Serum MicroRNAs as Diagnostic Biomarkers for Macrosomia

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Abstract

Background: Macrosomia is defined as an infant's birth weight of more than 4000 g. Although microRNAs (miRNAs) have been implicated in the pathogenesis of various diseases, the associations between serum miRNAs and macrosomia have been rarely reported. **Methodology:** We used the Taqman Low Density Array followed by quantitative reverse transcriptase polymerase chain reaction assays to screen for miRNAs associated with macrosomia using serum samples collected 1 week before delivery. **Results:** Profiling results showed that 1 miRNA was significantly upregulated and 10 miRNAs were significantly downregulated in serum samples of macrosomia ($\Delta\Delta C_t > 3$ -fold). The expression levels of miR-21 were significantly decreased in macrosomia as compared to the controls in the third trimester. Receiver operating characteristic (ROC) curve analyses showed that the area under the ROC curve for miR-21 was 67.7% (sensitivity = 66.7% and specificity = 70.0%). **Conclusions:** miR-21 in maternal serum is differentially expressed between macrosomia and controls, and miR-21 could be used as a candidate biomarker to predict macrosomia.

Keywords

microRNA, serum, macrosomia, TLDA, biomarker

Introduction

Macrosomia is defined as an infant's birth weight of more than 4000 g at term due to excessive intrauterine fetal growth occurrence. It has been reported that the incidence rate of macrosomia is 7.83% in 2005 in Southeast China and is 6.5% in 2006 in China, respectively.¹ Furthermore, the rapid increase in the rate of macrosomia is a worldwide problem,² and macrosomia is a challenge in current obstetrics. Fetal macrosomia is associated with increased risk of complications both for the mother and the newborn. Macrosomia is associated with higher rates of cesarean section² and sequential use of vacuum and obstetric forceps for vaginal delivery for the mother.³ In addition, the association between macrosomia and subsequent obesity in the offspring also has been confirmed.⁴ Increasing prevalence of obesity in women and offspring overweight/obesity in many parts of the world could be associated with a parallel increase in macrosomic births. In China, from 1992 to 2010, the prevalence of overweight in women aged 18 to 44 years increased from 16.8% to 26.4%, and that of obesity increased from 3.1% to 9.0%, respectively.⁵ Meanwhile, the prevalence of overweight and obesity among Chinese children and adolescents has increased steadily from 5.2% in 1991 to 13.2% in 2006.⁶ It is a vicious circle.

The causes of macrosomia include both genetic and environmental factors. Previous research focused on the rapid increase in the prevalence of macrosomia has identified the

underlying environmental factors such as prenatal nutrients, maternal body mass index (BMI) at early pregnancy, and gestational weight gain.⁷⁻¹⁰

In recent years, more and more evidence has supported that the intrauterine environment is critical in "programming" the fetus, leading to diverse health and disease outcomes throughout its life.¹¹ The intrauterine environment can influence gene expression, resulting into epigenetic alterations.¹²

MicroRNAs (miRNAs) are short (19-25 nucleotides), single-stranded, and nonprotein-coding RNAs that regulate gene expression by binding to the 3'-untranslated region of the target messenger RNAs (mRNAs) and function in diverse biological processes, including development, differentiation, apoptosis, and oncogenesis.¹³ It is an important epigenetic regulatory mechanism. Growing evidence indicates that miRNAs are involved in the pathogenesis of gestational diabetes mellitus (GDM),¹⁴ preeclampsia, and intrauterine growth restriction.¹⁵ Our previous

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research had found that the levels of placental miR-21 was up-regulated in macrosomia.¹⁶ These studies suggest that miRNAs may serve as clinical biomarkers for pregnancy's complication.

After the initial identification of serum miRNAs in 2008,¹⁷ several studies have shown that miRNAs present in human serum/plasma can predict the prognosis of diseases, such as nonsmall-cell lung cancer,¹⁸ breast cancer,¹⁹ and GDM, and so on. But few studies have investigated the relationship between the levels of circulating miRNAs in pregnant woman and macrosomia.

To develop this novel class of markers for clinical use and to investigate the detailed pathogenic mechanism of macrosomia, we systematically screened serum miRNAs associated with macrosomia using the Taqman Low Density Array (TLDA) chips followed by individual quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays. The aim of the present study was to explore the possibility of using a class of serum miRNAs as biomarkers for the prediction of macrosomia.

Methods

Ethics Statement

The study was approved by the Human Research Ethics Committees of Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University. Written informed consent was obtained from every patient and control individual before blood was taken.

Study Design

We designed a retrospective nest case-control study to determine whether serum miRNAs profiling could predict macrosomia. Partial pregnant women provided blood samples when they received prenatal care in the time frame of 16 to 20 gestational weeks and 1 week before delivery in Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University. These pregnant women were divided into 2 groups according to neonates' birth weight. The neonates with macrosomia were defined as those with birth weight equal to or greater than 4000 g in this study. Those neonates with birth weight between 2500 and 4000 g were classified as controls. The mothers had no diabetes during pregnancy, confirmed by negative results of oral glucose tolerance test of 75 g in 24th to 28th week of pregnancy. The mothers in this study had no smoking during pregnancy. Exclusion criteria also included multiple gestation, preterm labor, preeclampsia, and other complications during pregnancy. To control sample heterogeneity, cases and controls were frequency matched for pregnant women's age, BMI before pregnancy, gestational age, and infant gender in this study. Medical records were used to obtain information on risk factors, pregnancy history, and perinatal outcome by using a self-defined questionnaire.

We first collected the blood samples, 1 week before delivery, from 60 pregnant women with macrosomia and 60 pregnant women with normal birth weight from September 1, 2011 to June 30, 2012. Among these participants, the blood samples

collected in the period of 16 to 20 gestational weeks of 30 pairs of women with either macrosomic pregnancy or normal pregnancy were found in the biobank. Second, we collected blood samples in early second trimester from 30 pairs of women either with normal pregnancy or with macrosomia.

This study comprised of 3 case-control studies. At discovery stage, we performed miRNA profiling using TLDA chips in 2 pooled samples from 30 cases and 30 controls, respectively. Individual qRT-PCR was conducted for the discovery phase samples to further filter signals of the screened miRNAs due to heterogeneity in the participants. Subsequently, a 2-stage validation study (qRT-PCR of selected miRNAs), including an expanded sample validation and base upon early second-trimester serum sample validations, was conducted to confirm the results from the discovery stage (Figure 1).

Samples

Venous blood samples (fasting; 5.0 mL per pregnant women) were collected from cases and controls during prenatal care in Changzhou Maternity and Child Health Care Hospital affiliated to Nanjing Medical University using procoagulant drying tube. The whole blood was separated into serum and cellular fractions by centrifugation at 4000 rpm for 10 minutes, followed by 12 000 rpm for 15 minutes to completely remove cell debris. The supernatant serum was stored at -80°C until analysis.

RNA Extraction

Total RNA containing small RNA was extracted from serum samples using the miRVana PARIS Kit (Applied Biosystems Inc, California) according to the manufacturer's protocol. To control sample to serum variability during the RNA extraction and/or purification procedures, after the initial denaturing step, we spiked-in synthetic *C.elegans* miR-39 (cel-mir-39; Takara, Japan) to a final concentration of 10^{-4} pmol/ μL .

Taqman Low Density Array Chip Assays and qRT-PCR

In the discovery stage, we used TLDA chips (human microRNA panel V2.0, Applied Biosystems Inc) to screen differentially expressed miRNAs from the 2 pooled samples. A total of 600 μL serum from each pooled samples was used. Megaplex RT reactions and preamplification reactions were run according to the manufacturer's instructions. Then, 75 μL 0.1 $\times$ Tris-EDTA was added to dilute the PreAmp product. A 9 μL diluted PreAmp product was used to run the RT-PCR reactions by dispensing 100 μL of the PCR reaction mix into each port of the TaqMan MicroRNA Array. The default PCR procedure was used and the analysis was performed using RQ manager software (Applied Biosystems Inc). According to our laboratory experience by testing large sample, we selected cel-miR-39 as a normalizer in miRNA expression experiment.^{14,20}

ΔC_T and $\Delta\Delta\text{C}_\text{T}$ were calculated using the following mathematical formula:

Figure 1. Schematic diagram of the workflow of this study. TLDA indicates TaqMan Low Density Array; qRT-PCR, quantitative reverse-transcriptase PCR assay; ROC, receiver operating characteristic curve.

$\Delta C_T = C_{T\text{sample}} - C_{T\text{cel-miR-39}}$, $\Delta\Delta C_T = \Delta C_{T\text{case}} - \Delta C_{T\text{control}}$. A miRNA was considered altered if $CT < 35$ in the one of the 2 groups and $|\Delta\Delta C_T| > 2$, meaning 4-fold altered expression between the macrosomia and control groups.

We used TaqMan microRNA probes (Applied Biosystems Inc) to perform qRT-PCR assays according to the manufacturer's protocol. Equal volume of serum sample was processed in each step from serum purification to qRT-PCR. The total RNA was reversely transcribed to complementary DNA using TaqMan microRNA RT Kit and stem-loop RT primers (Applied Biosystems Inc). We performed RT-PCR by the TaqMan PCR kit on the ABI 7900 Real-Time PCR System (Applied Biosystems Inc). The reactions were initiated in a 384-well optical plate at 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The sample numbers of cases and controls were same on each plate (number of cases and controls displayed in Figure 1). The RT-PCR was examined for target miRNAs and cel-miR-39 simultaneously. All reactions were run in triplicate. The CT values were determined using the fixed threshold settings. The relative expression levels of target miRNAs were determined by the equation $2^{-\Delta C_T}$, in which $\Delta C_T = C_{T\text{sample}} - C_{T\text{cel-39}}$.

Statistical Analysis

Group comparisons of basic characteristics were performed using the Student *t* test or the Wilcoxon rank sum test, as appropriate. Simple and multiple logistic regression models were

used for analyses. The area under the curve (AUC) was calculated for miRNAs, in order to assess the miRNAs on macrosomia predicting. All analyses were performed with Stata 10.0 (Stata Corp, College Station, Texas). A *P* value of $< .05$ was considered statistically significant.

Results

Characteristics of the Sample Population

We selected serum samples 1 week before delivery and early second-trimester of maternal for this prospectively planned miRNA-biomarker study. About 30 pairs of serum samples were analyzed for the TLDA chip screening and individual qRT-PCR, another 30 pairs of serum samples 1 week before delivery, and 30 pairs of serum samples early second-trimester were detected to further confirm the results.

Two groups were well matched on maternal age, BMI before pregnancy, gestational weeks, and infant gender in this study. Study population characteristics are described in Table 1.

Micro RNA TLDA Chip Data Analysis

In all participants, 32 miRNAs showed $\Delta\Delta C_T > 2$ -fold, which included 26 downregulated miRNAs and 6 upregulated miRNAs. A total of 11 miRNAs showed $\Delta\Delta C_T > 3$ -fold, which

Table 1. Characteristics of the Study Population.^a

Variable	Discovery Stage Validation I (Blood Samples I Week Before Delivery)				Validation II (Blood Samples I Week Before Delivery)				Validation III (Blood Samples Early Second trimester)			
	Case (n = 30)	Control (n = 30)	t	P	Case (n = 60)	Control (n = 60)	t	P	Case (n = 30)	Control (n = 30)	t	P
Age, years	26.767 ± 2.825	27.167 ± 2.628	-0.568	.572	26.95 ± 2.971	27.117 ± 2.823	-0.315	.753	26.467 ± 2.488	26.933 ± 2.572	-0.714	.478
BMI before pregnancy, kg/m ²	21.255 ± 2.541	21.052 ± 2.386	0.32	.751	21.208 ± 2.364	21.105 ± 2.343	0.239	.811	21.014 ± 2.255	20.989 ± 2.221	0.043	.966
Gestational week, w	39.703 ± 0.861	39.397 ± 0.805	1.425	.16	39.5 ± 0.865	39.277 ± 0.798	1.47	.144	39.4 ± 0.817	39.19 ± 0.713	1.061	.293

Abbreviation: BMI, body mass index.

^at test was used to examine difference in continuous variables. *P* < .05, significant differences from controls. The mothers had no cigarette smoking during pregnancy. Both patients and controls are cesarean section. To control sample heterogeneity, cases and controls were frequency matched for pregnant women's age, BMI before pregnancy, gestational age, and infant gender in this study.

Table 2. Serum miRNA Expression in Macrosomia and Controls (TLDA Screening Results).^a

MicroRNA	Macrosomia	ΔC_T^a	Control	ΔC_T^b	$\Delta\Delta C_T^c$
miR-122	33.531	7.345	26.706	2.162	5.183
miR-923	34.313	8.127	27.573	3.029	5.098
miR-25	34.175	7.989	28.511	3.967	4.022
miR-20a	32.208	6.022	26.839	2.295	3.727
miR-223	25.917	-0.269	20.597	-3.947	3.678
miR-132	34.481	8.295	29.197	4.653	3.642
miR-192	32.575	6.389	27.393	2.849	3.54
miR-451	33.306	7.12	28.171	3.627	3.493
let-7b	34.758	8.572	29.624	5.08	3.492
miR-93	32.167	5.981	27.112	2.568	3.413
miR-760	30.722	4.536	32.623	8.079	-3.543
miR-144*	34.353	8.167	29.758	5.214	2.953
miR-21	34.931	8.745	30.573	6.029	2.716
miR-106a	29.125	2.939	24.842	0.298	2.641
miR-99b	34.233	8.047	30.26	5.716	2.331
miR-29a	32.395	6.209	28.484	3.94	2.269
miR-7-1*	34.641	8.455	30.73	6.186	2.269
miR-30c	30.663	4.477	26.797	2.253	2.224
let-7e	34.563	8.377	30.704	6.16	2.217
miR-200c	33.93	7.744	30.076	5.532	2.212
miR-142-3p	33.04	6.854	29.199	4.655	2.199
miR-19a	33.142	6.956	29.354	4.81	2.146
miR-30e*	32.49	6.304	28.733	4.189	2.115
miR-140-5p	32.977	6.791	29.24	4.696	2.095
miR-19b	27.971	1.785	24.247	-0.297	2.082
miR-30a	29.227	3.041	25.542	0.998	2.043
miR-30b	31.056	4.87	27.401	2.857	2.013
miR-99b*	31.357	5.171	32.447	7.903	-2.732
miR-526b*	32.657	6.471	33.674	9.13	-2.659
miR-139-3p	31.96	5.774	32.504	7.96	-2.186
miR-509-3p	33.454	7.268	33.878	9.334	-2.066
miR-483-5p	25.908	-0.278	26.27	1.726	-2.004

Abbreviations: TLDA, Taqman Low Density Array; miRNA, microRNA.

^a $\Delta C_T^a = C_{T \text{ case}} - C_{T \text{ case- cel-39}}$; $\Delta C_T^b = C_{T \text{ control}} - C_{T \text{ control- cel-39}}$; $\Delta\Delta C_T^c = \Delta C_T^a - \Delta C_T^b$; $C_{T \text{ case- cel-39}} = 26.186$; $C_{T \text{ control- cel-39}} = 24.544$. *, represents a way of naming of miRNA.

included 10 downregulated miRNAs and 1 upregulated miRNA. $\Delta\Delta C_T$ of miR-144 $\times$ ($\Delta\Delta C_T = 2.953$) is about 3-fold (Table 2).

Validation Result of miRNA Gene Expression Using qRT-PCR

We selected 12 miRNAs with great than or equal to 3-fold of change for individual qRT-PCR analyses on 30 pairs of discovery-stage samples. As our previous research had found that the levels of placental miR-21 was upregulated in macrosomia, we are interested in serum miR-21 expression in macrosomia, we also added miR-21 to individual qRT-PCR analyses. MiR-21 ($P = .011$) and miR-20a ($P = .015$) showed significant differential expression between macrosomia and controls (Table 3 and Figure 2). The qRT-PCR results were concordant with the miRNA microarray results, although miR-132 and miR-760 did not pass in the first validation.

Table 3. Results of 11 miRNAs in the Discovery Stage.^a

microRNA	Macrosomia median	Control median	$\Delta\Delta C_T^b$	Z	P^c
miR-122	0.012	0.016	5.183	1.685	.092
miR-923	0.024	0.047	5.098	1.703	.089
miR-25	0.010	0.026	4.022	0.894	.371
miR-20a	0.041	0.065	3.727	2.434	.015 ^d
miR-223	0.847	2.056	3.678	1.796	.073
miR-192	0.004	0.006	3.54	1.387	.166
miR-7b	0.033	0.049	3.492	1.501	.133
miR-451	0.040	0.055	3.495	0.070	.944
miR-93	0.059	0.115	3.413	0.785	.432
miR-144*	0.002	0.003	2.953	-0.049	.961
miR-21	0.036	0.073	2.716	2.556	.011 ^d

Abbreviations: TLDA, Taqman Low Density Array; miRNA, microRNA.

^aTLDA results of 30 pooled samples.^b $\Delta\Delta C_T = \Delta C_{T \text{ case}} - \Delta C_{T \text{ control}} - \Delta C_{T \text{ cel-39}}$ from TLDA data.^cDifference between groups was tested by Wilcoxon rank sum test.^d $P < .05$, significant differences from controls.

To further evaluate the diagnostic value of miR-21 and miR-20a, the levels of miR-21 and miR-20a were measured on a total 120 serum samples, including another 30 pairs of serum samples at 1 week before delivery and 30 pairs of serum samples among early second trimester. The levels of the miR-21 were significantly lower in serum samples at 1 week before delivery of macrosomia (Table 4 and Figure 2), but the expression levels of the 2 miRNAs were no different in the early second-trimester serum sample of macrosomia cases compared with controls (Table 4 and Figure 2).

We further plotted the receiver operating characteristic (ROC) curves for macrosomia predicting. The AUC analysis was only performed in the samples from the discovery and internal validation stages (30 pairs). Receiver operating characteristic curve analyses showed that the ROC curve areas for miR-21 was 67.7% (sensitivity = 66.7% and specificity = 70.0%; Figure 3).

Discussion

Macrosomia is defined as an infant's birth weight of more than 4000 g at term. It was diagnosed at postpartum. It has been implicated with many delivery complications including shoulder dystocia, traumatic birth injuries, and asphyxia.²¹ To improve pregnancy outcome, numerous studies have explored the possibility of early stage diagnosis to reduce the morbidity of macrosomia. Ultrasound is currently used as a noninvasive technique for macrosomia. Canavan TPetal find the AC in the >90th percentile at 28 to 34 weeks' gestation is the best sonographic predictor of macrosomia at birth.²² Little is known about the serum biomarkers for the diagnosis of macrosomia. The goal of the present study was to find a class of serum miRNAs as biomarkers for the prediction of macrosomia.

Our previous research used the TLDA followed by individual qRT-PCR assays to screen miRNAs in serum samples of early second trimester and found serum miR-376a may serve as a potential non-invasive biomarker in detecting macrosomia

Figure 2. Expression levels of miRNAs. VI indicates discovery stage blood samples 1 week before delivery, n = 30; VII, validation stage blood samples 1 week before delivery, n = 60; VIII, validation stage blood samples early second trimester, n = 30. VI, MiRNA-21 ($P = .011$) and miRNA-20a ($P = .015$) were significantly differentially expressed among macrosomia cases compared with controls (30 pairs). VII, The levels of the miRNA-21 ($P = 0.028$) was significantly lower in serum samples at 1 week before delivery of macrosomia, but the expression levels of the miRNA-20a ($P = .312$) were no different among the 60 pairs. VIII, The expression levels of the 2 miRNAs were no different among early second-trimester serum sample of macrosomia cases compared with controls.

Table 4. Expression of the Identified 2 miRNAs in the Validation Stages.^a

Validation	miRNAs	Macrosomia		Control		P^b	P^c
		N	Median	N	Median		
VII validation (blood samples one week before deliver)	miR-20a	60	0.033	60	0.038	.312	.321
	miR-21	60	0.021	60	0.023	.028 ^d	.014 ^d
VIII validation (blood samples early second- trimester)	miR-20a	30	0.062	30	0.062	.594	.738
	miR-21	30	0.164	30	0.150	.847	.592

Abbreviation: miRNA, microRNA.

^aSimple and multiple logistic regression models were used to analyze the data.

^bA model was constructed with simple logistic regression, miR-21 (continuous) or miR-20a (continuous) were candidate factors in the analysis.

^cB model was constructed with multiple logistic regression, the maternal old (continuous), maternal BMI before pregnancy (continuous), maternal gestational week (continuous), miR-21 or miR-20a (continuous) were candidate factors in the analysis.

^d $P < .05$, significant differences from controls.

(Manuscript submitted to “BioMed Research International”). Su et al²³ had demonstrated the differential expression patterns of miRNAs in different pregnancy stages. In this microarray study, we evaluated differential serum miRNAs expression in pregnant women of macrosomia at 1 week before delivery using 667 probes that represented both characterized and novel miRNAs. Our results showed that miR-376a did not present differential expression between macrosomia and controls at 1 week before delivery. However, we identified 32 miRNAs with differential expression patterns between macrosomia and controls at 1 week before delivery, with 26 downregulated miRNAs and 6 upregulated miRNAs. After a multistage validation, we found

Figure 3. Receiver-operating characteristic of the miR-21 signature to predict macrosomia between macrosomia pregnant women and controls: area under the curve (AUC) = 0.677, sensitivity = 0.667, specificity = 0.7.

aberrant miR-21 expression in serum samples of women with macrosomic pregnancy at 1 week before delivery.

Serum/plasma miRNAs was first identified in 2008.¹⁷ Results from recent studies have revealed the remarkable stability of miRNAs in various clinical samples.²⁴⁻²⁷ It was found that endogenous serum/plasma miRNAs exist in a form that is resistant to 4°C or 37°C incubation, freeze–thaw cycles, and even to RNase activity.^{24,27} It is regarded as the biomarker in the clinical diagnostics because of these important features of miRNAs.

A recent report has shown miRNA expressed in the human placenta.^{28,29} Chim et al¹³ suggested that several placental miRNAs were highly expressed in maternal plasma during

pregnancy. These results suggest miRNAs could act as potential serum markers for pregnancy monitoring.

We have used a 3-stage study to investigate the role of serum miRNAs in predicting macrosomia. We provide the first evidence suggesting that miR-21 is involved in the pathology of macrosomia. Our data suggest that low expression of miR-21 in the serum of third stage pregnant woman is associated with macrosomia. MiR-21 is encoded by a single gene, which locates at the fragile site FRA17B on 17p23.2 and is overlapped with the gene encoding transmembrane protein 49 (TMEM49, also known as VMP1).³⁰ MiR-21 contains 22 nucleotides and is separated from a 3400 nt pretranscript.³¹ As a typical multi-functional miRNA, miR-21 has distinct expression profiles and plays a crucial role in various physiological and pathological processes such as cancer,³² type 2 diabetes,³³ obesity,³⁴ and fetal growth.³⁵ These findings suggest that miR-21 promotes cell proliferation and induces cell dedifferentiation. Maccani et al.³⁵ analyzed 107 human placenta samples for the expression of 6-candidate miRNAs. They found that placental miR-21 expression was reduced in infants with the lowest birth weights. Our previous research found that the levels of placental miR-21 was upregulated in macrosomia. However, the miRNA level in tissue was not completely consistent with that in serum. Mouillet et al.³⁶ showed that plasma levels of miRNA are regulated in pregnancy and that fetal growth restriction may be associated with an increase in circulating miRNA levels. In this study, serum miR-21 expression was significantly lower in pregnant women with macrosomia as compared with the control group, and the satisfactory ROC values of miR-21 suggest that plasma miR-21 can be used as an effective, noninvasive marker for macrosomia forecasting. MiR-21 has been shown to target a number of key regulators of these processes, such as PLAG1, PTEN, and TGFBR2. However, the biological function of miR-21 in macrosomia is not clear. Further study on miR-21 is required to understand the pathogenesis of macrosomia.

In this study, we demonstrated that miR-20a was low expression in the serum of third-stage pregnant woman of the macrosomia group. But according to our observation, there was significant statistical difference at the discovery stage and the first validation stage ($P = .015$), while there was no statistical difference at the second validation stage with regard to the expression of miR-20a mRNA in the serum between the macrosomia group and the control group. Further studies with large sample sizes are warranted.

Many studies have shown the differential expression patterns of miRNAs in different stages of pregnancy. Our study also confirms this observation. The expression patterns of miR-21 and miR-20a in serum samples collected in the early second trimester were validated by qRT-PCR and there was no statistical difference at the early second trimester between the 2 groups.

In summary, we used miRNA microarray technology to analyze the miRNA expression profiles of macrosomia pregnant woman compared to normal birth weight pregnant woman from serum sample and validated the results with qRT-PCR in serum samples of third-stage pregnant women and in early second

trimester pregnant women. Our data suggest that low expression of miR-21 in serum samples of pregnant women in the third trimester is associated with macrosomia. Our study suggests that miRNAs have the potential to predict macrosomia. Future work investigating the roles of miR-21 in specific pathways leading to altered fetal growth is critical for a better understanding of the molecular mechanism of macrosomia.

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Authors' Note

Hua Jiang and Yang Wen contributed equally to the study and they should be regarded as joint first authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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