
The Georgia Cardiovascular Twin Study: Influence of Genetic Predisposition and Chronic Stress on Risk for Cardiovascular Disease and Type 2 Diabetes

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The Georgia Cardiovascular Twin Study is a longitudinal study of biobehavioral antecedents of cardiovascular disease in youth and young adults, including around 500 twin pairs with roughly equal numbers of African Americans and European Americans. Focus of study includes the longitudinal change in relative influence of genetic and environmental factors (especially chronic stress) on development of risk factors for cardiovascular disease. Approaches include quantitative genetic modeling of phenotypic twin data as well as the examination of the influence of polymorphic variation in candidate genes and their potential interaction with environmental factors on these risk factors. Future work will expand the scope of the study to investigating the impact of chronic stress as measured by indices of the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system on preclinical markers of cardiovascular disease, essential hypertension and type 2 diabetes.

Major Research Focus

The primary aim of the Georgia Cardiovascular Twin Study is to investigate longitudinal changes in relative influence of genetic and environmental factors on development of biobehavioral risk factors for cardiovascular disease in both European American (EA) and African American (AA) youth during their transition into young adulthood (Snieder & Treiber, 2002). This includes the examination of the influence of polymorphic variation in candidate genes and their potential interaction with environmental factors on these risk factors. Unique aspects of this study include its longitudinal design and biethnic sample. The evaluation over a crucial time span capturing the transition from childhood into adulthood permits examination of potential changes over time in the contributions of environmental (e.g., chronic stress) and genetic factors upon key components of our model of stress-induced essential hypertension (Snieder et al.,

2002a). This model includes both short- and long-term blood pressure (BP) regulatory pathways by which repeated exposure to stress may lead to increased BP at rest and eventually to overt manifestation of essential hypertension. The two primary intermediate components of the model were BP reactivity to acute behavioral stress and (impaired) stress-induced sodium excretion ($U_{Na}V$), representing the cardiovascular and renal stress response, respectively.

Chronic stress, in conjunction with genetic predisposition, contributes to dysregulation of regulatory pathways required for adaptation to environmental demands. The resulting cumulative burden on the body, or 'allostatic load', has been linked to the development of chronic diseases (McEwen, 1998; McEwen & Seeman, 1999). Continued follow-up of the Georgia Cardiovascular Twin Study provides an opportunity to test the allostatic load hypothesis that an overactive hypothalamus pituitary adrenal (HPA) axis is a precursor of the metabolic syndrome (Bjorntorp & Rosmond, 1999; Chrousos, 2000; McEwen & Seeman, 1999). To this end, we have expanded our biobehavioral model of stress-induced essential hypertension as presented in Figure 1. First, in addition to the sympathetic nervous system (SNS) we have included the HPA axis as another major mediator of the stress response (Henry & Grim, 1990) as described recently (Imumorin et al., 2005). Second, we have added the metabolic syndrome (ATP III, 2002) as a major intermediate outcome in the pathway from chronic stress to the clinical endpoints of cardiovascular disease and type 2 diabetes.

Recruitment and Zygosity Determination

The twin pairs were recruited through announcements in local media and flyers distributed to public

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middle and high schools within 120 miles of the study location (Augusta, Georgia, United States). Twins have been seen three times between 1997 and 2005, and we intend to see the participants on two additional occasions in the next 5 years (2007–2012). New recruitment will be limited to compensate for potential dropouts. Zygosity of all same-sex pairs has been determined by DNA fingerprinting (Jackson et al., 2001).

Major Achievements

Quantitative Genetics: Cross-Sectional Findings

We have investigated the relative influence of genetic and environmental factors for a number of components of our model outlined in Figure 1.

In the first twin study on lipid profile to include African Americans (Iliadou et al., 2005), heritabilities ranged from 69% to 92%, showing no ethnic differences except for low-density lipoprotein (LDL), where AAs exhibited higher estimates.

Pulse Wave Velocity (PWV) is an accurate, noninvasive measure of arterial stiffness and an independent predictor of cardiovascular morbidity and mortality (Asmar et al., 1995; Safar et al., 2002). We showed substantial heritabilities of .43 and .53 for radial and foot PWV, respectively, which could be partially explained by genes for diastolic blood pressure (DBP). Heritability

estimates did not show any differences between blacks and whites or males and females (Snieder et al., 2005).

In another study we examined the genetic and/or environmental origin of variation and covariation of perceived stressful life events and two stress-related coping styles, anger expression and John Henryism (Wang, Trivedi, et al., 2005). We found individual differences in coping styles and life events in youth can be explained by moderate genetic and substantial environmental influences, of which most are idiosyncratic to the individual. The association between anger expression and life events is largely the result of common genes.

Stress-induced $U_{Na}V$ is a key component of our model of stress-induced essential hypertension (Imumorin et al., 2005) and can be measured as stress $U_{Na}V$ level and as the $U_{Na}V$ response to stress ($\Delta U_{Na}V = \text{stress } U_{Na}V - \text{baseline } U_{Na}V$). For these variables we showed substantial heritabilities ranging from .38 to .57. Thus, we established these two measures as heritable phenotypes that may be used to study the genetic etiology of salt sensitivity in relation to the early development of hypertension.

Quantitative Genetics: Longitudinal Findings

Longitudinal analyses of data collected from our Georgia Cardiovascular Twin Study has recently yielded unique results for hemodynamic variables (Kupper et al., 2006), as well as for left ventricular

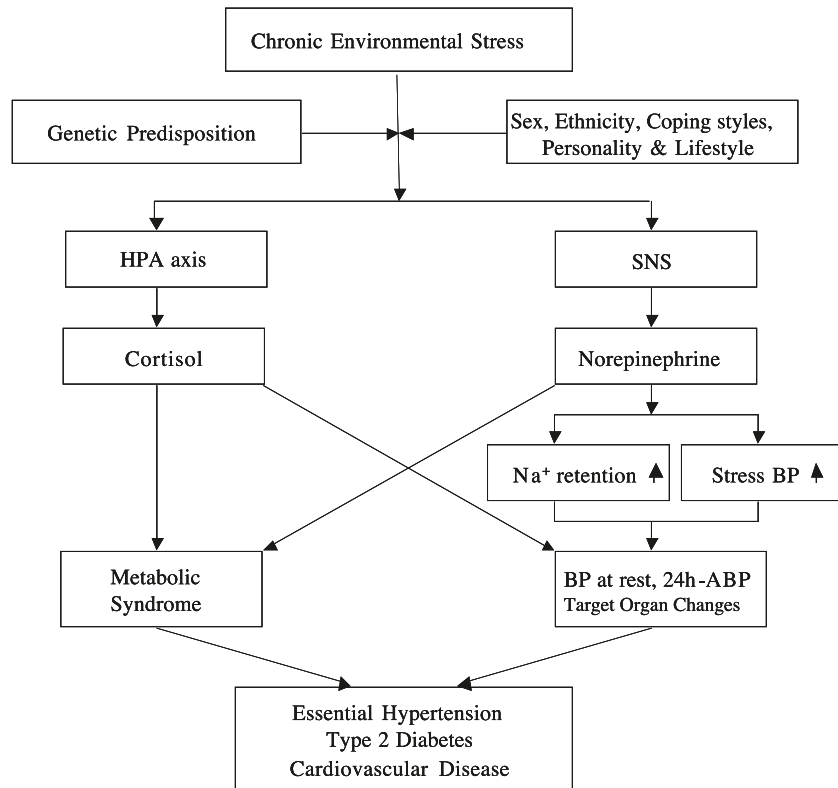


Figure 1

Effects of chronic stress on essential hypertension, type 2 diabetes and cardiovascular disease. HPA axis, hypothalamus pituitary adrenal axis; SNS, sympathetic nervous system; BP, blood pressure; ABP, ambulatory BP; Na⁺, sodium.

mass, a measure of target organ change (Ge et al., 2005) for the period between 14 and 18 years of age, showing the emergence of substantial new genetic variance in both AAs and EAs. For the hemodynamic variables, the magnitude of heritability estimates was relatively stable over time (around 50%), however, for both systolic blood pressure (SBP) and DBP environmental influences became larger in AAs compared to EAs. Although our primary focus is on environmental influences, particularly on chronic stress and how its impact may vary by ethnicity, these results also have important implications for gene finding studies and suggest new avenues of future investigation.

Heart Rate Variability

We have recently proposed that the parasympathetic nervous system may be an important additional system involved in stress-mediated risk of essential hypertension (Imumorin et al., 2005). Heart rate variability (HRV), a simple noninvasive measure of cardiac autonomic function, has been used as an indicator of cardiovascular health. Reduced HRV, reflecting a shift in cardiac sympathovagal balance from parasympathetic to sympathetic control of the heart rhythm (La Rovere et al., 2002; Schwartz et al., 1988), is a predictor of all-cause mortality, arrhythmic events and sudden death after acute myocardial infarction (Bigger et al., 1993; Kleiger et al., 1987; Reinhardt et al., 1996) as well as in the general population (Dekker et al., 1997; Tsuji et al., 1996). Previous studies in twins (Boomsma et al., 1990; Busjahn et al., 1998; De Geus et al., 2003; Kupper et al., 2004; Snieder et al., 1997) and families (Singh et al., 1999; Sinnreich et al., 1999) in Caucasian populations have found that up to 65% of the variance in HRV can be explained by genetic influences. As part of our Georgia Cardiovascular Twin Study, we recently conducted the first comparison of heritability of HRV at rest between black and white Americans. We measured HRV parameters in 42 black and 61 white twin pairs over 256 heart beats (i.e., RR intervals) in a supine position. Three HRV indices, SDNN (standard deviation of normal RR intervals), RMSSD (root mean square of successive differences in RR intervals) and HF (high frequency component of the power spectrum), showed strong correlations (greater than .8) so we created a combined HRV score by factor analysis. Heritability of this combined HRV score was 70%, (95% confidence interval [CI]: 57%–80%) and did not differ between blacks and whites (Wang, Thayer, et al., 2005). Recent evidence suggests that the effect of genes influencing HRV at rest are amplified under behavioral stress (De Geus et al., 2006). We aim to capitalize on these finding and use HRV measured at both rest and stress for the identification of genetic variants underlying cardiac autonomic function.

Candidate Gene Studies

We have performed a number of candidate gene studies investigating the genetic basis of BP regulation and hypertension risk using the Georgia cardiovascular twin

cohort. We first investigated the effects of variation of candidate genes in the sympathetic nervous system such as two polymorphisms (Arg16Gly and Gln27Glu) in the β 2-adrenergic receptor gene (ADRB2). For the Arg16Gly polymorphism, carriers of one or two Gly alleles exhibited significantly higher levels of SBP and pulse pressure in EAs. Carriers of the Glu allele of the Gln27Glu polymorphism showed an elevated SBP and DBP, mean arterial pressure, total peripheral resistance index, and a lower stroke volume in EAs. These findings suggested that vasodilatory related genetic factors play a particularly important role in BP control in EA youths (Snieder et al., 2002b).

Dopamine facilitates vasodilatation and natriuresis, exerting antihypertensive function. The dopaminergic signaling pathway consists of two key components: dopamine receptors (DRD) and G proteins. Therefore, we studied whether the A-48G polymorphism of the DRD1 gene and the T393C polymorphism of the Gs protein α subunit (GNAS) gene influenced BP levels at rest and in response to stress. A dominant BP lowering effect of the -48G allele was observed on DBP at rest and in response to a car driving stressor. The 393C allele was associated with higher SBP levels during a social competence interview and the car driving stressor ($p = .016$; Lu et al., 2006).

G protein-coupled receptor kinase 4 (GRK4) is involved in activity of dopamine receptors in renal proximal tubules and thus mediates sodium reabsorption and BP regulation. We evaluated the impact of the GRK4 gene variants on BP levels in normotensive adolescents and young adults and our data indicated that the R65L polymorphism of the GRK4 gene plays a role in BP regulation in these subjects (Zhu et al., 2006). Furthermore, in AAs only, compared to R65R homozygotes, individuals with R65L or L65L genotype had significantly lower levels of stress-induced $U_{Na}V$ (9.85 ± 0.37 vs. 8.42 ± 0.63 mEq/h, $p = .01$). As such, the 65L allele of the GRK4 gene seems to be associated with impaired stress-induced $U_{Na}V$ in this ethnic group (Zhu et al., 2006).

Future Plans

Continued follow-up of our biethnic cohort of twins will enable investigation of potential changes over time in the contributions of chronic environmental stress and genetic factors upon longitudinal development of key components of our biobehavioral model of stress-induced essential hypertension. Our expanded model outlined in Figure 1 will enable us to determine whether activation of HPA axis and SNS are pathways through which chronic environmental stress may exert its influence on preclinical markers of essential hypertension, target organ changes and the metabolic syndrome.

Ultimately, increased understanding of the role of environmental stress-related factors and their underlying physiological mechanisms as potential contributors to health disparities between AAs and

EAs will assist in earlier and better identification of youth and young adults at increased risk for cardiovascular disease and type 2 diabetes and will aid the development of more personalized primary prevention programs involving lifestyle interventions (e.g., low sodium diets, physical activity programs, stress management programs) in which the role of chronic stress will be taken into account to prevent early onset of these diseases (Barnes et al., 2001; Barnes et al., 2004a; Barnes et al., 2004b), similar to the way this is currently being done in secondary prevention trials (Koertge et al., 2003).

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Appendix A

Georgia Cardiovascular Twin Study

Name of register	Georgia Cardiovascular Twin Study
Country	Georgia, USA
Kind of Ascertainment	Schools
Opposite-sex twins (yes or no)	Yes
Number of pairs (separated by birth range and sex)	European Americans: 272 (102 mm, 103 ff, 67 mf) African Americans: 219 (73 mm, 99 ff, 47 mf)
Primary interest	Longitudinal development of biobehavioral antecedents of cardiovascular disease and type 2 diabetes in youth
Variables measured (+number of pairs in total)	Biobehavioral risk factors of cardiovascular disease measured in all twins
DNA/blood samples (+number of pairs)	DNA and blood samples in all twins
Contact	Harold Snieder
Institution	Georgia Prevention Institute, Medical College of Georgia
Address	Building HS-1640
Tel	(706) 721 4534
Fax	(706) 721 7150
E-mail	hsnieder@mcg.edu or dge@mcg.edu
Webpage	http://www.mcg.edu/institutes/gpi
Major publications	Kupper et al. (2006) Emergence of novel genetic effects ... <i>Hypertension</i> , 47, 948–954 Zhu et al. (2006) The G protein-coupled receptor kinase 4 gene... <i>Am J Hypertens</i> , 19, 61–66. Wang, Trivedi, et al. (2005) Genetic and environmental influences... <i>Psychosom Med</i> , 67, 16–23. Snieder et al. (2003) Heritability of blood pressure... <i>Hypertension</i> , 41, 1196–1201
Major sources of funding	National Heart Lung and Blood Institute (HL56622)
Comments	Twins are followed longitudinally

