.86 (0.08)	0.82 (0.1)	0.83 (0.07)	0.83 (0.1)	0.88 (0.05)	0.85 (0.05)	0.88 (0.06)
Fasting insulin	10.5 [6.7-22.3]	8.7 [6.4-20.7]	10.2 [7.07-16.56]	9.8 [6.72-19.85]	18.36 [11.34-29.9]	15.81 [13.07-18.62]	17.08 [12.2-24.26]
Fasting glucose	95.8 (6.5)	93.41 (13.6)	90.6 (11.01)	93.7 (10.8)	102.23 (11.38)	94.22 (8.05)	99.52 (10.82)
HOMA-IR	2.4 [1.4-5.5]	2.1 [1.3-4.3]	2.1 [1.4-3.9]	2.2 [1.36-4.56]	4.1 [2.5-7.3]	3.8 [3-4.3]	3.95 [2.75-5.8]
Fructoseamine	220.1 (22.8)	226.3 (20.84)	222.8 (19.65)	223.46 (20.67)	218.76 (20.64)	222.11 (12.72)	220.52 (17.24)
HbA1c	5.8 (0.3)	5.64 (0.7)	5.64 (0.5)	5.68 (0.53)	6.15 (0.47)	5.67 (0.44)	5.97 (0.51)
Salivary cortisol	150 (60)	180 (10)	190 (160)	170 (10)	170 (90)	130 (70)	160 (80)
PSS	14.2 (5.6)	12.8 (8.6)	19.4 (12.42)	14.7 (8.5)	16 (6.16)	15.28 (4.11)	15.2 (5.76)
Elevated BP	7 (78%)	3 (25%)	0	10 (38%)	7 (54%)	4 (44%)	12 (52%)
Hyperlipidemia	4 (44%)	2 (17%)	1 (20%)	7 (27%)	2 (15%)	1 (11%)	4 (17%)

Comparison Caucasian vs African American			
Characteristics	p-value		
	CC	CG	Total
Age	0.019	0.413	0.057
BMI	0.229	0.055	0.012
Waist to hip ratio	0.788	0.594	0.277
Fasting insulin	0.270	0.286	0.092
Fasting glucose	0.227	0.569	0.071
HOMA-IR	0.216	0.255	0.058
Fructoseamine	0.736	0.749	0.595
HbA1c	0.051	0.567	0.030
Salivary cortisol	0.525	0.200	0.724
PSS	0.521	0.463	0.554
Elevated BP	0.38	0.397	0.396
Hyperlipidemia	0.178	>0.999	0.506

* p value for unadjusted differences; Nonparametric Wilcoxon Rank Sum tests for continuous vars, Fisher's exact test for categorical
 * values in table are N (%) for categorical vars, median [IQR] for skewed continuous vars (HOMA-IR, Insulin), mean (SD) otherwise
 * only 1 African American GG genotype was observed; BP: blood pressure; PSS: perceived stress scale.

Table 3. Ethnicity Associations - Mediation Effect Regression Models

Outcome	Ethnicity Effect				
	Unadjusted	Model 1: Adjusted for age	Model 2: Model 1 + <i>BclI</i>	Model 3: Full Model	Model 4: Parsimonious
BMI	6.62 p=0.010 (1.65, 11.58)	7.89 p=0.001 (3.27, 12.51)	6.85 p=0.006 (2.09, 11.62)	1.29 p=0.488 (-2.48, 5.06)	3.13 p= 0.039 (0.16, 6.10)
Waist-to-hip ratio	0.04 p=0.081 (-0.005, 0.09)	0.053 p=0.035 (0.003, 0.10)	0.053 p=0.044 (0.001, 0.10)	0.029 p=0.282 (-0.025, 0.085)	0.023 p=0.276 (-0.019, 0.066)
Log (HOMA-IR)	0.37 p=0.081 (-0.048, 0.79)	0.42 p=0.050 (-0.007, 0.84)	0.37 p=0.100 (-0.08, 0.82)	-0.2 p=0.322 (-0.60, 0.20)	-0.07 p=0.654 (-0.383, 0.243)
Fructosamine	-2.93 p=0.593 (-13.96, 8.08)	-3.74 p=0.501 (-14.98, 7.49)	-2.87 p=0.637 (-14.80, 9.06)	1.63 p=0.781 (-10.54, 13.80)	2.67 p=0.552 (-6.31, 11.66)
HbA1c	0.28 p=0.059 (-0.011, 0.59)	0.34 p=0.021 (0.057, 0.64)	0.31 p=0.042 (0.009, 0.61)	0.097 p=0.513 (-0.20, 0.39)	0.012 p=0.918 (-0.224, 0.249)
Salivary cortisol	-0.02 p=0.535 (-0.076, 0.04)	-0.024 p=0.422 (-0.085, 0.036)	-0.022 p=0.496 (-0.087, 0.043)	-0.024 p=0.535 (-0.10, 0.054)	-0.014 p=0.616 (-0.07, 0.042)
PSS	0.49 p=0.827 (-4.04, 5.02)	0.76 p=0.747 (-3.86, 5.39)	1.03 p=0.671 (-3.88, 5.94)	2.56 p=0.414 (-3.83, 8.96)	0.73 p=0.759 (-4.05, 5.52)
Elevated BP*	1.74 p=0.331 (0.55, 5.44)	2.38 p=0.171 (0.67, 8.40)	1.73 p=0.422 (0.44, 6.74)	3.42 p=0.262 (0.38, 30.43)	1.57 p=0.498 (0.42, 5.85)
Hyperlipidemia*	0.57 p=0.426 (0.14, 2.27)	1.67 p=0.579 (0.27, 10.19)	1.72 p=0.573 (0.26, 11.23)	1.31 p=0.863 (0.06, 28.8)	1.72 p=0.570 (0.264, 11.24)

*Note: AA - Caucasian

*Elevated BP & Hyperlipidemia are binary variables, logistic regression Odds Ratios and 95% CI are reported

**Hyperlipidemia full model excluded Waist-to-Hip Ratio due to collinearity induced nonconvergence issues.

metabolic/ CVD outcomes. The initially supported statistically relationships were weakened for BMI (6.85, p=0.006), waist-to-hip ratio (0.053, p=0.044), and HbA1c (0.31, p=0.042) and just marginally important for log (HOMA-IR) (0.37, p=0.100).

We additionally constructed fully adjusted and parsimonious models to examine any additional relationships between the selected variables and ethnicity. Only the BMI showed any significant difference across ethnicities, with African Americans showing an estimated 3.13 kg/m² higher BMI after adjusting for log (HOMA-IR), fructosamine, and HbA1c. Waist-to-hip ratio, log (HOMA-IR), and HbA1c outcomes, which statistically supported differences in age and *BclI* adjusted models between CC and CG ethnic genotypes, have ceased to support these substantial changes after adjustment made in full and parsimonious models. The adjustments in the parsimonious regression model for the aforementioned three out-

comes were: BMI and hyperlipidemia for waist-to-hip ratio; fructosamine and HbA1c for log (HOMA-IR); BMI, log (HOMA-IR), and fructosamine for HbA1c. These variables have contributed significantly to the variance of these three outcomes. Full model and complete parsimonious model estimates are available from the authors on request.

As an additional part to our research project we investigated the effects of the C and G allele on metabolic/CVD outcomes within AA group. In Table 4, we have presented the results of our analyses for group 2 AA.

Our 23 AA females were analyzed for the prevalence of the *BclI* polymorphism. Allele and genotype frequencies of the *BclI* polymorphism in the GR gene identified in our AA group are displayed in Table 2. We found 13 homozygous CC (57%), 9 heterozygous CG (39%), and one homozygous GG.

Table 4. *BcII* Effect (CG versus CC) within African Americans

Outcome	Unadjusted	Model 1: Adjusted for Age	Model 2: Parsimonious
BMI	-4.52 p=0.176 (-11.23, 2.19)	-4.44 p=0.123 (-10.18, 1.31)	0.011 p=0.996 (-4.25, 4.28)
Waist to hip ratio	-0.027 p=0.264 (-0.077, .022)	-0.027 p=0.274 (-0.078, 0.023)	-0.0025 p=0.901 (-0.044, 0.039)
Log (HOMA-IR)	-0.21 p=0.417 (-0.72, 0.31)	-0.202 p=0.419 (-0.71, 0.31)	0.23 p=0.268 (-0.191, 0.646)
Fructosamine	3.34 p=0.671 (-12.85, 19.53)	3.32 p=0.681 (-13.31, 19.94)	13.99 p=0.001 (6.83, 21.16)
HbA1c	-0.48 p=0.029 (-0.898, -0.054)	-0.47 p=0.028 (-0.891, -0.056)	-0.31 p=0.037 (-0.604, -0.021)
Salivary cortisol	-0.049 p=0.214 (-0.128, 0.031)	-0.048 p=0.240 (-0.130, 0.035)	-0.061 p=0.062 (-0.125, 0.0035)
PSS	-0.71 p=0.789 (-6.26, 4.83)	-1.21 p=0.632 (-6.48, 4.06)	-3.56 p=0.217 (-9.44, 2.32)
Elevated BP**	0.686 p=0.665 (0.124, 3.78)	0.686 p=0.667 (0.123, 3.82)	0.686 p=0.665 (0.124, 3.78)
Hyperlipidemia*‡	0.688 p=0.775 (0.053, 8.96)	0.695 p=0.795 (0.045, 10.83)	

* The parsimonious model for hypertension was found to be the same as the unadjusted model

‡ The parsimonious model for hyperlipidemia failed to converge due to possible collinearity issues.

In the unadjusted and age adjusted models, the only statistically significant difference between CC and CG genotypes was observed for HbA1c: (-0.48, p=0.029) and (-0.47, p=0.028), respectively. None of the other reported relationships between the aforementioned genotypes and clinical parameters were confirmed in our study. However, after we constructed parsimonious models to correct for the effects of all physiological variables, other differences between our homozygous and heterozygous AA subjects were identified.

Although in the initial models (unadjusted and adjusted for age) fructosamine did not show any statistical importance between CC and CG genotypes, after correcting for BMI log (HOMA-IR), HbA1c and cortisol – the variables with significant contribution to variance in fructosamine – the relationship grew stronger to a significant statistical difference (13.99, p=0.001). The HbA1c difference between CC and CG genotypes remained statistically important also in our parsimonious model after correcting for the effects of BMI, log (HOMA-IR), and fructosamine (variables with significant contribution to variance in HbA1c) (-0.31, p=0.037).

The latest statistically significant relationship between CC and CG genotypes in regard to clinical parameters analyzed in our parsimonious model was observed for cortisol. After adjusting for fructosamine and elevated BP – variables with significant contribution to variance in cortisol – our statistical results have confirmed marginally this relationships for cor-

tisol (-0.061, p=0.062). None of the other variables analyzed (BMI, waist-to-hip ratio, log (HOMA-IR), PSS, elevated BP, and hyperlipidemia) in unadjusted, adjusted for age, and in parsimonious models have shown any significant relationships for the *BcII* polymorphism within the AA group.

DISCUSSION

Obesity and hypertension are increasing in many societies worldwide. Although most of this epidemic is simply attributable to miscalculating caloric intake and loss by behavioral and environmental issues, there are also genetic contributors. As is true for many complex traits, most likely a combination and corroboration of many altered genes increases the susceptibility for becoming overweight rather than a single gene alteration. However, to better understand mechanisms of disease, studying monogenic disorders or those in which one specific gene may be altered still represents a fruitful avenue for discovery and advancement of knowledge.³⁸ In this study, we attempted to assess the impact of polymorphisms N363S and *BcII* of the GR gene on various metabolic, anthropometric, and psychological parameters in relationship to obesity. AA seem to be more sensitive to glucocorticoids than Caucasian Americans, with greater peak insulin and postprandial insulin areas under the curve.¹⁹ AA also appear to have higher sweet taste perception scores when compared with Caucasians.³⁹ In previous studies not including AA individuals from Mississippi, a state with a high poverty level in addition to having

the highest percentage (68%) of overweight and obese individuals in the United States, these two SNPs of the GR have been found to be associated with a milieu of glucocorticoid hypersensitivity, leading to more body fat, less lean mass, higher insulin secretion, and higher cholesterol levels (reviewed in 33,34). Any impact of N363S was ruled out in our study group, as all participants were homozygous for N363N (wild-type). However, the *BclI* polymorphism with its C and G alleles was found in various frequencies in our two study groups.

Our data for C and G allele frequencies of the *BclI* polymorphism in white females are similar for the CG genotype and slightly different for the CC and GG genotypes compared with data published previously.³¹ In the AA group (group 2, n=23), the C and G allele proportions were different both for homozygotes (CC and GG) and heterozygotes (CG). A previously published report found allele frequencies of 5% CC (compared to ours: 57%), 31% CG (compared to ours: 39%), and 64% for GG.³¹ Whether this implies linkage disequilibrium which may alter the transcriptional activity of target genes that are important in glucose, insulin, and cholesterol metabolism, is unknown and we cannot make additional comments about these results due to the fact that we analyzed only female subjects and have incorporated only a small number of patients in our study.

Based on the paucity of data in the AA population, it is difficult to define precisely the role of the *BclI* polymorphism in our female population and associations with various metabolic/CVD outcomes. In Caucasian women, sequence variants in both the N363S and the *BclI* polymorphism may result in higher cholesterol levels and a tendency for higher blood pressure.⁴⁰

In the main part of our study, we intended to search for ethnic differences between homozygous and heterozygous genotypes related to the C and G allele of the *BclI* polymorphism.

We compared Caucasian and AA females (homozygous and heterozygous genotypes) using a set of metabolic/CVD and stress outcomes (physiological and psychological stresses).

Multiple studies published before have stressed

the role of *BclI* and N363S polymorphisms on various clinical parameters. In our subjects we studied the interethnic variability of nine clinical parameters: BMI, waist-to-hip ratio, log (HOMA-IR), fructosamine, HbA1c, salivary cortisol, PSS, elevated blood pressure/hypertension, and hyperlipidemia.

Previous studies have found statistically different outcomes for most of these parameters analyzed for both *BclI* and N363S SNPs within the same ethnic group but did not investigate further if the ethnicity factor plays a role here.

An interesting finding in our study working with different regression models was that adding the *BclI* polymorphism to model 1 (see Table 3) made the relationship grow weaker statistically for all of the nine outcomes investigated, suggesting a limited or no effect on these variables.

Some studies have shown an increased subcutaneous fat deposition in women who carry a *BclI* or N363S polymorphism. In our study, the only statistically significant interethnic difference was observed for BMI. Waist-to-hip ratio, although showing a statistical difference between these two ethnic groups in the adjusted model for age and *BclI*, failed to show any race differences after correcting for the interference of other parameters in parsimonious models. For parameters such as BMI, waist-to-hip ratio, and waist circumference, controversy exists regarding superiority in cardiometabolic risk prediction, and international experts recommend the use of population-specific and country-specific definitions for waist circumference and BMI. The new Asian BMI criteria include a lower cut-off of 23.0 kg/m² for defining overweight Asians and of 25.0 kg/m² for identifying obesity in Asians. Srivastava et al³⁵ concluded that the GG genotype of the *BclI* polymorphism in the GR gene modulates blood pressure and blood glucose in Northern Indians with a waist-to-hip ratio of 0.95. On the other hand, neither BMI nor waist circumference nor waist-to-hip ratio identified newly diagnosed type 2 diabetics or hypertensives with a high sensitivity or specificity in 2,477 men and 3,107 women, aged 20-79 years, from the Pudong new area of Shanghai in China.⁴¹ In the SABPA study of Sub-Saharan Africans, investigators suggested that the optimal cut-off point in waist circumference for males be set at 90 cm, as opposed to

the present 94 cm, and for females be set at 98 cm, compared to the current cut-off of 80 cm.⁴² NHANES data (1999-2006) of non-minority American adults aged 52-85 years showed much higher rates of type 2 diabetes and waist circumference at each level of BMI compared to an equivalent group in England (2004 English Longitudinal Study of Aging), suggesting that waist circumference in this group is superior in predicting cardiometabolic risk.⁴³ A prospective cohort study comprising 9,753 German men and 15,491 German women, aged 35-65 years, similarly concluded that waist circumference represents a better marker to predict diabetes risk than the BMI.⁴⁴

The addition of the *BcII* polymorphism to our regression models seemed to detect a possible association between this polymorphism and log (HOMA-IR) and HbA1c (model 2). These relationships between the metabolic outcomes of Caucasian and AA females from Mississippi lost their statistical significance in full and in parsimonious regression models for log HOMA-IR and for HbA1c. Using HbA1c for the diagnosis of diabetes in adults is problematic and even more so in children and adolescents.⁴⁵⁻⁴⁷ A HbA1c cut-off of 6.5% would miss approx. 50% of diabetic children and adolescents aged 12-18 years (area under curve value of 0.88 in ROC analysis, compared to 0.93 in adults).⁴⁸ In Chinese subjects, the sensitivity of HbA1c >6.5% for the diagnosis of diabetes was 48%, with a specificity of 98%.⁴⁹ A recent meta-analysis of genome-wide association studies including 46,000 Europeans without diabetes mellitus revealed that genetic factors affecting expression, turnover, and abnormal glycation of hemoglobin could be associated with increased levels of HbA1c.²⁰ Further complicating the assessment of HbA1c is the fact that one also has to consider that type 1 and type 2 diabetics may involve a dysfunction of the hypothalamic-pituitary-adrenal axis with flattened circadian cortisol rhythm.^{50,51} In patients with hypercortisolism caused by Cushing's disease, diabetes mellitus type 2 and hypertension are risk factors for worse outcome.⁵²

When we searched for interracial differences in our groups in relation to CVD outcomes, we found no difference in elevated BP and hyperlipidemia in the parsimonious model. Although these two CVD outcomes may play a role in women who carry these two polymorphisms – as reported in some studies –

they did not seem to differ between these two races. In regression model 2 after adding the *BcII* polymorphism, no statistical difference was observed for elevated BP and hyperlipidemia.

The last outcomes analyzed in our study were related to stress. Stress can affect many body functions and depends on numerous factors including stress perception, stress coping and behavior modification and adaptation, individual hormonal responses, and others.^{3,53,54} Of interest, the norm table for the PSS 10 item inventory reports a mean of 12.8 for white, of 14.7 for black, of 14.0 for Hispanic, and of 14.1 for other minorities. In our groups (see Table 2), PSS scores and cortisol levels did not differ (model 4). The presence of the *BcII* polymorphism in both groups seemed to attenuate interracial differences in coping with stress. With PSS mean scores of 14.7 for whites and of 15.2 for blacks, therefore only slightly above the reported norm mean, one cannot conclude that Mississippians experience/perceive more stress, although an environment with high poverty and low education levels certainly can cause stress.⁶ The question arises whether coping mechanisms are utilized in the sense of stress desensitization. Certainly, the right amount of stress sometimes referred to as eustress can have many positive effects, as opposed to chronic “kakostress” (distress). Simply put, a high BMI around other people with high BMI (68% of the population in Mississippi) may not be perceived as abnormal but rather as the norm, therefore not causing any kind of stress. This type of norm change (oversizing) is also reflected by industrial efforts, for instance by moving up the waist circumference defined for a certain clothing size (i.e. jeans size 32 in 1993 is now size 30, allowing people to keep gaining weight and psychologically think “I am still in good/normal shape”). To initiate behavior change, i.e. regarding lifestyle modifications including more physical activity and healthier food choices, a certain amount of eustress is necessary. Practically put, the time has now come to “downsize” and confront people with their body sizes/shapes by re-setting parameters such as definition of a normal BMI, normal waist and hip circumference including size for jeans, suits, dresses, and other clothing.

Although it is premature to draw any conclusion about interracial differences in homozygous and

heterozygous individuals with the *BclI* polymorphism, different ethnic background, and metabolic/CVD and stress outcomes, we did not observe a significant difference, at least in our Mississippian study population. The only positive interethnic outcome studied was BMI (higher in blacks compared to whites).

As an additional project to our study we investigated the impact of the *BclI* polymorphism within the AA ethnic group. Such analyses have not been reported extensively in the literature and the results for the AA group based on the influence of the *BclI* polymorphism on these nine outcomes are not known. We analyzed the differences between the hetero- (CG) and homozygous (CC) AA females based on these nine different study parameters. We had only one case carrying the GG genotype and we dropped this case from our calculations.

In our study we found significant differences between our hetero- and homozygous participants for fructosamine, HbA1c, and salivary cortisol levels (see Table 4).

We cannot report any difference between AA hetero- and homozygotes for C and G alleles for: BMI, waist-to-hip ratio, log (HOMA-IR), PSS, elevated blood pressure (parsimonious regression model, Table 4).

Although in Caucasian women included in this type of analysis BMI, hyperinsulinemia, elevated BP, and hyperlipidemia have been considered to be altered by the presence of the *BclI* polymorphism, in our AA group we could not find these associations.

We are aware of the difficulties of extrapolating these data to the general AA, African, and Caucasian female populations due to the small number of subjects incorporated in our investigation. We hope larger studies, particularly on African populations, will be performed in the future to better define metabolic and psychological characteristics in relation to the N363S and *BclI* GR gene polymorphisms. Perhaps this information may then facilitate the identification of a new biomarker, analogous to SNPs in the CRP (C-reactive protein) gene.⁵⁵

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