Identification of Genetic and Environmental Factors Predicting Metabolically Healthy Obesity in Children: Data From the BCAMS Study

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Context: Available data related to the metabolically healthy obesity (MHO) phenotype are mainly derived from studies in adults because studies during childhood are very limited to date.

Objective: The objective of the study was to determine the prevalence of MHO in Chinese children and to investigate environmental and genetic factors impacting on MHO status.

Design: This was a cross-sectional study.

Participants: A total of 1213 children with a body mass index at the 95th percentile or greater aged 6–18 years were included in this study. Participants were classified as MHO or of metabolically unhealthy obesity based on insulin resistance (IR) or cardiometabolic risk (CR) factors (blood pressure, lipids, and glucose). Twenty-two genetic variants previously reported from genome-wide association studies of obesity and diabetes plus the environmental factors of lifestyle, socioeconomic status, and birth weight was assessed.

Results: The prevalence of MHO-IR and MHO-CR were 27.1% and 37.2%, respectively. Waist circumference was an independent predictor of MHO, regardless of definitions, whereas walking to school and *KCNQ1*-rs2237897 were independent predictors of MHO-CR. Acanthosis nigricans, birth weight, the frequency of soft drink consumption, the mother's education status, and *KCNQ1*-rs2237892 were independent predictors of MHO-IR. Multiplicative interaction effects were found between *KCNQ1*-rs2237897 and walking to school on MHO-CR (odds ratio 1.31 [95% confidence interval 1.05–1.63]) and between rs2237892 and consumption of soft drinks on MHO-IR (odds ratio 0.80 [95% confidence interval 0.68–0.94]).

Conclusions: Approximately one-third of Chinese obese children can be classified as MHO. Both genetic predisposition and environment factors and their interaction contribute to the prediction of MHO status. This study provides novel insights into the heterogeneity of obesity and has the potential to impact the optimization of the intervention options and regimens in the management of pediatric obesity. (***J Clin Endocrinol Metab*** **101: 1816–1825, 2016)**

Obesity has reached epidemic proportions worldwide; at present, more than one-third of the global population is estimated to be overweight or obese (1). Obesity is strongly associated with the development of diabetes, cardiovascular disease (CVD), and certain kinds of cancer (2). However, not all obese individuals have the same prognosis; recent studies demonstrate that 10%–46% of obese adults may be free of metabolic complications, which was recognized as metabolically healthy obesity (MHO) (3). In light of being characterized as a favorable metabolic profile, with no greater risk of developing CVD than expected for their adiposity status (4), MHO has been the focus of recent research interest.

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Abbreviations: AN, acanthosis nigricans; BMI, body mass index; CI, confidence interval; CR, cardiometabolic risk; CVD, cardiovascular disease; DBP, diastolic blood pressure; FAT%, fat mass percentage; FG, fasting glucose; GPS, genetic predisposition score; GWAS, genome-wide association studies; HBW, higher birth weight; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; IR, insulin resistance; LBW, low birth weight; LDL-C, low-density lipoprotein-cholesterol; MHO, metabolically healthy obesity; MI, multiplicative interaction; MS, metabolic syndrome; MUO, metabolically unhealthy obesity; OR, odds ratio; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

The MHO phenotype is generally defined based on either the absence of insulin resistance (IR) (5) or the absence of some or all features of the metabolic syndrome (MS) in adults (6). Although there is still no consensus on the definition of MHO, much emphasis has been placed on identifying the factor underlying the pathogenesis of this trait. Currently it is well known that both genetic and environmental factors are involved in the development of obesity (7), but whether such factors contribute to the dissociation of obesity into subphenotypes of metabolically healthy and metabolically unhealthy obesity (MUO) has still to be elucidated (8). Recent meta-analysis and genome-wide association studies (GWAS) have identified single-nucleotide polymorphisms (SNPs) at loci that are associated with obesity (9) and type 2 diabetes phenotypes (10). Given the evidence for the genetic overlap between obesity and metabolic disorders (11, 12), we hypothesized that at least some of these genetic variants confer predisposition to MHO or MUO. On the other hand, considering environmental factors, recent studies in adults and children suggest lifestyle factors, such as physical activity and dietary behavior, have an influence on MHO pathogenesis (13, 14), but they were limited in the number and scope of the risk factors studied. Additionally, because accumulating evidence supports the long-term fetal programming effects on the risk for obesity and its comorbidities (15), it is speculated that intrauterine environment, albeit not being studied yet in MHO, represents an additional source of environmental factors that modulate determinants of MHO, especially in early age.

Until now, most available data related to the MHO phenotype were derived from studies in adults (5, 6). Because the peak of obesity rates is trending increasingly toward younger ages and the demand for weight management brings heavy economic burden, especially in developing countries like China, it is critically important to distinguish MHO early from the large population of pediatric obesity and to focus on those MUO children who should be directed to earlier intensive clinical care. To our knowledge, no study has yet investigated the contribution of both genetic and environmental factors to the pathogenesis of MHO. Therefore, leveraging the large Beijing Children and Adolescents Metabolic Syndrome study, we addressed two aspects in our study: 1) due to lack of a universal definition, we tackle the prevalence rates of MHO in Chinese children by leveraging two commonly used definitions, and 2) we explore the contribution of genetic and environmental factors to the pathogenesis of MHO, with particular emphasis on modifiable factors.

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Materials and Methods

Subjects

The Beijing Child and Adolescent Metabolic Syndrome study was designed as a population-based study of obesity and MS in a representative sample of Beijing school-age children (n = 19 593, aged 6–18 y, 50% boys) in 2004 (16–18). Within this cohort, 4500 subjects were identified as having one or more of the following metabolic disorders: overweight defined by body mass index (BMI) of the 85th percentile or greater, increased total cholesterol (TC) of 5.2 mmol/L or greater, triglycerides (TGs) of 1.7 mmol/L or greater or fasting glucose (FG) of 5.6 mmol/L or greater based on finger capillary blood tests. Among the subjects, a total of 1213 obese children (777 boys) completed further medical examination and were included in the current study. Obesity was defined by using the sex- and age-specific 95th percentile of BMI, recommended by the Working Group on Obesity in China (19). Informed consents from participants and/or parents/guardians were obtained before entering into the study. The Beijing Child and Adolescent Metabolic Syndrome study was approved by the Ethics Committee at the Capital Institute of Pediatrics in Beijing.

Anthropometric parameters and biochemical analyses

Height, weight, waist circumference (WC), and systolic and diastolic blood pressures (SBP and DBP, respectively) were measured according to our standard protocol (16–18). Fat mass percentage (FAT %) was determined using a bioimpedance analysis. Acanthosis nigricans (AN) of the neck and pubertal development were assessed by a pediatrician of the same gender as the child. Pubertal status was evaluated by Tanner stage of breast development in girls and testicular volume in boys. Venous blood samples were collected after an overnight fast (≥10 h) and were analyzed for plasma concentrations of FG, TG, TC, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and insulin. Insulin was measured by a specific ELISA, without cross-reactivity to proinsulin (<0.05%) (16, 18). Homeostasis model assessment-insulin resistance index (HOMA-IR) was calculated as fasting insulin (milliunits per liter) × FG (millimoles per liter)/22.5.

Assessment of environmental factors

Dietary records including 10 items as shown in Table 1 were assessed with the question, "What is your frequency of food intake?" Response options were ranged from seldom or never to every day. For ease of interpretation, dietary item was further dichotomized according to its median frequency.

Extracurricular physical activities such as cycling, running, swimming, dancing, team sports, etc was divided into two categories as low and moderate to vigorous physical activity

Table 1. Comparison of Characteristics Between MHO and MUO According to the Two Different Definitions

	CR Definition			IR Definition		
	MHO (n = 329)	MUO (n = 884)	*P* Value	MHO (n = 451)	MUO (n = 762)	*P* Value
Demography						
Boys, %	60.4	65.4	.11	66.7	62.5	.13
Urban, %	72.6	60.1	**6.01E-05**	63.4	63.5	.97
Tanner stage	2.2 ± 1.4	2.5 ± 1.4	**.002**	1.9 ± 1.3	2.7 ± 1.4	**2.82E-20**
Age, y	10.8 ± 2.9	11.5 ± 2.9	**3.28E-04**	10.2 ± 2.9	11.9 ± 2.6	**9.30E-25**
BMI z-score	1.94 ± 0.26	2.04 ± 0.29	**4.42E-07**	1.98 ± 0.30	2.03 ± 0.28	**.007**
WC z-score, cm	−0.11 ± 1.00	0.35 ± 1.02	**4.57E-12**	−0.32 ± 0.95	0.55 ± 0.95	**2.86E-49**
FAT% z-score, %	−0.01 ± 0.97	0.30 ± 1.00	**1.73E-06**	−0.10 ± 0.93	0.40 ± 0.99	**9.86E-18**
AN, %	12.7	20.9	**.001**	7.5	25.5	**2.34E-14**
Family history						
Father with obesity, %	9.1	10.4	.52	9.5	10.4	.64
Mother with obesity, %	6.4	6.2	.91	5.8	6.6	.58
Father with diabetes, %	2.7	3.6	.45	2.9	3.7	.46
Mother with diabetes, %	1.5	1.8	.74	1.3	2.0	.41
Father with hypertension, %	9.1	10.3	.55	6.9	11.8	**.006**
Mother with hypertension, %	3.7	5.1	.30	3.8	5.2	.24
Father with dyslipidemia, %	7.0	7.9	.60	6.9	8.1	.42
Mother with dyslipidemia, %	2.4	2.5	.96	2.9	2.2	.48
Birth weight, kg	3.47 ± 0.50	3.41 ± 0.52	.09	3.48 ± 0.50	3.39 ± 0.53	**.005**
Low, %	3.8	4.8	.19	1.9	6.1	**.004**
Normal, %	84.9	87.3		88.0	85.7	
High, %	11.3	7.9		10.1	8.2	
Lifestyle factors						
Breakfast ≥5 times/wk, %	71.8	73.9	.14	77.8	73.3	.08
Beans ≥3 times/wk, %	27.6	28.3	.82	30.1	27.0	.25
Meat ≥5 times/wk, %	44.5	40.2	.16	37.6	44.0	**.03**
Seafood ≥1 times/wk, %	48.6	43.1	.09	49.2	41.9	**.01**
Dairy ≥5 times/wk, %	60.2	51.7	**.009**	59.7	50.6	**.002**
Vegetables ≥5 times/wk, %	87.5	88.8	.52	88.9	88.2	.72
Fruit ≥5 times/wk, %	57.7	51.6	.07	52.0	53.8	.55
Soft drinks ≥1 time/wk, %	47.3	50.8	.29	44.2	53.7	**.002**
Snack ≥1 time/wk, %	50.6	53.9	.32	48.4	46.1	.44
Fast food ≥1 times/wk, %	26.9	23.1	.16	23.0	25.0	.43
MVPA, %	57.0	52.0	.13	59.6	49.7	**.001**
Walk to school, %	54.9	47.5	**.023**	52.6	47.8	.11
Sedentary time ≥2 h/d, %	50.3	49.9	.90	49.3	50.3	.73
Sleep duration, h/d	8.62 ± 1.09	8.57 ± 1.11	.46	8.81 ± 1.10	8.45 ± 1.08	**5.11E-08**
Socioeconomic factors						
Father's education university or higher, %	19.2	16.8	.35	20.1	16.0	.08
Mother's education university or higher, %	16.1	11.9	.06	17.0	10.7	**.002**
Unemployed father, %	6.7	6.5	.91	4.6	7.6	**.04**
Unemployed mother, %	11.9	10.9	.65	10.4	11.6	.52
Cardiometabolic risk						
SBP, mm Hg	105 ± 9	118 ± 12	**5.77E-58**	110 ± 13	117 ± 12	**1.72E-15**
DBP, mm Hg	66 ± 7	74 ± 9	**1.37E-40**	70 ± 9	73 ± 9	**2.30E-06**
TC, mmol/L	4.05 ± 0.67	4.10 ± 0.77	.22	4.02 ± 0.72	4.13 ± 0.76	**.01**
TGs, mmol/L	0.87 ± 0.25	1.32 ± 0.68	**3.52E-29**	1.02 ± 0.50	1.30 ± 0.67	**1.93E-11**
LDL-C, mmol/L	2.55 ± 0.59	2.65 ± 0.69	**.018**	2.55 ± 0.62	2.67 ± 0.68	**.002**
HDL-C, mmol/L	1.41 ± 0.23	1.22 ± 0.25	**6.17E-32**	1.33 ± 0.26	1.23 ± 0.25	**2.83E-11**
FG, mmol/L	4.99 ± 0.33	5.22 ± 0.51	**1.33E-13**	4.99 ± 0.42	5.25 ± 0.49	**2.83E-20**
Ln-insulin[a]	2.22 ± 0.62	2.59 ± 0.62	**1.56E-19**	1.87 ± 0.42	2.85 ± 0.44	**1.26E-210**
Ln-HOMA-IR[a]	0.71 ± 0.64	1.12 ± 0.65	**7.56E-22**	0.36 ± 0.44	1.39 ± 0.47	**3.37E-210**

BMI z-score is the BMI z-score by the standards of the Centers for Disease Control and Prevention 2000; WC z-score is the WC z-score per SD; FAT% z-score is the at mass percentage z-score per SD. Boldface type indicates nominally significant values ($P < .05$).

[a] Natural logarithm transformed.

(MVPA; ≥ 3 times/wk, at least 30 min/time) (20). In addition, the mode of transportation to school was recorded individually as following two divisions of walking and nonwalking. Sedentary lifestyle was assessed by the time of watching television or playing computer games and divided into screen time of 2 h/d or more and screen time of less than 2 h/d (20). Sleep duration was assessed with the question, "How many hours of sleep do you usually get at night?"

The socioeconomic factors included the parents' education (categorized as lower than university or university and higher) and vocation (categorized as employed and unemployed).

Family history including parental obesity, diabetes, dyslipidemia, and hypertension was collected via a questionnaire answered by parents. Intrauterine nutrition was represented by birth weight. In line with the World Health Organization definition, low birth weight (LBW) was less than 2.5 kg and high birth weight was 4.0 kg or greater, with normal birth weight in between.

SNP selection and genotyping

Genomic DNA was isolated from peripheral white blood cells using the QIAamp DNA blood midikits (Qiagen). SNPs were

selected from GWAS reports of obesity, diabetes, or birth weight conducted in east Asian ancestry populations. The 22 SNPs consisted of BMI-related loci (9), type 2 diabetes-related loci (10), and birth weight-related loci (21) were genotyped (Supplemental Table 1) on the Sequenom Mass Array iPLEX genotyping platform in BioMiao Biological Technology Co, Ltd (22). Repeated control samples were present in each genotyping plate, with the concordance rate being 100%. SNPs were excluded if they had a genotyping efficiency less than 0.95 or a Hardy-Weinberg equilibrium value of $P < .002$.

Definitions of metabolically healthy and unhealthy obesity

We applied two commonly used definitions to group subjects in the MHO and MUO categories. The first definition is more clinically relevant, in which, subjects were classified according to the presence/absence of the following traditional cardiometabolic risk (CR) factors (MHO-CR: 0 risk factors; MUO-CR: one or more risk factors): elevation in TG, FG, SBP, and/or DBP or decrease in HDL-C. The cut points by Jolliffe and Janssen (23) were used to define CR factors for individuals 12 years old or older because they are equivalent to those proposed for adults by the International Diabetes Foundation and Adult Treatment Panel III and are adjusted to age and gender based on population growth curves in youth, whereas criteria based on the modified Adult Treatment Panel III MS definition (14, 17) were applied for those aged below 12 years old. All details can be found in Supplemental Table 2. The second definition was based on the presence/absence of IR, in which MHO-IR was defined as HOMA-IR 2.3 or less, which was the optimal point for diagnosis of metabolic syndrome in our Beijing Child and Adolescent Metabolic Syndrome study (18), and MUO-IR was defined by HOMA-IR greater than 2.3.

Statistical analysis

All statistical analyses were performed using SPSS version 17.0 software for windows (SPSS Inc). All skewed distributions were natural logarithm transformed for analysis. Results are expressed as mean ± SD or percentage as appropriate. During the initial step, an independent-sample t test or a χ^2 test was used to analyze continuous or categorical variables relation to MHO, respectively. Genotype distributions in Hardy-Weinberg equilibrium were assessed using the χ^2 test with a Bonferroni-adjusted significance level of $P < .002$. The association of individual SNP and MHO was estimated using additive models and recessive and dominant genetic models, respectively. A score of 0, 1, or 2 was assigned to genotypes of associated SNPs according to the number of risk alleles in additive model. The genetic predisposition score (GPS) was calculated as the sum of risk alleles of all the associated variants on the basis of single SNP analysis with the additive model. Logistic regression analysis was applied to examine the associations between each of the variables (including SNPs) and MHO phenotype with adjustment for gender, age, Tanner stage, and residence. Subsequently, multivariate testing was performed by entering all the associated factors of MHO into the final logistic regression model.

Moreover, multiplicative interactions (MIs) between individual SNPs or the GPS and the associated lifestyle factors on MHO were analyzed using a logistic regression by adding interaction terms, in which the lifestyle factors were entered as categorical variables with adjustment for gender, age, Tanner stage, and residence. A two-tailed value of $P < .05$ indicated statistical significance.

Results

Subject characteristics by MHO categories

Table 1 shows the characteristics of participants by MHO/MUO categories according to the two definitions. There were 27.1% obese children defined as MHO by the CR definition and 37.2% by the IR definition, respectively, in which 181 of 1213 children (14.9%) were simultaneously identified as being both MHO-CR and MHO-IR, whereas 614 of 1213 (50.6%) were categorized as MUO by both definitions. The agreement between the two definitions of MHO was poor because the κ-coefficient was only 0.219 (Supplemental Table 3). Across both the CR and IR definitions, as expected, the MHO group showed a favorable cardiometabolic profile. Compared with their MUO peers, the MHO group trended toward being younger, more prepubertal, and leaner and had lower FAT% and lower proportion of AN. Moreover, the MHO group also had healthier dietary structure, higher amount of exercise including extracurricular physical activity, and walking to school. Furthermore, the MHO group under the IR definition had higher birth weight, longer sleep times, a higher proportion of a well-educated mother, a lower proportion of an unemployed father, and a lower incidence of a father's history of hypertension.

Table 2 shows the factors tested for MHO after adjusting for gender, age, Tanner stage, and residence. Of the anthropometric factors, BMI, WC, and FAT%, and the presence of AN were the strongly related markers of MHO under both definitions (all $P < .02$). Among the environmental factors, walking to school ($P < .05$) was the most strongly related factors of MHO under the CR definition, whereas the consumption of meat ($P = .05$), soft drinks ($P < .05$), the mother's education level ($P < .05$), having an unemployed father ($P < .05$), and birth weight ($P < .05$) were the strong independent predictors of MHO-IR after adjustment.

Regarding the genetic information, the entire data for all SNPs tested are shown in Supplemental Table 1. Among the 22 variants, three were excluded due to departure from the Hardy-Weinberg equilibrium. After adjusting for gender, age, Tanner stage, and residence, as shown in Supplemental Table 1 and Table 2, *KCNQ1*-rs2237892 and *KCNQ1*-rs2237897 were significantly associated with MHO under both definitions (all $P < .05$), whereas *GNPDA2*-rs16858082 ($P < .05$) and *MC4R*-rs2331841 ($P = .07$) had a nominal effect only on the MHO-CR status; however, the GPS consisting of these four variants were significantly associated with MHO-CR

Table 2. Associations Between Lifestyle and Socioeconomic Factors, Genetic Predisposition, and Adiposity and MHO According to the Two Definitions

	CR Definition			IR Definition		
	OR	(95% CI)	*P* Value	OR	(95% CI)	*P* Value
Anthropometry						
BMI, kg/m^2	0.82	(0.77–0.87)	**1.05E-09**	0.76	(0.72–0.79)	**6.39E-34**
WC z-score, cm	0.56	(0.46–0.68)	**6.85E-09**	0.37	(0.32–0.43)	**2.40E-37**
FAT% z-score, %	0.77	(0.67–0.89)	**3.33E-04**	0.66	(0.57–0.76)	**1.00E-08**
AN (yes)	0.64	(0.43–0.94)	**.02**	0.36	(0.24–0.55)	**1.59E-06**
Birth weight, kg	1.14	(0.87–1.50)	.33	1.33	(1.03–1.72)	**.03**
High	1.32	(0.83–2.11)	.24	1.15	(0.73–1.80)	.55
Low	0.90	(0.45–1.80)	.76	0.36	(0.16–1.80)	**.01**
Normal	1.00			1.00		
Lifestyle factors						
Breakfast ≥5 times/wk, %	1.10	(0.80–1.51)	.58	0.94	(0.69–1.27)	.68
Beans ≥3 times/wk, %	0.91	(0.68–1.22)	.53	1.12	(0.85–1.48)	.44
Meat ≥5 times/wk, %	1.25	(0.96–1.61)	.09	0.77	(0.60–1.00)	**.05**
Seafood ≥3 times/wk, %	1.08	(0.83–1.42)	.56	1.06	(0.82–1.37)	.65
Dairy ≥5 times/wk, %	1.26	(0.96–1.65)	.10	1.08	(0.83–1.41)	.55
Vegetables ≥5 times/wk, %	0.91	(0.61–1.36)	.65	1.32	(0.89–1.96)	.16
Fruit ≥5 times/wk, %	1.26	(0.96–1.64)	.09	0.94	(0.73–1.21)	.64
Soft drinks ≥1 times/wk, %	0.86	(0.66–1.12)	.26	0.73	(0.57–0.94)	**.016**
Snack ≥1 times/wk, %	1.09	(0.83–1.42)	.54	1.11	(0.86–1.43)	.42
Fast food ≥1 times/wk, %	0.98	(0.72–1.33)	.90	0.76	(0.56–1.02)	.07
MVPA	1.12	(0.85–1.46)	.43	1.26	(0.98–1.62)	.07
Walk to school	1.32	(1.01–1.72)	**.04**	0.97	(0.75–1.25)	.83
Sedentary time ≥2 h/d	0.96	(0.73–1.25)	.74	0.94	(0.73–1.21)	.63
Sleep duration, h/d	0.95	(0.83–1.10)	.51	1.09	(0.95–1.25)	.21
Socioeconomic factors						
Father's education university or higher	1.16	(0.82–1.64)	.39	1.26	(1.91–1.76)	.16
Mother's education university or higher	1.42	(0.97–2.06)	.07	1.53	(1.06–2.20)	**.02**
Unemployed father	1.16	(0.68–1.97)	.59	0.48	(0.27–0.85)	**.01**
Unemployed mother	1.10	(0.73–1.68)	.64	0.83	(0.55–1.25)	.37
SNPs						
*KCNQ-1*rs2237892-C	0.75	(0.62–0.92)	**.005**	0.77	(0.64–0.94)	**.009**
KCNQ1-rs2237897-C	0.75	(0.61–0.91)	**.004**	0.80	(0.66–0.97)	**.023**
GNPDA2-rs16858082-T	0.80	(0.66–0.97)	**.024**	0.97	(0.81–1.16)	.72
GPS	0.83	(0.76–0.91)	**3.72E-05**	0.86	(0.79–0.95)	**.002**

WC z-score is the WC z-score per SD; FAT% z-score is the fat mass percentage z-score per SD. OR was adjusted for gender, age, Tanner stage, and residence. Boldface type indicates nominally significant values ($P < .05$).

($P = 3.72E-05$). Similar trends were also evident for MHO-IR ($P = .002$).

Independent predictors of MHO status

To identify the independent factors associated with MHO, all the significant variables from the aforementioned logistic regression models (Table 2) were entered into the final models for multivariate testing. As shown in Table 3, after controlling for confounding factors, WC remained significant independent predictors of MHO under both definitions. For every SD increase (10.5 cm) in WC, independently of the BMI, there was a 27% or 51% reduction in the odds of being MHO-CR or MHO-IR, respectively.

For physical activity, compared with their peers, children who walked to school had a great increase (49%) in the odds of being MHO-CR ($P = .007$). As for the SNPs, subjects with each additional allele C in *KCNQ1*-rs227897 or *KCNQ1*-rs227892 had a significant decrease in the odds of being MHO-CR (23%) or MHO-IR (24%), respectively. When using GPS instead of single variant to enter into the modal (data not shown), the GPS was more strongly associated with MHO status (MHO-CR: per effect allele odds ratio [OR] 0.86 [95% confidence interval [CI] 0.78–0.94]; $P = .001$; MHO-IR: OR 0.87 [95% CI 0.78–0.96]; $P = .008$).

In addition, unlike MHO-CR, MHO-IR has several specific predictors such as AN ($P < .001$), birth weight ($P < .05$), consumption of soft drinks ($P < .05$) and the mother's education ($P < .05$). Compared with their peer group, children consuming soft drinks one time or more per week or suffering from AN had a 27%, and 60% decrease, respectively, in the odds of being MHO-IR, whereas children with a highly educated mother had a 59% increase in the odds of being MHO-IR. For each 1-kg increase in birth weight, there was a 40% increase in the odds of being MHO-IR.

Evidence of a gene-environment interaction on MHO phenotype

To explore the possibility of an interaction between gene and environment on MHO, we selected the strongest SNP, *KCNQ1*-rs2237897, *KCNQ1*-rs2237892, and GPS to study the interactions with lifestyle factors currently

Table 3. Independent Factors of MHO According to the Two Different Definitions

	OR	95% CI	*P* Value
CR definition			
BMI, kg/m^2	0.88	0.80–0.96	**.004**
WC z-score, cm	0.73	0.55–0.96	**.024**
Walking to school	1.49	1.12–1.98	**.007**
KCNQ1-rs2237897-C	0.77	0.63–0.95	**.015**
IR definition			
BMI, kg/m^2	0.87	0.79–0.96	**.004**
WC z-score, cm	0.49	0.36–0.66	**3.87E-06**
Birth weight, kg	1.40	1.04–1.87	**.024**
High, ≥4 kg	1.37	0.85–2.20	.191
Low, <2.5 kg	0.45	0.27–0.74	**.002**
Normal, 2.5–4 kg	1.00		
AN (yes)	0.40	0.24–0.66	**3.64E-04**
Mother's education university or higher	1.59	1.04–2.25	**.034**
Soft drink ≥1 time/wk	0.73	0.54–0.98	**.036**
KCNQ1-rs2237892-C	0.76	0.60–0.95	**.016**

WC z-score is the WC z-score per SD. ORs were adjusted for gender, age, Tanner stage, residence, family history, and the significant variables from Table 2. Boldface type indicates nominally significant values ($P < .05$).

shown to be associated with MHO. As shown in Table 4, rs2237897 had a MI with walking to school on MHO-CR (OR 1.31 [95% CI 1.05–1.63]; $P = .015$). Moreover, compared with the subjects with the diabetic risk genotype CC and nonwalking to school, the ones carrying genotype TT/TC and walking to school had an addictive effect on MHO-CR status (OR 2.00 [95% CI 1.35–2.95]; $P < .001$). That is, the positive association of walking to school on MHO appears to be strengthened in TT/TC genotype. It also suggests that although diabetic risk genotype CC is inversely associated with MHO when evaluated independently of lifestyle factors, and the protective association of walking to school may attenuate the inverse association between this variant and MHO. In addition, MIs between rs237892 and soft drinks (OR 0.80 [95% CI 0.68–0.94]; $P = .005$) were evident for MHO-IR. Compared with subjects in the group with the TT/TC genotype and consumption of fewer soft drinks, the subjects with diabetic risk genotype CC and consumption of soft drinks one or more times per week had addictive inverse effects on MHO-IR status (OR 0.60 [95% CI 0.41–0.86]; $P = .006$), implying that an unhealthy diet may strengthen the inverse association of CC genotype with MHO. Meanwhile, MIs were also evident between GPS and the above-mentioned lifestyle factors ($P = .001$–0.003) (Table 4).

Discussion

Several important observations emerged from our present analysis. First, about one in three obese children were at relative low cardiometabolic risk or IR despite with a high level of weight. Second, we found not only current lifestyle and socioeconomic factors such as walking to school and consumption of soft drinks but also early intrauterine nutrition environment could independently predict MHO. Finally, we have found that common variants such as *KCNQ1*-rs237897 and rs237892, interacting with modifiable lifestyle factors are associated with MHO, which suggests there is a partly genetic-environmental basis to the widely reported MHO phenotype.

Our study has several implications. First, with this large representative sample, we demonstrate that there are a relatively high proportion of Chinese children with MHO. As in adults, there is no homogenous definition of MHO for the pediatric population. The prevalence of MHO varied greatly from different definitions and samples (6, 24). Furthermore, the definition of MHO in youth is particularly complicated because abnormalities in the metabolic profile become more apparent in adulthood, and there is less consensus about how to define MS (17) or IR (18) in youth. However, the absence of IR (14) or criteria indic-

Table 4. Interactions Between Genetic Variants and Lifestyle Factors on MHO According to the Two Different Definitions (OR [95% CI])

	***KCNQ1*-rs237897 (CR) or *KCNQ1*-rs237892 (IR)**						**GPS**	
	TT/TC		**CC**		**MI**		**MI**	
	OR [95% CI]	***P* Value**	**OR [95% C]**	***P* Value**	**OR [95% CI]**	***P* Value**	**OR [95% CI]**	***P* Value**
MHO-CR								
Walk to school								
Yes	2.00 (1.35–2.95)	**.001**	1.45 (0.95–2.21)	.089	1.31 (1.05–1.63)	**.015**	1.00	
No	1.62 (1.09–2.40)	**.017**	1.00		1.00		0.91 (0.85–0.97)	**.003**
MHO-IR								
Soft drinks ≥1 time/wk								
Yes	0.73 (0.542–1.04)	.078	0.60 (0.41–0.86)	**.006**	0.80 (0.68–0.94)	**.005**	0.90 (0.84–0.96)	**.001**
No	1.00		0.77 (0.54–1.10)	.15	1.00		1.00	

OR was adjusted for gender, age, Tanner stage, and residence. MIs were calculated by logistic regression with adjustment for gender, age, and Tanner stage. Boldface type indicates nominally significant values ($P < .05$).

ative of MS (14, 25, 26) has also been proposed as diagnostic criteria for the MHO phenotype in children, with the prevalence rates varying between 21% and 68% (14, 25, 26). For comparison, we simultaneously chose those two definitions: the MHO-IR with emphasis on potential biological mechanisms and the MHO-CR with the clinical interesting.

As expected, our results showed that not only the prevalence rates but also the factors of MHO status varied, depending widely on the definition. With respect to MHO-CR, even when we chose the strictest standard without any classical MS components, the prevalence of MHO reached 27.1%, which is comparable with the rate of 25% in a small study consisting of 108 overweight and obese adolescents using the similar cut points (26) but quite different from the study in Austria (36%, n = 108 of 299) (27) and in Canada (21.5%, n = 39 of 181) (14) using the different criteria. Nevertheless, using the IR definition by a strict criterion as HOMA-IR of 2.3 or less (18), the prevalence rate of MHO-IR is 37.2% in our study, which was a little higher than that of 31.5% in the Canadian study using a HOMA-IR less than 3.16 (14). Notably, comparability of studies is difficult due to the different definitions used for MHO and the inclusion of overweight children in some studies (6), even in our study using different definitions as shown in Supplemental Table 4. However, regardless of definitions, the common existence of MHO in children of our study suggests that it deserves consideration in the management of pediatric obesity.

As in adult studies, it is unclear to date why some obese children do not develop any associated metabolic complications and some do. One explanation might be due to the specific healthy lifestyle factors. However, there was much debate on the influence of lifestyle factors on the MHO phenotype in adults (6). Phillips et al (13) reported that in the European adult, compliance with food pyramid recommendations and being MVPA increase the likelihood of MHO, depending on which criteria are used. Another study from the National Health and Nutrition Examination Survey shows that physical activity differs between MHO and MUO in adults but not in adolescents (25). Nevertheless, study in pediatric population is still lacking. Based on our large sample size study, we found exercise, particularly walking to school, or a healthy diet habit such as reduced consumption of soft drinks would have independently impact on MHO status, depending on the definitions. Walking to school is an affordable and simple way of increasing physical activity levels. In line with us, a study consisted of 229 Portuguese adolescents supported that those who walk to school have a better metabolic profile than passive travelers independent of MVPA and diet (28). On the other hand, less sugar in soft drink has been shown to reduce the prevalence of obesity and type 2 diabetes, especially in adolescents (29). Given the difficulty for children with obesity to lose and maintain weight loss over time, our findings may have an important implication: obese children should be particularly encouraged to increase exercise like walking to school and avoid sugar-sweetened beverages to maintain a healthy metabolic status.

Another explanation of healthy and unhealthy obesity might be due to the weight at birth and obesity rebound during early infancy (30). In our study, we demonstrated that higher birth weight (HBW) was more likely to be obesity with insulin sensitivity, whereas subjects with LBW were significantly associated with the likelihood of being obese with IR. To our knowledge, this is the first evidence of an association between fetal environment and MHO in childhood. It has been shown that LBW, no early catch-up weight, and subsequent fat accumulation are associated with increased risk for IR and CVD from childhood onward, whereas HBW and early weight gain may alter or even program insulin sensitivity and adipose tissue metabolism in later life (30); hence, both LBW and HBW might contribute to the development of the MUO phenotype. To agree with this finding, although we found higher birth weight is more likely to being MHO-IR, we did not find a higher proportion of MHO in the HBW group compared with normal birth weight group. However, given that both HBW and LBW are the categories of risk for obesity and IR (15) and we did not collect the data about early weight gain, other confirmative studies are required to establish whether the obesity originated from intrauterine nutritional deficiency or excess might be MUO and to elucidate the underlying mechanism of early-life programming on MHO.

In addition to environmental aspects, our study provides a novel finding on genetic evidence of MHO. There has been evidence of overlap between susceptibility loci for type 2 diabetes, obesity, and birth weight (11). Among the 22 selected variants, we found *KCNQ1*-rs2237897 and *KCNQ1*-rs2237892 had the strongest association with MHO in the CR and IR definition, respectively. As far as we know, our study was the first to identify the loci concerned with MHO. The associations of *KCNQ1*-rs2237897 and *KCNQ1*-rs2237892 with diabetes and obesity have been indicated in previous GWAS (9, 10, 31) in Asian and European populations and in other studies in the Chinese population (32). Because the R^2 of linkage disequilibrium between rs2237892 and rs2237897 was 0.677 (33), it is not surprising that our study found that they became the strongest loci associated with MHO after multifactor testing. In addition, another BMI-associated loci, *GNPDA2*, was observed to show correlation with

MHO-CR definitions. But after multifactor adjustment, this association disappeared. However, we cannot rule out that the lack of association of other variants may be due to insufficient statistical power in the current study.

It is not clear how those variants in the BMI/diabetes-associated genes could influence the risk of metabolic healthy or unhealthy obesity. *KCNQ1* encodes the pore-forming subunit of a voltage-gated K+ channel, mainly expressed in pancreatic islets, which is primarily important in triggering and maintaining glucose-stimulated insulin secretion (34). It is speculated that common variants in *KCNQ1* may gradually impair pancreatic β-cell function over time and thus link to type 2 diabetes (35). However, in our study, we found these loci were significantly associated with insulin resistance and lipids (Supplemental Table 5); thus, the associations of SNPs in *KCNQ1* and MHO might be mediated by mechanisms other than insulin secretion. Unlike with *KCNQ1*, variants in *GNPDA2* showed an association with MHO, depending on the definition and adjustment of covariates. *GNPDA2* located on chromosome 4p12 encodes an allosteric enzyme, which mainly participated in the metabolism of glucose. *GNPDA2* is highly expressed in the central nervous system and hence highlight a neuronal influence on the dissociation of obesity into subphenotypes of MUO and MHO (36).

Another novel finding in our study is that the genetic predisposition to MHO would be modified by interacting with lifestyle factors, which may also provide additional understanding of mechanisms related to MHO. The beneficial effect of exercise on obesity and metabolic disorder has been well established. However, even when exposed to the same level of exercise, responses are different from one individual to the next (20). Thus, the gene-lifestyle interactions might play important roles in the pathogenesis of metabolic diseases. In our study, the polymorphism rs2237897 had MI with walking to school for MHO-CR, and rs2237892 had MI with the consumption of soft drinks for MHO-IR. The novel interaction between the *KCNQ1* locus and lifestyle factors was first detected in this study. Because *KCNQ1* is also widely expressed in a variety of tissues including the heart, skeletal muscle, and liver and participates in the regulation of cell volume, which is, in turn, critically important for the regulation of metabolism (35), we speculated that the habit of diet and exercise might regulate the expression of *KCNQ1* in these tissues; thus, the adverse effect of the CC genotype on MHO might be attenuated by walking to school in rs2237897 or strengthened by a high consumption of soft drinks in rs2237892. Given the fact that GWAS often highlights intergenic or noncoding regions raises questions about the underlying mechanisms by which noncoding variation influences disease risk; we should allow for the possibility that certain epigenetic mechanisms that regulate gene expression, such as DNA methylation, could be directly affected by diet or exercise (37). However, determining the precise mechanism behind this interaction will require further experimentation. Nevertheless, our finding implies that maintaining the healthy diet habits and exercise in children who had the genetic predisposition to MHO would be more beneficial for being healthy.

Similarly to the previous study in adults (38) and children (14), we confirmed that WC was a strong predictor of MHO independently of BMI and total fat mass, supporting the notion that visceral fat accumulation is a strong and independent predictor of adverse health and should be paid more attention than weight or BMI in obesity management. Furthermore, as a feature of IR, AN was established as a formal risk factor for the development of diabetes in children; thus, it is not surprising that we find it as a powerful physical sign in distinguishing MHO-IR from obese youth.

There are several limitations that should be noted. First, due to the lack of consensus on the definition of MHO, our results may be different with other studies. Second, although we collected many aspects of the lifestyle and socioeconomic factors, our use of self-reported information was susceptible to reporting bias. Moreover, childhood obesity is much more complex than obesity in adults; except for lifestyle factors that we have already explored, other psychological factors may need to be considered in further study. Third, fitness was not included as a determinant for MHO. Given that some studies support the link between fitness and metabolic health in youth, further study should explore whether fitness is a characteristic of MHO among Chinese children (6, 39). Fourth, we just opened the door to explore the genetic evidence of MHO, by selecting only 22 SNPs related with BMI, diabetes, or birth weight; in future study, more attention should be paid to the genetic and epigenetic mechanisms regulating the function of adipose tissue (40). Finally, because of the cross-sectional nature of the study, the causal relationship cannot be determined currently, and our ongoing follow-up study would be expected to elucidate how long these influence factors could maintain the healthier metabolic status from childhood onward.

Until now, recommendations for obesity treatment do not consider differences between healthy and unhealthy obese phenotypes. The increasing rate of pediatric obesity highlights the importance of distinguishing MHO and MUO, which benefit the delivery of optimal health services for obesity management in a manner that is both efficient and effective. We have identified that the lifestyle and socioeconomic factors, genetic predisposition, and

birth weight all play important roles in predicting MHO, and we have found evidence of interactions between genetic factors and lifestyle factors that contribute to the MHO status. Our findings provide novel insights into the heterogeneity of obesity and may have an impact on intervention options and regimens for the management of pediatric obesity.

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