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Genetic variants in the renin-angiotensin system and blood pressure reactions to the cold pressor test

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Abstract

Objectives—The purpose of this study was to examine the association between genetic variants in the renin-angiotensin system (RAS) and blood pressure (BP) responses to cold pressor test (CPT).

Methods—The CPT was conducted among 1,998 Han Chinese participants. BP measurements were obtained before and after the CPT using a standard sphygmomanometer according to a standard protocol. The association between SNP genotypes and BP responses to the CPT was assessed using a mixed linear model.

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Results—Of 68 SNPs genotyped in 6 RAS genes, two were strongly associated with diastolic BP (DBP) responses to CPT ($P \leq 0.001$; false discovery rate $q\text{-value} < 0.05$): rs2006765 and rs943580 in the angiotensinogen (AGT) gene. Compared to C allele carriers of rs2006765, the TT homozygotes had a significantly decreased DBP response to the CPT. For participants with the TT genotype, percent DBP responses were 5.68% (4.25%, 7.10%), compared to corresponding responses of 9.17% (8.66%, 9.68%) among participants with the CC+CT genotype. In addition, SNP rs4681443 of the angiotensin type 1 receptor (AGTR1) gene was significantly associated with percent SBP responses to CPT ($P \leq 0.001$; $q\text{-value} < 0.05$).

Conclusion—Briefly, our study identified variants in the AGT and AGTR1 genes that may influence BP responses to CPT in Han Chinese population. These results show that genetic variants in the RAS play an important role in BP responses to CPT, and therefore in predicting future hypertension.

Keywords

blood pressure; cold pressor test; renin-angiotensin system; genetics; polymorphism

Introduction

Hypertension is a major risk factor for stroke and coronary heart disease and is the leading cause of death and premature disability in developed and developing countries [1-3]. Classical risk factors, including psychological and emotional stress, have been shown to predict approximately 50% of future hypertension cases [4,5]. The human body reacts to stress by activating many physiological and psychological responses. Specifically, the up-regulation of sympathetic activity plays a fundamental role in the pathogenesis of hypertension [4]. It is well known that the renin-angiotensin system (RAS) has crucial effects on blood pressure (BP) regulation and is involved in sympathetic-mediated stress response at both central and peripheral levels [6,7]. Angiotensin II, a potent vasopressor and aldosterone-stimulating peptide, is involved in the enhancement of central sympathetic tone and facilitation of the catecholamine release from cardiac sympathetic nerve terminals via the angiotensin II type 1 receptor (AGTR1) [8,9]. An increase of plasma renin activity (PRA) and concentration (PRC) after immobilization stress has been demonstrated in rats [10]. Stress increases the expression of AGTR1 in the hypothalamic paraventricular nucleus following stress and glucocorticoid administration in animal model [11]. In hypertensive humans, ACE inhibition attenuates forearm vasoconstriction induced by sympathetic stimulation [12].

As a tool to measure cardiovascular reactivity in response to environment stress, the cold pressor test (CPT) has been used extensively in previous research [13-15]. CPT stimulus involves both a cold and pain component and induces an α -adrenergic vasoconstriction with an increased total peripheral resistance [13]. Twin and family studies have suggested that individual difference in BP responses to CPT was significantly determined by genetic factors [14-18]. In a meta analysis, the heritability for reactivity to the CPT ranged from 0.21 to 0.55 [17]. The determinants of BP responses to CPT, including genetic factors, however, are largely unknown. Still, a few of studies have focused on candidate genes for cardiovascular reactivity to an acute stress. Kurnik and his colleagues performed several studies to determine the effect of the α_2A -adrenergic receptor (ADRA2C) gene del322–325 on physiologic and psychological responses to the CPT and found that this common variant was associated with greater heart rate and pain perception, but not with local vascular responses to cold [19-21].

These observations raise the possibility that genetic variation in the RAS might be associated with BP responses to the CPT. In the current study, we investigated the association between RAS candidate genes [angiotensin converting enzyme (ACE), angiotensinogen (AGT), AGTR1, angiotensin II type 2 receptor (AGTR2), renin (REN), and renin-binding protein (RENBP)] and BP responses to CPT.

Methods

Study Participants

The GenSalt study was conducted in a Han Chinese population in rural areas of northern China. A community-based BP screening was conducted among persons aged 18–60 years in the study villages to identify potential probands and their families. Those with a mean SBP between 130–160 mmHg and/or a DBP between 85–100 mmHg and no use of antihypertensive medications were identified as probands. Probands, along with their spouses, siblings and offspring, were invited to participate in this study. Detailed eligibility criteria for the probands and spouses/siblings/offspring have been presented elsewhere [22]. Individuals who had stage-2 hypertension, secondary hypertension, a history of clinical CVD or diabetes, used antihypertensive medications, or were pregnant, heavy alcohol drinking or older than 60 years were excluded from the cold pressor test. Among the 2006 eligible participants, 1998 (99.6%) completed the CPT and were included in the current analysis.

Institutional Review Boards at all of the participating institutions approved the GenSalt study. Written informed consents were obtained from each participant.

Phenotype Measurement

A standard questionnaire was administered by trained staff at the baseline examination to collect information on family structure, demographic characteristics, personal and family medical history, and lifestyle risk factors. Body weight and height were measured twice in light indoor clothing without shoes during the baseline examination. Body mass index (BMI) was calculated as kilograms per square meter (kg/m^2).

Cold Pressor Test

All participants were advised to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 minutes prior to their cold pressor test. After a participant was in the sitting position for 20 minutes, 3 BP measurements were obtained using a standard mercury sphygmomanometer before the ice water immersion. Then, the participant immersed his or her left hand in the ice water bath (3°C to 5°C) to just above the wrist for 1 minute. BP measurements at 0, 60, 120, and 240 seconds were obtained using a standard mercury sphygmomanometer after the CPT stimulus. The CPT was well tolerated by all subjects, and no adverse effects were reported.

Candidate Gene and SNP Selection and Genotyping

Six candidate genes in the RAS were selected based on their potential biological effect on BP regulation, and included ACE, AGT, AGTR1, AGTR2, REN, and RENBP. Based on linkage disequilibrium (LD) structure in the Chinese population HapMap sample, tagSNPs in these candidate genes were chosen for genotyping. We also selected SNPs that had previously reported associations with BP. Fifty-one SNPs were genotyped using SNPlex assays (Applied Biosystems) based on the oligonucleotide ligation assay (OLA) for capillary electrophoresis on an automated DNA Sequencer (ABI 3700 DNA Analyzer). To provide better coverage of the candidate genes, we included an additional 17 SNPs genotyped on the Affymetrix 6.0 platform (Affymetrix, Santa Clara, California, USA), whose minor allele

frequencies were no less than 0.05. In brief, a total of 68 SNPs were analyzed in this study (Supplementary Table 1).

Statistical Analysis

ASPEX and GRR were used to check for potential misreported relationships in the GenSalt family pedigrees [23,24]. Mendelian consistency of the SNP genotype data was assessed by PLINK and PedCheck [25,26], and Hardy-Weinberg Equilibrium (HWE) for each SNP was examined using the PEDSTATS procedure [27]. Haploview software (version 4.0, <http://www.broad.mit.edu/mpg/haploview>) was used to estimate the extent of pairwise linkage disequilibrium between SNPs.

All analyses were carried out separately for SBP and DBP. We used the average of the three BP measurements taken before the CPT as the baseline BP. BP measurements at 0, 60, 120, and 240 seconds after the hand has been removed from the ice water were denoted as time 0, 1, 2, and 4 min. Over 90% participants reached their maximum change in SBP by 0 min (i.e., immediately after removing the hand from ice water bath), and over 80% reached their maximum change in DBP by 0 min (Figure 1). Therefore, percent responses to the CPT were calculated by dividing the mean BP response to the CPT at 0 min by the baseline BP. The association between SNP genotypes and BP responses to CPT were examined in codominant, additive, dominant and recessive genetic models using a mixed linear model. A sandwich estimator was used to account for the non-independence of family members. This method assumes the same degree of dependency among family members. Age, gender, and study site were adjusted in multivariable analyses. A similar, gender-stratified analysis was conducted for those SNPs located on the X-chromosome. To adjust for multiple testing, the false discovery rate (FDR) q-value was calculated for all SNPs [28]. For those SNPs that were significant after adjustment for multiple comparisons for at least one BP response phenotype, we used a mixed linear model to estimate the mean effect size and 95% confidence interval (CI) of each genotype using the appropriate genetic model. These analyses were conducted using SAS statistical software (version 9.1; SAS Institute Inc).

Results

Table 1 describes the baseline characteristics and BP responses of the study participants. On average, study participants were 40.1 years old and had a mean BMI of 23.4 kg/m², mean baseline SBP of 120.1 mmHg and mean baseline DBP of 74.7 mmHg. Approximately 52.5% of the participants were male. Mean SBP and DBP responses to the CPT were 13.6 mmHg and 6.9 mmHg, respectively. Percent SBP and DBP responses to the CPT were 11.4% and 9.5%, respectively. BP responses were normally distributed and varied widely among study participants.

Figure 2 shows the association between each SNP and percent SBP and DBP responses to CPT in codominant and recessive genetic models. All SNPs that were significantly associated with BP responses to CPT after adjustment for multiple testing (FDR q-value<0.05) have been labeled, including two SNPs in the AGT gene (rs2006765 and rs943580) and SNP rs4681443 in the AGTR1 gene. SNPs with an unadjusted p-value < 0.05 for any response phenotype are shown in Table 2, and include 3 SNPs in the REN gene, 5 SNPs in the AGT gene, 6 SNPs in the AGTR1 gene and 1 SNP in the ACE gene. After adjustment for multiple testing, SNPs rs2006765 and rs943580 of the AGT gene were still significantly associated with DBP response to CPT in codominant and recessive models (all $P \leq 0.001$, q-value<0.05). Additionally, SNP rs4681443 was significantly associated with SBP response to CPT in codominant and recessive models ($P \leq 0.001$, q-value<0.05). Assessment of the p-values for these models and the mean change in BP level for each genotype suggested a potentially better fit of the recessive model for these 3 SNPs.

Figure 3 presents BP responses to the CPT according to the genotypes of SNP rs2006765 of the AGT gene. Compared with common C allele carriers, homozygotes of the minor T allele had a significantly decreased DBP response to the CPT, even after adjustment for multiple testing (q -values <0.0001). For participants with the TT genotype, percent DBP responses were 5.68% (4.25%, 7.10%), compared to corresponding responses of 9.17% (8.66%, 9.68%) among participants with the CC+CT genotype. Additionally, another SNP, rs943580, which belongs to the same haplotype block as rs2006765 ($r^2=0.94$) (Figure 4), also showed a significant relationship with DBP response to CPT [AA: 6.26% (4.80%, 7.73%), GG+GA: 9.13% (8.62%, 9.63%), q -value = 0.001]. Despite the low MAF of rs4681443 (5%), participants that were homozygous of minor A allele had a lower percent BP response to CPT [6.32% (3.84%, 8.80%)] compared with persons who carried with the common G allele [11.08% (10.59%, 11.56%)].

Discussion

Our study identified several novel genetic variants in the RAS genes which may play critical roles in BP responses to CPT. Participants who were homozygous for the rs2006765 T allele or the rs943580 A allele in the AGT gene had significantly lower DBP responses to CPT (even after adjustment for multiple testing). In addition, SNP rs4681443 in the AGTR1 gene was also significantly associated with SBP responses to CPT after adjustment for multiple testing in our analysis. To our knowledge, our study is the first investigation to report a relationship between genetic variants in the RAS and BP responses to the CPT. These findings may have important clinical and public health implications.

Cardiovascular responses to physical and mental stressors have been studied extensively, because they offer mechanistic and prognostic information beyond that provided by resting measures. For instance, enhanced BP responses to stress in young, healthy participants were associated with subsequent higher resting heart rate, BP, and hypertension [5,29]. As a classic experimental cardiovascular stressor, CPT elicits profound hemodynamic responses, greater than other physiological or psychological stress tests [5]. Cardiovascular responses to stress are heritable [14,15,18]. Therefore, genetic variation may contribute to inter-individual differences in cardiovascular reactivity. However, little is known about the effects of genetic factors on the cardiovascular reactivity to stress.

We identified a significant relationship between two SNPs in the AGT gene and BP responses to CPT. There have been no previous reports of genetic variants in AGT and their associations with BP responses to CPT. However, relationships between variants in the AGT gene and hypertension have been extensively studied. Most previous studies focused on two non-synonymous polymorphisms of marker M235T (rs699) and T174M (rs4762) [30,31]. The association of AGT M235T with hypertension has been reported in many populations [30,31]. A recent meta-analysis study [32] and a study in Hong Kong [33] revealed that AGT M235T contributed to the risk of hypertension in Chinese populations. In contrast, a linkage study conducted among Chinese participants showed no evidence for linkage of neither AGT M235T nor T174M to hypertension [34]. The current study also examined the association between rs699 and rs4762 and BP responses to CPT, and showed no significant relationship after adjustment for multiple testing. However, we found that two SNPs (rs2006765 and rs943580), which are located on the 3'-flanking region of the AGT gene and belong to the same haplotype block ($r^2 = 0.94$), were significantly associated with decreased DBP response to CPT.

The AGTR1 gene encodes the angiotensin II type 1 receptor which is thought to mediate the major cardiovascular effect of angiotensin II. Polymorphisms within this gene have been extensively studied in association with hypertension; however, findings are conflicting

[35,36]. Previous studies have focused on a single polymorphism of marker A1166C (rs5186). The current study also examined the association between rs5186 and BP responses to CPT, and no significant relationship was observed. However, we found that marker rs4681443 was significantly associated with SBP responses to CPT after adjustment for multiple testing. SNP rs4681443 is located at the intron 2 of the AGTR1 gene. SNPseek (<http://snp.wustl.edu/cgi-bin/SNPseek/index.cgi>) analysis could not provide functional support for the association of this marker. However, recent evidence has suggested that intronic polymorphisms may be etiologically involved in the development of complex disorders [37]. Additional studies designed to investigate the positive associations reported here are warranted, and functional studies are needed to elucidate the potential mechanism underlying the association of the RAS gene polymorphisms with BP responses to CPT.

Although the current study has a few limitations, such as no record of heart rate change before and after the CPT stimulus which is known to be an important index of cardiovascular response to stress, our study has several important strengths. To date, it is the largest study to examine the association between genetic variants and BP response to CPT. Study attributes, including use of a family design and recruitment of all Han Chinese participants, should make the analysis robust to population stratification. The study participants were also similar with respect to lifestyle and environmental risk factors, including diet and physical activity. Thus, confounding due to these exposures should be minimal.

Conclusion

In summary, we have described novel genetic variants in the AGT and AGTR1 genes that are significantly associated with BP responses to CPT in Han Chinese population. These findings may have important clinical and public health implications. Additional research should be conducted to determine whether these observations can be replicated in other populations with different ethnic backgrounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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APPENDIX

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Figure 1. Blood pressure response profile of cold pressor test participants. Average baseline BP is shown by the horizontal dashed line.

Figure 2.

–Log p-values for the association between 68 SNPs in 6 candidate genes and systolic blood pressure and diastolic blood pressure responses to cold pressor test in codominant (top) and recessive (bottom) genetic models. All labeled SNPs were significant after adjustment for multiple comparisons (q-value <0.05).

Figure 3. Percent change in systolic (SBP) and diastolic (DBP) for the cold pressor test by SNP rs2006765 genotypes of the AGT gene.

Figure 4.

–Log p-value for the association between AGT gene polymorphisms and BP responses to CPT (top), and LD structure of AGT gene (bottom). The numbers inside the squares are D' × 100 (D' values of 1.0 are not shown). The r^2 color scheme of Haploview was applied ($r^2=0$ shown in white, $0 < r^2 < 1$ shown in shades of gray, and $r^2=1$ shown in black). The solid spine linkage disequilibrium method, as implemented in Haploview software, was used to define linkage disequilibrium blocks.

Table 1

Characteristics and blood pressure responses to cold pressor test among 1998 study participant.

Variable	Mean $\pm$ SD or Percentage
Age, yrs	40.1 $\pm$ 10.3
Male, %	52.5
Cigarette smoking, %	34.6
Alcohol drinking, %	26.9
BMI, kg/m ²	23.4 $\pm$ 3.2
Pre-CPT BP, mm Hg	
Systolic	120.1 $\pm$ 15.6
Diastolic	74.7 $\pm$ 10.9
BP response to CPT, mm Hg	
Systolic	13.6 $\pm$ 10.6
Diastolic	6.9 $\pm$ 6.7
BP response to CPT, %	
Systolic	11.4 $\pm$ 9.0
Diastolic	9.5 $\pm$ 9.6

Table 2

Unadjusted p-values for SNPs in the renin-angiotensin system that were significantly associated with blood pressure response in at least one genetic model.

Candidate Gene	SNP	Systolic blood pressure				Diastolic blood pressure			
		Codominant	Additive	Dominant	Recessive	Codominant	Additive	Dominant	Recessive
REN	rs5707 [#]	0.519	0.673	0.867	0.304	0.067	0.270	0.993	0.026
	rs11571080 [#]	0.476	0.366	0.723	0.224	0.088	0.102	0.546	0.028
	rs12750834	0.559	0.367	0.287	0.856	0.012	0.396	0.688	0.009
	rs2006765	0.919	0.995	0.889	0.729	0.00002 *	0.202	0.855	0.000004 *
AGT	rs943580	0.951	0.752	0.769	0.854	0.001 *	0.147	0.576	0.0002 *
	rs2478545	0.855	0.683	0.603	0.944	0.089	0.055	0.188	0.041
	rs699	0.717	0.889	0.701	0.519	0.047	0.934	0.514	0.029
	rs11122577 [#]	0.316	0.150	0.202	0.331	0.011	0.014	0.052	0.010
AGTR1	rs4681443 [#]	0.001 *	0.293	0.384	0.0002 *	0.617	0.434	0.474	0.481
	rs718858	0.021	0.066	0.096	0.024	0.700	0.421	0.468	0.617
	rs389566	0.018	0.005	0.006	0.211	0.444	0.268	0.374	0.249
	rs6801836	0.011	0.026	0.005	0.669	0.989	0.881	0.886	0.944
ACE	rs1800766 [#]	0.112	0.079	0.039	0.891	0.528	0.264	0.319	0.396
	rs275646	0.026	0.071	0.140	0.015	0.178	0.107	0.153	0.175
	rs4968591	0.368	0.801	0.483	0.295	0.123	0.044	0.081	0.160

* FDR q-value < 0.05.

[#] Genotyped as part of the Affymetrix 6.0 platform.