DYSREGULATED PLACENTAL MICRORNAS IN EARLY AND LATE ONSET PREECLAMPSIA

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest .

ABSTRACT

Introduction: To determine the miRNA expression profile in placentas complicated by Preeclampsia (PE) and compare it to uncomplicated pregnancies.

Methods: Sixteen placentas from women with PE, [11 with early onset PE (EOPE) and 5 with late onset PE (LOPE)], as well as 8 placentas from uncomplicated pregnancies were analyzed using miRNA microarrays. For statistical analyses the MATLAB® simulation environment was applied. The over-expression of miR-518a-5p was verified using Quantitative Real-Time Polymerase Chain Reaction.

Results: Forty four miRNAs were found dysregulated in PE complicated placentas. Statistical analysis revealed that miR-431, miR-518a-5p and miR-124* were over-expressed in EOPE complicated placentas as compared to controls, whereas miR-544 and miR-3942 were down-regulated in EOPE. When comparing the miRNA expression profile in cases with PE and PE- growth restricted fetuses (FGR), miR-431 and miR-518a-5p were found over-expressed in pregnancies complicated by FGR.

Discussion: Since specific miRNAs can differentiate EOPE and LOPE from uncomplicated placentas, they may be considered as putative PE-specific biomarkers. MiR-518a-5p emerged as a potential diagnostic indicator for EOPE cases as well as for PE-FGR complicated placentas, indicating a potential link to the severity of the disease.

Keywords: miRNAs, placenta, microarrays, early onset Preeclampsia, late onset Preeclampsia

ABBREVIATIONS

ABBREVIATION	EXPLANATION
ADAM	A Disintegrin And Metalloproteinase
AUC	Area Under the Curve
CDK6	Cyclin Dependent Kinase 6
cDNA	Complementary Deoxyribonucleic Acid
EOPE	Early Onset PE
FDR	False Discovery Rate
FGR	Fetal Growth Restricted Fetuses
IGF1R	Insulin Like Growth Factor 1 Receptor
IUGR	Intrauterine Growth Restriction
LOPE	Late Onset PE
MAPK	Mitogen-Activated Protein Kinases
miRNAs	MicroRNAs
MMP	Matrix Metalloproteinase
PE	Preeclampsia
PTEN	Phosphatase and Tensin homolog
PU	Proteinuria
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
ROC curve	Receiver Operating Characteristic curve
RUNX2	Runt-Related Transcription Factor 2
TGF- β	Transforming Growth Factor beta
TNF	Tumor Necrosis Factor
TP53	Tumor Protein 53
VCAM1	Vascular Cell Adhesion Molecule 1
VEGFA	Vascular Endothelial Growth Factor A

INTRODUCTION

Preeclampsia (PE) is an obstetric complication affecting 2-8% of all pregnancies responsible for maternal, fetal and neonatal morbidity and mortality worldwide [1]. Increasing evidence suggests that both women who develop PE and their neonates have a higher risk of developing cardiovascular disease and metabolic syndromes including type II diabetes, later in life. Traditionally, PE is characterized by new onset hypertension and proteinuria after 20 weeks of gestation. Depending on the gestational age by which the symptoms develop, PE is classified as early-onset (EOPE-before 34 weeks of gestation) or late-onset (LOPE-at or after 34 weeks of gestation). Despite extensive efforts, the pathogenesis of the disease is not fully understood. EOPE shows a greater association with impaired trophoblast invasion, ischemia at the placenta and fetal growth restriction (FGR) as compared to LOPE, whereas LOPE is not often associated with placenta hypoperfusion and profound FGR [2].

MicroRNAs (miRNAs) belong to a highly conserved class of short non-coding molecules that regulate gene expression at post-transcriptional level [3]. It is estimated that 30% of human genes are transcriptionally negatively regulated by miRNAs, highlighting their crucial role in several cellular processes including cell growth, proliferation, differentiation and apoptosis [4]. Given the central role of the placenta in the development of PE, recent evidence suggests that miRNAs may be implicated in the pathogenesis of the disease regulating common pathways such as angiogenesis, hypoxia, ischemia and metabolism [5-14].

In the present study, we compared the placenta miRNA expression profiles of PE with those of uncomplicated pregnancies using a microarray hybridization based technology. The obtained miRNA patterns were correlated with clinical parameters known to be associated with PE including proteinuria, gestational age of disease onset and the presence of FGR fetus.

PATIENTS AND METHODS

Patient and tissue samples

Placental samples were collected from 16 women with PE (*n*=11 with EOPE and *n*=5 diagnosed with LOPE) and from 8 with uncomplicated term pregnancies at the time of cesarean section in the 3rd Department of Obstetrics and Gynecology at National and Kapodistrian University of Athens. Among the PE complicated cases, 8 carried a FGR fetus (PE-FGR): in the EOPE group, 6 out of 11 neonates had FGR confirmed by birth weight percentile below the 10th whereas in the LOPE group 1 neonate was confirmed as FGR by birth weight below the 5th percentile and the other was classified as FGR on the basis of a noted reduction of growth expressed by a static growth of abdominal velocity and abnormal placental Dopplers. Clinical characteristics of women participating in the study are summarized in **Table 1**.

For each case, two pieces of placental tissue approximately 1×1×1cm in size were randomly obtained from the central part of the placenta maternal phase, as previously described [9]. Tissues were washed with isotonic saline solution, to remove excess of blood cells and immediately stored at-80C° until further processing.

PE was defined as hypertension (blood pressure ≥140/90 mmHg) in two determinations, 4 hours apart associated with proteinuria (> 300 mg/24 h or ≥1+, according to a routine urinalysis) after 20 weeks of gestation in previously normotensive women [15]. In the absence of proteinuria, PE was characterized based on the new modified guidelines recommended by the task force on Hypertension in pregnancy [16]. In 2 out of 16 PE cases included in this study, the diagnosis was established on the presence of new onset hypertension and impaired liver enzymes concentration.

FGR was diagnosed when the estimated fetal weight, calculated using the Hadlock formula (Astraia Software GmbH), was below the 10th percentile for the evaluated gestational age [17].

Pregnant women with multiple pregnancies, congenital or chromosomal abnormalities of the fetus, renal disease, chronic hypertension, cardiovascular disease or other pregnancy complications were excluded from the study.

The study was approved by the hospital's ethics committee according to the Helsinki Declaration on ethical principles for medical research involving human subjects. A written consent form was obtained from all participants prior to surgery.

MicroRNA extraction and expression profiling

For total RNA extraction, 100mg of placental tissue were mechanically homogenized in TRIzol (Life Technologies, Foster City, CA), treated with DNase I (Ambion, Austin, USA) and enriched for small RNAs (siRNAs, miRNAs) using the mirVana microRNA Isolation kit (Ambion, Austin, USA). RNA quantity and purity were measured using a Nanodrop spectrophotometer (ND-1000, NanoDrop Technologies, Houston, TX, USA).

Labeling and hybridization were performed using the LabelIT miRNA labeling kit (Mirus Bio LLC, USA) according to the manufacturer's instructions. Samples were hybridized to an Applied MicroArrays (miRlink

Bioarray 300054-3PK) platform containing 1211 human miRNAs (Applied Biosystems, Foster City, CA). This platform consists of 8 arrays per slide and on each array miRNAs are represented by multiple replicates. Images were scanned using an Agilent Microarray Scanner (G2565CA).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The differential expression of miR-518a-5p in PE complicated placentas, as compared to the control group, was verified using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Complementary DNA (cDNA) synthesis and subsequent RT-PCR were performed, using the TaqMan miRNA Reverse Transcription kit and TaqMan universal PCR master mix (Applied Biosystems, Inc., USA) respectively. Specific primers and a TaqMan probe for miR-518a-5p (PN4427975, Applied Biosystems, Inc., USA) were used according to the manufacturer's instructions. All samples were analyzed in triplicate in an LC480 LightCycler system (Roche GmbH, Switzerland). Positive amplification of each sample was considered when a signal occurred before 40th threshold cycle. The miRNA levels were normalized to RNU44 (Applied Biosystems, Inc., USA) as an internal control and the relative abundance of each miRNA was calculated using the $\Delta\Delta Ct$ equation.

Data analyses and statistics

Microarray Data Extraction and Analysis

Microarray raw data were extracted, as previously described, using the Imagene 6.0 software (Biodiscovery Inc., USA) [18]. The MATLAB® simulation environment (The Mathworks, Inc, Natick, MA) was applied for multi-parameter analyses. Background correction was performed by subtracting the median local background from the signal intensity. Normalization was performed using the quantile normalization algorithm. The two-tailed student t-test was used to assess the mean differences between the two groups. Relative expression was estimated as the log_2-transformed ratio of the individual miRNA expression levels over the mean expression level of all control samples for the respective miRNA under investigation. Final miRNA values have been calculated as the mean of each specific miRNA replicates on each array. Continuous variables were expressed as median ± standard deviation, unless differently indicated. MiRNAs were considered significant if they had a p-value<0.05 and False Discovery Rate (FDR)≤0.05. Microarray platform as well as raw data have been uploaded on the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) with reference numbers GPL23980 and GSE103542 respectively.

Group-wise Comparison of miRNA expression and clinical data

MiRNA expression values were compared with clinical parameters, including PE (EOPE and LOPE), as well as the presence of proteinuria and FGR with the *Kruskal-Wallis* test. *Bonferroni* correction was used in order to adjust p-values for the statistical test performed and reduce the chances of obtaining false-positive results (type I errors) with the multiple pair-wise tests performed on the present dataset. Differences were considered significant at p<0.02.

ROC Analysis

Receiver Operating Characteristic (ROC) curves were performed with the MATLAB® simulation environment (The Mathworks, Inc, Natick, MA) to evaluate the diagnostic potential of differentially expressed miRNAs with 95% Standard Error (SE) and 95% Confidence Intervals (CI). ROC curves were considered significant when an Area Under the Curve (AUC) value > 0.8 at a $p < 0.05$ was obtained.

MiRNA Enrichment, Gene Ontology and Pathway Analysis

Differentially expressed miRNAs were further enriched and analyzed for known functions and pathway participation. Functional annotation was performed using Webgestalt web-tool [19]. RNA target prediction for selected miRNAs was performed with TargetScan v.7.0 [20], DIANA tools [21-23] and the miROB annotation web tool (http://mirob.interactome.ru/). Further on, we have used the GOmiR [24] multi-compare tool for a more in-depth functional annotation of investigated microRNAs.

RESULTS

MicroRNA expression profiles between PE and the control group

Overall, 44 miRNAs were dysregulated in PE-complicated placentas, as compared to the control cohort, with a two-fold change and statistical significance ($p < 0.05$) (**Figure 1**). The vast majority was up-regulated with a total of 27 miRNAs (61.4%) showing over-expression and 17 (38.6%) decreased expression.

MicroRNA expression in early-onset and late-onset PE

When comparing miRNA expression in samples obtained from women with EOPE (n=11) and LOPE (n=5) with controls (n=8), 9 miRNAs were found associated with the early or the late type of the disease (**Figure 2**). Specifically, miR-431 (p=0.003), miR-518a-5p (p=0.002) and miR-124* (p=0.001) were significantly over-expressed in EOPE complicated placentas, as compared to controls, whereas miR-544b (p=0.001) was significantly down-regulated and miR-3942 was marginally down-regulated (p=0.02) in EOPE when compared to controls. Further on, miR-130b (p=0.004) and miR-423-3p (p=0.001) were significantly up-regulated in EOPE placentas as compared to controls, while miR-383 was marginally up-regulated in EOPE (p=0.02) and significantly up-regulated (p=0.01) in LOPE samples. MiR-1183 was marginally up-regulated (p=0.02) in LOPE samples, as compared to controls. In the EOPE placentas, following *Bonferroni* correction, the over-expression of miR-1183 was not considered significant (p=0.03).

MicroRNA expression in pregnancies complicated by FGR

The miRNA expression profile was also compared in cases of PE and PE-FGR. Two miRNAs were found overexpressed in PE-FGR complicated placentas, including miR-431 and miR-518a-5p (**Figure 3**).

ROC analysis

ROC analysis revealed that 8 miRNAs up-regulated in PE complicated placentas (miR-500a, miR-383, miR-518a-5p, miR-431, miR-423-3p, miR-124*, miR-1183 and miR-130b) and 2 down-regulated (miR-3942, miR-544b) were associated with PE and miRNAs with $p<0.05$ and AUC>0.8 were considered as potential biomarkers. The ROC curves yielded the following AUCs: miR-500a (AUC=0.8), miR-3942 (AUC=0.8), miR-544b (AUC=0.84), miR-383 (AUC≈0.82), miR-518a-5p (AUC=0.83), miR-431 (AUC=0.82), miR-423-3p (AUC≈0.90), miR-124* (AUC=0.88), miR-1183 (AUC=0.80) and miR-130b (AUC=0.82) (**Figure 4**).

qRT PCR verification

The over-expression of miR-518a-5p in PE complicated placentas as compared to controls was verified using qRT-PCR in all samples. Consistent with microarray results the change of dysregulated miRNA was approximately two fold above **(Figure 1 suppl.)**.

MiRNA Enrichment, Gene Ontology and Pathway Analysis

Several genes have been reported as predicted or verified targets of the aforementioned miRNAs. Specifically, miR-126*, miR-885, miR-130B, miR-1915, miR-1204, miR-3928, miR-518a-5p, miR-631, miR-383, miR-19a, miR-302c, miR-155, miR-30a, miR-542 and miR-1248 manifested significant associations with genes including *SMAD1/2/4/5*, *ADAM9*, *VCAM1*, *TGFBP2*, *PTEN*, *TP53* and *RUNX2* **(Figure 2 suppl.).** Pathway analysis of significantly annotated miRNAs and their respective targets revealed that they participate in biological pathways associated with PE including MAPK, TNF signaling, T-cell receptor, B-cell receptor and TGF-β pathways **(Figure 5).** Further on, the GOmiR tool searches in the MiRanda, TargetScan, PicTar, Sanger, RNAhybrid databases unraveled common gene and functional targets for all the aforementioned miRNAs. In particular, GOmiR confirmed the results obtained by the other tools, revealing that miR-126* and miR-130B target genes participate in TNF-signaling. It also revealed *PPARG*, another target gene, which participates in obstetric diseases such as Polycystic Ovary Syndrome, Ovarian Cysts, Ovarian Diseases and Metabolic Syndrome. In addition, GOmiR confirmed that miR-518a-5p participates in MAPK signaling. Further on, miR-631 was found to target *TBX2*, *HDAC1* and *AXIN1* which participate in ectodermal placode formation. Target genes of the miR-631 were shown to participate in metabolic pathways and the insulin signaling pathway, whereas miR-30a target genes are involved in MAPK, JAK-STAT signaling pathways as well as in metabolic pathways.

DISCUSSION

Several studies have demonstrated dysregulation of placenta miRNAs in PE, implying their involvement in the pathogenesis of the disease by targeting a variety of genes and regulating crucial biological pathways [5-7, 10, 11, 14, 25, 26]. Despite extensive efforts, however, conflicting results exist which may be attributed to the small number of samples included in each study, the increased number of molecules analyzed, the position of the placenta from which samples are obtained, the gestational age at sampling and the differences in the platforms used for analysis [7].

In the present study, 44 miRNAs were found dysregulated in PE complicated placentas. The results obtained verified the differential expression of several miRNAs previously reported, including the over-expression of miR-155 and miR-130b, as well as the down-regulation of miR-126* [11,25, 27-35].

MiR-155 and miR-130b have been implicated in the pathogenesis of PE through their involvement in pathways regulating trophoblast cells such as TGF-β signaling [11, 27-30]. Recently, miR130b was reported as differentially expressed in relation to pre-pregnancy BMI and adipogenesis, both of which have been associated with increased risk for the development of pregnancy complications including PE [31-33].

Although conflicting results exist regarding the role of mir-126 in the pathogenesis of PE, miR-126 and its isoform 126* regulate many target genes mainly related to angiogenesis [25, 34, 35]. Notably, *in vitro* experiments, using endothelial cells, revealed that miR-126 positively regulates their migration and proliferation, by enhancing fibroblast growth during arterial development [35]. Lack of miR126 has a negative angiogenic response, through VEGFA, bFGF and MAP kinase [34, 36]. In the current study, *in silico* analysis also revealed that miR126* can potentially modulate *ADAM9*, *MMP7*, *MMP13*, *VEGFA* and *VCAM1*. Proteins encoded by these genes are currently under investigation as potential biomarkers for the identification of pregnant women destined to develop PE.

Over-expression of miR-423-3p, miR-124*, miR-500a, miR-431 and miR-1305 and down-regulation of miR-544 and miR-3942 were observed in PE complicated placentas, as compared to controls. These miRNAs have not been previously associated with the pathogenesis of PE. MiR-423-3p, has been linked to renal function, acute kidney injury and cell proliferation [37, 38]. MiR-124 has been implicated in angiogenesis through the renin-angiotensin system and is hence linked with the regulation of blood pressure [39]. MiR-1305 has been recently implicated in cell cycle regulation and apoptosis in pluripotent stem cells by targeting *CDK6*, *CYCLIND2* and *RUNX2* [40]. Mir-431, a member of C14MC placenta cluster, directly regulates the expression of *SMAD4* [41]. Over-expression may negatively regulate *IGF1R* and consequently inhibit cell proliferation through MAPK pathway, which plays an important role in the development of vascular lesions that are observed in diabetes and hypertension [42].

Results were also analyzed with respect to the gestational age of disease onset. In the literature, a limited number of studies have focused on comparisons between miRNA expression pattern in EOPE and LOPE. In the current study, when correlations between EOPE, LOPE and the control cohort were performed, 9 miRNAs were found aberrantly expressed. Specifically, miR-383 and miR-1183 were over-expressed in PE, higher in LOPE cases as compared to controls, possibly reflecting an association with a milder form of the disease. Additionally, higher levels of expression for miR-518a-5p, miR-423, miR-124* and miR-431 were

detected in EOPE as compared to controls. It seems likely that all four miRNAs are associated with the severity of the disease and the severity of the clinical symptoms. On the contrary, miR-544 and miR-3942 were down-regulated in the EOPE cases, indicating a putative PE-specific diagnostic marker. Mir-130b was found over-expressed in the EOPE cases when compared to the LOPE, signifying its potential use as a biomarker for severe PE.

When examining miRNA expression profiles in PE complicated by FGR, miR-518-5p and miR-431 were up-regulated in all PE-FGR cases, indicating their potential as unfavorable prognostic markers. It is noticeable that overexpression of both miRNAs was also observed in previous correlations, reinforcing the prediction about their possible linkage to poor PE outcomes when up-regulated.

Although the properties of miR-518a-5p remain to be elucidated, statistically significant over-expression was found in all associations performed in the present study, including PE diagnosis, EOPE, LOPE and PE-FGR following ROC analysis. Vashukova *et al.*, reported up-regulation of miR-518a-5p in superimposed PE on chronic hypertension [43]. The aforementioned observation, along with the data obtained in the present study, possibly indicate a common pathway in the pathogenesis of EOPE, superimposed PE and PE-FGR. Since miR-518a-5p is also expressed in maternal peripheral blood during pregnancy, it represents a potential noninvasive diagnostic biomarker for all types of PE [10]. These findings however, should be validated in larger PE cohorts.

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403 FIGURE LEGENDS

404 **Figure 1**. Histogram of the 44 dysregulated miRNAs in PE complicated placentas as compared to controls ($p<0.05$ and FDR$\leq$0.05). Over-expressed miRNAs are
405 indicated with upward bars and down-regulated with downward bars. Y axis values represent miRNA fold change (ratios presented include Early Onset
406 Preeclampsia (EOPE) (n=11) and Late Onset Preeclampsia (LOPE) (n=5).
407

408 **Figure 2**. Differences in miRNA expression in EOPE, LOPE and control placentas. Significant differential expression was identified for (**A**) miR-3942 between
409 EOPE and controls (p=0.02), (**B**) miR-544b between EOPE and controls (p=0.001), (**C**) miR-383 between EOPE and controls as well as LOPE and controls (p=0.02
410 and p=0.01 respectively), (**D**) miR-518a-5p between EOPE and controls (p=0.002), (**E**) miR-431 between EOPE and controls (p=0.003), (**F**) miR-423-3p between
411 EOPE and controls as well as LOPE and controls (p=0.001 and p=0.007 respectively), (**G**) miR-124* between EOPE and controls (p=0.001), (**H**) miR-1183 between
412 EOPE and controls as well as LOPE and controls (p=0.03 and p=0.02 respectively) and (**I**) miR-130b between EOPE and controls as well as LOPE and controls
413 (p=0.004 and 0.04 respectively) (**EOPE**: Early Onset Preeclampsia, **LOPE**: Late Onset Preeclampsia) (Expression levels concern the natural, $\log_2$ untransformed
414 miRNA expression values. * depicts a significance at the $p<0.05$ level and ** depicts a significance at the $p<0.01$ level).
415

416 **Figure 3.** Over-expression of (**A**) miR-518a-5p and (**B**) miR-431 in PE complicated placentas with FGR fetuses as compared to those with unaffected fetal growth
417 (PE: Preeclampsia, FGR: Fetal Growth Restriction) (Expression levels correspond to the natural log_2 untransformed expression values. Differences were significant
418 at $p<0.05$).
419

420 **Figure 4**. Receiver Operating Characteristic (ROC) curves of miRNAs differentiating controls from Preeclampsia complicated placentas. Ten miRNAs can
421 differentiate controls from Preeclampsia complicated placentas: (**A**) miR-500a, (**B**) miR-03942, (**C**) miR-544b, (**D**) miR-383, (**E**) miR-518a-5p, (**F**) miR-431, (**G**)
422 miR-423-3p, (**H**) miR-124*, (**I**) miR-1183 and (**J**) miR-130b manifested an Area Under the Curve (AUC) value greater than 0.8 (AUC>0.8) and $p<0.05$, suggesting
423 that they are possible candidates for Preeclampsia.

424 **Figure 5.** Selected significant signaling pathways associated with annotated differentially expressed miRNAs (miR-125b, miR-155-5p, miR-21-5p and miR-145).
425 MiRNA annotation was performed with the DIANA mIRNA web annotation tool http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=mirpath/index).

Table 1. Maternal and neonatal characteristics of Preeclampsia cases and controls included in the study.

	Controls (n=8)	EOPE* (n=11)	LOPE** (n=5)	p-value[1]	p-value[2]	p-value[3]	p-value[4]
Maternal Age (y)	35.7 [35-39]	35.1 [28-45]	28.4 [20-35]	0.051	0.820	0.036	0.055
Pre-pregnancy BMI (kg/m2)	29.2 [22.96-31.99]	29.9 [19.83-41.77]	28.6[24.02 –36.57]	0.942	0.835	0.867	0.778
Blood Pressure (mmHg)							
Systolic	121 [122-118]	197.75 [160-250]	172.25 [152-197]	0.702	0.889	0.612	0.412
Diastolic	75 [72-85]	113.5 [90-150]	107.75 [94-135]	0.966	0.962	0.923	0.798
Proteinuria (n)							
Yes	0	10 (91%)	4 (80%)	0.0002¥			
No	8 (100%)	1 (9%)	1 (20%)				
Fetal Growth Restriction (FGR)	None	6 (54.5%)	2 (40%)				
Gestational Age at delivery (wk)	36.3 [34.2 – 38.5]	31.2 [28 – 33.5]	35.6 [34.4 – 37.3]	0.051	0.0003	0.29	0.78
Fetal Birth Weight (grams)	2792.6 [2215 - 3560]	1335 [1025 - 1540]	2454 [1900 - 3020]	0.0003	0.0002	0.336	0.002
>10th percentile (n)	8 (100%)	5 (45.4%)	3 (60%)				
<10th percentile (n)	0	4 (36.4%)	1 (20%)				
<5th percentile (n)	0	2 (18.2%)	1 (20%)				

*EOPE: Early onset Preeclampsia ** LOPE: Late onset Preeclampsia

[1]Significance level between all groups

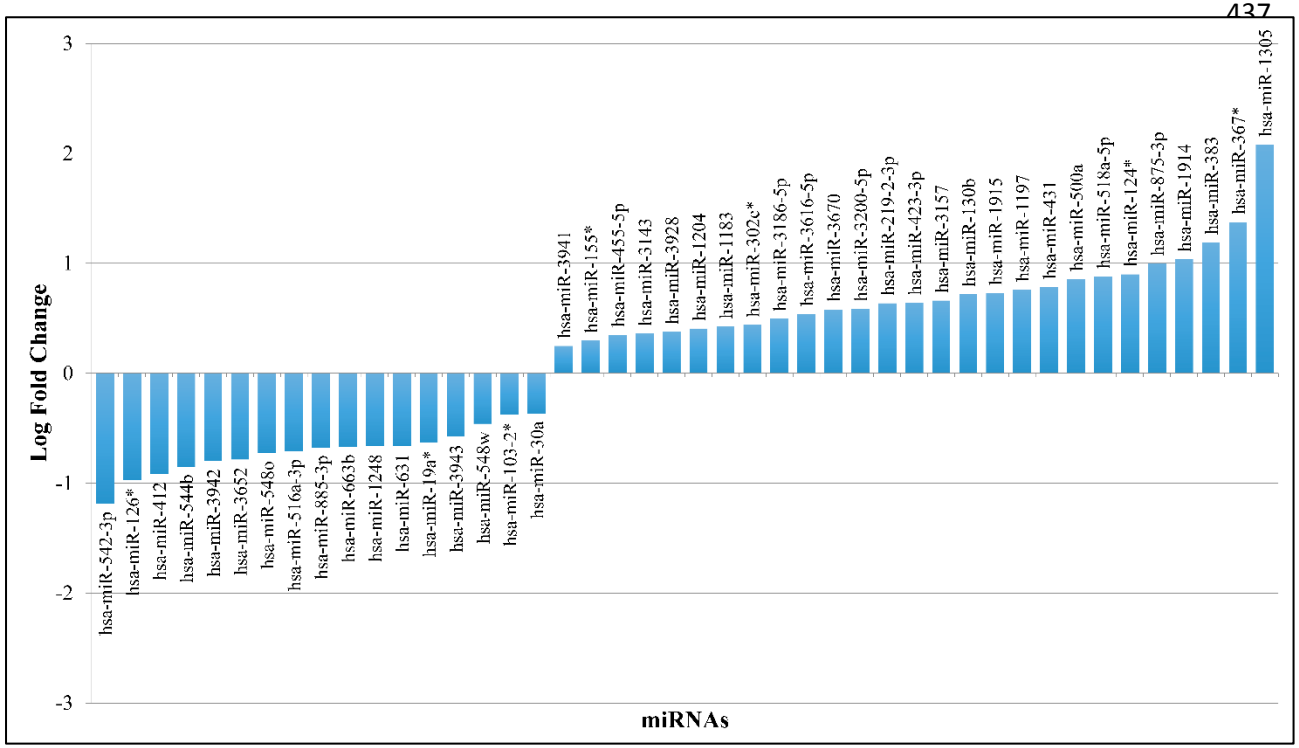
[2]Significance level between Control and EOPE placentas

[3]Significance level between Control and LOPE placentas

[4]Significance level between EOPE and LOPE placentas

¥p-value estimated with *chi-square* test for independence between proteinuria and early and late onset Preeclampsia.

Figure 1.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2
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hsa-miR-3652
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hsa-miR-516a-3p
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hsa-miR-1183
hsa-miR-302c*
hsa-miR-3186-5p
hsa-miR-3616-5p
hsa-miR-3670
hsa-miR-3200-5p
hsa-miR-219-2-3p
hsa-miR-423-3p
hsa-miR-3157
hsa-miR-130b
hsa-miR-1915
hsa-miR-1197
hsa-miR-431
hsa-miR-500a
hsa-miR-518a-5p
hsa-miR-124*
hsa-miR-875-3p
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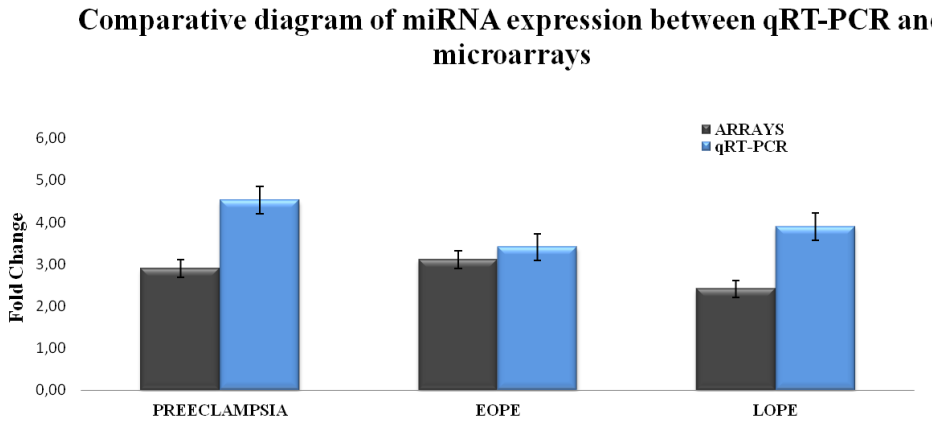
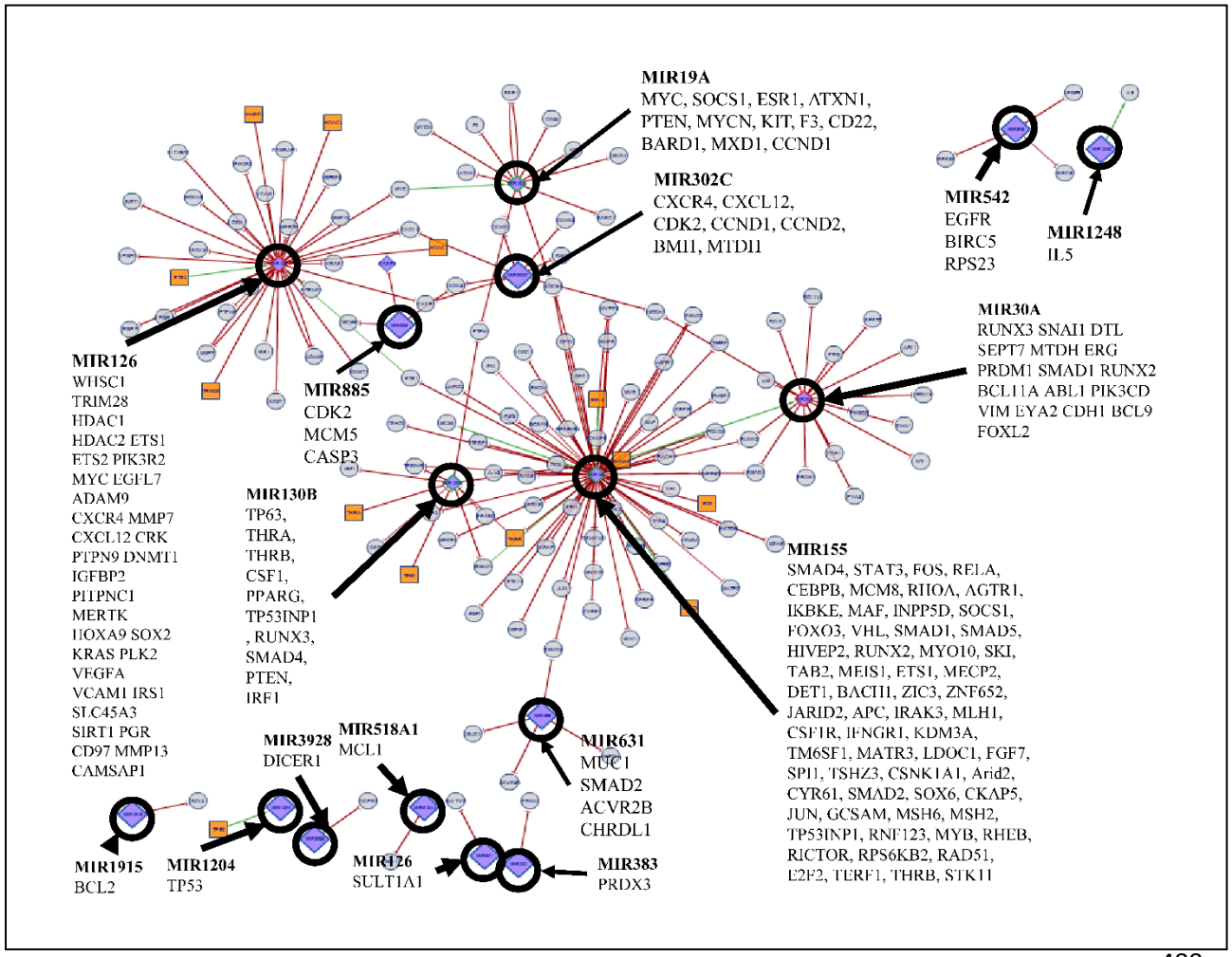
hsa-miR-367*
hsa-miR-1305
miRNAs

SUPPLEMENTARY FIGURES

Supplementary Figure 1.





Supplementary Figure 2. Networks of miRNAs and their association with target genes. In particular, we have found that miR-126, miR-885, miR-130b, miR-1915, miR-1204, miR-3928, miR-518a1, miR-126, miR-631, miR-383, miR-19a, miR-302c, miR-155, miR-30a, miR-542, miR-1248, manifested significant associations with gene groups. miRNA annotation was performed with the miROB web tool (http://mirob.interactome.ru/).