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A new risk locus in *CHCHD5* for hypertension and obesity in a Chinese child population: a cohort study

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ABSTRACT

Objective Coiled-coil-helix-coiled-coil-helix domain containing 5 (CHCHD5), a mitochondrial protein, is involved in the oxidative folding process in the mitochondrial intermembrane space. A previous study identified a hypertension-related single nucleotide polymorphism (SNP), rs3748024, in *CHCHD5* in adults, but there are no reports regarding the association between *CHCHD5* and obesity, which is a known risk factor for hypertension. The aim of the present study is to investigate the associations of the SNP rs3748024 with hypertension and obesity.

Design Cohort study.

Setting Institute of Pediatrics in China.

Participants We genotyped the SNP rs3748024 in the Beijing Child and Adolescent Metabolic Syndrome study. A total of 3503 children participated in the study.

Primary and secondary outcome measures Genotyping of rs3748024 was conducted using the TaqMan Allelic Discrimination Assay. Lipids and glucose were analysed by an automatic biochemical analyser using a kit assay. The levels of adipocytokines (leptin, adiponectin and resistin) were measured by ELISA techniques.

Results There was a statistically significant association between rs3748024 and systolic blood pressure (SBP) (β=−0.853, 95% CI −1.482 to −0.024, p=0.044) under an additive model adjusted for age, gender and body mass index (BMI) after correction for multiple testing. The SNP was also significantly associated with BMI (β=−0.286, 95% CI −0.551 to −0.021, p=0.043), obesity (OR=0.828, 95% CI 0.723 to 0.949, p=0.018) and triglycerides (β=−0.039, 95% CI −0.070 to −0.007, p=0.044) after correction for multiple testing.

Conclusions We demonstrate for the first time that the SNP rs3748024 in *CHCHD5* is associated with SBP, BMI, obesity and triglycerides in Chinese children. Our study identifies a new risk locus for hypertension and obesity in a child population. The function of CHCHD5 remains to be further studied to help elucidate the pathogenic role of CHCHD5 in hypertension and obesity.

Strengths and limitations of this study

- This study identifies a new risk locus for hypertension and obesity in a child population.
- This study is the first to demonstrate that the single nucleotide polymorphism rs3748024 in *CHCHD5* is associated with systolic blood pressure, body mass index, obesity and triglycerides in Chinese children.
- This study may not provide direct evidence that the expression of *CHCHD5* influences hypertension and obesity.

INTRODUCTION

In recent years, the prevalence of hypertension and obesity has been increasing in most parts of the world, and these two diseases are major threats to public health.[1 2] Childhood hypertension and obesity strongly predispose to adult hypertension and obesity.[3–5]

Previously, multiple single nucleotide polymorphisms (SNPs) related to hypertension or obesity have been identified by genome-wide association studies.[6–9] However, almost no SNPs are associated with both hypertension and obesity.

Coiled-coil-helix-coiled-coil-helix domain containing 5 (CHCHD5), a mitochondrial protein encoded by *CHCHD5*, is located at chromosome 2q13.[10] CHCHD5 is homologous to yeast Mic14, which affects mitochondrial oxygen consumption.[11] New evidence suggests that CHCHD5 is a substrate of the oxidoreductase Mia40, which promotes an oxidative folding process in the mitochondrial intermembrane space.[12] The maintenance of redox balance in the mitochondria depends on the mitochondrial oxidative folding pathway and is crucial for normal cell physiology.

Evidence from a previous study suggested that the SNP rs3748024 in *CHCHD5* was significantly associated with hypertension in Taiwanese adults,[13] but no study has been conducted to confirm that this SNP contributes to hypertension in other populations, especially in children.

Because obesity is a known risk factor for hypertension and CHCHD protein family members play a vital role in a wide variety of physiological and pathological processes,[14] we investigated the associations of the SNP rs3748024 with both hypertension and obesity.

We genotyped this SNP in Chinese children who had participated in the population-based Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study.[15] The present study attempts to provide an analysis of epidemiological and genetic data towards the associations of the SNP in *CHCHD5* with hypertension and obesity.

METHODS

Population

Subjects were recruited from a cross-sectional population-based survey, termed the BCAMS study, in 2004.[15] The survey included a questionnaire, medical examination and anthropometric measurement in a representative sample (n=19 593, 50% boys) of children in Beijing aged 6–18 years. Anthropometric measurements included height, weight, waist circumference and fat mass percentage. Within this large group of children in whom venepuncture blood samples were collected, 1229 were diagnosed as obese using the Chinese age-specific and sex-specific body mass index (BMI) cut-offs (online supplementary table S1).[16] We used BMI as a measure of obesity because BMI is significantly associated with adolescent subcutaneous fat and is an indicator of childhood and adolescent obesity.[17] An additional 2274 non-obese children, including 655 overweight and 1619 normal-weight children, were randomly selected by the SPSS statistical software (V.18.0; SPSS). The participants were then divided into two groups. One group comprised 1045 children with elevated blood pressure (EBP, including prehypertension and hypertension). EBP includes systolic blood pressure (SBP) or diastolic blood pressure (DBP) that is elevated. The other group comprised 2458 children with normal blood pressure as diagnosed by the blood pressure reference cut-offs for Chinese children and adolescents (online supplementary table S2).[18] The BCAMS study was approved by the ethics committee of the Capital Institute of Pediatrics. We obtained written informed consent from parents or guardians.

Measurement of BMI and blood pressure

BMI was calculated as the person's weight (kg) divided by the squared height in metres. After a rest period of 5 min, blood pressure (BP) was measured by auscultation using a standard clinical sphygmomanometer. DBP was determined by the fourth Korotkoff sound (K4), and SBP was determined by the onset of the 'tapping' Korotkoff sounds (K1). Three consecutive measurements were performed, and the mean of the three readings was used for analysis.

Measurement of biochemical analyses and genotyping

The level of fasting plasma glucose (FPG) was measured using the hexokinase method. The levels of lipids were measured using the enzymatic methods for triglycerides (TGs) and total cholesterol (TC) measurements, and the clearance methods for high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) measurements. The measurements were performed using a kit assay (SEKISUI Medical Technology, Tokyo, Japan) and an automatic biochemical analyser (Hitachi 7060). The levels of adipocytokines (leptin, adiponectin and resistin) were measured by ELISA techniques.[19]

Genomic DNA was isolated from peripheral white blood cells using the salt fractionation method. Genotyping of rs3748024 was conducted using the TaqMan Allelic Discrimination Assay with the GeneAmp 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The genotyping call rate for the SNP was 98.3%. We repeated 70 samples randomly for the SNP to validate the accuracy of the genotyping and observed 100% concordance between the results of the two tests. We also sent 30 samples to direct sequencing and observed 100% concordance between the two genotyping methods.

Statistical analyses

Categorical variables were presented as percentages and continuous variables were presented as mean±SD. The Hardy-Weinberg equilibrium was assessed using the χ^2 test. Adjusted ORs for EBP were performed by logistic regression with genotypes, age, gender and BMI as the independent variables. ORs for obesity were performed by logistic regression with genotypes, age and gender as the independent variables. A linear regression model was used to investigate the associations of the SNP with SBP, DBP, BMI, lipids, FPG and adipocytokines (leptin, adiponectin and resistin). The data were analysed using SPSS statistical software. p Value <0.05 was used to indicate statistically significant differences. The false discovery rate (FDR)[20] approach was used to correct for multiple testing. Supposing that there are a total of m p values, the original p value was arranged from small to large: p (1), p (2), …, p (m), stringent p (i)=(p (i)×m)/i. In brief, the stringent p value was considered statistically significant only if it was less than 0.05 for the FDR. Power calculation was performed using Quanto software (http://hydra.usc.edu/gxe/) according to the sample size, effect size (β or OR), inheritance mode and allele frequency.

RESULTS

The basic characteristics of the study participants are summarised in online supplementary table S3. The SNP rs3748024 (chromosome: 2:112588836) lies in an intron (location of the intron: 112584650–112588865) of the gene and three genotypes of the SNP are GG, GC and CC. The SNP occurs downstream of the transcription start site and is not within a splice sequence. The SNP is also an expression quantitative trait locus (eQTL) in adipose (subcutaneous), heart (left ventricle) or artery (aorta) (data from GTEx Portal, https://gtexportal.org/home/). The significance values and effect sizes of the *cis*-eQTLs are shown in online supplementary table S4. We genotyped the SNP rs3748024 in *CHCHD5* in the cohort, and the genotype of the SNP (the numbers of GG, GC and CC are 2190, 1113 and 140, respectively) was tested to be in Hardy-Weinberg

Table 1 Associations of SNP rs3748024 with SBP, DBP and EBP

(A) Associations of rs3748024 with SBP and DBP

Phenotype	Additive model	Mean±SD	β*	95% CI*	Stringent p value*	Power	β†	95% CI†	Stringent p value†	Power
SBP	GG	108±14								
	GC	107±14								
	CC	106±15	−1.260	−1.996 to −0.524	**0.005**	0.844	−0.853	−1.482 to −0.024	**0.044**	0.521
DBP	GG	68±10								
	GC	67±10								
	CC	67±10	−0.067	−1.225 to −0.019	**0.032**	0.056	−0.421	−0.982 to 0.087	0.286	0.280

(B) Association of rs3748024 with EBP

Additive model	N (EBP)	N (NBP)	OR*	95% CI*	Stringent p value*	Power	OR†	95% CI†	Stringent p value†	Power
GG	403	1781								
GC	183	928								
CC	24	116	0.876	0.768 to 0.999	**0.048**	0.970	0.991	0.790 to 1.051	0.319	0.058

Those highlighted in bold indicate that the associations showed statistical significance.
*Adjusted for age and gender.
†Adjusted for age, gender and body mass index.
DBP, diastolic blood pressure; EBP, elevated blood pressure, including prehypertension and hypertension; NBP, normal blood pressure; SBP, systolic blood pressure.

equilibrium (p=0.924). The associations of the SNP rs3748024 with SBP, DBP and EBP are shown in table 1A,B. There were statistically significant associations of rs3748024 with SBP, DBP and EBP after adjustment for age and gender. As obesity is a known risk factor for hypertension, we also adjusted for BMI besides age and gender. After correction for multiple testing, the SNP rs3748024 was significantly associated with SBP (β=−0.853, 95% CI −1.482 to −0.024, p=0.044) under an additive model adjusted for age, gender and BMI.

We further analysed the associations of the SNP with BMI and obesity. There were statistically significant associations of rs3748024 with BMI (β=−0.286, 95% CI −0.551 to −0.021, p=0.043) and obesity (OR=0.828, 95% CI 0.723 to 0.949, p=0.018) under an additive model after adjustment for age and gender (table 2). Online supplementary figure S1 shows the means of SBP, DBP, BMI and obesity (%) in groups with different genotypes of rs3748024.

We also analysed the associations of the SNP with lipids, plasma glucose and adipocytokines (online supplementary table S5). After correction for multiple testing, the SNP rs3748024 was significantly associated with TG (β=−0.039, 95% CI −0.070 to −0.007, p=0.044) under an additive model adjusted for age, gender and BMI. No statistical significance was found between the SNP and TC, HDL, LDL, FPG and adipocytokines after correction for multiple testing. Studies with greater sample size are needed to confirm these associations.

Table 2 Associations of rs3748024 with BMI and obesity

	BMI			Obesity			
Additive model	Mean±SD	Stringent p value (β, 95% CI)*	Power	N (obese)	N (NW)	Stringent p value (OR, 95% CI)*	Power
GG	22.02±4.92			798	974		
GC	21.73±4.87			364	540		
CC	21.67±5.67	**0.043** (−0.286, −0.551 to −0.021)	0.475	46	73	**0.018** (0.828, 0.723 to 0.949)	0.998

Those highlighted in bold indicate that the associations showed statistical significance.
*Adjusted for age and gender.
BMI, body mass index; NW, normal weight.

DISCUSSION

CHCHD5 belongs to the CHCHD protein family and is involved in the oxidative folding process in the mitochondrial intermembrane space. A previous study has shown that *CHCHD5* is related to hypertension in adults, but there are no reports regarding the association between *CHCHD5* and obesity, which is a known risk factor for hypertension.

Because childhood hypertension and obesity strongly predispose to adult hypertension, we investigated the genetic susceptibility of the SNP rs3748024 in children. Because obesity is a known risk factor for hypertension, we also adjusted for BMI besides age and gender. Our results indicated that the significant association between the SNP and SBP remained after adjustment for BMI. Therefore, we further investigated the associations of the SNP with BMI and obesity. The results showed positive correlations.

Because the CHCHD protein family members play a vital role in a wide variety of physiological and pathological processes and it is not clear whether the function of CHCHD5 affects endocrine and metabolic processes, we investigated the associations of the SNP in *CHCHD5* with lipids, glucose and adipocytokines. The results indicated that the SNP was significantly associated with TG after multiple testing.

Our results indicated that the SNP rs3748024 was significantly associated with BMI and obesity, but the significant associations of the SNP with SBP and TG were independent of BMI. It suggested that although obesity is a risk factor for hypertension and hyperlipidaemia, the SNP rs3748024, as a new risk locus in *CHCHD5* for SBP and TG, was not related to BMI. However, the molecular mechanism by which this SNP associates with these phenotypes remains to be studied. Given the marginal significance of the association in this study, we are cautious in assuming the biological/clinical relevance of this SNP. It is possible that this SNP is not the 'causal' mutation, and some other SNPs in the region might explain the associations.

In addition, a large percentage of children had been identified as hypertensive because of the high proportion of obesity in our participants. Obesity increases the prevalence of hypertension in children. However, in our study, 72.4% of the overweight/obese participants did not have EBP, and 6.4% of the normal weight participants had EBP. The blood pressure measurements were taken at the same time of day, and white coat hypertension might not have been completely avoided. This is a limitation of our study.

CONCLUSIONS

We demonstrate for the first time that the SNP rs3748024 in *CHCHD5* is associated with SBP, BMI, obesity and TG in Chinese children. These novel findings identify a new risk locus associated with hypertension and obesity in children. The function of CHCHD5 remains to be further studied to help elucidate the pathogenic role of CHCHD5 in hypertension and obesity.

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Contributors LW designed the study, collected the data and wrote the manuscript. LG performed the statistical analysis. XZ and MZ collected the DNA samples. JW reviewed the manuscript. All the authors reviewed and approved the final manuscript. JM directed the project.

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Competing interests None declared.

Patient consent Parental/guardian consent obtained.

Ethics approval The study was approved by the ethics committees of the Capital Institute of Pediatrics.

Provenance and peer review Not commissioned; externally peer reviewed.

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