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Circulating early- and mid-pregnancy microRNAs and risk of gestational diabetes

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Abstract

Aims—Epigenetic regulators, including microRNAs(miRNAs), are implicated in type 2 diabetes, but evidence linking circulating miRNAs in pregnancy and risk of gestational diabetes(GDM) is sparse. Potential modifiers, including pre-pregnancy overweight/obesity and offspring sex, are unexamined. We hypothesized that circulating levels of early-mid-pregnancy(range 7-23 weeks of gestation) candidate miRNAs are related to subsequent development of GDM. We also hypothesized that miRNA-GDM associations might vary by pre-pregnancy body-mass index(ppBMI) or offspring sex.

Methods—In a case-control analysis(36 GDM cases/80 controls) from the Omega study, a prospective cohort study of pregnancy complications, we measured early–mid-pregnancy plasma levels of 10 miRNAs chosen for potential roles in pregnancy course and complications(miR-126-3p, -155-5p, -21-3p, -146b-5p, -210-3p, -222-3p, -223-3p, -517-5p, -518a-3p, and 29a-3p) using qRT-PCR. Logistic regression models adjusted for gestational age at blood draw (GA) were fit to compare circulating miRNAs between cases and controls. We repeated analyses among overweight/obese(ppBMI≥25kg/m^2) or lean(ppBMI<25kg/m^2) women, and women with male or female offspring separately.

Results—Mean age was 34.3 years(cases) and 32.9 years(controls). GA-adjusted miR-155-5p(β=0.260/p=0.028) and - 21-3p(β=0.316/p=0.005) levels were positively associated

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with GDM. MiR-146b-5p(β=0.266/p=0.068) and miR-517-5p(β=0.196/p=0.074) were borderline. Associations of miR-21-3p and miR-210-3p with GDM were observed among overweight/obese but not lean women. Associations of six miRNAs(miR-155-5p, -21-3p, - 146b-5p, -223-3p, -517-5p, and -29a-3p) with GDM were present only among women carrying male fetuses(all p<0.05).

Conclusions—Circulating early–mid-pregnancy miRNAs are associated with GDM, particularly among women who are overweight/obese pre-pregnancy or pregnant with male offspring. This area has potential to clarify mechanisms underlying GDM pathogenesis and identify at-risk mothers earlier in pregnancy.

Keywords

Epidemiology; epigenetics; fetal; sex-specific disease associations; gestational diabetes mellitus; maternal pre-pregnancy overweight/obesity; microRNAs

1. Introduction

Gestational diabetes (GDM) is increasingly common, affecting 5-15% of pregnancies[1], and is implicated in adverse outcomes for both mother and child[2]. Controversy persists surrounding GDM screening, but current consensus recommendations support screening for GDM in mid pregnancy (24-28 weeks gestation)[3]. Changes related to maternal glycemia and fetal hyperinsulinemia, however, begin much earlier[4, 5]. The pathophysiology of GDM is not completely understood, and early-mid pregnancy biomarkers may facilitate progress in this area. Early-mid pregnancy biomarkers could help in investigations of mechanisms that define the role of well-known risk factors, such as pre-pregnancy overweight/obesity status, and novel risk factors, such as fetal sex, in GDM pathogenesis. For example, mothers of male offspring have been shown to have lower beta-cell function (as reflected by the disposition index) and higher risk of GDM[6], but mechanisms explaining these observations are unknown.

MiRNAs are small non-coding RNAs (approximately 20 nucleotides in length) that regulate gene expression and diverse cellular functions by directing degradation or inhibiting translation of messenger RNA transcripts[7]. Differential miRNA expression has been demonstrated in adipocytes, islet cells, endothelium, and hepatocytes, as well as smooth, skeletal, and cardiac muscle cells[8-17] among type 2 diabetes (T2D) cases, compared with controls. Identified miRNAs have been related to insulin secretion, inflammation, and insulin resistance. Differences have also been seen in circulating levels of miRNAs (e.g., miR-126, -146a, and -29a) comparing type 2 diabetes (T2D) cases to controls[13, 18-27]. Epigenetic regulatory mechanisms, including miRNAs, may also have important roles in GDM, a condition that shares similar pathophysiologic features to T2D[28-33]. Several small studies have demonstrated differences in placental or umbilical vein endothelial cell miRNA expression at delivery in GDM pregnancies vs. controls[28-33]. Only two previous studies, however, have compared second-trimester circulating miRNA levels in women with GDM to controls[34, 35]. These previous studies were small (n = 20 and n = 48) and were limited to Asian populations. In addition, to our knowledge, no previous study has examined

maternal pre-pregnancy overweight/obesity- or fetal–sex-specific associations of miRNAs with GDM.

In the current study, we selected candidate miRNAs that have previously been associated with pregnancy complications (e.g., GDM, preeclampsia, or intrauterine growth retardation) or pathophysiologic pathways (e.g., placental functions, oxidative stress, and inflammation) related to pregnancy complications.[23, 27, 34] We hypothesized that circulating levels of early-mid- (7-23 weeks) pregnancy candidate miRNAs are related to subsequent development of GDM. We also hypothesized that associations of these candidate miRNAs with GDM might vary by pre-pregnancy overweight and obesity or by offspring sex.

2. Materials and Methods

2.1. Study setting and study population

The study was conducted among participants of the Omega study, a pregnancy cohort study based at the Center for Perinatal Studies at Swedish Medical Center in Seattle, Washington. Study design and protocols have been published previously[36]. Briefly, the Omega study was designed to examine metabolic and dietary risk factors of preeclampsia, GDM, and other pregnancy complications/outcomes. From 1996 to 2008, participants were recruited from prenatal care clinics affiliated with Swedish Medical Center in Seattle and Tacoma General Hospital in Tacoma, Washington.

Pregnant women were eligible to participate in the Omega study if they were ≥18 years old at enrollment, initiated prenatal care prior to 16 weeks of pregnancy, were able to speak and read English, and planned to carry the pregnancy to term and deliver at one of the study hospitals. During the study period, approximately 80% of eligible women who were approached consented to participate and >95% were followed until delivery. Among 4,011 Omega study participants, a subcohort of 767 randomly selected participants was included in a traffic-related air-pollution case-cohort study investigating course and complications of pregnancy. For the current study, we included all GDM cases from the subcohort (n = 38) and 100 randomly selected control participants without GDM, pre-eclampsia, low birthweight, or preterm delivery. Participant characteristics for the subcohort were similar to characteristics of the Omega study population (Table 1). Of the randomly selected controls, 17 were missing plasma samples. After excluding non-singleton pregnancies, we were left with an analytic sample of 116 participants (36 GDM cases, 80 controls). Characteristics of the participants with missing plasma samples were similar to those included in the current study (Supplementary Table 1).

The institutional review boards of Swedish Medical Center and Tacoma General Hospital approved the study, and all study participants provided written informed consent.

2.2. Data collection

At an enrollment visit before 20 weeks of gestation, trained interviewers conducted in-person interviews (45-60 minutes in length) to collect data on expectant mothers' age, height, pre-pregnancy weight, socioeconomic characteristics, reproductive and medical histories, and tobacco consumption before and during pregnancy. Pre-pregnancy body mass

index (ppBMI), based on self-reported measures of weight and height, was calculated as weight in kg divided by height in meters squared. Expectant mothers with ppBMI≥25kg/m^2 were classified as overweight/obese and were compared to women with ppBMI<25kg/m^2 in analyses of potential effect modification by overweight/obese status. Gestational weight gain was calculated as the difference in weight between last recorded maternal weight within four weeks prior to delivery (abstracted from medical records) and self-reported pre-pregnancy weight during the three months prior to conception. Maternal race was classified as non-Hispanic white, non-Hispanic black, Asian, Hispanic, or other. Maternal peripheral blood was collected shortly after enrollment (see below). Mothers were followed through delivery, and trained personnel abstracted data on course and outcomes of the pregnancy (e.g., GDM, offspring sex) from maternal and infant medical records.

2.3. GDM diagnosis

A 50g glucose challenge test was administered between gestational weeks 24 and 28 to screen for GDM as part of routine follow-up of all women at participating clinics. Women testing positive on the screening test (≥140 mg/dL) completed an additional 100g, three-hour oral glucose tolerance test (OGTT) within two weeks of the first test. Women were diagnosed with GDM if two or more plasma glucose values during the three-hour OGTT exceeded the following American Diabetes Association (ADA) 2004 criteria cut-points: fasting ≥ 95 mg/dL; 1-hr ≥ 180 mg/dL; 2-hr ≥ 155 mg/dL; or 3-hr ≥ 140 mg/dL[37].

2.4. Sample collection, pre-processing, and RNA extraction

Peripheral blood samples, collected at 16.1 weeks of gestation on average (range 7.0-22.9), were kept at 4°C until processing that occurred within 1 hour of collection. Approximately 200μL of plasma was used for extracting small RNAs using the Exiqon miRCURY™ RNA Biofluids Isolation Kit (Exiqon, Woburn, MA). We assessed the integrity, purity, and quantity of purified miRNA using spectrophotometry and an Agilent 2100 Bioanalyzer capillary electrophoresis system (Agilent Technologies Inc, Palo Alto, CA). To further assess quality of extracted RNA, we measured spike-in values of cel-miR-39.

2.5. miRNA selection, profiling, data processing, and normalization

We chose the following miRNAs: miR-126-3p, -155-5p, -21-3p, -146b-5p, -210-3p, -222-3p, -223-3p, -517-5p, -518a-3p, and -29a-3p. The selected candidate miRNAs have previously been associated with type 2 diabetes (miR-126[27], -223[27], and -29a[24]); pregnancy complications including pre-eclampsia (miR-155[38], -210[39], 517[40], and -518a[41]), growth restriction (miR-21[42] and -517[40]), and GDM (miR-222[33]); or pathways related to pregnancy complications including oxidative stress (miR-21[43]), endothelial function (miR-126, -210, and -222[44]), or inflammation (miR-155[45] and -146b[46]). We constructed a custom targeted panel of the candidate miRNAs and two control miRNA assays using ExiqonLNA™ primers. An exogenous miRNA cel-miR-39 was added as a positive control for technical factors including RNA extraction, complementary DNA synthesis, and PCR amplification[47], as mentioned above, and an endogenous "housekeeping" miRNA, miR-423-3p was chosen for normalization, based on previous recommendations[48]. qPCR was conducted in duplicate using 96-well qPCR plates. Reactions were run on an ABI PRISM 7000 Real Time PCR machine (Applied Biosystems,

Foster City, CA), using default cycling conditions. We recorded threshold cycle (C_T) values on two measurements per sample. True replicates were done in the sense that the original plasma samples were split, completely independent RNA preps were done, independent RT reaction was performed for each replicate, and each replicate was run on a different qPCR 96-well plate. C_T values of the duplicates differing by greater than 0.2 times the standard deviation were re-tested, and replicates were averaged for analyses. Lab personnel was blinded to case-control status. Data from miRNA qRT-PCR arrays were imported into SDS Enterprise Software (V2.2.2, Applied Biosystems), and C_T values were calculated using a consistent thresholding value for each assay across all plates. Raw C_T values were scaled to values for cel-miR-39-3p. Then, $\Delta\Delta C_T$ values were expressed relative to values of miR-423-3p and used in subsequent analyses.

2.6. Statistical analyses

We examined distributions of selected characteristics, as well as gestational age at blood draw, among GDM cases and controls. MiRNA C_T values were log-transformed to achieve normal distribution. We used parallel coordinates plots of ln(miRNA) levels to visualize patterns of variability that might differ by case status. We then fit logistic regression models examining the association of each candidate miRNA with GDM, adjusting for gestational age at blood collection (Model 1), and adjusting for gestational age at blood collection, maternal age, and pre-pregnancy BMI (Model 2). Because we hypothesized that associations of circulating miRNA levels with GDM might differ by pre-pregnancy obesity or offspring sex, we fit gestational-age-adjusted models that were stratified by pre-pregnancy overweight/obesity or offspring sex. If there were differences in associations among strata, we fit models that included terms for miRNAs, pre-pregnancy overweight/obesity status or offspring sex, and an interaction term for pre-pregnancy obesity or offspring sex and miRNA, to assess statistical significance of the interactions. We used Stata version 13.1 (College Station, TX), R 3.3.1 (www.R-project.org), including the MASS package[49], and MATLAB Software Package (The MathWorks Corporation, Natick, MA) for the analyses. All tests were two-sided and $p<0.05$ was used to determine statistical significance.

3. Results

On average, GDM cases were 34.3 years old at enrollment, while controls were 32.9 years old. Blood samples were collected at 15.1 weeks among cases and 16.5 weeks among controls (Table 1). Cases had greater ppBMI on average than controls (median 25.5 kg/m^2 vs. 21.7 kg/m^2). Median values of untransformed miRNA C_T levels are shown in Table 2.

Parallel coordinates plots did not reveal systematic patterns of variability in miRNA expression profiles by GDM case status (not shown). In models adjusted for gestational age at blood collection, higher circulating levels of two miRNAs, miR-155-5p (β=0.260, p=0.028) and -21-3p (β=0.316, p=0.005), were associated with higher odds of GDM. In addition, associations of miR-146b-5p (β=0.266, p=0.068) and miR-517-5p (β=0.196, p=0.074) with GDM odds were borderline. The associations were attenuated in models adjusting for gestational age at blood draw, maternal age, and ppBMI; only miR-21-3p (β=0.262, p=0.029) remained significantly associated with higher odds of GDM (Table 3).

In analyses stratified by pre-pregnancy BMI category, associations between miRNA and GDM risk were similar between overweight/obese and lean women, except for associations of miR-21-3p and miR-210-3p with GDM risk. Associations of these two miRNAs, miR-21-3p (β =0.467, p=0.042) and miR-210-3p (β=0.514, p=0.036), were observed only among women who were overweight/obese during pre-pregnancy. In a logistic regression model with interaction between obesity status and miRNA, the interaction term was not significant for miR-21-3p and was borderline for miR-210-3p (Table 4). In stratified analyses by the sex of the fetus, among participants who were pregnant with male offspring, circulating levels of miR-155-5p (β=0.474, p=0.008), -21-3p (β=0.665, p=0.003), miR-146b-5p (β=0.418, p=0.037), -223-3p (β=0.421, p=0.047), -517-5p (β=0.377, p=0.028), and -29a-3p (β=0.345, p=0.047) were associated with risk of GDM (Table 5). Among participants who were pregnant with female offspring, none of the candidate circulating miRNAs were associated with risk of GDM. Tests for interaction effects between miRNA and offspring sex were statistically significant for miR-21-3p (p=0.020) and borderline (< 0.10) for several of the other miRNAs: miR-155-5p (p=0.059), -223-3p (p=0.060), -517-5p (p=0.076), or 29a-3p (p=0.059).

4. Discussion

In this study, we found that early–mid-pregnancy maternal plasma levels of two miRNAs (miR-155-5p and -21-3p) were associated with subsequent risk of GDM. Associations of miR-21-3p, along with miR-210-3p, with GDM odds was observed only among women who were overweight/obese prior to pregnancy. In sex-stratified analyses, associations of miR-155-5p, -21-3p, and four others (miR-146b-5p, -223-3p, -517-5p, and -29a-3p) with subsequent risk of GDM were present only among mothers bearing male offspring.

Two previous studies have measured circulating miRNA levels in women with GDM and controls[34, 35]. Zhao et al. compared serum miRNA levels at 16-19 weeks gestational age among 24 GDM cases (based on 75-g OGTT results) and 24 controls using Taqman Low Density array (human microRNA panel V2.0) and pooled samples, followed by confirmatory qRT-PCR in the primary study population and two separate populations[34, 35]. In that study, GDM cases and controls were frequency matched for age, gestational age, and pre-pregnancy BMI. Women older than 33 years or with BMI > 26kg/m^2 were excluded. They reported lower levels of miR-132, -222, and -29a among GDM cases compared with controls. Zhu et al. performed a pilot study using high-throughput sequencing on pooled plasma samples (collected at 16-19 weeks gestational age) from 10 GDM cases (based on 75-g OGTT results) and 10 age- and gestational-age–matched controls, followed by qRT-PCR confirmatory experiments[34, 35]. In the sequencing study, twelve miRNAs were upregulated and 20 were downregulated. On confirmatory qRT-PCR, five miRNAs (miR-16-5p, -17-5p, -19a-3p, -19b-3p, and 20a-5p) were seen at higher levels in GDM plasma, compared with controls. In our analysis, we did not see differences in miR-222 or -29a levels between GDM case and control groups, although miR-29a was associated with GDM risk in the analysis restricted to male offspring. We did not measure miR-192, nor the miRNAs that were identified in Zhu's pilot study. To our knowledge, no previous study has reported pre-pregnancy overweight/obesity- or offspring sex-specific associations of miRNAs with GDM risk.

There are some important differences among 1) the populations studied, 2) the study designs, and 3) the laboratory techniques that might explain the differing miRNAs identified in ours and previous studies. For example, both previous studies were performed in Chinese populations. The cohorts were also relatively younger and leaner than in the Omega study. In the Zhu study, although women were not excluded based on age or ppBMI, on average, GDM cases were younger (mean age 30 years) and leaner (mean ppBMI 24kg/m^2) than our population. Zhao excluded women 33 years or older or with BMI > 26kg/m^2. In both previous studies, samples were collected between 16 and 19 weeks. The authors did not additionally adjust for gestational age at the time of blood draw, potentially a key factor, as currently available evidence suggests that circulating miRNA levels may change across the course of pregnancy[50, 51]. In addition to adjusting for gestational age in all models, we also conducted a sensitivity analysis excluding participants with specimens collected at the extremes of gestational age, with quantitatively very similar results. Previous authors also did not comment on the prevalence of other pregnancy complications in their populations. Our controls did not have preterm delivery (PTD), pre-eclampsia (PE), or low birthweight (LBW). Of the GDM cases in our analytic sample, eight had one or more of these other pregnancy complications. (Three had LBW. Two had PE, and five had PTD. Two pregnancies had both LBW and PTD.) Pre-eclampsia and preterm delivery share common risk factors and mechanisms with GDM[52], so we included GDM cases with PE or PTD in our analytic sample. We conducted a sensitivity analysis excluding LBW cases, however. Findings were similar to the primary results we have reported in this manuscript.

Differing pre-processing and normalization strategies may also have contributed importantly to the varying results. Zhao normalized to spike-in levels of *C. elegans* miR-39[34, 35]. Zhu, however, normalized to endogenous miR-221 level[34, 35]. Although miR-221 is sometimes used for normalization, it has been related to inflammation[53], which has been linked to abnormal glucose metabolism and diabetes[54]. Differences in pre-analytic strategies, especially normalization, have been implicated as factors contributing to inconsistent results seen in circulating miRNA-T2D association studies[55, 56]. We, therefore, conducted multiple sensitivity analyses using two other normalization strategies: we normalized to cel-miR-39 level only and also performed quantile normalization. In general, findings from our sensitivity analyses were similar to the primary results we have reported in this manuscript with one exception. MiR-155 was not associated with GDM in quantilenormalized analyses using all participants, but it was associated with GDM when we restricted the quantile-normalized analysis to pregnancies with male fetuses only.

Our study identified several candidate miRNAs that are associated with risk of GDM either in overall analyses or analyses stratified by pre-pregnancy obesity or offspring sex. Several of these miRNAs have previously been implicated in the pathogenesis of T2D. For example, miR-29a is seen at higher levels in serum and whole blood of individuals with newly diagnosed and existing T2D[24]. Overexpression of miR-29a also leads to insulin resistance in adipocyte cell lines, possibly by targeting proteins in the PTEN-AKT pathway[57]. On the other hand, Zampetaki et al. identified *lower* levels of both miR-21 and -223 when they compared individuals with prevalent diabetes to controls[27]. miR-155 was also seen at lower levels in peripheral blood mononuclear cells of individuals with diabetes compared to controls[58], although in that sample, most of the patients with diabetes were treated with

metformin and/or a sulfonylurea, which may have influenced results. Several of the candidates we identified (miR-155-5p, -21-3p, and -223-3p) are highly expressed in a variety of tissues and have been associated with a number of malignant and non-malignant disease states[59], suggesting they might play roles in regulatory pathways common across cell types, including apoptosis, cell-cycle regulation, and response to inflammation. For example, upregulation of miR-155 in both umbilical vein endothelial cells and placenta tissues has been implicated in the pathogenesis of pre-eclampsia, possibly by inhibiting trophoblast invasion and proliferation via the cell-cycle regulator Cyclin D1[60, 61]. miR-155 and -146b, also identified in our study, are induced by inflammatory cytokines, including NF-κB[46, 62, 63]. In the case of miR-21, miR depletion in a pancreatic cancer model led to increased apoptosis of malignant cells, inhibiting tumor growth[64]. In pancreatic β-cells of mice with a model of T1D, NF-κB increased miR-21 levels, while miR-21 decreased the level of PDCD4, an inducer of apotosis[65]. In contrast to these widely expressed miRNAs, miR-517-5p is thought to be relatively specific to placenta. It has been linked to development of pre-eclampsia, possibly by regulating trophoblast proliferation[60]. Our findings support its potential role in GDM pathogenesis.

In the current study, the statistically significant miRNA-GDM associations we observed were limited to pregnancies in mothers with pre-pregnancy overweight or obesity. This may be due in part to the candidate miRNAs we selected, which were reflective of pathways that link obesity and Type 2 diabetes, highlighting potential differences in pathways that lead to GDM among obese and lean women. Gestational weight gain in early pregnancy might also influence the association of circulating miRNAs with GDM risk. In our dataset, ppBMI was correlated with gestational weight gain in the first 20 weeks of pregnancy (Pearson correlation coefficient -0.25, p=0.009). In a post-hoc analysis, we fit a model that adjusted for early pregnancy gestational weight gain and observed no meaningfully different results compared to the ones we report in this manuscript. It is also possible that overweight/obese mothers differed from lean mothers in other characteristics (e.g., diet and socio-economic status). Sample size limited our ability to fully account for these potential confounders. Additionally, miRNA-GDM associations were observed among pregnancies with male infants, but not female infants. Male fetal sex has been associated with both higher risk of maternal GDM and a lower disposition index[6] in the mother, suggesting the association of sex with GDM may be mediated in part by an effect of fetal sex on maternal beta-cell function. Sex-specific differences in placental development, placental hormone secretion and placental response to maternal factors have also been reported, including differences in expression of immune-regulating genes[66], which may reflect differences in maternal immune tolerance to the male fetus[67]. To the extent that these are related to risk of GDM, they may explain, at least in part, the sex-specific differences in miRNA-GDM associations that we observed. Placental miRNAs appear to regulate both trophoblast cell migration and maternal immune response[67], which may occur in a sex-dependent fashion. For instance, miR-517b, which was associated with GDM among pregnancies with male fetuses only, regulates expression of TNFS15[68], a pro-inflammatory, anti-angiogenic cytokine, which may reflect a sex-specific placental response to the maternal immune system. In line with recent National Institutes of Health statements highlighting the growing awareness of

differences in response to metabolic stressors by sex at all ages[69], future studies investigating fetal–sex-specific effects of miRNAs in pregnancy are warranted.

The current study has several strengths, most notably, the use of a well-characterized prospective cohort of pregnant women and adjustment for gestational age at blood collection —an important potential determinant of circulating miRNA levels[50, 51]. The fact that associations remained generally consistent across normalization strategies also lends credibility to our findings. Several limitations of the study deserve mention. First, we used a candidate-miRNA approach, which might exclude important miRNAs related to GDM pathogenesis. Our list of miRNAs was chosen based on thorough literature review, however, and these candidate biomarkers had biologically plausible functions regulating messenger RNA targets involved in putative cellular functions. Second, our study is of moderate size. Larger studies will need to replicate these findings in a variety of populations. Third, we did not assess downstream effects of the miRNAs we identified. Last, because we had a limited number of non-white participants or participants with lower education levels or SES, results may not be generalizable to more diverse populations. In a sensitivity analysis, we repeated our analyses among non-Hispanic white mothers. While there were some differences in specific estimates, in general, findings were similar to what was reported in the current manuscript including the fact that associations were observed only among participants who were overweight/obese pre-pregnancy or who were pregnant with male offspring.

In sum, our findings suggest that early-mid–pregnancy circulating miRNAs are associated with subsequent risk of GDM, particularly among women who were overweight/obese prior to the pregnancy or among women bearing male offspring. Larger follow-up studies in populations representative of a variety of ethnic groups and socio-economic backgrounds as well as profiling of larger set of candidate miRNAs or epigenome-wide profiling (e.g., using sequencing approaches) may clarify mechanisms underlying GDM pathogenesis as well as potential pre-pregnancy overweight/obesity- or offspring-sex–specific differences. Better mechanistic understanding of GDM pathogenesis may help identify mothers at high risk of GDM earlier in pregnancy and guide development of targeted preventive interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Evidence linking circulating miRNAs in pregnancy and risk of gestational diabetes (GDM) is sparse.
- Whether miRNA-GDM associations vary by pre-pregnancy body-mass index or offspring sex is unknown.
- Levels of circulating gestational age-adjusted miRNAs were associated with GDM risk.
- Associations varied by pre-pregnancy overweight/obesity and offspring sex.

Table 1

Baseline characteristics of Omega participants, environmental study subcohort participants, and gestational diabetes cases and controls in the current study

	Omega overall n=4,011	Environmental study subcohort n=767	GDM cases n=36	Controls n=80	p-value*
Mean maternal age, years (SD)	32.6 (4.6)	32.7 (4.4)	34.3 (3.6)	32.9 (4.4)	0.101
Mean gestational age at blood draw, weeks (SD)	15.6 (3.0)	16.0 (2.6)	15.1 (2.9)	16.5 (2.3)	0.0098
Median pre-pregnancy body-mass index, kg/m2 (Q1-Q3)	22.4 (4.6)	22.5 (4.6)	25.5 (6.7)	21.7 (4.1)	0.0003
Mean gestational weight gain**, kg (SD)	16.0 (6.1)	16.1 (6.0)	12.4 (7.8)	15.9 (5.6)	0.001
Mean gestational age at delivery, weeks	38.3 (4.0)	38.4 (3.3)	38.2 (1.7)	38.9 (1.3)	0.0196
Overweight/obese, % yes (n)	27 (1073)	27 (210)	53 (19)	16 (13)	<0.0001
Singleton pregnancy, % yes (n)	96 (3,807)	96 (733)	100 (36)	100 (80)	—
Primiparous, % yes (n)	38 (1,525)	36 (279)	39 (14)	34 (27)	0.592
Married, % yes (n)	85 (3,404)	88 (675)	86 (31)	86 (69)	0.984
Race, % white (n)	86 (3,412)	86 (655)	67 (24)	80 (63)	0.130
High school education or less, % (n)	4 (153)	2 (18)	3 (1)	3 (2)	0.898
Smoked during pregnancy, % (n)	6 (217)	5 (39)	9 (3)	8 (6)	0.835
Offspring sex, % male (n)	51 (1,903)	51 (373)	61 (22)	50 (39)	0.269

*p-value from t-test or chi-squared test comparing means or proportions among GDM cases and controls in the current study

Table 2

Median* values of circulating candidate microRNAs

	GDM cases	Controls
miR-126-3p (Q1-Q3)	1.0 (0.3-2.7)	0.8 (0.2-2.7)
miR-155-5p (Q1-Q3)	3.8 (0.8-13.4)	1.8 (0.4-4.9)
miR-21-3p (Q1-Q3)	12.2 (3.0-33.8)	3.4 (1.1-11.6)
miR-146b-5p (Q1-Q3)	3.9 (0.8-9.4)	1.4 (0.8-5.8)
miR-210-3p (Q1-Q3)	2.3 (0.7-4.6)	1.5 (0.3-3.9)
miR-222-3p (Q1-Q3)	1.2 (0.4-2.5)	1.1 (0.2-3.0)
miR-223-3p (Q1-Q3)	1.7 (0.5-2.7)	0.9 (0.3-3.3)
miR-517-5p (Q1-Q3)	5.2 (1.1-19.0)	2.7 (0.5-10.8)
miR-518a-3p (Q1-Q3)	6.1 (2.8-43.7)	5.1 (1.2-27.4)
miR-29a-3p (Q1-Q3)	1.0 (0.3-2.2)	0.7 (0.2-2.1)

* cel-miR-39-adjusted ΔΔCt levels relative to housekeeping miR (miR-423-3p)

Table 3

Coefficients and 95% confidence intervals from logistic regression models examining associations of circulating microRNAs with risk of gestational diabetes mellitus

	Model 1			*Model 2*		
miRNA	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
miR-126-3p	0.156	(-0.100 0.412)	0.232	0.131	(-0.139 0.402)	0.341
miR-155-5p	0.260	(0.028 0.493)	0.028	0.210	(-0.043 0.462)	0.103
miR-21-3p	0.316	(0.095 0.537)	0.005	0.262	(0.027 0.496)	0.029
miR-146b-5p	0.266	(-0.020 0.551)	0.068	0.190	(-0.106 0.487)	0.208
miR-210-3p	0.153	(-0.060 0.366)	0.159	0.150	(-0.083 0.382)	0.207
miR-222-3p	0.070	(-0.168 0.307)	0.566	0.080	(-0.172 0.331)	0.535
miR-223-3p	0.177	(-0.083 0.437)	0.181	0.135	(-0.138 0.408)	0.334
miR-517-5p	0.196	(-0.019 0.410)	0.074	0.113	(-0.118 0.344)	0.337
miR-518a-3p	0.077	(-0.090 0.243)	0.367	0.026	(-0.151 0.203)	0.774
miR-29a-3p	0.151	(-0.066 0.368)	0.173	0.130	(-0.096 0.356)	0.260

Coefficients and 95% confidence intervals from unadjusted and adjusted logistic regression models showing change in log odds of gestational diabetes mellitus associated with a one-unit increase in log-ΔΔCt level of candidate miRNAs

Model 1: Adjusted for gestational age only

Model 2: Adjusted for gestational age, maternal age at delivery, and pre-pregnancy body-mass index

Table 4

Coefficients and 95% confidence intervals from logistic regression models examining associations of circulating microRNAs with gestational diabetes mellitus, stratified by BMI

	BMI < 25kg/m² n = 84 (17 GDM cases, 67 controls)			*BMI ≥ 25kg/m²* n = 32 (19 GDM cases, 13 controls)			*Interaction effect** n = 116	*Interaction effect*** n = 116
miRNA	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**	**p-value**	**p-value**
miR-126-3p	0.158	(-0.193 0.510)	0.376	0.269	(-0.169 0.707)	0.228	0.691	0.883
miR-155-5p	0.276	(-0.059 0.611)	0.107	0.233	(-0.133 0.600)	0.213	0.875	0.984
miR-21-3p	0.186	(-0.088 0.459)	0.183	0.467	(0.017 0.916)	0.042	0.334	0.304
miR-146b-5p	0.209	(-0.189 0.607)	0.304	0.333	(-0.128 0.794)	0.156	0.679	0.716
miR-210-3p	0.026	(-0.242 0.294)	0.850	0.514	(0.033 0.995)	0.036	0.081	0.051
miR-222-3p	0.000	(-0.312 0.312)	1.000	0.321	(-0.114 0.757)	0.148	0.237	0.274
miR-223-3p	0.131	(-0.212 0.473)	0.456	0.314	(-0.145 0.773)	0.180	0.525	0.536
miR-517-5p	0.189	(-0.091 0.468)	0.186	0.275	(-0.147 0.698)	0.202	0.757	0.481
miR-518a-3p	-0.002	(-0.217 0.213)	0.987	0.142	(-0.154 0.439)	0.347	0.443	0.607
miR-29a-3p	0.083	(-0.224 0.389)	0.597	0.285	(-0.094 0.663)	0.140	0.401	0.315

Coefficients and 95% confidence intervals from logistic regression models showing change in log odds of gestational diabetes mellitus associated with a one-unit increase in log-ΔΔCt level of candidate miRNAs and p-values from interaction models, stratified by maternal pre-pregnancy body-mass index

Models were additionally adjusted for gestational age

* BMI included as overweight yes/no

** BMI modeled continuously

Table 5

Coefficients and 95% confidence intervals from sex-stratified logistic regression models examining associations of circulating microRNAs with gestational diabetes mellitus

	Male offspring only n = 61			*Female offspring only* n = 53			*Interaction effect* n = 114
miRNA	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**	**p-value**
miR-126-3p	0.343	(-0.038 0.724)	0.078	-0.034	(-0.395 0.327)	0.854	0.109
miR-155-5p	0.474	(0.121 0.826)	0.008	0.035	(-0.317 0.388)	0.844	0.059
miR-21-3p	0.665	(0.231 1.099)	0.003	0.126	(-0.170 0.422)	0.403	0.020
miR-146b-5p	0.418	(0.025 0.810)	0.037	0.037	(-0.386 0.459)	0.866	0.165
miR-210-3p	0.289	(-0.026 0.603)	0.072	0.046	(-0.284 0.377)	0.783	0.171
miR-222-3p	0.218	(-0.116 0.552)	0.201	-0.081	(-0.457 0.295)	0.673	0.130
miR-223-3p	0.421	(0.006 0.836)	0.047	-0.034	(-0.390 0.321)	0.850	0.060
miR-517-5p	0.377	(0.041 0.712)	0.028	-0.017	(-0.343 0.309)	0.919	0.076
miR-518a-3p	0.073	(-0.156 0.301)	0.533	0.058	(-0.200 0.317)	0.658	0.977
miR-29a-3p	0.345	(0.005 0.685)	0.047	-0.028	(-0.355 0.300)	0.869	0.059

Coefficients and 95% confidence intervals from sex-stratified logistic regression models showing change in log odds of gestational diabetes mellitus associated with a one-unit increase in log-ΔΔCt level of candidate miRNAs

Models were additionally adjusted for gestational age

Two pregnancies with data missing on infant sex were excluded from this analysis