Insulin-Like Growth Factor 2/H19 Methylation at Birth and Risk of Overweight and Obesity in Children

Ellen Perkins, MD[1], Susan K. Murphy, PhD[2,3], Amy P. Murtha, MD[4], Joellen Schildkraut, PhD[1,5], Randy L. Jirtle, PhD[6], Wendy Demark-Wahnefried, PhD[8], Michele R. Forman, PhD[9], Joanne Kurtzberg, MD[7], Francine Overcash, MPH[1,5], Zhiqing Huang, MD, PhD[2], and Cathrine Hoyo, PhD[1]

Objective To determine whether aberrant DNA methylation at differentially methylated regions (DMRs) regulating insulin-like growth factor 2 (*IGF2*) expression in umbilical cord blood is associated with overweight or obesity in a multiethnic cohort.

Study design Umbilical cord blood leukocytes of 204 infants born between 2005 and 2009 in Durham, North Carolina, were analyzed for DNA methylation at two *IGF2* DMRs by using pyrosequencing. Anthropometric and feeding data were collected at age 1 year. Methylation differences were compared between children >85th percentile of the Centers for Disease Control and Prevention growth charts weight-for-age (WFA) and children ≤85th percentile of WFA at 1 year by using generalized linear models, adjusting for post-natal caloric intake, maternal cigarette smoking, and race/ethnicity.

Results The methylation percentages at the *H19* imprint center DMR was higher in infants with WFA >85th percentile (62.7%; 95% CI, 59.9%-65.5%) than in infants with WFA ≤85th percentile (59.3%; 95% CI, 58.2%-60.3%; $P = .02$). At the intragenic *IGF2* DMR, methylation levels were comparable between infants with WFA ≤85th percentile and infants with WFA >85th percentile.

Conclusions Our findings suggest that *IGF2* plasticity may be mechanistically important in early childhood overweight or obese status. If confirmed in larger studies, these findings suggest aberrant DNA methylation at sequences regulating imprinted genes may be useful identifiers of children at risk for the development of early obesity. *(J Pediatr 2012;161:31-9)*.

The prevalence of obesity in children <5 years old has more than doubled since the 1990s, affecting 1 in 5 children in the United States, with minority populations disproportionately affected.[1,2] Early childhood obesity and excessive infant weight gain have been associated with higher blood pressure[3] and wheezing[4] in childhood and obesity and metabolic and cardiovascular diseases in adulthood.[5] Childhood obesity may be an early adaptive response, hypothesized to be largely driven by epigenetic mechanisms that guide expression of genes involved in energy balance, culminating in gene expression profiles that predispose children to overweight and obesity.

A commonly studied epigenetic mechanism is DNA methylation, in part because of its stability in conditions in which human specimens are collected. Animal evidence from the last decade indicates that DNA methylation alterations at susceptible loci link the early environment to obesity in later life. The monoallelic expression of imprinted genes-a class of genes that is over-selected for growth effectors[6] is regulated (and dysregulated) by DNA methylation at differentially methylated regions (DMRs). Aberrant methylation at these DMRs has been associated with aberrant changes in gene expression. Because imprinted genes occur in clusters throughout the genome[7] and their regulation may be networked,[6] a single DMR can regulate the expression of several genes; suggesting aberrant methylation at a single DMR can affect the expression of several of these growth effectors. The most studied imprinted gene is insulin-like growth factor 2 (*IGF2*). *IGF2* is a paternally expressed imprinted gene that encodes a potent mitogenic growth factor that plays a critical role in placental and fetal development. Aberrant DNA methylation at the *IGF2* DMRs has been associated with increased gene expression, and presumably, circulating *IGF2* levels and risk of overweight status, obesity, and overgrowth disorders.[8]

Numerous epidemiological studies have reported a small but consistently lower risk of rapid growth and obesity in breastfed children. Although epigenetic mechanisms have been proposed,[9] the mechanism by which breastfeeding

BMI	Body mass index
CpG	Cytosine-phosphate-Guanine
DMR	Differentially methylated region
IGF2	Insulin-like growth factor 2
NEST	Newborn Epigenetics Study
WFA	Weight-for-age

From the [1]Department of Community and Family Medicine, [2]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, [3]Department of Pathology, [4]Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, [5]Program of Cancer Detection, Prevention, and Control, [6]Department of Radiation Oncology, and [7]Department of Pediatrics, Duke University, Durham, NC; [8]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL; and [9]Department of Epidemiology, MD Anderson Cancer Center, Houston, TX

Supported by the National Institutes of Health (grants ES016772, R21ES014947, K01CA104517, R01DK085173, and R01 ES015165). The authors declare no conflicts of interest.

 10.1016/j.jpeds.2012.01.015

confers a lower risk of childhood obesity remains unknown. Because breastfeeding varies by race/ethnicity, we evaluated whether aberrant DNA methylation at two *IGF2* DMRs at birth increases the risk of overweight and obesity status in early childhood, and this association may vary by ethnic group and breastfeeding.

Methods

Study participants were children born to women who sought obstetric care at Duke Obstetrics and Durham Regional Hospital (Durham, North Carolina) between 2005 and 2009, as part of the Newborn Epigenetics Study (NEST). NEST is a prospective, perinatal epidemiologic study aimed at determining how the in utero environment influences epigenetic profiles and phenotypes in children. Procedures for participant enrollment have been detailed elsewhere.[10] In brief, between 2005 and 2009, women who attended prenatal care at Duke's Maternal Fetal Medicine and one affiliated clinic and intended to use Duke or Durham Regional Hospitals for their obstetrics care were enrolled in the study. Inclusion criteria were age ≥18 years and English speaking. We excluded women who planned to give offspring up for adoption and women who were human immunodeficiency virus-positive, because the effect of anti-retroviral medications on the methylation profile is still unknown. Most women (>50%) were from Durham County, North Carolina, although the catchment area also included contiguous counties.

Between 2005 and 2009, 940 of the 1101 women (85%) approached consented to participate and were enrolled in NEST. Methylation analyses were performed on cord blood leukocyte DNA of the first 438 neonates (46%). The final sample includes the first 204 offspring who had methylation data and had reached 1 year of age by August 2010 and whose mothers completed a follow-up questionnaire. Follow-up is ongoing. The distribution of factors that may affect overweight status or obesity, including maternal age ($P = .54$), education ($P = .94$), race/ethnicity ($P = .43$), and sex of infant ($P = .80$) were comparable between the 940 infant-mother pairs enrolled and the 438 infant-mother pairs with DNA methylation data. These factors were also comparable between the first 204 infants in whom follow-up data have been collected to date and the 428 in whom methylation analyses were conducted. Most questionnaires (78%) were completed via mailed survey, 16% were interviewer-administered during a pediatric office visit, and 6% were telephone-administered.

To characterize the in utero environment, pregnant women completed a questionnaire soliciting information on sociodemographic characteristics, maternal lifestyle factors, and morbidity at recruitment. Women self-reported maternal pre-pregnancy anthropometric measurements, race, level of education, and cigarette smoking status. Self-reported height and usual pre-pregnancy weight were used to calculate maternal body mass index (BMI). At delivery, medical records were abstracted to obtain maternal age at delivery and parturition data, including morbidity during pregnancy, mode of delivery, infection in labor, gestational age at birth, and infant data including birth-weight, head circumference, and Apgar score. Approximately 1 year after the child's birth, a 1-year questionnaire was completed to obtain data on anthropometric measures, temperament, and use of childcare. We also estimated the infant's caloric intake from a single 24-hour dietary recall by using the University of Minnesota's Nutrition Data System for Research (2008). Although a single 24-hour recall cannot adequately reflect total energy intake in the first year of life, we used this information as an indicator to adjust for, in the investigation of *IGF2* DMR methylation at birth and overweight and obesity in early childhood.

To estimate breastfeeding status, we used additional dietary information collected in the 1-year questionnaire, which asked mothers to report—for each of the first 12 months of life—whether their child was fed breast milk, cow's milk formula, and/or soy milk. Specifically, mothers were asked, "How did you feed your baby during his/her first year? (Please go month by month)"; for each month, mothers responded either "yes" or "no" individually to breast milk, formula, and soy milk. Questionnaires were completed when the child was between 12 and 29 months of age. The median age of the child when the first follow-up questionnaire was mailed was 14 months, and the median time to returning the questionnaire was 1 month.

To verify the accuracy of the infant weights reported by mothers in the questionnaire, anthropometric measurements at child age 1 year were abstracted from the medical records of 72 infants who had the data available within 1 month of receipt of their 1-year questionnaire. The Pearson correlation co-efficient between anthropometric measurements abstracted from the medical record and weights reported by mothers was 0.94 ($P < .0001$), suggesting that mothers accurately reported their offspring's weight.

At delivery, cord blood specimens were collected in ethylenediaminetetraacetic acid-treated tubes within minutes of delivery. Specimens were processed to obtain plasma and buffy coat for DNA extraction (Qiagen, Valencia, California); samples were stored at −80°C until processed. DNA was extracted by using Puregene reagents according to the manufacturer's protocol (Qiagen).

DNA methylation from leukocytes of umbilical cord blood samples is generally used as a surrogate measure of genomic stability in the study of prenatal exposures and epigenetic response to these exposures, because they contribute to long-term health in humans.[11-16] Genomic DNA was modified by treatment with sodium bisulfite by using high throughput methods as previously described.[17] Bisulfite treatment of denatured DNA converts unmethylated cytosines to uracils and leaves methylated cytosines unchanged. Pyrosequencing was performed by using a Biotage Pyromark MD pyrosequencing instrument (Qiagen). We evaluated two regions, including 3 cytosine-phosphate-Guanine (CpG) sites comprising the intragenic *IGF2* DMR, upstream of exon 3 (chr11p15.5, site 1: 2 109 519; site 2: 2 109 516; and site 3: 2 109 500; NCBI Human Genome Build 37.1; National Institutes of Health,