Gene 535 (2014) 299–302

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

An obesity genetic risk score is associated with metabolic syndrome in Chinese children

Xiaoyuan Zhao [a,1], Bo Xi [b,1], Yue Shen [a], Lijun Wu [a], Dongqing Hou [a], Hong Cheng [a], Jie Mi [a,*]

[a] Department of Epidemiology, Capital Institute of Pediatrics, Beijing 100020, China
[b] Department of Maternal and Child Health Care, School of Public Health, Shandong University, Jinan 250012, China

ARTICLE INFO

Article history:
Accepted 3 November 2013
Available online 19 November 2013

Keywords:
Metabolic syndrome
Obesity
Polymorphism
Children
Chinese

ABSTRACT

Recent genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) associated with body mass index (BMI)/obesity. In this study, we aim to examine the associations of obesity related loci with risk of metabolic syndrome (MetS) in a children population from China. A total of 431 children with MetS and 3046 controls were identified based on the modified ATPIII definition. 11 SNPs (*FTO* rs9939609, *MC4R* rs17782313, *GNPDA2* rs10938397, *BDNF* rs6265, *FAIM2* rs7138803, *NPC1* rs1805081, *SEC16B* rs10913469, *SH2B1* rs4788102, *PCSK1*rs6235, *KCTD15* rs29941, *BAT2* rs2844479) were genotyped by TaqMan 7900. Of 11 SNPs, *GNPDA2* rs10938397, *BDNF* rs6265, and *FAIM2* rs7138803 were nominally associated with risk of MetS (*GNPDA2* rs10938397: odds ratio (OR) = 1.21, 95% confidence interval (CI) = 1.04–1.40, $P = 0.016$; *BDNF* rs6265: OR = 1.19, 95% CI = 1.03–1.39, $P = 0.021$; *FAIM2* rs7138803: OR = 1.20, 95% CI = 1.02–1.40, $P = 0.025$); genetic risk score (GRS) was significantly associated with risk of MetS (OR = 1.09, 95% CI = 1.04–1.15, $P = 5.26 \times 10^{-4}$). After further adjustment for BMI, none of SNPs were associated with risk of MetS (all $P > 0.05$); the association between GRS and risk of MetS remained nominally (OR = 1.02, 95%CI = 0.96–1.08, $P = 0.557$). However, after correction for multiple testing, only GRS was statistically associated with risk of MetS in the model without adjustment for BMI. The present study demonstrated that there were nominal associations of *GNPDA2* rs10938397, *BDNF* rs6265, and *FAIM2* rs7138803 with risk of MetS. The SNPs in combination have a significant effect on risk of MetS among Chinese children. These associations above were mediated by adiposity.

1. Introduction

Metabolic syndrome (MetS) is characterized by a cluster of factors, including central obesity, hypertension, hypertriglyceridemia, decreased plasma high-density lipoprotein cholesterol, and elevated glucose (Bruce and Hanson, 2010). Since MetS predicts risk of type 2 diabetes (T2D) and cardiovascular diseases, it is important to clarify the causal risk factors to prevent and control for MetS. Epidemiological studies have reported that environmental factors such as high-fat food intake and lack of physical activity contribute to the development of MetS. However, genetic factors also play important roles in the pathogenesis of MetS.

Recently, several genome-wide association studies (GWASs) identified many single nucleotide polymorphisms (SNPs) associated with higher body mass index (BMI) or increased risk of obesity (Benzinou et al., 2008; Berndt et al., 2013; Frayling et al., 2007; Loos et al., 2008; Meyre et al., 2009; Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2009) in populations of European ancestry. In 2007, Frayling et al. (2007) initially reported that rs9939609 in fat mass- and obesity- associated (*FTO*) gene was significantly associated with increased risk of T2D. However, the association disappeared after adjustment for BMI, suggesting that the effect of *FTO* on T2D was completely mediated through BMI. In other words, *FTO* is identified as the first obesity related loci. Then, the second one is Melanocortin-4 Receptor (*MC4R*) gene (Loos et al., 2008). The most recent loci include SNPs in/near *GNPDA2*, *BDNF*, *FAIM2*, *NPC1*, *SEC16B*, *SH2B1*, *PCSK1*, *KCTD15* and *BAT2* genes (Benzinou et al., 2008; Meyre et al., 2009; Speliotes et al., 2010; Thorleifsson et al., 2009 and Willer et al., 2009). In East Asians, most obesity loci identified in Europeans were replicated, and new additional loci were reported (Okada et al., 2012; Wen et al., 2012).

Since obesity is highly correlated with MetS, they may share similar genetic background. Actually, many studies have investigated the association between variants in *FTO* gene and risk of MetS in adult populations. However, the results have been inconsistent. Two similar meta-

Abbreviations: BCAMS, the Beijing Child and Adolescent Metabolic Syndrome; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; FG, fasting glucose; FTO, fat mass- and obesity associated; GRS, genetic risk score; GWAS, genome-wide association study; HDL-C, HDL-cholesterol; HWE, Hardy–Weinberg equilibrium; LDL-C, LDL-cholesterol; MetS, metabolic syndrome; OR, odds ratio; SNP, single nucleotide polymorphism; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

* Corresponding author at: Department of Epidemiology, Capital Institute of Pediatrics, 2 Ya Bao Road, Beijing 100020, China. Tel.: +86 10 8569 5591; fax: +86 10 8563 2799.
E-mail address: jiemi@vip.163.com (J. Mi).

[1] Xiaoyuan Zhao and Bo Xi contributed equally to this work.

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.11.006

analyses (Wang et al., 2012; Zhou et al., 2012) demonstrated that variants in *FTO* gene were associated with increased risk of MetS in adults. However, as mentioned by Zhou et al. (2012), the majority of the previous studies did not adjust for BMI. Thus, it is still unclear whether variants in *FTO* gene can influence MetS independently, or mediate through BMI. Moreover, no related studies have been performed in children population. Thus, based on Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study, we aim to examine the associations of 11 obesity related loci with risk of MetS and the effect of BMI on the associations among Chinese children population.

2. Subjects and methods

2.1. Subjects

Subjects were recruited from the cross-sectional population-based BCAMS study (Shan et al., 2010). The survey included completion of a questionnaire, a medical examination, anthropometric measurements, and finger capillary blood tests from a representative sample of Beijing school-aged children ($N = 19{,}593$, age range 6–18 years, 50% boys) in 2004. Of them, 3518 children who participated in a 12 hour overnight fasting venous blood testing were recruited. The detailed information has been described elsewhere (Wu et al., 2010 and Xi et al., 2010). However, 41 individuals were excluded because of missing data on BMI, waist circumference (WC), blood pressure (BP), triglyceride (TG), HDL-cholesterol (HDL-C) and/or fasting glucose (FG). Thus, 3477 children were used for final data analysis. 431 children were diagnosed as MetS cases and 3046 children were defined as controls based on the modified ATPIII definition (Cook et al., 2003). 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.

2.2. Definition of metabolic syndrome

The modified ATPIII definition was employed in which MetS was defined by the presence of three or more of the following five components (Cook et al., 2003): (1) central obesity defined as WC ≥90th percentile for age and gender (established based on the BCAMS study); (2) elevated systolic and/or diastolic blood pressure ≥90th percentile for age, sex and height (according to the BCAMS study); (3) hypertriglyceridemia defined as TG ≥1.24 mmol/L, equal to the 90th percentile of the reference population; (4) Low-HDL defined as ≤1.03 mmol/L, and (5) impaired FG (IFG) defined as ≥5.6 mmol/L.

2.3. Measurement of anthropometric parameters

All instruments were validated following the manufacturers' protocols (World Health Organization, 1995). Height without shoes was measured using wall-mounted stadiometers to the nearest 0.1 cm. Body weight was measured with underwear and no shoes to the nearest 0.1 kg using beam scales with a maximum weight of 140 kg. BMI (kg/m^2) was calculated as body weight/height^2. WC was measured midway between the lowest rib and the superior border of the iliac crest with an inelastic measuring tape at the end of normal expiration to the nearest 0.1 cm. BP was measured with mercury manometer. Systolic BP (SBP) was indicated by Korotkoff's first phase and diastolic BP (DBP) by Korotkoff's fourth phase.

2.4. Measurement of biochemical markers

All biochemical measurements were performed using commercially available kits. Serum TC and TG concentrations were determined using standard enzymatic methods. FG was determined by the glucose oxidase method. HDL-C and LDL-C were measured directly. The serum lipid levels and FG were assayed using 7060 chemistry analyzer (Hitachi, Tokyo, Japan). All experiments were carried out according to standard operating procedures.

2.5. Selection of SNPs and genotyping

To achieve a statistical power of more than 0.75, only SNPs in obesity related genes with minor allele frequencies >0.15 in Chinese individuals in the HapMap database were selected, assuming the odds ratio of the studied SNP being 1.3 and the population risk being 10% using Quanto software (http://hydra.usc.edu/gxe/). Base on these criteria, in our previous studies to replicate the obesity related loci (Wu et al., 2010; Xi et al., 2010, 2013a), we chose the 11 SNPs (*FTO* rs9939609, *MC4R* rs17782313, *GNPDA2* rs10938397, *BDNF* rs6265, *FAIM2* rs7138803, *NPC1* rs1805081, *SEC16B* rs10913469, *SH2B1* rs4788102, *PCSK1*rs6235, *KCTD15* rs29941, *BAT2* rs2844479) that have been shown to be significantly associated with the risk of obesity by the GWAS (Benzinou et al., 2008; Frayling et al., 2007; Loos et al., 2008; Meyre et al., 2009; Thorleifsson et al., 2009; Willer et al., 2009). The statistical power of the present case–control study on the association between 11 SNPs and MetS was more than 0.75 using Quanto software.

Genomic DNA was isolated from peripheral blood white cells using the salt fractionation method. All genotyping were performed by TaqMan probes Allelic Discrimination Assays with the GeneAmp 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Genotyping call rates for all variants were greater than 99%. Duplicate samples were assayed with a concordance rate of 100%.

2.6. Statistical analysis

Quantitative variables were expressed as means ± standard deviation, and differences between groups were assessed with Student's *t*-test. Categorical variables were represented as percentages and were tested by the χ^2 test. Hardy–Weinberg equilibrium (HWE) was assessed using the χ^2 test. The risk alleles of 11 SNPs were determined based upon the recent GWASs. The weighted genetic risk score (GRS) was calculated by the sum of risk alleles of 11 SNPs (Qi et al., 2012). The associations of 11 SNPs and GRS with risk of MetS were estimated using multivariate logistic regression model, assuming an additive model and with adjustment for sex, age and BMI (when appropriate). The Bonferroni correction was used to control for multiple testing ($0.05/11 = 0.0045$). Statistical analyses were performed with SPSS, version 13.0 (SPSS, Inc., Chicago, Illinois).

3. Results

A total of 431 MetS cases and 3046 controls were included in the present study. Sex, age, BMI, SBP, DBP, TC, TG, HDL-C, LDL-C, and FG

Table 1
Characteristics of the study population.

	Metabolic syndrome (n = 431)	Controls (n = 3046)	*P* value
Male (%)	61.0	49.3	<0.001
Age, years	12.8 ± 2.9	12.3 ± 3.1	0.002
BMI, kg/m^2	27.6 ± 4.1	21.1 ± 4.5	<0.001
SBP, mm Hg	120.6 ± 11.4	105.8 ± 13.2	<0.001
DBP, mm Hg	76.1 ± 18.5	66.6 ± 9.6	<0.001
TC(mmol/L)	4.20 ± 0.84	4.07 ± 0.79	0.003
TG (mmol/L)	1.66 ± 0.79	0.94 ± 0.46	<0.001
HDL-C(mmol/L)	1.10 ± 0.22	1.45 ± 0.31	<0.001
LDL-C(mmol/L)	2.77 ± 0.73	2.51 ± 0.72	<0.001
FG (mmol/L)	5.40 ± 0.89	5.05 ± 0.56	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FG, fasting glucose.
Data are presented as mean ± SD.