Cerrahpaşa Med J 2024; 48(2): 146-151 ORIGINAL ARTICLE

Analysis of miR-1183 Expression Level and Its role in Preeclampsia Pathogenesis via the Regulation of Its Target Gene *CHURC1*

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Cite this article as: Altındirek D, Gümüşoğlu-Acar E, Benian A, Günel T, Aydınlı K. Analysis of miR-1183 expression level and its role in preeclampsia pathogenesis via the regulation of its target gene *CHURC1*. *Cerrahpaşa Med J.* 2024;48(2):146-151.

Abstract

Objective: Preeclampsia (PE) is a multi-systemic disease in pregnancy and a leading cause of maternal and fetal mortality and morbidity worldwide. Elevated expression levels of certain circulating microRNAs (miRs) can be considered potential biomarkers, and have been reported in maternal plasma of pregnant women. We aimed to evaluate the expression profiles of circulating miR-1183 and its putative target mRNA in preeclampsia and their utility for prenatal diagnosis of preeclampsia.

Methods: Plasma samples were obtained from pregnant women between 26 and 32 weeks of gestation. Circulating miR-1183 and its target mRNA expression levels were determined by qRT-PCR in maternal plasma of 31 patients with preeclampsia and 22 normotensive pregnancies patient as control. Bioinformatics tools were used to predict the target gene of miR-1183.

Results: The expression level of circulating miR-1183 was significantly increased in plasma of preeclampsia patients compared to controls (P = .002). The expression level of *CHURC1* gene, the target gene of miR-1183, is dramatically decreased in PE cases compared to controls ($P < .001$). Spearman's correlation between miR-1183 and CHURC1 expression levels shows an r-value of −0.37, suggesting a moderate inverse relationship between the 2 parameters but it was not statistically significant ($P = .08$). By Receiver Operating Curve (ROC) analysis, miR-1183 and *CHURC1* showed high accuracy in discriminating PE from controls. The area under the curve (AUC) was found in miR-1183 and *CHURC1* at 0.79 (95% CI, 0.62-0.91) and 0.96 (95% CI, 0.78-0.99), respectively.

Conclusion: Circulating miR-1183 may be involved in the pathogenesis of preeclampsia via the regulation of its target mRNA, CHURC1.

Keywords: Biomarker, circulating microRNA, *CHURC1*, miR-1183, preeclampsia

Introduction

Preeclampsia (PE) is a multisystemic disorder of pregnancy, defined as hypertension accompanied by proteinuria, and complicates 5%-8% of all gestations and develops after 20 weeks of gestation.[1] One-quarter of stillbirths and neonatal deaths in developing countries are associated with preeclampsia and eclampsia complications.[2] Even though the exact mechanism of PE remains largely elusive, it is confirmed that PE results from abnormal placentation which in turn leads to insufficient placental perfusion and ischemia.[3] The concept of early and late is more modern and it has been suggested that these are 2 entities with different pathophysiologies.[4] Early-onset PE (before 34 weeks) comprises 5%-20% of all PE cases worldwide. It is commonly associated with inadequate and incomplete trophoblastic invasion of maternal spiral arteries, fetal growth restriction and considerable additional maternal morbidity and mortality. Late-onset PE (after 34 weeks) constitutes more than 80% of all preeclampsia cases worldwide and is associated with a normally grown baby with no signs of growth restriction and, normal or only slightly altered behavior of the uterine spiral arteries.[5] Despite the lack of therapeutic treatment, predicting preeclampsia is an urgent and essential issue for the management of maternal and fetal complications for both mother and fetus.

MicroRNAs (miRNAs) are small noncoding RNAs that act at a posttranscriptional level to degrade or rarely activate the expression of target mRNA molecules by complementary base pairing in the 3′ untranslated region (3′UTR) of the mRNA.[6] MiRNAs, called circulating miRNA or cell-free miRNA, also exist in circulation and a number of extracellular fluids.[7] They may serve as non-invasive biomarkers for cancer, diabetes, central nervous system disorders, and pregnancy-associated disorders.[8,9] In pregnancy, miRNAs which are synthesized by human trophoblast cells can be secreted into the maternal circulation.[10,11] They have the potential of serving as biomarkers in the diagnosis of pregnancy-associated disorders. Their exact roles in the pathogenesis of PE still need further investigation.

Trophoblast cells are specialized cells of the placenta. The proliferation, differentiation, and apoptosis of trophoblasts may contribute to the development of PE. Multiple signaling pathways are involved in regulating the proliferation and apoptosis

Received: June 20, 2023 **Revision requested:** December 18, 2023
Last revision received: December 22, 2023 **Accepted:** January 12, 2024
Publication Date: July 11, 2024
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DOI: 10.5152/cjm.2024.23061

of trophoblasts during the placentation process, including the TGF-β signaling pathway.[12] Our group has recently reported that miR-1183 is one of the upregulated circulating microRNAs in PE cases compared to controls with microarray analysis.[13] Herewith, the aims of this study were to compare the expression level of circulating miR-1183 in preeclampsia patients and controls by qPCR, to investigate the putative target mRNA of miR-1183 via in silico target analysis tools, and to detect expression level of the target gene in the maternal plasma. It is aimed to evaluate the miRNA and the target gene's expression profiles as biomarkers and their potential pathological role in the development of early-onset PE.

Methods

Subjects

Maternal peripheral blood samples were collected (n = 53) from both the early-onset preeclamptic patients between 26 and 32 weeks of gestation (n = 31) as the study group and from women with normotensive pregnancies (NP) as the control group (n = 22). From all these samples, 18 preeclampsia patient samples were used for miRNA expression analysis, and the remaining 13 samples were used for gene expression analysis. For the control group, 15 samples and 10 samples (3 of them used in both groups) were analyzed for miRNA and mRNA expression analysis, respectively. All samples were collected in Department of Obstetrics and Gynecology at İstanbul University with ethical approval taken at İstanbul University, İstanbul Faculty of Medicine Clinical Research Ethics Committee (Approval no: 2012/983-1111, Date: April 14, 2012). All the study participants provided written informed consent in accordance with the ethical principles for medical research stated in the Declaration of Helsinki. Diabetes mellitus, chronic hypertension, acute or chronic infectious diseases or other chronic illnesses are our exclusion criteria and continual drug use was not reported. Preeclampsia patients did not have previous preeclampsia history.

Plasma MicroRNA Quantification

Peripheral blood samples were collected in EDTA tubes (Becton Dickinson, Cat. No. 366643). Each peripheral blood sample was centrifuged within 4 hours of collection at 1600 *g* for 15 min, and plasma samples were stored at −80°C until the RNA isolation step.

Total RNA that contained circulating miRNAs was isolated from maternal plasma (500 µL) using the mirVana miRNA Isolation Kit (#AM1560, Life Technologies, Carlsbad, Calif, USA). During the miRNA isolation, 1.6×10^8 copies of a synthetic miRNA mimic that is only expressed in *Caenorhabditis elegans* and not in mammals were spiked into each plasma sample prior to the RNA isolation for precise quantification (*cel-mir-39*, Qiagen, Valencia, Calif, USA). The circulating miRNA concentration was determined by the NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, Mass, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, Calif, USA). Hsa-mir-1183 (Assay ID: 002841, Thermo Scientific, Waltham, Mass, USA) from each sample was reverse transcribed into cDNA using the TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, Lithuania) and a miRNA specific 5X stem-loop primer according to the manufacturer's protocol. The real-time PCR was performed using the TaqMan miRNA qPCR Assay (Applied Biosystems, Universal PCR Master Mix, USA) and a 20× primer which includes FAM dye specific probes for each miRNA. Each sample was run in duplicate for analysis.

Plasma mRNA Quantification

Plasma samples were thawed on ice and centrifuged at 2000 *g* for 5 minutes at 8°C in order to remove cellular debris. The RNeasy Mini Kit (Qiagen, Hilden, Germany) was used to extract total RNA from 500 µL of plasma from each sample, by adding 1000 µL of TRIzol LS (Life Technologies, CA, USA), and finally the samples were eluted in 20 µL of nuclease-free water. cDNA was synthesized using Random Hexamers (pdN_6) (Roche Diagnostics, Penzberg,Germany) and M-MLV Reverse Transcriptase (Life Technologies, CA, USA) from 500 ng of total RNA input. The cDNA samples were stored at −20°C until further steps.

mRNA expression was performed using the LightCycler 480 instrument (Roche Applied Sciences, Germany). The specific primer-probe sets were designed using the Universal Probe Library System Assay Design for humans (Roche Molecular Systems, Inc., CA, USA). The primer sequences for *CHURC1* are 5′-GGACATT CCCTGTTGACTGC-3′ (forward) and 5′-TGCACAGCCTGTAAAGT TCAGT-3′ (reverse), with UPL probe no. 80. *GAPDH* was used as the reference gene for expression analysis, and primer sequences for GAPDH are 5′-GAAGGTGAAGGTCGGAGT-3′ (forward) and 5′-GAAGATGGTGATGGGATTTC-3′ (reverse).

Statistical Analysis

Continuously distributed demographic and clinical variables are presented as mean with SD and were compared using an unpaired *t*-test. Plasma miRNA levels among different groups of subjects were normalized by exogenous control, *cel-miR-39*. The relative quantification method ($2^{-\Delta\Delta Cq}$) was used to evaluate quantitative variations based on the mathematical model described by Livak et al.[14] Statistical analyses for gene expressions were carried out by Mann–Whitney *U*-test. Sample distributions were analyzed by D'Agostino–Pearson normality test and non-parametric Spearman correlation coefficients were calculated to examine the correlation between circulating miRNA and the target gene expressions. The receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were used to evaluate the sensitivity and specificity of miRNA and mRNA biomarkers for the diagnosis of PE. ROC analyses were done by the MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium). All statistical evaluations and graphs, except for the ROC analysis, were performed using the GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, Calif, USA). *P* values of $< .05$ were considered statistically significant.

Results

Clinical characteristics of the PE and NP groups are shown in Table 1. The mean age of the subjects was 31.23±0.75 in the study group and 31.91 ± 0.8 in the control group. Student's *t*-test showed no significant difference between the 2 groups in terms of age ($P = .531$). Gestational age at delivery in the PE group was significantly earlier than in the NP group ($P < .0001$). Besides, fetal birthweight in the PE group was significantly lower than in the NP group ($P < .0001$) (Table 1). Descriptive statistics comparisons of the expression levels of miRNA-1183 and CHURC1 between groups are also shown in Table 2. A correlation of miR-1183 expression with systolic blood pressure in 15 patients revealed only a slight trend towards an inverse correlation (Figure 1).

Target Gene Identification and Bioinformatic Analysis

In order to determine the target gene of miR-1183, we employed bioinformatics approaches and used *in silico* target prediction tools, including miRanda,[15] TargetScan,[16] DIANA-microT-CDS.[17] *CHURC1* was identified as a potential targeted gene of miR-1183

Table 1. Demographic and Clinical Characteristics of Normotensive Pregnancies and Preeclampsia

Demographic and Clinical Characteristics	NP (n = 22)	PE (n = 31)	*P*
Maternal age (years)	31.91 ± 0.8	31.23 ± 0.75	NS
BMI (kg/m^2)	ND	31.3 ± 0.68	ND
Gestational age at delivery (weeks)	37.91 ± 0.5	29.39 ± 0.41	<.0001
Systolic blood pressure (mmHg)	ND	159 ± 3.81	ND
Diastolic blood pressure (mmHg)	ND	100 ± 2.00	ND
Fetal birthweight (g)	3190 ± 128.5	1284 ± 104.7	<.0001

ND, not determined; NS, not significant; NP, normotensive pregnancies; PE, preeclampsia.

Table 2. Descriptive Statistics for miRNA-1183 and ***CHURC1***

Descriptive Statistics	miRNA-1183		*CHURC1*	
	Patients	Controls	Patients	Controls
Number of values	18	15	13	10
Mean of relative quantification	0.0031	0.0004	0.0517	0.1444
SD	0.0064	0.0002	0.0357	0.0464

with a perfect match at 9 base pairs and 2 G-U wobble base pair, which stabilizes the miRNA oligonucleotide-mRNA duplex and enhances silencing (Figure 2A).

The results of expression level analysis of miR-1183 and CHURC1

Plasma samples collected from 18 preeclamptic and 15 normotensive women were subjected to the qPCR process. The circulating level of miR-1183 in maternal plasma significantly increased (*P* = .002*)* in the PE group compared to controls (Figure 2B). Following the discovery of miR-1183 dysregulation in women who went on

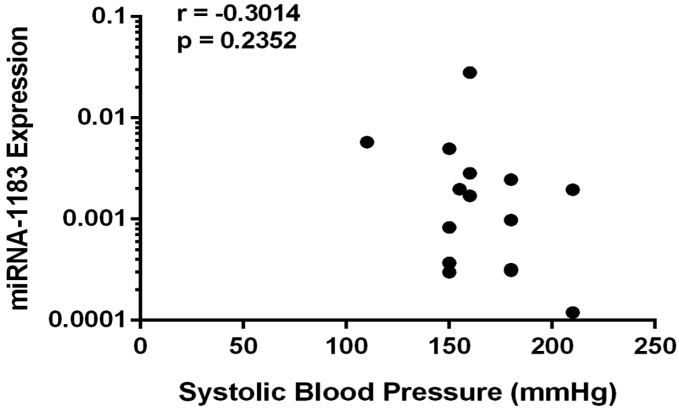

Figure 1. Correlation of miR-1183 expression with systolic blood pressure. A slight trend towards inverse correlation was observed for miR-1183 with systolic blood pressure (number of XY pairs = 15). Significance was assessed by Spearman's correlation.

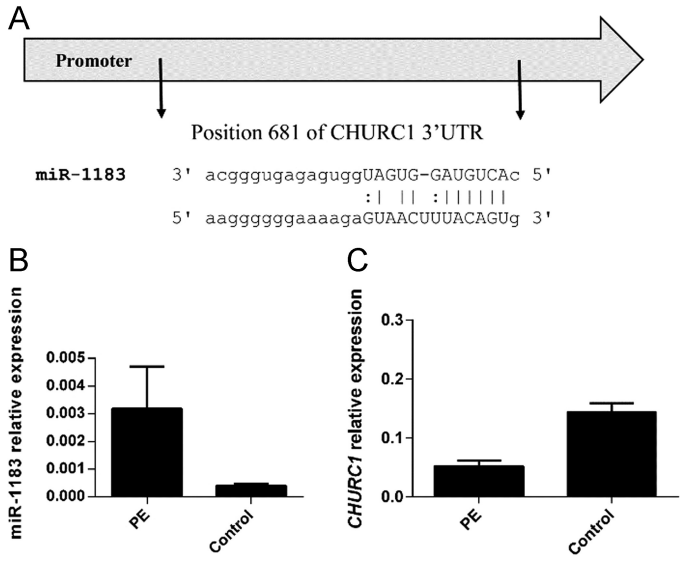

Figure 2. Differences in expression of circulating miR-1183 and *CHURC1* in the preeclampsia (PE) and control groups. A) The target site for miR-1183 sequences in the 3'UTR of *CHURC1*. B) Relative expression level of circulating miR-1183, *P* = .002 by Mann–Whitney test. miR-1183 expression was normalized with *cel-mir-39*. C) Relative expression level of circulating *CHURC1*, *P* < .0001 by Mann–Whitney test. *CHURC1* expression was measured in comparison to *GAPDH* by qPCR. Results are presented as Mean ± SD.

to develop preeclampsia, the relative expression of *CHURC1* in maternal plasma was tested. The expression level of *CHURC1* significantly decreased (*P* < .0001) in PE cases compared to controls (Figure 2C). Spearman correlation coefficient was examined between the circulating miR-1183 and *CHURC1*. Even though there was no significant correlation (*P* = .528), plasma expression levels of miR-1183 and *CHURC1* represented a moderate inverse correlation (*r* = −0.1384, number of XY pairs = 22). The results are compatible with our previous work in the PE and NP groups, which is performed by microarray.[13] The expression of miR-1183 was chosen as the most diminishing miRNA, and according to the microarray results, the upregulation of miR-1183 was statistically significant (*P* < .05).

Diagnostic Role of miR-1183 and CHURC1

In order to evaluate the discriminative value of the circulating miRNA and its target gene for PE, the ROC curves were established for miR-1183 and *CHURC1*'s elevated expression levels. The AUC for miR-1183 is 0.798 (*P* < .001), indicating a powerful prediction for PE. The AUC for *CHURC1* gene is 0.962 (*P* < .001), almost 1.0, indicating perfect accuracy of the data (Figure 3, Table 3).

Discussion

Preeclampsia remains a leading cause of maternal and fetal morbidity and mortality. Accurate prediction of PE is a significant challenge in maternal and fetal medicine. There are several studies showing that many miRNAs are selectively elevated in maternal plasma/serum of women with PE,[7,11,13,18-20] suggesting a pivotal role of this epigenetic mechanism.

In the present study, the results indicate that the levels of circulating miR-1183 significantly increased in the maternal plasma of preeclamptic women. miR-1183 studies in the literature are extremely limited. One study has reported that miR-1183 overexpression may

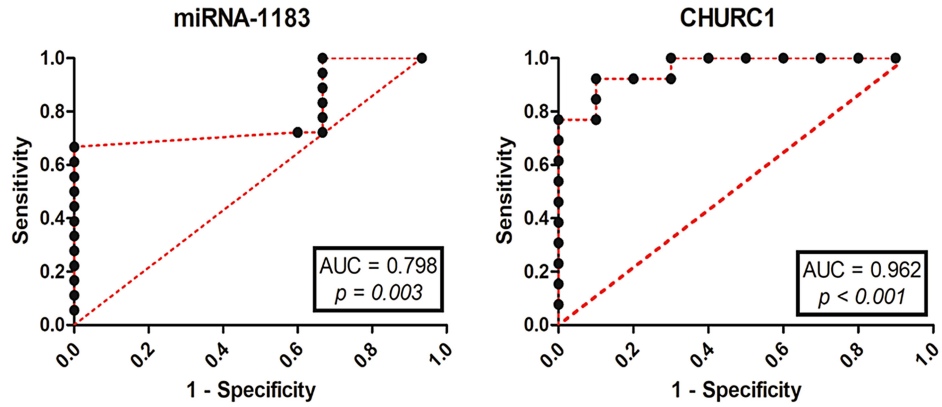

Figure 3. Receiver Operating Characteristic (ROC) curves representing PE diagnostic tests by miRNA-1183 and *CHURC1* in PE and control subjects. ROC curve for miRNA-1183 is a good test since the area under the curve (AUC) is 0.798 ($P < .001$), with 18 samples for PE and 15 for control. The ROC curve analysis for the *CHURC1* gene is an excellent test with an AUC value of 0.962 ($P < .001$), number of samples for PE = 13 and control = 10. The dashed diagonal line represents a reference line showing zero sensitivity and zero specificity.

reflect pulmonary artery remodeling in rheumatic heart disease (RHD) patients with secondary pulmonary hypertension (PAH).[21] Another study has shown that ox-LDL increases the ROCK2 gene expression by reducing miR-1183 levels. A single nucleotide polymorphism at the 3'UTR of the ROCK2 gene could affect the miR-1183 binding site and consequently increased ROCK2 expression is related to arterial stiffness.[22] miR-1183 has also been reported to be involved in cancer progression. It has been shown that miR-1183 expression is altered in rectal cancer and Kaposi's sarcoma samples.[23,24] Another study has reported that miR-1183 has a relationship with a functional polymorphism in the EpCAM gene in cervical cancer patients.[25]

Our analysis has identified *CHURC1* as a predicted target gene of miR-1183 and shown the downregulation of gene expression in the maternal plasma of preeclamptic women. A moderate inverse correlation between circulating miR-1183 and *CHURC1* expression levels has suggested that *CHURC1* is one of the target genes of miR-1183. Our results also showed that this inverse correlation between miRNA and *CHURC1* is not statistically significant (Figure 1). This can be mainly caused by the expression differences between miRNA and mRNA regulated by various factors such as transcription factors and endogenous long non-coding RNAs. Also, the correlation coefficient could be affected due to the small sample size.

CHURC1 gene encodes a zinc finger protein that acts as a transcriptional activator. In the case of preeclampsia, only 1 study has evaluated the expression of *CHURC1* in the peripheral blood mononuclear cells (PBMC) from preeclamptic women and demonstrated downregulation of *CHURC1*.[26] In our study, we have used the maternal plasma instead of PBMC as the source of *CHURC1*. Notably, under fibroblast growth factor control, *CHURC1* represses cell migration in gastrulation and it has been shown that *CHURC1* represses BMP (bone morphogenetic protein) signaling through bone morphogenetic protein receptor type 2 (BMPR2) [27,28,29]. *BMPR2* encodes a transmembrane serine/threonine kinase receptor which belongs to the transforming growth factor beta (TGF-β) superfamily, and initiates BMP signaling.[30] Bone morphogenetic protein 2 belong to TGF-β superfamily and are highly expressed in human endometrium and placenta. Transforming growth factor betasignaling pathway is a known regulator of placental trophoblastic invasion and migration, and plays an autocrine role in regulating the gene expression in human trophoblast cell lines.[31] Lee et al studied the mice with a conditional deletion of BMP2, one of the ligands for BMPR2, demonstrate a defect in uterine decidualization at the beginning of pregnancy.[32] Bone morphogenetic protein receptor type 2 mediated signaling and CORIN (corin, serine peptidase) gene has a major role on the development of preeclampsia by inducing trophoblast invasion. Nagashima et al[33] demonstrated that signaling through BMPR2, directly or indirectly regulates CORIN expression and controls trophoblastic invasion (Figure 4). Corin is a cardiac protease which is important in regulating blood pressure. And interestingly, it's expression was detected in the pregnant uterus.[34] Cui et al[35] identified a novel function of CORIN gene in promoting trophoblast invasion and spiral artery remodeling. They showed that corin-deficient mice developed high blood pressure and proteinuria features of preeclampsia. Consequently, impaired CORIN expression or function in pregnant uterus is associated with preeclampsia. Moreover, corin variants in the frizzled 2 domain that impaired corin function have been reported in African Americans,[36] a high-risk population for preeclampsia. In the case of preeclampsia, where there is increased trophoblastic invasion and placental blood flow, trophoblasts might repress CHURC1 expression and stimulate the overexpression of miR-1183.

As the alterations identified in this study were seen at 26-32 weeks of gestation, miR-1183 upregulation may be a response to the trophoblastic invasion characteristic of preeclampsia. We hypothesize that after the occurrence of the disease, trophoblasts attempt to suppress *CHURC1* expression to increase trophoblast invasion in the placenta. For this purpose, trophoblast cells may increase their negative regulator, miR-1183. Thus, it cannot be the factor that causes the disease, but it might have a regulating effect

Table 3. Receiver Operating Characteristic Parameters for Circulating miR-1183 and *CHURC1*

ROC Curve	miRNA-1183	*CHURC1*
Area under curve (AUC)	0.798	0.962
95%CI*	0.622-0.917	0.788-0.999
P	0.0002	<0.0001
Sensitivity	66.67	92.31
Specifity	100.00	90.00
Std Error	0.08	0.03

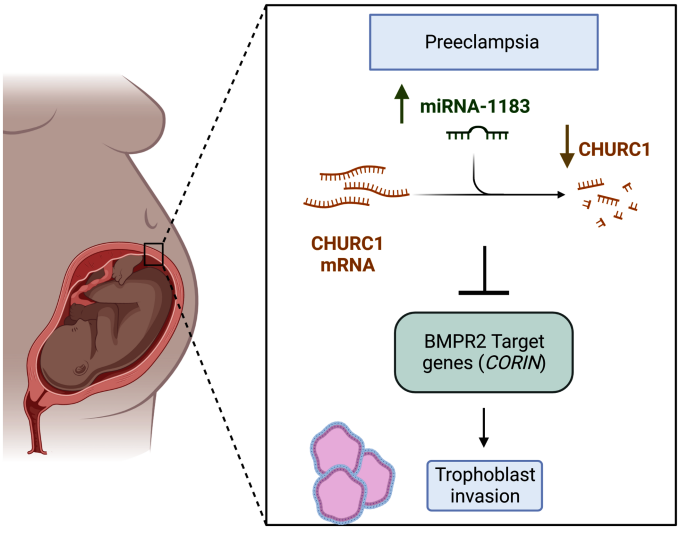

Figure 4. A model suggesting that increased circulating miR-1183 expression represses target *CHURC1,* and *CHURC1* represses *BMPR2* genes which directly or indirectly regulate *CORIN*.

once PE occurs. This has led us to suggest that the increased miR-1183 might be overexpressed to reduce *CHURC1* expression, as suggested in our model presented in Figure 4.

In conclusion, the circulating miR-1183 levels were up-regulated, and *CHURC1* was identified as a putative target of miR-1183 and down-regulated in maternal plasma of preeclamptic women. Since miR-1183 and *CHURC1* have a high discriminative value and significant differential expression levels, they might be potential biomarkers for the prediction of PE. To our knowledge, this is the first study dealing with these relationships in the maternal plasma of preeclamptic women. The mechanisms by which *CHURC1* modulates the PE pathogenesis need to be further studied, and functional cell culture studies in trophoblast cell lines are planning to be done.

Ethics Committee Approval: Ethics approval for this study was granted from İstanbul Faculty of Medicine Clinical Research Ethics Committee (Approval no: 2012/983-1111, Date: April 16, 2012).

Informed Consent: All the study participants provided written informed consent in accordance with the ethical principles for medical research stated in the Declaration of Helsinki.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – T.G., D.A.; Design – T.G., D.A., A.B., K.A.; Supervision – T.G.; Resources – A.B., K.A.; Materials – D.A., E.G.A.; Data Collection and/or Processing – D.A., E.G.A., T.G.; Analysis and/or Interpretation – D.A., T.G.; Literature Search – D.A., T.G., E.G.A.; Writing Manuscript – D.A., T.G., E.G.A.; Critical Review – T.G., A.B., K.A.

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This study was funded by the Research Fund of İstanbul University (Grant no: 25666).

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