Heritability of Hemodynamic Reactivity to Laboratory Stressors in a Homogenous Arab Population: 'Oman Family Study'

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Background: Exaggerated cardiovascular reactivity to stressful stimuli may be a risk factor for the development of hypertension. The genetic influence on blood pressure (BP) reactivity to stress and its control mechanisms has been receiving considerable support. This study aims at examining the heritability of BP and its intermediate hemodynamic phenotypes to acute stress in a homogeneous Arab population. *Methods:* Parameters were computed from continuous BP, electrocardiography and impedance cardiography measurements, during rest, word conflict (WCT) and cold pressor (CPT) tests. Heritability estimates (h^2) were obtained using the variance components-based approach implemented in the SOLAR software package. *Results:* Reactivity scores for WCT and CPT increased significantly ($P < .05$) for systolic (SBP), diastolic (DBP), heart rate (HR), cardiac output (CO), and total peripheral resistance (TPR). They decreased significantly ($P < .05$) for stroke volume (SV), left ventricular ejection time (LVET), end diastolic (EDI) and cardiac contractility (IC) indices. Univariate analysis detected heritability estimates that ranged from 0.19–0.35 for rest, 0.002–0.40 for WCT and 0.08–0.35 for CPT. *Conclusion:* In this unique cohort, resting as well as challenged cardiovascular phenotypes are significantly influenced by additive genetic effects. Heritability estimates for resting phenotypes are in a relatively narrow range, while h^2 for their reactivity is somewhat broader with lower estimates. Further analyses of this study may offer important opportunities for gene finding in hypertension. *What is Known About the Topic:* (1) cardiovascular reactivity to stress predicts cardiovascular disease; (2) genetic susceptibility plays an important role in stress reactivity. Family studies using the cold pressure test reported significant heritability for blood pressure. *What this Study Adds:* (1) this cohort is from five highly consanguineous isolated Arab pedigrees with genetically verified genealogical records and environmental homogeneity; (2) This is the first study to estimate heritability of detailed intermediate hemodynamic phenotypes that make up normal blood pressure.

Keywords: heritability, hemodynamics, Arab population

Stress reactivity, defined as an exaggerated cardiovascular response (Δ) to a behavioral or psychological challenge, may play a role as a marker or mechanism in the prediction and pathogenesis of essential hypertension and cardiovascular disease (Bacon et al., 2006; Manuck et al., 1996). The concept of stress reactivity has a long history (Folkow, 1987; Obrist et al., 1974); however, the realization that genetic susceptibility plays an important role in stress reactivity is more recent (Light, 2001; Snieder et al., 2002; Treiber et al., 2003). Characterizing genetic influences of blood pressure reactivity to a specific short-term environmental stressor came largely from twin cohorts and from a few family studies, results of which varied across studies and across tasks within the same study. These differences were attributed to study design, cohort size and analytical methods (Turner et al., 1992). Family studies using the cold pressor test (CPT), reported significant heritability (h^2) for systolic BP reactivity (ΔCPT SBP; Choh et al., 2005) and for Δ CPT SBP and DBP (Mitchell et al., 2008). Twin studies using mental stress tests reported significant h^2 for ΔSBP but not for Δ DBP (Busjahn et al., 1996; de Geus et al., 2007). Studies using stress to expose genetic variance focused mainly on the reactivity of BP in normal and /or probands of hypertensive subjects (Gottesman & Gould, 2003; Hassan et al., 2001; Rice et al., 1999). As BP itself is a complex phenotype controlled by several regulatory mechanisms, it was proposed that the study of intermediate traits, as compared to the final disease state, may have several advantages for the genetic dissection of complex diseases such as hypertension (Sing et al.,

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2003). In the Georgia Cardiovascular Twin Study (GCTS) of European (EA) and African–American (AA) youths, h^2 of hemodynamic intermediate phenotypes was estimated twice after an interval of 4 years (Kupper et al., 2006). However, using acute mental stress in the same cohort of the GCTS, Snieder et al. (2005) showed an inconsistent pattern of reactivity h^2 of the same hemodynamic parameters to different stressors within the same ethnic group and across ethnic groups.

The main aim of the first phase of Oman Family Study (Hassan et al., 2005) was to determine genetic influences on the response of BP and its intermediate phenotypes to acute mental and physical stress in large homogeneous isolated pedigrees. Detailed h^2 of ambulatory BP, anthropometric and metabolic phenotypes were reported earlier (Bayoumi et al., 2007). Here we report h^2 for resting and for cold pressor and mental stress reactivity of beat-to-beat hemodynamic phenotypes.

Study Area and Pedigrees

Five large, extended and highly consanguineous families, each living in a separate village, were selected within a perimeter of 20 km around Nizwa (Table 1). The number of subjects interviewed and found eligible for the study in the five pedigrees was 327, 160, 230, 279 and 281, totaling 1277 which represented roughly 10–15% of the total number of individuals in these 5 pedigrees. They were 16–80 years old and all voluntarily took part in the study, appeared healthy and had no clinical complaints as administered in the questionnaire. First cousin marriages represent > 50% of all marriages (Hassan et al., 2005; Sulaiman et al., 2001). Polygamy is widely practiced with some men marrying up to 4 wives. The consequent rapid population growth produced these fairly young isolates of 7–12 generations each. A more detailed description of the stratification of the cohort and the Oman Family study design can be found in earlier report (Bayoumi et al., 2007; Hassan et al., 2005). Prevalence of hypertension was 22% (Males 22%, Females 18%) with 2% of both genders on medication. Exclusion criteria were pregnancy, malignancy, renal failure, heart failure and myocardial infarction/stroke within 6 months. A written and signed or thumb print rubber-stamped consent was obtained from each subject. The Study was approved by the Medical Research and Ethics Committee of Sultan Qaboos University.

Phenotype Definitions

Anthropometric Measurements

After explanation, a 20-minute questionnaire was then administered by trained male and female volunteers from each village. Body mass index and waist circumference were measured using standard methods. Body fat percentage (Fat%) was estimated using electrical impedance (Tanita, Japan).

Hemodynamic Measurements

Hemodynamic measurements were compiled using direct and derived signals computed within the Task Force Monitor (TFM, CNSystems, Austria). Non-derived direct signals of the TFM were HR obtained from lead II of a 6-lead electrocardiogram (ECG), beat-to-beat BP and the impedance signal. Beat-to-beat BP was acquired by the vascular unloading technique. Finger cuff readings were automatically counterchecked and corrected every minute by the oscillometric BP measurements recorded from the contra lateral upper arm (Gratze et al., 2005; Skrabal et al., 2004). The TFM displayed beat-by-beat hemodynamic parameters and their average values in graphical and digital format.

Impedance Cardiography

Derived hemodynamic parameters were computed from continuous BP, HR and the impedance signal (Fortin et al., 2001; Gratze et al., 2005; Skrabal et al., 2004).

The impedance signal was acquired from a small constant sinusoidal alternating current passing through the thorax between an electrode placed around the neck and another placed at the lower end of the sternum. The voltage between the electrodes is proportional to the thorax impedance. Left ventricular ejection time (LVET msec), the time between points 'B' and 'X' (opening and closure of aortic valve, respectively) of the impedance signal, was considered in further calculations of hemodynamic parameters using the standard Kubisek's formula (Kubicek et al., 1974). Hemodynamic parameters calculated and indexed for body surface area were stroke volume and index (SV, SI), cardiac output and index (CO,CI), total peripheral resistance and index (TPR, TPRI), end-diastolic index (EDI) and index of cardiac contractility(IC).

Laboratory Stress Tests

Word Conflict Test (WCT)

The word conflict test involves sensory rejection of names of a spectrum of colors but written in colors different from that of the color itself (Stroop, 1935). The right cerebral hemisphere recognizes the colors and the left names the word. The verbal narration of the conflict of words and colors forms the basis of the WCT. This creates cerebral confusion and invokes cardiovascular responses through central cerebral stimulation (Fauvel et al., 1996).

The original English names of colors were translated into Arabic using the same incongruent colors and were displayed on a monitor. The observer selected the words at a constant speed of one word/sec for a period of 3 minutes. The subject was asked to vocalize the color of the word and not read the word.

Cold Pressor Test (CPT)

The CPT is based on stimulation of pain receptors which induces cardiovascular reactions (Wolff, 1951). The left foot was immersed in cold water with crushed ice (4° C) up to the ankle joint for 3 minutes. Foot

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rather than hand immersion in this study was because both hands were used for BP measurements (Houben et al., 1982). All but 5 subjects completed the CPT protocol of 3 minutes, due to intolerable pain.

Experimental Protocol

After an overnight fast subjects reported to the field research centre at 07:00 hours. After explaining the procedure, the TFM electrodes were attached and subjects were made to rest supine for 10 minutes on a comfortable bed in a quiet room with a temperature of 24–26^{0}C, Recordings were then acquired in the supine position as follows: 10 minutes of rest, 3 minutes of WCT, 3 minutes of recovery or until recording returned to baseline, and 3 minutes of CPT. Tests were administered by the same male and female research assistants for the respective gender throughout the study.

Statistics

Beat-to-beat measurements were averaged for rest and stress periods. Reactivity (Δ) was the difference between average resting and average stress values.

Descriptive and comparative analyses were performed using SPSS package (version 13.0). Parametric data were expressed as means ± *S*D. Probability value of < .05 was considered statistically significant.

The Student's paired *t* test was used to calculate differences between hemodynamic parameters at rest and during stress conditions and their gender differences. Pearson's correlation test was used to correlate age, ΔWCT and ΔCPT.

Heritability Analysis

Table 1 shows the total number of relative pairs used in analysis. The large number of relative pairs of 25104 is due to the very high degree of inbreeding and complexity of the pedigrees. Heritability for all hemodynamic parameters during rest and reactivity was computed using the maximum likelihood variance decomposition method implemented in SOLAR 2.1.4 (Almasy & Blangero, 1998). The covariance matrix for each continuously distributed quantitative trait in a pedigree is given by:

$$\Omega = 2\Phi\sigma^2_G + I\,\sigma^2_E$$

Where Φ is the n × n matrix of kinship coefficients that structures σ^2_G, the variance due to additive genetic effects; and I is the identity matrix of order n that serves as the structuring matrix for σ^2_E which is the variance due to unmeasured, non-genetic factors. Heritability, defined as the proportion of the phenotypic variance attributable to additive genetic effects, is estimated as:

$$h^2 = \sigma^2_G / \sigma^2_P$$

where σ^2_G is the additive genetic variance and σ^2_P is the phenotypic variance.

The trait mean, and mean effects of age, sex, age^2, age*sex and age^2*sex were also simultaneously estimated. Significance of h^2 was determined using likelihood ratio test. Because of intermarriages between the 5 pedigrees SOLAR considered all subjects in the cohort as one family. The trait mean, and mean effects of age, sex, age^2, age*sex and age^2*sex were also simultaneously estimated for heritability of resting and reactivity values. Significance of h^2 was determined using likelihood ratio test.

Table 1

Relative Pairs in the Five Pedigrees

Relationships	Size
Pedigree members	1851
Parent-offspring	2482
Siblings	1278
Grandparent-grandchild	3774
Avuncular	2815
Half-siblings	322
Grand avuncular	2928
Half avuncular	918
1st cousins	2610
1st cousins, 1 removed	932
Half 1st cousins, 1 removed	441
2nd cousins	4363
Other relationship	390
Total	25104

Results

Table 2 shows anthropometric and average beat-to-beat values of cardiac and hemodynamic parameters during rest, WCT and CPT interventions. It is important to note the younger age and the smaller number of participants during WCT which is due to illiteracy, especially of older females. Reactivity during WCT and CPT is shown as a change score ΔWCT and ΔCPT, respectively. Significant increases in SBP, DBP and MBP during WCT and CPT mirror the increases of the BP components; HR, CO and TPR. Conversely, cardiac parameters; SV, SI, LVET, EDI and IC were significantly reduced during both tests.

Table 3 shows gender differences of the WCT and CPT groups. There were significant gender difference between ΔWCT and ΔCPT groups for fat%, HR, SBP, CO (P <0.05) and no differences for TPR and IC.

Correlations between ΔWCT and ΔCPT for HR, SBP, DBP, MBP, CO, CI, TPR, TPRI and LVET were weak (r = 0.30–0.40; P = .0001) while correlations for SV, SI, EDI and IC were moderate (r = 0.47–0.49; P = .0001). Except for BP which was not significant, all ΔWCT and ΔCPT hemodynamic phenotypes were weakly correlated with age (r = 0.06–0.17, P < .05).

Table 4 shows the univariate results of the heritability estimates, significance levels and covariate effects of the hemodynamic phenotypes during rest, ΔWCT and ΔCPT. With the exception of a few, significant (P < .05) h^2 during rest, ΔWCT and ΔCPT were

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Table 2

Anthropometric and Hemodynamic Parameters at Rest, WCT, CPT and Their Respective Reactivity Scores (**WCT, CPT**)

Variable	Rest *N* = 683	WCT *N* = 683	Δ WCT	Rest *N* = 1277	CPT *N* = 1277	Δ CPT
Age (years)	23. 2 (6.7)*$			33.5 (15.8)		
Waist (cm)	74.9 (12.5)	—		80.9 (14.4)	—	
BMI (kg/m^2)	23.5 (5.1)	—		25.0 (5.3)	—	
Body fat (%)	19.6 (9.8)	—		23.4 (10.5)	—	
HR (beats/min)	72.0 (11.1)	79.0 (12.5)**	7.6	71.0 (10.7)	77.0 (12.1)*	6.2
SBP (mmHg)	112.0 (14.7)	120.0 (15.3)**	8.7	112. (15.2)	127.0 (17.30)***	14.8
DBP (mmHg)	70.0 (11.8)	77.0 (12.7)**	7.0	71.0 (12.1)	84.0 (13.9)***	13.2
MBP (mmHg)	82.0 (13.1)	90.0 (13.9)**	8.1	83.0 (13.2)	98.0 (15.2)***	14.7
SV (ml)	82.6 (16.8)	80.9 (16.3)**	–1.6	75.1 (18.4)	72.2 (16.9)***	–2.9
SI (ml/beat/m^2)	51.4 (10.2)	50.3 (9.4)**	–1.1	46.5 (11.4)	44.6 (10.1)***	–1.9
CO (L/min)	5.9 (1.4)	6.4 (1.6)**	0.5	5.3 (1.4)	5.5 (1.4)***	0.2
CI (L/min/m^2)	3.7 (0.9)	4.0 (1.0)**	0.3	3.3 (0.9)	3.4 (0.9)***	0.1
TPR, (dyne*s*/cm^2)	1130.5 (302.8)	1150.0 (303.8)**	18.3	1297.7 (407.5)	1463.1(432.4)***	167.3
TPRI (dyne*s*m^2/cm^2)	1841.0 (574.0)	1868.6 (564.5)**	25.8	2122.1 (735.8)	2392.3 (783.1)***	275.9
LVET (ms)	311.1 (16.8)	300.4 (17.9)***	–10.7	311.9 (17.7)	306.1 (17.7)***	–5.6
EDI (ml/m^2)	80.0 (15.0)	78.4 (13.8)***	–1.6	72.5 (16.6)	70.1 (15.1)***	–2.4
IC (1000/sec)	67.8 (21.3)	67.7 (20.8)	–0.1	57.9 (22.9)	55.1 (20.6)***	–2.8

Note: Values are means (± *SD*); $; Age difference of WCT and CPT subjects. *P = .01; ** P = .001; ***P = .0001

Abbreviations: WCT, word conflict test; CPT, cold pressor test; HR, Heart rate; SBP: Systolic BP; DBP: Diastolic BP; MBP: Mean BP; SV: Stroke volume; SI: Stroke index; CO: Cardiac output; CI: Cardiac index; TPR: Total peripheral resistance; TPRI: Total peripheral resistance index; LVET: Left ventricular ejection time; EDI: End diastolic index; IC: Index of cardiac contractility; WCT: Difference at WCT from rest; CPT: Difference at CPT from rest

Table 3

Gender Difference in Age, Anthropometric Parameters and in Reactivity to WCT and CPT

Variable	WCT group (*N* = 683)		*P*	CPT group (*N* = 1277)		*P*
	Male (*N* = 330)	Female (*N* = 353)		Male (*N* = 562)	Female (*N* = 715)	
Age (years)	23. 3 (8.0)	23.0 (5.2)	NS	32.3 (16.1)	34.3 (15.0)	0.03
Waist (cm)	76.2 (12.8)	73.6 (12.1)	0.014	80.9 (14.4)	80.7 (14.5)	NS
BMI (kg/m^2)	23.6 (4.9)	23.4 (5.3)	NS	24.8 (5.0)	25.2 (5.7)	NS
Body fat (%)	15.1 (7.8)	23.7 (9.5)	0.0001	17.9 (8.5)	27.7 (9.9)	0.0001
		Δ WCT			Δ CPT	
HR (beats/min)	9.0 (7.9)	6.0 (6.5)	0.0001	5.0 (8.0)	7.0 (8.5)	0.0001
SBP (mmHg)	10.0 (10.7)	8.0 (9.5)	0.006	14.0 (14.4)	16.0 (13.4)	0.01
DBP (mmHg)	8.0 (8.2)	6.0 (7.8)	0.02	13.0 (11.8)	14.0 (11.1)	NS
MBP (mmHg)	9.0 (9.2)	7.0 (8.6)	0.004	14.0 (13.1)	15.0 (12.1)	NS
SV (ml)	–2.4 (9.6)	–0.9 (7.5)	0.03	–3.2 (9.4)	–2.7 (7.9)	NS
SI (ml/beat/m^2)	–1.5 (5.6)	–0.7 (4.8)	0.04	–1.9 (5.3)	–1.8 (5.1)	NS
CO (L/min)	0.6 (0.9)	0.4 (0.7)	0.004	0.1 (0.7)	0.3 (0.6)	0.001
CI (L/min/m^2)	0.4 (0.5)	0.3 (0.4)	NS	0.08 (0.4)	0.1 (0.4)	0.0001
TPR, (dyne*s*/cm^2)	9.8 (188.7)	28.6 (155.6)	NS	168.2 (253.1)	163.1 (232.7)	NS
TPRI (dyne*s*m^2/cm^2)	13.9 (321.1)	40.2 (236.8)	NS	293.3 (431.5)	251.5 (361.6)	NS
LVET (ms)	–11.4 (11.7)	–9.9 (10.6)	NS	–3.5 (12.9)	–7.6 (15.6)	0.0001
EDI (ml/m^2)	–2.8 (7.6)	–0.6 (7.3)	0.0001	–2.7 (7.5)	–2.1 (7.2)	NS
IC (1000/sec)	–0.9 (9.6)	0.6 (9.6)	NS	–3.3 (8.7)	–2.3 (9.0)	NS

Note: Abbreviations: WCT, word conflict test; CPT, cold pressor test; HR, Heart rate; SBP: Systolic BP; DBP: Diastolic BP; MBP: Mean BP; SV: Stroke volume; SI: Stroke index; CO: Cardiac output; CI: Cardiac index; TPR: Total peripheral resistance; TPRI: Total peripheral resistance index; LVET: Left ventricular ejection time; EDI: End diastolic index; IC: Index of cardiac contractility; ΔWCT: Difference at WCT from rest; ΔCPT: Difference at CPT from rest.

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Table 4

Heritability Estimates (h^2) of Anthropometric, Resting and Hemodynamic Reactivity Phenotypes

	Rest				ΔWCT				ΔCPT			
	h^2	SEM	*P*	Cov	h^2	SEM	*P*	Cov	h^2	SEM	*P*	Cov
Anthropometric parameters												
Waist	0.31	0.056	$1.0x10^{-6}$	0.24								
Weight	0.68	0.052	$4.3x10^{-24}$	0.22								
BMI	0.68	0.051	$2.1x10^{-12}$	0.2								
Fat %	0.56	0.056	$1.0x10^{-18}$	0.05								
Hemodynamic parameters												
HR	0.31	0.056	$6.6 x10^{-17}$	37.2	0.40	0.088	$3.3x10^{-9}$	8.5	0.35	0.065	$1.3x10^{-13}$	6.5
SBP	0.19	0.054	$3.8x10^{-6}$	18	0.22	0.083	$9.0x10^{-4}$	0.6	0.13	0.048	0.001	1.6
DBP	0.19	0.051	$1.2x10^{-6}$	21.1	0.09	0.068	0.07	1.3	0.10	0.047	$7.4x10^{-3}$	2.2
MBP	0.19	0.051	$1.0x10^{-6}$	21.2	0.12	0.072	0.03	0.9	0.13	0.049	$8.0x10^{-4}$	1.7
SV	0.30	0.055	$3.2x10^{-15}$	38.6	0.15	0.079	$1.5x10^{-2}$	5.0	0.15	0.051	$2.0x10^{-4}$	7.7
SI	0.32	0.054	$1.1x10^{-15}$	33.9	0.15	0.079	$1.4x10^{-2}$	5.2	0.15	0.051	$2.0x10^{-4}$	7.6
CO	0.26	0.054	$6.1x10^{-13}$	31.6	0.30	0.094	$5.9x10^{-5}$	0	0.11	0.047	$2.0x10^{-3}$	5.3
CI	0.26	0.051	$1.9x10^{-11}$	33.6	0.30	0.094	$5.9x10^{-5}$	0	0.12	0.048	$2.0x10^{-3}$	5.2
TPR	0.19	0.041	$6x10^{-7}$	29.8	0.01	0.076	0.4	0.6	0.10	0.051	0.011	3.1
TPRI	0.21	0.044	$2x10^{-7}$	35.2	0.002	0.075	0.5	1.0	0.08	0.049	0.036	3.0
LVET	0.35	0.055	$2.4x10^{-17}$	2.9	0.17	0.075	$3.1x10^{-3}$	2.7	0.22	0.057	$8.0x10^{-7}$	3.7
EDI	0.32	0.054	$2.9x10^{-16}$	37.2	0.21	0.082	$9.0x10^{-4}$	6.5	0.18	0.054	$4.7x10^{-5}$	8.1
IC	0.32	0.053	$3.4x10^{-16}$	37.5	0.20	0.088	$4.5x10^{-3}$	3.4	0.11	0.078	$3.4x10^{-3}$	8.4

Note: ΔWCT: world conflict test reactivity, ΔCPT: cold pressor test reactivity, Cov: covariates

detected for most of the phenotypes listed. It is important to note that h^2 ΔWCT and ΔCPT for the phenotypes associated with cardiac contractility and systolic blood pressure; HR, SV, SI, CO, CI, LVET and EDI were significant ($P < .05$) and ranged from 0.11–0.40 as compared to those related to diastolic blood pressure; MBP, TPR and TPRI which ranged from 0.002–0.13. Heritability estimates for ΔWCT DBP, TPR and TPRI were, however not significant. Covariates of all the phenotypes accounted for 18.0–38.6% of the variation during rest, for 0.00–8.5% during ΔWCT and for 1.6–8.4% during ΔCPT.

Discussion

This study was conducted in isolated, highly consanguineous and multigenerational Arab pedigrees of 1277 individuals with a mean age of (33.5 years), 60% of whom were below 30 years of age. The advantage that isolated populations offer over the general population is a more uniform environment of their living conditions and access to their genealogical records (Almasy & Blangero, 1998; Hassan et al., 2005; Samani, 2003).

We have applied stringent criteria for extensive phenotyping of cardiovascular traits contributing to the regulation of blood pressure. Using continuous non-invasive BP recordings and impedance-derived hemodynamics, our study differs from most other studies by dissecting blood pressure into its primary and intermediate phenotypes in a supposedly normal population. In addition, the entire battery of phenotypes was studied during rest and during two laboratory stress tests. The weak correlations between most of the ΔWCT and ΔCPT phenotypes indicate that the responses to the two stress tests were not affected by each other.

Several other studies have used CPT, WCT and other stress tasks to elicit BP reactivity, mostly in twins, sib-pairs, extended families and offspring of hypertensive and hypertensive subjects (Carmelli et al., 1991; Choh et al., 2005; de Geus et al., 2006; Halliwill et al., 1997; Wolff, 1951; Yamamoto et al., 1992). Studies using laboratory stress tests share the common finding of the steady increase in TPR and HR during pressor tests. The increase in TPR was associated with a concomitant increase in muscle sympathetic nerve activity and increased levels of catecholamines (Halliwill et al., 1997; Yamamoto et al., 1992). The link between sympathetic activation and the genesis of hypertension, as assessed by shortening of the pre-ejection period (PEP; de Geus et al., 2007) or increased epinephrine levels during mental stress, received considerable support (Dimsdale & Moss, 1980; Floras, 1992).

Heritability Estimates

Resting Hemodynamics

Few studies reported h^2 for impedance-derived resting hemodynamic phenotypes. Using a multivariate model in twin cohorts, the GCTS (Kupper et al., 2006) estimated total, time and ethnic-specific resting h^2 of

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around 0.25–0.64 for SBP, DBP, HR, SV, CI and TPRI compared with 0.19–0.32 of the same resting parameters in this study. Using univariate analysis in European American families, Choh et al. (2005) estimated significant h^2 for resting SBP and DBP of 0.35 and 0.20, respectively, compared with 0.19 of same parameters in our study. Busjahn et al. (1996) reported significant h^2 for SBP and DBP of 0.53 but not for resting HR. However, McCaffery et al. (2002) reported significant h^2 for resting SBP, DBP and HR ranging from 0.39 to 0.52.

Mental Stress

We report significant h^2 for ΔWCT HR, SBP, SV and CI ranging from 0.15–0.40 while h^2 for ΔWCT DBP and TPRI of 0.09 and 0.002 respectively, were nonsignificant. Few studies, using different physiological stressors and designs, estimated h^2 of impedance-derived hemodynamics. In the GCTS (Kupper et al., 2006) after an intervening period of 4 years, significant ethnic specific h^2 for Δ HR, SBP, DBP, SV ranged from 0.17–0.34. In the same cohort of EA and AA of the GCTS, Snieder et al. (2005) studied the same parameters under acute mental stress of 5 minutes of virtual reality car driving tests and 10 minutes of social stressor interviewing. In EA the car driving tests produced significant h^2 ranging from 0.28–0.60 for ΔSBP and HR, but not for ΔDBP, SV, CO and TPR. However, social stress produced similar h^2 in EA and AA for all the above parameters but not for ΔDBP. In contrast, in AA, car driving tests produced significant h^2 for ΔSBP, HR, SV, CO and TPR, while social stress produced significant h^2 only for ΔSBP and ΔDBP. The GCTS studies showed that h^2 for reactivity estimates varied across studies, across task within the same study and across ethnic groups. Compared to the later study, h^2 ΔWCT for most of the phenotypes in our study, though lower, shared the same h^2 trends for social stressors in EA and the same trends in car driving in AA. It is important to note that in our study h^2 ΔWCT was significant for SBP but not for DBP. Similarly, McCaffery et al. (2002) reported significant h^2 for ΔSBP WCT but not for ΔDBP; they attributed this difference to the increased influence of covariates on DBP responses to WCT which are similar to those found in our study. In addition these differences may be ascribed to the different control mechanisms for SBP and DBP for CPT (Choh et al., 2005).

Cold Pressor Test

In Table 4 we present significant h^2 ranging from 0.08–0.40. It is important to note that h^2 of ΔSBP, HR, SV and LVET ranged from 0.13–0.35 while those of ΔDBP and TPR and TPRI ranged from 0.08–0.10. To our knowledge only two studies on extended pedigrees reported h^2 for ΔCPT SBP and DBP. Choh et al. (2005) reported h^2 of 0.37 and 0.08 for ΔCPT SBP and DBP respectively, while the respective values by Mitchell et al. (2008) were 0.16 and 0.24. The differences in h^2 between ΔSBP and ΔDBP related phenotypes described above were attributed to contractility, a feature of SBP regulation that does not influence DBP regulation (Choh et al., 2005). Other h^2 of ΔCPT SBP and DBP came solely from twin studies and they ranged from 0.30–0.70 for ΔCPT SBP and 0.38–0.62 for ΔCPT DBP (Busjahn et al., 1996; Matthews et al., 2004). In our study the modest h^2 respective values of 0.13 and 0.10 for ΔCPT SBP and DBP, fell below the lower end of those studies.

In our study, although not comparable to studies using bivariate, multivariate and model fitting, the laboratory stressors used detected significant genetic variance using univariate analysis. The most important current and expected outcomes of this study are: (1) the information inherent to these inbred pedigrees with ascertained genealogical records may help accurate estimations of the degree to which traits are determined by genetic factors and the appropriate model of linkage analysis, such as hypothesized in the HAPI Heart Study (Mitchell et al. 2008) and other homogeneous populations (Charlesworth & Hughes, 2000; Matthews et al., 2004); (2) a novel addition in our study is the dissection of blood pressure into its intermediate physiological components. Apart from two twin studies on the heritability of impedance-derived hemodynamic phenotypes (Kupper et al., 2006; Snieder et al., 2005), most other studies used normal blood pressure or hypertension as phenotypes. Normal and high blood pressure are controlled at different levels by the same short- and long-term neurohormonal and renal regulatory mechanisms which are in turn modulated by genetic and environmental factors. Characterizing genetic influences of the intermediate phenotypes that make up and control blood pressure require the use of specific short-term environmental stressors to illicit reactivity (Sing et al., 2003). When analyzed as a change score, the heritability of reactivity will reflect an inseparable mix of an amplification or de-amplification of genetic (or environmental) influences already present at rest and newly emerging genetic (or environmental) influences during stress. Amplified genes are genes that have an effect on individual differences in a cardiovascular trait at rest and these effects become stronger under stress. Emerging genes are genes that are only expressed during stress and only contribute to the heritability of a cardiovascular trait when it is measured under stress conditions (de Geus et al., 2007).

We conclude that since shared environment in this cohort is common to all and therefore minimal, environmental influence occurs primarily via variation in non-shared environment unique to the individual. We have therefore demonstrated that heritability estimates of reactivity of the intermediate hemodynamic phenotypes tested capture the main genetic influences on resting levels. The current ongoing bivariate and multivariate analyses may help uncover genetic and environmental influences that may emerge from amplification or de-amplification of genes. The increased power achieved by the exploitation of

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shared genetic effects will ultimately lead to a higher likelihood of mapping genes related to BP regulation. Linkage studies using these results may home more accurately in on loci that may help in gene finding studies. Finding novel genes of relevance to (for) BP and hypertension may have considerable clinical impact.

Future studies in the offspring of these isolated pedigrees, coupled with the rapidly changing environment may help understand gene-gene and gene-environment interactions.

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