

# Genetic influences on blood pressure response to the cold pressor test: results from the Heredity and Phenotype Intervention Heart Study

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**Objectives** Blood pressure (BP) response to the cold pressor test (CPT) has been found to predict the development of hypertension and cardiovascular disease in prospective studies. The determinants of BP response to the CPT, including the role of genetic factors, however, are largely unknown. Additionally, to our knowledge, no study has examined the genetics of BP recovery from the CPT, including whether shared genetic factors influence both reactivity and recovery.

**Methods** As part of the Heredity and Phenotype Intervention Heart Study, we administered a 2.5 min hand CPT to 835 participants from 18 extended Amish families. We estimated the heritability of BP reactivity and recovery (measured by the incremental area under the curve) and the genetic correlations between baseline, reactivity, and recovery BP phenotypes.

**Results** After adjusting for relevant covariates, including baseline BP, the heritability estimates for both systolic BP (SBP) and diastolic BP (DBP) reactivity and recovery differed significantly from zero ( $P < 0.01$ ), with 12–25% of the total variation in BP response attributable to additive genetic effects. The genetic correlations between baseline DBP and response phenotypes were not significantly different from zero, whereas the genetic correlation between DBP reactivity and recovery (0.74) was significantly different from zero and 1 ( $P < 0.005$ ). The genetic correlation between SBP reactivity and recovery was similar (0.81;  $P < 0.05$ ).

## Introduction

Hypertension is a major risk factor for both stroke and coronary heart disease, the leading causes of death and premature, permanent disability in the United States [1]. Genetic influences on blood pressure (BP) have been clearly established, with most reported heritability estimates of 20–40%, although environmental factors also contribute greatly to BP variation [2–6]. The relative importance and types of genes influencing BP variation are likely to depend on the environmental context. Characterizing genetic influences on BP variation in response to a specific environmental stressor

**Conclusion** We conclude that, independent of baseline BP, BP response to CPT is heritable, and that both shared and unshared genetic factors influence BP reactivity and recovery, thus stressing the importance of identifying genetic variants that influence both traits. *J Hypertens* 26:729–736 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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**Abbreviations:** BMI, Body Mass Index; BP, Blood Pressure; bpm, Beats per minute; CPT, Cold Pressor Test; DBP, Diastolic Blood Pressure; HAPI, Heredity and Phenotype Intervention; iAUC, Incremental Area Under the Curve; SBP, Systolic Blood Pressure;  $\rho_P$ , Phenotypic correlation;  $\rho_G$ , Genetic correlation;  $\rho_E$ , Environmental correlation

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may help focus the search for hypertension susceptibility genes relevant to cardiovascular disease and its treatment and prevention.

In 1932, Hines and Brown [7,8] introduced the cold pressor test (CPT) as a tool to measure cardiovascular reactivity, specifically BP changes, in response to stress. The CPT stimulus involves both a cold and pain component, which induces a thermoregulatory reflex and global sympathetic activation, producing several physiological responses, including vasoconstriction and BP elevation [9,10]. Prospective studies have demonstrated

that normotensive individuals with a heightened BP reactivity response to the CPT have higher ambulatory BP 3 years later [11], are at a two- to four-fold increased risk of developing hypertension [12–15], and are also more likely to develop clinical coronary heart disease [16]. Recently, the importance of measuring BP during recovery from the CPT has also been emphasized [17,18], and higher BP levels during recovery have been found to predict increases in BP levels 3 years later, independent of BP reactivity [19].

Twin studies have suggested a genetic basis for BP reactivity to the CPT [20–22], and a recent family study of BP reactivity reported heritability estimates of 12–37% for systolic BP (SBP) and 2–8% for diastolic BP (DBP) [4]. In addition, results from the latter study suggested that the genetic determinants of baseline and reactivity SBP to the CPT might be different. To our knowledge, the genetics of BP recovery from the CPT, including whether shared genetic factors influence both reactivity and recovery, has not been studied. Such studies would provide insight into the pathophysiology of BP response to the CPT and guidance for future genetic studies involving the CPT.

As part of the Heredity and Phenotype Intervention (HAPI) Heart Study, we administered a 2.5 min hand CPT to 835 participants from 18 extended, Old Order Amish families from Lancaster County, Pennsylvania. The overall goal of the HAPI Heart Study is to identify genes that interact with specific environmental exposures to influence risk factors for cardiovascular disease. In this study, we examined the heritability of phenotypes measuring BP reactivity to and recovery from the CPT. In addition, we investigated whether the same or different genes influence baseline, reactivity, and recovery BP phenotypes.

## Methods

### Participants

Between 2003 and 2006, we recruited study participants aged 20 years and older from Amish families living in Lancaster County, Pennsylvania. We initially selected participants on the basis of their prior participation in earlier family studies on bone mineral density and coronary artery calcification. Recruitment guidelines were subsequently broadened to allow participation of other interested individuals from the Amish community and their family members. Consequently, the resulting sample of HAPI Heart Study participants represents a community-based convenience sample of relatively healthy Amish individuals aged 20–80 years old (see health exclusions below).

Pregnant women were excluded from the HAPI Heart Study. They were, however, eligible to participate 1 year after delivery. Other study-wide exclusion criteria included

severe hypertension (SBP/DBP > 180/105 mmHg), coexisting malignancy, creatinine level greater than 2 mg/dl, aspartate transaminase or alanine transaminase levels greater than twice the normal levels, hematocrit levels less than 32%, and/or abnormal levels of thyroid-stimulating hormone. We also excluded individuals who were taking medications that could potentially affect any outcome measures that could not be discontinued for 1 week prior to the initiation of the protocol. These medications included all antihypertensive medications, nitrates, lipid-lowering agents, antidiabetic medications (with the exception of insulin), adrenergic or cholinergic acting agents (including cold formulas and antidepressants), and diet or weight loss agents. Antiplatelet agents and vitamins were discontinued 14 and 7 days, respectively, prior to the start of the study. In addition to these study-wide exclusion criteria, participants suffering from Raynaud's disease were excluded from the CPT intervention.

Eight hundred and sixty-eight participants were enrolled in the HAPI Heart Study. Of these, 835 completed the CPT and were included in our analyses. These participants were distributed across 518 nuclear families. For the purpose of these analyses, we used the extensive genealogical information available from the Amish Genealogical Database [23] to combine nuclear families into 18 extended families by including genealogical information on grandparents of the participants even if they did not participate in the study. All participants gave written, informed consent as approved by the Institutional Review Board at the University of Maryland and collaborating institutions prior to participation.

### Study design

Variables assessing medical history and lifestyle were obtained during a home visit by field staff through questionnaires. During the participant's first visit to the Amish Research Clinic, a physical examination was performed. Height (cm), weight (kg), and waist and hip circumference (cm) were measured. BMI was calculated as weight (kg) divided by the square of height (m). Radial pulse (bpm) was measured three times and resting heart rate was calculated as the average of the last two measurements.

The CPT was performed during the first clinic visit as described by Corretti *et al.* [24]. Stimuli other than the CPT (e.g. pharmacological agents) can be used to trigger cardiovascular response and several measures of cardiovascular response (e.g. forearm blood flow or epicardial coronary artery dysfunction assessed by quantitative coronary angiography) exist. We chose to measure BP response to the CPT because it is well suited for the rural clinic setting of the HAPI Heart Study.

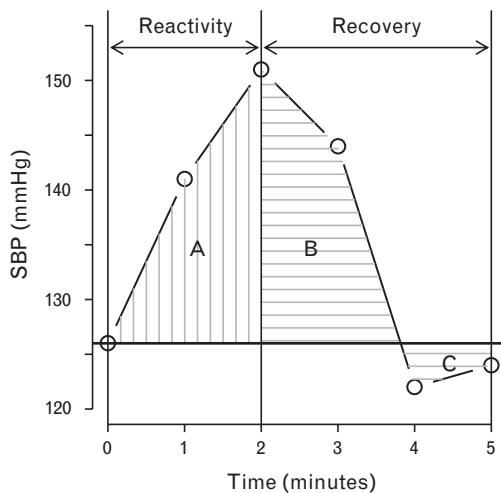
All participants were studied in a temperature-controlled room (22°C) in the resting, supine state in the morning

(following a 12-h fast). Participants were instructed not to engage in exercise or any vigorous activity on the morning of the study. BP and heart rate were measured with a properly fitted automated BP cuff (Datascoper Acutor 3SAT). BP measurements were made before the CPT every 5 min for at least 20 min or until a stable baseline BP was obtained (i.e. no more than 3 mmHg difference between three consecutive readings for both SBP and DBP). The right hand and wrist were immersed in ice water for 2.5 min and then withdrawn. Nine blood pressure measurements were taken during and after the cold pressor stimulus at 1, 2, 3, 4, 5, 7.5, 10, 15, and 20 min.

### Statistical analysis

All analyses were carried out separately for SBP and DBP. We used the average of the last two BP measurements taken before the CPT as baseline BP, denoted as time 0. Over 90% of the participants reached their maximum change in BP by 2 min, and the majority had a stable BP after 5 min. Therefore, measurements at 1 and 2 min were considered to reflect reactivity to the CPT, whereas measurements at 3, 4, and 5 min were considered to reflect recovery. To measure BP response, we calculated the incremental area under the curve (iAUC) separately for the reactivity and recovery periods. Within each period, the iAUC was defined as the difference between the area under the response curve and the area below baseline and calculated by first subtracting baseline from all subsequent measurements before calculating the area

Fig. 1



Response profile from one HAPI Heart Study participant illustrating the blood pressure (BP) phenotypes derived from the cold pressor test. Baseline BP is shown by the bold, horizontal line. The reactivity (0–2 min) and recovery (2–5 min) periods are delimited by vertical lines at times 0, 2, and 5 min. We used the incremental area under the curve (iAUC) to measure the two periods of BP response, that is, reactivity and recovery, separately. Within each period, the iAUC was defined as the difference between the area under the response curve and the area below the baseline. Hence, reactivity iAUC corresponds to area A (vertical lines), whereas recovery iAUC is represented by the difference between areas B and C (horizontal lines).

(Fig. 1) [25,26]. We used the trapezoidal rule to calculate the areas [27].

We performed heritability analyses using maximum likelihood variance components methods as implemented in the program SOLAR [28]. For a particular phenotype  $y$ , the value of  $y$  for individual  $i$  is modeled as  $y_i = \mu + \sum \beta_j X_{ij} + g_i + e_i$ , where  $\mu$  is the mean of  $y$ ,  $X_{ij}$  is the  $j$ th covariate with associated regression coefficient  $\beta_j$ ,  $g_i$  is an additive genetic effect normally distributed with mean 0 and variance  $\sigma_g^2$ , and  $e_i$  is a random residual effect normally distributed with mean 0 and variance  $\sigma_e^2$ . Any nonadditive genetic and unmeasured nongenetic effects (as well as random errors) are incorporated into  $e_i$ . The heritability of the phenotype is estimated by the ratio of the variance attributable to additive genetic effects,  $\sigma_g^2$ , and the total phenotypic variance. We used likelihood-ratio tests to assess the significance of a parameter of interest by comparing the log-likelihood of the model in which the parameter is estimated with that of the model in which the parameter is fixed at 0 [29]. We adjusted all heritability estimates for covariates that were associated with at least one of the phenotypes (at  $P < 0.1$ ). We performed covariate screening in SOLAR. Significant covariates were age, age<sup>2</sup>, sex, age-by-sex, and age<sup>2</sup>-by-sex interactions, BMI, and resting heart rate, a surrogate for cardiovascular fitness (that was measured during the clinical examination). For the response traits, we also included baseline BP as a covariate. We estimated the total proportion of variance explained by additive genetic effects as the product of the heritability estimate and 1 minus the proportion of the variance explained by covariates.

We also performed bivariate analyses using maximum likelihood methods as implemented in the program SOLAR [30–32] to assess the presence of pleiotropic genetic effects influencing baseline and reactivity, baseline and recovery, and reactivity and recovery. We partitioned the phenotypic correlation ( $\rho_P$ ) between two traits into components attributable to shared genetic effects (genetic correlation,  $\rho_G$ ) and shared environmental factors (environmental correlation,  $\rho_E$ ). If  $h_1^2$  and  $h_2^2$  are the heritabilities of the two traits, then

$$\rho_P = \rho_G \sqrt{h_1^2 h_2^2} + \rho_E \sqrt{(1 - h_1^2)(1 - h_2^2)}$$

Using likelihood-ratio tests, we tested two different hypotheses:  $\rho_G = 0$  and  $\rho_G = 1$  (or  $-1$ ). Rejecting  $\rho_G = 0$  provides evidence that shared genetic effects exist, whereas rejecting  $\rho_G = 1$  (or  $-1$ ) indicates that one or more genetic factors are specific to each trait. We also tested the hypothesis that  $\rho_E = 0$  to assess the presence of shared environmental factors affecting the two traits. Covariates included in the bivariate analyses were the same as in the univariate analyses. Baseline BP

**Table 1 Characteristics of the 835 HAPI Heart Study participants who underwent the cold pressor test**

	Men (n = 448)		Women (n = 387)	
	Mean ± SD	Range	Mean ± SD	Range
Age (years)	42 ± 13	[20, 80]	45 ± 14	[20, 80]
Height (cm)	173 ± 6	[147, 194]	161 ± 6	[141, 175]
Weight (kg)	77 ± 11	[54, 110]	72 ± 14	[42, 122]
BMI (kg/m <sup>2</sup> )	25.6 ± 3.2	[18.4, 37.9]	27.8 ± 5.5	[17.2, 46.8]
Resting heart rate (bpm)	62 ± 7	[41, 92]	66 ± 7	[52, 96]

was included as a covariate when the bivariate analysis included both response traits.

After adjusting for covariates, the BP phenotypes were close to normally distributed except for the presence of outliers. We assessed the impact of outliers on the estimates of heritability and genetic and environmental correlation by examining the change in the estimates when extreme values were excluded. All analyses (except where noted above) were conducted using version 8.2 of the Statistical Analysis System programming language (SAS Institute, Cary, North Carolina, USA).

## Results

The 835 HAPI Heart Study participants (387 women) who underwent the CPT intervention had a mean age of 44 ± 14 years (±SD). Selected characteristics of these participants are shown in Table 1 by sex. Mean BMI was 25.6 ± 3.2 kg/m<sup>2</sup> for men and 27.8 ± 5.5 kg/m<sup>2</sup> for women, and mean resting heart rate was 62 ± 7 bpm for men and 66 ± 7 bpm for women. The proportion of participants with hypertension (defined by baseline SBP > 140 mmHg or DBP > 90 mmHg) was 3.6% for men and 11.1% for women (data not shown). Less than 4% of the participants reported that they currently smoke (16 men and 0 women, data not shown). The participants were distributed across 518 nuclear families from 18 extended families, including 581 sibling pairs, 302 parent-offspring pairs, and 10 113 more distantly related pairs.

Maximum BP response was attained within 2 min for over 90% of the participants. On average, participants' BP

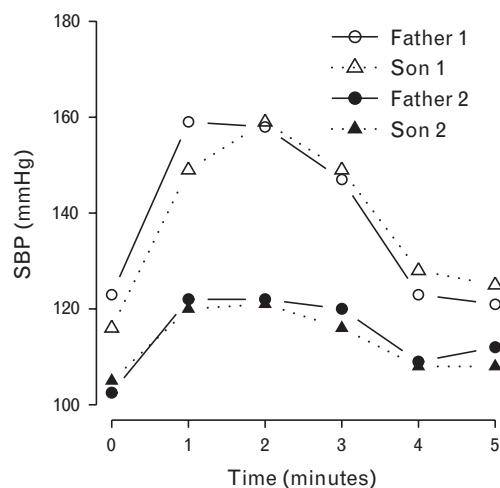
**Table 2 Summary of baseline blood pressure and blood pressure response to the cold pressor test**

	Men (n = 448)		Women (n = 387)	
	Mean ± SD	Range	Mean ± SD	Range
Systolic blood pressure				
Baseline (mmHg)	116 ± 11	[80, 173]	117 ± 15	[89, 175]
iAUC (mmHg <sup>2</sup> )				
Reactivity (0–2 min)	27 ± 16	[–13, 88]	29 ± 17	[–19, 84]
Recovery (2–5 min)	31 ± 29	[–52, 186]	25 ± 28	[–44, 124]
Diastolic blood pressure				
Baseline (mmHg)	71 ± 7	[52, 103]	70 ± 8	[55, 95]
iAUC (mmHg <sup>2</sup> )				
Reactivity (0–2 min)	17 ± 13	[–17, 69]	15 ± 11	[–14, 61]
Recovery (2–5 min)	13 ± 19	[–45, 79]	6 ± 16	[–42, 48]

iAUC, incremental area under the curve.

returned to baseline levels by 5 min. Few participants (4.1%) had a negative BP response to the CPT, meaning that no BP measurement during the reactivity part of the CPT was greater than or equal to their baseline BP. With the exception of self-reported diabetes, there were no significant differences in baseline clinical or demographic characteristics (e.g. age, sex, body size, baseline BP, and hypertension status) between negative and nonnegative responders ( $P > 0.05$ , data not shown). In fact, after comparing 17 characteristics, only the proportion of individuals who self-reported diabetes (in total, three negative and 11 nonnegative responders) was significantly different between the two groups ( $P = 0.02$  for Fisher's exact test). In addition, the majority of negative responders were not closely related to one another (data not shown).

Mean baseline SBP and DBP were 117 ± 13 and 71 ± 8 mmHg, respectively. Mean reactivity, as measured by the iAUC from 0 to 2 min, was 28 ± 17 mmHg<sup>2</sup> for SBP and 16 ± 12 mmHg<sup>2</sup> for DBP. Descriptive statistics of baseline BP and the response phenotypes are shown in Table 2 by sex. For most phenotypes, BP response was greater in men than in women and was positively associated with age and BMI and negatively associated with heart rate and baseline BP ( $P < 0.05$ , data not shown). As

**Fig. 2**

Familial resemblance of response profiles: individual profiles of systolic blood pressure (SBP) response to the cold pressor test for two different father-son pairs from the HAPI Heart Study.

**Table 3 Heritability estimates for baseline blood pressure and blood pressure response to the cold pressor test**

Trait <sup>a</sup>	Heritability estimate (SE)	P value	Percentage of variance explained by	
			Measured covariates <sup>a</sup>	Additive genetic effects
Systolic blood pressure				
Baseline	0.27 (0.08)	<0.0001	26	20
Response (iAUC)				
Reactivity (0–2 min)	0.15 (0.07)	0.005	14	13
Recovery (2–5 min)	0.14 (0.06)	0.006	15	12
Diastolic blood pressure				
Baseline	0.21 (0.08)	0.0008	23	17
Response (iAUC)				
Reactivity (0–2 min)	0.27 (0.08)	<0.0001	5	25
Recovery (2–5 min)	0.20 (0.07)	0.0003	10	18

iAUC, incremental area under the curve. <sup>a</sup> Adjusted for age, age<sup>2</sup>, sex, age-by-sex and age<sup>2</sup>-by-sex interactions, BMI, resting heart rate, and for response phenotypes only, baseline blood pressure.

an illustration of both the familial resemblance of response patterns and the variation in the magnitude of response, Fig. 2 shows the SBP response profiles for two different father–son pairs from the HAPI Heart Study.

After adjusting for covariates, heritability estimates for baseline SBP and DBP were 0.27 and 0.21, respectively ( $P < 0.001$ ; Table 3). After adjusting for covariates, including baseline BP, all heritability estimates of BP responses (as measured by the iAUC) were also significantly different from zero ( $P < 0.01$ ), ranging from 0.14 (for SBP recovery) to 0.27 (for DBP reactivity). Compared with measured covariates, additive genetic factors accounted for a greater proportion of the total variance in DBP response and for a similar proportion of the variance in SBP response. For example, additive genetic effects and covariates accounted for 25 and 5%, respectively, of the total variance in DBP reactivity and for 13 and 14%, respectively, of the total variance in SBP reactivity (Table 3).

After adjusting for covariates, the estimates of phenotypic correlation ( $\rho_P$ ) between baseline and response BP phenotypes were negative and ranged from  $-0.13$  (SBP reactivity) to  $-0.23$  (DBP recovery). Table 4 shows the

partition of  $\rho_P$  into genetic and environmental correlations. The genetic correlation ( $\rho_G$ ) between baseline and response DBP phenotypes was significantly different from 1 ( $P < 0.001$ ) but not zero ( $P > 0.4$ ), suggesting that different sets of genes may be influencing baseline and response DBP phenotypes. For SBP,  $\rho_G$  estimates between baseline and response phenotypes were 0.43 and 0.86 for reactivity and recovery, respectively, and neither was significantly different from 1 ( $P > 0.08$ ). Environmental correlation ( $\rho_E$ ) estimates between all baseline and response BP phenotypes were negative and differed significantly from 0 ( $\rho_E \leq -0.25$ ,  $P < 0.001$ ), except for baseline DBP and reactivity ( $\rho_E = -0.13$ ,  $P = 0.08$ ). Hence, unmeasured environmental factors may influence both baseline and response BP phenotypes in opposite directions.

The reactivity and recovery periods of the BP response to the CPT were positively correlated ( $\rho_P = 0.73$  for SBP and 0.68 for DBP). Estimates of the genetic correlation between reactivity and recovery were significantly different from zero and 1 ( $\rho_G = 0.81$ ,  $P < 0.05$  for SBP;  $\rho_G = 0.74$ ,  $P < 0.005$  for DBP), providing evidence that shared (i.e. pleiotropic) and unique genetic effects influence both reactivity and recovery. Estimates of the

**Table 4 Genetic ( $\rho_G$ ) and environmental ( $\rho_E$ ) correlation between baseline blood pressure and blood pressure response phenotypes to the cold pressor test**

Pairs of traits	$\rho_G$ (SE)	P value		$\rho_E$ (SE)	P value
		H <sub>0</sub> : $\rho_G = 0$	H <sub>0</sub> : $\rho_G = 1$ (or $-1$ )		
Systolic blood pressure					
Baseline–reactivity iAUC <sup>a</sup>	0.43 (0.35)	0.18	0.087	$-0.26$ (0.07)	0.0002
Baseline–recovery iAUC <sup>a</sup>	0.86 (0.45)	0.013	0.39	$-0.38$ (0.06)	<0.0001
Reactivity iAUC–recovery iAUC <sup>b</sup>	0.81 (0.14)	0.024	0.041	0.72 (0.03)	<0.0001
Diastolic blood pressure					
Baseline–reactivity iAUC <sup>a</sup>	$-0.18$ (0.23)	0.45	0.0007	$-0.13$ (0.07)	0.084
Baseline–recovery iAUC <sup>a</sup>	$-0.13$ (0.26)	0.62	0.0007	$-0.25$ (0.07)	0.0004
Reactivity iAUC–recovery iAUC <sup>b</sup>	0.74 (0.12)	0.002	0.003	0.66 (0.04)	<0.0001

The genetic and environmental correlation between a pair of traits (expressed as  $\rho_G$  and  $\rho_E$ , respectively) captures the extent to which the same genes and the same environmental factors influence both traits. Rejection of the hypothesis that  $\rho_G = 0$  suggests that one or more of the same genetic factors influences both traits. Rejection of the hypothesis that  $\rho_G = 1$  (or  $-1$ ) suggests that one or more unique genetic factors influences one trait but not the other (i.e. all of the genetic factors that influence one trait are not the same as all of the genetic factors that influence the other trait). Rejection of the hypothesis that  $\rho_E = 0$  indicates that one or more of the same environmental factors influences both traits. iAUC, incremental area under the curve. <sup>a</sup> Adjusted for age, age<sup>2</sup>, sex, age-by-sex interactions, BMI, and resting heart rate. <sup>b</sup> Adjusted for age, age<sup>2</sup>, sex, age-by-sex interactions, BMI, resting heart rate, and baseline blood pressure.

environmental correlation differed significantly from zero ( $\rho_E = \sim 0.70$ ,  $P < 0.0001$ ), indicating that unmeasured environmental factors influence both reactivity and recovery in the same direction.

We repeated all heritability and genetic and environmental correlation analyses after removing individuals with values higher than 3 SD from the mean (3–14 participants). All heritability and correlation estimates from these analyses were within 1 SD of the original estimates.

## Discussion

Our study is the largest family study of BP response to the CPT to date and is the only family study to obtain multiple measurements before, during, and after the CPT. To our knowledge, we are also the first to demonstrate that BP recovery from the CPT is heritable and to estimate the genetic correlation between the reactivity and recovery periods of BP response to the CPT. We found that both BP reactivity and recovery were significantly heritable and estimated that additive genetic effects explained 12–25% of the total variance in BP response. In other words, although no inferences can be made regarding specific individuals or families in the present study, these estimates suggest that in the population from which the pedigrees were sampled, genetic differences among individuals explain one eighth to one fourth of the observed variation in the CPT responses. Both SBP and DBP reactivities were previously found to be heritable in twin studies [20–22], whereas only SBP reactivity was found to be heritable in the family study by Choh *et al.* [4].

Our results suggest that DBP response to the CPT captures a physiological phenomenon that is different from baseline DBP, that is, there may be genetic factors relevant to the etiology of cardiovascular disease that influence CPT-derived BP response but not baseline BP. We also found evidence that both shared and unique genetic factors influence DBP reactivity and recovery. For SBP response, we could not reject the hypothesis of complete pleiotropy of genetic effects on baseline and response SBP phenotypes ( $P = 0.09$ ). The genetic correlation between baseline and reactivity SBP (0.43), however, was also not significantly different from zero ( $P = 0.18$ ). Interestingly, Choh *et al.* [4] found no evidence for pleiotropic effects of genes on baseline and reactivity SBP to the CPT, which is in agreement with previous twin studies [21,22]. Further investigation is needed to determine whether both shared and unshared genetic factors influence baseline, reactivity, and recovery SBP phenotypes.

We found that a large proportion of the variation in BP response was left unexplained after accounting for measured covariates and unmeasured additive genetic effects. Part of this unexplained variation could be due

to measurement error, although previous studies have found that CPT-derived BP responses were reproducible, especially when the responses were derived from several measurements during the test [33–35]. Other possible factors that may explain the residual variation in BP response include dietary intake [36] and physical activity/cardiovascular fitness [37,38]. Variation in dietary intake, however, is likely to be limited in the Amish owing to their communal lifestyle. In addition, resting heart rate (a measure of cardiovascular fitness) was included in our analyses but explained less than 3 and 1% of the variation in SBP and DBP response, respectively.

The Old Order Amish is a non-Hispanic white population with a relatively homogeneous lifestyle and similar environmental exposures. For example, the Amish rarely use prescription medications or smoke. The homogeneity of environmental exposures in the Amish represents a strength of our study by minimizing the effects of important, potential confounders. It also permits a more controlled investigation of the role of genetic factors in BP response to the CPT than has been possible in previous studies. As the Amish are derived from the same overall gene pool as the US and European non-Hispanic white populations, we believe that the genes influencing BP response in the Amish will be relevant to the general population.

Differences in BP response to the CPT are likely to be influenced by several physiological pathways and different from those controlling baseline BP. For example, in addition to physiological pathways related to global sympathetic activation, endothelial function and psychological components (related to the perception of pain and the degree of difficulty of the test) may influence BP response [39,40]. In addition, Busjahn *et al.* [22] suggested that long-term regulatory systems influence baseline BP, whereas systems controlling acute sympathetic activation are involved in BP reactivity. Our results provide new information implicating both shared and unique pathways/genes in the reactivity and recovery periods of BP response to the CPT. For example, systems involved in catecholamine reuptake are likely to be specific to BP recovery [41]. Still, at present, little is known about the specific pathways involved in and the genetic factors that contribute to and/or distinguish BP reactivity and recovery.

Importantly, BP response to the CPT predicts the development of hypertension and coronary heart disease, even in normotensive individuals [12–16]. Moreover, as described above, we found that a significant proportion of the variation in BP response to the CPT is independent of baseline BP and likely due to a heritable (genetic) component that is also independent of baseline BP. In fact, the BP response profiles in our sample were remarkably similar between individuals who would be classified

[42] as normotensive (baseline SBP and DBP less than 120 and 80 mmHg, respectively) versus hypertensive (baseline SBP or DBP greater than or equal to 140 or 90 mmHg, respectively). For example, after adjusting for relevant covariates, including baseline BP, mean BP reactivity was not significantly different between normotensive and hypertensive individuals ( $P=0.44$  and  $P=0.23$  for SBP and DBP reactivity, respectively). Thus, there are normotensive individuals who are at increased risk of developing hypertension and coronary heart disease on the basis of their BP reactivity to the CPT, yet who would not come to clinical attention on the basis of their baseline BP alone. Together, these findings emphasize the importance of understanding how genetic factors influence BP response to the CPT as this information may provide valuable insight into the pathophysiology of BP, that is, insight that could not be surmised by simply studying baseline BP.

## Conclusion

Our results provide new evidence for a genetic basis for BP recovery from the CPT and add to the existing evidence that BP reactivity to the CPT is heritable. Our results also emphasize the importance of considering both reactivity to and recovery from the CPT since both shared and unique genetic factors may influence the reactivity and recovery periods of the BP response to the CPT. Findings from this study will inform and guide our search for the specific genes that underlie BP response to the CPT. Identifying genes that influence BP response to the CPT is clinically relevant given the accumulating evidence that BP measured under environmental stressors may predict the risk of developing hypertension. In turn, understanding the genetic basis of cardiovascular reactivity may facilitate future efforts to improve cardiovascular health.

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There are no conflicts of interest.

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