JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
SHORT REPORT

Investigation of umbilical cord serum miRNAs associated with childhood obesity: A pilot study from a birth cohort study

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Keywords
Adiposity rebound, Childhood obesity, Micro RNA

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J Diabetes Investig 2022; 13: 1740–1744

doi: 10.1111/jdi.13863

ABSTRACT

We investigated umbilical cord serum microRNA (miRNA) profiles to identify biomarkers of a risk for obesity later in life. Participating children were divided into high- and low-risk groups of obesity based on the timing of adiposity rebound and the body mass index (BMI) at 5 years and randomly selected from each group for this study. 3D-Gene® Human miRNA Oligo Chip was performed using cord serum in five children of both groups. The most relevant miRNAs were confirmed in 33 children of the groups using the TaqMan® microRNA assay. We detected five cord serum miRNAs differentially expressed in children at high risk of obesity compared with the levels in children at low risk, namely, miR-516-3p and miR-130a-3p with increased levels and miR-1260b, miR-4709-3p, and miR194-3p with decreased levels. This study provides the first identification of altered umbilical cord serum miRNAs in childhood obesity.

INTRODUCTION

Childhood obesity is increasing globally and has become a serious problem worldwide[1,2]. It is reported that the incidence of obesity from 5 to 14 years was related to the body weight at the age of 5 years[3]. Furthermore, early adiposity rebound (AR) has become the focus of attention as a factor related to adult obesity[4]. Body mass index (BMI) rises from birth to around 1 year of age and then declines. After that, it generally rises again at the age of 6–7 years. This second rise is called AR.

MicroRNAs (miRNAs) are now attracting substantial attention as useful biomarkers of various diseases[5–7]. The fetal environment is thought to influence the likelihood of developing diseases later in life. To predict childhood obesity and subsequent adulthood obesity, it is important to explore useful markers reflecting the fetal environment. We hypothesized that umbilical cord serum miRNAs could be biomarkers acting as early predictors of childhood obesity. Here, we examined the association between umbilical cord serum miRNAs and the risk of obesity.

Received 6 December 2021; revised 5 May 2022; accepted 31 May 2022

MATERIALS AND METHODS

Participants

This study was performed as an adjunct study of the Japan Environment and Children's Study (JECS). The JECS protocol has been described previously[8]. The JECS is a birth cohort study comprising 15 study regions including Chiba. In this study, the participants followed by the Chiba Regional Center were included. We extracted groups at high and low risk of obesity. Early AR was considered to reflect a high risk of obesity. Those who had a higher BMI at 3 years than at 1.5 years were identified as having early AR[9]. Children with early AR and whose BMI at 5 years of age was over the 95th percentile were considered as the high-risk group. Children without early AR and whose BMI at the age of 5 years was within the 25th–75th percentile were classified into the low-risk group. The BMI percentile was determined according to the BMI percentile chart for Japanese children[10]. Of the 2,716 children whose BMI

data were available at ages 1.5, 3, and 5, 66 (2.5%) were in the high-risk group for obesity and 1,279 (47%) were in the low-risk group. Five children were randomly selected from each group for comprehensive miRNA analysis. Moreover, the study included 28 children for whom umbilical cord serum could be prepared in the high-risk obesity group and 28 children who were randomly selected from the low-risk obesity group. Quantitative miRNA PCR was performed on these 33 children in each group.

Umbilical cord serum miRNA extraction

Umbilical cord blood was obtained immediately after birth. Cord blood was centrifuged, and cord serum was obtained by JECS and then stored at −20°C. miRNA was isolated from some of these samples using the miRNeasy Mini Kit (Qiagen, Hilden, Germany).

Comprehensive umbilical cord serum miRNA profiling

The serum levels of miRNAs were analyzed using the 3D-Gene® Human miRNA Oligo Chip (Toray Industries, Inc., Tokyo, Japan), which was designed to detect 2,632 miRNA sequences registered in miRBase release 20. The expression levels of miRNAs were globally normalized using the background-subtracted signal intensity of the entire miRNAs in each microarray[11,12]. Serum levels of miRNA were compared between the high- and low-risk groups. The top 10 miRNAs with the most significant findings (in terms of the *P*-value) were used for further analysis.

Analysis of individual miRNAs

The serum levels of selected miRNAs were examined by quantitative real-time PCR. The TaqMan® microRNA assay (Thermo Fisher Scientific, San Jose, CA, USA), TaqMan® Reverse Transcription kit (Thermo Fisher Scientific), and TaqMan® Universal PCR Master Mix II (Applied Biosystems, Foster City, CA, USA) were used in accordance with the manufacturers' instructions. Real-time PCR was performed in 96-well plates using ABI StepOne Plus Thermal Cycler (Applied Biosystems). Each PCR reaction was performed in triplicate. Fold changes in miRNA levels were calculated using the $2^{-\Delta\Delta C_t}$ method and Spike-In cel-miR-39 was used as a normalization control[13].

Statistical analysis

All the reported data are expressed as mean ± standard deviation. To compare the data between groups, we performed a *t*-test or Wilcoxon's rank-sum test according to their distribution, using GraphPad Prism 7. The Benjamini–Hochberg method was used to control for multiple testing.

RESULTS

Participants' characteristics

The clinical characteristics of the children enrolled in this study are shown in Table 1. At 5 years, height was 106.6 ± 4.7 and

Table 2 | Profile of differentially expressed miRNAs in cord serum from children at high and low risk of obesity

miRNA	Fold change	*P*-value
hsa-miR-516b-3p	1.34085	0.0036
hsa-miR-6721-5p	1.26432	0.0255
hsa-miR-4672	1.41865	0.0260
hsa-miR-130a-3p	3.05748	0.0284
hsa-miR-3065-3p	0.63102	0.0292
hsa-miR-1260b	1.8545	0.0310
hsa-miR-4709-3p	0.87565	0.0349
hsa-miR-194-3p	0.86472	0.0371
hsa-miR-3907	1.73265	0.0399
hsa-miR-612	1.26418	0.0426
hsa-miR-671-5p	0.77169	0.0442
hsa-miR-8055	0.74269	0.0457
hsa-miR-4,286	1.44223	0.0480

Fold change: log2 fold change (children at high vs low risk of obesity).

Table 1 | Clinical characteristics in children at low and high risk of obesity

	Low-risk group $n = 33$	High-risk group $n = 33$	*P*-value
Female, %	61	64	
Maternal BMI before pregnancy (kg/m^2)	21.2 ± 2.3	23.3 ± 3.9	**0.0116**
Weight gain during pregnancy (kg)	11.7 ± 3.6	8.0 ± 4.7	**0.0011**
Gestation period (weeks)	38.9 ± 1.2	38.8 ± 1.3	0.7036
Maternal age at delivery (years)	34.6 ± 5.0	33.0 ± 3.9	0.1574
Birth weight (g)	3,042 ± 268	3,073 ± 420	0.7326
Birth height (cm)	48.8 ± 1.4	48.6 ± 2.1	0.7362
BMI at 1.5 years (kg/m^2)	16.5 ± 0.9	16.6 ± 1.5	0.9508
BMI at 3 years (kg/m^2)	15.7 ± 0.7	17.7 ± 1.0	**6.54E-12**
Height at 5 years (cm)	106.6 ± 4.7	107.5 ± 4.4	0.4483
Body weight at 5 years (kg)	17.5 ± 1.7	22.1 ± 2.0	**3.77E-14**
BMI at 5 years (kg/m^2)	15.4 ± 0.5	19.1 ± 1.5	**3.01E-16**

Data are expressed as mean ± standard deviation. *P*-values are from Welch's two-sample *t*-test. *P*-values <0.05 are shown in bold.

Table 3 | Cord serum miRNA expression in children at low and high risk of obesity

	Low-risk group $n = 33$	High-risk group $n = 33$	*P*-value	*q*-value
miRNAs				
miR-130a-3p	0.93 ± 0.78	1.46 ± 1.06	**0.0136**	**0.0272**
miR-516-3p	1.56 ± 0.82	2.47 ± 1.33	**0.0009**	**0.0045**
miR-1260b	4.61 ± 3.50	2.81 ± 2.14	**0.0090**	**0.0225**
miR-4709-3p	2.60 ± 2.55	1.14 ± 0.50	**0.0008**	**0.0080**
miR-4672	1.25 ± 1.12	1.34 ± 0.90	0.1342	0.2237
miR-194-3p	1.07 ± 0.90	0.55 ± 0.24	**0.0017**	**0.0057**

Data are expressed as mean ± standard deviation. miRNA values were obtained by quantitative PCR and are shown as expression relative to sample 1. *P*-values were determined by Wilcoxon's rank-sum test. *P*-values <0.05 are shown in bold. *q*-values were determined by Benjamini-Hochberg method. *q*-values <0.05 are shown in bold.

107.5 ± 4.4 cm, while BMI was 15.4 ± 0.5 and 19.1 ± 1.5 kg/m^2 in the low- and high-risk groups, respectively. Birth weight was 3,042 ± 268 and 3,073 ± 420 g, respectively.

Selection of candidate umbilical cord serum miRNAs from the profiling

To select candidate miRNAs for subsequent examination, the serum levels of the 2,632 miRNAs were evaluated and compared between five subjects from the high-risk group and five from the low-risk group. Of these miRNAs, 13 showed a crude (unadjusted for multiple tests) *P*-value <0.05 (Table 2).

miRNA expression levels measured by qRT-PCR

We selected the top 10 most significant miRNAs for qRT-PCR assay. However, miR-6721-5p, miR-3065-3p, miR-3907, and miR-612 could not be detected using this protocol. Therefore, we evaluated the expression levels of miR-516b-3p, miR-4672,

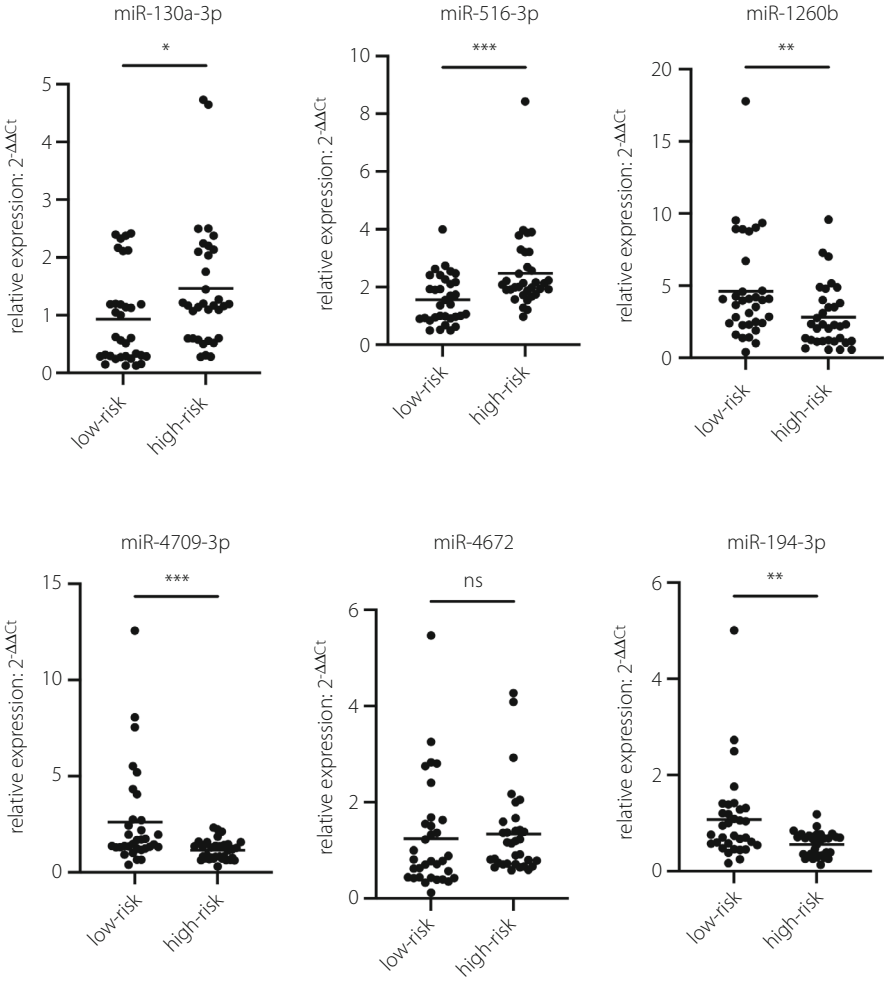

Figure 1 | Expression levels of six candidate miRNAs in cord serum from children at high ($n = 33$) and low risk of obesity ($n = 33$). miRNA levels were normalized to spike-in cel-miR-39 and are represented in scatter plots. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

miR-130a-3p, miR-1260b, miR-4709-3p, and miR194-3p. The serum levels of miR-516-3p and miR-130a-3p were higher in the high-risk group than in the low-risk group. In contrast, the serum levels of miR-1260b, miR-4709-3p, and miR194-3p were lower in the high-risk group than in the low-risk group (Table 3, Figure 1). For miR-1260b, the results of comprehensive analysis and quantitative PCR showed the opposite patterns.

DISCUSSION

The present study identified five umbilical cord serum miRNAs that were differentially expressed between groups at high and low risk of obesity. Recently, many studies have revealed that nutrient and environmental exposure during the fetal period impacts on postnatal growth and diseases such as metabolic syndrome later in life through epigenetic mechanisms including altered microRNA expression[14]. It is reported that a specific placental miRNA profile was related to prenatal and postnatal growth parameters[15]. In addition, Marcondes *et al.*[16] demonstrated that altered miR-181a in umbilical cord blood cells could be adopted as a biomarker for childhood obesity. Moreover, in the past few years, it has been reported that circulating miRNAs may also play a variety of biological roles, such as in energy homeostasis and metabolic processes[6,17,18]. Three of the candidate miRNAs in this study have been reported to be associated with metabolism. Interestingly, the hepatic exosome-derived miR-130a-3p regulates energy metabolism in adipose tissues[19]. Moreover, miR-1260b directly targets the 3'UTR of growth differentiation factor 11[20]. miR-194 was also reported to suppress the synthesis of glucagon-like peptide-1 in L cells[21]. Taken together, our data suggest that umbilical cord serum miRNAs may be associated with the biological process of childhood obesity and could be new biomarkers for the early identification of future obesity. Our study this time has a limited number of samples. Therefore, future studies with larger sample sizes are required to verify our findings.

ACKNOWLEDGMENTS

The authors thank the families that participated in the JECS study. The authors also thank Edanz (https://jp.edanz.com/ac) for editing the English text of a draft of this manuscript. This adjunct study was funded by JSPS KAKENHI Grant Number JP20K19670, the Japanese Society for Pediatric Endocrinology Future Development Grant supported by Novo Nordisk Pharma Ltd, and Kashiwado Medical Research Grant. The JECS was done using the budget of the Ministry of the Environment.

DISCLOSURE

This study was supported by a grant from the Yamada Bee Company Inc. The sponsor had no control over the interpretation, writing, or publication of this work.

Approval of the research protocol: The JECS protocol was approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies (Registration number: 2021-012) and by the ethics committees of all participating institutions. Approval date of registry of this adjunct study by Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University was August 30, 2019 and registry no. of the study trial: 995(958).

Informed consent: Written informed consent was obtained from all participating women in accordance with the Declaration of Helsinki. In conducting this adjunct study, we confirmed the consent of the participants by opt-out.

Animal studies: Not applicable.

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