Prenatal metabolomic profiles mediate the effect of maternal obesity on early childhood growth trajectories and obesity risk: the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study

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ABSTRACT

Background: Maternal prepregnancy obesity is an important risk factor for offspring obesity, which may partially operate through prenatal programming mechanisms.

Objectives: The study aimed to systematically identify prenatal metabolomic profiles mediating the intergenerational transmission of obesity.

Methods: We included 450 African-American mother-child pairs from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study pregnancy cohort. LC-MS was used to conduct metabolomic profiling on maternal plasma samples of the second trimester. The childhood growth outcomes of interest included BMI trajectories from birth to age 4 y (rising-high-, moderate-, and low-BMI trajectories) as well as overweight/obesity (OWO) risk at age 4 y. Mediation analysis was conducted to identify metabolite mediators linking maternal OWO and childhood growth outcomes. The potential causal effects of maternal OWO on metabolite mediators were examined using the Mendelian randomization (MR) method.

Results: Among the 880 metabolites detected in the maternal plasma during pregnancy, 14 and 11 metabolites significantly mediated the effects of maternal prepregnancy OWO on childhood BMI trajectories and the OWO risk at age 4 y, respectively, and 5 mediated both outcomes. The MR analysis suggested 6 of the 20 prenatal metabolite mediators might be causally influenced by maternal prepregnancy OWO, most of which are from the pathways related to the metabolism of amino acids (hydroxyasparagine, glutamate, and homocitrulline), sterols (campesterol), and nucleotides (N2,N2-dimethylguanosine).

Conclusions: Our study provides further evidence that prenatal metabolomic profiles might mediate the effect of maternal OWO on early childhood growth trajectories and OWO risk in offspring. The metabolic pathways, including identified metabolite mediators, might provide novel intervention targets for preventing the intrauterine development of obesity in the offspring of mothers with obesity. *Am J Clin Nutr* 2022;116:1343–1353.

Keywords: childhood obesity, growth trajectory, maternal obesity, mediation, metabolomics

Introduction

The escalating epidemic of childhood obesity remains an important challenge in the USA. One-third of children and adolescents aged 2–19 y had overweight (16.1%) or obesity

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Supplemental Figure 1 and Supplemental Tables 1–7 are available from the "Supplementary data" link in the online posting of the article and from the same link in the table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early Childhood; FDR, false discovery rate; IVW, inverse-variance weighted analysis; MR, Mendelian randomization; OWO, overweight/obesity; QC, quality control; SNP, single nucleotide polymorphism.

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(19.3%) in 2017–2018 (1). Children born to mothers with obesity are more likely to develop obesity in childhood (2–5). This intergenerational transmission may partly explain the exacerbated epidemic of obesity. Although previous studies have identified some prenatal factors, such as gestational weight gain and placental weight (6–9), that may mediate the association of maternal obesity with child obesity, the exact intrauterine molecular mechanisms are still not well characterized. Some obesity-related metabolic factors, such as gestational diabetes (10), elevated circulating triglycerides (11, 12), and leptin (13), have been associated with higher birth weight and adiposity in offspring. However, their relative contributions to mediating the effect of maternal obesity on child growth are not yet clarified. Additionally, a previous study did not observe significant mediation effects of gestational diabetes, which was highly related to maternal obesity, on the association between maternal obesity and fetal growth, suggesting other effects of maternal obesity on fetal growth beyond the glucose/insulin pathway, and beyond other known obesity-related pathways, may exist (8). Thus, it is of crucial importance to untangle the biological mechanisms of the intergenerational cycle of obesity to develop effective methods to prevent its early development.

Metabolomics, a global chemical phenotyping approach that quantitatively measures a wide array of endogenous and exogenous features, can provide a comprehensive snapshot of metabolic status at a given time point (14, 15). Metabolic biomarkers, the ultimate step in the cellular response and the key links between genes and phenotypes, may act as mediators in the intergenerational associations of obesity. Only 1 study examined 23 acylcarnitine species, intermediates of fatty acid oxidation, of maternal plasma samples collected soon after delivery and identified that β-hydroxybutyryl-carnitine mediated 27% of the effect of maternal prepregnancy overweight/obesity (OWO) on child OWO (16). More studies are required to elucidate the mediation effects of the global metabolomics profiles instead of candidate metabolites during pregnancy.

We have observed significant associations between maternal prepregnancy OWO and early childhood BMI trajectories and OWO risk in the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study (3). The BMI trajectories, integrating repeated measurements during early childhood, may provide additional prediction values for obesity risk in later life beyond the measurement at a single time (17). In this study, we conducted global/untargeted metabolomic profiling on the prenatal plasma samples from the CANDLE mothers to better understand the molecular mechanisms, overcome the limitations of previous studies, and maximize our ability to identify novel prenatal metabolite mediators for the intergenerational transmission of obesity.

Methods

Study cohort

All study participants were from the CANDLE Study, which is a prospective pregnancy cohort of mother-child dyads in Shelby County, Tennessee (Memphis and surrounding areas). Accrued between 2006 and 2011, a total of 1503 participants aged 16–40 y during their second trimester of pregnancy with 1455 live births were enrolled in the CANDLE Study (3, 18, 19). Primarily composed of African-American (65.5%) and European-American participants (32.4%), the CANDLE sample demographically represents Shelby County, with many families experiencing high levels of socioeconomic adversity. Considering the high prevalence of obesity among African-American subjects and less metabolomics studies have been conducted in this population, we randomly selected and included 450 African-American mother-child pairs in this substudy (20). The participant selection flow chart has been published previously (**Supplemental Figure 1**) (21). The characteristics of this sample were similar to the full sample ($N = 953$) of African-American mothers and children in the CANDLE Study (21). The CANDLE Study was conducted in accordance with the Helsinki Declaration and approved by the Institutional Review Board of the University of Tennessee Health Science Center. Informed consent was given by participants aged 18 y or older, whereas assent was given by those <18 y and consent was provided by their legally authorized representative prior to enrollment.

Maternal measures

The mother's sociodemographic information (age, race, education, insurance type, and marital status), health behaviors (cigarette smoking and alcohol use during pregnancy), parity, and medical history were collected by self-administered questionnaires at enrollment. Self-reported height and weight before pregnancy were collected at enrollment and used to calculate prepregnancy BMI as weight (in kgs) divided by the square of height (in meters). Prepregnancy overweight and obesity were defined as 25 kg/m^2 $\leq$ BMI <30, and BMI $\geq$30, respectively.

Child measures

The children's birth weight and length were extracted from medical charts by research assistants. The body weight and length/height were also measured at each annual visit until the age of 4 y using the methods guided by the NHANES protocol (22). The sex- and age-specific BMI z-scores for each child were calculated based on the WHO growth standards (<2 y) and the CDC growth charts ($\geq$2 y) as recommended by the CDC (23). Information on breastfeeding was collected at the 4-y visit.

Childhood growth outcomes

Both BMI trajectories from birth to age 4 y and OWO status at age 4 y were the early childhood growth outcomes of interest in this study. *1*) Three BMI z-score trajectories (rising-high-, moderate-, and low-BMI) among the 450 children were identified using the latent class growth modeling approach as reported and published previously (3, 21). Briefly, children in the rising-high-BMI trajectory were characterized as having an average birth size followed by a rapid weight gain during the first year and staying at a stable high BMI level until the age of 4 y. Those in the moderate-BMI trajectory had an average size at birth, experienced a moderate growth rate, and stayed at an average level of BMI thereafter. The low-BMI trajectory had a relatively lower birth size and rapid growth during the first year but still stayed at a relatively lower BMI level. *2*) Childhood overweight and obesity at age 4 y were defined according to CDC criteria

TABLE 1 Characteristics of the CANDLE mother-child dyads included in the current study

Variables	Mean ± SD or percentage ($N = 450$)
Mother	
Maternal age, y	24.5 ± 5.1
Education (≤12 y), %	76.4
Marital status (single), %	62.7
Insurance (Medicaid or Medicare), %	80.0
Smoking during pregnancy, %	9.1
Alcohol use during pregnancy, %	5.8
Parity (primiparous), %	27.1
Prepregnancy BMI, kg/m^2	28.3 ± 8.3
Prepregnancy overweight, %	24.2
Prepregnancy obesity, %	33.6
Prepregnancy overweight/obesity, %	57.8
Gestational weight gain, kg	14.4 ± 8.3
Gestational diabetes mellitus, %	4.5
Child	
Gestational age at birth, wk	38.5 ± 2.3
Male, %	51.8
Birth weight, kg	3.1 ± 0.6
Birth length, cm	49.4 ± 3.4
Ever breastfed (yes), %	47.2
BMI-*z* at birth	−0.7 ± 1.4
BMI-*z* at age 1 y	0.7 ± 1.2
BMI-*z* at age 2 y	0.2 ± 1.2
BMI-*z* at age 3 y	0.3 ± 1.3
BMI-*z* at age 4 y	0.5 ± 1.2
Overweight at age 4 y, %	13.1
Obesity at age 4 y, %	15.5
Overweight/obesity at age 4 y, %	28.6
Rising-high-BMI trajectory, %	9.8
Moderate-BMI trajectory, %	68.2
Low-BMI trajectory, %	22.0

CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early Childhood Study.

(24). Childhood overweight was defined as a BMI at or above the 85th percentile and below the 95th percentile, and obesity was defined as a BMI at or above the 95th percentile for children of the same age and sex.

Metabolomics analysis

Maternal plasma samples collected in the second trimester (at recruitment) and stored at –80°C were used to conduct prenatal metabolomic profiling. The untargeted metabolomics analysis was performed using the Metabolon Discovery HD4TM Platform (Metabolon Inc.), which includes 4 UHPLC-MS methods; further details of metabolomics analysis have been previously reported (21). A total of 949 metabolites with known structural identity (named biochemicals) were identified in the study samples. After excluding 69 metabolites with missing/below-the-detection-limit >80% of the samples, 880 metabolites were included in the present study.

Genome-wide genotyping

Among the 450 CANDLE mothers, 351 had available genotype data obtained from the Affymetrix Axiom® Genome-Wide AFR 1 Array Set (Affymetrix) after the quality control (QC) procedures were performed, including removing individuals with sex discordance, high genotype missing rates (>3.0%), cryptic relatedness (identity by descent >0.1875), or a very high or low heterozygosity rate (> mean+3SD or < mean–3SD). We also excluded single nucleotide polymorphisms (SNPs) with minor allele frequencies <0.01, genotype call rates <95%, or Hardy–Weinberg equilibrium testing P values $<10^{-6}$. A total of 802,649 autosomal SNPs passed QC. Principal component analysis was performed using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (25), and the first 10 principal components were calculated for subsequent analysis in an attempt to correct for the potential genetic structure of the study subjects.

Statistical analysis

We imputed the missing values for the metabolites with missing rates <20% using the K-nearest neighbor imputation method implemented in the R package "impute." After imputation, the values were natural log-transformed, followed by median normalization and auto-scaling using the R package "specmine" (26). For metabolites with missing rates between 20% and 80%, the abundance of metabolites was coded as: missing values were coded as 0; values below the median of the nonmissing values were coded as 1; and values above the median of the nonmissing values were coded as 2.

TABLE 2 Significant metabolite mediators for the association between maternal prepregnancy OWO and early childhood rising-high-BMI trajectory

Metabolite	Class	Pathway	Associations between prepregnancy OWO and prenatal metabolites		Associations between prenatal metabolites and childhood rising-high-BMI trajectory		Mediation of prenatal metabolites		
			β (SE)	*P* value	OR (95% CI)[1]	*P* value	Mediation effect (95% CI)	Mediation proportion[2] (95% CI)	*P* value
Hydroxyasparagine	Amino acid	Alanine and aspartate metabolism	0.31 (0.03)	2.03×10^{-18}	4.69 (1.92, 11.50)	7.20×10^{-4}	0.0276 (0.0042, 0.0546)	37.2% (3.2%, 172.2%)	0.0232[4]
4-Hydroxyglutamate	Amino acid	Glutamate metabolism	0.31 (0.10)	2.65×10^{-3}	1.47 (1.10, 1.96)	9.00×10^{-3}	0.0085 (0.0007, 0.0200)	10.8% (0.7%, 47.3%)	0.0258[4]
Glutamate	Amino acid	Glutamate metabolism	0.20 (0.05)	2.04×10^{-5}	2.62 (1.34, 5.12)	4.90×10^{-3}	0.0116 (0.0007, 0.0260)	15.2% (−0.1%, 67.1%)	0.0376[4]
Homocitrulline	Amino acid	Urea cycle; arginine and proline metabolism	−0.26 (0.09)	2.45×10^{-3}	0.62 (0.43, 0.90)	1.10×10^{-2}	0.0082 (0.0003, 0.0200)	10.8% (−;0.1%, 56.6%)	0.0372[4]
FAD	Flavin nucleotide	Riboflavin metabolism	−0.16 (0.07)	1.27×10^{-2}	0.51 (0.31, 0.82)	6.10×10^{-3}	0.0084 (0.0008, 0.0198)	10.5% (0.7%, 46.1%)	0.0238[4]
Carotene diol (3)	Carotenoid	Vitamin A metabolism	−0.27 (0.08)	6.04×10^{-4}	0.56 (0.38, 0.83)	4.20×10^{-3}	0.0113 (0.0021, 0.0245)	14.9% (2.0%, 69.0%)	0.0104[4]
Carotene diol (2)	Carotenoid	Vitamin A metabolism	−0.27 (0.07)	2.15×10^{-4}	0.54 (0.36, 0.81)	3.20×10^{-3}	0.0109 (0.0018, 0.0237)	14.6% (1.6%, 71.2%)	0.0132[4]
Glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Lipid	Hexosylceramides	−0.20 (0.06)	1.11×10^{-3}	0.54 (0.32, 0.90)	1.90×10^{-2}	0.0087 (0.0004, 0.0207)	10.8% (0.1%, 47.9%)	0.0386[4]
1,2-Dilinoleoyl-GPC (18:2/18:2)	Lipid	Phosphatidylcholine	−0.22 (0.04)	2.73×10^{-7}	0.41 (0.20, 0.82)	1.20×10^{-2}	0.0136 (0.0011, 0.0295)	18.2% (0.5%, 88.8%)	0.0332[4]
1-Stearoyl-2-linoleoyl-GPI (18:0/18:2)	Lipid	Phosphatidylinositol	−0.15 (0.04)	1.14×10^{-4}	0.31 (0.14, 0.68)	3.40×10^{-3}	0.0130 (0.0029, 0.0271)	17.0% (3.0%, 74.4%)	0.0076[4]
1-Stearoyl-2-oleoyl-GPI (18:0/18:1)	Lipid	Phosphatidylinositol	−0.28 (0.07)	2.46×10^{-5}	0.56 (0.36, 0.87)	1.10×10^{-2}	0.0113 (0.0011, 0.0251)	15.2% (0.7%, 76.7%)	0.0274[4]
Campesterol	Lipid	Sterol	−0.43 (0.10)	3.18×10^{-5}	0.55 (0.41, 0.74)	1.00×10^{-4}	0.0183 (0.0067, 0.0340)	24.5% (7.6%, 102.3%)	0.0001[3]
β-Sitosterol	Lipid	Sterol	−0.34 (0.11)	1.61×10^{-3}	0.54 (0.40, 0.72)	2.70×10^{-5}	0.0154 (0.0046, 0.0298)	20.8% (5.7%, 88.7%)	0.0016[3]
2,6-Dihydroxybenzoic acid	Organic acid	Xenobiotics	−0.39 (0.11)	7.50×10^{-4}	0.66 (0.51, 0.87)	3.00×10^{-3}	0.0106 (0.0018, 0.0235)	13.7% (1.8%, 60.9%)	0.0128[4]

OWO, overweight/obesity.

[1]Associated with a 1-SD increase in the abundance of metabolites.

[2]The mediation proportion is calculated as the ratio of the mediation effect of a metabolite mediator and the total effect of maternal OWO on early childhood rising-high-BMI trajectory.

[3]False discovery rates <0.05.

[4]False discovery rates <0.15.

All analysis adjusted for maternal age, education, insurance type, alcohol intake, and tobacco use during pregnancy and parity.

TABLE 3 Significant metabolite mediators for the association between maternal prepregnancy overweight/obesity and child obesity at age 4 y

Metabolite	Class	Pathway	Associations between prepregnancy OWO and prenatal metabolites		Associations between prenatal metabolites and child obesity at age 4 y		Mediation of prenatal metabolites		
			β (SE)	*P* value	OR (95% CI)[1]	*P* value	Mediation effect (95% CI)	Mediation proportion[2] (95% CI)	*P* value
Hydroxyasparagine	Amino acid	Alanine and aspartate metabolism	0.31 (0.03)	2.03×10^{-18}	3.02 (1.54, 5.92)	1.30×10^{-3}	0.0413 (0.0020, 0.0829)	29.9% (1.0%, 105.8%)	0.0406[4]
4-Hydroxyglutamate	Amino acid	Glutamate metabolism	0.31 (0.10)	2.65×10^{-3}	1.4 (1.13, 1.73)	2.30×10^{-3}	0.0168 (0.0020, 0.0376)	11.6% (1.3%, 41.1%)	0.0182[4]
FAD	Flavin nucleotide	Riboflavin metabolism	− 0.16 (0.07)	1.27×10^{-2}	0.53 (0.37, 0.76)	4.90×10^{-4}	0.0198 (0.0036, 0.0424)	13.9% (2.6%, 47.0%)	0.0080[4]
Androsterone sulfate	Lipid	Androgenic steroids	− 0.47 (0.11)	2.11×10^{-5}	0.73 (0.60, 0.90)	2.40×10^{-3}	0.0249 (0.0053, 0.0503)	17.6% (3.4%, 59.2%)	0.0082[4]
Epiandrosterone sulfate	Lipid	Androgenic steroids	− 0.45 (0.12)	1.27×10^{-4}	0.77 (0.64, 0.93)	7.00×10^{-3}	0.0185 (0.0023, 0.0407)	12.9% (1.5%, 46.8%)	0.0204[4]
Androstenediol (3α, 17α) monosulfate (3)	Lipid	Androgenic steroids	− 0.43 (0.11)	6.60×10^{-5}	0.77 (0.63, 0.94)	1.20×10^{-2}	0.0183 (0.0011, 0.0409)	12.9% (0.6%, 47.6%)	0.0358[4]
Undecenoylcarnitine (C11:1)	Lipid	Fatty acid metabolism	0.39 (0.09)	1.13×10^{-5}	1.49 (1.15, 1.94)	2.80×10^{-3}	0.0271 (0.0046, 0.0545)	19.2% (2.8%, 64.2%)	0.0180[4]
Campesterol	Lipid	Sterol	− 0.43 (0.10)	3.18×10^{-5}	0.65 (0.52, 0.81)	1.80×10^{-4}	0.0260 (0.0072, 0.0508)	18.5% (5.0%, 59.7%)	0.0014[3]
β-Sitosterol	Lipid	Sterol	− 0.34 (0.11)	1.61×10^{-3}	0.64 (0.52, 0.79)	5.20×10^{-5}	0.0208 (0.0031, 0.0442)	14.7% (2.4%, 48.2%)	0.0190[4]
N2,N2-Dimethylguanosine	Nucleotide	Purine metabolism, guanine containing	0.25 (0.03)	3.45×10^{-14}	3.1 (1.52, 6.35)	2.00×10^{-3}	0.0383 (0.0064, 0.0734)	27.4% (4.0%, 89.7%)	0.0210[4]
(S)-a-Amino-ω-caprolactam	Amino acid derivative	Xenobiotics	0.19 (0.03)	7.92×10^{-8}	2.39 (1.22, 4.68)	1.10×10^{-2}	0.0224 (0.0002, 0.0492)	16.0% (−0.1%, 57.6%)	0.0468[4]

OWO, overweight/obesity.

[1]Associated with 1-SD increase in the abundance of a metabolite.

[2]The mediation proportion is calculated as the ratio of the mediation effect of a metabolite mediator and the total effect of maternal OWO on child obesity at age 4 y.

[3]False discovery rates <0.05.

[4]False discovery rates <0.15.

All analysis adjusted for maternal age, education, insurance type, alcohol intake, and tobacco use during pregnancy and parity.

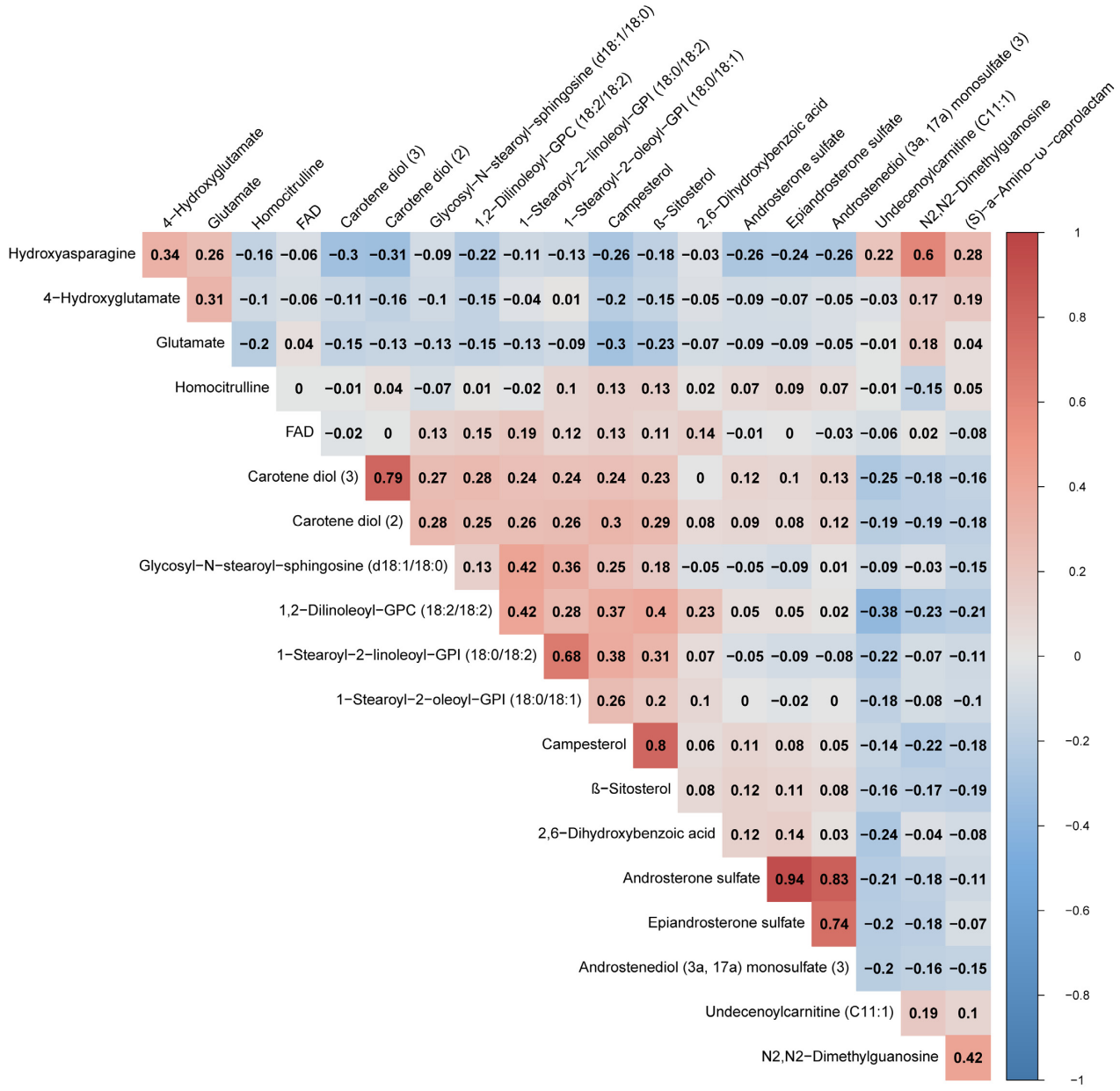

FIGURE 1 Pairwise correlation coefficients among the potential prenatal metabolite mediators ($n = 450$).

The associations of maternal prepregnancy OWO and childhood growth outcomes were confirmed using logistic regression. The mediation analysis usually needs to satisfy the following assumptions: *1*) a significant association between the mediator and the exposure, and *2*) a significant association between the mediator and the health outcome (27). A prescreening process was performed to select prenatal metabolites associated with both prepregnancy OWO and childhood growth outcomes. The associations between prepregnancy OWO and prenatal metabolites were examined using linear regression models for metabolites with missing rates <20% (continuous Y) and logistic regressions for those with missing rates ≥20% (ordinal Y). The associations between prenatal metabolites and childhood growth trajectories (the rising-high-BMI trajectory compared with the combined moderate-BMI and low-BMI trajectories) and OWO risk at age 4 y were examined using logistic regression models. Potential confounding factors, including maternal demographic (age), socioeconomic (education and insurance type), health behaviors (alcohol drinking and tobacco use during pregnancy), and reproductive history (parity), were adjusted in these association analyses. The metabolites significantly associated with prepregnancy OWO and either childhood growth outcome ($P < 0.05$) were included in the mediation analysis. The mediation effects of prenatal metabolites for the associations between prepregnancy OWO and childhood growth outcomes were estimated using the R package "mediation," with CIs estimated using the quasi-Bayesian Monte Carlo method with 10,000 simulations (28). In this mediation analysis method, the

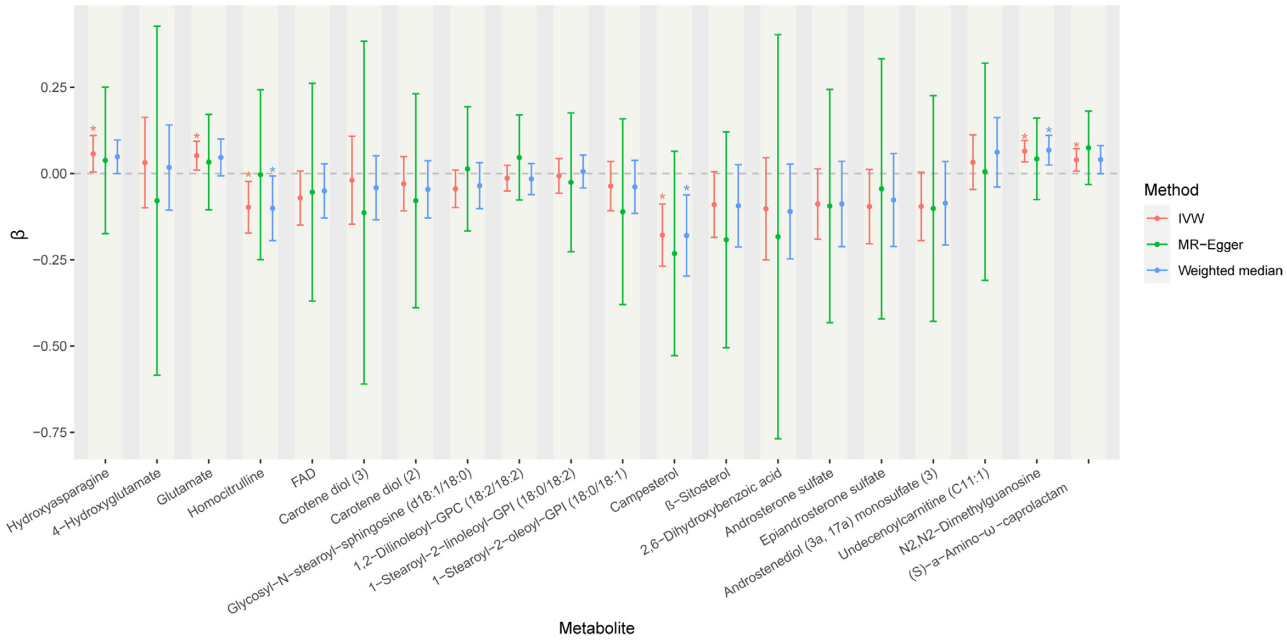

FIGURE 2 The results of Mendelian randomization (MR) ($n = 450$). $*$ indicates $P < 0.05$. IVW, inverse-variance weighted analysis.

total (T) effect of exposure on outcome is decomposed into a sum of a direct (D) effect of exposure on outcome and an indirect (I) effect of exposure on outcome via the mediator, the indirect effect is also known as a mediation effect. The mediation proportion is defined as the proportion of the effect of exposure on outcome mediated through the mediator and calculated as the ratio of the indirect (I) effect and the total (T) effect (mediation proportion $= \frac{I}{T} = \frac{I}{I+D}$) (29). The mediation proportion reflects the magnitude of the mediation effect, which could be over 100% if the indirect and direct effects are in opposite directions. The covariates mentioned above were also included in the mediation analysis. The false discovery rate (FDR) method was used to adjust for multiple testing for the metabolites that passed the prescreening and were examined for mediation effects. Gestational weight gain, gestational diabetes mellitus, birth weight, and gestational age at birth were not adjusted for because they may be on the

TABLE 4 Mendelian randomization analysis results of the IVW method

Metabolite	β (SE)	P value
Hydroxyasparagine	0.057 (0.027)	3.50×10^{-2}[a]
4-Hydroxyglutamate	0.032 (0.067)	6.40×10^{-1}
Glutamate	0.052 (0.021)	1.60×10^{-2}[b]
Homocitrulline	−0.098 (0.038)	1.00×10^{-2}[b]
FAD	−0.071 (0.040)	7.50×10^{-2}
Carotene diol (3)	−0.020 (0.065)	7.60×10^{-1}
Carotene diol (2)	−0.030 (0.040)	4.60×10^{-1}
Glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	−0.044 (0.028)	1.10×10^{-1}
1,2-Dilinoleoyl-GPC (18:2/18:2)	−0.013 (0.019)	4.80×10^{-1}
1-Stearoyl-2-linoleoyl-GPI (18:0/18:2)	−0.007 (0.026)	7.90×10^{-1}
1-Stearoyl-2-oleoyl-GPI (18:0/18:1)	−0.037 (0.037)	3.20×10^{-1}
Campesterol	−0.179 (0.046)	1.00×10^{-4}[b]
β-Sitosterol	−0.090 (0.049)	6.30×10^{-2}
2,6-Dihydroxybenzoic acid	−0.102 (0.075)	1.80×10^{-1}
Androsterone sulfate	−0.089 (0.052)	8.90×10^{-2}
Epiandrosterone sulfate	−0.096 (0.055)	8.10×10^{-2}
Androstenediol (3α, 17α) monosulfate (3)	−0.095 (0.050)	5.80×10^{-2}
Undecenoylcarnitine (C11:1)	0.033 (0.040)	4.20×10^{-1}
N2,N2-Dimethylguanosine	0.065 (0.016)	4.20×10^{-5}[b]
(S)-a-Amino-ω-caprolactam	0.040 (0.016)	1.60×10^{-2}[b]

IVW, inverse-variance weighted.
[a]False discovery rates <0.10.
[b]False discovery rates <0.05. *P* values in bold refer to false discovery rates <0.10.

same pathways from prenatal metabolites to childhood growth outcomes; in other words, they may be mediators but not confounders for the effects of prenatal metabolites on childhood growth outcomes. Additionally, breastfeeding is not considered a confounding factor because it may be related to or caused by birth outcomes (e.g. breastfeeding recommended for low-birth-weight infants) and still on the same pathways from prenatal metabolites to childhood outcomes. After identifying significant prenatal metabolite mediators for prepregnancy OWO, we further examined their associations with these factors using linear regression models for continuous variables (gestational weight gain, birth weight, and gestational age at birth) and logistic regression models for categorical variables (gestational diabetes and breastfeeding) with the adjustment for the same set of covariates as those in the mediation analysis.

The potential causal relation between prepregnancy OWO and significant metabolite mediators was further examined using Mendelian randomization (MR) analysis (30). For the implementation of MR, independent genetic variants ($r^2 \leq 0.001$) associated with prepregnancy OWO (P values $< 1 \times 10^{-5}$) were selected as the instrumental variables. The inverse-variance weighted analysis (IVW), MR-Egger, and MR weighted median methods were used to conduct causal inference between prepregnancy OWO and prenatal metabolites. Because the presence of horizontal pleiotropy may bias MR estimates, the intercept that deviates from the MR-Egger method was used to examine the presence of horizontal pleiotropy (31). R packages "TwoSampleMR" and "MendelianRandomization" were used for MR analysis, which was adjusted for maternal age and the first 10 principal components. Two-sided statistical significance was used for all statistical inferences. Data analyses were performed using R (version 3.6.2, https://www.R-project.org/) and PLINK (version 1.9).

Results

Characteristics of the study subjects

The characteristics of the mothers and children included in this study are listed in **Table 1**. The average age (SD) of the mothers at recruitment was 24.5 (5.1) y, with relatively low education (76.4% of ≤12 y of education) and a high rate of being covered by Medicaid or Medicare (80.0%) during pregnancy. Nearly 60% of the mothers had overweight (24.2%) or obesity (33.6%) before pregnancy. Almost 30% of the children had overweight (13.1%) or obesity (15.5%) at age 4 y. A total of 9.8%, 68.2%, and 22.0% of the children were categorized into the rising-high-, moderate-, and low-BMI growth trajectories, respectively.

Mediation results

In the 450 CANDLE mother-child pairs, we observed significant associations between maternal prepregnancy OWO and the studied childhood growth outcomes. Compared with children of the mothers with normal weight, children of the mothers with prepregnancy OWO had 2.57 times increased risk of being classified into the rising-high-BMI trajectory group and 2.10 times increased risk of having OWO at age 4 y (**Supplemental Table 1**).

We first analyzed the associations of the 880 prenatal metabolites with prepregnancy OWO and childhood growth outcomes (**Supplemental Table 2**). We identified 37 prenatal metabolites associated with both prepregnancy OWO and childhood rising-high-BMI trajectory and 47 metabolites associated with both prepregnancy OWO and child OWO at age 4 y (P <0.05). These prenatal metabolites passed the prescreening for the mediation analysis and were included in the mediation analysis (**Supplemental Tables 3** and **4**). Among these prenatal metabolites for the mediation analysis, 14 and 11 metabolites significantly mediated the effect of prepregnancy OWO on childhood rising-high-BMI trajectory and OWO at age 4 y, respectively (P <0.05) (**Tables 2** and **3**). Of these metabolites, 5 metabolites, hydroxyasparagine, 4-hydroxyglutamate, FAD, campesterol, and β-sitosterol, mediated the effect of prepregnancy OWO on both early childhood growth outcomes. Most of the metabolite mediators belong to the chemical classes of amino acids, lipids, nucleotides, and carotenoids, and are involved in the metabolic pathways of alanine and aspartate metabolism, glutamate metabolism, riboflavin and vitamin A metabolism, androgenic steroids, and sterol metabolism. After adjusting for multiple testing (among all the metabolites included in the mediation analysis), the mediation effects of 2 metabolites (campesterol and β-sitosterol) and 1 metabolite (campesterol) were still significant for childhood BMI trajectories and OWO at age 4 y outcomes, respectively (Tables 2 and 3) (FDR <0.05). The rest of the metabolite mediators still had FDR values <0.15. **Figure 1** shows the pairwise correlations among the 20 unique metabolite mediators. Some of them were highly correlated, such as campesterol and β-sitosterol and the 3 androgenic steroids [androsterone sulfate, epiandrosterone sulfate, and androstenediol (3α, 17α) monosulfate (3)]. There were 3, 1, and 2 metabolite mediators associated with gestational weight gain, gestational diabetes, and birth weight, respectively, and no metabolite mediators associated with gestational age at birth and ever breastfed (**Supplemental Table 5**), suggesting the effects of most of the metabolite mediators on childhood growth outcomes were independent of these variables.

MR results

The selected SNP instruments for MR analysis and their associations with prepregnancy OWO and metabolite mediators are listed in **Supplemental Tables 6** and **7**, respectively. Most of the significant MR results were observed in the IVW method (**Figure 2**). MR analysis by the IVW method identified 6 prenatal metabolite mediators, hydroxyasparagine, glutamate, homocitrulline, campesterol, N2,N2-dimethylguanosine, and (S)-a-amino-ω-caprolactam, that might be causally influenced by maternal prepregnancy OWO (**Table 4**). Three of them, homocitrulline, campesterol, and N2,N2-dimethylguanosine, were also significant in the weighted median method. Although none of them showed significant results in the MR-Egger method, the effect directions for the 6 metabolites were consistent across the 3 methods (Figure 2). All P values of the intercept from the MR-Egger method were >0.05, indicating that horizontal pleiotropy was not likely to bias the MR results.

Discussion

Our study identified 20 prenatal metabolites that appeared to statistically mediate the effect of maternal OWO on early childhood growth outcomes, highlighting the potential importance of several prenatal metabolic pathways in early child development. MR analysis provided evidence supporting potential causal relations between maternal OWO and 6 prenatal metabolite mediators, which are involved in the metabolism of multiple amino acids, sterol, and purine. These findings may provide novel insights into the intrauterine mechanisms linking maternal obesity with early childhood growth and obesity risk in offspring.

Many cohort studies and large-scale meta-analyses have provided strong evidence that maternal obesity before pregnancy is an important risk factor for child obesity (32, 33). The possible mechanisms underlying this association include inherited genetic susceptibility to obesity; diet, physical activity, or other health behavior patterns shared by mothers and their children; and effects of maternal adiposity on the intrauterine environment and subsequent fetal development (34). Many genomic loci and health behaviors (e.g. physical inactivity and high energy intake) have been identified for increased risk of obesity (35, 36). However, the possible intrauterine mechanisms linking maternal obesity and child obesity are still largely unknown.

One of the most interesting findings was that prenatal plasma campesterol and β-sitosterol, the 2 most common plant sterols present in humans, which are completely derived from food, significantly mediated the effect of maternal obesity on child growth outcomes even after adjusting for multiple testing. MR analysis also supports that maternal prepregnancy OWO may influence the metabolism of campesterol. We found maternal prepregnancy OWO was associated with a decreased concentration of plasma campesterol. This finding is in line with a previous report that sterol intestinal absorption was reduced in patients with obesity and insulin resistance (37). A possible mechanism is that obesity-induced upregulated cholesterol transporters ABC G-5 and G-8 export absorbed sterols back into the intestinal lumen (37). In utero exposure to excess maternal cholesterol may cause epigenetic changes of pathways in developing organs, such as the liver, skeletal muscle, adipose tissue, brain, and pancreas, resulting in metabolic abnormalities in offspring, including obesity (38). Plant sterols share the same transport mechanisms as cholesterol and could reduce both the intestinal absorption of cholesterol and the transplacental transportation of cholesterol from the mother to the fetus (39). Therefore, the effect of prenatal campesterol on childhood growth outcomes may be through its impact on fetal exposure to cholesterol. Our study may support further investigations of the potential application of increasing maternal campesterol concentrations during pregnancy to prevent the development of obesity in offspring.

Three amino acids, hydroxyasparagine, glutamate, and homocitrulline, were identified as potential mediators and also significant in the MR analysis. Obesity might influence the metabolism of glutamate, such as the increased circulating glutamate concentration identified as a biomarker of visceral obesity (40) and the synthesis of glutamate increased in obese adipose tissue (41). Furthermore, glutamate metabolism is known to be important for the growth and development of human fetuses (42). Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and is involved in metabolic processes, such as protein synthesis, energy metabolism, and ammonia fixation. Glutamate transporters are distributed in the human placenta, regulating the exchange of glutamate between mother and fetus (42). All these lines of evidence may support, to a certain degree, the mediation role of prenatal glutamate in the intergenerational transmission of obesity. Both hydroxyasparagine and homocitrulline are metabolites of the posttranslational modification of proteins. Hydroxyasparagine is found in fibrillin which is essential for the formation of elastic fibers in connective tissue. Mutations of the coding gene of fibrillin lead to connective tissue disorders and adverse fetal cardiovascular development (43). Plasma homocitrulline has recently been reported to be negatively associated with liver fat measured by MRI in humans (44). Unfortunately, there are few studies regarding the biological functions of these 2 amino acids related to obesity and fetal development. However, our study may provide new directions for further investigations of these metabolites in the pathogenesis of obesity.

N2,N2-dimethylguanosine, related to purine metabolism, constitutes tRNA molecules and can stabilize the cloverleaf structure of the tRNA (45). It is thought to be an indicator for tRNA half-life in tissues and circulating tRNA fragments have been associated with oxidative stress (46), which is increased in obesity (47). N2,N2-dimethylguanosine is strongly correlated with BMI (48, 49) and involved in the development of type 2 diabetes (49). N2,N2-dimethylguanosine was also shown to be associated with the risk of childhood obesity (50). Our study found maternal OWO might increase prenatal plasma N2,N2-dimethylguanosine concentrations, which was further associated with increased risk of child OWO. (S)-a-amino-ω-caprolactam is another potential metabolite mediator for the impact of maternal obesity on child obesity, further supported by MR analysis, and is exogenously derived from ingestion of plant-based food sources. Unfortunately, the biological function of this metabolite is still largely unknown. Some metabolites from vitamins A [carotene diol (3) and carotene diol (2)] and B (FAD) metabolic pathways were identified as potential mediators and negatively associated with maternal and child OWO. Both vitamins A and B are crucial micronutrients for pregnant females and fetuses (51–53); however, MR analysis did not support their causal relation with prepregnancy OWO.

Our study has several strengths. First, to the best of our knowledge, this study is the first to systematically investigate the mediating role of prenatal metabolomic profiles in the association of maternal OWO with early childhood growth outcomes. Second, we used an untargeted/global metabolomics platform, a hypothesis-free approach, to maximize the study's ability to identify novel prenatal metabolite mediators. Third, a comprehensive set of potential confounding factors was controlled to satisfy the assumptions of mediation analysis, such as no unmeasured confounders of the relations among exposures, mediators, and outcomes (54), although it is difficult to prove if residual confounding still exists. Furthermore, MR analysis improved causal inference for understanding the identified associations. Finally, our study included an urban, predominantly low-income sample of African-American subjects with a high risk of obesity. Of course, caution is still needed when generalizing the findings to other populations because previous studies have shown potential racial differences in metabolic profiles, including those associated with OWO (55, 56). In addition, maternal

prepregnancy OWO status was based on self-reported BMI, which might introduce moderate misclassification (57). However, self-reported and measured prepregnancy weights were similarly associated with perinatal outcomes (58). Therefore, it is not very likely that the associations between prepregnancy OWO and prenatal metabolites have been substantially influenced by self-reported pregnancy weight measures in this study. Another potential limitation is that the MR analysis was conducted among a subgroup of the study sample with available genome-wide genotype data, potentially limiting the statistical power. However, multiple MR analysis methods were used, and no horizontal pleiotropy was observed. Finally, because there is still a lack of studies in this area, it was difficult to reach a conclusion regarding biological pathways linking prepregnancy obesity and child obesity through the identified metabolite mediators.

In conclusion, the findings of our study indicate that prenatal metabolomic profiles might mediate the effect of maternal prepregnancy obesity on early childhood growth trajectories and obesity risk in offspring. They also provided potential metabolic pathway targets during pregnancy for preventing the intergenerational transmission of obesity. Future studies are warranted to replicate these findings and further elucidate the underlying biological mechanisms of the identified metabolite mediators.

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The authors' responsibilities were as follows—QZ: contributed to the concept and design of the study; ZH, JL, DK, and QZ: contributed to metabolomics data generation; KZL, NRB, and WAM: contributed to the provision of clinical and biosamples; ZH, LH, and QZ: contributed to data analysis, result interpretation, and manuscript preparation; JHF, JCH, KZL, NRB, and WAM: provided critical review and revisions of the manuscript; QZ: had primary responsibility for final content; and all authors: reviewed and approved the final manuscript.

The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request and approval by the CANDLE Study.

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