Can Proopiomelanocortin Methylation Be Used as an Early Predictor of Metabolic Syndrome?

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OBJECTIVE

The objectives of this study were to compare early predictive marker of the metabolic syndrome with proopiomelanocortin (POMC) methylation status and to determine the association among birth weight, ponderal index, and cord blood methylation status.

RESEARCH DESIGN AND METHODS

We collected pregnancy outcome data from pregnant women, cord blood samples at delivery, and blood from children (7–9 years old; *n* = 90) through a prospective cohort study at Ewha Womans University, MokDong Hospital (Seoul, Korea), from 2003–2005. POMC methylation was assessed by pyrosequencing. We divided subjects into three groups according to cord blood POMC methylation: the low methylation (<10th percentile), mid-methylation, and high methylation (>90th percentile) groups. We analyzed the association of POMC methylation status at birth with adiposity and metabolic components using ANCOVA and multiple linear regression analysis.

RESULTS

Birth weights (*P* = 0.01) and ponderal indices (*P* = 0.01) in the high POMC methylation group were significantly lower than in the mid-POMC methylation group. In terms of metabolic components of childhood, blood triglycerides (57.97, 67.29 vs. 113.89 mg/dL; *P* = 0.03, 0.01) and insulin (7.10, 7.64 vs. 10.13 μIU/mL; *P* = 0.05, 0.02) at childhood were significantly higher in the high POMC methylation group than in the low and mid-POMC methylation group.

CONCLUSIONS

High POMC methylation in cord blood was associated with lower birth weight, and children with high POMC methylation in cord blood showed higher triglycerides and higher insulin concentrations in blood. Thus, POMC methylation status in cord blood may be an early predictive marker of future metabolic syndrome.

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The prevalence of obesity has increased worldwide in recent decades (1,2). South Korea had relatively low prevalences of overweight and obesity (3,4). However, in recent years, the prevalence of obesity in childhood has increased dramatically. The rates of overweight and obesity in childhood almost doubled between 1997 (9.7%) and 2005 (19.0%) in Korea (5). Childhood obesity often persists into adulthood and is associated with the risk of chronic diseases, such as type 2 diabetes, cardiovascular disease, hypertension, and metabolic syndrome (1,6). Many researchers suggested that the fetus period is a crucial time for the metabolic programming, leading to a high risk of the offspring developing early-onset overweight and obesity, called "fetal programming" or the "Barker hypothesis" (7–9).

Epigenetic mechanisms, such as DNA methylation, are thought to be factors by which the fetal environment influences the adult phenotype (10). The establishment and alteration of DNA methylation have been studied with respect to various environmental and lifestyle exposures (11). The ability of epigenetic markers to persist during development and potentially be transmitted to offspring may be necessary for generation of a range of phenotypes from the same genotype (12). In addition to natural diversity, alterations in epigenetic markers are closely associated with pathogenic mechanisms in offspring, such as metabolic syndrome in childhood (11).

The malnutrition may undergo a nutritional programming event, leading to altered physiology of the hypothalamic pathways on leptin and proopiomelanocortin (POMC) gene (9,13). The POMC gene is located at chromosome 2p23 and spans 7,665 base pairs (14). The POMC gene has two cytosine guanine dinucleotide (CpG) islands: one at exon 1 and the associated promoter region and the other, downstream, at exon 3 (14,15). The POMC gene is expressed highly in immature neurons at early embryonic stages (16). Small litters from animal studies were associated with hypomethylation at the promoter of the hypothalamic POMC gene, via interfering with leptin- and insulin-induced POMC expression (15,17). Additionally, in obese children, methylation in this CpG-hypermethylated region was extended into the nonmethylated CpG island region (intron 2 and exon 3) (18). Thus, it is important to investigate early markers of the metabolic syndrome with POMC methylation status.

We hypothesized that POMC methylation status in cord blood may be an early predictive marker of metabolic syndrome. The purposes of this study were to compare early marker of the metabolic syndrome with POMC methylation status and to determine the association among birth weight, ponderal index, and cord blood methylation status.

RESEARCH DESIGN AND METHODS

Study Design

This study was conducted in the Ewha Birth & Growth Cohort. Details of the cohort design have been reported previously (19). Briefly, the Ewha Birth & Growth Cohort was established in 2001–2006 and consists of pregnant women who visited Ewha Womans University Hospital, Seoul, Korea, for prenatal care during gestational weeks 24–28 of their offspring. All participants gave their informed consent to participate in the study. The study was approved by the Institutional Review Board of the Ewha Womans University Hospital (ECT 13–01A-13).

In August 2011, we contacted 90 subjects in the age range 7–9 years who were part of the Ewha Birth & Growth Cohort (recruitment period was September 2001 to July 2003). During follow-up, we collected anthropometric data and blood samples. For this study, we excluded those who have experienced gestational diabetes, preeclampsia, chronic diseases, smoking history, and a history of any medication affecting growth. These 90 children had no specific diseases and congenital anomalies.

Anthropometric Measurements: BMI and Ponderal Index

All anthropometric measurements data were collected by the same examiners. The height, waist circumference, and weight of each child were measured to the nearest 0.1 cm and 0.1 kg while wearing light clothing without shoes using an automatic electronic scale (DS-102; Dong-Sahn Jenix Co. Ltd, Seoul, Korea). BMI was calculated as weight divided by height squared (kg/m^2). The BMI *z*-score was calculated for each child at the follow-up age using a sex- and age-specific criteria reference source from the 2007 Korean Children and Adolescents Growth Standards (20). The ponderal index was calculated as weight divided by height in cubic meter (kg/m^3). We also measured percentage of body fat mass using an automatic body composition analyzer (Inbody 230; GE Healthcare, Madison, WI).

Blood Collection and Biochemical Assessments

We collected cord blood samples after delivery. Blood samples during childhood were collected from the median cubital vein into Vacutainer tubes containing EDTA or serum tubes after an overnight fasting period (BD Vacutainer, Franklin Lakes, NJ). All blood samples obtained from the subjects were stored at −70°C. Concentrations of glucose, triglycerides, cholesterol, and HDL cholesterol were measured using an automatic analyzer (Model 7180; Hitachi, Tokyo, Japan). Serum insulin was measured using an immunoradiometric assay kit (BioSource Europe, Nivelles, Belgium). Insulin resistance was determined by the widely used homeostasis model assessment (HOMA) of insulin resistance method, which was calculated as (plasma glucose [mmol/L] × insulin [μIU/mL])/22.5.

DNA Extraction and Quantitative DNA Methylation Analysis by Pyrosequencing

Genomic DNA samples from the cord blood and blood samples of children were extracted from 250 μL of whole blood using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA), according to the manufacturer's protocol. The purity and concentration of isolated DNA were determined using a spectrophotometer (Model ND-2000; Nanodrop Technologies, Wilmington, DE).

The downstream exon 3 region of POMC was amplified using a forward primer

and a biotinylated reverse primer designed by PSQ Assay Design (Biotage AB, Uppsala, Sweden) (Supplementary Table 1). Each genomic DNA sample (20 ng) was treated with sodium bisulfite using the EZ DNA Methylation kit (ZYMO Research, Irvine, CA), according to the manufacturer's instructions. Each target region of bisulfite-converted DNA was amplified in a 25-μL reaction with the primer sets and 5 units of Taq polymerase (Solgent Co., Ltd., Daejeon, Korea). Briefly, DNA samples were heated to 94°C for 10 min and then amplified for 45 cycles consisting of 94°C for 30 s, the annealing temperature for 30 s, and 72°C for 30 s. For the annealing step, the temperature was set at 56, 58, and 55°C for regions 2, 3, and 4, respectively. All reactions were then incubated at 72°C for 10 min and cooled to 4°C. The PCR products were visualized on a 1.5% agarose gel by ethidium bromide staining for verification.

Pyrosequencing reactions of POMC methylation were conducted with sequencing primers on the PSQ HS 96A System (Biotage AB) according to the manufacturer's specifications (Supplementary Fig. 1). The percentage of methylation was calculated by dividing the peak area of the methylation cytosine (mC) peak by the combined peak areas of the non-methylation cytosine (C). The methylation index of each sample was calculated as the average value of mC(mC+C) for all examined CpGs in the target region. POMC methylation in cord blood and blood samples from children are expressed as percentages. Because of the high positive correlation of methylation among CpG regions, we applied the average POMC methylation value.

Statistical Analyses

Because there is no cutoff point for POMC methylation in cord blood, we defined those <10th percentile and >90th percentile of POMC methylation as the low and high methylation groups, respectively, according to a previous report (21–25). To assess the relationship of cord blood and current POMC methylation, a correlation analysis of the parameters studied was also carried out, and the Pearson correlation index was calculated. Birth weight and maternal features during pregnancy in terms of POMC methylation status at birth were compared using one-way ANOVA. We assessed the association of POMC methylation status at birth with adiposity or metabolic components using ANCOVA and multiple linear regression analysis, adjusted for the sex, birth weight, follow-up age, and current POMC methylation (percentage) of children as a continuous value. For group differences, we carried out the pairwise comparisons under considering covariates. To satisfy statistical assumptions of normality, insulin and triglycerides values were log-transformed, and then the results are presented as back-transformed values. Statistical analyses were conducted using the SAS software (version 9.3; SAS Institute Inc., Cary, NC). All analyses were two-tailed, and a P value of <0.05 was considered to indicate statistical significance.

RESULTS

The basic characteristics of the subjects are provided in Table 1. Among the 90 subjects, there were 43.33% boys. Also, the mean of BMI z-score and the percent body fat mass were −0.21 and 22.83%, respectively.

The PCR products of the POMC gene were 109 bp in length. PCR products were analyzed using the pyrosequencing method for CpG island detection. Methylated cytosine was detected as a thymine peak; nonmethylated cytosine was detected as a cytosine peak (Supplementary Fig. 1). The average cord blood methylation value for the POMC gene was 49.53; by methylation site: 52.48 (site 1), 50.30 (site 2), 47.57 (site 3), and 47.78 (site 4). The average methylation rate of 49.51 in blood in childhood was similar to that in cord blood (Supplementary Table 2). Additionally, cord blood POMC methylation status was strongly correlated with POMC methylation values in childhood (r = 0.80; P = 0.0001; Supplementary Fig. 2).

Table 2 shows comparisons of birth outcomes, maternal features during pregnancy, and POMC methylation in childhood according to cord blood POMC methylation status. In the high

Table 1—Basic characteristics of study subjects

	Overall (n = 90)
Maternal features	
Age at delivery (years)	31.19 ± 3.67
Prepregnancy BMI (kg/m^2)	20.71 ± 3.37
Weight gain during pregnancy (kg)	13.21 ± 4.24
Birth outcome	
Birth weight (kg)	3.29 ± 0.42
Ponderal index (kg/m^3)	26.90 ± 1.88
Gestational age (weeks)	39.4 ± 1.30
Preterm (n)	2 (2.22%)
Postterm (n)	1 (1.11%)
Small for gestational age (n)	7 (7.78%)
Large for gestational age (n)	1 (1.11%)
Offspring features included with adiposity or metabolic indices	
Boys (n)	39 (43.33%)
Age (years)	7.80 ± 0.77
BMI z-score	−0.21 ± 1.25
PBF (%)	22.83 ± 6.59
TGs (mg/dL)	72.50 (49.00–97.00)
HDL cholesterol (mg/dL)	59.42 ± 10.89
Total cholesterol (mg/dL)	164.23 ± 22.94
Insulin (μIU/mL)	7.67 (6.23–9.07)
Glucose (mmol/L)	4.36 ± 0.37
HOMA index	1.60 ± 0.62
Average of cord blood POMC methylation	49.53 ± 7.77

Data are means ± SD or median (interquartile range), unless otherwise indicated. PBF, percent body fat mass; TG, triglyceride.

Table 2—Comparisons of birth outcomes, maternal features during pregnancy, and POMC methylation in childhood according to cord blood POMC methylation status

	Cord blood POMC methylation					
	≤10th (1) (n = 10)	11th–89th (2) (n = 70)	≥90th (3) (n = 10)	P value† (1) vs. (2)	P value† (2) vs. (3)	P value† (1) vs. (3)
Birth outcome						
Average of POMC methylation in children (%)	37.57 ± 3.24	49.77 ± 5.83	59.60 ± 10.84	<0.0001	<0.0001	<0.0001
Gestational age (weeks)	39.3 ± 1.2	39.4 ± 1.0	38.5 ± 2.60	0.83	0.08	0.24
Birth weight (kg)	3.44 ± 0.37	3.33 ± 0.36	2.89 ± 0.64	0.40	<0.01	<0.01
Ponderal index (kg/m^3)	26.66 ± 1.53	27.18 ± 1.77	25.18 ± 2.13	0.40	<0.01	0.07
Maternal features						
Maternal age at delivery (years)	31.80 ± 3.55	31.01 ± 3.60	31.80 ± 4.52	0.53	0.53	>0.99
Prepregnancy BMI (kg/m^2)	21.51 ± 2.75	20.64 ± 3.51	20.45 ± 3.15	0.45	0.87	0.49
Weight gain during pregnancy (kg)	13.00 ± 4.40	13.42 ± 3.55	11.98 ± 7.74	0.77	0.32	0.60
Weight change rate (%)	22.32 ± 8.49	25.95 ± 8.02	24.07 ± 14.14	0.23	0.53	0.66

Data are means ± SD. †Using the ≤10th percentile group or ≥90th percentile group as dummy variables (reference: 11th–89th group); P value obtained from multiple linear regression analysis.

POMC methylation group, birth weight (P = 0.01) and ponderal index (P = 0.01) were significantly lower than in the mid-POMC methylation group (Table 2). However, birth weight and ponderal index in the low POMC methylation group were not significantly different than those in the mid-POMC methylation group. The maternal characteristics, such as gestational age, maternal age, BMI, and weight gain during pregnancy did not differ significantly ($P > 0.05$).

Table 3 shows body composition and metabolic parameters in children aged 7–9 years according to cord blood POMC status. Regarding biochemical parameters in children, triglyceride concentrations were significantly higher in the high POMC methylation group than the mid-POMC methylation group (113.89 mg/dL, 95% CI 78.00–166.30 vs. 67.29 mg/dL, 95% CI 59.96–75.52; P = 0.01). The insulin concentrations in the high POMC methylation group were also significantly higher than in the mid-POMC methylation group (10.13 μIU/mL, 95% CI 8.09–10.31 vs. 7.64 μIU/mL, 95% CI 7.13–8.19; P = 0.02). When comparing between low and high POMC methylation group, triglycerides showed statistically significant difference (P = 0.03), and HDL cholesterol, insulin, and HOMA showed marginally significant differences (P = 0.09 in HDL cholesterol, P = 0.05 in insulin, and P = 0.09 in HOMA, respectively). There was no significant difference in BMI z-score, percentage of body fat mass, total cholesterol, or glucose among the groups ($P > 0.05$). However, HDL cholesterol and HOMA index showed marginally significant differences between the mid-POMC methylation group and high POMC methylation group (P = 0.06, respectively).

CONCLUSIONS

In this prospective cohort study, high POMC methylation in cord blood was associated with lower birth weight, and children with higher POMC methylation in cord blood showed higher triglycerides and higher insulin levels in blood. We investigated whether perinatal epigenetic analyses using cord

Table 3—Body composition and metabolic components in children aged 7–9 years according to cord blood POMC methylation status

	Cord blood POMC methylation					
	≤10th (1) (n = 10)	11th–89th (2) (n = 70)	≥90th (3) (n = 10)	P value† (1) vs. (2)	P value† (2) vs. (3)	P value† (1) vs. (3)
BMI z-score	−0.45 (−1.43 to 0.52)	−0.26 (−0.56 to 0.05)	0.31 (−0.68 to 1.31)	0.70	0.28	0.34
PBF (%)	22.07 (17.06–27.08)	22.60 (21.04–24.16)	25.24 (20.13–30.36)	0.84	0.33	0.44
TGs (mg/dL)‡	57.97 (40.02–83.97)	67.29 (59.96–75.52)	113.89 (78.00–166.30)	0.45	0.01	0.03
HDL cholesterol (mg/dL)	63.04 (54.78–71.30)	60.04 (57.47–62.61)	51.50 (43.07–59.94)	0.49	0.06	0.09
Total cholesterol (mg/dL)	161.75 (143.95–179.55)	166.32 (160.78–171.86)	152.10 (133.91–170.28)	0.63	0.14	0.51
Insulin (μIU/mL)‡	7.10 (5.70–8.86)	7.64 (7.13–8.19)	10.13 (8.09–12.70)	0.53	0.02	0.05
Glucose (mmol/L)	4.44 (4.16–4.73)	4.36 (4.27–4.45)	4.29 (4.00–4.58)	0.57	0.65	0.51
HOMA index	1.39 (0.94–1.85)	1.57 (1.43–1.71)	2.03 (1.57–2.50)	0.46	0.06	0.09

Data are presented as least squares means with 95% CIs. HOMA index was calculated as: (plasma glucose [mmol/L] × insulin [μIU/mL])/22.5. PBF, percent body fat mass; TG, triglyceride. †Using the ≤10th percentile group or ≥90th percentile group as dummy variables (reference: 11th–89th group); P value obtained from multiple linear regression analysis with adjusted for sex, birth weight, age, and POMC methylation (%) in blood at childhood. ‡To satisfy the normal distribution, TGs and insulin were analyzed as log-transformed scale and then results presented as back-transformed value.

blood may have use in identifying susceptibility to subsequent obesity and metabolic syndrome.

The high POMC methylation in cord blood group had significantly lower birth weights. They also showed higher triglycerides and insulin concentrations in blood during childhood. Animal experiments suggested that lower birth weight may indicate significantly higher concentrations of cholesterol, HDL cholesterol, and triglycerides (26). It may be that low birth weight predisposes individuals to type 2 diabetes and metabolic syndrome, called "fetal programming" or the "Barker hypothesis" (8,27). Thus, we suggest that lower-birth-weight infants with high POMC methylation are likely to have increased risks of metabolic syndrome, obesity, type 2 diabetes, and cardiovascular disease (15,28,29).

A hypermethylation variant targeting individual CpGs at the intron 2 and exon 3 boundary of the POMC gene, as determined by bisulfite sequencing, was significantly associated with childhood obesity (18). The hypermethylation variant showed reduced mRNA expression of the POMC transcript containing exon 3 regions (30). In addition, the activation of POMC in neurons resulted in the cleavage of the prohormone, POMC, to α-melanocyte stimulation hormone (MSH) and the release of α-MSH in the paraventricular nucleus (15). In the paraventricular nucleus, the orexigenic pathway acts to increase food intake, in part, by agouti-related peptide, preventing α-MSH from binding to the melanocortin-4 receptor and melanocortin-3 receptor (31). One of the key players in obesity and metabolic syndrome is POMC, a precursor of a variety of neuropeptides (15,32). Different polypeptide products of POMC, which act in the brain to control food intake, involving the MC4R gene, are mediators of leptin action (33). Although we did not measure POMC gene expression, we suggest that high POMC methylation likely affects POMC gene expression. Such a change in POMC gene expression could induce higher levels of triglycerides and insulin.

Our findings also indicated that cord blood POMC methylation status and POMC methylation status in childhood were significantly correlated. High expression of the POMC gene affects immature neurons in early embryonic stages (16). The fetal hypothalamic appetite regulation network is a prime candidate for epigenetic fetal programming (14). It may be that the fetal period is an important time for neural development, affected by fetal programming. First, the level of POMC methylation at the developmental stage of the fetus is determined. Then, the continuous influence of high POMC methylation would induce highly correlated POMC methylation levels in peripheral blood throughout the childhood period.

Generally, DNA methylation levels are tissue-specific. However, analysis of methylation using cell-free circulating DNA can facilitate the development of accurate biomarkers for detection and prediction of prognosis and outcomes (34). In another study, a POMC CpG methylation variant was associated with body weight and was detected in DNA from peripheral blood leukocytes (18). Ultimately, we suggest that POMC methylation, assessed using cord blood, may be an early predictor of metabolic syndrome.

This is a prospective cohort study in which we evaluated cord blood POMC methylation as an early predictive marker of metabolic syndrome. We identified at first that high POMC methylation may be associated with metabolic syndrome in childhood.

This study had some limitations. First, the statistical power is low because of the small number of subjects; only 10 subjects were included in each of the low and high methylation groups. Second, we did not assess any joint effect of POMC single nucleotide polymorphism and methylation status because of the small numbers of subjects. Third, we did not obtain target organ tissues from the subjects and measured only POMC methylation using cord blood and peripheral blood in children, although DNA methylation is tissue-specific. Future study is needed with more samples.

Despite these limitations, this study has the strength of including human cord blood, which is a material of considerable value that is not readily obtained, and DNA methylation data, which are costly to obtain but of great value.

In conclusion, a high POMC methylation status in cord blood was associated with lower birth weight, and children with higher POMC methylation in cord blood showed higher triglycerides and insulin levels in blood. Thus, POMC methylation status in cord blood may be an early predictive marker of metabolic syndrome.

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