Interaction between early environment and genetic predisposition instigates the metabolically obese, normal weight phenotype in children: findings from the BCAMS study

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Abstract

Objective: A subset of normal-weight individuals appears predisposed to obesity-related cardiometabolic abnormalities. Studies of this metabolically obese, normal weight (MONW) phenotype in youth are scarce. We aimed to identify early environmental and genetic factors associated with MONW in children.

Methods: Overall, 1475 normal-weight Chinese children aged 6–18 were recruited from the Beijing Children and Adolescents Metabolic Syndrome study cohort. Birthweight, childhood lifestyle, socio-economic factors, and 20 genetic variants previously shown to be associated with BMI or glucose metabolism in East Asian adults were examined for their association with the MONW phenotype. MONW was defined by exhibiting any metabolic syndrome component.

Results: After adjusting for covariates including BMI, low birthweight and low levels of physical activity, fruit consumption, parental education and household income, as well as *CDKAL1* rs2206734 genotype were independent predictors of the MONW phenotype (all $P < 0.05$). Moreover, rs2206734 interacted with birthweight to predict the MONW phenotype ($P_{\text{interaction}} = 0.0008$). Among high (>75th percentile) birthweight individuals, each C allele at this locus was associated with a 62% reduced risk of MONW (OR = 0.38; 95% CI = 0.26–0.58; $P = 5.71 \times 10^{-6}$), while no such genetic associations were found in intermediate or low birthweight individuals ($P > 0.1$). This *CDKAL1*-MONW relationship in high birthweight individuals was especially strong in the presence of favorable childhood environmental factors (high levels of physical activity, fruit consumption, parental education and household income) ($P_{\text{interaction}} = 0.013$).

Conclusions: Our findings provided the novel evidence that early environment (especially birthweight) and genetics, along with their interaction with one another, play important roles in predicting the MONW phenotype among children.

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Introduction

Obesity is a well-known cardiometabolic risk factor. However, some individuals, despite a healthy BMI, are at increased risk of cardiometabolic disorders, including type 2 diabetes (T2D), hypertension and cardiovascular disease (CVD). These people are referred to as 'metabolically obese, normal weight' (MONW) (1). Both genetic and environmental factors are known to contribute to the development of the MONW phenotype (1); however, unlike metabolic defects in subjects with obesity, the factors underlying the pathogenesis in normal-weight individuals are largely unknown. Previous studies have shown that Asians are at greater risk for cardiometabolic abnormalities compared to their Western counterparts, even at relatively low BMI levels (2, 3). Yet, the factors underlying this increased CVD risk remain unclear. Because children exhibit metabolic plasticity, and are relatively free of co-morbidities and confounding treatment effects, they represent an ideal population in which to study the pathogenesis of MONW. Nevertheless, we can find no studies which assess environmental and genetic factors contributing to the MONW phenotype in Asian children.

While the pathogenesis of MONW remains unclear, recent evidence suggests that adipose tissue dysregulation may contribute via the 'adipose expandability' hypotheses, which states that inadequate s.c. adipose tissue expansion in response to ingested triglycerides may lead to visceral and ectopic fat deposition with inherent insulin resistance and cardiometabolic dysfunction, even in the face of normal body weight (1, 4, 5). A genetic basis for MONW can be inferred from the myriad of genome-wide association studies (GWAS) which identify various genetic variants associated with fat mass or body fat distribution, and which involve pathways relevant to adipose tissue dysregulation (4, 6). In addition, a number of genetic variants from GWAS have been associated with carbohydrate and lipid metabolism disorders and are worthy of investigation for their ability to confer a predisposition to MONW (7, 8).

Increasing evidence suggests that childhood environmental exposures, such as lack of physical activity, unfavorable dietary patterns and low socioeconomic status, play a significant role in metabolic health (9, 10, 11). However, no study has systematically assessed the individual and combined effects of environmental factors in early-life or the interaction between environmental patterns and genetic susceptibility in determining the pediatric MONW phenotype. While it is well established

that the intrauterine environment has a persistent impact on metabolism and gene expression in later life (12), relevant studies examining the early origins of the MONW phenotype are scarce.

Leveraging the large dataset from the Beijing Children and Adolescents Metabolic Syndrome (BCAMS) study, we aimed to explore the contribution of genetic and environmental factors to the pathogenesis of the MONW phenotype in childhood. In addition, we examined whether individual or a multiplex combination of environmental factors during critical periods of development can modify the genetic predisposition to the MONW phenotype. Our study may shed light on underlying mechanisms and help distinguish between those children who are most and least prone to developing cardiometabolic impairment despite their body weight being within the normal range, and identify a path toward optimization of early prevention and treatment strategies.

Subjects and methods

Subjects

Study participants were from the BCAMS study, which is a longitudinal study of cardiometabolic risk factors from childhood to young adulthood in a representative sample of school-age children ($n=19,593$, aged 6–18 years, 50% boys) from Beijing (13, 14). The baseline survey began in 2004 and has been described in detail elsewhere (14). Within this cohort, 4500 participants were recognized as having risk factors defined by the presence of at least one of the following: overweight (defined as BMI > 85th percentile), total cholesterol (TC) ≥ 5.2 mmol/L, triglyceride (TG) ≥ 1.7 mmol/L or fasting blood glucose ≥ 5.6 mmol/L based on capillary blood tests. Along with a parallel reference sample of 1095 children, these participants were invited to undergo further medical examinations for verification based on venipuncture blood samples and a clinical examination. Among these cohorts, 3235 children completed further medical examination and provided complete data, including information about lifestyles, family history, and parental education. In the current analysis, we focused on the 1475 normal-weight individuals (594 boys) from the 3235 children with complete data (Supplementary Fig. 1, see section on [supplementary materials](#) given at the end of this article). Children with a known history of type 1 or type 2 diabetes were excluded, and normal weight was defined by a sex- and age-specific BMI between the 5th

and 85th percentiles. The study protocol was approved by the Ethics Committee at the Capital Institute of Pediatrics and Peking Union Medical College Hospital. Signed informed consent was obtained from all participants and/or their parents or guardians. The study protocol was also registered at www.clinicaltrials.gov (NCT03421444).

Anthropometric and biochemical parameters

Weight, height, waist circumference (WC), blood pressure and fat mass percentage (FAT%) by bio-impedance analysis were measured by trained staff as previously reported (14, 15). Pubertal development was scored by Tanner stage for breast development in girls and testicular volume in boys. This assessment was performed by two pediatricians of the same gender as the child. Venous blood samples were collected after an overnight (≥ 12 h) fast. Fasting plasma concentrations of glucose (FPG), TG, TC, HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) were assayed by a Hitachi 7060 C automatic biochemistry analysis system. Fasting insulin was measured by an ELISA developed at the Key Laboratory of Endocrinology, Peking Union Medical College Hospital, without cross-reactivity to proinsulin ($<0.05\%$) (13, 14). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (mU/L) $\times$ FPG (mmol/L)/22.5 (16).

Assessment of environmental factors

The intrauterine environment was assessed from birthweight, with <25 th, 25th–75th and >75 th percentiles equated with low, intermediate and high birthweights, respectively. Questionnaires were used to obtain information on lifestyle factors including diet, physical activity, sleep duration, and mode of transportation to school as previously described (13, 15). Dietary factors were assessed with a food frequency intake assessment of ten items that were dichotomized according to their median frequency. Physical activity was assessed by the frequency of vigorous exercise including cycling, hiking, batting, running, swimming and other extracurricular physical activities performed for at least 30 min in duration and was classified as low (<3 times/week) or high (≥ 3 times/week) levels of moderate-to-vigorous physical activity (MVPA). Additionally, the mode of transportation to school was divided into two categories as ‘walking’ and ‘nonwalking’. Sleep duration was recorded as the response to ‘How many hours of sleep do you usually get at night?’ Socioeconomic status was assessed by maximum parental

education level attained and annual household income (13). Highest level of parental education was classified as $<$ University or $\geq$ University. Annual household income was categorized in quartiles as low ($<15,600$ yuan, <25 th percentiles), moderate (15,600–58,000 yuan, 25th–75th percentiles), or high ($>58,000$ yuan, >75 th percentiles).

In order to combine the multiplex of childhood environmental factors into a single element for analysis, we derived a composite score by enumerating the independent protective environmental factors. Every independent protective environmental factor including MVPA, high fruit consumption, parental education $\geq$ university, or high annual household income was assigned a score of 1, while low physical activity, low fruit consumption, parental education $<$ university, low or moderate annual household income was ranked as a 0, according to their presumed effects on metabolic health. Furthermore, low and high composite scores were defined as 0–1 and 2–4 protective factors, respectively.

SNP selection and genotyping

Genomic DNA was extracted from blood samples using the QIAamp DNA Blood Midi Kits. We selected 20 SNPs including BMI-related loci (13) and glucose metabolism-related loci (7) previously reported in GWAS studies of East Asian populations (Supplementary Table 1). Genotyping was performed on the Sequenom Mass Array iPLEX genotyping platform in BioMiao Biological Technology (Beijing) Co, Ltd. (17). Repeated control samples were present in each genotyping plate with the concordance rate being 100%. All SNPs had a genotyping efficiency of greater than 95%, but two SNPs were excluded due to deviation from Hardy-Weinberg equilibrium with a Bonferroni-adjusted significance level of $P < 0.0025$ (0.05 divided by 20).

Definitions of metabolically obese, normal weight

The MONW phenotype was defined by the presence of any classic component of the pediatric metabolic syndrome (MS) including (14): (1) hypertension (SBP and/or DBP ≥ 90 th percentile for age, sex and height according to BCAMS); (2) hypertriglyceridemia (TG ≥ 1.24 mmol/L); (3) low serum HDL-C (defined as ≤ 1.03 mmol/L), or (4) impaired FPG (defined as ≥ 5.6 mmol/L), occurring in a normal-weight child. The metabolically healthy normal-weight (MHNW) phenotype was defined by the absence of any such features of MS.

Statistical analysis

Any parameters whose values were not normally distributed underwent logarithmic transformation before analysis. All results are expressed as mean $\pm$ s.d., unless otherwise stated. Group differences of continuous or categorical variables in children with or without the MONW phenotype were evaluated by independent sample *t* tests or χ^2 tests, respectively. The associations between individual SNPs and MONW were tested using additive models. Each SNP was recoded as 0, 1, or 2 according to the number of high risk alleles. Logistic regression analysis was used to assess the associations between each of the variables (including SNPs) and the MONW phenotype. Variables found to be significant in the age-, gender-, puberty- and BMI-adjusted analyses were added to a multivariable logistic regression model to evaluate the independent risk factors of MONW. Moreover, interactions between individual SNPs and individual environmental factors (or combined factor patterns) on MONW were examined using logistic regression analysis by adding the relevant interaction terms into the models. For example, birthweight $\times$ SNP, with inclusion of the main effects in the models. Power calculation was performed by Quanto software version 1.2.3 (University of Southern California, Los Angeles, CA, USA). All statistical analyses were performed using SPSS version 22.0 software for windows (SPSS Inc.).

Results

Subject characteristics according to metabolic health status in normal weight children

Table 1 shows the characteristics of normal-weight subjects with or without metabolic abnormalities. There were 531 (36.0%) children defined as MONW. Factors associated with the MONW phenotype after adjusting for age, gender, pubertal status and BMI are shown in Table 2. Increased FAT% was related to MONW ($P<0.001$). Among the lifestyle factors evaluated, consumption of bean, meat, seafood and fruit, as well as increased physical activity (whether from walking to school or extracurricular MVPA) were associated with a reduced risk of MONW after adjustment for covariates (all $P<0.05$). Furthermore, a lower birthweight was linked to greater risk of MONW ($P<0.05$). Finally, lower levels of parental education and household income were associated with the MONW phenotype (all $P<0.05$). The statistics for all genetic variants analyzed are listed in Supplementary

Table 1. Only *CDKAL1*-rs2206734 showed a significant association with the MONW phenotype after Bonferroni correction ($P=0.002$).

Independent predictors of the MONW phenotype

To identify the independent factors related to metabolic aberration in normal weight individuals, all the significant variables in Table 2 were included in the final models for multivariate analysis. Regarding the intrauterine environment, participants with the highest birthweight (>75th percentile) had the lowest risk of MONW, independent of childhood BMI (Table 3). Compared to less physically active individuals, children with greater amounts of exercise had a significant reduction (26%) in the likelihood of exhibiting metabolically obesity (95% CI=0.58–0.93, $P=0.011$). Higher consumption of fruit was also a protective factor for MONW (OR=0.75, 95% CI=0.59–0.95, $P=0.018$). Analysis of the contribution of combined childhood environmental factors revealed that the composite score (which involves a combination of protective factors including physical activity, fruit consumption, parental education and annual household income) was strongly related to the MONW phenotype with an odds ratio (OR) per factor increase of 0.68 (95% CI=0.62–0.76, $P<0.001$). As for genetic associations, after controlling for the aforementioned risk variables, only the *CDKAL1*-rs2206734 genotype showed an association with protection from the MONW phenotype, with each additional C allele in rs2206734 having a 21% decrease in the odds of exhibiting MONW (95% CI=0.67–0.93, $P=0.005$).

Gene-environment interaction effects on the MONW phenotype

Since the *CDKAL1* rs2206734 locus had the strongest association with the MONW phenotype, we further examined the association between this variant and the environmental factors influencing the risk of MONW, and no significant association was evident in our study population (data not shown). We therefore examined whether the significant environmental factors listed in Table 3 could modify the effect of this key genetic variant on MONW risk by testing for their interactions. As shown in Fig. 1A, a significant interaction between the *CDKAL1* locus and birthweight was observed in determining the MONW phenotype, even after adjusting for age, gender, pubertal status, gestational age, BMI, physical activity level, fruit consumption, parental education, and household

Clinical Study	G Li and others	Nature vs nurture in metabolic health	182:4	397
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Table 1 Comparison of characteristics between MONW and MHNW. Data are shown as the mean $\pm$ S.D. or median (interquartile range) or percentage.

Variables	MHNW	MONW	P value
Demography			
<i>n</i>	944	531	
Age (years)	12.2 $\pm$ 3.1	12.9 $\pm$ 3.1	<0.001
Male (%)	41.6	37.9	0.156
Tanner stage (%)			<0.001
1	30.9	21.5	
2	15.8	15.7	
3	14.8	13.8	
4	28.4	33.7	
5	10.1	15.3	
BMI Z-score	-0.31 $\pm$ 0.86	-0.14 $\pm$ 0.81	<0.001
BMI (kg/m ²)	17.54 $\pm$ 2.39	18.26 $\pm$ 2.45	<0.001
WC (cm)	61.67 $\pm$ 7.09	63.25 $\pm$ 7.06	<0.001
FAT%	17.48 $\pm$ 5.49	19.21 $\pm$ 5.87	<0.001
Family history			
Father, diabetes (%)	1.3	2.1	0.234
Mother, diabetes (%)	0.8	0.8	0.847
Father, hypertension (%)	6.7	7.5	0.534
Mother, hypertension (%)	2.8	4.0	0.208
Father, dyslipidemia (%)	4.8	4.5	0.829
Mother, dyslipidemia (%)	1.3	1.3	0.939
Birthweight (kg)	3.35 $\pm$ 0.53	3.22 $\pm$ 0.47	<0.001
Gestational age (%)			0.264
Premature	5.4	5.9	
Full-term	90.8	88.5	
Overdue	3.8	5.7	
Lifestyle factors			
Breakfast $\geq$ 5 times/week (%)	81.0	76.8	0.059
Bean $\geq$ 3 times/week (%)	37.4	27.7	<0.001
Meat $\geq$ 5 times/week (%)	51.8	37.5	<0.001
Seafood $\geq$ 1 time/week (%)	21.1	16.0	0.017
Dairy $\geq$ 5 times/week (%)	59.3	42.9	<0.001
Vegetable $\geq$ 5 times/week (%)	89.7	86.6	0.075
Fruit $\geq$ 5 times/week (%)	63.7	50.7	<0.001
Fried food $\geq$ 1 time/week (%)	33.4	33.6	0.931
Soft drink $\geq$ 1 time/week (%)	45.1	42.1	0.284
Fast food $\geq$ 1 time/week (%)	13.8	10.0	0.035
MVPA (%)	63.1	53.7	<0.001
Walk to school (%)	69.0	56.2	<0.001
Sleep duration (hours/day)	8.59 $\pm$ 1.24	8.52 $\pm$ 1.22	0.336
Socioeconomic factors			
Parental education $\geq$ University (%)	33.5	17.9	<0.001
Annual household income			<0.001
Low (<25th percentiles)	20.4	35.8	
Intermediate (25th–75th percentiles)	46.6	46.9	
High (>75th percentiles)	32.9	17.3	
Cardiometabolic risk			
SBP (mmHg)	97.9 $\pm$ 10.5	107.9 $\pm$ 12.8	<0.001
DBP (mmHg)	61.6 $\pm$ 8.0	69.0 $\pm$ 10.3	<0.001
Total cholesterol (mmol/liter)	4.10 $\pm$ 0.90	4.17 $\pm$ 0.93	0.117
Triglycerides (mmol/liter)*	0.71 (0.56–0.90)	0.98 (0.69–1.44)	<0.001
HDL-C (mmol/liter)	1.58 $\pm$ 0.29	1.45 $\pm$ 0.34	<0.001
LDL-C (mmol/liter)	2.44 $\pm$ 0.73	2.57 $\pm$ 0.87	0.020
FPG (mmol/liter)	4.88 $\pm$ 0.38	5.23 $\pm$ 0.53	<0.001
Insulin (mU/L)*	5.09 (3.14–7.74)	7.16 (4.88–10.02)	<0.001
HOMA-IR*	1.12 (0.65–1.73)	1.65 (1.11–2.40)	<0.001

*Natural logarithm-transformed. Boldface type indicates nominally significant values ($P < 0.05$).

DBP, diastolic blood pressure; FAT%, fat mass percentage; FPG, fasting plasma concentrations of glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; LDL-C, low-density lipoprotein cholesterol; MHNW, metabolically healthy normal weight; MONW, metabolically obese, normal weight; MVPA, moderate-to-vigorous physical activity; SBP, systolic blood pressure; WC, waist circumference.

Clinical Study	G Li and others	Nature vs nurture in metabolic health	182:4	398
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Table 2 Associations between lifestyle and socioeconomic factors, genetic predisposition and metabolically obese, normal weight.

	OR	95% CI	P value
Anthropometry			
WC (cm)	0.97	0.93–1.01	0.091
FAT%	1.04	1.02–1.07	<0.001
Birthweight (kg)	0.55	0.44–0.70	<0.001
Intermediate (25th–75th percentiles)	1	–	–
Low (<25th percentiles)	1.38	1.08–1.78	0.010
High (>75th percentiles)	0.64	0.48–0.86	0.003
Lifestyle factors			
Breakfast $\geq$ 5 times/week (%)	0.86	0.66–1.13	0.284
Bean $\geq$ 3 times/week (%)	0.65	0.51–0.82	<0.001
Meat $\geq$ 5 times/week (%)	0.55	0.44–0.68	<0.001
Seafood $\geq$ 1 time/week (%)	0.73	0.55–0.97	0.027
Vegetable $\geq$ 5 times/week (%)	0.74	0.53–1.03	0.072
Fruit $\geq$ 5 times/week (%)	0.60	0.48–0.75	<0.001
Soft drink $\geq$ 1 time/week (%)	0.83	0.66–1.04	0.106
Fast food $\geq$ 1 time/two week (%)	0.71	0.51–1.00	0.053
MVPA (%)	0.71	0.57–0.88	0.002
Walk to school (%)	0.60	0.48–0.75	<0.001
Sleep duration (h/day)	1.08	0.97–1.19	0.168
Socioeconomic factors			
Parental education $\geq$ University	0.43	0.33–0.56	<0.001
Annual household income			
Low (<25th percentiles)	1	–	–
Intermediate (25th–75th percentiles)	0.55	0.43–0.71	<0.001
High (>75th percentiles)	0.30	0.22–0.41	<0.001
SNP			
<i>CDKAL1</i> -rs2206734-C*	0.78	0.66–0.91	0.002

*Additive genetic model was used. ORs were adjusted for age, gender, puberty and BMI. Boldface type indicates nominally significant values ($P < 0.05$). MHNW, metabolically healthy normal weight; MONW, metabolically obese, normal weight; MVPA, moderate-to-vigorous physical activity; OR, odds ratio; WC, waist circumference.

income (P for interaction=0.0008). Notably, every C allele at the *CDKAL1* locus reduced the risk of MONW by 62% in people with high birthweight (95% CI=0.26–0.58, $P=5.71 \times 10^{-6}$) as compared to the (insignificant)

associations observed in children of intermediate and low birthweight ($P > 0.100$).

As the genetic effects of *CDKAL1* were clearly modified by the intrauterine environment, we explored whether

Table 3 Independent factors of metabolically obese, normal weight.

Variable	OR	95% CI	P value
Birthweight (kg)			
Low (<25th percentiles)	1	–	–
Intermediate (25th percentiles–75th percentiles)	0.80	0.61–1.04	0.093
High (>75th percentiles)	0.56	0.40–0.78	0.001
Parental education $\geq$ University	0.63	0.47–0.86	0.003
Annual household income			
Low (<25th percentiles)	1	–	–
Intermediate (25th–75th percentiles)	0.65	0.49–0.86	0.003
High (>75th percentiles)	0.43	0.30–0.62	<0.001
Fruit $\geq$ 5 times/week (%)	0.75	0.59–0.95	0.018
MVPA (%)	0.74	0.58–0.93	0.011
<i>CDKAL1</i> -rs2206734-C*	0.79	0.67–0.93	0.005

Variables adjusted for age, gender, puberty and BMI and a P value ≤ 0.05 was selected in the model.

*Additive genetic model was used. ORs were adjusted for age, gender, puberty, BMI and gestational age. Boldface type indicates nominally significant values ($P < 0.05$).

OR, odds ratio.

Figure 1

The effects of the C allele of *CDKAL1*-rs2206734 in the stratification of birthweight (A) or birth weight and childhood environmental patterns (B) on the metabolically obese, normal weight phenotype. The data are shown as OR (95% CI). The ORs for each C allele of *CDKAL1*-rs2206734 in the stratification of birthweight were adjusted for age, gender, puberty, gestational age, BMI, physical activity, fruit consumption, parental education and annual household income. The composite score of protective childhood environmental factors was the combination of high physical activity, fruit consumption, parental education and annual household income. The ORs for each C allele in the stratification of birth weight and childhood environmental factors were adjusted for age, gender, puberty, gestational age, and BMI. *P* values for the gene-environment interaction on the MONW phenotype were adjusted for the above variables.

childhood environmental factors could further modify the impact of intrauterine environment on *CDKAL1* effects. A significant interaction between birthweight, childhood environments and the *CDKAL1* locus was found (*P* for interaction=0.013; Fig. 1B). Within the high birthweight strata, every C allele of the rs2206734 variant was linked to an 80% reduction in MONW risk in children with favorable childhood environments, while in children with unfavorable childhood environments, every C allele of rs220673 was associated with only a 35% reduced risk of MONW, a difference of only marginal significance (OR per C allele=0.20, 95% CI=0.09–0.44 vs OR=0.65, 95% CI=0.41–1.03). These findings suggest that a favorable childhood environment can further amplify the protective effect of the *CDKAL1* locus in children with high birthweight. Within the strata of intermediate and low birthweight, however, the contribution of C alleles

within rs2206734 was not significant in both favorable and unfavorable childhood environments (OR=1.08, 95% CI=0.75–1.55 and OR=0.85, 95% CI=0.69–1.05). This finding suggests that improving childhood environmental factors does not amplify the effect of the *CDKAL1* locus on MONW risk in intermediate and low birthweight individuals.

Discussion

In this cohort of Chinese children, we found that lifestyle and socioeconomic factors, as well as low birthweight, were independent risk factors for the MONW phenotype. In addition, we identified an association in this population between the MONW phenotype and SNPs at the obesity susceptibility locus (*CDKAL1*) within the gene encoding cyclin dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1. Moreover, we provide evidence that the protective effects of the *CDKAL1*-rs2206734 gene variant could be strengthened by a favorable intrauterine nutritional environment and amplified further by the combined impact of protective childhood environmental factors like physical activity, fruit consumption, advanced parental education and high household income. Our study provides the first evidence of gene–environment interactions predisposing to the MONW phenotype, suggesting the importance of identifying genetically susceptible individuals for targeted fetal and childhood environmental modifications to achieve disease prevention.

In this study, we were able to take into account several potential risk factors for MONW based on our large well-characterized cohort of children. We found not only behavioral factors such as physical activity and fruit consumption, but also socioeconomic components including parental education and household income have an independent impact on childhood MONW status. Consistent with our results, a prior pediatric study demonstrated an association between physical activity and metabolic health in children with normal weight and obesity (18). Furthermore, socioeconomic status has been shown to influence food choices, access to health care, and exposure to environmental stressors that directly impact metabolic health (11, 19, 20), which emphasizes the role of childhood environment as a modifiable risk factor for MONW.

In addition to postnatal environment, low birthweight strongly predicted childhood MONW status in our study. Birthweight is a reflection of the intrauterine

Clinical Study	G Li and others	Nature vs nurture in metabolic health	182:4	400
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nutritional environment, which may permanently affect the function of various organ systems, including the endocrine pancreas and adipose tissue, through epigenetic modifications which thereby impact the risk of subsequent development of cardiovascular disease (21, 22). Furthermore, our research evinces for the first time that low (≤ 25 th percentile) birthweight contributes to the development of childhood MONW, while high (> 75 th percentile) birthweight was more likely to impart a protective effect against metabolic disorders in children who subsequently assume a normal weight. It has been reported that children with high birthweight exhibit higher peripheral versus central fat distribution, and higher insulin sensitivity than their normal birthweight peers. This occurs in spite of similar percentages of total body fat mass (23). Meanwhile, low birthweight has been associated with reduced lean tissue mass and a greater relative body fat, particularly the truncal fat accumulation which may in turn lead to insulin resistance and adverse metabolism (24). It is possible that an unfavorable intrauterine environment may generate the adaptation of adipose tissue's function and distribution to favor MONW. However, the mechanisms underlying this adaptation warrant further investigation.

To date, limited data exist with regard to genetic predisposition to MONW (25). Although a limited number of genetic variants (20 SNPs) were examined, our study presents the first convincing evidence that *CDKAL1* rs2206734 is associated with protection from the MONW phenotype. This is consistent with previous reports of the C allele of *CDKAL1* rs2206734 being associated with higher BMI but reduced risk of T2D in East Asian adults (26). Similarly, recent studies have identified common genetic variants, including that near insulin receptor substrate 1 (*IRS1*), where specific alleles were associated with elevated BMI and body fat percentage, but reduced cardiometabolic risk (4, 27, 28). Given the well-established association between obesity and cardiovascular risk, these results seem counterintuitive. However, these 'high BMI alleles' have all been associated with disproportionate s.c.–to–visceral adipose tissue ratios, gynoid fat distribution, and greater insulin sensitivity (4, 28). These loci may affect metabolic health by regulating the capacity of s.c. adipose tissue to store excess lipid and support the 'adipose tissue expandability' hypothesis which states that when adipose tissue is unable to undergo healthy hyperplasia and hypertrophy to cope with an increased demand for lipid storage, the excess lipid accumulates in the visceral compartment, thus predisposing to insulin resistance and cardiometabolic risk. It is interesting to note that *CDKAL1*

is a methylthiotransferase that enhances translational efficiency and is broadly expressed in metabolic tissues, including adipose tissue and pancreatic β cells (29, 30). Thus, *CDKAL1* rs2206734 may protect against MONW through pathways of adipose tissue expansion or β cell function by influencing translation, given that the MONW protective C allele was also associated with lower TG ($P=0.011$) and glucose levels ($P=0.005$) in our study (Supplementary Table 2). In contrast to a previous study of 62,245 East Asian adults (26), but consistent with a study in children from Greece (31), the observed relationship between rs2206734 and metabolic disorders was not accompanied by an association with BMI in our children, although we had more than 80% power to detect a per-allele effect on BMI of 0.035 and 0.039 kg/m² with allele frequency of 43% in an additive model. This contradiction may result from the genetic association with body fat being less penetrant in children. In this case, the associated mechanisms in children warrant further study.

Another novel and important finding of our study is that the genetic association of *CDKAL1* rs2206734 with the MONW phenotype was altered by birthweight. Given that another locus at *CDKAL1*, i.e. rs7756992, has been recognized as a potential link between low birthweight and T2D according to the fetal insulin hypothesis (32, 33), we speculate that the significant association between *CDKAL1* rs2206734 and the MONW phenotype might be due to its association with birthweight, although these two variants are not in perfect linkage disequilibrium with one another ($r^2_{\text{CHB}}=0.620$). However, there was no direct association between *CDKAL1* rs2206734 and birthweight in our study; but rather, an interaction between the rs2206734 locus and birthweight was discovered. Interestingly, a stratified analysis according to birthweight categories revealed that the protective association between the rs2206734 locus and MONW was most pronounced in children with high birthweight, while this association was lost in children with low and intermediate birthweight. This suggests that exposure to an adverse intrauterine environment may contribute to the loss of the protective cardiometabolic effect at this locus, whereas maternal and/or placental factors which increase birthweight may be necessary for this gene variant to exert its cardio-protective effects.

In addition to the effects beginning *in utero*, the effects of environmental exposures on gene expression were perceived to extend through childhood development, but the mechanisms responsible for these complex interactions between genetic and environmental factors remain unclear (34). Our study revealed that the effect of high birthweight to strengthen the protective influence of

the *CDKAL1* locus on MONW appears to be augmented by a favorable childhood (postnatal) environment, whereas an adverse childhood environment could weaken the protective effect at *CDKAL1* by more than 50%. Given that *CDKAL1* is broadly expressed in adipose tissue, the possibility that epigenetic regulation of adipose tissue function during the adaptation to intrauterine and childhood environments may be involved in these gene-environment interactions warrants further investigation. Nonetheless, our study demonstrates that modification of the intrauterine and early childhood environments has important implications for prevention of MONW, particularly in genetically susceptible individuals.

Our study can claim several strengths. The principal strength of our analysis was the availability of a large, well-characterized cohort of Chinese children as it afforded an opportunity to examine not only the participants but also the combined effects of numerous environmental factors. The collective examination of genetic and environmental factors (as well as the interactions thereof) is another strength. Notwithstanding, several limitations in our study should be noted. Firstly, our examination encompassed only a limited number of genetic variants associated with adult BMI or glucose metabolism. Our novel finding that the BMI-related allele in *CDKAL1* showed a protective association against MONW, along with findings from recent studies in adults (4, 25), suggests that future studies in children should focus on genes relevant to adipose tissue expandability and storage. Secondly, the lack of consensus on the definition of MONW may result in difficulty comparing our results with those of previous and subsequent pediatric studies. Thirdly, because Asians tend to have cardiometabolic abnormalities at relatively low levels of BMI, our study may have more significant public health implications in Asian populations than other ethnicities. Finally, due to the cross-sectional design of our study, it is not possible to establish causality. Our ongoing prospective study will more definitively elucidate the role of these various factors in the development and persistence of MONW from childhood onward.

In conclusion, our study demonstrates that early environmental exposures, including lifestyle and socio-economic factors during childhood, as well as birthweight, play important roles in determining the MONW phenotype. Our findings furthermore suggest that the protective *CDKAL1* rs2206734 allele may be a novel and promising locus for future research into the genetic origins of cardiovascular disease, and a deeper understanding of the MONW phenotype in particular. Specifically, the

observation that the genetic protection at the *CDKAL1* locus could be mitigated by birthweight suggests the possibility of an epigenetic mechanism in determining the MONW phenotype. Furthermore, our finding that favorable socioeconomic status could strengthen this genetically determined protective effect suggests that environmental modifications may be disproportionately effective at preventing cardiovascular disease in individuals with the protective *CDKAL1* rs2206734 allele. Given that the prevention and detection of metabolic abnormalities is largely unnoticed in normal-weight individuals until the onset of a chronic diseases like T2D, our study provides new insights into potential prevention strategies for the MONW phenotype, especially in genetically susceptible individuals. Future work is needed to elucidate the genetic and epigenetic mechanisms at work to determine the increased risk of cardiovascular disease in metabolically obese, normal weight individuals.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-0755>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

M Li, J Mi and S Gao contributed equally to this study.

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Clinical Study	G Li and others	Nature vs nurture in metabolic health	182:4	403
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