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Genetic variations in *SEC16B*, *MC4R*, *MAP2K5* and *KCTD15* were associated with childhood obesity and interacted with dietary behaviors in Chinese school-age population

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ABSTRACT

Both genetic predisposition and lifestyle factors are associated with the risk for obesity. Multiple obesity loci have been identified using genome-wide association studies mainly in European populations. The aims of this study were to examine the associations of these loci with obesity and gene × dietary behavior interactions among Chinese children and adolescents. Nineteen candidate SNPs were genotyped using Sequenom technology in the Chinese children (N = 2977, 853 obese and 2124 controls, aged 7–17). Dietary behaviors were assessed using self-administered questionnaires. After adjusting for age, sex and multiple testing, *MC4R* rs17782313, *SEC16B* rs543874, *MAP2K5* rs2241423 and *KCTD15* rs11084753 were associated with obesity and obesity-related traits (all $P < 0.005$), with odd ratios ranging from 1.22 to 2.15. Dose–response association was significant between genetic risk score, which was calculated by summing the risk alleles, and the risk of obesity ($P < 0.001$). Multiplicative interaction was found between rs543874 and salt preference on obesity with an OR of 4.40 (95% CI, 1.12–17.30). Additive interactions with salt preference were found in rs17782313 and rs11084753. Our findings indicated that rs17782313, rs543874, rs2241423 and rs11084753 were associated with the risk for children obesity in China, and interaction of genetic variants with diet behaviors on obesity.

1. Introduction

Childhood and adolescent obesity rapidly becomes a serious public health problem worldwide including the developing countries like China (Bradford, 2009; Lanigan et al., 2010; Kuhne, 2011). The worldwide prevalence of childhood obesity has dramatically increased from 4.2% in 1990 to 6.7% in 2010 (de Onis et al., 2010). This rapidly rising tendency turned out to be more alarming with the prevalence of Chinese childhood obesity climbing from 0.2% in 1985 to 8.1% in 2012 (Song et al., 2013). Previous studies indicated that childhood obesity is associated with the risk of adult obesity and increases the risk of common chronic disease such as type 2 diabetes, cardiovascular diseases and strokes, rheumatoid arthritis, obstructive sleep apnea, and some cancers (Haslam and James, 2005). The current obesity prevalence may obviously overload the national economy and medical institutions, so particular measures are necessary to prevent obesity in children and adolescents.

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; WC, waist circumference; WtHR, waist-to-height ratio; SD, standard deviations; GRS, genetic risk score; FDR, false discovery rate; MI, multiplicative interaction; AP, attributable proportion due to interaction; *HOXB5*, homeobox protein Hox-B5; *OLFM4*, olfactomedin-4; *AMD1*, adenosylmethionine decarboxylase 1; *PAX5*, paired box 5; *TMEM18*, transmembrane protein 18; *MTCH2*, mitochondrial carrier 2; *LYPLAL1*, lysophospholipase-like 1; *SEC16B*, SEC16 homolog B; *BDNF*, brain-derived neurotrophic factor; *MSRA*, methionine sulfoxide reductase A; *MC4R*, melanocortin 4 receptor; *MAP2K5*, mitogen-activated protein kinase kinase 5; *KCTD15*, potassium channel tetramerization domain containing 15; *CDKAL1*, CDK5 regulatory subunit associated protein 1-like 1; *NEGR1*, neuronal growth regulator 1; *RPL27A*, ribosomal protein L27A; *ETV5*, ets variant 5; *TFAP2B*, transcription factor AP-2 beta; *GNPDA2*, glucosamine-6-phosphate deaminase 2.

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Individual susceptibility to obesity is determined by the interaction between genetic and environmental factors (Han et al., 2010; Robiou-du-Pont et al., 2013). Indeed, according to monozygotic twins reared apart study, the heritability of body mass index (BMI) ranges between 0.50 and 0.70 (Allison et al., 1996). Recent genome-wide association studies (GWASs) have identified a range of obesity susceptibility loci in European populations since the first GWAS in obesity was reported in 2006 (Herbert et al., 2006). Subsequently, *FTO* and *MCR4* have been replicated in children and adolescents (Dina et al., 2007; Haworth et al., 2008; Loos et al., 2008; Willer et al., 2009; Hardy et al., 2010; Liem et al., 2010; Liu et al., 2010a,b). *TMEM18*, *MTCH2*, *GNPDA2*, *KCTD15* and *NEGR1* are highly expressed in the central nervous system, and in particular the hypothalamus, to influence energy intake and appetite, thereby associated with the risk of obesity (Willer et al., 2009). *LYPLAL1*, *MSRA* and *TFAP2B* have been found in GWAS associated with waist circumference (WC) and waist–hip ratio (WHR) (Lindgren et al., 2009). A most recent meta-analysis has reported two new loci (*HOXB5 and OLFM4*) associated with adult obesity (Bradfield et al., 2012). The majority of previous studies are focused on populations of European Ancestry and adults. Subsequent studies have attempted to identify the associations in Chinese population, however, the results of *GNPDA2*, *KCTD15*, *NEGR1*, *MC4R*, *SEC16B* and *ETV5* are inconsistent (Cheung et al., 2010; Wang et al., 2012; Hong et al., 2013; Xi et al., 2013). In addition, ten other obesity-related genes (*LYPLAL1*, *MSRA*, *TFAP2B*, *MAP2K5*, *RPL27A*, *PAX5*, *CDKAL1*, *AMD1*, *HOXB5* and *OLFM4*) have not been examined in Chinese children. There are significant differences in the prevalence of obesity and the frequencies of the genetic variants among different ethnic populations (Lyon et al., 2007). It is very important to explore the effects of these loci in Chinese especially children.

Environmental factors especially high-calorie dietary behaviors and low physical activities have consistently contributed to the increasing children obesity. Accumulating evidences have demonstrated that dietary behaviors contributed to the risk of children obesity. The Mediterranean diet, characterized by high consumption of plant foods, high-fiber foods and low intake of sweetened beverages and red meat, is a health-promoting factor in children obesity (Lazarou et al., 2010). By contrast, the Western dietary pattern, which is high consumption of the high-fat foods and refined foods, has been related to children obesity (Song et al., 2010). Some individuals were more sensitive to the deleterious effects by unhealthy lifestyles. However, there is limited insight into the interactions between genetic and environmental factors.

In this study, we carried out a population-based case–control study in Chinese children and adolescents and examined the associations of nineteen single-nucleotide polymorphisms (SNPs) with obesity and the biological interactions between genetic variation and dietary behaviors.

2. Materials and methods

2.1. Subjects

A total of 853 obese cases and 2124 healthy controls aged 7–17 years were recruited from a cross-sectional study on metabolic syndrome of children and adolescents in six cities in China (Beijing, Shanghai, Tianjin, Hangzhou, Chongqing, and Nanjing) in 2010. The inclusion criterion for obesity cases was defined as the body mass index (BMI) exceeding 95% of the Chinese BMI references for children and adolescents according to the age and gender (WGOC, 2004). The children who had chronic heart, liver, lung or renal diseases, cancer or other serious diseases were excluded. Healthy control children and adolescents were randomly recruited from normal weight children with 15th to 85th percentile BMIs and were frequency-matched by gender, age and living area. The study protocols were approved by the Research Ethics Committees of School of Public Health and Medical Ethics Committees of Children's Hospital of College of Medicine, Zhejiang University.

2.2. Anthropometric measurements and epidemiologic investigation

Anthropometric indices, including weight, height and waist circumference, were measured by trained physicians or investigators, following to a standard protocol. Height was measured without shoes to the nearest 0.1 cm. Weight with light clothing was measured to the nearest 0.1 kg. BMI was calculated as the individual's body mass in kilograms divided by the square of their height in meters (kg/m^2). Waist circumference (WC) was measured midway between the iliac crest and the lower costal margin at the end of normal exhalation to the nearest 0.1 cm. Waist-to-height ratio (WtHR) was calculated as WC in centimeters divided by the height in centimeters.

The epidemiological data, including demographic characteristics, health status and dietary intake and behavior, was collected with a face to face interview by trained investigators. Dietary behaviors including diet preference (meat-based diet, balanced diet and plant-based diet), salty flavor (like, dislike and no strong preference) and sweet flavor (like and dislike) were collected through the use of a validated questionnaire. Prior to the formal interview, the questionnaires were assessed for validity and reliability in 58 subjects. Test–retest reliability was assessed by re-administering the questionnaire 4 weeks later, and validity was assessed by comparing 3-day food records. The reliability (Cronbach's α) and content validity of the questionnaire were 0.86 and 0.88, respectively (Yang et al., 2014).

2.3. SNP selection and genotyping

The GWAS identified some loci associated with obesity and overweight. In our study, to achieve a power of not less than 80%, the SNPs which were reported significantly associated with BMI or WC in GWAS ($P \leq 10 \times 10^{-8}$) and minor allele frequencies >0.10 in Chinese population in the HapMap database, were selected. Nineteen SNPs (rs6548238(*TMEM18*) (Willer et al., 2009), rs10838738(*MTCH2*) (Willer et al., 2009), rs10938397(*GNPDA2*) (Willer et al., 2009), rs11084753(*KCTD15*) (Willer et al., 2009), rs2815752(*NEGR1*) (Willer et al., 2009), rs2605100(*LYPLAL1*) (Lindgren et al., 2009), rs545854(*MSRA*) (Lindgren et al., 2009), rs987237(*TFAP2B*) (Lindgren et al., 2009), rs17782313(*MC4R*) (Meyre et al., 2009), rs2241423(*MAP2K5*) (Speliotes et al., 2010), rs4929949(*RPL27A*) (Speliotes et al., 2010), rs6265(*BDNF*) (Thorleifsson et al., 2009), rs543874(*SEC16B*) 32, rs7647305(*ETV5*) (Thorleifsson et al., 2009), rs16933812(*PAX5*) (Graff et al., 2013), rs9356744(*CDKAL1*) (Wen et al., 2012), rs2796749(*AMD1*) (Tabassum et al., 2012), rs9299(*HOXB5*) (Bradfield et al., 2012) and rs9568856(*OLFM4*) (Bradfield et al., 2012)) were included in this study (Supplementary Table 1).

Genomic DNA was extracted from peripheral blood using the TOYOBO MagExtractor Genomic DNA Purification Kit (Toyobo, Osaka, Japan) following the manufacturer's protocol. All candidate SNPs were genotyped on Sequenom MassARRAY platform (Gabriel et al., 2009) in Bio-X Institutes, Shanghai. The repeated control samples were set in every genotyping plate and the concordance was more than 99%.

2.4. Statistical analysis

Continuous variables were expressed as mean ± standard deviations (SD) and categorical variables as frequencies (percentages). Statistical significances for continuous variables were assessed using Student's t test and for categorical variables using Chi-square test. Hardy–Weinberg equilibrium tests were performed using Pearson's Chi-square for each SNP among control subjects. The genetic risk score (GRS) was computed as the sum of risk alleles of the four significantly associated SNPs on the basis of the best-fitting genetic model from single SNP analysis. A score of 0, 1 or 2 was assigned to genotypes of associated SNPs according to the number of risk alleles in additive model, whereas scores of 0 or 1 were assigned for the recessive and dominant models. Multivariate logistic (continuous dependent variables) or