Identification of microRNA signatures in umbilical cord blood associated with maternal characteristics

Jaroslav Juracek ¹, Pavel Piler ², Petr Janku ³, Lenka Radova ¹, Ondrej Slaby ¹

¹ Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic
² Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Brno, Czech Republic
³ Department of Gynecology and Obstetrics, Institutions shared with the Faculty Hospital Brno, Institutions of Reproductive Medicine, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Corresponding Author: Ondrej Slaby
Email address: ondrej.slaby@ceitec.muni.cz

Background. Umbilical cord blood could serve as useful source of blood markers enabling more efficient and reliable prenatal and neonatal diagnostics. MicroRNAs (miRNAs) are ubiquitous in body fluids where they were used for detecting and monitoring various physiological and pathological conditions. In this descriptive study, we aimed to identify changes in miRNA expression profiles associated with basic maternal somatic and epidemiological characteristics.

Methods. Study is based on 24 mothers from the Pilot phase of CELSPAC: TNG (Central European Longitudinal Studies of Parents and Children: The Next Generation) study. Cord blood was collected at time of delivery and global miRNA profiling was performed using microRNA Ready-to-use PCR Human Panel I+II TaqMan microarrays. Expression profiles were statistically evaluated in relation to maternal age, BMI, pregnancy weight gain, blood type, Rh factor status, allergies during pregnancy, addictive substance abuse and smoking status.

Results. We analyzed expression of 752 human mature miRNAs in 24 samples of umbilical cord blood. For all maternal characteristics tested we described a specific signature of significantly deregulated miRNAs (P<0.05). Analysis revealed 7 miRNA associated with maternal age (3 increased and 4 decreased in women younger than 35 years), 14 miRNAs associated with BMI status (5 miRNAs increased and 9 miRNAs decreased in women with BMI>25) and 9 miRNAs associated with maternal weight gain during pregnancy (8 miRNAs increased, and 1 miRNA decreased in women with weight gain < 12 kg). Additionally, 17 miRNAs correlated to blood type (2 miRNAs decreased in blood type A, 11 increased in blood type B, 2 miRNAs increased in blood type AB and 2 miRNAs increased in blood type 0) and 17 miRNAs to Rh status of mother. We also detected 7 miRNAs deregulated in umbilical cord blood of women with allergy (4 increased and 3 decreased in women with allergy), 4 miRNAs associated to addictive substance abuse status (2 up- and 2 downregulated in women with addictive substance abuse) and 8 miRNAs associated with maternal cigarette smoking.
Conclusions. We successfully described differences in miRNA profiles in umbilical cord blood associated with basic characteristics connected with mother. Our data suggest that miRNAs in umbilical cord blood are detectable and associated with a wide range of maternal characteristics. These results indicate that miRNAs could potentially serve, and should be studied, also as biomarkers for screening and diagnosis of pregnancy-associated complications and pathologies.
Identification of microRNA signatures in umbilical cord blood associated with maternal characteristics

Jaroslav Juracek¹, Pavel Piler², Petr Janku³, Lenka Radova¹, Ondrej Slaby¹*

¹Masaryk University, Central European Institute of Technology (CEITEC), Brno, Czech Republic.
²Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Brno, Czech Republic.
³Department of Gynecology and Obstetrics, Institutions shared with the Faculty Hospital Brno, Institutions of Reproductive Medicine, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

Corresponding author:
Prof. Ondrej Slaby, Ph.D.
Central European Institute of Technology (CEITEC), Masaryk University, University Campus Bohunice, Building A35, Kamenice 753/5, 625 00 Brno, Czech Republic
Tel. +420549497574
Email: on.slaby@gmail.com

Abstract

Background. Umbilical cord blood could serve as useful source of blood markers enabling more efficient and reliable prenatal and neonatal diagnostics. MicroRNAs (miRNAs) are ubiquitous in body fluids where they were used for detecting and monitoring various physiological and pathological conditions. In this descriptive study, we aimed to identify changes in miRNA expression profiles associated with basic maternal somatic and epidemiological characteristics.

Methods. Study is based on 24 mothers from the Pilot phase of CELSPAC: TNG (Central European Longitudinal Studies of Parents and Children: The Next Generation) study. Cord blood was collected at time of delivery and global miRNA profiling was performed using microRNA Ready-to-use PCR Human Panel I+II TaqMan microarrays. Expression profiles were statistically evaluated in relation to maternal age, BMI, pregnancy weight gain, blood type, Rh factor status, allergies during pregnancy, addictive substance abuse and smoking status.

Results. We analyzed expression of 752 human mature miRNAs in 24 samples of umbilical cord blood. For all maternal characteristics tested we described a specific signature of significantly deregulated miRNAs (P<0.05). Analysis revealed 7 miRNA associated with maternal age (3
increased and 4 decreased in women younger than 35 years), 14 miRNAs associated with BMI status (5 miRNAs increased and 9 miRNAs decreased in women with BMI>25) and 9 miRNAs associated with maternal weight gain during pregnancy (8 miRNAs increased, and 1 miRNA decreased in women with weight gain < 12 kg). Additionally, 17 miRNAs correlated to blood type (2 miRNAs decreased in blood type A, 11 increased in blood type B, 2 miRNAs increased in blood type AB and 2 miRNAs increased in blood type 0) and 17 miRNAs to Rh status of mother. We also detected 7 miRNAs deregulated in umbilical cord blood of women with allergy (4 increased and 3 decreased in women with allergy), 4 miRNAs associated to addictive substance abuse status (2 up- and 2 downregulated in women with addictive substance abuse) and 8 miRNAs associated with maternal cigarette smoking during pregnancy.

Conclusions. We successfully described differences in miRNA profiles in umbilical cord blood associated with basic characteristics connected with mother. Our data suggest that miRNAs in umbilical cord blood are detectable and associated with a wide range of maternal characteristics. These results indicate that miRNAs could potentially serve, and should be studied, also as biomarkers for screening and diagnosis of pregnancy-associated complications and pathologies.

Introduction

Umbilical cord blood (UCB) is blood that remains in the placenta and umbilical cord after birth [1]. Apart from common blood elements cord blood is rich source of primitive, undifferentiated hematopoietic stem cells [2]. Though it was originally considered as a waste product it has developed into an important allogeneic donor source in transplantation in pediatrics and a novel source of blood markers [3]. Especially in neonatal diagnostics, where blood from peripheral veins is used, UCB might be a suitable alternative and valuable source of blood biomarkers thanks to noninvasive and painless collection. Recent studies show that certain acute phase reactants are elevated in umbilical cord blood of premature infants with early onset sepsis [4]. Other studies described the distribution of immune biomarkers in cord blood across gestational age and show the association between biomarker level patterns and preterm birth [5]. Similarly, growth factors levels in cord blood can correlate with birth weight and postnatal growth in premature infants and was also associated with risk for postnatal growth failure [6]. One of the most abundant groups of biomarkers are microRNAs (miRNAs). They are ubiquitous in most of the body fluid types, where they may have functional roles associated with the surrounding tissues [7]. In addition, the changes in levels of specific miRNAs in body fluids were used for detecting and monitoring various somatic and pathological conditions [8 9]. The role of circulating miRNAs has been reported also in the context of neonatal diagnostics. Higher expression levels of miR-615-3p were observed in neonatal peripheral blood where this miRNA promoted acute respiratory distress syndrome (ARDS) development [10]. Similarly decrease in levels of miR-132 and miR-223 was associated with neonatal sepsis [11]. First description of miRNA profiling in cord blood was reported in 2015. In this study, downregulation of miR-374a-5p was observed in infants with hypoxic ischemic encephalopathy (HIE) [12]. In subsequent study altered miRNA levels were detected also in umbilical cord blood
of neonates with perinatal asphyxia (PA) suggesting their potential role in early detection of this
disease [13]. Moreover, previous research has shown that not only pathologies but also somatic
characteristic such as birth weight modifies the expression of miRNAs [14]. Based on these
observations we hypothesize that specific miRNA patterns in umbilical cord blood can be
associated with physiological as well as pathological conditions of mother or fetus.
In this pilot study, we aimed to perform high-capacity screening of miRNA levels in umbilical
cord blood in order to describe miRNA signatures associated with basic maternal somatic and
epidemiological characteristics.

Materials & Methods

Patient Cohorts

Study data and samples were obtained from the Pilot phase of CELSPAC: TNG (Central
European Longitudinal Studies of Parents and Children: The Next Generation Study).
CELSPAC: TNG is designed as a new prospective birth cohort which will follow up on 10 000
children from their prenatal period to adolescence with the aim of assessing multiple factors
potentially affecting children’s health. The Ethical committee of University Hospital Brno,
Czech Republic approved this study (No. 20140409-01). All mothers gave their written informed
consent.
The Pilot phase of CELSPAC: TNG was initiated in April 2015 to evaluate feasibility of the
protocol for collection, processing and storing of biological samples (cord blood; venous blood,
urine and buccal smear from mothers; stool, dry blood spot and buccal smear from babies); to
estimate future study response rates; to evaluate on-line distribution and respond rate of
questionnaires.
The current study included 24 mothers from whom we collected umbilical cord blood (UCB) at
time of delivery. We also used the data from medical records related to mother somatic
characteristics, pregnancy and birth. Women with non-physiological pregnancy including
medical and obstetrical complications or comorbid conditions that could affect fetus were
excluded.

Sample Collection and Processing

Cord blood was collected to S-Monovette® K3E (S-Monovette® 9 ml, K3 EDTA) after the
second stage of labor from the umbilical cord vein. Plasma was prepared by centrifugation (2
500g for 10 minutes at 22°C) and aliquoted into tubes as 250 µL samples and stored at -80°C.

miRNA Quantification

Prior to RNA isolation umbilical cord blood plasma samples were centrifuged at 4°C at 1000g
for 5 min. Total RNA from 200 µl of UCB plasma was isolated using miRNeasy Serum/Plasma
Kit (Qiagen, Hilden, Germany). RNA quality and quantity was evaluated using Nanodrop 2000
Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Whole-genome miRNA
profiling was performed by use of Human panel I+II, V4, miRCURY LNA miRNA miRNome
PCR Panel (Exiqon by Qiagen, Hilden, Germany) accordingly to the manufacturers protocol. On
each plate for each sample we included interplate calibrator enabling compensation of signal
variations between instrument runs (inter-plate calibrator assay UniSp3). Expression levels of miRNA represented by Ct values were normalized on U6 reference gene expression level using the $2^{-\Delta \text{Ct}}$ method where $\Delta \text{Ct} = (\text{Ct}_{\text{Target miRNA}} - \text{Ct}_{\text{U6}})$. Relative miRNA expression levels were correlated with selected epidemiological and somatic characteristics of mothers. Only miRNAs having non-zero expression values within more than 50% of samples were included in statistical analysis. Statistical analyses were performed within R/Bioconductor environment. The Mann-Whitney and Kruskal-Wallis test were applied for two or more categorical variables. In all comparisons, p-values <0.05 were set as statistically significant.

**Results**

Global profiling performed using TaqMan array enabling detection of 752 human mature miRNAs was performed in 24 umbilical cord blood plasma samples (Dataset S1). From this number, 656 miRNAs were detectable in at least one sample and 491 miRNAs had non-zero expression values within more than 50% of samples. For subsequent statistical analysis samples were regrouped by selected maternal characteristics including age, BMI, pregnancy weight gain (PWG), blood type, Rh factor status, allergies during pregnancy, addictive substance abuse and smoking status (summarized in Table 1). Genome-wide microRNA expression data have been deposited in the GEO repository (Gene Expression Omnibus, www.ncbi.nlm.nih.gov/geo/) under accession number GSE128943.

Global expression analysis revealed pattern of seven miRNA associated with maternal age namely miR−137, miR−665, miR−770−5p were increased and miR−625−3p, miR−377−3p, miR−224−3p and miR−671−3p decreased in women younger than 35 years. Next, we analyzed miRNA changes in relation to a maternal BMI. Analysis identified 14 miRNAs with differential levels between women with overweight (BMI>25) and women with normal BMI status (BMI 18.5-25). Five of these miRNAs were increased and nine miRNAs decreased in women with overweight (BMI>25). Similarly, levels of nine miRNAs were amended in umbilical cord blood in association with maternal weight gain during pregnancy (eight miRNAs increased, and one miRNA decreased in women with pregnancy weight gain lesser than 12 kg). Additionally, we identified 17 miRNAs with levels correlated to blood type of mother (two miRNAs showed decreased levels in blood type A group, 11 showed increased levels in blood type B group, two miRNAs were increased in blood type AB group and two miRNAs were increased in blood type 0 group). Moreover, 17 miRNAs were significantly increased when samples of Rh positive and Rh-negative women were compared. In mothers with unspecified allergies we detected pattern of seven miRNAs with deregulated levels; miR−181d−5p, miR−545−3p, miR−153−3p, miR−632 increased in women with allergy, miR−371a−3p, miR−96−5p, miR−216a−5p decreased in women with allergy. Next, we described four miRNAs with levels associated to addictive substance abuse status. Subsequent multivariate statistical analysis revealed increased levels of six miRNAs (miR−129−5p, miR−30b−3p, miR−187−3p, miR−507, miR−520b and miR−33b−3p) associated with maternal cigarette smoking during pregnancy. Similarly, we identified significantly higher level of miR-138-1-3p and decreased level of miR-760 in mothers
who quit smoking during pregnancy. Complete list of deregulated miRNAs associated with monitored maternal characteristics is summarized in Table 2 (detailed information is Supplementary Tables S1-S8).

Discussion

Circulating miRNAs are currently widely accepted as promising markers of both pathological and physiological conditions [9 15 16]. In reproductive medicine specific miRNA profiles in peripheral blood were found to relate to complications of pregnancy, such as placental abruption [17], ectopic pregnancy [18] or preeclampsia [19]. Moreover, expression patterns of circulating miRNA are promising solution for noninvasive prenatal testing of Down Syndrome and other genetic diseases [20]. However, considering direct connection with fetus, umbilical cord blood could serve as valuable source of biomarkers in prenatal diagnostics and screening.

In our pilot study, we successfully described aberration in miRNA profiles in umbilical cord blood plasma associated with basic characteristics connected with mother. So far publications focused on the identification of miRNA expression profiles in umbilical cord blood were connected mainly to pathophysiology of a particular disease or pathological conditions. As in the case of infants with hypoxic ischemic encephalopathy (HIE) where miR-374a revealed significant down-regulation in cord blood of infants with perinatal asphyxia and subsequent HIE [12]. Similarly study of Rager et al. highlight miRNAs as novel responders to prenatal arsenic exposure that may contribute to associated immune response perturbations [21]. Despite diagnostic potential of UBC there is lack of descriptive studies which demonstrate miRNA deregulation in association with basic somatic and epidemiological characteristics of mothers and newborns. Ghaffari et al. investigated whether maternal obesity is associated with alterations in expression of fetal miRNAs [22]. Despite negative results, this study delineated role of miRNA within delivery course and success rate.

Since umbilical cord blood flow is dynamic and progressive process where exchange of blood elements and nutrients occurs, we expected that possible deregulation should be influenced by both the mother’s and the newborn’s environment. Currently there are no comparable studies supporting our findings, however, we found overlap in identified miRNAs within studies focusing on miRNAs functioning. For example, miR-625-3p and miR-671-3p showing significant association with maternal age in our study were described also in study of Huan et al. as age-associated miRNAs [23]. Moreover, miR-671-3p seems to be differentially expressed between keratinocytes prepared from child skin and aged skin [24]. Similarly, we identified miRNAs indicating association with maternal BMI or weight gain during pregnancy. In accordance with our findings, miR-143-3p was depicted as regulator of adipocyte differentiation [25] and its upregulation in mesenteric fat in mice was associated with body weight [26]. MiR-450-5p, miR-203a, miR-141-3p and miR-205-5p were found to be differentially expressed in subcutaneous adipose tissue of obese individuals and normal-weight subjects [27]. Other miRNAs such as miR-551a or miR-138-5p were already associated with BMI [28] or weight gain [29]. Regarding to mothers with allergies, we identified miR-181d-5p, member of miR-181
family, which has a central role in vascular inflammation by controlling critical signaling pathways and regulates immune cell homeostasis [30]. Other identified molecule, miR-371a-3p was suggested to modulate the Th1/Th2 balance in asthma [31]. Among miRNAs deregulated within mothers who did smoke during pregnancy we described miR-129-5p which was observed to be upregulated in lung cancer patients with a smoking history [32 33]. Further, miR-33b-3p was differentially expressed between rectal cancer tissue and normal rectal mucosa and associated with smoking and miR-520b was significantly differentially expressed with cigarette smoking and associated with CIMP and/or MSI status in colon and rectal cancer [34].

Conclusion

The data obtained in this pilot study indicated that miRNA levels in umbilical cord blood plasma are related to somatic and epidemiological characteristics of mother and newborn infants. Therefore, UCB miRNAs should be studied also as biomarkers for screening and diagnosis of pregnancy-associated complications and pathologies.

Acknowledgements

Many thanks go to the participating families as well as the physicians and medical staff of The University Hospital Brno, and the entire study team.

References

1. Waller-Wise R. Umbilical cord blood: information for childbirth educators. The Journal of perinatal education 2011;20(1):54-60 doi: 10.1891/1058-1243.20.1.54[published Online First: Epub Date]].
2. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. British journal of haematology 2000;109(1):235-42
3. O'Brien TA, Tiedemann K, Vowels MR. No longer a biological waste product: umbilical cord blood. The Medical journal of Australia 2006;184(8):407-10
4. Mithal LB, Palac HL, Yogev R, Ernst LM, Mestan KK. Cord Blood Acute Phase Reactants Predict Early Onset Neonatal Sepsis in Preterm Infants. PloS one 2017;12(1):e0168677 doi: 10.1371/journal.pone.0168677[published Online First: Epub Date]].
5. Matoba N, Yu Y, Mestan K, Pearson C, Ortiz K, Porta N, Thorsen P, Skogstrand K, Hougaard DM, Zuckerman B, Wang X. Differential patterns of 27 cord blood immune biomarkers across gestational age. Pediatrics 2009;123(5):1320-8 doi: 10.1542/peds.2008-1222[published Online First: Epub Date]].
6. Voller SB, Chock S, Ernst LM, Su E, Liu X, Farrow KN, Mestan KK. Cord blood biomarkers of vascular endothelial growth (VEGF and sFlt-1) and postnatal growth: a preterm birth cohort study. Early human development 2014;90(4):195-200 doi: 10.1016/j.earlhumdev.2014.01.003[published Online First: Epub Date]].
7. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. Clinical chemistry 2010;56(11):1733-41 doi: 10.1373/clinchem.2010.147405[published Online First: Epub Date]].
8. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nature reviews. Clinical oncology 2011;8(8):467-77 doi: 10.1038/nrclinonc.2011.76[published Online First: Epub Date]].
9. Velu VK, Ramesh R, Srinivasan AR. Circulating MicroRNAs as Biomarkers in Health and Disease.
Journal of clinical and diagnostic research : JCDR 2012;6(10):1791-5 doi: 10.7860/jcdr/2012/4901.2653[published Online First: Epub Date]].

10. Wu YQ, Ding YJ. Overexpressed microRNA-615-3p promotes progression of neonatal acute respiratory distress syndrome by inhibiting differentation of mesenchymal stem cells to alveolar type II epithelial cells. European review for medical and pharmacological sciences 2018;22(14):4625-33 doi: 10.26355/eurrev_201807_15521[published Online First: Epub Date]].

11. Dhas BB, Dirisala VR, Bhat BV. Expression Levels of Candidate Circulating microRNAs in Early-Onset Neonatal Sepsis Compared With Healthy Newborns. Genomics insights 2018;11:1178631018797079 doi: 10.1177/1178631018797079[published Online First: Epub Date]].

12. Looney AM, Walsh BH, Moloney G, Grencham S, Fagan A, O'Keeffe GW, Clarke G, Cryan JF, Dinan TG, Boylan GB, Murray DM. Downregulation of Umbilical Cord Blood Levels of miR-374a in Neonatal Hypoxic Ischemic Encephalopathy. The Journal of pediatrics 2015;167(2):269-73.e2 doi: 10.1016/j.jpeds.2015.04.060[published Online First: Epub Date]].

13. O'Sullivan MP, Looney AM, Moloney GM, Finder M, Hallberg B, Clarke G, Boylan GB, Murray DM. Validation of Altered Umbilical Cord Blood MicroRNA Expression in Neonatal Hypoxic-Ischemic Encephalopathy. JAMA neurology 2018 doi: 10.1001/jamaneurol.2018.4182[published Online First: Epub Date]].

14. Rodil-Garcia P, Arellanes-Licea EDC, Montoya-Contreras A, Salazar-OLivo LA. Analysis of MicroRNA Expression in Newborns with Differential Birth Weight Using Newborn Screening Cards. International journal of molecular sciences 2017;18(12) doi: 10.3390/ijms18122552[published Online First: Epub Date]].

15. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer science 2010;101(10):2087-92 doi: 10.1111/j.1349-7006.2010.01650.x[published Online First: Epub Date]].

16. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America 2008;105(30):10513-8 doi: 10.1073/pnas.0804549105[published Online First: Epub Date]].

17. Miura K, Higashijima A, Murakami Y, Fuchi N, Tsukamoto O, Abe S, Hasegawa Y, Miura S, Masuzaki H. Circulating Levels of Pregnancy-Associated, Placenta-Specific microRNAs in Pregnant Women With Placental Abruptio. Reproductive sciences (Thousand Oaks, Calif.) 2017;24(1):148-55 doi: 10.1177/1933719116653837[published Online First: Epub Date]].

18. Zhao Z, Zhao Q, Warrick J, Lockwood CM, Woodworth A, Moley KH, Gronowski AM. Circulating microRNA miR-323-3p as a biomarker of ectopic pregnancy. Clinical chemistry 2012;58(5):896-905 doi: 10.1373/clinchem.2011.179283[published Online First: Epub Date]].

19. Gunel T, Zeybek YG, Akcakaya P, Kallelioglu I, Benian A, Ermis H, Aydiniyi K. Serum microRNA expression in pregnancies with preeclampsia. Genetics and molecular research : GMR 2011;10(4):4034-40 doi: 10.4238/2011.November.8.5[published Online First: Epub Date]].

20. Erturk B, Karaca E, Akyut A, Durmaz B, Guler A, Buke B, Yeniel AO, Ergenoglu AM, Ozkinay F, Ozeren M, Kazandi M, Akerca F, Sagol S, Gunduz C, Cogulu O. Prenatal Evaluation of MicroRNA Expressions in Pregnancies with Down Syndrome. BioMed research international 2016;2016:5312674 doi: 10.1155/2016/5312674[published Online First: Epub Date]].

21. Rager JE, Bailey KA, Smeester L, Miller SK, Parker JS, Laine JE, Drobna Z, Currier J, Douillet C, Olshan AF, Rubio-Andrade M, Styblo M, Garcia-Vargas G, Fry RC. Prenatal arsenic exposure and the epigenome: altered microRNAs associated with innate and adaptive immune signaling in newborn cord blood. Environmental and molecular mutagenesis 2014;55(3):196-208 doi: 10.1002/em.21842[published Online First: Epub Date]].

PeerJ reviewing PDF | (2019:01:34024:1:1:NEW 16 Apr 2019)
22. Ghaffari N, Parry S, Elovitz MA, Durnwald CP. The Effect of an Obesogenic Maternal Environment on Expression of Fetal Umbilical Cord Blood miRNA. Reproductive sciences (Thousand Oaks, Calif.) 2015;22(7):860-4 doi: 10.1177/1933719114565032[published Online First: Epub Date]]

23. Huan T, Chen G, Liu C, Bhattacharya A, Rong J, Chen BH, Seshadri S, Tanriverdi K, Freedman JE, Larson MG, Murabito JM, Levy D. Age-associated microRNA expression in human peripheral blood is associated with all-cause mortality and age-related traits. Aging cell 2018;17(1) doi: 10.1111/acel.12687[published Online First: Epub Date]]

24. Muther C, Jobeili L, Garion M, Heraud S, Thepot A, Damour O, Lamartine J. An expression screen for aged-dependent microRNAs identifies miR-30a as a key regulator of aging features in human epidermis. Aging 2017;9(11):2376-96 doi: 10.18632/aging.101326[published Online First: Epub Date]]

25. Esau C, Kang X, Peralta E, Hanson E, Marcusson EG, Ravichandran LV, Sun Y, Koo S, Perera RJ, Jain R, Dean NM, Freier SM, Griffin R. MicroRNA-143 regulates adipocyte differentiation. The Journal of biological chemistry 2004;279(50):52361-5 doi: 10.1074/jbc.C400438200[published Online First: Epub Date]]

26. Takanabe R, Ono K, Abe Y, Takaya T, Horie T, Wada H, Kita T, Satoh N, Shimatsu A, Hasegawa K. Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. Biochemical and biophysical research communications 2008;376(4):728-32 doi: 10.1016/j.bbrc.2008.09.050[published Online First: Epub Date]]

27. Kurylowicz A, Wicik Z, Owczarz M, Jonas MI, Kotlarek M, Swierniak M, Lisik W, Jonas M, Noszczyk B, Pazunowska-Kuznicka M. NGS Reveals Molecular Pathways Affected by Obesity and Weight Loss-Related Changes in miRNA Levels in Adipose Tissue. International journal of molecular sciences 2018;19(1) doi: 10.3390/ijms19010066[published Online First: Epub Date]]

28. Iacomino G, Russo P, Marena P, Lauria F, Venezia A, Ahrens W, De Henauw S, De Luca P, Foraita R, Gunther K, Lissner L, Molnar D, Moreno LA, Tornaritis M, Veidebaum T, Siani A. Circulating microRNAs are associated with early childhood obesity: results of the I.Family Study. Genes & nutrition 2019;14:2 doi: 10.1186/s12263-018-0622-6[published Online First: Epub Date]]

29. Zhao H, Shen J, Daniel-MacDougall C, Wu X, Chow WH. Plasma MicroRNA signature predicting weight gain among Mexican-American women. Obesity (Silver Spring, Md.) 2017;25(5):958-64 doi: 10.1002/oby.21824[published Online First: Epub Date]]

30. Sun X, Sit A, Feinberg MW. Role of miR-181 family in regulating vascular inflammation and immunity. Trends in cardiovascular medicine 2014;24(3):105-12 doi: 10.1016/j.tcm.2013.09.002[published Online First: Epub Date]]

31. Qiu YY, Zhang YW, Qian XF, Bian T. miR-371, miR-138, miR-544, miR-145, and miR-214 could modulate Th1/Th2 balance in asthma through the combinatorial regulation of Runx3. American journal of translational research 2017;9(7):3184-99

32. Mullany LE, Herrick JS, Wolff RK, Stevens JR, Slattery ML. Association of cigarette smoking and microRNA expression in rectal cancer: Insight into tumor phenotype. Cancer epidemiology 2016;45:98-107 doi: 10.1016/j.canep.2016.10.011[published Online First: Epub Date]].
List of maternal characteristics used in specific statistical analysis
Table 1: List of maternal characteristics used in specific statistical analysis

| Maternal characteristics       | Specific characteristics (Number of subjects) |
|--------------------------------|-----------------------------------------------|
| Age                           | <35 years (15)  > 35 years (9)               |
| BMI                           | <25 (19)  >25 (5)                               |
| Pregnancy weight gain         | <12 kg (12)  >12 kg (12)                       |
| Blood type                    | A (10)  B (4)  0 (2)  AB (5)                   |
| Rh factor                     | Negative (6)  Positive (18)                    |
| Allergies                     | Yes (12)  No (12)                              |
| Addictive substance abuse     | Yes (9)  No (15)                               |
| Smoking status                | Non-smoker (15)  Smoker (3)  Stop-smoker (6) |
Table 2

Table 2

List of umbilical cord blood miRNAs significantly associated with individual maternal characteristics (P<0.05).
Table 2: List of umbilical cord blood miRNAs significantly associated with individual maternal characteristics (P<0.05).

| Maternal characteristics | List of associated miRNAs |
|--------------------------|---------------------------|
| Age (≤ 35 years)         | ↑ miR−137, miR−665, miR−770−5p; ↓ miR−625−3p, miR−377−3p, miR−224−3p, miR−671−3p. |
| BMI (> 25)               | ↑ miR-1203, miR-143-3p, miR-582-5p, miR-510-5p, miR-450a-5p; ↓ miR-604, miR-205-5p, miR-551a, miR-203a, miR-548l, miR-424-5p, miR-627-5p, miR-629-3p, miR-141-3p. |
| Pregnancy weight gain (<12 kg) | ↑ miR−138−5p, miR−760, miR−9−3p, miR−548c−5p, miR−1260a, miR−145−3p, miR−34a−3p, miR−320d; ↓ miR−1224−3p. |
| Blood type               | A: ↓ miR−380−5p, miR−92a−1−5p; B: ↑ miR−760, miR−10b−5p, miR−34b−3p, miR−145−5p, miR−153−3p, miR−548c−5p, miR−511−5p, miR−330−5p, miR−24−1−5p, let−7b−3p, let−7f−2−3p; AB: ↑ miR−595, miR−431−3p; 0: ↑ miR−641, miR−548h−5p. |
| Rh factor status (positive) | ↑ miR−141−3p, miR−188−5p, miR−211−5p, miR−205−5p, miR−150−5p, miR−181c−5p, miR−124−3p, miR−142−5p, miR−15b−5p, miR−1269a, miR−1260a, miR−518d−3p, miR−27a−5p; ↓ miR−514a−3p, miR−449b−5p, miR−641, miR−548l. |
| Allergies (Yes)          | ↑ miR−181d−5p, miR−545−3p, miR−153−3p, miR−632; ↓ miR−371a−3p, miR−96−5p, miR−216a−5p. |
| Addictive substance abuse (Yes) | ↑ miR−138−1−3p, miR−33b−3p; ↓ miR−760, miR−377−3p. |
| Smoking status (Yes)     | ↑ miR−129−5p, miR−30b−3p, miR−187−3p, miR−507, miR−520b, miR−33b−3p, miR−138-1-3p; ↓ miR-760. |