ORIGINAL ARTICLE

Influence of Obesity on Association Between Genetic Variants Identified by Genome-Wide Association Studies and Hypertension Risk in Chinese Children

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BACKGROUND
Childhood hypertension is a complex disease influenced by both genetic and environmental factors. We aimed to examine how obesity status influences the association of 6 single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWASs) with systolic/diastolic blood pressure (SBP/DBP) and hypertension in Chinese children.

METHODS
We recruited 619 hypertensive case subjects and 2,458 individuals with normal blood pressure from the Beijing Child and Adolescent Metabolic Syndrome study, a population-based case–control study. We selected 6 SNPs from earlier GWASs of hypertension and genotyped them using TaqMan assay.

RESULTS
In the normal weight group, we did not observe any significant association of 6 SNPs and the genetic risk score (GRS) with SBP/DBP and hypertension (all $P > 0.05$). Only *STK39* rs3754777 was significantly associated with higher DBP ($P = 0.02$) in the overweight subjects. In the obese group, 3 SNPs and the GRS were significantly associated with higher SBP (*ATP2B1* rs17249754: $P = 0.02$; *CSK* rs1378942: $P = 0.003$; *CYP17A1* rs1004467: $P = 0.04$; GRS: $P = 0.0002$). We also observed a significant association of 4 SNPs and the GRS with hypertension (*ATP2B1* rs17249754: $P = 0.02$; *CSK* rs1378942: $P = 0.02$; *CYP17A1* rs1004467: $P = 0.02$; *MTHFR* rs1801133: $P = 0.03$; GRS: $P = 0.0004$). Correction for multiple testing had no influence on the statistical significance of the association of GRS with SBP/hypertension.

CONCLUSIONS
This study shows a significant association of hypertension susceptibility loci only in obese Chinese children, suggesting a likely influence of childhood obesity on the risk of hypertension.

Keywords: blood pressure; Chinese children; genetic risk score; hypertension; obesity; polymorphism.

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Hypertension is a major risk factor for cardiovascular diseases (CVDs) including stroke and coronary heart disease.[1] It is well established that hypertension at childhood can continue into adulthood.[2] Thus, prevention and control of childhood hypertension may reduce the incidence of CVDs in adulthood. Hypertension is a complex disease influenced by both genetic and environmental factors,[3] and epidemiological studies have established that obesity is significantly associated with risk of hypertension.[4] Although genetic factors play an important role in the development of hypertension, nongenetic risk factors including obesity can influence blood pressure (BP)/hypertension through the influence on gene expression or through interaction with gene products.[5] Therefore, research on both genetic and nongenetic risk factors for hypertension is necessary to comprehensively understand the etiology of this disease.

Recent genome-wide association studies (GWASs), conducted mostly in Europeans, have identified >30 genomic loci associated with systolic/diastolic BP (SBP/DBP).[6–11] However, subsequent studies have reported inconsistent results, especially among East Asians.[10,12,13] These controversial observations might be due to the gene–environment interaction, which significantly contributes to the increased risk of hypertension. To our knowledge, there are very few reports on the influence of nongenetic risk factors such as obesity on association between GWAS-identified loci and

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risk of hypertension. Hence, we investigated the effect of obesity status on the association of 6 single nucleotide polymorphisms (SNPs) with SBP/DBP and hypertension in the Chinese children from the Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study.

METHODS

Subjects

Subjects were recruited from the cross-sectional population-based BCAMS study conducted in 2004.[14] The survey included completion of a structured questionnaire, detailed medical examination including SBP/DBP, anthropometric measurements such as height and weight, and finger capillary blood tests from a representative sample of Beijing school-aged children (N = 19,593; age range = 6–18 years; 50% boys). Based on the age- and sex-specific BP criteria for Chinese children (Supplementary Table S1),[15] prevalence of hypertension was 8.5% (n = 1,662 hypertensive cases among 19,593 BCAMS children aged 6–18 years). Informed consent was requested from all the children and/or their parents/guardians; a total of 3,077 children comprising 619 hypertensive children and 2,458 normotensive children participated in a venous blood test after a 12-hour overnight fast. Distribution of age, sex, BP values, and severity of hypertension were comparable between the 619 hypertensive children who participated in the study and the 1,043 hypertensive children who refused to participate in the study (all $P > 0.05$). None of the hypertensive children were on antihypertensive medications. We excluded cases of secondary hypertension identified through the questionnaire or clinical examination by the pediatric cardiologist and that based on color Doppler ultrasound. According to the age- and sex-specific body mass index criteria for Chinese children (Supplementary Table S2),[16] all participating children were grouped into 3 categories: normal weight, overweight, and obese. All participating children and their parents gave written informed consent under protocols provided by the Capital Institute of Paediatrics that clearly stated that blood samples would be used for scientific research purposes, including genetic studies. The BCAMS study was approved by the Ethics Committee and Institutional Review Board of the Capital Institute of Paediatrics.

Measurement of BP

Resting BP was measured in accordance with standard procedures and recommendations.[17] BP measurements among observers were standardized by a physician, and the difference between individual readings by each observer and the physician was kept at ≤4 mm Hg. After 5 minutes of rest, BP was measured by auscultation using a standard clinical sphygmomanometer. Measurements were made on the right arm in sitting position with the elbow at the level of the right atrium using an appropriately sized cuff. SBP was determined by the onset of the "tapping" Korotkoff sounds and DBP by the fourth Korotkoff sound. Three consecutive measurements were performed, and their mean was used for further analysis. Hypertension was defined as elevated BP measured on at least 2 separate occasions.

Selection of SNPs and genotyping

To achieve adequate power, we selected only SNPs in/near hypertension-related genes with minor allele frequencies ≥0.30 in Chinese individuals as available in the HapMap database. We chose 6 SNPs (*ATP2B1* rs17249754, *CSK* rs1378942, *CYP17A1* rs1004467, *STK39* rs3754777, *FGF5* rs16998073, and *MTHFR* rs1801133) that have been shown to be significantly associated with the risk of hypertension.[6–9,18] The power calculation for the study and for different subgroups based on obesity status was performed using Quanto software (http://hydra.usc.edu/gxe/). Overall, our study had approximately 80% power for each variant to detect an odds ratio (OR) of 1.30 for hypertension under an additive model, assuming a significance of 0.05, an allele frequency of 0.30, and hypertension prevalence of 10% in the school-age children of Beijing. However, based on obesity status, the power for each variant to detect an OR of 1.30 was about 45%, 37%, and 77%, in the normal weight, overweight and obese subgroups, respectively.

Genomic DNA was isolated from peripheral blood white cells using the salt fractionation method. SNPs were genotyped by TaqMan Allelic Discrimination Assays on the GeneAmp 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) using specific TaqMan probes. Genotyping call rates for all 6 SNPs were 100%. To validate the accuracy of genotyping, we repeated genotyping on 70 randomly selected samples for each SNP and observed 100% concordance between the results of the 2 tests.

Statistical analysis

Quantitative variables are expressed as means ± SD, and differences between groups were assessed using the Student *t* test. Categorical variables are represented as percentages and were tested by the χ^2 test. Hardy–Weinberg equilibrium was assessed using the χ^2 test. The risk alleles of 6 SNPs were determined based upon the results of earlier GWASs. The weighted SBP or DBP risk score was a weighted sum across 6 variants combining beta coefficients and doses of risk alleles, rounded to 1 mm Hg for SBP (groups <3 to >6) and 0.5 mm Hg for DBP (groups <2 to >3.5).[10] The weighted hypertension risk score was assessed as the sum of doses of the risk alleles weighted by the logarithm of the ORs at each SNP.[10] We used the multiple linear regression model (continuous variable) and multiple logistic regression model (categorical variable), assuming an additive model and additional adjustment for sex and age to investigate the association of 6 SNPs and genetic risk score (GRS) with SBP/DBP and hypertension within each group defined by body mass index. The Bonferroni correction was used to control for multiple testing (0.05/54 = 0.000926). Statistical analyses were performed with SPSS version 13.0 (SPSS, Chicago, IL).

RESULTS

Table 1 shows various characteristics of the study population. We observed statistically significant differences in sex, BP, body mass index, and weight status between hypertensive case subjects and normotensive control subjects (all

Table 1. Characteristics of the study population

Characteristics	All (n = 3,077)	Hypertension (n = 619)	Controls (n = 2,458)	*P* value
Male, %	50.5	61.2	47.8	<0.001
Age, years	12.3 ± 3.1	12.3 ± 3.2	12.4 ± 3.0	0.77
SBP, mm Hg	106 ± 14	126 ± 11	102 ± 10	<0.001
DBP, mm Hg	67 ± 10	80 ± 8	64 ± 8	<0.001
BMI, kg/m^2	21.7 ± 4.9	25.4 ± 5.1	20.7 ± 4.4	<0.001
Normal weight, %	48.6	16.6	56.6	
Overweight, %	18.0	17.5	18.2	
Obesity, %	33.4	65.9	25.2	<0.001

Data are presented as mean ± SD unless otherwise noted. Hypertension and control subjects were defined using the Chinese age- and sex-specific blood pressure standards.

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

$P < 0.001$), but the mean age of the 2 groups were similar ($P = 0.77$). There were 1,494 children in the normal weight group, 556 children in the overweight group, and 1,027 children in the obese group. The genotypes at all the SNPs were in Hardy–Weinberg equilibrium in the control group (all $P > 0.05$).

We performed association analysis of 6 SNPs with SBP/DBP in the 3 groups defined by obesity status (Table 2). In the normal weight group, there was no significant association of any SNP and the GRS with SBP/DBP (all $P > 0.05$). In the overweight group, *STK39* rs3754777 ($\beta = 1.37$, 95% confidence interval (CI) = 0.26–2.47; $P = 0.02$) was associated with DBP, but the 5 other SNPs and the GRS were not. However, GRS was significantly associated with SBP in the overweight category ($P = 0.02$). Interestingly, 3 SNPs and the GRS were significantly associated with SBP in obese individuals (*ATP2B1* rs17249754: $\beta = 1.29$, 95% CI = 0.23–2.35, $P = 0.02$; *CSK* rs1378942: $\beta = 2.29$, 95% CI = 0.78–3.81, $P = 0.003$; *CYP17A1* rs1004467: $\beta = 1.10$, 95% CI = 0.05–2.16, $P = 0.04$; GRS: $\beta = 1.85$, 95% CI = 0.78–2.92, $P = 0.0002$), whereas no significant association was found for the remaining 3 SNPs with SBP and for all 6 SNPs with DBP (all $P > 0.05$) in this group. After Bonferroni correction for multiple testing, the statistical associations of GRS with SBP retained significance. Similar results were obtained on analyzing the associations of 6 SNPs with SBP/DBP *z* scores (Supplementary Table S3).

Table 3 shows the association of 6 SNPs with hypertension by obesity status. None of the 6 SNPs or the GRS was significantly associated with risk of hypertension in both the normal weight and overweight groups. However, in the obese group, 4 SNPs and the GRS were significantly associated with hypertension (*ATP2B1* rs17249754: OR = 1.26, 95% CI = 1.04–1.52, $P = 0.02$; *CSK* rs1378942: OR = 1.39, 95% CI = 1.06–1.83, $P = 0.02$; *CYP17A1* rs1004467: OR = 1.25, 95% CI = 1.03–1.50, $P = 0.02$; *MTHFR* rs1801133: OR = 1.22, 95% CI = 1.02–1.46, $P = 0.03$; GRS: OR = 1.32, 95% CI = 1.12–1.57, $P = 0.0004$), whereas no significant association was found for 2 other SNPs (*STK39* rs3754777: OR = 0.93, 95% CI = 0.76–1.14, $P = 0.51$; *FGF5* rs16998073: OR = 1.03, 95% CI = 0.86–1.23, $P = 0.77$). The statistical significance of association of GRS with hypertension was not influenced by the Bonferroni correction for multiple testing. Besides age and sex, we further adjusted for the region and physical activity but did not observe any substantial change (data not shown).

DISCUSSION

In this study, we investigated the effect of obesity on association of 6 genetic loci recently identified by GWASs with BP/hypertension in the Chinese children. The results indicated that in obese Chinese children, *ATP2B1* rs17249754, *CSK* rs1378942, *CYP17A1* rs1004467, and the GRS based on 6 genetic variants were associated with both SBP and the risk of hypertension. However, only 1 variant, *STK39* rs3754777, was associated with DBP, and only *MTHFR* rs1801133 was associated with the risk of hypertension. After multiple testing corrections, only the statistical association of GRS with SBP/hypertension persisted.

The epidemic of overweight/obesity has become a serious public health issue worldwide, mainly owing to imbalance between dietary energy intake and physical activity. Avoiding unhealthy weight gain is indicated to be an effective and safe way to prevent and treat hypertension and CVDs. Although hypertension is attributed to both genetic and lifestyle/environment factors, few studies have been conducted so far that investigate this aspect. Previous studies have reported influence of obesity on the association of BP/hypertension with candidate genes, including the endothelin-1 gene (Lys198Asn variant),[19] the angiotensinogen gene (A20C variant),[20] the E-selectin gene (Leu554Phe variant),[21] and the *GNB3* (C825T variant).[22] However, whether the recent identified loci by GWASs are also associated with BP/hypertension in overweight/obese subjects is unknown. Our results probably provide the first evidence that obesity may influence the associations of genetic variants identified by GWASs and hypertension.

The biological mechanisms by which obesity may modulate the association between genetic variants and BP/hypertension still remain unclear and can at best be speculated. The gene *ATP2B1* codes for an ATPase that is a member of the family of plasma membrane calcium-pumping ATPases and regulates homeostasis of cellular calcium levels and plays an important role in controlling the contraction and dilation of vascular smooth muscles.[23] It has also been reported to be linked with sodium retention.[24] The cytochrome P450, family 17, subfamily A, polypeptide 1 encoded by *CYP17A1* mediates 17α hydroxylase activity that regulates biosynthesis of mineralocorticoids and glucocorticoids that affect sodium handling in the kidney and 17,20 lyase activity that is involved in the sex-steroid biosynthesis.[25] CSK encodes a cytoplasmic tyrosine kinase that is rapidly activated by angiotensin II and thus plays a key role in signaling events associated with vascular smooth muscle cell contraction, growth, and migration.[26] STK39 is a serine-threonine kinase that may alter renal sodium excretion.[9] Future studies are necessary to investigate the mechanisms of theses SNPs in the pathogenesis of hypertension.

Table 2. Influence of obesity on association of 6 SNPs with SBP and DBP in Chinese children

Gene	Variant	Chr.	MAF	Risk/ nonrisk	Normal weight			Overweight			Obese		
					β	95% CI	*P* value	β	95% CI	*P* value	β	95% CI	*P* value
SBP													
ATP2B1	rs17249754	12	0.373	G/A	0.66	−0.14–1.47	0.11	1.17	−0.15–2.50	0.08	1.29	0.23–2.35	0.02
CSK	rs1378942	15	0.158	C/A	0.74	−0.33–1.81	0.17	−0.53	−2.32–1.25	0.56	2.29	0.78–3.81	0.003
CYP17A1	rs1004467	10	0.362	A/G	0.51	−0.28–1.31	0.20	0.24	−1.11–1.59	0.73	1.10	0.05–2.16	0.04
STK39	rs3754777	2	0.259	T/C	0.03	−0.86–0.92	0.95	0.18	−1.28–1.64	0.81	−0.75	−1.89–0.39	0.20
FGF5	rs16998073	4	0.392	T/A	0.38	−0.41–1.17	0.34	1.31	−0.01–2.63	0.05	−0.38	−1.42–0.67	0.48
MTHFR	rs1801133	1	0.444	A/G	−0.02	−0.79–0.75	0.96	−0.43	−1.73–0.88	0.52	0.68	−0.33–1.69	0.19
Genetic risk score		—	—	—	0.69	−0.11–1.50	0.09	1.55	0.22–2.87	0.02	1.85	0.78–2.92	0.0002
DBP													
ATP2B1	rs17249754	12	0.373	G/A	0.03	−0.61–0.67	0.92	0.60	−0.41–1.60	0.24	0.50	−0.34–1.33	0.24
CSK	rs1378942	15	0.158	C/A	0.17	−0.68–1.02	0.70	−0.01	−1.36–1.34	0.99	1.02	−0.18–2.21	0.10
CYP17A1	rs1004467	10	0.362	A/G	0.20	−0.44–0.83	0.54	0.03	−0.99–1.05	0.95	0.65	−0.18–1.48	0.12
STK39	rs3754777	2	0.259	T/C	−0.26	−0.97–0.45	0.48	1.37	0.26–2.47	0.02	−0.32	−1.22–0.58	0.48
FGF5	rs16998073	4	0.392	T/A	0.37	−0.26–1.00	0.25	0.90	−0.10–1.90	0.08	−0.16	−0.98–0.66	0.70
MTHFR	rs1801133	1	0.444	A/G	0.31	−0.31–0.92	0.33	0.78	−0.20–1.77	0.12	0.48	−0.32–1.28	0.24
Genetic risk score		—	—	—	0.05	−0.54–0.65	0.87	0.82	−0.15–1.79	0.10	0.56	−0.19–1.31	0.14

β and 95% confidence intervals were calculated using a multiple linear regression model adjusted for age and sex.
Abbreviations: Chr., chromosome; CI, confidence intervals; DBP, diastolic blood pressure; MAF, minor allele frequency; SBP, systolic blood pressure.

Table 3. Influence of obesity on association of 6 single nucleotide polymorphisms with hypertension in Chinese children

Gene	Variant	Chr.	MAF	Risk/ nonrisk	Normal weight			Overweight			Obese		
					OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
ATP2B1	rs17249754	12	0.373	G/A	1.06	0.78–1.42	0.72	1.34	0.97–1.84	0.08	1.26	1.04–1.52	0.02
CSK	rs1378942	15	0.158	C/A	1.19	0.79–1.79	0.42	0.94	0.62–1.40	0.75	1.39	1.06–1.83	0.02
CYP17A1	rs1004467	10	0.362	A/G	0.96	0.72–1.29	0.80	1.05	0.77–1.43	0.78	1.25	1.03–1.50	0.02
STK39	rs3754777	2	0.259	T/C	0.91	0.65–1.28	0.59	1.16	0.83–1.62	0.37	0.93	0.76–1.14	0.51
FGF5	rs16998073	4	0.392	T/A	1.22	0.92–1.63	0.17	1.21	0.89–1.63	0.22	1.03	0.86–1.23	0.77
MTHFR	rs1801133	1	0.444	A/G	1.13	0.85–1.50	0.40	0.96	0.71–1.30	0.79	1.22	1.02–1.46	0.03
Genetic risk score		—	—	—	1.06	0.80–1.40	0.68	1.04	0.77–1.40	0.80	1.32	1.12–1.57	0.0004

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a multiple logistic regression model adjusted for age and sex.
Abbreviations: Chr., chromosome; MAF, minor allele frequency.

Although this study was adequately powered to investigate the associations of 6 genetic variants with childhood hypertenstion, it had limited power for subgroup analysis, especially in the normal weight and overweight children. However, observation of a strong association in the obese children indicates the importance of childhood obesity in predicting the risk of childhood hypertension. Further well-designed and adequately powered studies may better confirm these observations in the normal weight and overweight children.

In conclusion, this study indicates that obesity status modifies the association between BP and hypertension-associated SNPs identified by recent GWASs and childhood hypertension.

SUPPLEMENTARY MATERIAL

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

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B.X. and X.Z. contributed equally to this work. J.M. and X.W. conceived and designed the experiments. Y.S. performed the experiments. B.X., X.Z., and G.R.C. analyzed the data. H.C. and D.H. contributed reagents/materials/analysis tools. B.X. and X.Z. wrote the manuscript. B.X., G.R.C., and J.M. revised the manuscript.

DISCLOSURE

The authors declared no conflict of interest.

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