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Maternal dietary cholesterol intake, genetic variations of cholesterol metabolism genes and the association with birth weight

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ABSTRACT: This study aimed to explore association of maternal dietary cholesterol and polymorphisms of genes coding for cholesterol metabolism pathway proteins with birth weight. A total of 1941 mother-infant pairs were included based on Tongji Birth Cohort. Dietary cholesterol was assessed by a validated food frequency questionnaire. Genotyping was performed using time-of-flight mass spectrometry. The genetic risk score (GRS) was calculated using genetic variants from 21 cholesterol metabolism-related single-nucleotide polymorphisms (SNPs). The results showed that the dietary cholesterol intake was linearly associated with the odd ratio (OR) of large-for-gestational-age (LGA) ($P = 0.012$), but nonlinearly associated with small-for-gestational-age (SGA) ($P = 0.014$). Compared to pregnant women with an egg intake of <7.0 eggs/week, participants who consumed ≥ 7 egg/week had an increased risk of LGA (OR = 1.62, 95%CI 1.00, 2.62). LDL-C mediated the association between dietary cholesterol and LGA. The variants of the *ABCA1* rs1883025 C $\rightarrow$ T showed a significant association with birth weight and LGA, and *CYP7A1* rs7833904 A $\rightarrow$ T presented a significant association with SGA. The GRS of these 21 variant SNPs was also linked to SGA and LGA, respectively. The OR was 2.13 (1.12, 4.07) in the highest categories of both dietary cholesterol and GRS compared with individuals with the lowest strata. Both dietary cholesterol intake and genetic variants of cholesterol metabolism genes are associated with risk of SGA and LGA.

Keywords: Dietary cholesterol; genetic risk score; birth weight; cholesterol metabolism related genes

1. Introduction

Birth weight is a commonly used index of evaluating fetal wellbeing, which may be affected by environmental factors and genetic factors^[1]. Studies have shown that abnormal birth weight, regardless of whether large-for-gestational-age (LGA) reflecting nutrition surplus, or small-for-gestational-age (SGA) reflecting the intrauterine growth restriction, was associated with short-term adverse outcomes (e.g. increased neonatal mortality risk) and long-term metabolic abnormalities, including type 2 diabetes mellitus (T2DM) and cardiovascular disease in later life^[2, 3]. The incidence of SGA in China was 6.4%^[4], while a survey including 12,992,558 singleton live births revealed that the incidence of LGA was 8.6% in China^[5].

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Cholesterol is an important part of the cell membrane, maintaining stability of membrane-related signal transduction pathways, and is also a precursor of bile acid and steroid hormones^[6], so a certain level of cholesterol is essential for cell growth and body development. Pregnant women are encouraged to consume cholesterol enriched foods such as eggs and meats in China. Dietary cholesterol intake increases dramatically among pregnant women^[7]. Our previous study demonstrated that higher dietary cholesterol intake and adhering to a cholesterol-rich dietary pattern during pregnancy increased the risk of gestational diabetes mellitus (GDM)^[7, 8], which is also observed in the other studies^[9–11]. GDM is a risk factor for adverse pregnancy outcomes including macrosomia, and LGA^[12]. However, studies on dietary cholesterol and birth weight are limited, only 2 prior studies showed that higher dietary cholesterol was a risk factor for developing LGA^[13, 14].

The homeostasis of cholesterol metabolism *in vivo* is affected by regulation of cholesterol synthesis, absorption and bile excretion^[15]. Whether serum cholesterol levels are sensitive to dietary cholesterol intake has remained a long-standing controversial issue. Because available evidence showed no appreciable relationship between consumption of dietary cholesterol and serum cholesterol, the 2015 Dietary Guidelines Advisory Committee commented that “cholesterol was not a nutrient of concern for overconsumption” and removed the previous restriction of limiting cholesterol intake to less than 300 mg/d^[16]. However, recent large-scale cohort studies have shown that increased dietary cholesterol was considered to raise serum total cholesterol levels (TC), and low-density lipoprotein-cholesterol (LDL-C), which suggests that serum lipid profiling can be used as an intermediate variable between dietary cholesterol and disease^[17, 18]. A prospective cohort study also found that a high-protein, high-cholesterol, and low-carbohydrate dietary pattern in pregnancy was associated with an elevated blood TC concentrations in pregnant Chinese women^[19]. Pregnancy is a unique state in which blood lipid levels physiologically increase to meet the needs of fetal growth, particularly in the mid to late stages of pregnancy. Pregnant women are prone to develop lipid metabolism disorders, but whether dietary cholesterol is related to blood lipid levels during pregnancy and thereby affects the risk of abnormal birth weight outcomes has not been reported. Cholesterol metabolism exhibits high genetic susceptibility, and recent GWAS studies have identified that single-nucleotide polymorphisms (SNPs) encoding cholesterol-metabolizing proteins such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), Niemann-Pick C1-like protein (NPC1L1), low-density lipoprotein receptor (LDLR), proprotein convertase subtilisin kexin type 9 (PCSK9), ATP-binding cassette subfamily-A transporters 1 (ABCA1), and cholesterol 7 α -hydroxylase (CYP7A1) were associated with blood cholesterol levels^[20, 21]. So far, few studies evaluated the association of cholesterol metabolism genes polymorphisms with birth weight.

Thus, the aim of the present study was to determine the association between pregnancy dietary cholesterol and genetic variations of cholesterol metabolism genes with fetal growth outcomes.

2. Materials and methods

2.1. Study design and subjects

The study population was drawn from participants of the Tongji Birth Cohort (TJBC, Wuhan, China)

study, which is an ongoing cohort. Details of the study design of TJBC have been described previously^[22]. Women were eligible if they were ≥ 18 years of age, 8-16 weeks of gestation at their first prenatal visit, singleton pregnancy, no infectious disease. All participants provided written informed consent upon recruitment. This study was approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology (No. of the ethics committee: 2021IEC[S044]).

This study adopted a prospective cohort study design. Based on the prior research^[13], the incidence of LGA in the control group (low dietary cholesterol intake group) was 9.9%, while that in the high dietary cholesterol intake group was 22.7%, with an odds ratio (OR) of 2.48. The sample size was calculated using the formula for comparing two proportions: $n = \frac{(Z_{\alpha} \sqrt{2pq} + Z_{\beta} \sqrt{p_0q_0 + p_1q_1})^2}{(p_1 - p_0)^2}$ (with two-tailed $\alpha = 0.05$, $Z_{\alpha} = 1.96$, and $1 - \beta = 0.90$, $Z_{\beta} = 1.28$). Assuming an equal allocation ratio (exposed : non-exposed = 1:1), the total sample size was estimated to be $172 \times 2 = 346$. After accounting for a 20% attrition rate, the required sample size was adjusted to $346 / (1 - 0.20) \approx 433$ participants. The TJBC participants were included in this analysis if they provide fasting blood samples. Participants who reported without birth outcomes ($n = 186$), with stillbirths or abortions ($n = 44$), and an implausible total energy intake (< 600 kcal/day or > 4000 kcal/day, $n = 9$) were excluded. The final sample size for this study was 1941 mother-infant pairs (**Fig.S1**).

2.2 Dietary assessment

Dietary intakes of participants were assessed face to face by trained interviewers, using the semi-quantitative food frequency questionnaire (FFQ) with good reliability and validity^[23]. This FFQ encompassed 61 food items across 10 categories (including cereals, meats, fish, eggs, legumes, vegetables, fruits, nuts, dairy products, and beverages), representing over 200 specific food types. Daily intakes of nutrient and energy were calculated based on the China Food Composition Database. The dietary intake of cholesterol and other nutrients (such as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA)) was adjusted by total energy using the nutrient residual method^[24].

2.3 Serum lipids levels

Serum levels of TC, triglycerides (TG), HDL-C, and LDL-C were measured using enzymatic assays by corresponding assay kits (BioSino Bio-Technology & Science Inc., Beijing, China). Non-HDL-C was calculated as TC minus HDL-C.

2.4 SNP genotyping

2.4.1 DNA extraction from samples

DNA was extracted from maternal blood clot samples using the DNA Rapid Extraction Kit (Tiangen, China). The extracted DNA samples were analyzed by using the NanoDrop2000 (Thermo Fisher Scientific Inc, USA). Samples meeting the following criteria were considered acceptable: DNA concentration: > 20 ng/ μ L; OD260/280 value: 1.6-2.2; OD260/230 value: ≥ 1.8 .

2.4.2 Gene loci information for detection

This study selected genes related to cholesterol metabolism, including *HMGCR*, which is involved in cholesterol synthesis; *NPC1L1*, which participates in cholesterol absorption in the intestine; *LDLR* and *PCSK9*,

which are involved in cholesterol uptake from the bloodstream; *ABCA1*, which is responsible for cholesterol reverse transport; and *CYP7A1*, which is related to the conversion of cholesterol into bile acids. A total of 21 SNPs of cholesterol metabolism genes were ultimately selected (**Table S1**).

2.4.3 Genotyping of SNPs

Genotyping of the target SNPs was conducted using the Sequenom MassArray platform based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The genotyping success rate for each SNP exceeded 95%. To ensure rigorous quality control, blind replicate genotyping was performed on 5% of the samples.

2.5 Outcomes

LGA was defined as an infant with birth weight above the 90th percentile for his/her gestational age and sex, whereas SGA was defined as an infant with birth weight below the 10th percentile for his/her gestational age and sex.

2.6 Calculation of genetic risk score

The genetic risk score (GRS) was calculated by combining the weighted contributions of the variant alleles, with weights determined by the strength of their association with outcomes. The GRS was calculated using the following equation: $GRS = \sum_{i=1}^{21} \ln(OR_i) * SNP_i$, where OR_i represented the effect size (odds ratio) for the corresponding SNP, and SNP_i indicated the number of risk alleles (0, 1, or 2) for each SNP. GRS-SGA was calculated according to the above method when the outcome was SGA. GRS-LGA was calculated according to the above method when the outcome was LGA.

2.7 Covariates

Information on maternal socio-demographic characteristics, including maternal age, average personal income (<5000, 5000-9999, 10000-14999, ≥ 15000 CNY/month), education years (≤ 12 , 13-15, ≥ 16 years), gravidity (1, 2, ≥ 3), primiparity (yes/no), family history of diabetes (yes/no), drinking before pregnancy (yes/no), smoking before pregnancy (yes/no), was obtained from a standardized and structured questionnaire at enrollment. Pre-pregnancy body mass index (BMI, kg/m^2) was calculated using pre-pregnancy weight and height (weight divided by the square of height) and was divided into four categories (<18.5, 18.5-23.9, 24.0-27.9, ≥ 28.0) according to the Chinese BMI cutoffs^[25]. Gestational weight gain (GWG) was calculated as the latest prenatal weight minus pre-pregnancy weight. The information of GDM was obtained from medical records.

2.8 Statistical analyses

The means $\pm$ standard deviations (SD) and median (interquartile range, IQR) were used to describe normally and non-normally distributed continuous variables respectively. Categorical variables were presented as frequencies and percentages. Comparisons of maternal characteristics were performed using one way ANOVA or non-parametric Kruskal-Wallis tests, χ^2 test for categorical variables.

Logistic regression analyses were used to evaluate the association of maternal dietary cholesterol, serum

lipid levels, and SNPs/GRS with the risk of LGA and SGA, and the results were presented as odd ratios (ORs) with 95% confidence intervals (CIs). Dietary cholesterol was assessed as either a categorical (quartile of energy-adjusted cholesterol) or a continuous variable (per 100 mg/d increase). The significance of liner trends across categories of dietary cholesterol was performed by assigning the median value for each quartile as a continuous parameter. Egg consumption was divided into two groups (<7 egg/week; ≥7 egg/week). Moreover, a restricted cubic spline model (RCS) with three knots (located at the 5th, 50th and 95th percentiles) was employed to investigate the potential dose-response association between maternal dietary cholesterol, GRS and the incidence of LGA and SGA. Generalized linear regression (GLM) models were fitted with RCS using the “rms” package in R software. Non-linearity was evaluated via Wald’s test. The linearity of associations was determined by two criteria: (1) the *P* value for the coefficient of the spline term in the non-linearity test was > 0.05, indicating no significant deviation from linearity; (2) visual inspection of the RCS curve revealed a roughly straight line across the entire exposure range, with no apparent inflection points. Lipid concentrations were natural ln transformed to meet a normal distribution for the following analysis. The generalized linear regression model was used to the correlations between dietary cholesterol and serum lipids. The partial correlation coefficients (β) mean an increase of each ln-transformed serum lipid with each additional 100 mg dietary cholesterol. A mediation analysis was performed to examine how TC or LDL-C or non-HDL-C as mediator variables affect the relationship between dietary cholesterol and LGA^[26]. Participants were classified into joint categories based on dietary cholesterol and GRS to assess their combined effect.

All analyses were conducted by using R software (version 4.2.2). A two-tailed *P* value < 0.05 was regarded as statistically significant.

3. Results

3.1 Basic characteristics of the study population

Of the 1941 women, 129 cases of SGA and 263 cases of LGA were identified, with an incidence of 6.6% and 13.5% respectively. The maternal age, pre-pregnancy BMI, GWG, TG, LDL-C of LGA mothers were higher than those of appropriate for gestational age (AGA) mothers (*P* < 0.05). SGA mothers presented lower pre-pregnancy BMI in comparison with AGA (*P* < 0.05). Compared with the AGA group, LGA group had a higher percentage of GDM and cesarean delivery, and had higher consumption of rice and wheat products, eggs, vegetables, nuts, they also had higher intake of total energy and cholesterol. No significant differences in other basic characteristics were observed (**Table 1**). The average dietary cholesterol intake of the study participants was 321.9 mg/day. Eggs were the primary cholesterol source (198.4 mg/day, 57.2%), followed by meat (49.6 mg/day, 17.4%), fish and shellfish (37.9 mg/day, 12.4%), dairy products (25.2 mg/day, 8.8%), and other foods (10.7 mg/day, 4.2%). Detailed information was presented in **Table S2** and **Fig.S2**.

Table 1 Basic characteristics of the participant

Variable	All	SGA	AGA	LGA	<i>P</i> value
n (%)	1941	129 (6.6)	1549 (79.8)	263 (13.5)	
Maternal characteristics					
Age (y)	29.6 ± 3.5	29.1 ± 3.6	29.5 ± 3.4	30.4 ± 3.8*	<0.001

Pre-pregnancy BMI (kg/m ²)	21.3 ± 3.0	20.4 ± 2.8 ⁺	21.2 ± 3.0	22.3 ± 3.1 [*]	<0.001
<18.5	307 (15.8)	35 (27.1)	250 (16.1)	22 (8.4)	
18.5-23.9	1307 (67.3)	80 (62.0)	1053 (68.0)	174 (66.2)	
24.0-27.9	257 (13.2)	9 (7.0)	198 (12.8)	50 (19.0)	
≥28.0	70 (3.6)	5 (3.9)	48 (3.1)	17 (6.5)	
GWG (kg)	15.0 ± 5.2	14.5 ± 5.1	14.8 ± 5.1	16.7 ± 5.7 [*]	<0.001
Education (y)					0.117
≤12	413 (21.3)	18 (14.0)	332 (21.4)	63 (24.0)	
13-15	600 (30.9)	46 (35.7)	467 (30.1)	87 (33.1)	
≥16	928 (47.8)	65 (50.4)	750 (48.4)	113 (43.0)	
Average personal income (CNY/month)					0.854
<5000	247 (12.7)	15 (11.6)	200 (12.9)	32 (12.2)	
5000-9999	1038 (53.5)	72 (55.8)	834 (53.8)	132 (50.2)	
10000-14999	457 (23.5)	27 (20.9)	360 (23.2)	70 (26.6)	
≥15000	153 (7.9)	10 (7.8)	120 (7.7)	23 (8.7)	
missing	46 (2.4)	5 (3.9)	35 (2.3)	6 (2.3)	
Drinking before pregnancy (yes)	105 (5.4)	5 (3.9)	87 (5.6)	13 (4.9)	0.659
Smoking before pregnancy (yes)	51 (2.6)	3 (2.3)	38 (2.5)	10 (3.8)	0.439
Gravidity					<0.001
1	1115 (57.4)	93 (72.1)	904 (58.4)	118 (44.9)	
2	530 (27.3)	25 (19.4)	417 (26.9)	88 (33.5)	
≥3	296 (15.2)	11 (8.5)	228 (14.7)	67 (21.7)	
Primiparity (yes)	1518 (78.2)	115 (89.1) ⁺	1221 (78.8)	182 (69.2) [*]	<0.001
Family history of diabetes (yes)	235 (12.1)	8 (6.2) ⁺	188 (12.1)	39 (14.8)	0.019
GDM (yes)	419 (21.6)	26 (20.2)	318 (20.5)	75 (28.5) [*]	0.013
Gestational age at blood sampling (week)	22.3 ± 4.5	22.0 ± 4.9	22.3 ± 4.5	22.6 ± 4.3	0.886
TC (mmol/L)	5.51 (4.74, 6.36)	5.40 (4.68, 6.42)	5.51 (4.73, 6.33)	5.67 (4.90, 6.55)	0.172
TG (mmol/L)	2.05 (1.49, 2.79)	2.03 (1.48, 2.89)	2.03 (1.47, 2.74)	2.38 (1.60, 3.08) [*]	0.008
HDL-C (mmol/L)	1.74 (1.33, 2.21)	1.80 (1.37, 2.31)	1.74 (1.32, 2.21)	1.75 (1.38, 2.16)	0.586
LDL-C (mmol/L)	2.74 (1.96, 3.57)	2.65 (1.91, 3.37)	2.73 (1.92, 3.57)	2.91 (2.19, 3.71) [*]	0.060
Non-HDL-C (mmol/L)	3.69 (2.83, 4.63)	3.43 (2.58, 4.55)	3.68 (2.83, 4.61)	3.89 (2.90, 4.83)	0.092
Dietary intake					
Rice and wheat products (g/d)	261.6 ± 82.0	261.5 ± 76.2	260.0 ± 80.9	275.3 ± 90.8 [*]	0.045
meats (g/d)	50.3 ± 33.2	50.9 ± 35.3	50.0 ± 33.0	51.7 ± 33.2	0.789
Fish and shellfish (g/d)	31.1 ± 27.9	27.3 ± 24.6	31.3 ± 28.2	31.9 ± 27.0	0.311
Eggs (g/d)	33.9 ± 20.3	34.8 ± 25.0	33.1 ± 19.4	38.8 ± 22.6 [*]	0.001
Beans and bean products (g/d)	7.6 ± 6.7	7.4 ± 6.5	7.5 ± 6.5	8.2 ± 7.7	0.366
Vegetables (g/d)	319.0 ± 148.8	315.0 ± 145.8	314.9 ± 147.1	349.8 ± 158.6 [*]	0.009
Fruits (g/d)	438.8 ± 229.7	437.6 ± 238.4	436.4 ± 226.1	456.3 ± 249.4	0.527
Nuts (g/d)	14.2 ± 16.8	11.5 ± 12.0	13.9 ± 15.3	17.6 ± 25.8 [*]	0.004
Milks and dairies (g/d)	196.9 ± 147.1	187.4 ± 131.4	197.1 ± 149.9	201.2 ± 135.2	0.733
Energy-adjusted nutrient intake					
Total energy (kcal/d)	2069.9 ± 509.2	2022.0 ± 483.3	2058.3 ± 502.2	2177.2 ± 558.6 [*]	0.006
Protein (g/d)	60.4 ± 7.8	59.8 ± 7.6	60.5 ± 7.8	60.4 ± 8.1	0.725
Carbohydrate (g/d)	297.6 ± 34.3	297.3 ± 38.9	297.3 ± 33.4	299.9 ± 37.7	0.627
Fat (g/d)	74.2 ± 14.4	74.6 ± 16.1	74.3 ± 14.0	73.1 ± 16.1	0.565
Cholesterol (mg/d)	321.9 ± 132.1	328.0 ± 157.3	318.2 ± 127.7	344.0 ± 144.9 [*]	0.035
Total fiber (g/d)	14.5 ± 3.5	14.5 ± 3.6	14.5 ± 3.5	14.6 ± 3.4	0.941
SFA (g/d)	16.6 ± 4.3	17.0 ± 4.8	16.6 ± 4.2	16.4 ± 4.5	0.511
MUFA(g/d)	25.0 ± 8.0	25.8 ± 8.2	25.1 ± 8.0	24.4 ± 8.1	0.290
PUFA(g/d)	23.7 ± 9.1	24.1 ± 9.4	23.6 ± 9.0	24.0 ± 9.5	0.782
Fetal characteristics					
Cesarean delivery (yes)	920 (47.6)	43 (33.3) ⁺	701 (45.5)	176 (67.4) [*]	<0.001
Gestational week of birth (week)	39.1 ± 1.3	39.2 ± 1.3	39.1 ± 1.3	38.9 ± 1.4	0.139
Boy (yes)	1024 (52.8)	69 (53.5)	815 (52.6)	140 (53.2)	0.968
Birth length (cm)	50.0 ± 1.6	48.4 ± 1.6 ⁺	49.9 ± 1.5	51.2 ± 1.4 [*]	<0.001
Birth weight (g)	3290.9 ± 432.3	2631.1 ± 292.9 ⁺	3245.0 ± 331.5	3885.3 ± 333.3 [*]	<0.001

Abbreviations: SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; BMI, body mass index; GWG, gestational weight gain; CNY, China Yuan; GDM, gestational diabetes mellitus; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; LBW, low birth weight; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

⁺ $P < 0.05$ SGA compared to AGA; ^{*} $P < 0.05$ LGA compared to AGA.

3.2 Association of pregnancy dietary cholesterol intake, serum lipids and risks of SGA and LGA

Per 100 mg/d increase in maternal dietary cholesterol was associated with an increase of 15.14 g (95%CI: 0.90, 29.38) in the infant birth weight (**Table S3**). There was a significant nonlinear relationship between dietary cholesterol and SGA (P for overall association = 0.034, P for non-linearity = 0.014) (**Fig.S3**). In the fully adjusted model, relative to the third quartile (Q3), the fourth quartile (Q4) of dietary cholesterol was associated with an increased risk of SGA (OR = 1.94, 95%CI 1.05, 3.59) (**Table 2**). The dietary cholesterol intake was linearly associated with OR of LGA (P for overall association = 0.012, P for non-linearity = 0.415) (**Fig.S3**). Relative to Q1, Q4 of dietary cholesterol was associated with a significantly increased risk of LGA (OR = 1.93, 95%CI 1.18, 3.15). Participants who consumed ≥ 7 egg/week versus those who had < 7 egg/week had an increased risk of LGA (OR = 1.62, 95%CI 1.00, 2.62) (**Table 2**).

Table 2 Associations between maternal dietary cholesterol intake, egg consumption and birth outcomes ^a.

	Quartile of energy-adjusted cholesterol intake					Per 100mg/d increase	Egg consumption (eggs/week)	
	Q1	Q2	Q3	Q4	P for trend		<7.0	≥ 7.0
SGA	31 (8.2)	26 (7.3)	18 (4.9)	34 (9.7)			94 (7.1)	15 (11.1)
Model 1	1.74 (0.96, 3.17)	1.52 (0.82, 2.82)	1.00	2.07 (1.15, 3.74)	0.752	1.06 (0.91, 1.23)	1.00	1.63 (0.92, 2.90)
Model 2	1.73 (0.92, 3.23)	1.48 (0.78, 2.80)	1.00	1.98 (1.08, 3.64)	0.921	1.06 (0.90, 1.24)	1.00	1.81 (0.98, 3.33)
Model 3	1.72 (0.92, 3.25)	1.47 (0.78, 2.79)	1.00	1.94 (1.05, 3.59)	0.980	1.03 (0.87, 1.22)	1.00	1.82 (0.98, 3.40)
LGA	36 (9.6)	54 (13.8)	45 (11.7)	59 (15.4)			167 (12.0)	27 (18.4)
Model 1	1.00	1.51 (0.96, 2.44)	1.25 (0.79, 1.98)	1.71 (1.10, 2.65)	0.040	1.16 (1.04, 1.30)	1.00	1.65 (1.05, 2.58)
Model 2	1.00	1.63 (1.01, 2.61)	1.35 (0.81, 2.24)	1.93 (1.21, 3.09)	0.017	1.19 (1.05, 1.34)	1.00	1.67 (1.04, 2.69)
Model 3	1.00	1.66 (1.03, 2.68)	1.35 (0.81, 2.24)	1.93 (1.18, 3.15)	0.028	1.21 (1.06, 1.37)	1.00	1.62 (1.00, 2.62)

^a Logistic regression models were as followed: Model 1, crude model. Model 2 adjusted for age, pre-pregnancy BMI, monthly household income, maternal education, primiparity, family history of diabetes, smoking before pregnancy, drinking before pregnancy, GWG, GDM. Model 3 further adjusted for gestational age at FFQ, total energy intake, saturated fatty acid, monounsaturated fatty acids, polyunsaturated fatty acids.

Comparing with Q1, risk of LGA increased by 66%, 96%, 108%, and 67% in the Q4 of Ln (TC), Ln (TG), Ln (LDL-C), and Ln (non-HDL-C), respectively (**Table S4**). There was no significant association between maternal lipids levels and SGA. The β between dietary cholesterol and serum TC, LDL-C and non-HDL-C were 0.011 (95%CI: 0.002-0.021), 0.019 (95%CI: -0.001-0.039), and 0.022 (95%CI: 0.007-0.038), respectively (**Table 3**). LDL-C significantly mediated the association between dietary cholesterol and LGA, with the estimated mediation proportion by LDL-C at 7.1% (**Fig.S4**).

Table 3 Association between dietary cholesterol and maternal lipid levels ^a.

Variable	Model 1		Model 2	
	β (95% CI)	P value	β (95% CI)	P value
Ln (TC)	0.016 (0.008, 0.04)	<0.001	0.011 (0.002, 0.021)	0.018
Ln (TG)	0.016 (-0.001, 0.033)	0.065	0.007 (-0.013, 0.027)	0.504
Ln (HDL-C)	-0.001 (-0.013, 0.012)	0.895	-0.004 (-0.019, 0.010)	0.563
Ln (LDL-C)	0.028 (0.011, 0.045)	0.001	0.019 (-0.001, 0.039)	0.056
Ln (non-HDL-C)	0.029 (0.016, 0.042)	<0.001	0.022 (0.007, 0.038)	0.004

^a Generalized linear regression models were used to estimate β and 95% CIs. β represents the increase of each ln-transformed serum lipid concentration by each additional 100 mg dietary cholesterol intake. Model 1, crude model. Model 2 adjusted for age, pre-pregnancy BMI, monthly household income, maternal education, primiparity, family history of diabetes, smoking before pregnancy, drinking before pregnancy, GWG, GDM, total energy intake, saturated fatty acid, monounsaturated fatty acids, polyunsaturated fatty acids.

3.3 Effect of genetic variants of cholesterol metabolism genes on risks of SGA and LGA

All SNPs conformed to Hardy-Weinberg equilibrium ($P > 0.01$) in the control group (**Table S5**). Birth weight increased by 33.81 g (95%CI: 5.03, 62.59) with one additional mutated base of the *ABCA1* rs1883025 C $\rightarrow$ T (**Table S6**). As shown in **Table 4**, the variant allele A carriers (CA + AA) of *HMGCR* rs10045497 C $\rightarrow$ A had a 35% decreased risk of SGA compared with the CC genotype (OR = 0.65, 95%CI 0.43, 0.98). The prevalence of TT genotypes for the *CYP7A1* rs7833904 A $\rightarrow$ T polymorphisms were significantly higher among women with SGA ($P = 0.025$). The TT genotype in the *CYP7A1* rs7833904 A $\rightarrow$ T had a 81% increased risk of SGA compared with the AA genotype (OR = 1.81, 95%CI 1.07, 3.05). The SGA risk increased by 31% with one additional mutated base of the *CYP7A1* rs7833904 A $\rightarrow$ T (**Table S7**). The variant allele T carriers (CT + TT) of the *ABCA1* rs1883025 C $\rightarrow$ T had a 33% increased risk of LGA compared with the CC genotype (OR = 1.33, 95%CI 1.01, 1.78). LGA risk increased by 25% with one additional mutated base of the *ABCA1* rs1883025 C $\rightarrow$ T (**Table S7**). No significant associations were observed among the other genetic polymorphisms and SGA and LGA risk.

Table 4 Associations between genetic variants of cholesterol metabolism genes and birth outcomes ^a.

Genotype	Control	SGA	LGA	P_1	P_2	SGA		LGA	
						Model 1	Model 2	Model 1	Model 2
HMGCR									
rs10045497				0.130	0.431				
CC	348 (22.6)	39 (30.5)	65 (24.9)			1	1	1	1
CA	775 (50.4)	58 (45.3)	135 (51.7)			0.67 (0.44, 1.02)	0.67 (0.43, 1.04)	0.93 (0.68, 1.29)	0.96 (0.68, 1.36)
AA	415 (27.0)	31 (24.2)	61 (23.4)			0.67 (0.41, 1.09)	0.61 (0.37, 1.03)	0.79 (0.54, 1.15)	0.89 (0.60, 1.34)
CA+AA						0.67 (0.45, 0.99)	0.65 (0.43, 0.98)	0.88 (0.65, 1.20)	0.94 (0.67, 1.30)
rs3846662				0.243	0.646				
AA	323 (21.1)	35 (27.3)	58 (22.2)			1	1	1	1
AG	748 (48.9)	56 (43.8)	132 (50.6)			0.69 (0.44, 1.08)	0.70 (0.44, 1.11)	0.98 (0.70, 1.37)	0.96 (0.67, 1.38)
GG	460 (30.0)	37 (28.9)	71 (27.2)			0.74 (0.46, 1.20)	0.69 (0.42, 1.14)	0.86 (0.59, 1.25)	0.94 (0.63, 1.40)
AG+GG						0.71 (0.47, 1.07)	0.70 (0.46, 1.06)	0.94 (0.68, 1.28)	0.95 (0.68, 1.34)
rs3846663				0.293	0.535				
CC	315 (20.5)	33 (25.8)	55 (21.1)			1	1	1	1
CT	761 (49.6)	58 (45.3)	135 (51.7)			0.73 (0.47, 1.14)	0.75 (0.47, 1.19)	1.02 (0.72, 1.43)	1.01 (0.70, 1.45)
TT	459 (29.9)	37 (28.9)	71 (27.2)			0.77 (0.47, 1.26)	0.72 (0.43, 1.20)	0.89 (0.61, 1.30)	0.98 (0.65, 1.47)
CT+TT						0.74 (0.49, 1.13)	0.74 (0.48, 1.14)	0.97 (0.70, 1.34)	1.00 (0.70, 1.41)
rs12654264				0.381	0.822				
AA	321 (20.7)	33 (26.0)	55 (21.0)			1	1	1	1
AT	772 (49.9)	59 (46.5)	135 (51.5)			0.74 (0.48, 1.16)	0.76 (0.48, 1.21)	1.02 (0.73, 1.43)	1.02 (0.70, 1.46)
TT	454 (29.3)	35 (27.6)	72 (27.5)			0.75 (0.46, 1.23)	0.70 (0.42, 1.18)	0.93 (0.63, 1.35)	1.02 (0.68, 1.54)
AT+TT						0.75 (0.49, 1.13)	0.74 (0.48, 1.14)	0.99 (0.71, 1.36)	1.02 (0.72, 1.44)
NPC1L1									
rs2073547				0.263	0.202				
AA	640 (41.4)	50 (39.1)	103 (39.3)			1	1	1	1
AG	687 (44.4)	53 (41.4)	130 (49.6)			0.99 (0.66, 1.48)	0.97 (0.64, 1.47)	1.18 (0.89, 1.56)	1.17 (0.86, 1.58)
GG	220 (14.2)	25 (19.5)	29 (11.1)			1.46 (0.88, 2.41)	1.42 (0.84, 2.38)	0.82 (0.53, 1.27)	0.87 (0.55, 1.39)
AG+GG						1.10 (0.76, 1.59)	1.08 (0.74, 1.58)	1.09 (0.83, 1.42)	1.10 (0.82, 1.46)
rs2072183				0.410	0.214				
GG	643 (42.1)	49 (38.6)	104 (39.7)			1	1	1	1

GC	671 (43.9)	55 (43.3)	129 (49.2)			1.08 (0.72, 1.61)	1.05 (0.70, 1.59)	1.19 (0.90, 1.57)	1.17 (0.86, 1.58)
CC	213 (13.9)	23 (18.1)	29 (11.1)			1.42 (0.84, 2.38)	1.37 (0.80, 2.34)	0.84 (0.54, 1.31)	0.90 (0.57, 1.44)
GC+CC						1.16 (0.80, 1.68)	1.13 (0.77, 1.66)	1.11 (0.85, 1.44)	1.10 (0.83, 1.47)
rs17725246				0.270	0.217				
TT	642 (41.7)	51 (39.5)	104 (39.7)			1	1	1	1
TC	680 (44.2)	53 (41.1)	129 (49.2)			0.98 (0.66, 1.46)	0.95 (0.63, 1.44)	1.17 (0.89, 1.55)	1.16 (0.86, 1.57)
CC	218 (14.2)	25 (19.4)	29 (11.1)			1.44 (0.87, 2.39)	1.39 (0.83, 2.34)	0.82 (0.53, 1.27)	0.88 (0.55, 1.39)
TC+CC						1.09 (0.76, 1.58)	1.06 (0.73, 1.55)	1.09 (0.83, 1.42)	1.09 (0.82, 1.46)
LDLR									
rs8110695				0.122	0.280				
TT	571 (37.2)	39 (30.7)	110 (41.8)			1	1	1	1
TA	714 (46.5)	59 (46.5)	109 (41.4)			1.21 (0.80, 1.84)	1.10 (0.72, 1.69)	0.79 (0.60, 1.06)	0.78 (0.57, 1.06)
AA	252 (16.4)	29 (22.8)	44 (16.7)			1.69 (1.02, 2.79)	1.47 (0.88, 2.48)	0.91 (0.62, 1.33)	0.92 (0.61, 1.38)
TA+AA						1.33 (0.90, 1.97)	1.20 (0.80, 1.79)	0.82 (0.63, 1.07)	0.82 (0.61, 1.08)
rs1010679				0.387	0.358				
TT	475 (30.7)	35 (27.6)	88 (33.6)			1	1	1	1
TC	758 (49.1)	60 (47.2)	116 (44.3)			1.07 (0.70, 1.66)	0.98 (0.63, 1.53)	0.83 (0.61, 1.12)	0.80 (0.58, 1.11)
CC	312 (20.2)	32 (25.2)	58 (22.1)			1.39 (0.84, 2.30)	1.26 (0.75, 2.11)	1.00 (0.70, 1.44)	0.98 (0.67, 1.44)
TC+CC						1.17 (0.78, 1.75)	1.06 (0.70, 1.61)	0.88 (0.67, 1.16)	0.86 (0.64, 1.15)
rs688				0.727	0.794				
CC	1074 (69.4)	87 (68.0)	183 (69.6)			1	1	1	1
CT	431 (27.9)	36 (28.1)	71 (27.0)			1.03 (0.69, 1.55)	1.08 (0.72, 1.64)	0.97 (0.72, 1.30)	1.01 (0.74, 1.39)
TT	42 (2.7)	5 (3.9)	9 (3.4)			1.47 (0.57, 3.81)	1.63 (0.61, 4.33)	1.26 (0.60, 2.63)	0.97 (0.41, 2.28)
CT+TT						1.07 (0.73, 1.58)	1.13 (0.76, 1.68)	0.99 (0.75, 1.32)	1.01 (0.74, 1.37)
PCSK9									
rs10888897				0.352	0.309				
TT	57 (3.7)	4 (3.1)	5 (1.9)			1	1	1	1
CT	488 (31.5)	33 (25.8)	88 (33.5)			0.96 (0.33, 2.82)	0.86 (0.29, 2.57)	2.06 (0.80, 5.27)	1.59 (0.61, 4.20)
CC	1002 (64.8)	91 (71.1)	170 (64.6)			1.29 (0.46, 3.65)	1.23 (0.43, 3.51)	1.93 (0.76, 4.90)	1.60 (0.62, 4.14)
CT+CC						1.19 (0.42, 3.32)	1.11 (0.39, 3.16)	1.97 (0.78, 4.97)	1.60 (0.62, 4.12)
rs11206514				0.233	0.327				
CC	57 (3.7)	3 (2.3)	5 (1.9)			1	1	1	1
CA	478 (30.9)	32 (25.0)	85 (32.4)			1.27 (0.38, 4.29)	1.16 (0.34, 3.97)	2.03 (0.79, 4.20)	1.52 (0.58, 4.01)
AA	1010 (65.4)	93 (72.7)	172 (65.6)			1.75 (0.54, 5.70)	1.68 (0.51, 5.53)	1.94 (0.77, 4.91)	1.56 (0.60, 4.05)

CA+AA						1.60 (0.49, 5.17)	1.52 (0.46, 4.97)	1.97 (0.78, 4.96)	1.55 (0.60, 4.00)
rs2483205				0.130	0.911				
CC	891 (57.6)	78 (60.9)	149 (57.1)			1	1	1	1
CT	564 (36.5)	38 (29.7)	98 (37.5)			0.77 (0.52, 1.15)	0.78 (0.51, 1.18)	1.04 (0.79, 1.37)	0.94 (0.70, 1.27)
TT	91 (5.9)	12 (9.4)	14 (5.4)			1.51 (0.79, 2.87)	1.57 (0.79, 3.11)	0.92 (0.51, 1.66)	0.97 (0.52, 1.82)
CT+TT						0.87 (0.60, 1.26)	0.88 (0.60, 1.29)	1.02 (0.78, 1.33)	0.95 (0.71, 1.26)
rs499883				0.690	0.782				
GG	84 (5.4)	8 (6.2)	16 (6.1)			1	1	1	1
AG	551 (35.7)	41 (32.0)	88 (33.7)			0.78 (0.35, 1.72)	0.80 (0.34, 1.88)	0.84 (0.47, 1.50)	0.76 (0.41, 1.41)
AA	910 (58.9)	79 (61.7)	157 (60.2)			0.91 (0.43, 1.95)	0.92 (0.41, 2.09)	0.91 (0.52, 1.49)	0.88 (0.48, 1.60)
AG+AA						0.86 (0.41, 1.82)	0.88 (0.39, 1.97)	0.88 (0.51, 1.53)	0.83 (0.46, 1.50)
ABCA1									
rs2230806				0.987	0.291				
CC	477 (30.8)	40 (31.5)	92 (35.2)			1	1	1	1
CT	777 (50.2)	63 (49.6)	127 (48.7)			0.97 (0.64, 1.46)	1.04 (0.67, 1.60)	0.85 (0.63, 1.13)	0.88 (0.64, 1.20)
TT	293 (18.9)	24 (18.9)	42 (16.1)			0.98 (0.58, 1.65)	1.12 (0.65, 1.93)	0.74 (0.50, 1.10)	0.78 (0.51, 1.18)
CT+TT						0.97 (0.66, 1.43)	1.11 (0.70, 1.59)	0.82 (0.62, 1.08)	0.85 (0.63, 1.15)
rs1800977				0.060	0.174				
GG	702 (45.5)	67 (52.8)	134 (51.1)			1	1	1	1
GA	697 (45.2)	44 (34.6)	110 (42.0)			0.66 (0.45, 0.98)	0.69 (0.46, 1.03)	0.83 (0.63, 1.09)	0.85 (0.64, 1.14)
AA	143 (9.3)	16 (12.6)	18 (6.9)			1.17 (0.66, 2.08)	1.13 (0.61, 2.10)	0.66 (0.39, 1.11)	0.72 (0.42, 1.23)
GA+AA						0.75 (0.52, 1.08)	0.76 (0.52, 1.10)	0.80 (0.61, 1.04)	0.83 (0.63, 1.09)
rs1883025				0.968	0.197				
CC	983 (63.8)	80 (63.0)	152 (58.0)			1	1	1	1
CT	502 (32.6)	42 (33.1)	99 (37.8)			1.03 (0.70, 1.52)	1.04 (0.69, 1.56)	1.28 (0.97, 1.68)	1.34 (1.00, 1.80)
TT	55 (3.6)	5 (3.9)	11 (4.2)			1.12 (0.44, 2.87)	1.16 (0.44, 3.04)	1.29 (0.66, 2.53)	1.27 (0.61, 2.65)
CT+TT						1.04 (0.71, 1.51)	1.05 (0.71, 1.55)	1.28 (0.98, 1.67)	1.33 (1.01, 1.78)
CYP7A1									
rs7833904				0.025	0.738				
AA	583 (37.9)	42 (32.8)	93 (35.4)			1	1	1	1
AT	742 (48.2)	57 (44.5)	132 (50.2)			1.07 (0.71, 1.61)	1.08 (0.70, 1.64)	1.12 (0.84, 1.49)	1.16 (0.85, 1.58)
TT	214 (13.9)	29 (22.7)	38 (14.4)			1.88 (1.14, 3.10)	1.81 (1.07, 3.05)	1.11 (0.74, 1.68)	1.16 (0.75, 1.80)
AT+TT						1.25 (0.85, 1.83)	1.23 (0.83, 1.84)	1.12 (0.85, 1.47)	1.16 (0.87, 1.55)
rs3808607				0.310	0.641				

GG	302 (19.6)	23 (18.0)	53 (20.3)			1	1	1	1
GT	749 (48.7)	56 (43.8)	119 (45.6)			0.98 (0.59, 1.62)	0.96 (0.57, 1.60)	0.91 (0.64, 1.29)	0.96 (0.66, 1.40)
TT	488 (31.7)	49 (38.3)	89 (34.1)			1.32 (0.79, 2.21)	1.25 (0.74, 2.12)	1.04 (0.72, 1.50)	1.11 (0.74, 1.65)
GT+TT						1.12 (0.70, 1.78)	1.08 (0.67, 1.73)	0.96 (0.69, 1.33)	1.02 (0.72, 1.44)
rs8192870				0.342	0.024				
TT	64 (4.2)	2 (1.6)	21 (8.0)			1	1	1	1
TG	517 (33.6)	43 (33.6)	83 (31.7)			2.66 (0.63, 11.25)	2.49 (0.58, 10.65)	0.49 (0.28, 0.84)	0.59 (0.33, 1.07)
GG	957 (62.2)	83 (64.8)	158 (60.3)			2.78 (0.67, 11.54)	2.58 (0.61, 10.87)	0.50 (0.30, 0.85)	0.60 (0.34, 1.06)
TG+GG						2.74 (0.66, 11.31)	2.55 (0.61, 10.66)	0.50 (0.30, 0.83)	0.60 (0.34, 1.04)
rs8192871				0.486	0.408				
GG	508 (32.9)	40 (31.5)	79 (30.3)			1	1	1	1
GA	751 (48.6)	58 (45.7)	125 (47.9)			0.98 (0.65, 1.49)	0.99 (0.65, 1.52)	1.07 (0.79, 1.45)	1.11 (0.80, 1.54)
AA	286 (18.5)	29 (22.8)	57 (21.8)			1.29 (0.78, 2.12)	1.22 (0.73, 2.06)	1.28 (0.89, 1.86)	1.30 (0.87, 1.93)
GA+AA						1.07 (0.72, 1.57)	1.06 (0.71, 1.58)	1.13 (0.85, 1.50)	1.16 (0.86, 1.58)

^a Logistic regression models were as followed: Model 1, crude model. Model 2 adjusted for age, pre-pregnancy BMI, monthly household income, maternal education, primiparity, family history of diabetes, smoking before pregnancy, drinking before pregnancy, GWG, GDM. P_1 : SGA compared to AGA; P_2 : LGA compared to AGA.

3.4 Association between GRS and risks of SGA and LGA

The GRS distribution was presented in **Fig.S5**. SGA group presented higher GRS-SGA in comparison with AGA ($P = 0.05$). The GRS-LGA of LGA mothers were higher than AGA group ($P = 0.001$). GRS-SGA was linearly associated with OR of SGA (P for overall association = 0.003, P for non-linearity = 0.077); there was a significant linear relationship between GRS-LGA and LGA (P for overall association = 0.005, P for non-linearity = 0.232) (**Fig.1**). Women in the Q4 of GRS-SGA had higher SGA risk compared with the lowest GRS-SGA group (OR: 1.91, 95%CI: 1.11, 3.29). Relative to Q1, Q4 of GRS-LGA was associated with a significantly increased risk of LGA (OR = 1.60, 95%CI 1.09, 2.36) (**Table 5**).

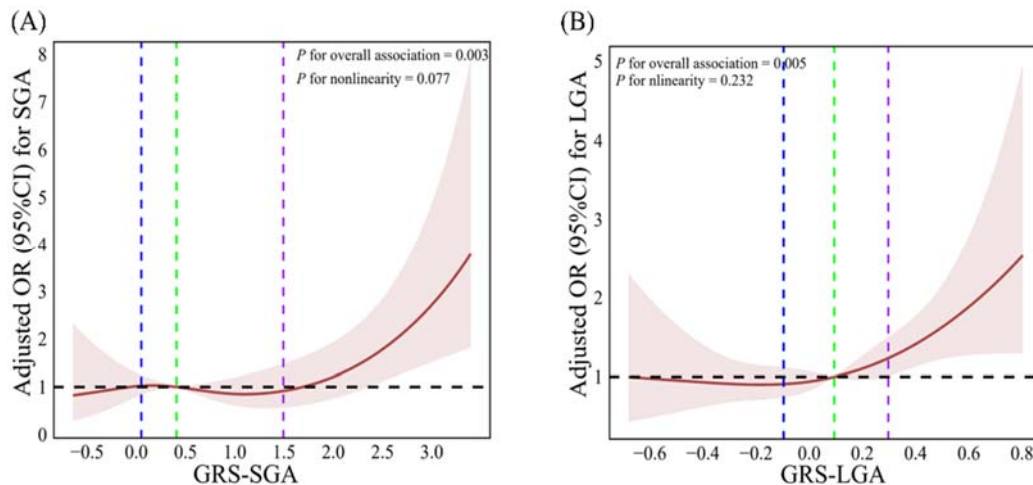


Fig. 1 Restricted cubic spline analyses illustrating the shapes of GRS and birth outcomes. The dashed blue line represents the 25th percentile (P25), the dashed green line represents the 50th percentile (P50), and the dashed purple line represents the 75th percentile (P75). (A) The RCS of GRS and SGA. P25=0.05, P50=0.41, P75=1.49. (B) The RCS of GRS and LGA. P25=-0.10, P50=0.09, P75=0.30. Adjusted for age, pre-pregnancy BMI, monthly household income, maternal education, primiparity, family history of diabetes, smoking before pregnancy, drinking before pregnancy, GWG, GD

Table 5 Associations between GRS and birth outcomes ^a.

	GRS ^b			
	Q1	Q2	Q3	Q4
SGA (%)	24 (5.5)	35 (8.4)	30 (7.2)	39 (9.5)
Model 1	1.00	1.57 (0.92, 2.69)	1.33 (0.76, 2.32)	1.81 (1.07, 3.06)
Model 2	1.00	1.61 (0.93, 2.77)	1.28 (0.72, 2.27)	1.91 (1.11, 3.29)
LGA (%)	56 (12.3)	57 (12.4)	64 (14.4)	86 (18.9)
Model 1	1.00	1.02 (0.69, 1.51)	1.21 (0.82, 1.77)	1.67 (1.16, 2.40)
Model 2	1.00	0.92 (0.60, 1.40)	1.18 (0.79, 1.78)	1.60 (1.09, 2.36)

^a Logistic regression models were as followed: Model 1, crude model. Model 2 adjusted for age, pre-pregnancy BMI, monthly household income, maternal education, primiparity, family history of diabetes, smoking before pregnancy, drinking before pregnancy, GWG, GDM.

^b When the analysis outcome is SGA, GRS is GRS-SGA; When the analysis outcome is LGA, GRS is GRS-LGA.

3.5 Joint association between dietary cholesterol and GRS on risks of SGA and LGA

Participants were divided into 8 strata according to the joint categories of dietary cholesterol and GRS (**Fig.2**) (**Table S8**). Individuals in the highest categories of both dietary cholesterol and GRS-LGA had a 1.13-fold increased risk of LGA (95%CI: 1.12, 4.07) compared to those in the lowest categories. No significant association was observed between dietary cholesterol and GRS-SGA on SGA risk.

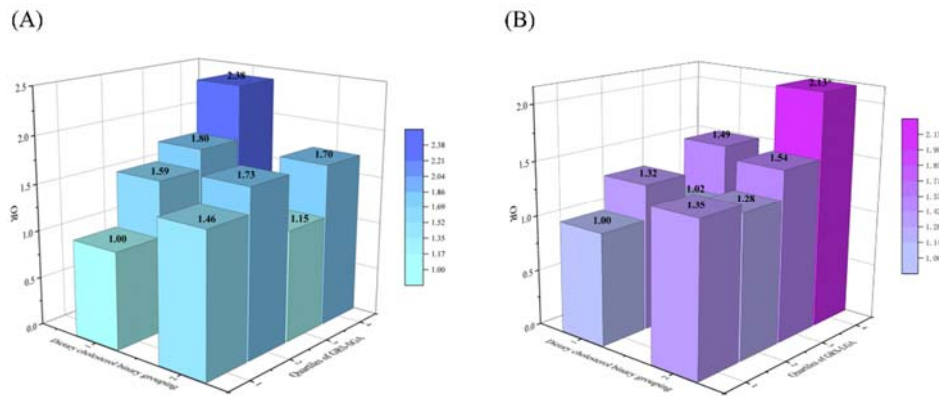


Fig. 2 The joint effect of dietary cholesterol and GRS on birth outcomes. The X-axis is the quartile of GRS. The Y-axis is the dietary cholesterol binary grouping, group 1: dietary cholesterol intake < 300 mg/d, group 2: dietary cholesterol intake ≥ 300 mg/d. The Z-axis is the odd ratio (OR) for SGA/LGA. Adjusted for age, pre-pregnancy BMI, monthly household income, maternal education, primiparity, family history of diabetes, smoking before pregnancy, drinking before pregnancy, GWG, GDM. (A) The joint effect of dietary cholesterol and GRS on SGA. (B) The joint effect of dietary cholesterol and GRS on LGA.

4. Discussion

In the current cohort study, we observed a significant and positive association between maternal dietary cholesterol and the risk of LGA and SGA. Dietary cholesterol was positively linear with serum TC, LDL-C and non-HDL-C. LDL-C mediated the association between dietary cholesterol and LGA. A total of 21 SNPs related to cholesterol-metabolism were included in the genetic analysis, among which *ABCA1* rs1883025 C → T showed the significant association with birth weight and LGA, *CYP7A1* rs7833904 A → T presented a significant association with SGA. The GRS of these 21 variant SNPs was also linked to SGA and LGA, respectively. Moreover, a significant combined effect of dietary cholesterol and GRS on LGA was observed.

Our finding that higher cholesterol intake and egg consumption were associated with the increased risk of LGA was generally consistent with two prior studies^[13, 14]. A cross-sectional study of 297 pairs of mothers/children from Brazil found that women with cholesterol intake within the fourth quartile were 2.48 times more likely to have an LGA infant than those in the 1-3 quartile^[13]. A cohort study conducted in China, showed that the risk of LGA increased in pregnant women whose egg consumption were 8-10 eggs/week and > 10 eggs/week as compared to those ≤ 7 eggs/week^[14]. In addition, our results found a "U-shaped" non-linear relationship between dietary cholesterol and SGA risk, with high cholesterol intake significantly increasing SGA risk. An animal experiment also found that high cholesterol intake during pregnancy reduces birth weight and leads to intrauterine growth restriction^[27]. Based on the findings of the present study, a range of cholesterol intake during pregnancy would be appropriate to consider in the future.

The correlations observed between serum lipids levels and LGA risk were consistent with previous research^[28, 29]. In addition, our study observed that increased non-HDL-C levels were correlated with elevated risks of LGA, which was rarely studied previously. Non-HDL-C encompasses all plasma lipoproteins like LDL, triglyceride-rich lipoproteins (TRL), and TRL-remnants, except HDL^[30], which has gained attention as a primary goal in dyslipidemia management. Lipid metabolism can be influenced by a number of dietary

factors, including total energy intake, fat composition (saturated vs. unsaturated fatty acids), and cholesterol intake^[19]. It is difficult to distinguish between the effect of dietary cholesterol *per se* and the effect of dietary saturated fat. Evidence from observational studies examining the relationship between dietary cholesterol and serum lipid levels is inconsistent: dietary cholesterol was shown to correlate positively with serum TC and LDL-C levels in Chinese populations^[17, 18]; by contrast, no significant association between dietary cholesterol and serum lipid levels was observed in American populations^[31]. It is worth highlighting that studies that reported a positive correlation between dietary cholesterol intake and serum cholesterol levels did not account for SFA as a confounding variable, whereas the study documenting a negative association incorporated SFA into its statistical adjustment model. A recent meta-regression analysis involving 55 randomized controlled dietary intervention trials demonstrated a dose-response relationship between dietary cholesterol and serum LDL-C levels after adjustment for dietary fat types^[32]. Another meta-regression analysis of controlled feeding studies - with matched PUFA:SFA ratios in comparison diets - indicated that dietary cholesterol led to a significant increase in serum TC levels^[33]. In the current study, we found that dietary cholesterol was positively linear with serum TC, LDL-C and non-HDL-C in pregnant women, after adjusting total energy intake, SFA, MUPA, and PUFA. The impact of dietary cholesterol on serum lipid profiles is more complex than historically recognized, with marked interindividual variability. While some studies posited that dietary cholesterol directly elevated serum TC and LDL-C levels, accumulating contemporary evidence reveals pronounced heterogeneity in this response: approximately 25% of the population are classified as “high responders” (exhibiting synchronized increases in LDL-C levels), whereas the remaining ~75% are “low responders” (displaying minimal to no alterations in serum lipid parameters following dietary cholesterol exposure)^[34]. This variability is driven by genetic factors, metabolic status, and intestinal absorption rate (ranging from 20% to 80% across individuals)^[35]. Notably, insulin-sensitive individuals - a subgroup relevant to pregnancy-related metabolic adaptations - have been shown to exhibit more robust lipid responses to dietary cholesterol, such as greater increments in LDL-C following increased cholesterol intake^[34, 36]. This subgroup-specific responsiveness may partially explain the positive association between maternal dietary cholesterol and serum TC and LDL-C levels observed in our cohort, as pregnancy-associated metabolic shifts could enhance maternal susceptibility to lipid alterations induced by dietary cholesterol. The results showed that LDL-C significantly mediated the association between dietary cholesterol and LGA. In human beings, LDL particles are the main carrier of cholesterol to peripheral tissues where they are internalized through the LDL receptor, a crucial mediator of plasma LDL concentrations^[36], which may partially explain the mediating effect of LDL-C.

Taking genetic heterogeneity into account, we examined the relationship between genetic polymorphisms involved in encoding cholesterol-metabolizing proteins and birth weight. In the univariate SNP analysis, we identified that genetic variants of *CYP7A1* rs7833904 were linked to an increased risk of SGA and genetic variants of *ABCA1* rs1883025 were associated with higher LGA risk. The potential role of genetic

polymorphisms involved in encoding cholesterol-metabolizing proteins in the development of SGA and LGA has not been previously investigated. *CYP7A1* is the initial enzyme in the classic conversion pathway that transforms cholesterol into bile acids (BA)^[37]. The functional role of the rs7833904 polymorphism remains unclear; however, it is worth noting that another polymorphism in the *CYP7A1* gene, rs1023652, is in complete linkage disequilibrium with rs7833904. The T allele of rs7833904 is co-inherited with the C allele of rs1023652^[38]. Xiang et al. found that the plasma concentration of hyodeoxycholic BA was higher in individuals with the rs1023652 CG or CC genotype than in those with GG genotype^[39]. It is reasonable to speculate that the T allele of the rs7833904 polymorphism enhance the ability of the enzyme to convert cholesterol to BA, which may lead to increase in BA level. A cohort study found that higher maternal serum BA levels were associated with an increased risk of SGA^[40]. Reports *in vivo* and *in vitro* indicated that cholestasis is associated with inflammation and oxidative stress^[41], which could lead to the occurrence of SGA. ABCA1, also referred to as the cholesterol efflux regulatory protein (CERP), is a cAMP-dependent ATP-binding anion transporter. It plays a key role in regulating cellular cholesterol and phospholipid levels by facilitating their transport to apolipoproteins, leading to the formation of HDL^[42]. The *ABCA1* rs1883025 is a widely recognized genetic variant known to modulate circulating HDL-C levels. Some studies identified that mutations of rs1883025 were associated with lower HDL-C levels^[43, 44] and higher TG levels^[45]. The GRS has been employed to represent heritability and the cumulative impact of genetic polymorphisms on diseases. In the present study, we utilized GRS as an instrumental variable to examine the impact of multiple genetic variations in cholesterol-metabolizing genes on birth weight and discovered that the higher GRS was associated with the increased risk of SGA and LGA. Moreover, our study also revealed a significant combined effect of dietary cholesterol and GRS on LGA, indicating that higher dietary cholesterol intake increased LGA risk in women with a higher genetic risk.

The principal strength of this study was that it was the first time considering the combined effects of dietary cholesterol and cholesterol metabolism genes polymorphisms on birth weight. Secondly, this study was a prospective cohort design, which could provide strong evidence and avoid possible recall biases and reverse causality biases. However, this study also had some limitations. Firstly, the dietary intake and serum lipid levels investigated in this study were only determined in the second trimester. The measurements of maternal serum lipid levels at three-time points across pregnancy allow a better understanding of the adaptive process through which maternal lipids impact on fetal growth. Secondly, this study focused solely on SNPs related to cholesterol metabolism that have been previously reported. Conducting a genome-wide association study in the Asian population is recommended to identify additional susceptibility loci for dietary cholesterol metabolism and to explore the causal relationships with birth weight.

5. Conclusion

In conclusion, the findings indicated that both dietary cholesterol during pregnancy and genetic variations of cholesterol metabolism genes were associated with SGA and LGA. LDL-C served as a significant mediator

in the relationship between dietary cholesterol and LGA. Our results highlighted the necessity of restricting daily dietary cholesterol intake and eggs consumption in pregnant individuals. Personalized and precise dietary recommendations and guidance should be provided to populations susceptible to abnormal birth weight.

Ethics approval

All participants provided written informed consent before participation. This study was approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology (No. of the ethics committee: 2021IEC[S044]).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence (AI) tools

AI tools (Grammarly) were used for improving readability and grammar. The authors reviewed and edited the content as needed and take full responsibility for the final manuscript.

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Conflict of interest

The authors report there are no competing interests to declare. References

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