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The G Protein-Coupled Receptor Kinase 4 Gene Modulates Stress-Induced Sodium Excretion in Black Normotensive Adolescents

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ABSTRACT: Approximately 20–40% of adolescents have shown a reduction of urinary sodium excretion ($U_{Na}V$) in response to blood pressure (BP) increase during behavioral stress. G protein-coupled receptor kinase 4 (GRK4) mediates the pressure and natriuresis relation. The present study investigated the impact of GRK4 genetic variants on $U_{Na}V$ under stress. A total of 664 normotensive adolescents including whites and blacks (17.6 ± 3.3 yrs, 43.4% blacks) were recruited. Participants were subjected to a stress-protocol including three 10-min tasks (a social competence interview, a virtual reality car driving simulation test, and a video game challenge), concluded by a urine collection. Three functional polymorphisms including R65L, A142V and A486V were genotyped. Given blacks compared with whites had significantly higher systolic BP (SBP) levels during rest ($p < 0.001$) and stress ($p \leq 0.001$), there was no statistical difference in $U_{Na}V$ in response to stress between the two ethnic groups. In blacks, compared with R65R homozygotes, individuals with R65L or L65L genotype had significantly lower levels of stress-induced $U_{Na}V$ (8.42 ± 0.63 *versus* 9.85 ± 0.37 mEq/h, $p = 0.01$). In summary, BP elevation seems uncoupled with $U_{Na}V$ increase during behavioral stress in black adolescents. The 65L allele of the GRK4 gene is associated with stress-induced $U_{Na}V$ reduction, suggesting impaired sodium handling in affected black youth. (***Pediatr Res* 60: 440–442, 2006**)

Essential hypertension is determined by gene and environment interactions, and has its origin in childhood. Blood pressure (BP) increase during a sustained period of behavioral stress is typically accompanied by a compensatory increase in urinary sodium excretion ($U_{Na}V$) (1,2). Our previous data show that an inadequate compensatory increase in $U_{Na}V$ in response to stress occurs in 20–40% of adolescents aged 15–19 y (Harshfield *et al.* Annual Meeting of Society of Behavioral Medicine, April 13–16, 2005, Boston), which may predict the development of hypertension (1,2). In particular, we have recently demonstrated that $U_{Na}V$ in response to stress is a heritable phenotype in a multi-ethnic cohort of adolescents (Ge *et al.*, Annual meeting of the American Society of Human Genetics, October 25–29, 2005, Salt Lake City). However, no candidate gene association study has been performed to date.

Dopamine enhances renal sodium excretion, diuresis and natriuresis *via* dopamine receptors D (DRD) in the renal proximal tubules (3–5). G protein-coupled receptor kinase 4 (GRK4) regulates DRD1 phosphorylation, desensitization and internalization (6,7). Although functional single nucleotide polymorphisms (SNPs) of the GRK4 gene have been associated with hypertension in adult populations (8–11), the impact of these variants on sodium homeostasis in humans remains unknown. Thus we conducted association analyses of the GRK4 polymorphisms in relation to stress-induced $U_{Na}V$ in a sample of young normotensive white and black twins.

METHODS

Study population. A total of 664 normotensive twin subjects including whites and blacks (43.4% blacks) were available for this study. There were 330 monozygotic (MZ) and 334 dizygotic (DZ) twin individuals. Recruitment of twin pairs and zygosity determination has been previously described in the Georgia Cardiovascular Twin Study (12–14). The Institutional Review Board at the Medical College of Georgia approved the study. Informed consent was obtained from subjects or from parents if subjects were under 18 y.

Measurements. Overnight urine was collected for baseline evaluation of $U_{Na}V$. Upon arrival the subjects voided and rested in a supine position for 15 min. Participants were then subjected to a two-hour experimental protocol consisting of an echocardiographic evaluation (15) and three 10-min stress tasks, a social competence interview (16), a virtual reality car driving simulation test (17), and a video game challenge (18) interspersed with recovery periods, and concluded by a urine collection. $U_{Na}V$ was denoted as an excretion rate, *i.e.*, excretion of urinary sodium per unit of time. Systolic BP (SBP) and diastolic BP (DBP) were recorded every other minute during the three stress tasks and averaged to represent stress BP levels (1). SBP and DBP reactivity were calculated as percentage change on SBP and DBP, respectively.

Genotyping. The three functional SNPs, R65L, A142V and A486V, were detected by polymerase chain reaction with restriction fragment length polymorphism analysis. Information about primers and digestion enzymes are available from the corresponding author.

Statistical analyses. All regular association analyses were performed using Generalized Estimating Equations, which take the non-independency of twin data into account and yields unbiased *p*-values (19,20). We first modeled the effects of age, ethnicity, gender and body mass index (BMI) on $U_{Na}V$ variation. After

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Abbreviations: BP, blood pressure; **DBP,** diastolic blood pressure; **GRK4,** G protein-coupled receptor kinase 4; **HTR,** haplotype trend regression; **LD,** linkage disequilibrium; **SBP,** systolic blood pressure; **$U_{Na}V$,** urinary sodium excretion

arriving at the most parsimonious full environmental model including only significant terms, polymorphisms or haplotypes were then added to test for main effects and interactions. Simultaneously modeling gender and ethnicity as factors in our model is more powerful than subgroup analyses, and it also allows us to statistically test for gender and ethnic specific effects of the polymorphisms and haplotypes. Overnight sodium excretion rate was put into the model as a covariate to adjust for the potential effects of different sodium intake. The haplotype trend regression (HTR) approach was used to test for associations of statistically inferred haplotypes with $U_{Na}V$ levels (21–23).

Hardy-Weinberg equilibrium and ethnic differences in allele and genotype frequencies were tested by a χ^2 test in subjects including only one of each twin pair chosen randomly to prevent inflated significance. $U_{Na}V$ during stress was log-transformed to obtain better approximations of the normal distribution. Single locus association analyses and HTR were performed with STATA 8 (StataCorp, College Station, Texas).

RESULTS

General characteristics of 664 study subjects and descriptive statistics by ethnicity and gender are shown in Table 1. Age and height were similar in the two ethnic groups. We found significant interactions between ethnicity and gender for weight ($p = 0.002$) and BMI ($p = 0.004$). In females, weight and BMI were greater in blacks than in whites. Compared with whites, blacks had significantly higher SBP/DBP levels at rest ($p < 0.001$) and stress ($p \leq 0.001$) as well as higher level of overnight $U_{Na}V$ ($p = 0.025$). SBP and DBP reactivity were greater in whites than in blacks, respectively ($p < 0.001$). Of note, there was no statistical difference in $U_{Na}V$ during stress between the two ethnic groups. In comparison with females, males had higher levels of SBP ($p < 0.001$) at rest and under stress. Similarly, overnight and stress levels of $U_{Na}V$ were higher in males than in females ($p \leq 0.005$).

Table 2 shows the allele and genotype distributions in whites and blacks. Allele and genotype frequencies differed significantly between whites and blacks. Strong linkage disequilibrium (LD) was observed between all three SNPs, but none of the SNP pairs were in complete LD ($D' = 0.515 \sim 0.830$). None of the three loci deviated from Hardy Weinberg equilibrium in whites or blacks (all ps$>$0.05).

In single locus association analyses, under the assumption of a dominant effect of the 65L allele, a significant interaction was uncovered between ethnicity and the R65L polymorphism for $U_{Na}V$ in response to stress ($p = 0.01$). As shown in Fig. 1, only in blacks, carriers of the 65L allele compared with R65R homozygotes had a significantly lower level of $U_{Na}V$ during stress (8.42 ± 0.63 *versus* 9.85 ± 0.37 mEq/h, $p = 0.01$). Further, the R65L polymorphism explained about 2% of the total variance in stress $U_{Na}V$ in the blacks. Adjustment for overnight $U_{Na}V$, weight, BMI, gender, and SBP /DBP during stress did not change the significance ($p < 0.05$). No significant main effects of haplotypes were observed on overnight or stress $U_{Na}V$ (results not shown).

DISCUSSION

The observation that blacks had significantly higher SBP/DBP at rest and during stress is consistent with our previous findings and those in the literature. A higher night time $U_{Na}V$ in blacks compared with whites may be due to the higher dietary salt intake. There was no difference in $U_{Na}V$ in response to stress between the two ethnic groups, although stress levels of SBP and DBP were higher in blacks than whites. These suggest an uncoupling between pressure and natriuresis during stress in blacks. Despite higher SBP/DBP at baseline, blacks exhibited lower SBP/DBP reactivity (%) compared with whites, suggesting ethnicity-dependent BP regulatory mechanisms (24).

Of interest, in blacks, even after adjustment for SBP and DBP during stress, and overnight $U_{Na}V$, the 65L allele was significantly associated with a reduction of $U_{Na}V$ in response to stress. As such, sodium handling in the kidney might be impaired in black adolescents with R65L or L65L genotype. The genetic effects were independent from the effects of overnight sodium excretion, a proxy of sodium intake. Of note, overnight urine might not be an ideal specimen for baseline $U_{Na}V$. However, we also collected a urine sample before the stress protocol and found that this prestress $U_{Na}V$ was not significantly different from the overnight $U_{Na}V$ (data not shown). On the other hand, overnight $U_{Na}V$ collected over a longer period of time may provide a more stable measurement. Our finding suggests the need for further research of the influence of the GRK4 gene variation on renal natriuresis in subjects with a sodium-controlled diet.

Table 1. *General characteristics of study subjects*

	Whites		Blacks			
	Males	Females	Males	Females	Ethnicity (*p*)	Gender (*p*)
N (subjects)	195	181	131	157		
Age (years)	17.5 ± 3.4	17.7 ± 3.2	17.3 ± 2.9	17.2 ± 3.5	NS	NS
Height (m)†	1.73 ± 0.10	1.63 ± 0.07	1.74 ± 0.09	1.62 ± 0.06	NS	<0.001
Weight (kg)†	70.2 ± 20.0	59.6 ± 15.1	72.2 ± 18.9	67.4 ± 20.8	*<0.001	<0.001
BMI (kg/m^2)†	23.3 ± 5.1	22.4 ± 4.8	23.7 ± 5.1	25.5 ± 7.1	*<0.001	0.006§
Supine SBP (mmHg)†	113.6 ± 10.6	106.8 ± 8.3	119.1 ± 10.2	111.5 ± 10.5	<0.001	<0.001
Supine DBP (mmHg)†	56.4 ± 6.2	58.9 ± 5.9	60.4 ± 6.8	62.2 ± 7.4	<0.001	<0.001
Stress SBP (mmHg)†	126.7 ± 12.8	116.1 ± 10.9	130.5 ± 11.6	119.0 ± 11.7	0.001	<0.001
Stress DBP (mmHg)†	66.9 ± 7.5	68.4 ± 8.1	69.4 ± 7.3	70.3 ± 7.5	<0.001	NS
SBP reactivity (%)	11.7 ± 6.8	8.4 ± 6.2	10.2 ± 6.4	6.8 ± 6.9	<0.001	<0.001
DBP reactivity (%)	18.8 ± 9.8	15.9 ± 9.1	16.4 ± 9.8	13.5 ± 8.9	<0.001	<0.001
UNaV_n† (mEq/h)	6.05 ± 4.05	4.89 ± 3.40	7.12 ± 5.08	5.48 ± 3.65	0.025	<0.001
UNaV_s† (mEq/h)	9.58 ± 5.02	8.81 ± 4.71	9.94 ± 4.94	7.85 ± 3.56	NS	0.005

Mean ± SD is shown unless indicated otherwise.

NS, not significant; SBP, systolic BP; DBP, diastolic BP; $U_{Na}V\_n$, $U_{Na}V$ overnight; $U_{Na}V\_s$, $U_{Na}V$ during stress.

* Significant only in females; § significant only in blacks.

† Age was adjusted for the evaluation of ethnicity and gender effects.

Table 2. *Genotype and Allele Frequencies of the three SNPs in whites and blacks*

SNPs	Ethnicity	N	Genotype 11	Genotype 12	Genotype 22	*P**	Allele Freq.	*P**
R65L	Whites	271	117	116	38	<0.001	0.653/0.347	<0.001
	Blacks	216	49	121	46		0.507/0.493	
A142V	Whites	274	120	121	33	<0.001	0.659/0.341	<0.001
	Blacks	220	34	110	76		0.405/0.595	
A486V	Whites	274	107	132	35	<0.001	0.631/0.369	<0.001
	Blacks	219	176	40	3		0.895/0.105	

N is the number of subjects including one of each MZ twin pair and both DZ twins of a pair; * p-values are based on tests in one twin of each pair

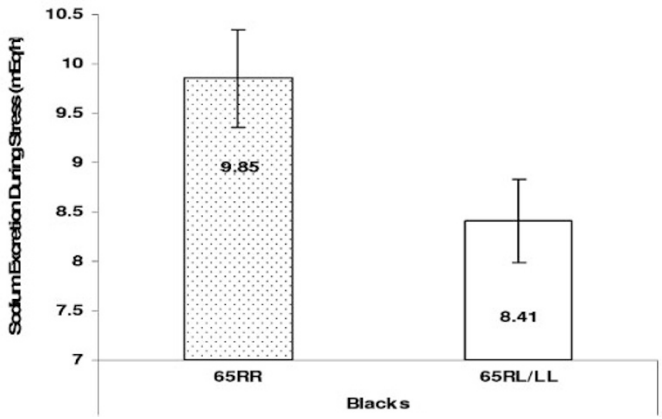

Figure 1. The effect of the 65L allele on $U_{Na}V$ during stress in 216 blacks. $p = 0.01$.

In Chinese hamster ovary cells *in vitro*, the 65L allele was involved in a decrease in cAMP production as well as an increase in DRD1 phosphorylation, which may subsequently inhibit the diuretic and natriuretic effects of renal dopamine (6,7). Further, it was shown that this allele tracked with BP elevation in adult hypertensives (11). Given the high frequency of the 65L allele in blacks (49%), it is likely to have an impact on the development of hypertension in blacks at the population level. A longitudinal study is required to reveal the effects of the GRK4 R65L polymorphism on renal natriuresis and BP development in these black young individuals. It is unclear why the effect of the R65L polymorphism on sodium excretion was not observed in whites. Our recent data indicate that impaired SIPN is approximately twice as prevalent in black as in white youth (40% *versus* 20%) (Harshfield *et al.*, Annual Meeting of Society of Behavioral Medicine, April 13–16, 2005, Boston). The genetic and environmental determinants of salt sensitivity and pressure natriuresis are speculated to be race-related (25).

A genetic predisposition to retain sodium is hypothesized as one possible reason for salt sensitive hypertension, especially in the black population. This present study has taken this hypothesis a step further by examining the interaction between genes and environmental stress in this process. $U_{Na}V$ in response to stress is a new phenotype of sodium homeostasis and BP, which can be used in candidate gene association studies. Increased understanding of the contribution of GRK4 will further elucidate the importance of the dopaminergic pathway in BP regulation. Screening GRK4 gene variants might identify a subset of individuals that are particularly susceptible as early as possible, so preventative strategies, *e.g.*, low-salt diets and stress management training can be taken.

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