Variant Near *FGF5* Has Stronger Effects on Blood Pressure in Chinese With a Higher Body Mass Index

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BACKGROUND
The objective of this study was to investigate the genetic association of 4 candidate variants with blood pressure and test the modifying effects of environmental factors including age, sex, and body mass index (BMI).

METHODS
We used a linear mixed-effects model to test for variant main effects and variant interactions with age, sex, and BMI on systolic (SBP) and diastolic (DBP) blood pressure in 7,319 Chinese adults from the China Health and Nutrition Survey (CHNS). We attempted to replicate our significant interaction findings in 1,996 Chinese men from the Fangchenggang Area Male Health and Examination Survey (FAMHES).

RESULTS
Two variants (rs11105378 near *ATP2B1* and rs1458038 near *FGF5*) were significantly associated ($P < 0.00625 = 0.05/8$) with both SBP and DBP in CHNS. Variant rs1378942 near *CSK* was nominally associated with SBP ($P = 0.01$). The signal at rs1458038 exhibited a genotype-by-BMI interaction affecting blood pressure ($P_{interaction} = 0.0018$ for SBP; $P_{interaction} = 0.049$ for DBP), with the strongest variant effects in those with the highest BMI. In FAMHES, rs1458038 also showed stronger effects on SBP and DBP among men with the highest BMI.

CONCLUSIONS
Our findings suggest high BMI increases the effect of the blood pressure-increasing allele at rs1458038 near *FGF5*, further highlighting the importance of obesity prevention in reducing hypertension risk.

Keywords: blood pressure; BMI; FGF5; hypertension; interaction.

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Hypertension is an important risk factor for cardiovascular disease, the leading cause of mortality all over the globe.[1] Hypertension is a worldwide problem[2] and its prevalence is increasing in China.[3] According to the data from the 2007–2008 China National Diabetes and Metabolic Disorders Study, 26.6% of Chinese adults have hypertension,[3] causing considerable health and economic burden.[4,5]

The etiology of high blood pressure involves the interplay among many factors. Risk factors include body mass index (BMI), tobacco use, salt and alcohol intake, and physical activity.[6]

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An estimated 30–60% of blood pressure variation is explained by genetic factors.[7] Many genetic variants have been identified by genome-wide association studies conducted in multiple populations. Blood pressure is a complex phenotype, and the effects of some variants may be stronger in individuals exposed to specific environmental factors. However, relatively few studies have investigated the possible interaction between genetic variants and environmental factors in affecting blood pressure.

Several loci first identified in subjects of European descent, including *ATP2B1* on chromosome 12q21.33, *FGF5* on 4q21.21, *CYP17A1* on 10q24.32, and *CSK* on 15q24.1 have been validated in East Asians.[8,9] In this study, we genotyped index variants from these 4 candidate loci and examined whether the associations with systolic (SBP) and diastolic (DBP) blood pressure could be replicated in 7,319 adults (18 years and older) from 9 provinces in the China Health and Nutrition Survey (CHNS). We further tested whether the associations were influenced by environmental factors including age, sex, and BMI. Finally, we attempted to replicate specific findings in 1,996 men from the Fangchenggang Area Male Health and Examination Survey (FAMHES).

METHODS

Study design: CHNS. CHNS is a nationwide longitudinal survey designed to examine a series of economic, sociological, demographic, and health questions in the Chinese population. The design of CHNS has been described in detail elsewhere.[10] This study was conducted in 9 provinces in China that vary significantly in terms of socioeconomic, health-related, and nutritional status. The sample from each province was drawn using a multistage, random cluster procedure, designed to select a stratified probability sample in each province, with selection of larger cities and smaller suburban and rural villages, from which households were randomly selected. Approximately 19,000 individuals from ~4,400 households participated in the overall survey. The first round of data was collected in 1989, followed by 8 additional waves of data collected in 1991, 1993, 1997, 2000, 2004, 2006, 2009, and 2011. The study was approved by the ethics committee at the National Institute of Nutrition and Food Safety at the China Center for Disease Control and Prevention and the Institutional Review Board at the University of North Carolina at Chapel Hill. Written consent was obtained from subjects surveyed in 2009.

Data collection and phenotypes. For this study, we used dietary, clinical, and anthropometric data collected from participants who were age 18 years or older and not pregnant at the time of the 2009 survey. Blood pressure was measured 3 consecutive times on the same day, with 10 minutes of seated rest before the first measurement and 3–5 minutes intervals between each measurement. SBP and DBP were determined by the first and fifth phase Korotkoff sounds,[11] respectively. The average of the 3 measurements was used for analyses. For participants who take antihypertensive medications, 10 and 5 mm Hg was added to the average SBP and DBP, respectively.[12,13] The smoking responses were dichotomized to define current smoking status. Total salt intake (grams) was estimated based on a combination of 3 consecutive 24-hour food recalls at the individual level and a food inventory at the household level.[14]

Genotyping. Fasting venous blood was collected and stored at −80 °C, and DNA was extracted from buffy coat using the FlexiGene DNA kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. Four candidate variants for blood pressure,[9] including rs11105378 at *ATP2B1*, rs1458038 at *FGF5*, rs1004467 at *CYP17A1*, and rs1378942 at *CSK-CYP1A1*, were genotyped using TaqMan chemistry (Applied Biosystems). TaqMan genotyping assays with probes labeled with the fluorophores FAM and VIC were purchased from Applied Biosystems. The Universal PCR Master Mix from Applied Biosystems was used in a 5 µl total reaction volume with 10 ng DNA per reaction. Allelic discrimination was measured automatically on ABI Prism 7900HT (Applied Biosystems) with Sequence Detection Systems. Among 8,221 individuals genotyped, the success rate for each variant was >98%. Genotype distributions were consistent with Hardy–Weinberg equilibrium expectations ($P > 0.05$). The concordance rate between 301 duplicate pairs across all variants was >99.9%.

Statistical analyses. A total of 7,319 nonpregnant adults, age 18 years or older at the time of blood pressure measurement, with complete phenotype, covariate, and genotype data were included in tests of association between variants and SBP or DBP. The distributions of SBP and DBP, after accounting for age, sex, and BMI, showed no significant deviation from normality; untransformed traits were analyzed. Linear mixed-effects models,[15] with a random effect for household to account for the correlation between blood pressure measurements from members in the same family, were used to test the variant additive genetic main effects. The base models included adjustment for covariates significantly ($P < 0.05$) associated with SBP or DBP; namely, age, sex, province, and BMI. Additional models also included covariate adjustment for current smoking status and total salt intake. Each variant that showed evidence for a main effect association ($P < 0.05$) with either SBP or DBP was further analyzed for variant-by-environment interactions with age, sex, and BMI by including an interaction term to the above base linear mixed-effects model. Stratified analyses were performed for variants demonstrating evidence of a significant interaction ($P_{\text{interaction}} < 0.05$) by categorizing participants according to quartiles of the environmental factor and testing main effects of the variants within each quartile.

Follow-up sample: FAMHES. We followed up our genotype-by-BMI interaction finding at rs1458038 near *FGF5* in 1,996 men from the FAMHES study using linear regression models with covariate adjustment for age and the main effects for genotype and BMI. As with CHNS, we also performed stratified analyses for the main effects of rs1458038 on SBP and DBP in strata defined by BMI quartiles of the FAMHES participants. The FAMHES project was conducted in Fangchenggang city, Guangxi, southern China in 2009.[16] The men were 20–69 years old (average age 37.5 ± 11.1 years) and of self-reported southern Chinese Han ethnicity.[17] About 50.8% of men were reported smokers and the average BMI

was 23.1 ± 3.4 kg/m^2. Written informed consent to participate in the study was provided by all participating men; the study was approved by the Wake Forest University Institutional Review Board. All samples were genotyped on the Illumina HumanOmni1-Quad BeadChip. A single measure of blood pressure on each FAMHES participant was taken by applying a mercury sphygmomanometer to the right arm of the participants *after* they were seated in a comfortable sitting position *for* an at least 5-minute rest period.

RESULTS

Overall, 7,319 CHNS subjects (3,987 females and 3,332 males) with complete phenotype (average SBP and DBP), essential covariates (age, sex, BMI, and province), and genotype data were included in the analyses (Table 1). Higher age, male sex, larger BMI, and current smoking were each associated with higher SBP and DBP (data not shown, $P < 0.05$). Higher salt intake was associated with higher DBP. Blood pressure levels were higher in residents from Northern provinces than in residents from Southern provinces (Supplementary Table 1), which is consistent with the observed north-south gradient in the prevalence of hypertension in China.[18] When all covariates were included in a single model, current smoking status and total salt intake no longer showed evidence for association with SBP or DBP. Our base model, model 1, included covariates for age, sex, BMI, and province while our secondary model, model 2, had additional covariates for current smoking status and total salt intake.

Four candidate variants that were reported to be associated with SBP and/or DBP in prior studies[7–9,19,20] were tested for association in CHNS. For the base model, 2 variants (rs11105378 near *ATP2B1* and rs1458038 near *FGF5*) were significantly associated ($P < 0.00625 = 0.05/8$) with both SBP and DBP (Table 2). Variant rs1378942 near *CSK* was nominally ($P < 0.05$) associated with SBP ($P = 7.0 \times 10^{-3}$). The direction and effect sizes for all 8 association tests were consistent with the previous reports. Additional covariate adjustment for current smoking status and total salt intake resulted in very similar findings compared to the base model (Table 2).

The 3 variants associated with SBP and 2 variants associated with DBP were tested for interaction with each of 3 environmental factors (age, sex, and BMI) that significantly affected blood pressure when controlling for all other covariates. Among the 15 tests performed, 4 showed nominally significant results ($P_{interaction} < 0.05$). The observed interaction between rs1458038 and BMI on SBP was significant ($P_{interaction} = 0.0018$) after Bonferroni correction for multiple tests ($P_{interaction} < 0.0033$, 0.05/15 tests). The interaction between rs1458038 and BMI was also nominally associated ($P_{interaction} = 0.049$) with DBP. The 2 remaining nominally significant interactions were between rs1378942 and sex on SBP ($P_{interaction} = 0.045$) and between rs11105378 and age on DBP ($P_{interaction} = 0.026$).

Further analyses were conducted in CHNS to interrogate the interactions between rs1458038 and BMI on SBP and DBP. We stratified the samples according to BMI quartiles and performed main-effects regression analyses of rs1458038 on SBP and DBP, adjusting for the base model covariates. The T allele of rs1458038 was significantly associated with higher SBP in the highest quartile of BMI, but the association was not significant in lower quartiles (Table 3). The magnitude of the effect size estimates increased with BMI. A similar pattern of stronger effects of this variant in subjects with the highest BMI was observed for DBP (Table 3). The interaction between rs1458038 and BMI was only nominally associated with DBP (females: $P_{interaction} = 0.0076$; males: $P_{interaction} = 0.99$) and SBP (females: $P_{interaction} = 0.0022$; males: $P_{interaction} = 0.16$) in CHNS females (Supplementary Tables 2 and 3), though the differences in effects were not statistically significant (3-way interaction between rs1458038, BMI and sex including all pairwise 2-way interactions in the model) for either DBP ($P_{3\text{-way-interaction}} = 0.12$) or SBP ($P_{3\text{-way-interaction}} = 0.33$).

In FAMHES, there was a trend ($P \leq 0.10$) with consistent direction of effects to CHNS, for main effects of rs1458038 on both DBP ($P = 0.058$) and SBP ($P = 0.10$) (Supplementary Table 4). The interaction effects between rs1458038 and BMI affecting SBP and DBP were in the same direction as CHNS but did not reach statistical significance in FAMHES ($P_{interaction} = 0.36$ for DBP; $P_{interaction} = 0.62$ for SBP). Consistent with the sex-combined results from CHNS, the estimates of

Table 1. Characteristics of the China Health and Nutrition Survey participants analyzed

	Female (n = 3,987)		Male (n = 3,332)		Total (n = 7,319)	
Characteristics	**Mean ± SD**	**Median (range)**	**Mean ± SD**	**Median (range)**	**Mean ± SD**	**Median (range)**
SBP (mm Hg)	124.3 ± 20.8	120.0 (76.7–266.7)	126.8 ± 18.2	122.0 (80.0–229.3)	125.4 ± 19.7	121.0 (76.7–266.7)
DBP (mm Hg)	79.4 ± 11.6	80.0 (44.0–152.0)	82.5 ± 11.3	80.7 (50.0–136.0)	80.8 ± 11.6	80.0 (44.0–152.0)
Age (years)	50.9 ± 15.0	51.1 (18.0–98.9)	50.7 ± 15.1	51.0 (18.0–92.3)	50.8 ± 15.0	51.0 (18.0–98.9)
BMI (kg/m^2)	23.4 ± 3.5	23.0 (13.4–38.8)	23.4 ± 3.4	23.2 (13.4–37.2)	23.4 ± 3.5	23.1 (13.4–38.8)
Current smoker (%)	3.70%	–	55.00%	–	27.04%	–
Total salt intake (grams)	4.5 ± 2.6	3.9 (0.1–22.2)	4.9 ± 2.7	4.3 (0.2–21.3)	4.7 ± 2.6	4.1 (0.1–22.2)

Values are means ± SD, medians (range), or %. The average of the 3 measurements of SBP or DBP was used for analysis. Total salt intake was based on a combination of 3 consecutive 24-hour food recalls at the individual level and a food inventory at the household level. The sample size is 7,315 with data on current smoking status and 7,063 with data on total salt intake.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 2. Main effect association of variants with SBP and DBP in CHNS

						Model 1				Model 2			
Variant	Chr	Nearby Gene	EA	EAF	Trait	Sample	β	SE	*P*	Sample	β	SE	*P*
rs1458038	4	*FGF5*	T	0.42	SBP	7,272	1.29	0.28	2.8E-06	7,013	1.28	0.28	5.9E-06
					DBP		0.70	0.17	5.2E-05		0.68	0.18	1.1E-04
rs1004467	10	*CYP17A1*	A	0.65	SBP	7,308	0.37	0.29	0.20	7,048	0.44	0.29	0.13
					DBP		0.11	0.18	0.54		0.13	0.18	0.49
rs11105378	12	*ATP2B1*	C	0.66	SBP	7,187	1.22	0.29	3.3E-05	6,929	1.29	0.30	1.8E-05
					DBP		0.73	0.18	6.9E-05		0.80	0.19	1.8E-05
rs1378942	15	*CSK*	C	0.84	SBP	7,276	1.02	0.38	7.0E-03	7,018	0.97	0.39	0.012
					DBP		0.35	0.24	0.14		0.36	0.24	0.14

β coefficients represent the estimated change in the level of blood pressure associated with each additional copy of the effect allele, designated as the blood pressure raising allele. Covariates for model 1 were age, gender, province, and BMI. Covariates for model 2 were age, gender, province, BMI, current smoking status, and total salt intake.

Abbreviations: BMI, body mass index; CHNS, China Health and Nutrition Survey; DBP, diastolic blood pressure; EA, effect allele; EAF, effect allele frequency; SBP, systolic blood pressure; SE, standard error.

Table 3. Mean levels of SBP and DBP by rs1458038 genotype and BMI quartile in CHNS

	All participants	CC	CT	TT	*N*	β (SE)	*P*
SBP							
Q1	118.9 ± 18.6	118.7 ± 18.3	118.7 ± 18.2	120.1 ± 20.0	1,818	0.21 (0.54)	0.69
Q2	122.8 ± 18.2	121.7 ± 17.4	123.0 ± 18.1	124.7 ± 20.2	1,818	0.88 (0.55)	0.11
Q3	127.1 ± 18.7	126.2 ± 18.6	127.2 ± 18.1	128.6 ± 20.4	1,818	1.05 (0.55)	0.057
Q4	132.8 ± 20.4	130.8 ± 19.2	133.4 ± 20.7	135.1 ± 21.6	1,818	2.93 (0.61)	**1.9E-06**
DBP							
Q1	76.4 ± 10.9	76.0 ± 10.6	76.4 ± 10.8	77.1 ± 11.6	1,818	0.33 (0.34)	0.34
Q2	78.9 ± 10.8	78.7 ± 10.8	79.1 ± 10.6	78.9 ± 11.4	1,818	0.12 (0.34)	0.72
Q3	82.2 ± 10.8	81.4 ± 11.0	82.5 ± 10.4	83.0 ± 11.3	1,818	0.80 (0.34)	**0.021**
Q4	85.7 ± 11.7	85.1 ± 11.7	85.6 ± 11.9	87.4 ± 11.2	1,818	1.23 (0.38)	**1.0E-03**

Q1–Q4: the lowest BMI quartile to the highest BMI quartile. Q1: BMI < 20.93; Q2: 20.93 ≤ BMI < 23.11; Q3: 23.11 ≤ BMI < 25.65; Q4: BMI ≥ 25.65. Values for SBP and DBP are mean ± SE.

Abbreviations: BMI, body mass index; CHNS, China Health and Nutrition Survey; DBP, diastolic blood pressure; SBP, systolic blood pressure; SE, standard error.

the main effects of rs1458038 on SBP and DBP were strongest in men grouped in the highest quartile of BMI (Table 4).

DISCUSSION

In this study, we tested the association of 4 candidate variants with SBP and DBP in 7,319 Chinese individuals, from 9 provinces, participating in CHNS and followed up our top findings in 1,996 Chinese men from the province of Guangxi participating in FAMHES. rs1458038, 23 kb upstream of *FGF5* on chromosome 4, and rs11105378, 41 kb upstream of *ATP2B1* on chromosome 12, were significantly associated with both SBP and DBP ($P < 0.00625$) in CHNS while rs1378942, an intronic variant in *CSK*, was nominally associated with SBP ($P = 7.0 \times 10^{-3}$). We also detected a significant interaction between rs1458038 and BMI affecting SBP and a nominally significant interaction between this same variant and BMI affecting DBP in CHNS. Specifically, we showed that the effect of the rs1458038 risk genotype is stronger in subjects in the heaviest quartile of the BMI distribution.

rs1458038, and nearby variant rs16998073, were identified to be associated with DBP and SBP in Europeans[19,20] and in East Asians.[8,9,21,22] We found no published evidence supporting rs1458038 to be an important regulatory variant (RegulomeDB (http://www.regulomedb.org) score: "minimal binding evidence"; no evidence for being an eQTL in MuTHER (http://www.muther.ac.uk), GTEx (http://www.gtexportal.org), or blood eQTL(http://genenetwork.nl/bloodeqtlbrowser). rs11105378 was identified by the CHARGE and Global BPgen Consortia,[7] and confirmed in Japanese.[9] Several other variants that are in high linkage disequilibrium with rs11105378 ($r^2 > 0.8$ in CEU) were also identified in different ethnicities.[7,8,19,23] rs11105378 was reported to be associated with expression of neighboring *WDR51B* ($P = 7.1 \times 10^{-15}$) in blood (blood eQTL). Differences in *ATP2B1* mRNA expression levels in umbilical artery

Table 4. Mean levels of SBP and DBP by rs1458038 genotype and BMI quartile in FAMHES

	All participants	CC	CT	TT	*N*	β (SE)	*P*
SBP							
Q1	113.3 ± 13.2	112.7 ± 13.6	112.8 ± 13.1	115.1 ± 12.9	499	1.10 (0.82)	0.18
Q2	116.6 ± 14.4	116.4 ± 15.7	116.6 ± 14.2	117.1 ± 12.2	499	0.37 (0.92)	0.69
Q3	118.6 ± 14.8	117.7 ± 14.0	118.8 ± 14.8	119.7 ± 16.6	499	1.02 (0.94)	0.28
Q4	124.3 ± 17.1	122.9 ± 15.8	124.8 ± 16.8	126.0 ± 20.6	499	1.63 (1.12)	0.15
DBP							
Q1	73.5 ± 8.8	73.3 ± 9.3	73.2 ± 8.7	74.3 ± 8.6	499	0.44 (0.55)	0.43
Q2	75.0 ± 9.3	74.2 ± 9.9	75.6 ± 9.4	75.3 ± 8.2	499	0.68 (0.60)	0.26
Q3	77.5 ± 9.2	77.0 ± 9.1	77.8 ± 8.8	77.9 ± 10.4	499	0.49 (0.58)	0.40
Q4	81.6 ± 11.5	80.1 ± 10.3	82.2 ± 11.3	83.0 ± 14.0	499	1.59 (0.75)	0.034

Q1–Q4: the lowest BMI quartile to the highest BMI quartile. Q1: BMI < 20.80; Q2: 20.80 ≤ BMI < 23.04; Q3: 23.04 ≤ BMI < 25.50; Q4: BMI ≥ 25.50.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FAMHES, Fangchenggang Area Male Health and Examination Survey; SBP, systolic blood pressure; SE, standard error.

smooth muscle cells have also been observed among different rs11105378 genotypes.[9] rs1378942, an intronic variant 2,306 bp from a splice site in *CSK*, was significantly associated with SBP and DBP in Europeans[19]; however, only nominal association was observed in East Asians[8] and Japanese.[9] In our study, we only replicate ($P < 0.05$) its association with SBP, although the effect size estimates for both SBP and DBP were similar in both direction and size to the previous study in Japanese. There is some evidence for rs1378942 being an important regulatory variant (RegulomeDB score: "likely to affect binding and linked to gene expression of gene target"). rs1378942 has been reported to be associated with gene expression of a number of nearby genes, including *CSK* (in blood ($P = 1.3 \times 10^{-129}$) (in blood eQTL)) and *ULK3* (in adipose tissue ($P = 6.3 \times 10^{-8}$), lymphocytes (1.0×10^{-20}), skin (3.1×10^{-8}) (in MuTHER) and blood ($P = 5.7 \times 10^{-33}$) (in blood eQTL)). We failed to replicate blood pressure associations with rs1004467, an intronic variant 35 bp from a splice-site in *CYP17A1,* in CHNS. Here too, our directions of effect were consistent with the previous Japanese study. However, the size of the effects for DBP and SBP were about half of those reported previously for this variant.

An interesting finding of this study is the evidence for interaction between rs1458038 and BMI in affecting both SBP and DBP in CHNS. BMI is a strong risk factor that affects blood pressure; the prevalence of hypertension and mean levels of SBP and DBP increase as BMI increases.[24,25] While variants 5′ of *FGF5* at 4q21 are associated with blood pressure in the Chinese population,[21,22] the interaction between these 2 risk factors on affecting blood pressure has not been previously reported. The strongest evidence for association between rs1458038 and blood pressure measures in CHNS were observed in participants in the highest quartile of BMI. In FAMHES, the strongest effects of rs14358038 for both blood pressure traits were also observed in the highest quartile of BMI.

When assessing the overall impact of the FAMHES results, some differences between CHNS and FAMHES are worth noting. FAMHES includes only males from the province of Guangxi, while CHNS includes both males and females from 9 provinces, including Guangxi. The sample size of FAMHES (n = 1,996) is considerably smaller than that of CHNS (n = 7,319). FAMHES men were also younger, on average, than CHNS individuals. Both the average SBP and DBP measures were lower in FAMHES men compared to CHNS men; however, these results are consistent with the younger ages in FAMHES and the lower blood pressure values observed in CHNS men from Guangxi (Supplementary Table 1). SBP and DBP were measured only once in FAMHES compared to 3 measurements (we applied the average) in CHNS. Finally, no hypertensive medication history was available in FAMHES. In CHNS, 399 subjects were known to be on hypertensive medication and an offset was applied to the SBP and DPB measures in CHNS to account for medication use. Given these sampling differences between FAMHES and CHNS participants, it is possible that the interactions between BMI and rs14358038 affecting blood pressure may not be the same in the 2 cohorts. Interestingly, though the results were not significantly different, the observed interactions between rs1458038 and BMI on the blood pressure measures were stronger in women than in men in CHNS.

Four genes including *ANTXR2*, *PRDM8*, *FGF5*, and *C4orf22* are located near rs1458038. *ANTXR2* encodes anthrax toxin receptor 2, a protein involved in angiogenesis.[26] A recent functional study,[27] using *in vivo* small interfering RNA silencing in mice, suggested *ANTXR2* is the most likely causative gene in the 4q21 region that regulates individual differences in blood pressure in humans. The study proposed that the lower *Antxr2* expression can lead to a decrease in the proliferation of endothelial cells, prevent the formation of capillary network, and result in microvascular rarefaction and increase of BP. Many studies observed a developing microvascular rarefaction within skeletal muscle during the metabolic syndrome including obesity, insulin resistance/type II diabetes mellitus, dyslipidemia, and hypertension.[28,29] Microvascular dysfunction is a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension.[30,31] In addition to *ANTXR2*,

FGF5 is also possibly involved in the metabolic syndrome. *FGF5* encodes fibroblast growth factor 5, a member of the fibroblast growth factor (FGF) family. Although FGF5 has not yet been shown to have a direct effect on BMI, several members of the FGF family have been shown to affect obesity by regulating fatty acid oxidation and lipid metabolism. Treatment with exogenous recombinant human FGF21 protein via infusion or injection can lead to weight loss and improvement of lipid profiles in diet-induced obese mice[32] and diabetic rhesus monkeys.[33] Transgenic mice expressing human *FGF19* display increased metabolic rate and decreased adiposity.[34] The roles of *PRDM8* and *C4orf22* in the pathogenesis of obesity and hypertension are not known.

In conclusion, the association of rs11105378 near *ATP2B1* and rs1458038 near *FGF5* with both SBP and DBP were replicated in CHNS. In addition, the magnitude of the associations between rs1458038, 5′ of *FGF5*, and blood pressure were modified by BMI in CHNS individuals. While we were unable to formally replicate the interaction in a second Chinese cohort, evidence from both studies implicate that the risk genotype at rs1458038 is particularly important in Chinese individuals with higher BMI. To our knowledge, this is the first reported interaction between a variant in or near *FGF5* and BMI on blood pressure. Further studies in Chinese and other populations are needed to confirm this finding.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Executive summary: heart disease and stroke statistics–2013 update: a report from the American Heart Association. *Circulation* 2013; 127:143–152.
2. Joffres M, Falaschetti E, Gillespie C, Robitaille C, Loustalot F, Poulter N, McAlister FA, Johansen H, Baclic O, Campbell N. Hypertension prevalence, awareness, treatment and control in national surveys from England, the USA and Canada, and correlation with stroke and ischaemic heart disease mortality: a cross-sectional study. *BMJ Open* 2013; 3:e003423.
3. Gao Y, Chen G, Tian H, Lin L, Lu J, Weng J, Jia W, Ji L, Xiao J, Zhou Z, Ran X, Ren Y, Chen T, Yang W. Prevalence of hypertension in China: a cross-sectional study. *PLoS One* 2013; 8:e65938.
4. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005; 365:217–223.
5. Alcocer L, Cueto L. Hypertension, a health economics perspective. *Ther Adv Cardiovasc Dis* 2008; 2:147–155.
6. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; 42:1206–1252.
7. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; 41:677–687.
8. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, Sim X, Tay WT, Chen CH, Zhang Y, Yamamoto K, Katsuya T, Yokota M, Kim YJ, Ong RT, Nabika T, Gu D, Chang LC, Kokubo Y, Huang W, Ohnaka K, Yamori Y, Nakashima E, Jaquish CE, Lee JY, Seielstad M, Isono M, Hixson JE, Chen YT, Miki T, Zhou X, Sugiyama T, Jeon JP, Liu JJ, Takayanagi R, Kim SS, Aung T, Sung YJ, Zhang X, Wong TY, Han BG, Kobayashi S, Ogihara T, Zhu D, Iwai N, Wu JY, Teo YY, Tai ES, Cho YS, He J. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. *Nat Genet* 2011; 43:531–538.
9. Tabara Y, Kohara K, Kita Y, Hirawa N, Katsuya T, Ohkubo T, Hiura Y, Tajima A, Morisaki T, Miyata T, Nakayama T, Takashima N, Nakura J, Kawamoto R, Takahashi N, Hata A, Soma M, Imai Y, Kokubo Y, Okamura T, Tomoike H, Iwai N, Ogihara T, Inoue I, Tokunaga K, Johnson T, Caulfield M, Munroe P, Umemura S, Ueshima H, Miki T. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. *Hypertension* 2010; 56:973–980.
10. Popkin BM, Du S, Zhai F, Zhang B. Cohort Profile: The China Health and Nutrition Survey–monitoring and understanding socio-economic and health change in China, 1989–2011. *Int J Epidemiol* 2010; 39:1435–1440.
11. Chio SS, Urbina EM, Lapointe J, Tsai J, Berenson GS. Korotkoff sound versus oscillometric cuff sphygmomanometers: comparison between auscultatory and DynaPulse blood pressure measurements. *J Am Soc Hypertens* 2011; 5:12–20.
12. Johnson AD, Newton-Cheh C, Chasman DI, Ehret GB, Johnson T, Rose L, Rice K, Verwoert GC, Launer LJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, Caulfield M, van Duijn CM, Ridker PM, Munroe PB, Levy D. Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. *Hypertension* 2011; 57:903–910.
13. Montasser ME, Shimmin LC, Hanis CL, Boerwinkle E, Hixson JE. Gene by smoking interaction in hypertension: identification of a major quantitative trait locus on chromosome 15q for systolic blood pressure in Mexican-Americans. *J Hypertens* 2009; 27:491–501.
14. Batis C, Gordon-Larsen P, Cole SR, Du S, Zhang B, Popkin B. Sodium intake from various time frames and incident hypertension among Chinese adults. *Epidemiology* 2013; 24:410–418.
15. Pinheiro JC, Bates DM. *Mixed-Effects Models in S and SPLUS*. Springer: New York, 2000.
16. Yang X, Sun J, Gao Y, Tan A, Zhang H, Hu Y, Feng J, Qin X, Tao S, Chen Z, Kim ST, Peng T, Liao M, Lin X, Zhang Z, Tang M, Li L, Mo L, Liang Z, Shi D, Huang Z, Huang X, Liu M, Liu Q, Zhang S, Trent JM, Zheng SL, Xu J, Mo Z. Genome-wide association study for serum complement C3 and C4 levels in healthy Chinese subjects. *PLoS Genet* 2012; 8:e1002916.

17. Tan A, Gao Y, Yang X, Zhang H, Qin X, Mo L, Peng T, Xia N, Mo Z. Low serum osteocalcin level is a potential marker for metabolic syndrome: results from a Chinese male population survey. *Metabolism* 2011; 60:1186–1192.
18. Reynolds K, Gu D, Muntner P, Wu X, Chen J, Huang G, Duan X, Whelton PK, He J. Geographic variations in the prevalence, awareness, treatment and control of hypertension in China. *J Hypertens* 2003; 21:1273–1281.
19. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sober S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjogren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimaki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kahonen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Kottgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grassler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Soler Artigas M, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stancakova A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT Jr, Mosley TH, Seshadri S, Shrine NR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikainen LP, Soininen P, Tukiainen T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Jarvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 2011; 478: 103–109.
20. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Döring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvänen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dörr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Völker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Völzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB; Wellcome Trust Case Control Consortium. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009; 41:666–676.
21. Liu C, Li H, Qi Q, Lu L, Gan W, Loos RJ, Lin X. Common variants in or near FGF5, CYP17A1 and MTHFR genes are associated with blood pressure and hypertension in Chinese Hans. *J Hypertens* 2011; 29:70–75.
22. Niu W, Zhang Y, Ji K, Gu M, Gao P, Zhu D. Confirmation of top polymorphisms in hypertension genome wide association study among Han Chinese. *Clin Chim Acta* 2010; 411:1491–1495.
23. Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Lee JK, Oh B, Kim HL. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet* 2009; 41:527–534.
24. Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res* 2000; 8:605–619.
25. Kotsis V, Stabouli S, Bouldin M, Low A, Toumanidis S, Zakopoulos N. Impact of obesity on 24-hour ambulatory blood pressure and hypertension. *Hypertension* 2005; 45:602–607.
26. Bell SE, Mavila A, Salazar R, Bayless KJ, Kanagala S, Maxwell SA, Davis GE. Differential gene expression during capillary morphogenesis in 3D collagen matrices: regulated expression of genes involved in basement membrane matrix assembly, cell cycle progression, cellular differentiation and G-protein signaling. *J Cell Sci* 2001; 114:2755–2773.
27. Park SY, Lee HJ, Ji SM, Kim ME, Jigden B, Lim JE, Oh B. ANTXR2 is a potential causative gene in the genome-wide association study of the blood pressure locus 4q21. *Hypertens Res* 2014; 9:811–817.
28. Irving RJ, Walker BR, Noon JP, Watt GC, Webb DJ, Shore AC. Microvascular correlates of blood pressure, plasma glucose, and insulin resistance in health. *Cardiovasc Res* 2002; 53:271–276.
29. Frisbee JC. Hypertension-independent microvascular rarefaction in the obese Zucker rat model of the metabolic syndrome. *Microcirculation* 2005; 12:383–392.
30. De Boer MP, Meijer RI, Wijnstok NJ, Jonk AM, Houben AJ, Stehouwer CD, Smulders YM, Eringa EC, Serné EH. Microvascular dysfunction: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Microcirculation* 2012; 19:5–18.
31. Jonk AM, Houben AJ, de Jongh RT, Serné EH, Schaper NC, Stehouwer CD. Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Physiology (Bethesda)* 2007; 22:252–260.
32. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* 2008; 149:6018–6027.
33. Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, Hansen BC, Shanafelt AB, Etgen GJ. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology* 2007; 148:774–781.
34. Tomlinson E, Fu L, John L, Hultgren B, Huang X, Renz M, Stephan JP, Tsai SP, Powell-Braxton L, French D, Stewart TA. Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology* 2002; 143:1741–1747.