Original Article

Weather and Birth Weight: Different Roles of Maternal and Neonatal *GPR61* Promoter Methylation*

LI Zhi Yuan[1], GONG Yong Xiang[1], YANG Meng[1], CHAI Jian[2], SUN Ren Jie[1], LI Qin Yang[1], HE Ya Nan[1], HUANG Hui[1,3], ZHANG Ya Wei[4], BA Yue[1,3], and ZHOU Guo Yu[1,3,#]

1. Department of Environmental Health & Environment and Health Innovation Team, School of Public Health, Zhengzhou University, Zhengzhou 450001, Henan, China; 2. National Health Commission Key Laboratory of Birth Defects Prevention, Henan Key Laboratory of Population Defects Prevention, Henan Institute of Reproduction Health Science and Technology, Zhengzhou 450002, Henan, China; 3. Yellow River Institute for Ecological Protection & Regional Coordinated Development, Zhengzhou University, Zhengzhou 450001, Henan, China; 4. Department of Environment Health Science, Yale University School of Public Health, New Haven, CT, USA

Abstract

Objectives It is unclear whether G protein-coupled receptor 61 (GPR61) affecting body weight, plays a role in the association between birth weight and weather. This study aimed to assess the effects of prenatal weather and GPR61 on birth weight.

Methods A total of 567 mother-newborn pairs were recruited in Houzhai Center Hospital during 2011–2012. We detected the maternal and neonatal *GPR61* promoter methylation levels, and obtained meteorological and air pollution data.

Results A positive association was observed between maternal and neonatal *GPR61* methylation levels, and both of them were affected by precipitation, relative humidity (RH) and daily temperature range (DTR). Birth weight was associated negatively with RH and positively with DTR (P < 0.05). A significant association was observed between birth weight and neonatal *GPR61* methylation. We observed that maternal *GPR61* methylation seemed to modify associations between weather and birth weight ($P_{interaction}$ < 0.10), while neonatal *GPR61* methylation mediated the effects of RH and DTR on birth weight (P < 0.05).

Conclusions Our findings revealed the significant associations among prenatal weather, *GPR61* methylation and birth weight. Maternal *GPR61* methylation may modify the susceptibility of birth weight to prenatal weather conditions, while neonatal *GPR61* methylation may be a bridge of the effects of prenatal RH and DTR on birth weight.

Key words: Pregnancy; Weather; Birth weight; *GPR61* gene; DNA methylation; Mother-newborns

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#Correspondence should be addressed to ZHOU Guo Yu, PhD & Lecturer, Tel: 86-15515772923, E-mail: zhouguoyu@zzu.edu.cn

Biographical note of the first author: LI Zhi Yuan, male, born in 1963, Master & Senior Experimentalist, majoring in environmental health.

INTRODUCTION

Numerous adverse health outcomes, including neonatal deaths, post-neonatal mortality and many adult-onset non-communicable diseases, are reported to be related to birth weight[1]. Epidemiologic studies have proved that multiple factors can affect birth weight, including maternal life-style factors and objective environmental factors[2]. However, the mechanism for effects of abnormal birth weight has not been fully elucidated.

Increasing studies have paid attention to the relationship between birth weight and exposure to environmental conditions during pregnancy, and found that adverse changes in the intrauterine environment, such as air pollution and persistent organic pollutants, can affect birth weight[3,4]. In recent years, with the rapid climate change, it was reported that the effect of weather changes can increase the risk of low birth weight[5], while low birth weight can cause public health problems, such as the increasing incidence of diabetes[6]. A study surveyed two million people in Taiwan China and found that newborns delivered by women exposed to extreme temperatures had greater risks for low birth weight[7]. Moreover, a study covering two mother-child cohorts suggested that increased humidity was associated with decreased birth weight[8]. These findings indicated that weather conditions may influence birth weight.

G protein-coupled receptor 61 (GPR61) is a member of the G protein-coupled receptor family, which is the largest and most functionally diverse superfamily of cell surface receptors in mammals and plays a critical role in cell signal transduction[9,10]. The *GPR61* gene sequence is highly similar to that of the biogenic amine receptor, which plays an important role in energy absorption and metabolism[11]. Although the function of GPR61 has not been fully elucidated, the relationship between GPR61 and body weight has been studied continuously[11,12]. Nambu and colleagues found that the risk of obesity in GPR61-deficient mice was higher than that of wild-type mice[11]. Additionally, a meta-analysis including 35,668 children in the discovery phase from 20 studies and 11,873 children in the replication phase of 13 studies showed that childhood body mass index (BMI, kg/m^2) was related to mutations in the *GPR61* genotype[12]. However, there is no epidemiological evidence for the relationship between birth weight and GPR61. Prenatal weather condition can affect maternal and infant methylation status, which in turn, affects protein expression[13]. Notably, the methylation levels of the *GPR61* promoter can be affected by air pollution[14]. However, the association between weather and *GPR61* methylation remains unclear. Here, we speculated that *GPR61* methylation may be involved in the changes in birth weight related to weather conditions during pregnancy. A retrospective pilot study based on Houzhai Center Hospital data during 2010–2012 was conducted in Zhengzhou to evaluate the relationships among weather, *GPR61* methylation levels, and birth weight.

MATERIALS AND METHODS

Study Population

Houzhai town (size = 83 square kilometers) is located in the south of Zhengzhou city [the coordinate of latitude and longitude: (113.59, 34.67)] and has 55,000 residents. We recruited pregnant women who delivered from January 2010 to January 2012 in the Houzhai Center Hospital by cluster sampling. Houzhai Center Hospital is a government hospital in Houzhai town and serves most local residents[15]. Basic maternal information including maternal age, height, education background, disease history during pregnancy, folic acid intake from three months before pregnancy to delivery, active and passive smoking status during pregnancy and pre-pregnancy weight were collected by trained investigators through face-to-face interviews within one week after delivery. Maternal pregestational BMI was calculated by dividing maternal pre-pregnancy weight by the square of the height, and maternal net weight gain during pregnancy was calculated by the difference between maternal pre-pregnancy and delivery weight. Information including the sex of the newborn, neonatal weight, height, and gestational week at birth was obtained from the medical record.

The inclusion and exclusion criteria were described previously[15]. Briefly, we recruited Han minorities, being > 16 years of age, living in the locality during pregnancy, and excluded women who were exposed to occupational toxic substances (such as heavy metals, organic compounds, acids and alkaline chemicals) during pregnancy, actively smoked during pregnancy (smoking refers to at least once a day for a month), abused drugs, had hepatitis B infection, hypertension or diabetes during pregnancy. Women with multiple births, stillbirths and births at gestational weeks of < 28 or > 44 weeks were excluded[16]. A total of 582 mothers met the inclusion criteria in this study. Fifteen participants who had incomplete information, lacked blood samples, or had no neonatal weight data were excluded. Finally, 567 (97.42%) mothers were enrolled. All subjects provided written informed consent in accordance with the requirements of the Institutional Review Board at Zhengzhou University.

Exposure Assessment

Meteorological data on daily mean temperature (degrees Celsius, °C), atmospheric pressure (hPa) and relative humidity (%), 1-h maximum temperature (°C), 1-h minimum temperature (°C), daily sunlight duration (h), and 24-h precipitation (mm) from one monitoring station located in Zhengzhou city were obtained from the China Meteorological Data Sharing Service System (http://data.cma.cn/). We calculated the daily temperature range with maximum and minimum temperatures (maximum temperature − minimum temperature)[17]. The monitoring station data represents the exposure level in Zhengzhou city. The participants' residences were an average of 35 km from the meteorological monitoring station. In addition, ambient air pollutants data were obtained from the Environmental Science Research Institute in Zhengzhou University and the Environmental Monitoring Center Station in Zhengzhou, which collected 24-h average concentrations for particulate matter 10 (PM_{10}), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) through an automated data reporting system during the study period from two stations located in Houzhai town. The exposure levels of air pollution for each individual were represented by the nearest monitoring station data. The permanent residences of the participants were an average of 2.9 km from the nearest air pollution monitoring station. No exposure data were missing. After confirming the pregnancy status using results from an ultrasonic examination performed during the first trimester of pregnancy, the conception date was determined according to the last menstrual period. We estimated the exposure level for each individual during the entire pregnancy by averaging the daily meteorology and air pollutant data. Then, the average daily mean temperature (T), atmospheric pressure (AP), and relative humidity (RH); daily sunlight duration (SSD); daily temperature range (DTR); 24-h precipitation, and PM_{10} were estimated to explore the effect of weather fluctuation. Additionally, we stratified the pregnancies into three trimesters, including trimester 1 (< 13 gestational weeks), trimester 2 (13–27 gestational weeks), and trimester 3 (≥ 28 gestational weeks), and the exposure levels during each trimester were also estimated.

Collection of Biological Samples

Maternal fasting peripheral blood (5 mL) and umbilical cord blood (5 mL) were collected immediately by a professional nurse when the mothers gave birth. No blood samples were collected before delivery. The details were described previously[15]. The blood samples were collected into vacuum anticoagulant tubes at 4 °C, then transported to the laboratory. The white blood cells were separated from whole blood after centrifugation for subsequent analysis.

Measurement of GPR61 Promoter Methylation Level

DNA samples was extracted from the white blood cells with a genomic DNA extraction kit (Bioteke Corporation, Beijing China). A Nanodrop ND-1000 spectrophotometer (Thermo, Waltham, MA, USA) was used to measure the concentrations of the DNA samples. We treated the DNA with sodium bisulfite using an EZ DNA Methylation Kit (Zymo Research, CA, USA). The *GPR61* gene sequence was obtained using UCSC/Ensembl (http://genome.ucsc.edu/), and the *GPR61* promoter sequence was predicted according to a previous study[18]. The methylation levels of the *GPR61* promoter were determined by quantitative methylation-specific polymerase chain reaction (QMSP). The specific QMSP primers used in the current study were designed using online MethPrimer software (http://www.urogene.org/cgibin/methprimer/methprimer.cgi), and the primer sequences are shown in Supplementary Table S1, available in www.besjournal.com. QMSP was carried out in a 15 μL reaction mixture, containing 2×SYBR Green Mixtrue (7.5 μL), 1.25 μmol/L primer (2 μL), DNA template (60 ng), and ddH_2O water. The cycling parameters for PCR were as follows: denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s. Melting curve analysis was performed at the end of each run to confirm amplification specificity and the absence of primer dimers. The methylation levels were calculated according to formula $1/[1 + 2\text{^}(Ct_{methylated} - Ct_{unmethylated})] \times 100\%$[19], where Ct is the number of PCR cycle whose fluorescent signal increases to the threshold.

Statistical Analyses

A multiple linear regression model was used to assess the associations among birth weight, *GPR61* methylation in maternal and cord blood at birth, and weather conditions during different exposure windows, and each exposure window was determined in a separate model. The median of each methylation level quartile was treated as a continuous independent variable to determine linear trends across increasing quartiles of the methylation levels and birth weight as dependent variables[20]. Furthermore, we classified methylation levels into a binary variable according to the median of the methylation levels. Then the effects modification of *GPR61* methylation on the associations between weather and birth weight were explored with an interactive term[21]. If there were significant pairwise correlations among weather conditions, *GPR61* methylation levels, and birth weight, a mediation analysis would be performed using an SAS macro described in a previous study[22] to quantitatively evaluate the role of the *GPR61* promoter methylation levels in the association between weather conditions and birth weight. Briefly, two models were required. The first model estimated the association (the total effect) between exposure and the outcome without adjusting for the mediator, and the second model estimated the association (the direct effect) between exposure and the outcome with adjusting for the mediator. The mediation effect was obtained by the following formula: (total effect – direct effect) / (total effect) × 100%.

Based on previous reports, gestational age, the sex of the newborn, maternal age, maternal education, family income, pregestational BMI, passive smoking, and birth season were adjusted in the models[8,23]. Additionally, if the change in the estimated effect exposure was over 10% after adjusting for a single covariate in the primary analysis, we further adjusted for the covariate in the final models[24]. Furthermore, we did not find significant collinearity in the models. Hence, each model in the current study followed a basic covariate adjustment, including sex of the newborn, maternal age, gestational age, maternal education attained, family income, pregestational BMI, maternal net weight gain, smoking status, folic acid intake, and birth season (October–March defined as the cold season, April–September defined as the warm season). Furthermore, previous studies reported the necessity of mutually adjusting for precipitation and RH when evaluating the health effects of precipitation and RH[25], hence, we adjusted them for each other in the relevant models when we analyzed their relationship with *GPR61* methylation and birth weight. We did not adjust for other weather levels in one model. To comprehensively reveal the health risk of weather and avoid significant collinearity interference among the above air pollutants, we further analyzed the health risks of weather after adjusting for PM_{10}.

All analyses were performed with SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) and all *P* values were two-sided with a significant level of 0.05.

RESULTS

Of the mothers, the mean age, pregestational weight, and net weight gain were 26.55 years, 54.46 kg, and 14.72 kg, respectively. Of the newborns, the mean birth weight was 3262.32 ± 428.72 g. The methylation levels of the *GPR61* promoter in maternal and cord blood were 71.55% ± 11.19% and 73.93% ± 11.94%, respectively (Table 1), and no significant difference of methylation level was found between different sexs (Supplementary Table S2, available in www.besjournal.com).

The characteristics and correlation of weather and air pollutants during each exposure window are summarized in Supplementary Tables S3–S5, available in www.besjournal.com, respectively. We observed significant seasonal trends of AP, SSD, RH and T (Supplementary Figure S1, available in www. besjournal.com). The level of AP peaked in cold season, and the level of SSD, RH, and T peaked in warm season. As shown in Figure 1, increased birth weight was observed with a decrease in RH or an increase in DTR during the entire pregnancy (the exposures in first and second trimesters showed the greater differences) (all $P < 0.05$). Near-null associations were observed between other weather conditions and birth weight ($P > 0.05$).

In addition, a positive linear correlation between maternal and neonatal *GPR61* methylation levels was revealed (Table 2). Neonatal *GPR61* methylation showed an upward trend with increases in maternal methylation. Multiple linear regression analysis suggested that neonatal *GPR61* methylation increased by 0.55% (95% *CI*: 0.44–0.65) with each 1% increase in maternal methylation ($P < 0.001$).

Then, we analyzed the relationships between weather and *GPR61* methylation (Table 3) and observed positive associations between precipitation (during the entire pregnancy and the first and third

Table 1. Characteristics of study population (*n* = 567)

Characteristics	Mean ± SD or *n* (%)
Maternal	
Age (year)	26.55 ± 5.28
Height (cm)	160.74 ± 4.52
Pregestational weight (kg)	54.46 ± 7.88
Net weight gain (kg)	14.72 ± 5.37
Pregestational BMI (kg/m^2)	
< 18.5	92 (16.82)
18.5–24.0	367 (67.09)
≥ 24.0	88 (16.09)
Folic acid intake	
Yes[a]	46 (8.73)
No	481 (91.27)
Passive smoking	
Yes[b]	131 (23.10)
No	436 (76.90)
Family income (RMB/capita)	
< 1,000	348 (69.60)
1,000–2,000	130 (26.00)
> 2,000	22 (4.40)
Education attainment	
Middle school and below	402 (73.76)
High school	111 (20.37)
Junior college and above	32 (5.87)
Maternal *GPR61* promoter methylation (%)	71.55 ± 11.19
Newborns	
Birth weight (g)	3262.32 ± 428.72
Gestational age (week)	
< 37	5 (0.88)
37–40	495 (87.30)
> 40	67 (11.82)
Sex	
Boys	320 (56.44)
Girls	247 (43.56)
Birth season[c]	
Cold season	268 (47.27)
Warm season	299 (52.73)
Neonatal *GPR61* promoter methylation (%)	73.93 ± 11.94

Note. [a]Folic acid intake was defined as the intake of folic acid and its derivatives (such as tetrahydrofolate and 5-methyl-tetrahydrofolate) at least once a day, for more than 30 days from 3 months before pregnancy to delivery. [b]Passive smoke exposure was defined as exposure to a smoking environment for at least 15 min a day, for more than a month. [c]October to March and April to September were defined as the cold and warm season, respectively. Abbreviations: RMB, China Yuan.

trimesters) and *GPR61* methylation in maternal and cord blood, and between DTR (during the entire pregnancy and the second trimester) and *GPR61* methylation in maternal and cord blood. We also observed the negative effects of RH on maternal and neonatal *GPR61* methylation (during the entire pregnancy and the second and third trimesters). A near null association was observed between other weather conditions during the entire pregnancy and *GPR61* methylation levels in maternal and cord

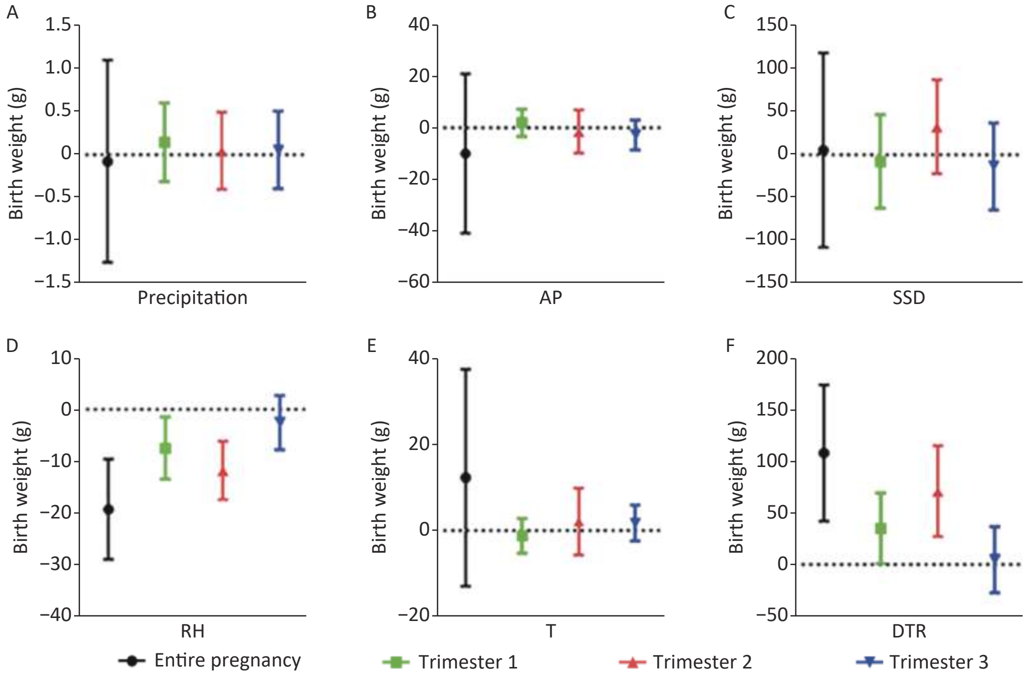

Figure 1. Association between weather and birth weight. The Y-axis represents the change of birth weight with 1 unit (mm for precipitation, hPa for AP, h for SSD, % for RH and °C for T and DTR) increment of weather conditions. (A) Adjustments: RH, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. (B, C, E, F) Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. (D) Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C).

Table 2. Association between maternal and neonatal *GPR61* promoter methylation levels[a]

Maternal methylation (%)	*n*	Neonatal methylation (%)	
		β (95% *CI*)	*P*
Quartile 1	145	Reference	–
Quartile 2	141	2.08 (–0.90, 5.06)	0.172
Quartile 3	140	4.58 (1.92, 7.23)	< 0.001
Quartile 4	141	16.05 (13.20, 18.90)	< 0.001
Trend test			< 0.001
Continuous	567	0.55 (0.44, 0.65)	< 0.001

Note. [a]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season.

blood (Table 3).

Next, we observed a significant correlation between birth weight and neonatal *GPR61* methylation ($P < 0.05$ for trend over quartiles), and a linear association that an elevation of 4.01 g of birth weight was observed with each 1% increase in the levels of neonatal *GPR61* methylation after adjusting for potential confounding factors (Table 4).

We further estimated the effects modification of maternal/neonatal *GPR61* methylation on the association between weather and birth weight. We found that there were marginally significant effects modification of maternal *GPR61* methylation on the associations between almost all meteorological conditions (except for DTR) and birth weight ($0.05 < P$ for interaction < 0.10) (Table 5), but no effects modification of neonatal methylation (Table 6).

Mediation analysis showed that the association between birth weight and RH during the entire pregnancy (the exposures in first trimester showed the greatest difference) and between birth weight and DTR during the entire pregnancy (the exposures in second trimester showed the greatest difference) were partially mediated by alterations in neonatal *GPR61* methylation ($P < 0.05$) (Table 7).

No significant change in the relationship between weather and *GPR61* methylation (Supplementary Table S6, available in www.besjournal.com), and between weather and birth weight (Supplementary Table S7, available in www.besjournal.com) was observed after further

Table 3. Association between weather and maternal/neonatal *GPR61* promoter methylation levels[a]

Meteorological conditions	*n*	Entire pregnancy		Trimester 1		Trimester 2		Trimester 3	
		β (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	*P*
Maternal methylation									
Precipitation (mm)[b]	567	0.07 (0.04, 0.11)	< 0.001	0.02 (0.00, 0.03)	0.010	−0.01 (−0.03, 0.01)	0.085	0.03 (0.01, 0.04)	< 0.001
AP (hPa)[c]	567	0.95 (0.20, 1.71)	0.014	−0.12 (−0.29, 0.04)	0.151	0.21 (−0.03, 0.46)	0.091	0.15 (−0.01, 0.31)	0.074
SSD (h)[c]	567	0.07 (−2.75, 2.89)	0.962	−1.31 (−2.66, 0.05)	0.059	1.14 (−0.48, 2.77)	0.168	0.30 (−1.07, 1.68)	0.665
RH (%)[d]	567	−0.64 (−0.88, −0.40)	< 0.001	−0.15 (−0.32, 0.03)	0.105	−0.22 (−0.40, −0.04)	0.016	−0.29 (−0.43, −0.14)	< 0.001
T (°C)[c]	567	−0.45 (−1.13, 0.23)	0.197	0.12 (0.00, 0.25)	0.046	−0.17 (−0.38, 0.05)	0.136	−0.12 (−0.25, 0.01)	0.053
DTR (°C)[c]	567	2.89 (1.19, 4.59)	0.001	0.23 (−0.74, 1.21)	0.640	2.10 (0.96, 3.24)	< 0.001	0.65 (−0.27, 1.56)	0.165
Neonatal methylation									
Precipitation (mm)[b]	567	0.11 (0.08, 0.15)	< 0.001	0.03 (0.01, 0.04)	< 0.001	−0.01 (−0.02, 0.01)	0.295	0.03 (0.02, 0.04)	< 0.001
AP (hPa)[c]	567	0.79 (−0.01, 1.59)	0.054	−0.17 (−0.35, 0.01)	0.067	0.22 (−0.01, 0.38)	0.065	0.18 (−0.01, 0.38)	0.066
SSD (h)[c]	567	−1.29 (−4.23, 1.67)	0.394	−1.24 (−2.56, 0.08)	0.065	1.84 (0.09, 3.60)	0.040	−1.04 (−2.54, 0.44)	0.169
RH (%)[d]	567	−0.82 (−1.07, −0.56)	< 0.001	−0.27 (−0.43, −0.10)	0.002	−0.24 (−0.42, −0.06)	0.009	−0.29 (−0.44, −0.14)	< 0.001
T (°C)[c]	567	−0.33 (−1.01, 0.35)	0.334	0.16 (0.02, 0.29)	0.023	−0.13 (−0.34, 0.08)	0.232	−0.16 (−0.30, −0.02)	0.023
DTR (°C)[c]	567	2.36 (0.48, 4.25)	0.014	0.01 (−0.97, 0.98)	0.990	2.81 (1.67, 3.96)	< 0.001	0.12 (−0.87, 1.10)	0.817

Note. [a]The assessments of *β* and the 95% *CI* were for 1 unit (mm for precipitation, hPa for AP, h for SSD, % for RH and °C for T and DTR) increment in weather conditions. [b]Adjustments: RH, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [c]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [d]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C).

adjusting for PM_{10}, suggesting that *GPR61* methylation and birth weight were still closely related to weather after eliminating the effects of air pollutants.

DISCUSSION

In this study, we found significant associations among weather conditions (especially RH and DTR), *GPR61* methylation, and birth weight. Neonatal *GPR61* methylation could mediate the effect of RH (during the entire pregnancy, especially the first trimester) and DTR (during the entire pregnancy, especially the second trimester) on birth weight. Maternal *GPR61* methylation seemed to modify the effect of weather on birth weight. This suggests that DNA methylation may play an important role in the effect of weather fluctuation on humans, especially newborns.

During the past few years, increasing attention has been paid to the relationship between weather and birth weight[26]. In this study, we observed negative associations between birth weight and RH. The effect of high-level humidity on heat stress causes extra energy consumption[27], which means a decrease in the energy used for fetal development. In addition, high-level humidity can increase the deposition of air pollutants[28] and induce psychological and physical discomfort[29], causing many adverse health outcomes including low birth weight[30]. Similar reasons may be responsible for our findings. We additionally found that birth weight was positively correlated with DTR. A previous study found that in a high latitude area with a large DTR, birth weight was higher than that in the low latitude area[31]. This finding supports our results to some extent. In addition, it was reported that high ambient temperature could increase the risk of low birth weight *via* disturbing endocrine system[32]. We speculated that high-level of DTR will produce a compensation response for fetal development when the mother enters the low temperature environment from the high temperature environment. This compensation mechanism makes the embryo shows a “catch-up” situation and accelerate the development in the low temperature environment to make up for the adverse effects of the ambient high temperature[33]. Interestingly, we observed that birth weight was mainly affected by the exposure to RH and DTR during the first and second trimesters of pregnancy, but not the third trimester of pregnancy. A lag effect has been observed in the relationship between environmental conditions and birth weight[34], and it is well-known that fetal weight

Table 4. Associations between birth weight and *GPR61* promoter methylation levels in maternal and cord blood[a]

GPR61 methylation	*n*	Birth weight (g)	
		β (95% *CI*)	*P*
Maternal			
Quartile 1	145	Reference	–
Quartile 2	141	21.05 (–75.43, 117.54)	0.669
Quartile 3	140	–2.58 (–99.17, 94.02)	0.958
Quartile 4	141	37.32 (–65.10, 139.74)	0.475
Trend test			0.495
Continuous	567	1.61 (–1.68, 4.91)	0.338
Neonatal			
Quartile 1	142	Reference	–
Quartile 2	142	84.71 (–17.18, 186.60)	0.103
Quartile 3	140	153.92 (58.10, 249.74)	0.002
Quartile 4	143	135.47 (31.19, 239.75)	0.011
Trend test			0.002
Continuous	567	4.01 (0.60, 7.42)	0.021

Note. [a]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season.

Table 5. Effect modification of maternal *GPR61* methylation on the associations between weather and birth weight

Meteorological conditions		Maternal methylation				Effect modification	
		Low-level (n = 286)		High-level (n = 281)			
		β (95% *CI*)	P	β (95% *CI*)	P	β (95% *CI*)	$P_{interaction}$
Precipitation (mm)[a]	Entire pregnancy	0.79 (−0.92, 2.49)	0.364	−1.04 (−3.02, 0.945)	0.305	−1.51 (−3.25, 0.23)	0.089
	Trimester 1	0.46 (−0.14, 1.07)	0.130	−0.41 (−1.14, 0.31)	0.266	−0.14 (−0.71, 0.42)	0.615
	Trimester 2	0.74 (0.09, 1.38)	0.026	0.02 (−0.74, 0.77)	0.964	−0.55 (−1.31, 0.21)	0.155
	Trimester 3	−0.22 (−0.86, 0.43)	0.507	0.49 (−0.25, 1.22)	0.194	0.01 (−0.70, 0.72)	0.984
AP (hPa)[b]	Entire pregnancy	−47.90 (−85.85, −9.95)	0.013	34.77 (−10.60, 80.14)	0.133	43.37 (6.35, 80.39)	0.022
	Trimester 1	3.64 (−4.72, 11.99)	0.394	2.03 (−6.20, 10.27)	0.628	2.87 (−8.06, 13.80)	0.606
	Trimester 2	−10.33 (−22.03, 1.38)	0.084	5.78 (−6.78, 18.33)	0.367	10.27 (−0.72, 21.26)	0.067
	Trimester 3	−5.66 (−14.34, 3.01)	0.201	−1.27 (−10.09, 7.55)	0.778	−1.74 (−12.80, 9.32)	0.758
SSD (h)[b]	Entire pregnancy	46.16 (−117.54, 209.86)	0.581	−44.42 (−178.49, 89.65)	0.516	−107.93 (−314.66, 98.80)	0.306
	Trimester 1	16.70 (−52.10, 85.49)	0.634	−41.49 (−115.04, 32.06)	0.269	−92.36 (−183.94, −0.79)	0.048
	Trimester 2	101.04 (20.88, 181.19)	0.014	−29.08 (−101.95, 43.78)	0.434	−96.55 (−204.62, 11.52)	0.080
	Trimester 3	−68.50 (−138.67, 1.68)	0.056	21.41 (−39.87, 82.68)	0.494	63.97 (−10.76, 138.70)	0.093
RH (%)[c]	Entire pregnancy	−23.544 (−38.10.−8.98)	0.002	−13.47 (−26.69, −0.25)	0.046	−1.69 (−15.56, 12.18)	0.811
	Trimester 1	−14.45 (−22.40, −6.50)	< 0.001	1.62 (−7.42, 10.66)	0.725	7.08 (−0.20, 14.37)	0.057
	Trimester 2	−12.06 (−20.94, −3.18)	0.008	−10.61 (−18.17, −3.06)	0.006	−4.71 (−12.53, 3.11)	0.238
	Trimester 3	2.35 (−4.68, 9.37)	0.513	−8.08 (−15.68, −0.49)	0.037	−4.65 (−12.00, 2.69)	0.214
T (°C)[b]	Entire pregnancy	41.00 (7.31, 74.69)	0.017	−15.72 (−53.87, 22.43)	0.419	−30.37 (−59.40, −1.33)	0.040
	Trimester 1	−3.50 (−9.55, 2.55)	0.257	0.13 (−6.28, 6.54)	0.968	0.54 (−8.12, 9.20)	0.902
	Trimester 2	12.42 (1.86, 22.98)	0.021	−7.52 (−18.87, 3.82)	0.194	−9.23 (−17.79, −0.66)	0.035
	Trimester 3	3.79 (−2.61, 10.19)	0.246	0.97 (−5.61, 7.55)	0.772	0.69 (−8.04, 9.41)	0.878
DTR (°C)[b]	Entire pregnancy	91.01 (−5.05, 187.08)	0.063	107.06 (17.17, 196.95)	0.020	12.57 (−109.46, 134.59)	0.840
	Trimester 1	45.15 (−5.08, 95.38)	0.078	23.59 (−20.67, 67.85)	0.296	−35.27 (−102.07, 31.54)	0.301
	Trimester 2	80.08 (20.96, 139.20)	0.008	45.63 (−15.95, 107.22)	0.146	1.76 (−77.89, 81.41)	0.965
	Trimester 3	−22.16 (−67.46, 23.13)	0.338	28.38 (−15.15, 71.91)	0.201	37.15 (−23.85, 98.15)	0.233

Note. [a]Adjustments: RH, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [b]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [c]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C).

Table 6. Effect modification of neonatal *GPR61* methylation on the associations between weather and birth weight

Meteorological conditions		Neonatal methylation				Effect modification	
		Low-level (*n* = 284)		High-level (*n* = 283)			
		β (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	$P_{interaction}$
Precipitation (mm)[a]	Entire pregnancy	−0.10 (−1.92, 1.71)	0.910	−1.54 (−3.50, 0.43)	0.125	−1.31 (−3.07, 0.45)	0.145
	Trimester 1	−0.01 (−0.65, 0.62)	0.966	−0.40 (−1.15, 0.36)	0.305	0.01 (−0.57, 0.59)	0.967
	Trimester 2	0.61 (−0.06, 1.28)	0.073	0.58 (−0.16, 1.33)	0.125	−0.28 (−1.04, 0.48)	0.475
	Trimester 3	−0.04 (−0.68, 0.61)	0.911	−0.29 (−1.05, 0.48)	0.459	−0.45 (−1.15, 0.24)	0.203
AP (hPa)[b]	Entire pregnancy	−35.67 (−73.93, 2.58)	0.068	11.17 (−33.60, 55.94)	0.625	26.77 (−9.71, 63.25)	0.150
	Trimester 1	3.07 (−5.69, 11.84)	0.492	4.22 (−3.53, 11.96)	0.286	1.97 (−8.99, 12.92)	0.725
	Trimester 2	−6.04 (−17.74, 5.65)	0.311	0.45 (−11.97, 12.87)	0.944	4.91 (−5.92, 15.73)	0.374
	Trimester 3	−5.78 (−14.97, 3.41)	0.218	−3.75 (−11.91, 4.41)	0.368	0.36 (−10.68, 11.40)	0.950
SSD (h)[b]	Entire pregnancy	−3.05 (−171.93, 165.84)	0.972	19.16 (−110.57, 148.88)	0.772	33.50 (−172.58, 239.57)	0.750
	Trimester 1	26.18 (−46.31, 98.67)	0.479	−32.98 (−102.50, 36.55)	0.353	−52.33 (−143.77, 39.11)	0.262
	Trimester 2	56.47 (−26.13, 139.06)	0.180	4.44 (−65.61, 74.49)	0.901	−65.84 (−173.51, 41.83)	0.231
	Trimester 3	−67.97 (−138.38, 2.44)	0.059	33.74 (−26.31, 93.78)	0.271	78.66 (4.89, 152.42)	0.037
RH (%)[c]	Entire pregnancy	−15.23 (−30.17, −0.29)	0.046	−13.66 (−27.35, 0.02)	0.050	−6.12 (−19.84, 7.61)	0.382
	Trimester 1	−8.52 (−16.65, −0.38)	0.040	−0.66 (−9.81, 8.49)	0.887	4.48 (−2.79, 11.74)	0.228
	Trimester 2	−8.28 (−18.19, 1.63)	0.102	−12.97 (−19.82, −6.12)	< 0.001	−4.25 (−12.09, 3.59)	0.288
	Trimester 3	0.55 (−6.47, 7.56)	0.879	−1.67 (−9.57, 6.24)	0.680	−5.29 (−12.46, 1.89)	0.149
T (°C)[b]	Entire pregnancy	30.04 (−4.84, 64.92)	0.091	−4.09 (−40.31, 32.12)	0.825	−17.29 (−46.13, 11.55)	0.240
	Trimester 1	−2.58 (−8.77, 3.61)	0.415	−2.52 (−8.60, 3.56)	0.416	−0.18 (−8.75, 8.40)	0.968
	Trimester 2	7.11 (−3.27, 17.49)	0.179	−1.77 (−13.27, 9.73)	0.763	−4.28 (−12.75, 4.19)	0.322
	Trimester 3	3.29 (−3.33, 9.91)	0.330	2.78 (−3.42, 8.98)	0.380	0.00 (−8.67, 8.68)	0.999
DTR (°C)[b]	Entire pregnancy	63.23 (−35.80, 162.26)	0.211	127.54 (40.24, 214.84)	0.004	69.64 (−51.18, 190.46)	0.259
	Trimester 1	52.74 (−1.60, 107.08)	0.057	27.17 (−14.57, 68.92)	0.202	−22.22 (−90.11, 45.66)	0.521
	Trimester 2	40.52 (−23.07, 104.10)	0.212	79.07 (20.09, 138.06)	0.009	32.54 (−47.54, 112.62)	0.426
	Trimester 3	−18.36 (−65.24, 28.51)	0.443	18.47 (−22.69, 59.63)	0.379	45.79 (−14.32, 105.91)	0.135

Note. [a]Adjustments: RH, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [b]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [c]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C); PM_{10}, particular matter 10; SO_2, sulfur dioxide; NO_2, nitrogen dioxide.

increases mainly in the second trimester[35]. These factors may make the fetal birth weight more susceptible to weather in the first and second trimesters of pregnancy. Additionally, we further analyzed the effect of RH and DTR on birth weight after adjusting for exposures during all trimesters of pregnancy. We observed consistent results for RH and DTR with the results without adjusting for exposure during all trimesters of pregnancy (Figure 1 and Supplementary Table S8, available in www.besjournal.com), further indicating the susceptibility of fetal development to RH and DTR during the first and second trimesters of pregnancy.

In this study, positive associations between precipitation/DTR and *GPR61* methylation, and negative associations between RH and *GPR61* methylation, were observed. There are few studies on the potential mechanism of gene methylation induced by meteorological factors. Previous research indicated that DNA methyltransferase which is responsible for methylation could be affected by meteorological factors[36]. Hence, we speculate that weather fluctuations interfere with DNA methyltransferase enzymes, resulting in methylation changes in *GPR61* promoter. In addition, we found that weather conditions showed a consistent effect on *GPR61* methylation in maternal and cord blood, and this effect occurred at almost all stages of pregnancy. Similar results have been reported in previous studies[13]. We speculated that the complex physiological changes during pregnancy and the increased sensitivity of mothers and newborns to the external environment, resulting that weather fluctuation during almost all stages of pregnancy could affect methylation levels. Given that DNA methylation is closely related to gene expression[37], the change in *GPR61* methylation may be an important reason for the close association between weather and birth weight.

Here, we found a positive association between *GPR61* methylation in maternal and cord blood. DNA methylation is heritable[38], and some environmental substance can pass through the placental barrier, simultaneously affecting the level of maternal and infant methylation[13]. These may be responsible for the close relationship between *GPR61* methylation in maternal and cord blood.

Given the complex associations among weather, *GPR61* methylation, and birth weight in this study, we used interactive analysis and mediated analysis to assess the role of *GPR61* methylation in the association between weather and birth weight. After comprehensive analyses, we found the effect modification of maternal *GPR61* methylation on the association between birth weight and weather during almost all stages of pregnancy, which may result from the susceptibility of methylation change during pregnancy. In addition, the mediation analysis showed that the effects of RH (during the entire pregnancy and the first trimester) and DTR (during the entire pregnancy and the second trimester) on birth weight were partially mediated by alterations in neonatal *GPR61* methylation. These findings suggest that *GPR61* methylation in maternal and

Table 7. Mediation analysis for the role of neonatal *GPR61* methylation in the effects of RH and DTR on birth weight

Meteorological conditions	*n*	Total effect, *β* (95% *CI*)	Direct effect, *β* (95% *CI*)	Proportion mediated by methylation (95% *CI*)	*P*
RH (%)[a]					
Entire pregnancy	567	−19.34 (−20.04, −9.64)	−15.59 (−26.04, −5.14)	19.4 (6.1, 47.0)	0.014
Trimester 1	567	−7.51 (−13.53, −1.49)	−5.35 (−11.05, 0.95)	28.8 (8.0, 65.3)	0.003
Trimester 2	567	−11.80 (−17.47, −6.13)	−10.57 (−16.66, −4.83)	8.9 (1.7, 35.9)	0.097
DTR (°C)[b]					
Entire pregnancy	567	108.67 (42.40, 174.94)	93.57 (26.20, 160, 94)	13.9 (3.7, 40.7)	0.041
Trimester 2	567	71.28 (27.14, 115.42)	58.15 (11.60, 104.69)	18.4 (4.7, 50.9)	0.033

Note. [a]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season. [b]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season. Abbreviations: RH, relative humidity (%); DTR, daily temperature range (°C).

cord blood may play different roles in the birth weight changes caused by weather. Maternal *GPR61* methylation changes are more likely to affect the susceptibility of birth weight to weather, whereas neonatal *GPR61* methylation changes are the bridge between weather conditions and birth weight. A previous study indicated that maternal and neonatal methylation could affect birth weight independently[39]. Considering that GPR61 was reported to be related to a variety of physiological and biochemical processes, such as energy metabolism[11], we speculated that *GPR61* methylation changes induced by weather influenced maternal and neonatal energy metabolism. The changes in maternal *GPR61* methylation directly influence maternal energy metabolism, and indirectly influence the nutrition supply to embryo. This maternal change may modify the vulnerability of neonatal development to weather fluctuation[40]. Accordingly, neonatal methylation change directly affects neonatal energy metabolism and neonatal growth.

Attention has been directed at the effect of climate and hereditary changes on human health[41], especially birth outcomes. In the current study, we observed a significant relationship between weather fluctuation and birth weight, in which *GPR61* methylation in maternal and cord blood played important roles. Maternal *GPR61* methylation seemed to modify the susceptibility of weather fluctuation on birth weight, whereas neonatal *GPR61* methylation may be a bridge between weather fluctuation and birth weight. According to our findings, further studies should notice the difference in health effect caused by maternal and neonatal genetic change, and it is best to use high-throughput methods to verify such associations more comprehensively, which is of great significance for the prevention of adverse birth outcomes. DNA methylation was an important mechanism for stable gene repression[42]. High-level methylation could reduce expression of critical proteins, further affect normal physiological functions and result adverse health outcomes. In the current study, high level *GPR61* methylation was associated with increased birth weight, this finding provides a new sight for prevention of abnormal birth weight and targeted therapy for specific gene methylation[43].

Our study still had two limitations. Firstly, due to the retrospective study design, our research may have recall bias in the professional interviews, although we checked and obtained consistent results between the questionnaires and hospital records. Meanwhile, due to the inherent weakness of retrospective study and the small sample size of this study, our findings should be cautious to generalize for more vulnerable infants. Hence, prospective studies with large sample population should be done in future. Secondly, we conducted this study in Houzhai Center Hospital in Houzhai town, and observed a low preterm birth rate. Houzhai Center Hospital is a primary hospital with low-level medical services. Therefore, parents with poor health in the area usually do not give birth in this hospital. This may be the reason for the relatively low preterm birth rate in our current study.

CONCLUSIONS

Birth weight was associated with weather, especially RH and DTR. Maternal and neonatal *GPR61* methylation appear to have different roles in the associations between weather and birth weight. Maternal *GPR61* methylation seems to modify the susceptibility of birth weight to weather. Changes in neonatal *GPR61* methylation can mediate the effect of RH and DTR on birth weight. Our findings provide epidemiological evidence for the cause of birth weight changes and provide a preliminary basis for preventing adverse health outcomes.

COMPETING FINANCIAL INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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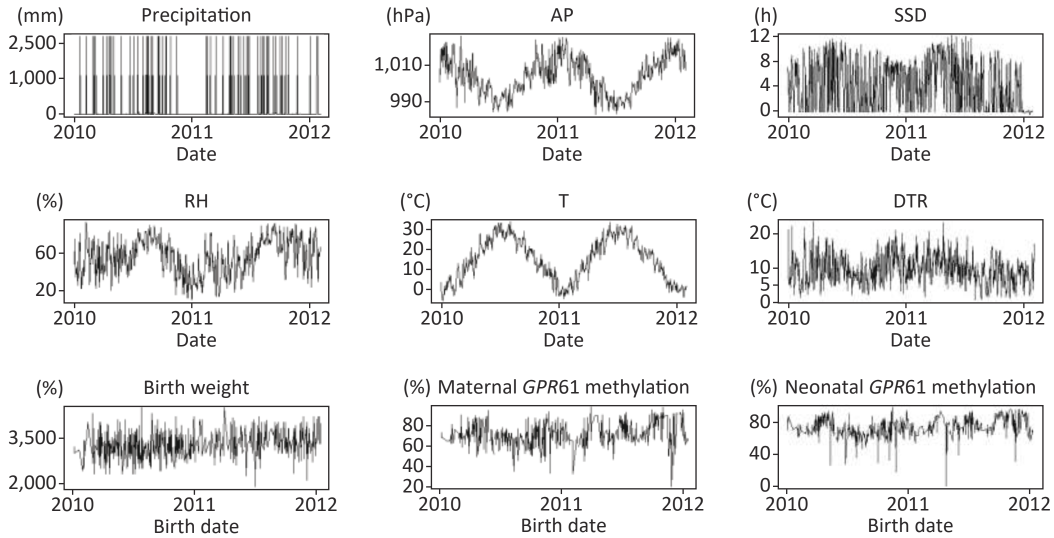

Supplementary Figure S1. Distribution of weather conditions during study period, birth weight, maternal and neonatal *GPR61* methylation levels. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C).

Supplementary Table S1. Primers for *GPR61* gene for QMSP

Primers	Sequences (5'–3')	Tm (°C)	Lengths of PCR products (bp)
MF	TGTGTTAGGTTATTGGAGAGGTTAC	53	166
MR	CCCTATTTTATAAATAAAAAACCGAA		
UF	TTGTGTTAGGTTATTGGAGAGGTTAT	53	167
UR	CCCTATTTTATAAATAAAAAACCAAA		

Note. M: Methylation; U: Unmethylation; F: Forward; R: Reverse.

Supplementary Table S2. The distribution of methylation levels by sex of the newborn

	Boys	Girls	*t*	*P*
Maternal *GPR61* promoter methylation (%)	71.98 ± 11.11	70.99 ± 11.29	1.052	0.293
Neonatal *GPR61* promoter methylation (%)	73.94 ± 12.24	73.93 ± 11.58	0.009	0.993

Supplementary Table S3. Characteristics of weather conditions and air pollutants

Weather conditions	Entire pregnancy (mean ± SD)	Trimester 1 (mean ± SD)	Trimester 2 (mean ± SD)	Trimester 3 (mean ± SD)
Precipitation (mm)	278.81 ± 40.90	283.78 ± 129.74	257.85 ± 95.88	294.81 ± 103.75
AP (hPa)	1003.53 ± 1.99	1004.20 ± 6.85	1003.73 ± 6.99	1002.66 ± 6.78
SSD (h)	4.81 ± 0.34	4.89 ± 0.87	4.87 ± 0.74	4.67 ± 1.02
RH (%)	55.83 ± 5.14	56.29 ± 10.51	54.76 ± 9.46	56.44 ± 10.48
T (°C)	15.24 ± 2.58	14.44 ± 8.72	14.83 ± 8.75	16.44 ± 8.49
DTR (°C)	9.78 ± 0.58	9.84 ± 1.11	9.86 ± 0.95	9.64 ± 1.26
PM_{10} (μg/m^3)	106.72 ± 5.50	105.49 ± 15.64	107.43 ± 10.23	107.04 ± 10.96
SO_2 (μg/m^3)	50.15 ± 7.28	51.96 ± 20.39	51.24 ± 19.74	47.22 ± 18.97
NO_2 (μg/m^3)	44.21 ± 2.77	44.64 ± 8.16	44.60 ± 7.53	43.36 ± 7.22

Note. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C); PM_{10}, particular matter 10; SO_2, sulfur dioxide; NO_2, nitrogen dioxide.

Supplementary Table S4. Spearman correlation of weather conditions and air pollutants

		Precipitation	AP	SSD	RH	T	DTR	PM_{10}	SO_2	NO_2
Entire pregnancy	Precipitation	1.00	−0.52	−0.47	0.74	0.52	−0.60	−0.58	−0.51	−0.58
	AP		1.00	0.18	−0.29	−0.97	0.29	0.42	0.84	0.85
	SSD			1.00	−0.48	−0.19	0.71	0.17	0.19	0.41
	RH				1.00	0.24	−0.80	−0.52	−0.01	−0.38
	T					1.00	−0.21	−0.41	−0.86	−0.83
	DTR						1.00	0.44	0.06	0.46
	PM_{10}							1.00	0.19	0.26
	SO_2								1.00	0.76
	NO_2									1.00
Trimester 1	Precipitation	1.00	−0.82	−0.02	−0.69	0.79	−0.52	−0.59	−0.83	−0.82
	AP		1.00	−0.32	−0.61	−0.98	0.31	0.65	0.93	0.95
	SSD			1.00	−0.24	0.29	0.64	0.00	−0.14	−0.16
	RH				1.00	0.67	−0.66	−0.66	−0.58	−0.56
	T					1.00	−0.28	−0.68	−0.93	−0.90
	DTR						1.00	0.45	0.36	0.41
	PM_{10}							1.00	0.65	0.60
	SO_2								1.00	0.93
	NO_2									1.00
Trimester 2	Precipitation	1.00	−0.76	−0.06	0.53	0.72	−0.43	−0.50	−0.73	−0.73
	AP		1.00	−0.23	−0.51	−0.97	0.33	0.67	0.91	0.91
	SSD			1.00	−0.39	0.21	0.67	0.01	−0.10	−0.10
	RH				1.00	0.59	−0.73	−0.60	−0.47	−0.47
	T					1.00	−0.32	−0.72	−0.90	−0.90
	DTR						1.00	0.40	0.41	0.41
	PM_{10}							1.00	0.68	0.57
	SO_2								1.00	0.93
	NO_2									1.00
Trimester 3	Precipitation	1.00	−0.76	−0.06	0.53	0.72	−0.43	−0.50	−0.74	−0.73
	AP		1.00	−0.23	−0.51	−0.97	0.33	0.67	0.94	0.91
	SSD			1.00	−0.39	0.21	0.67	0.01	−0.20	−0.10
	RH				1.00	0.59	−0.73	−0.60	−0.42	−0.47
	T					1.00	−0.32	−0.72	−0.94	−0.90
	DTR						1.00	0.40	0.29	0.41
	PM_{10}							1.00	0.68	0.57
	SO_2								1.00	0.93
	NO_2									1.00

Note. All $P < 0.05$. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C); PM_{10}, particular matter 10; SO_2, sulfur dioxide; NO_2, nitrogen dioxide.

Supplementary Table S5. Spearman correlation of trimesters of pregnancy exposures to weather conditions and air pollutants

Meteorological conditions	Trimester 1 *vs*. trimester 2	Trimester 1 *vs*. trimester 3	Trimester 2 *vs*. trimester 3
Precipitation	–0.35	–0.28	–0.25
AP	–0.10	–0.95	–0.08
SSD	–0.52	0.08	–0.47
RH	0.15	–0.57	0.08
T	–0.10	–0.97	–0.03
DTR	–0.24	0.11	–0.15
PM_{10}	–0.06	–0.48	–0.10
SO_2	–0.12	–0.90	–0.01
NO_2	–0.10	–0.83	–0.04

Note. All P < 0.05. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C); PM_{10}, particular matter 10; SO_2, sulfur dioxide; NO_2, nitrogen dioxide.

Supplementary Table S6. Analysis of the relationship between weather conditions and maternal/neonatal *GPR61* promoter methylation levels after adjusting PM_{10}[a]

Meteorological factors	*n*	Entire pregnancy		Trimester 1		Trimester 2		Trimester 3	
		β (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	*P*
Maternal methylation									
Precipitation (mm)[b]	567	0.07 (0.02, 0.11)	0.003	0.02 (0.00, 0.03)	0.024	–0.02 (–0.03, –0.00)	0.033	0.03 (0.01, 0.04)	< 0.001
AP (hPa)[c]	567	0.78 (0.08, 1.47)	0.030	–0.16 (–0.47, 0.15)	0.315	0.28 (0.03, 0.59)	0.031	0.27 (–0.05, 0.58)	0.093
SSD (h)[c]	567	1.37 (–2.13, 4.87)	0.443	–1.41 (–2.80, –0.01)	0.049	1.18 (–0.48, 2.84)	0.164	0.45 (–1.00, 1.90)	0.539
RH (%)[d]	567	–0.67 (–0.94, –0.40)	< 0.001	–0.17 (–0.35, 0.02)	0.075	–0.28 (–0.47, –0.08)	0.005	–0.31 (–0.45, –0.16)	< 0.001
T (°C)[c]	567	–0.34 (–0.96, 0.29)	0.294	0.20 (–0.01, 0.40)	0.059	–0.23 (–0.46, –0.01)	0.043	–0.19 (–0.38, –0.00)	0.047
DTR (°C)[c]	567	4.08 (2.23, 5.93)	< 0.001	0.66 (–0.39, 1.71)	0.219	2.59 (1.41, 3.77)	< 0.001	0.68 (–0.29, 1.66)	0.170
Neonatal methylation									
Precipitation (mm)[b]	567	0.11 (0.07, 0.15)	< 0.001	0.02 (0.01, 0.04)	0.003	–0.01 (–0.03, 0.01)	0.197	0.03 (0.02, 0.04)	< 0.001
AP (hPa)[c]	567	0.57 (–0.11, 1.24)	0.102	–0.06 (–0.40, 0.30)	0.762	0.27 (0.03, 0.51)	0.026	0.46 (0.19, 0.73)	< 0.001
SSD (h)[c]	567	–0.62 (–4.08, 2.84)	0.727	–1.45 (–2.79, –0.12)	0.033	1.85 (0.14, 3.56)	0.034	–0.78 (–2.37, 0.80)	0.332
RH (%)[d]	567	–0.87 (–1.12, –0.62)	< 0.001	–0.35 (–0.52, –0.17)	< 0.001	–0.29 (–0.48, –0.11)	0.002	–0.32 (–0.47, –0.17)	< 0.001
T (°C)[c]	567	–0.11 (–0.67, 0.45)	0.697	0.13 (–0.12, 0.37)	0.305	–0.19 (–0.41, 0.03)	0.087	–0.34 (–0.52, –0.15)	< 0.001
DTR (°C)[c]	567	3.85 (1.85, 5.85)	< 0.001	0.75 (–0.33, 1.84)	0.174	3.29 (2.10, 4.48)	< 0.001	0.15 (–0.92, 1.23)	0.783

Note. [a]The assessments of *β* and the 95% *CI* were for 1 unit (mm for precipitation, hPa for AP, h for SSD, % for RH and °C for T and DTR) increment of weather conditions. [b]Adjustments: RH, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season and PM_{10}. [c]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season and PM_{10}. [d]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season and PM_{10}. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C); PM_{10}, particular matter 10; SO_2, sulfur dioxide; NO_2, nitrogen dioxide.

Supplementary Table S7. Analysis of the relationship between weather conditions and birth weight after adjusting PM_{10}[a]

Meteorological factors	*n*	Entire pregnancy		Trimester 1		Trimester 2		Trimester 3	
		β (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	*P*	*β* (95% *CI*)	*P*
Precipitation (mm)[b]	567	−0.40 (−1.77, 0.97)	0.567	0.04 (−0.44, 0.51)	0.883	0.43 (−0.05, 0.90)	0.077	0.14 (−0.13, 0.60)	0.536
AP (hPa)[c]	567	−3.49 (−30.49, 23.51)	0.800	−4.50 (−13.67, 4.67)	0.336	−1.69 (−10.44, 7.06)	0.705	7.60 (−1.93, 17.14)	0.118
SSD (h)[c]	567	40.67 (−93.78, 175.12)	0.553	−5.89 (−60.60, 48.83)	0.833	30.11 (−26.09, 86.31)	0.294	2.46 (−51.85, 56.78)	0.929
RH (%)[d]	567	−19.21 (−29.20, −9.21)	< 0.001	−8.18 (−15.24, −1.12)	0.023	−11.25 (−17.21, −5.30)	< 0.001	−7.70 (−13.44, −1.96)	0.009
T (°C)[c]	567	3.90 (−18.96, 26.76)	0.738	3.73 (−3.01, 10.47)	0.278	2.30 (−6.04, 10.64)	0.589	−5.14 (−11.37, 1.09)	0.106
DTR (°C)[c]	567	121.93 (50.30, 193.56)	< 0.001	28.40 (−11.12, 67.91)	0.159	67.28 (19.66, 114.91)	0.006	28.57 (−9.95, 67.09)	0.146

Note. [a]The assessments of *β* and the 95% *CI* were for 1 unit (mm for precipitation, hPa for AP, h for SSD, % for RH and °C for T and DTR) increment of weather conditions. [b]Adjustments: RH, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season and PM_{10}. [c]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season and PM_{10}. [d]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake, birth season and PM_{10}. Abbreviations: AP, atmospheric pressure (hPa); SSD, sunshine duration (h); RH, relative humidity (%); T, average daily mean temperature (°C); DTR, daily temperature range (°C); PM_{10}, particular matter 10; SO_2, sulfur dioxide; NO_2, nitrogen dioxide.

Supplementary Table S8. Association between weather conditions and birth weight with further adjusting exposures during all trimesters of pregnancy

Weather conditions		*n*	Birth weight	
			β (95% *CI*)	*P*
RH[a]	Trimester 1	567	−6.26 (−12.17, −0.36)	0.038
	Trimester 2	567	−8.86 (−16.40, −1.32)	0.021
	Trimester 3	567	−2.99 (−7.87, 1.89)	0.230
DTR[b]	Trimester 1	567	49.27 (15.74, 82.76)	0.004
	Trimester 2	567	92.09 (45.70, 138.49)	< 0.001
	Trimester 3	567	14.86 (−20.11, 49.83)	0.405

Note. [a]Adjustments: precipitation, gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. [b]Adjustments: gestational age, sex of the newborn, maternal age, maternal education, family income, pregestational BMI, net weight gain, passive smoking, folic acid intake and birth season. Abbreviations: RH, relative humidity (%); DTR, daily temperature range (°C).