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Maternal Pre-pregnancy Body Mass Index and Circulating MicroRNAs in Pregnancy

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Abstract

Background—Maternal pre-pregnancy overweight and obese status has been associated with a number of pregnancy complications and adverse offspring outcomes. Mechanisms for observed associations, however, are largely unknown. We investigated associations of pre-pregnancy body mass index with early-mid pregnancy epigenetic biomarkers, circulating microRNAs.

Methods—Peripheral blood was collected from participants (16–27 weeks gestation) of two multi-racial pregnancy cohorts, the Omega Study and the Pregnancy Outcomes and Community Health Study. Plasma miRNA expression was characterized using epigenome-wide (319 miRNAs) profiling among 20 pregnant women in each cohort. Cohort-specific linear regression models that included the predictor (pre-pregnancy body mass index), the outcome (microRNA expression), and adjustment factors (maternal age, gestational age at blood collection, and race) were fit.

Results—Expression of 27 miRNAs was positively associated with pre-pregnancy body mass index in both cohorts (p -values <0.05). A number of these differentially expressed miRNAs have previously been associated with adipogenesis (e.g. let-7d*, miR-103-2*, -130b, -146b-5-p, -29c, and -26b). Identified miRNAs as well as their experimentally validated targets participate in pathways that involve organismal injury, reproductive system disease, connective tissue disorders, cancer, cellular development, growth and proliferation.

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Conclusion—Pre-pregnancy body mass index is associated with circulating miRNAs in early-mid pregnancy.

Keywords

microRNAs; epigenetics; pre-pregnancy obesity; pregnancy

Introduction

Obesity, a largely unabated epidemic affecting close to a third of the population, is associated with numerous cardiovascular and metabolic disorders among the general population.^{1–2} Maternal pre-pregnancy overweight and obesity status have also been associated with a number of pregnancy complications (including preeclampsia, gestational diabetes, and urinary tract infection) and adverse offspring outcomes (including suboptimal fetal growth and later life obesity and cardiometabolic diseases).^{3–5} Biological mechanisms underlying observed associations, however, are largely unknown. While available evidence points to the role of gene-environment interactions in the relationships between high pre-pregnancy body-mass index, pregnancy complications, and adverse outcomes, previous research has been limited by inadequate assessment of biomarkers that represent these interactions, such as biomarkers of epigenetic processes.

Characterizing microRNA (miRNA) expression, part of the epigenetic regulatory mechanism, can provide unique opportunities to assess gene-environment interactions.⁶ MiRNAs have broad regulatory roles in obesity-related pathways such as low-grade chronic inflammation, oxidative stress, insulin resistance, dyslipidemia, endothelial dysfunction, and nutrient/energy imbalances.^{7–9} Therefore, miRNA investigations can enhance mechanistic understanding of the effects of high pre-pregnancy body mass index on pregnancy and parturition. In addition, miRNAs have been shown to be important preventative and therapeutic targets in several research areas, highlighting their potential significance in perinatal clinical care and research.^{10–11} Several studies, primarily experimental, have investigated miRNA expression in adipose and other tissues, in relation to obesity.^{12–18} Overall, findings from these obesity-miRNA studies suggest differential expression of miRNAs (e.g. over-expression of miR-143 and miR-103)^{16–18} in relation to obesity or obesity-related parameters. Studies investigating miRNA expression in relation to pre-pregnancy body mass index among pregnant women or their offspring, however, are few.^{19–20} To our knowledge, only one recent study conducted in Europe, investigated circulating miRNAs in pregnancy in relation to pre-pregnancy body mass index (BMI).²¹ In two well-characterized cohorts, the Omega study and the Pregnancy Outcomes and Community Health (POUCH) study, based in Washington State and Michigan, respectively, we conducted secondary analyses of epigenome-wide data to examine whether pre-pregnancy BMI is associated with early-mid pregnancy circulating miRNA expression.

Subjects

The current report is based on secondary analyses of data collected as part of a multi-stage, nested case-control study of preterm delivery and miRNA expression among participants in

the Omega and the POUCH studies. The objective of the Omega study (1996–2008) was to investigate risk factors for pregnancy complications among attendants of prenatal clinics affiliated with the Swedish Medical Center (SMC), in Seattle, WA.²² The POUCH study (1998–2004) was designed to investigate risk factors and mechanisms of preterm delivery among pregnant women recruited from 52 clinics in five Michigan communities.²³

Materials & Methods

The parent preterm delivery-miRNA study was conducted in two stages, epigenome-wide and candidate miRNA profiling, among self-reported Black and White Omega and POUCH study participants. For epigenome-wide profiling, a total of 11 White and 9 Black preterm delivery cases and an equivalent number of controls, frequency-matched on maternal race, age, and gestational age at blood collection were selected from each cohort. For the current study, epigenome-wide expression data collected from control participants, N=40 (N=20 from each cohort) were analyzed. Since the candidate miRNA selection in the candidate profiling experiments of the parent study was based on preterm delivery, we did not include the candidate miRNA expression data in the current analyses. Study participants provided written informed consent, and institutional review boards of the SMC and Michigan State University approved study protocols in the Omega and POUCH studies, respectively.

Data Collection

Information on maternal socio-demographic characteristics, family/medical histories, occupation, reproductive and medical histories, height, weight and other maternal characteristics were collected in early to mid-pregnancy using interviewer-administered questionnaires, in the Omega study, and both interviewer-administered and self-administered questionnaires, in the POUCH study. Pre-pregnancy BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Participants also provided peripheral blood samples (8–20 weeks in the Omega study and 16–27 weeks in the POUCH study) from which plasma was isolated for miRNA expression measurements. At the end of pregnancy, medical records were reviewed to abstract information on course and outcomes of the pregnancy.

RNA Isolation and Quality Control

Total RNA was extracted from 0.25–1.40 ml of plasma using TRI Reagent® BD (Molecular Research Center, Cincinnati, OH) according to manufacturer's instructions with minor modifications. To determine the quality of RNA isolated from plasma samples, RNA, equivalent to 40ul of plasma samples randomly selected from each isolation batch, was reverse transcribed (RT) using known miRNA-specific RT primers (Life Technologies, Carlsbad, CA). Polymerase chain reactions (PCR) were performed on the synthesized and diluted cDNA product in duplicate wells using known miRNA-specific Taqman PCR primers. RNA quality was assessed using threshold cycle (Ct) measurement of two endogenous miRNAs (miR-150 and miR-451) and a synthetic miRNA sequence, cel-miR-257, in randomly selected samples from each batch. Samples with Ct<35 for endogenous miRNAs and Ct<26.5 for cel-miR-257 were deemed of satisfactory quality,

based on previous reports and guidelines^{24–26}. All RNA samples were labeled using the same standardized protocols.

Microarray-based Expression Profiling and Data Pre-processing

Microarray-based epigenome-wide miRNA profiling experiments were performed at Ocean Ridge Biosciences (Palm Beach Gardens, FL) using the multispecies miRBase version 16.0 microarrays produced by Microarrays Inc. (Huntsville, AL). Total RNA was 3'-end labeled with Oyster-500 fluorescent dye using the Flash Tag RNA labeling Kit (Genisphere, Hatfield, PA). A synthetic miRNA was added at 200 amoles per sample prior to labeling and hybridization. Labeled RNA samples were hybridized to the miRNA microarrays according to conditions recommended in the Flash Tag RNA labeling kit manual. Microarrays were scanned using an Axon GenePix 4000B scanner (Molecular Devices Corp., Sunnyvale, CA), and data were extracted using GenePix V4.1 software (Molecular Devices Corp., Sunnyvale, CA).

Spot intensities were obtained by subtracting the median local background from the median local foreground for each spot. The spot intensities and 95th percentiles of negative controls (TPT95) were transformed by taking the log (base 2) of each value. Sensitivity of hybridization was confirmed by detection of hybridization signal ($>TPT95$) for all spikes that were added during isolation and labeling. Specificity of hybridization was confirmed by comparing the hybridization signal for perfect match probes with double mismatch and shuffled version probes for three endogenous miRNAs. Reproducibility of arrays was determined by monitoring the hybridization intensity for the triplicate human spots on each array. Prior to analyses, average probe intensities (not including spots with poor quality) were calculated from triplicate spots. The normalization factor (F_j) for each microarray (j) was determined by obtaining the 20% trim mean of the human miRNA probe intensities that were detected above $TPT95+1$ in all samples, with standard deviation of probe intensities less than 1.0 across samples, and not saturated in $> 90\%$ of the samples^{27, 28}. Probe intensities were normalized by subtracting F_j from each spot intensity on microarray j , and scaled by adding the grand mean of the F_j 's across all microarrays. Human miRNAs ($N=319$) that were represented by probes (with intensity $>TPT95+1$) in $\geq 10\%$ of the samples were selected for statistical analyses.

Statistical Analyses

All analyses were conducted separately within each cohort. Distributions of maternal socio-demographic, medical and clinical characteristics according to pre-pregnancy BMI (<18 , $18-25$ kg/m² or ≥ 25 kg/m²) were examined. Analyses of cohort site-specific epigenome-wide miRNA expression profiling data were conducted separately. Overall objectives of the statistical analyses were to identify miRNAs whose expression is associated with pre-pregnancy BMI. For these analyses, linear regression models were used to evaluate the association between pre-pregnancy BMI (predictor modeled as a continuous variable) and miRNA expression (outcome modeled as a continuous variable), adjusting for other factors (maternal age, gestational age at blood collection, and race). Beta estimates and standard errors (along with p-values) from these models were used to determine whether expression of miRNAs was associated with pre-pregnancy BMI. The set of differentially expressed

miRNAs consisted of miRNAs with significant p-values (p-value < 0.05) in both Omega and POUCH cohorts. We examined functions and functional relationships of differentially expressed miRNAs as well as their experimentally validated targets using the Ingenuity Pathway Analysis software (IPA, Ingenuity, Redwood, CA). In IPA, each miRNA or target gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These miRNAs or target genes were then overlaid onto a global molecular network developed from information contained in the IPKB. Network enrichment was assessed using a network score (negative log of p-values of Fisher tests).

All statistical tests were 2-tailed and statistical significant was defined at alpha of 0.05. R software package was used for the analyses.

Results

Omega study participants were older than POUCH study participants (29.8 vs. 26.0 years, respectively) (Table 1). POUCH study participants had higher pre-pregnancy BMI compared with Omega study participants (26.92 kg/m² vs. 24.22 kg/m², respectively). The average gestational age at blood collection for Omega study participants was 16 weeks gestation while the average gestational age at blood collection for POUCH study participants was 22.5 weeks of gestation.

Overall, we identified 27 miRNAs with expression that was associated (all positively) with pre-pregnancy BMI in both cohorts (p-values<0.05) (Table 2). A number of these differentially expressed miRNAs have been previously associated with adipogenesis (e.g. let-7d*, miR-103-2*, -130b, -146b-5-p, -29c, and -26b). The full list of miRNAs that were differentially expressed (p-values<0.05) in at least one of the cohorts in the epigenome-wide profiling experiments is shown in the Supplemental Table.

In pathway analyses of functions and functional relationships of differentially expressed miRNAs in both cohorts (N=27), using IPA, the top two networks (scores 32 and 20) were overrepresented by miRNAs that regulate organismal injury and abnormalities, reproductive system diseases, connective tissue disorders, and cancer. The top network (score 32) is shown in Figure 1. The top network (score 34) overrepresented by experimentally validated gene (mRNA) targets of differentially expressed miRNAs in our set included genes with functions that involve cellular development, cellular growth and proliferation, and cancer (Figure 2).

Discussion

In this study, we identified a set of circulating miRNAs in early-mid pregnancy that are associated with pre-pregnancy BMI. Identified miRNAs include well-described adipogenesis related miRNAs as well as novel miRNAs which participate directly or through their gene targets, in pathways that involve organismal injury, reproductive system disease, connective tissue disorders, cancer, cellular development, growth, and proliferation.

To our knowledge, only one recent study investigated associations of circulating miRNA expression profiles in pregnancy in relation to pre-pregnancy and gestational obesity.²¹ This

study by Carreras-Badosa et al. was conducted in the setting of prenatal primary care in Girona (Spain) among 70 pregnant women who had uncomplicated pregnancies. The authors identified 18 miRNAs that were differentially expressed in plasma (during the second trimester of pregnancy), in relation to maternal obesity. Seven of these miRNAs or miRNAs in their families (miR-29c, -103, -128a, 130a, -221, 423-5p, and -652) were also differentially expressed in relation to pre-pregnancy BMI in the current study. Nardelli et al. examined miRNA expression in amnion (at-delivery) among five obese and ten control women.¹⁹ In that study, seven miRNAs (miR-422b, -219, -575, -523, -579, -618, and -659) were exclusively expressed in the amnion of obese women, while 13 miRNAs were upregulated and 12 downregulated in amnion of obese women, compared with amnion of non-obese women.¹⁹ These included the let-7d (upregulated) miRNA which was upregulated in our study. Several studies have also examined circulating miRNAs as well as miRNA expression in adipose and other related tissues, in relation to obesity or BMI in non-pregnant populations.^{12–18, 29–31} Ortega et al. examined circulating miRNA among morbidly obese White men (N=32 discovery and N=80 replication cohort) and changes after weight loss.²⁹ In that study, miR-140-5p, -142-3p and -222 were upregulated while miR-532-5p, -125b, -130b, -221, -15a, -423-5p, and -520c-3p were downregulated among the obese group.²⁹ In addition, a discriminant function of 3 miRNAs (miR-15A, -520c-3p, and -423-5p) was specific for morbid obesity, with an accuracy of 93.5%. In a study by Pescador et al., expression of three miRNAs (miR-138, -15b, and -376a) in serum was found to have the potential as predictive biomarkers of obesity with area under the curve ranging from 0.888 to 0.995.³⁰ Martinelli et al. have demonstrated differential expression of 42 miRNAs, including miR-519d (upregulation) and miR-376 (upregulated), in adipose tissue of obese individuals, compared with non-obese individuals.³¹

Of miRNAs that were previously identified in studies that examined circulating miRNAs or tissue miRNA expression in relation to BMI or obesity, several were associated with pre-pregnancy BMI in our study. These included miR-130b, (targeting PPARG), let-7 (targeting HMGA2, E2F6, and CDC34), -103 (targeting ARNT, FZD1, RUNX1T1, ETO, MTG8, PDK1 and WNT3A), -221/222 (targeting CDKN1B and p27), -423-5p (targeting CD1C), -26 (targeting EZH2 and SMAD1), -128-2 (targeting TXNIP), -146b (targeting NFKB), -29 (targeting HDAC4), -425 (targeting PTEN) and -376 (targeting ATG4C and BECN1).^{12–21, 29–38} It is important to note that the direction of associations (upregulation or downregulation) for some of these miRNAs was not similar in several of the studies, including our own. This may be due to differences in samples used for the expression measurements, and needs further investigation. In addition to these miRNAs, we identified a number of novel miRNAs that are involved in adipogenesis including miR-139-5p (targeting FOXO1) and -199a-5p (targeting HIF1A and SIRT1). On the other hand, several miRNAs (e.g. miR-27, -143, -122, and -519d)^{37–38} that were previously identified were not associated with pre-pregnancy BMI in our study. Some of these were significantly associated with pre-pregnancy BMI in only one of our cohorts (e.g. miR-27 and -143 in the POUCH cohort), and did not make the list of differentially expressed miRNAs.

In our study, both differentially expressed miRNAs and their experimentally validated targets had broad functions involving cellular development, growth and proliferation that have been related to reproductive system disease, connective tissue disorders, and cancer. In

network analyses of miRNA targets, we identified many genes that play central roles in the top network, including RUNX1, SMAD1/4, STAT5a/b, FSH, TGF-beta, TGFBR1, Cg, FOXO1/3, VEGF, KLF4, and ADRB. These genes are well described in pathways (e.g. inflammation and endothelial dysfunction) that are dysregulated in pregnancy complications, including preeclampsia, gestational diabetes, and preterm delivery.

Circulating miRNAs can have useful potential roles as biomarkers. MiRNAs can enter the circulation through passive leakage (necrosis, inflammation, and injury), through active secretion via cell-derived membrane vesicles (e.g. exosomes), and through active secretion in protein-miRNA complexes (e.g. HDL-AGO2).^{39–40} Circulating miRNAs could be footprints of tissue level (e.g. placenta) changes, reflecting physiological or pathological conditions,^{41–42} or biomarkers that mediate cell-to-cell communications.⁴³ MiRNAs have also been shown to have utility as clinical predictive biomarkers.^{12, 44} Milagro et al. investigated peripheral blood miRNA expression among responders and non-responders in a weight-loss trial and reported that two miRNAs (miR-935 and -4772) were upregulated and three miRNAs (miR-223, -224, and -376b) were downregulated in the non-responder group.⁴⁴ Our focus on circulating miRNAs in early-mid pregnancy will facilitate the effort to leverage the use of miRNAs in obesity-related mechanistic studies and preventive, diagnostic or therapeutic perinatal applications.

Some strengths of our study deserve mention. The timing of miRNA expression profiling, in early-mid pregnancy, minimizes potential miRNA expression changes that follow common pregnancy complications (e.g. preeclampsia or gestational diabetes mellitus) and/or their treatment. Our approach involving discovery and replication in two well-characterized cohorts that differ in important characteristics (e.g. age, pre-pregnancy BMI, gestational age at blood collection, and racial make-up) highlight the potential generalizability and significance of our findings^{45,46}. While most previous studies evaluated differences between extremes (e.g. obese and non-obese), our analyses examined miRNA expression among participants who were mostly overweight or obese women. In our analyses, we adjusted for gestational age at blood collection, which has been shown to be an important determinant of miRNA expression^{47, 48}. On the other hand, our study had several limitations. The small sample size in the epigenome-wide miRNA expression profiling may have increased imprecision of statistical estimates. Our study, however, is similar in size to the lone previous analysis of miRNA levels in maternal plasma in relation to obesity²¹. The large number of tests we conducted in the analyses of the epigenome-wide profiling data may increase the likelihood of false positives. We chose not to correct for multiple testing in these exploratory secondary data analyses. Selection of miRNAs that were significant in both cohorts served to mitigate some concerns. Our microarray results were not validated by confirmatory testing such as qPCR. Therefore, future studies that involve larger number of samples and sensitive/specific validation platforms are needed. While we adjusted for some potential confounders (e.g. maternal age, race, and gestational age at blood collection), we did not evaluate the potential effect (confounding or effect modification) of other risk factors (e.g. diet and physical activity).

In sum, we report that miRNAs are differentially expressed in early-mid pregnancy plasma in relation to pre-pregnancy BMI. We replicated a number of miRNAs that have been

associated with BMI or obesity in non-pregnant populations. The broad functions of identified miRNAs and their targets mirror the extensive adverse outcomes that have been related to excess maternal pre-pregnancy body mass index. Similar studies, including compilation of miRNAs⁴⁹ related to obesity can enhance identification of novel pathways underlying adverse effects of excess maternal pre-pregnancy body mass index, motivate and guide design of studies that test targeted hypotheses, and facilitate clinical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Yanovski SZ, Yanovski JA. Obesity prevalence in the United States--up, down, or sideways? *N Engl J Med*. 2011; 364:987–989. [PubMed: 21410367]
2. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet*. 2011; 378:815–825. [PubMed: 21872750]
3. Hedderson MM, Williams MA, Holt VL, Weiss NS, Ferrara A. Body mass index and weight gain prior to pregnancy and risk of gestational diabetes mellitus. *Am J Obstet Gynecol*. 2008; 198:409.e1–409.e7. [PubMed: 18068138]
4. Samuels-Kalow ME, Funai EF, Buhimschi C, Norwitz E, Perrin M, Calderon-Margalit R, et al. Prepregnancy body mass index, hypertensive disorders of pregnancy, and long-term maternal mortality. *Am J Obstet Gynecol*. 2007; 197:490.e1–490.e6. [PubMed: 17714679]
5. Drake AJ, Reynolds RM. Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. *Reproduction*. 2010; 140:387–398. [PubMed: 20562299]
6. Zhang C. MicroRNomics: a newly emerging approach for disease biology. *Physiol Genomics*. 2008; 33:139–147. [PubMed: 18303086]
7. Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. *Obes Rev*. 2010; 11:354–361. [PubMed: 19793375]
8. Ge Q, Brichard S, Yi X, Li Q. microRNAs as a new mechanism regulating adipose tissue inflammation in obesity and as a novel therapeutic strategy in the metabolic syndrome. *J Immunol Res*. 2014; 2014:987285. [PubMed: 24741638]
9. Xie H, Lim B, Lodish HF. MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. *Diabetes*. 2009; 58:1050–1057. [PubMed: 19188425]
10. Olson EN. MicroRNAs as therapeutic targets and biomarkers of cardiovascular disease. *Sci Transl Med*. 2014; 6:239ps3.
11. Osaki M, Okada F, Ochiya T. miRNA therapy targeting cancer stem cells: a new paradigm for cancer treatment and prevention of tumor recurrence. *Ther Deliv*. 2015; 6:323–337. [PubMed: 25853308]
12. Hsieh CH, Rau CS, Wu SC, Yang JC, Wu YC, Lu TH, et al. Weight-reduction through a low-fat diet causes differential expression of circulating microRNAs in obese C57BL/6 mice. *BMC Genomics*. 2015; 16:699. [PubMed: 26377847]

13. Marques-Rocha JL, Milagro FI, Mansego ML, Zulet MA, Bressan J, Martínez JA. Expression of inflammation-related miRNAs in white blood cells from subjects with metabolic syndrome after 8 wk of following a Mediterranean diet-based weight loss program. *Nutrition*. 2016; 32:48–55. [PubMed: 26421388]
14. Yan X, Huang Y, Zhao JX, Rogers CJ, Zhu MJ, Ford SP, et al. Maternal obesity downregulates microRNA let-7g expression, a possible mechanism for enhanced adipogenesis during ovine fetal skeletal muscle development. *Int J Obes (Lond)*. 2013; 37:568–575. [PubMed: 22614057]
15. Nicholas LM, Rattanatrak L, Morrison JL, Kleemann DO, Walker SK, Zhang S, et al. Maternal obesity or weight loss around conception impacts hepatic fatty acid metabolism in the offspring. *Obesity (Silver Spring)*. 2014; 22:1685–1693. [PubMed: 24719305]
16. McGregor RA, Choi MS. microRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med*. 2011; 11:304–316. [PubMed: 21506921]
17. Jordan SD, Krüger M, Willmes DM, Redemann N, Wunderlich FT, Bronneke HS, et al. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nat Cell Biol*. 2011; 13:434–446. [PubMed: 21441927]
18. Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A, Zavolan M, et al. MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature*. 2011; 474:649–653. [PubMed: 21654750]
19. Nardelli C, Iaffaldano L, Ferrigno M, Labruna G, Maruotti GM, Quaglia F, et al. Characterization and predicted role of the microRNA expression profile in amnion from obese pregnant women. *Int J Obes (Lond)*. 2014; 38:466–469. [PubMed: 23817051]
20. Xi Y, Jiang X, Li R, Chen M, Song W, Li X. The levels of human milk microRNAs and their association with maternal weight characteristics. *Eur J Clin Nutr*. 2016; 70:445–449. [PubMed: 26486303]
21. Carreras-Badosa G, Bonmati A, Ortega FJ, Mercader JM, Guindo-Martinez M, Torrents D, et al. Altered circulating miRNA expression profile in pregestational and gestational obesity. *J Clin Endocrinol Metab*. 2015; 100:E1446–E1456. [PubMed: 26406295]
22. Enquobahrie DA, Williams MA, Butler CL, Frederick IO, Miller RS, Luthy DA. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. *Am J Hypertens*. 2004; 17:574–581. [PubMed: 15233976]
23. Holzman C, Bullen B, Fisher R, Paneth N, Reuss L. Prematurity Study Group. Pregnancy outcomes and community health: the POUCH study of preterm delivery. *Paediatr Perinat Epidemiol*. 2001; 15(Suppl 2):136–158. [PubMed: 11520406]
24. Plieskatt JL, Feng Y, Rinaldi G, Mulvenna JP, Bethony JM, Brindley PJ. Circumventing qPCR inhibition to amplify miRNAs in plasma. *Biomark Res*. 2014; 2:13. [PubMed: 25075309]
25. Kotlabova K, Doucha J, Chudoba D, Calda P, Dlouha K, Hromadnikova I. Extracellular chromosome 21-derived microRNAs in euploid & aneuploid pregnancies. *Indian J Med Res*. 2013; 138(6):935–943. [PubMed: 24521639]
26. Amplification Efficiency for TaqMan Gene Expression Arrays. [Retrieved September 18, 2016] (n.d.). from http://www3.appliedbiosystems.com/cms/groups/-mcb_marketing/documents/generaldocuments/cms_040377.pdf.
27. Pradervand S, Weber J, Thomas J, Bueno M, Wirapati P, Lefort K, Dotto GP, Harshman K. Impact of normalization on miRNA microarray expression profiling. *RNA*. 2009; 15(3):493–501. [PubMed: 19176604]
28. Folkersen L, Kurtovic S, Razuvaev A, Agardh HE, Gabrielsen A, Paulsson-Berne G. Endogenous control genes in complex vascular tissue samples. *BMC Genomics*. 2009; 10:516. [PubMed: 19900295]
29. Ortega FJ, Mercader JM, Catalán V, Moreno-Navarrete JM, Pueyo N, Sabater M, et al. Targeting the circulating microRNA signature of obesity. *Clin Chem*. 2013; 59:781–792. [PubMed: 23396142]
30. Pescador N, Pérez-Barba M, Ibarra JM, Corbatón A, Martínez-Larrad MT, Serrano-Ríos M. Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. *PLoS One*. 2013; 8:e77251. [PubMed: 24204780]

31. Martinelli R, Nardelli C, Pilone V, Buonomo T, Liquori R, Castano I, et al. miR-519d overexpression is associated with human obesity. *Obesity (Silver Spring)*. 2010; 18:2170–2176. [PubMed: 20057369]
32. Ling H, Li X, Yao CH, Hu B, Liao D, Feng S, et al. The physiological and pathophysiological roles of adipocyte miRNAs. *Biochem Cell Biol*. 2013; 91:195–202. [PubMed: 23859012]
33. McGregor RA, Choi MS. microRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med*. 2011; 11:304–316. [PubMed: 21506921]
34. Abente EJ, Subramanian M, Ramachandran V, Najafi-Shoushtari SH. MicroRNAs in obesity-associated disorders. *Arch Biochem Biophys*. 2016; 589:108–119. [PubMed: 26416722]
35. Thomé JG, Mendoza MR, Cheuiche AV, La Porta VL, Silvello D, Dos Santos KG, et al. Circulating microRNAs in obese and lean heart failure patients: A case-control study with computational target prediction analysis. *Gene*. 2015; 574:1–10. [PubMed: 26211628]
36. Li J, Zhou C, Li J, Su Z, Sang H, Jia E, et al. Global correlation analysis for microRNA and gene expression profiles in human obesity. *Pathol Res Pract*. 2015; 211:361–368. [PubMed: 25701361]
37. Perri R, Nares S, Zhang S, Barros SP, Offenbacher S. MicroRNA modulation in obesity and periodontitis. *J Dent Res*. 2012; 91:33–38. [PubMed: 22043006]
38. Peng Y, Yu S, Li H, Xiang H, Peng J, Jiang S. MicroRNAs: emerging roles in adipogenesis and obesity. *Cell Signal*. 2014; 26:1888–1896. [PubMed: 24844591]
39. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, et al. Serum microRNAs are promising novel biomarkers. *PLoS One*. 2008; 3:e3148. [PubMed: 18773077]
40. Rebane A, Akdis CA. MicroRNAs: Essential players in the regulation of inflammation. *J Allergy Clin Immunol*. 2013; 132:15–26. [PubMed: 23726263]
41. Zhao E, Keller MP, Rabaglia ME, Oler AT, Stapleton DS, Schueler KL, et al. Obesity and genetics regulate microRNAs in islets, liver, and adipose of diabetic mice. *Mamm Genome*. 2009; 20:476–485. [PubMed: 19727952]
42. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, et al. Detection and characterization of placental microRNAs in maternal plasma. *Clin Chem*. 2008; 54:482–490. [PubMed: 18218722]
43. Indrakusuma I, Sell H, Eckel J. Novel Mediators of Adipose Tissue and Muscle Crosstalk. *Curr Obes Rep*. 2015; 4:411–417. [PubMed: 26349436]
44. Milagro FI, Miranda J, Portillo MP, Fernandez-Quintela A, Campión J, Martínez JA. High-throughput sequencing of microRNAs in peripheral blood mononuclear cells: identification of potential weight loss biomarkers. *PLoS One*. 2013; 8:e54319. [PubMed: 23335998]
45. Luque-Fernandez MA, Gelaye B, VanderWeele T, Ferre C, Siega-Riz AM, Holzman C, Enquobahrie DA, Dole N, Williams MA. Seasonal variation of 25-hydroxyvitamin D among non-Hispanic black and white pregnant women from three US pregnancy cohorts. *Paediatr Perinat Epidemiol*. 2014; 28(2):166–176. Epub 2013 Dec 20. [PubMed: 24354847]
46. Tian Y, Holzman C, Siega-Riz AM, Williams MA, Dole N, Enquobahrie DA, Ferre CD. Maternal Serum 25-Hydroxyvitamin D Concentrations during Pregnancy and Infant Birthweight for Gestational Age: a Three-Cohort Study. *Paediatr Perinat Epidemiol*. 2016; 30(2):124–133. Epub 2015 Nov 17. [PubMed: 26575943]
47. Whitehead CL, Teh WT, Walker SP, Leung C, Larmour L, Tong S. Circulating MicroRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero. *PLoS One*. 2013; 8(11):e78487. [PubMed: 24282500]
48. Farina A, Chan CW, Chiu RW, Tsui NB, Carinci P, Concu M, Banzola I, Rizzo N, Lo YM. Circulating corticotropin-releasing hormone mRNA in maternal plasma: relationship with gestational age and severity of preeclampsia. *Clin Chem*. 2004; 50(10):1851–1854. [PubMed: 15388664]
49. Kunej T, Jevsinek Skok D, Zorc M, Ogrinc A, Michal JJ, Kovac M, et al. Obesity gene atlas in mammals. *J Genomics*. 2013; 1:45–55. [PubMed: 25031655]

Figure 1. Top microRNA network represented by microRNAs significantly associated with pre-pregnancy body mass index

Using Ingenuity Pathway Analysis software (IPA, Ingenuity, Redwood, CA), each miRNA was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These miRNAs or target genes were then overlaid onto a global molecular network developed from information contained in the IPKB. Network enrichment was assessed using a network score (negative log of p-values of Fisher tests). The figure represents the top network with network score of 32.

Figure 2. Top network represented by experimentally validated gene (mRNA) targets of microRNAs significantly associated with pre-pregnancy body mass index

Using Ingenuity Pathway Analysis software (IPA, Ingenuity, Redwood, CA), identifier of each target gene was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These miRNAs or target genes were then overlaid onto a global molecular network developed from information contained in the IPKB. Network enrichment was assessed using a network score (negative log of p-values of Fisher tests). The figure represents the top network with network score of 34.

Table 1

Selected Study Participant Characteristics

	Omega Study				POUCH Study				
Characteristics	All	ppBMI <18kg/m ²	ppBMI 18–25kg/m ²	ppBMI 25kg/m ²	All	ppBMI <18kg/m ²	ppBMI 18–25kg/m ²	ppBMI 25kg/m ²	p-value**
Epigenome-Wide Profiling									
Number	20 (100.0)	1 (5.0)	12 (60.0)	7 (35.0)	20 (100)	1 (5.0)	9 (45.0)	10 (50.0)	—
Maternal Age, years*	29.75 (6.0)	32.0 (–)	28.58 (5.4)	31.43 (7.5)	26.01 (6.3)	16.50 (–)	26.67 (7.5)	26.36 (4.9)	0.0621
Non-Hispanic White	11 (55.0)	1 (100.0)	7 (58.3)	3 (42.9)	11 (55.0)	0 (100.0)	7 (77.8)	4 (40.0)	1.000
ppBMI, kg/m ² *	24.22 (5.4)	17.94 (–)	21.55 (1.8)	29.70 (5.7)	26.92 (7.6)	16.70 (–)	21.43 (2.2)	32.87 (6.0)	0.2031
GA at blood collection, wks*	16.62 (4.7)	17.14 (–)	16.16 (1.7)	17.35 (1.7)	22.40 (1.9)	20.44 (–)	22.72 (19.9)	22.31 (1.6)	<0.0001
Smoked									
Never	15 (75.0)	1 (100.0)	9 (75.0)	4 (57.1)	14 (70.0)	1 (100.0)	6 (66.7)	7 (70.0)	0.105
Quit	4 (25.0)	0 (0.0)	3 (25.0)	1 (14.3)	1 (5.0)	0 (0.0)	1 (11.1)	0 (0.0)	
Smoked	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (25.0)	0 (0.0)	2 (33.3)	3 (30.0)	

Abbreviations: ppBMI:pre-pregnancy body mass index; GA: gestational age

* mean (standard deviation), otherwise number (%)

** p-value for Student's t-test or chi-squared test comparing Omega and POUCH participants

Table 2
Significant microRNAs* associated with maternal pre-pregnancy body mass index

miRNA	Omega Study			POUCH Study		
	Estimate	Std. Error	p-value	Estimate	Std. Error	p-value
hsa-miR-28-3p	0.115	0.039	0.011	0.101	0.036	0.012
hsa-let-7d*	0.117	0.041	0.011	0.081	0.022	0.002
hsa-miR-3137	0.125	0.044	0.012	0.105	0.034	0.007
hsa-miR-584	0.120	0.042	0.013	0.110	0.043	0.021
hsa-miR-28-5p	0.130	0.046	0.013	0.118	0.043	0.016
hsa-miR-4286	0.096	0.035	0.015	0.104	0.027	0.002
hsa-miR-376a	0.174	0.065	0.017	0.141	0.062	0.037
hsa-miR-423-5p	0.082	0.032	0.021	0.079	0.031	0.023
hsa-miR-425	0.138	0.054	0.022	0.099	0.039	0.023
hsa-miR-199a-5p	0.185	0.073	0.022	0.147	0.050	0.010
hsa-miR-652	0.133	0.053	0.024	0.117	0.039	0.008
hsa-miR-151-3p	0.130	0.053	0.027	0.115	0.038	0.009
hsa-miR-221	0.185	0.077	0.029	0.124	0.044	0.014
hsa-miR-891a	0.116	0.048	0.030	0.093	0.036	0.020
hsa-miR-103-2*	0.080	0.034	0.030	0.076	0.031	0.028
hsa-miR-361-5p	0.126	0.054	0.035	0.112	0.042	0.018
hsa-miR-151-5p	0.164	0.072	0.038	0.145	0.050	0.012
hsa-miR-130b	0.119	0.052	0.038	0.103	0.045	0.037
hsa-miR-146b-5p	0.161	0.072	0.041	0.160	0.055	0.010
hsa-miR-377	0.153	0.069	0.042	0.129	0.059	0.045
hsa-miR-128	0.129	0.058	0.042	0.121	0.039	0.007
hsa-miR-139-5p	0.090	0.041	0.042	0.070	0.031	0.039
hsa-miR-423-3p	0.116	0.053	0.043	0.100	0.035	0.013
hsa-miR-487b	0.130	0.060	0.046	0.114	0.051	0.042
hsa-miR-191	0.130	0.060	0.046	0.130	0.053	0.027

miRNA	Omega Study			POUCH Study		
	Estimate	Std. Error	p-value	Estimate	Std. Error	p-value
hsa-miR-29c	0.112	0.052	0.049	0.120	0.040	0.010
hsa-miR-26b	0.060	0.027	0.043	0.165	0.063	0.019

Note: Beta estimates, standard errors, and p-values corresponding to pre-pregnancy body mass index in linear regression models where the outcome is microRNA expression, and adjusted for maternal age, gestational age at blood collection, and race.

* MicroRNAs that were significant (p-values <0.05) in both cohorts are shown. All microRNAs are from the epigenome-wide profiling experiments.