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MATERNAL-FETAL MEDICINE

Circulating levels of specific members of chromosome 19 microRNA cluster are associated with preeclampsia development

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Abstract

Purpose To perform serum microRNA expression profiling to identify members of chromosome 19 miRNA cluster involved in preeclampsia development.

Methods Serum chromosome 19 miRNA cluster microRNA expression profiling was evaluated at 12, 16, and 20 gestational weeks and at the time of preeclampsia diagnosis, in women who developed preeclampsia (WWD-PE; $n = 16$) and controls ($n = 18$) using TaqMan low density array plates.

Results A total of 51 chromosome 19 microRNA cluster members were evaluated. The circulating hsa-miRs 512-3p, 518f-3p, 520c-3p, and 520d-3p, were differentially expressed between groups ($P < 0.05$). Compared with controls, serum levels of hsa-miR-518f-3p at 20 GW were useful for identifying WWD-Mild-PE ($P = 0.035$) and WWD-Severe-PE ($P = 0.007$).

Conclusions Serum hsa-miRs 512-3p, 518f-3p, 520c-3p, and 520d-3p, are differentially expressed between WWD-PE and controls and their role in the development of preeclampsia should be investigated further.

Keywords Biomarkers · Preeclampsia · Placenta · Signaling pathway · MicroRNA

Introduction

Micro-RNAs (miRNA) are non-coding, single-stranded RNAs of 17–25 nucleotides in length that are highly conserved during evolution and that function to negatively regulate gene expression by mechanisms such as translational repression, mRNA cleavage, and deadenylation [1–3]. Mechanistically, miRNAs exert their regulatory effects by binding to the complementary untranslated regions on their mRNA targets [2, 3]. One miRNA can regulate the mRNA degradation of many genes and thus has the ability to alter multiple signaling pathways and impact essential biological processes such as human embryogenesis and placental

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development [4, 5]. The placenta is a vital transient organ during pregnancy that is involved in the exchange of gases, nutrients, and waste products between the mother and the fetus. In a normal pregnancy, the placenta expresses ubiquitous and specific miRNAs that participate in the regulation of trophoblast cell differentiation, proliferation, migration, invasion, angiogenesis, and apoptosis [6–9]. Numerous miRNAs expressed during pregnancy are clustered in chromosomal regions, may be controlled by the same promoters, may have similar seed regions and targets, and work synergistically. The three most eminent clusters are the chromosome 19 miRNA cluster (C19MC), miR-371-3 cluster, which is also localized on chromosome 19, and the chromosome 14 miRNA cluster (C14MC) [10]. The C19MC locus maps to chromosome 19q13.41, spans about 100 kb of genomic DNA and contains 46 pri-miRNA genes, yielding 59 mature miRNAs that are found exclusively in primates, attesting to their recent evolution [10, 11]. Although the expression of C19MC is mainly restricted to the reproductive system, the expression of hsa-miR-498 has been reported in fetal brain, and some C19MC members are highly expressed in human embryonic stem cells [10]. Based on microarray technology, miRNAs within the C14MC, miR-371-3 cluster, and C19MC were found to be significantly upregulated in first trimester placentas [12]. Following on from this it was demonstrated that trophoblast and stromal cells of the villi release exosomes (small membrane microvesicles) into the maternal circulation [10, 13]. The exosomes miRNA composition strongly resembled that of the primary cells from which they were secreted. In both cases C19MC members accounted for the majority of mature miRNAs and six of them were among the top-ten exosomal miRNAs [13]. Based on these findings it has been assumed that these miRNAs play an important role in placental–maternal communication, possibly directing maternal adaptation to pregnancy [13, 14]. In pathological pregnancy conditions such as preeclampsia (PE; defined as hypertension and proteinuria after 20 weeks of pregnancy), in which abnormal trophoblast invasion is the most important issue, recent studies suggest that deregulation of miRNA expression in placental trophoblasts may contribute to the development of the pathology [8, 13, 15, 16]. MiRNAs, including C19MC members have shown aberrant expression in case–control studies consisting of individuals with PE and healthy pregnancy controls [8, 15, 17–20]. While studies initially focused on placental samples from term deliveries, pre-term circulatory samples from PE cases were soon revealed to also harbor detectable quantities of differentially expressed C19MC miRNAs [13, 16]. Although the use of these circulating placental C19MC miRNAs as biomarkers for pregnancy-related disorders has been suggested, there is no conclusive evidence regarding the presence of a differential expression profile of the C19MC associated with the development of PE. The aim of this study was to identify circulating members of C19MC at 12, 16, and 20 gestational weeks (GW) and at the time of PE diagnosis in serum samples from pregnant women who developed PE (WWD-PE) and from controls, to evaluate their association with the development of PE.

Methods

Patients and study design

We performed a nested cohort case–control study in which the participants were drawn from a cohort of 588 pregnant women who were followed from the first trimester to delivery as part of a screening study for adverse pregnancy outcomes in the Centro de Salud Urbano de Zacatecas "José Castro Villagrana" and the Hospital de la Mujer Zacatecana, from Zacatecas' Health Services, in Zacatecas, Mexico between November 2011 and January 2014. Sixteen women who developed PE during the follow-up period were selected and individually matched to 18 women in the cohort who had healthy pregnancies without complications (normotensive controls). The criteria for PE diagnosis and its severity/onset sub-classifications were established as follows: PE was considered as severe if the patient had a blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic on two occasions at least 6 h apart while the patient was at bed rest, and a proteinuria of 5 g or more in a 24-h urine specimen, or 3 + or greater in two random urine samples collected at least 4 h apart. Early PE was considered if present before 34 GW and late PE as that occurring with more than 34 + 1 GW [21–23]. The study exclusion criteria included gestational hypertension, gestational diabetes mellitus, and underlying medical diseases such as chronic hypertension, diabetes mellitus type 2, hypo/hyperthyroidism, chronic renal disease, rheumatoid arthritis, and systemic lupus erythematosus. This protocol complied with the Declaration of Helsinki. The patients in the study provided written informed consent for their participation, and Institutional Review Board approvals were obtained from the participating institutions, as follows: Comité de Bioética del Área de Ciencias de la Salud-UAZ (ID numbers: ACS/UAZ. Ofc. Nos. 0072009, 0062010) and Comité de Enseñanza, Investigación, Capacitación y Ética from Hospital de la Mujer Zacatecana (ID numbers: HMZ-520/281/11 and HMZ-5020/318/11).

Biological samples

All participants (initially normotensive pregnant women) provided peripheral blood at the 12th, 16th and/or 20th GW upon enrolling in the study. Patients who had a PE diagnosis during follow-up provided an additional blood sample at the time of diagnosis. Women with gestational ages that were

different from 12, 16, or 20 GW ranging between 13 and 19 GW were also included in the study (although these participants had lower sample numbers). Blood samples were collected in tubes without anticoagulant and centrifuged at 2000 rpm for 10 min at room temperature. The serum was collected, aliquoted, and stored at − 80 °C until assayed. Blood samples with hemolytic processes or from patients with a urinary tract infection at the time of sampling were not considered for the study.

MiRNA isolation from serum samples

Before miRNA isolation, the serum samples were thawed on ice and centrifuged at 13,300 rpm for 5 min to remove any trace of cellular debris or sediment. Total RNA was isolated from 200 µl of serum using the miRNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions and eluted in 50 µl of nuclease-free water. The RNA quality and concentration were assessed using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., USA).

Maternal serum miRNA profiling using a TaqMan low density array (TLDA)

A total of 99 miRNA samples from the 34 participants were included, with 24 from samples collected at 12 GW (6 from WWD-PE and 18 from controls), 28 at 16 GW (10 from WWD-PE and 18 from controls), 32 at 20 GW (14 from WWD-PE and 18 from controls), and the remaining 15 from the WWD-PE group at the time of PE diagnosis (Fig. 1). The C19MC miRNA expression was quantified for each of the 45 PE samples, while only 25 serum samples from five normotensive pregnant women were individually quantified at 12, 16, and 20 GW for the control group. One pooled sample from the 13 additional controls was also included for each time point (Fig. 1). Quantification of the serum levels of 51 C19MC members (Online Resource 1) was performed using Megaplex Pools from the TaqMan Human MicroRNA Array set v2.0 (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. cDNAs for the mature miRNAs were synthesized using 40–180 ng of total RNA from 99 serum samples. A 12-cycle pre-amplification round was included to enhance the ability to detect miRNAs with low expression. The cDNA synthesis and pre-amplification steps were performed on a Veriti® 96-Well Thermal Cycler (Applied Biosystems). Quantitative real-time polymerase chain (qRT-PCR) reactions were performed according to the manufacturer's instructions, replacing the suggested pre-amplified product diluent (buffer TE 1×) with nuclease-free water. The qRT-PCR reactions were performed on an Applied Biosystems ViiA™ 7 Real-Time PCR System using the default cycling conditions. Quantification cycle (C_q) values were calculated using ViiA™ 7 Software, and the automatic baselines and thresholds were homogenized across multiple runs using Expression Suite software v1.0.3 (Applied Biosystems). A sample was considered positive if the amplification signal occurred before the 37th C_q.

Data analysis

Comparisons of the risk factors and personal characteristics among the groups were performed using the Chi-squared test or Fisher's exact test. Continuous variables were compared between the groups using the Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks coupled to Dunn's method as a multiple comparison procedure. At each evaluated pregnancy time point, the relative quantity (RQ) of circulating miRNAs was determined from the qRT-PCR data using the global normalization method, the control group as a reference, and a confidence level of 95%. At the time of PE diagnosis, the comparison between the mild and severe PE groups was performed using the mild PE data as the reference. ΔC_q values and standard errors were calculated for the data generated using ExpressionSuite software v1.0.3 (Applied Biosystems).

Results

A total of 34 patients were selected for the study, including 16 who developed PE during the follow-up and were among the women who developed PE (WWD-PE) (cases). The control group included 18 women with healthy pregnancies who

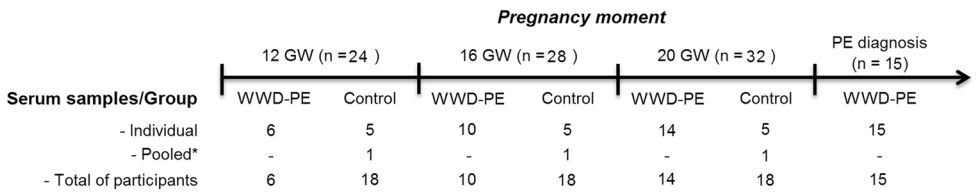

Fig. 1 Summary of the serum samples included in the study. A total of 99 serum samples from the 34 participants were considered. In the controls one pooled sample was included for each stage of pregnancy. Each pooled sample (*) was prepared using 20 µl of serum from 13 additional controls

were individually paired with the WWD-PE patients based on parameters such as maternal age, nulliparity, personal and family histories of PE, and body mass index (Table 1). The mean maternal age was 23.5 years for the WWD-PE group and 23.4 years for the control group. The nulliparity variable was present in 37.5% of the WWD-PE patients and 50% of the controls ($P = 0.699$). No differences in other known PE risk factors or in clinical data were observed between the groups (Table 1 and Online Resource 2). At the time of PE diagnosis, the patients showed a mean systolic blood pressure of 151.3 mm/Hg and a mean diastolic blood pressure of 100 mm/Hg, and their urine protein values ranged between 300 and 619.3 mg/dl. Of the cases, 25% of patients received a PE diagnosis before 34 GW (early PE), and the remaining 75% had late-onset disease (range 34–37 GW). Thirty-one per cent of the cases were sub-classified as severe PE.

The expression of 51 C19MC members was evaluated in the study (Online Resource 1). Among the miRNAs evaluated 10 miRNAs were detected ($C_q < 37$) in serum of the participants at 12 and 16 GW, while the amplification of 13 C13MC members was detected at 20 GW (Table 2).

At 16 GW, the levels of circulating hsa-miR-520c-3p differed between the WWD-PE and control group (Table 2). At 20 GW, the serum concentration of hsa-miR-518f, hsa-miR-512-3p, and hsa-miR-520d-3p was higher in WWD-PE than that observed in pregnancies without complications (Table 2). The RQ values observed for the miRNAs at each time point were 2.4 for hsa-miR-520c-3p at 16 GW ($P = 0.029$), 3.7, 7.2, and 4.4 for hsa-miR-518f ($P = 0.004$), hsa-miR-512-3p ($P = 0.023$), and hsa-miR-520d-3p ($P = 0.024$) at 20 GW, respectively. There were no differences in the serum C19MC signatures between study groups at 12 GW ($P > 0.05$).

To identify differences in circulating miRNA associated with the severity of clinical manifestations of PE, the participants were classified as women who developed mild PE (WWD-Mild-PE), women who developed severe PE (WWD-Severe-PE), and controls. Table 3 displays the members of C19MC with significant differences in serum

Table 2 Serum expression levels of members of C19MC at the pregnancy time points evaluated

Time	Target name	RQ	RQ min	RQ max	*P* value
12 GW	hsa-miR-520d-3p	2.6	1.110	6.018	0.260
	hsa-miR-518f	2.5	0.543	11.821	0.309
	hsa-miR-520a	3.2	0.509	20.726	0.318
	hsa-miR-518b	0.0	0.007	0.130	0.423
	hsa-miR-520c-3p	0.8	0.114	6.270	0.425
	hsa-miR-519a	0.2	0.008	4.412	0.524
	hsa-miR-520f	0.5	0.000	725.01	0.575
	hsa-miR-521	0.4	0.051	3.214	0.713
	hsa-miR-523	4.3	2.093	8.672	0.742
	hsa-miR-518a-3p	0.6	0.059	7.053	0.958
16 GW	hsa-miR-520c-3p	2.4	1.206	4.673	**0.029**
	hsa-miR-520d-3p	10.2	0.887	118.47	0.064
	hsa-miR-520a	4.8	1.775	13.003	0.104
	hsa-miR-518b	0.0	0.001	0.007	0.188
	hsa-miR-523	0.4	0.101	1.346	0.626
	hsa-miR-516-3p	0.5	0.186	1.550	0.652
	hsa-miR-515-3p	0.5	0.046	5.130	0.668
	hsa-miR-518a-3p	0.7	0.097	5.666	0.699
	hsa-miR-518d	0.6	0.017	17.751	0.788
	hsa-miR-518f	1.0	0.421	2.220	0.920
20 GW	hsa-miR-518f	3.7	1.566	8.834	**0.004**
	hsa-miR-512-3p	7.2	1.671	31.23	**0.023**
	hsa-miR-520d-3p	4.4	2.175	9.088	**0.024**
	hsa-miR-517a	1.2	0.603	2.384	0.131
	hsa-miR-516-3p	0.3	0.189	0.600	0.151
	hsa-miR-520c-3p	1.9	1.050	3.278	0.186
	hsa-miR-515-3p	0.3	0.057	1.576	0.212
	hsa-miR-519a	3.6	1.004	12.703	0.271
	hsa-miR-518b	16.8	1.012	277.79	0.350
	hsa-miR-520a	1.4	0.638	3.077	0.384
	hsa-miR-521	1.0	0.372	2.581	0.760
	hsa-miR-523	2.0	0.743	5.294	0.816
	hsa-miR-518a-3p	0.6	0.151	2.065	0.950

Significant *P* values are highlighted in bold

RQ relative quantification, *GW* gestation week

Table 1 General characteristics of the study population

Characteristics	WWD-PE ($n = 16$)	Control ($n = 18$)	*P* value
Maternal age (mean ± SD)	23.5 ± 5.1	23.4 ± 5.8	0.913
Body mass index (mean ± SD)	28.1 ± 5.5	27.2 ± 4.9	0.639
Number of pregnancies (median, range)	2 (1–5)	1 (1–4)	0.387
Nulliparous, *n* (%)	6 (37.5)	9 (50.0)	0.699
Personal history of PE, *n* (%)	1 (6.3)	1 (5.5)	1.0
Family history of PE, *n* (%)	1 (6.3)	1 (5.5)	1.0
Family history of hypertension, *n* (%)	13 (81.2)	13 (72.2)	1.0
Family history of diabetes mellitus type II, *n* (%)	8 (50)	13 (72.2)	0.176
Smoking during pregnancy, *n* (%)	0 (0.0)	0 (0.0)	1.0

SD standard deviation

Table 3 Members of C19MC differentially expressed between healthy pregnancies and WWD-PE, classified as mild or severe PE

Target	Regulation group	GW	RQ	RQ min	RQ max	*P* value
hsa-miR-520d-3p	Mild-PE	16	92.2	35.9	236.6	0.040
hsa-miR-518f	Severe-PE	20	6.43	4.05	10.22	0.007
hsa-miR-517a	Severe-PE	20	2.12	1.31	3.43	0.027
hsa-miR-518f	Mild-PE	20	5.34	1.96	14.58	0.035
hsa-miR-520d-3p	Mild-PE	20	6.37	1.66	24.49	0.044

Data were calculated using the control group as the reference

levels between study groups. Using the PE severity criteria as a classifier (see "Methods" for details), at 16 GW, the hsa-miR-520d-3p was differentially expressed between the WWD-Mild-PE and the control group (RQ 92.2, $P = 0.04$). At 20 GW, the level of hsa-miR-518f varied according PE severity, the highest RQ differences being between the WWD-Severe-PE and controls (RQ 6.43, $P = 0.007$).

At the time of PE diagnosis and considering the WWD-Mild-PE group as a reference, the expression of the 51 circulating miRNAs evaluated did not differ between the WWD-Mild-PE and WWD-Severe-PE groups ($P > 0.05$; data not shown).

Discussion

Members of the C19MC are highly enriched in the placenta, particularly in term trophoblast cells where they account for a significant proportion of cellular miRNA content and demonstrate increased expression from the first to third trimester [13, 24, 25]. Because increased expression is mirrored in the maternal circulation and C19MC miRNAs comprise the majority of exosomal miRNAs, it is recognized that C19MC play an important role in the physiology of a normal pregnancy and therefore their deregulation may be used as a biomarker for pregnancy-related disorders including PE. Accordingly, in this study, we performed serum miRNA expression profiling to identify C19MC members involved in the development of PE. The circulating miRNA expression profile was evaluated at 12, 16, 20 GW, and at the time of PE diagnosis. Our results showed the differential expression of four members of the C19MC cluster between the WWD-PE and healthy pregnant women: one at 16 GW (hsa-miR-520c-3p) and the remaining three at 20 GW (hsa-miRs 518f-3p, 512-3p, and 520d-3p). A dynamic determination of the circulating C19MC expression profiles at different time points in early pregnancy has not yet been performed; however, using a transversal nested case–control study, Hromadnikova et al. compared pregnant women enrolled at 10–13 GW to evaluate risk for PE development based on six serum C19MC members. Circulating miR-517-5p, miR-518b, and miR-520h were up-regulated in patients destined to develop PE [26]. Similarly, to identify circulating miRNA signatures associated with PE development, Ura et al. analyzed 754 miRNAs in serum samples obtained at 12–14 GW from pregnant women who later developed severe PE and controls [20]. Their results showed up-regulation of miR-520a and miR-518b in women who developed severe PE. Although our study is not entirely comparable with these previous reports, and the miR-517-5p, and miR-518b expression was detected at the three time points evaluated, there were no significant differences between study groups. The "apparent" dissimilarities between studies may be explained in part by the stage of pregnancy at which the miRNA levels were quantified (study design), and the differences in the PE stratification criteria used in the studies [20, 27]; more specifically in our study the time in pregnancy at which the circulating CM19C miRNA levels were evaluated was very strict and only women with the correct GW (12, 16 or 20 GW) were included. Accordingly, we consider that there are specific serum miRNA signatures according to the gestational age of the participants and that this should be considered in future studies focused on validating circulating miRNA associated with PE development. In our study, at 20 GW and according to PE severity, the serum levels of hsa-miR-518f were useful to separately identify WPD-Mild-PE, WPD-Severe-PE, and controls, demonstrating the differential modulation of miRNAs closely associated with miR-518 in early pregnancy, according to the current PE stratification criteria.

The presence of specific members of the C19MC in the maternal circulation has been associated with existing PE. At least 11 independent studies have discovered members of the C19MC to be among the most represented across PE case–control studies [25]. The most frequently cited C19MC members (and their specific variants) that are differentially expressed in PE are miR-518 (b/c/e/f), -517 (a/b/c/*), -519 (a, b-3p, d, e, e*), and -520 (a, a-3p, c-3p, f, g, h) [25, 28]. In our study, no blood sample was taken from healthy pregnancies (paired with the PE cases) at the time of PE diagnosis, and therefore no comparison of circulating C19MC signatures between PE and controls was possible. Although the absence of a control group at the time of PE diagnosis was a disadvantage of the study, there were no differences in serum C19MC miRNAs between women diagnosed with mild-PE and severe-PE. However, it is important to mention

that these results should be considered carefully because the mild/severe PE cases were not paired according to risk factors and this may have introduced some bias in the statistical analysis. Finally, some study limitations must be highlighted: the number of women who developed PE and who were available for the study was low and therefore validation of the miRNAs that differed between study groups is needed in an independent cohort. Similarly, the determination of parameters to evaluate the diagnostic test (cutoffs, predictive values, sensitivity, specificity, etc.) was not considered due to the low number of participants at each stage of pregnancy evaluated.

In summary, to the best of our knowledge, this study is the first to analyze the signatures of C19MC circulating miRNAs at four pregnancy time points in order to assess their association with the development and severity of PE. Our results provide evidence of the early differential modulation of the C19MC cluster in WWD-PE. This knowledge provides a basis for understanding the complex etiopathogenesis of PE and for developing early screening tests for the disease.

Conclusion

Serum levels of hsa-miRs 520c-3p at 16 GW, and the circulating levels of hsa-miR-512-3p, 518f-3p, and 520d-3p at 20 GW, were differentially expressed between WWD-PE and controls. The serum levels of hsa-miR-518f-3p at 20 GW were useful to identify WWD-Mild-PE, WWD-Severe-PE, and controls. Our results support the differential modulation of the C19MC cluster during early pregnancy in WWD-PE and thus their participation in the development of should be considered.

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Author contributions All authors participated throughout the study and during the preparation of the manuscript, as follows: Protocol/project development: MLM-F, VF-M, YO-C. Data collection or management: MLM-F, IG-V, CG-A, OYB-C, IPR-S, RC-M. Data analysis: MLM-F, CG-A, ID-E, JIB-A, JMO-R, LOS-S. Manuscript writing/editing/approval: MLM-F, IG-V, CG-A, ID-E, OYB-C, VF-M, GPH-D, MRR-P, IPR-S, RC-M, JMO-R, LOS-S, JIB-A, YO-C.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

References

1. Aslam MI (2009) MicroRNAs are novel biomarkers of colorectal cancer. Br J Surg 96(7):702–710
2. Maccani MA (2009) Epigenetics in the placenta. Am J Reprod Immunol (New York, NY: 1989) 62(2):78–89
3. Kim VN (2005) MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 6(5):376–385. https://doi.org/10.1038/nrm1644
4. Ji L, Brkic J, Liu M, Fu G, Peng C, Wang YL (2013) Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Mol Aspects Med 34(5):981–1023. https://doi.org/10.1016/j.mam.2012.12.008
5. Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K, Williams MA (2011) Placental microRNA expression in pregnancies complicated by preeclampsia. Am J Obstet Gynecol 204(2):178 e112-121. https://doi.org/10.1016/j.ajog.2010.09.004
6. Fu G, Brkic J, Hayder H, Peng C (2013) MicroRNAs in human placental development and pregnancy complications. Int J Mol Sci 14(3):5519–5544. https://doi.org/10.3390/ijms14035519
7. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, Draghici S, Espinoza J, Kusanovic JP, Mittal P, Hassan SS, Kim CJ (2007) Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol 196 e261-266(3):261. https://doi.org/10.1016/j.ajog.2007.01.008
8. Chen DB, Wang W (2013) Human placental microRNAs and preeclampsia. Biol Reprod 88(5):130. https://doi.org/10.1095/biolreprod.113.107805
9. Doridot L, Miralles F, Barbaux S, Vaiman D (2013) Trophoblasts, invasion, and microRNA. Front Genet 4:248. https://doi.org/10.3389/fgene.2013.00248
10. Morales-Prieto DM, Ospina-Prieto S, Chaiwangyen W, Schoenleben M, Markert UR (2013) Pregnancy-associated miRNA-clusters. J Reprod Immunol 97(1):51–61. https://doi.org/10.1016/j.jri.2012.11.001
11. Ouyang Y, Mouillet JF, Coyne CB, Sadovsky Y (2014) Review: Placenta-specific microRNAs in exosomes—good things come in nano-packages. Placenta 35(Suppl):S69–S73. https://doi.org/10.1016/j.placenta.2013.11.002
12. Gu Y, Sun J, Groome LJ, Wang Y (2013) Differential miRNA expression profiles between the first and third trimester human placentas. Am J Physiol Endocrinol Metab 304(8):E836–E843. https://doi.org/10.1152/ajpendo.00660.2012
13. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y (2012) The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod 18(8):417–424. https://doi.org/10.1093/molehr/gas013
14. Bullerdiek J, Flor I (2012) Exosome-delivered microRNAs of "chromosome 19 microRNA cluster" as immunomodulators in pregnancy and tumorigenesis. Mol Cytogenet 5(1):27. https://doi.org/10.1186/1755-8166-5-27

15. Xu P, Zhao Y, Liu M, Wang Y, Wang H, Li YX, Zhu X, Yao Y, Qiao J, Ji L, Wang YL (2014) Variations of microRNAs in human placentas and plasma from preeclamptic pregnancy. Hypertension 63(6):1276–1284. https://doi.org/10.1161/HYPERTENSIONAHA.113.02647
16. Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT, Sadovsky Y (2010) The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. Placenta 31(9):781–784. https://doi.org/10.1016/j.placenta.2010.07.001
17. Li H, Ge Q, Guo L, Lu Z (2013) Maternal plasma miRNAs expression in preeclamptic pregnancies. Biomed Res Int 2013:970265. https://doi.org/10.1155/2013/970265
18. Wu L, Zhou H, Lin H, Qi J, Zhu C, Gao Z, Wang H (2012) Circulating microRNAs are elevated in plasma from severe preeclamptic pregnancies. Reproduction 143(3):389–397. https://doi.org/10.1530/REP-11-0304
19. Yang Q, Lu J, Wang S, Li H, Ge Q, Lu Z (2011) Application of next-generation sequencing technology to profile the circulating microRNAs in the serum of preeclampsia versus normal pregnant women. Clin Chim Acta Int J Clin Chem 412(23–24):2167–2173. https://doi.org/10.1016/j.cca.2011.07.029
20. Ura B, Feriotto G, Monasta L, Bilel S, Zweyer M, Celeghini C (2014) Potential role of circulating microRNAs as early markers of preeclampsia. Taiwan J Obstet Gynecol 53(2):232–234. https://doi.org/10.1016/j.tjog.2014.03.001
21. Herzog EM, Eggink AJ, Willemsen SP, Slieker RC, Wijnands KPJ, Felix JF, Chen J, Stubbs A, van der Spek PJ, van Meurs JB, Steegers-Theunissen RPM (2017) Early- and late-onset preeclampsia and the tissue-specific epigenome of the placenta and newborn. Placenta 58:122–132. https://doi.org/10.1016/j.placenta.2017.08.070
22. Garza-Veloz I, Castruita-De la Rosa C, Cortes-Flores R, Martinez-Gaytan V, Rivera-Munoz JE, Garcia-Mayorga EA, Meza-Lamas E, Rojas-Martinez A, Ortiz-Lopez R, Martinez-Fierro ML (2011) No association between polymorphisms/haplotypes of the vascular endothelial growth factor gene and preeclampsia. BMC Pregnancy Childbirth 11:35. https://doi.org/10.1186/1471-2393-11-35
23. Martinez-Fierro ML, Garza-Veloz I, Carrillo-Sanchez K, Martinez-Gaytan V, Cortes-Flores R, Ochoa-Torres MA, Guerrero GG, Rodriguez-Sanchez IP, Cancela-Murrieta CO, Zamudio-Osuna M, Badillo-Almaraz JI, Castruita-De la Rosa C (2014) Expression levels of seven candidate genes in human peripheral blood mononuclear cells and their association with preeclampsia. Hypertens Pregnancy 33(2):191–203. https://doi.org/10.3109/10641955.2013.853777
24. Morales-Prieto DM, Chaiwangyen W, Ospina-Prieto S, Schneider U, Herrmann J, Gruhn B, Markert UR (2012) MicroRNA expression profiles of trophoblastic cells. Placenta 33(9):725–734. https://doi.org/10.1016/j.placenta.2012.05.009
25. Sheikh AM, Small HY, Currie G, Delles C (2016) Systematic review of micro-RNA expression in pre-eclampsia identifies a number of common pathways associated with the disease. PLoS ONE 11(8):e0160808. https://doi.org/10.1371/journal.pone.0160808
26. Hromadnikova I, Kotlabova K, Ivankova K, Krofta L (2017) First trimester screening of circulating C19MC microRNAs and the evaluation of their potential to predict the onset of preeclampsia and IUGR. PLoS ONE 12(2):e0171756. https://doi.org/10.1371/journal.pone.0171756
27. Luque A, Farwati A, Crovetto F, Crispi F, Figueras F, Gratacos E, Aran JM (2014) Usefulness of circulating microRNAs for the prediction of early preeclampsia at first-trimester of pregnancy. Sci Rep 4:4882. https://doi.org/10.1038/srep04882
28. Hromadnikova I, Kotlabova K, Ondrackova M, Kestlerova A, Novotna V, Hympanova L, Doucha J, Krofta L (2013) Circulating C19MC microRNAs in preeclampsia, gestational hypertension, and fetal growth restriction. Mediat Inflamm 2013:186041. https://doi.org/10.1155/2013/186041