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# Downregulation of miR-424 in placenta is associated with severe preeclampsia

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ABSTRACT

Previous studies have suggested that altered miRNA expression in the placenta is associated with preeclampsia. The aim of this study was to investigate the expression of miR-424 in placental samples of severe preeclampsia (sPE) and uncomplicated pregnancy patients. miRNA was isolated from placentas obtained from 30 sPE patients and 30 healthy women. Quantitative real-time polymerase chain reaction was used to analyze the expression of miR-424. The prediction of target genes of miR-424 was performed using miRGen database. The function of these target genes was analyzed further by DAVID and Gorilla software. The expression of miR-424 was significantly lower in patients with sPE than in healthy controls. Changed expression of miR-424 in the case of pregnancy-related hypertensive disorders might affect the Wnt signaling pathway. These factors have a strong correlation with the development of PE. Expression of miR-424 in placenta was lower in patients with sPE, suggesting its role in the pathology of sPE.

## 1. Introduction

Preeclampsia (PE), a syndrome among pregnant women characterized by elevated maternal blood pressure and proteinuria after 20 weeks of gestation, is one of the leading causes of pregnancy-related maternal and fetal morbidity and mortality [1]. PE is characterized as severe according to hypertension degree (arterial pressure 160/110 mmHg on two occasions 6 h apart); proteinuria (5 g/24 h or 3 in two urine samples); or any of the following: cerebral/visual disturbances, abdominal pain, abnormal liver function, oliguria, pulmonary edema, thrombocytopenia, or fetal growth restriction [2]. Severe PE (sPE) is associated with fetal growth restriction, indicating placental dysfunction, and with preterm birth and perinatal death. Most of the pathological conditions associated with PE appear to resolve after delivery. However, there is growing evidence that women with a history of PE are more likely to develop cardiovascular disease (CVD) later in life [3]. Although several factors may cause PE, it is generally agreed that one of the origins of PE is the placenta itself [4]. It has long been found that the placenta plays an important role in the development of PE, in which poor placentation, shallow invasion, and abnormal angiogenesis are the main pathological manifestations. Evidence has demonstrated that hypertensive disorders in pregnancy are associated with alterations in the expression of different microRNAs (miRNAs) [5–7].

miRNAs are small, noncoding RNAs that regulate gene expression at the posttranscriptional level through mRNA degradation and translational repression [8]. They act by targeting the RNA-induced silencing complex to complementary sites within the 3′-untranslated region (UTR) of their target mRNAs. Depending on the degree of base pairing between the miRNA and the 3′-UTR, either degradation or translational repression of the targeted mRNA occurs. Although they account for < 1% of all human genes, miRNAs have been estimated to regulate up to 30% of all protein-encoding genes [9]. They have important roles in diverse biological processes, such as development [10], differentiation [11], apoptosis [12], and oncogenesis [13]. miRNAs have been connected with human disease including cancer [14], heart disease [15],

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fetal growth restriction [16], macrosomia [17], and male infertility [18]. Recent miRNA expression profile studies in PE placenta and serum showed seven different expressed miRNAs, indicating their potential roles in PE development [19–21].

Although the precise mechanism is still unknown, miRNAs expression features within the placenta have been implicated in the pathogenesis of PE [22]. Mouillet et al. [23] demonstrated that miR-424 is uniquely down-regulated in primary human trophoblasts by hypoxia or chemicals known to hinder cell differentiation. MicroR-424 is a mammalian-specific miRNA that is particularly abundant in placental trophoblasts [24]. In the present study, we examined the expression level of miR-424 in placentas of patients with severe preeclampsia and normal controls. Additionally, the pathways and enrichment of target genes of miR-424 were analysed using bioinformatics methods.

## 2. Material and methods

### 2.1. Sample collection

This study was approved by the Institutional Ethics Committee of Nanjing Medical University. Written informed consent was obtained from each participant. Our study was designed using a case-controlled approach. All study subjects were non-smokers. Thirty sPE patients and 30 healthy pregnant women with uncomplicated pregnancies were involved in the study. The placenta samples, delivered at 37–42 weeks, were collected from the 60 pregnant women undergoing cesarean section in the Department of Obstetrics and Gynecology. The tissue samples were collected 2–3 cm in radial distance from the margin of the placenta. PE was regarded as severe if any of the following criteria were present: blood pressure ≥ 160 mmHg systolic or ≥110 mmHg diastolic, or proteinuria ≥ 5 g/24 h (or ≥3+ on dipstick). Exclusion criteria were multifetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease, angiopathy, renal disorder, maternal or fetal infection, and fetal congential anomaly. All placental samples were frozen within a half hour after delivery and frozen in liquid nitrogen and stored at –80 °C until extraction of RNA.

### 2.2. RNA extraction and quantitative real-time PCR

Total RNAs were extracted from sPE and normal control tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the protocol of the manufacturer. The concentration and purity of RNA were determined spectrophotometrically by Nano Drop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The expression level of miR-424 was measured using commercially available TaqMan microRNA Assays (Applied Biosystems, Valencia, CA, USA) on an Applied Biosystems 7900HT Real-Time PCR system. Fast PCR instrument using 1 μg total RNA as the template and specific reverse primers at 16 °C, 30 min, 42 °C, 30 min and 85 °C, 5 min of reverse transcription. The resulting cDNAs were amplified by real-time PCR using TaqMan® Gene Expression Master Mix (Applied Biosystems, Valencia, CA, USA). Cycling conditions were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Quantification cycle (Ct) values were recorded with SDS version 2.4 software. All reactions were run in triplicate on 384-well plates. Total RNA input was normalized using RNU6B RNA as an endogenous control. The mean Ct value was determined from three PCR replicates.

### 2.3. miR-424 target predictions and bioinformatic analysis

To better understand the function of the aberrantly expressed miR-424, putative miR-424-directed target genes were predicted by miRGen database [25]. For an overview of the aberrantly expressed miR-424 in placentas of sPE patients, functional analysis of pathway and enrichment analysis were performed using DAVID tools (http://david.abcc.ncifcrf.gov) [26] according to the KEGG database (http://www.genome.jp/kegg/) and Gorilla (http://cbl-gorilla.cs.technion.ac.il) [27], respectively.

### 2.4. Statistical analysis

Statistical analyses for miR-424 expression levels were performed by the Mann-Whitney test. All statistical analyses were carried out using Stata (Version 9.0, StataCorp LP, TX, USA), and $P \leq 0.05$ were considered to be significant.

## 3. Results

### 3.1. Clinical characteristics

No significant difference in maternal age or parity was found between the groups (Supplementary Table 1). Gestational age was significantly lower in patients with preeclampsia compared to the controls. Systolic and diastolic blood pressure and proteinuria were significantly higher in patients with preeclampsia compared to the controls (Supplementary Table 1).

### 3.2. Expression of miR-424 in the placenta of sPE patients and controls

We analyzed 30 sPE and 30 normotensive placentas for expression of miR-424 reported to be expressed in the placenta and to regulated cell growth and development pathways. The expression level of miR-424 was significantly decreased in patients with sPE compared with normal controls ($P = 0.010$) (Fig. 1).

### 3.3. Bioinformatics analysis of target gene of miR-424

To better understand the function of the aberrantly expressed miR-424, putative miRNA-directed target genes were predicted by miRGen v3 [25]. miRGen is an integrated database with targets interface facilities that provide access to unions and intersections of four widely used target prediction programs: PicTar, TargetScan, MiRanda, and DIANA-microT algorithm, as well as the experimentally supported targets from TarBase. We assembled the lists of the miRNA target genes, as determined either by the four-algorithm combinations or the TarBase. Eighty genes were identified by two or more algorithms (score ≥ 2, Supplementary Table 2).

As presented in Fig. 2, four possible pathways were listed with the $P$ value < 0.001. They were Wnt signaling pathway, neurotrophin

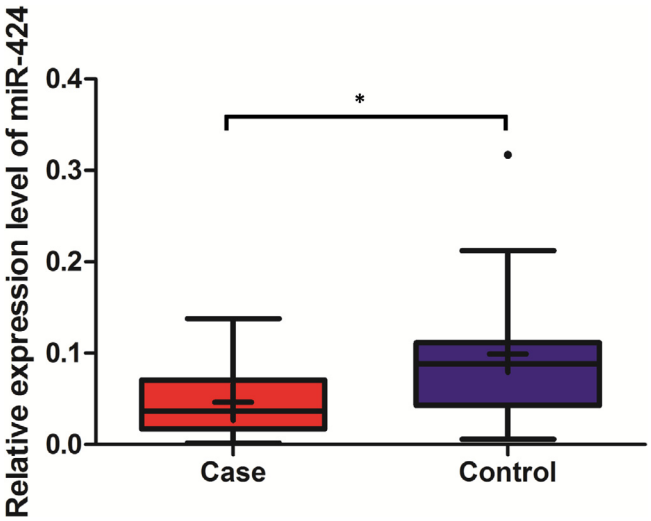


**Fig. 1.** Expression level of miR-424 in placentas of patients with sPE and normal controls. Data are given in Tukey Box plots showing median (−) and mean (+) values. Significant difference is marked with *$P < 0.05$.

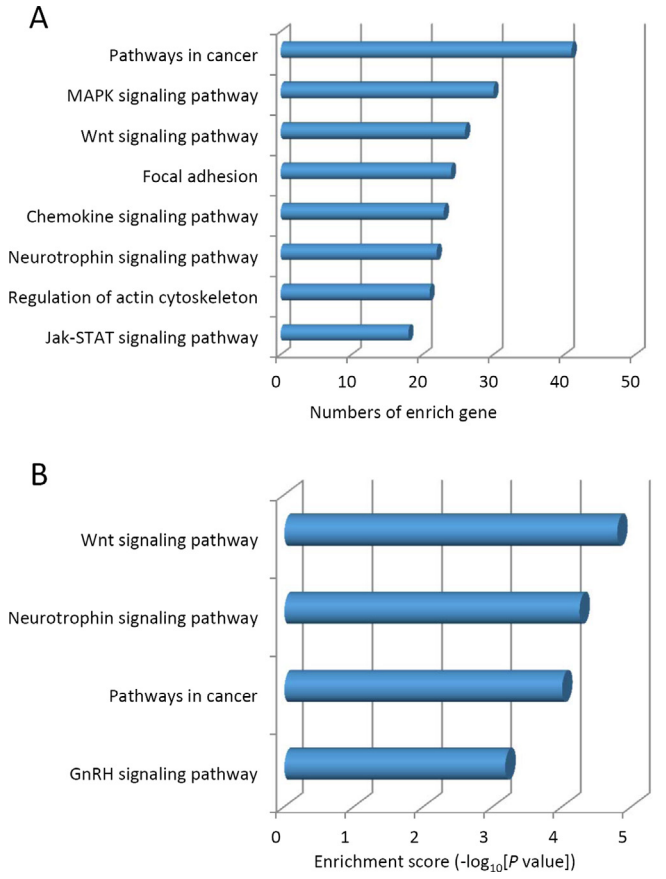


**Fig. 2.** Functional classification of aberrantly expressed miR-424 in severe PE using DAVID data base (http://david.abcc.ncifcrf.gov/). (A) KEGG analysis of the pathway with the number of target gene included. (B) KEGG analysis of the pathway with the *P* value. The functional classifications presented here are significant.

signaling pathway, pathways in cancer, and GnRH signaling pathway.

### 3.4. Enrichment analysis

A total of 80 target genes of miR-424 were entered into the enrichment analyses. As many as 5 enriched molecular functions (Supplementary Table 3) met the threshold of significance ($P < 0.05$). The most enriched molecular functions were 'signaling receptor activity' with an enrichment of 10.67. A total of 6 enriched biological processes (Supplementary Table 4) were identified. Among the most enriched processes, we found 'response to inorganic substance' with an enrichment of 20.00. Other processes of interest among the most enriched are 'response to oxygen-containing compound', 'chemical homeostasis', 'response to lipid', 'response to organic cyclic compound' and 'single-organism cellular process'.

## 4. Discussion

PE is characterized by vasospasm, reduced placental perfusion, and abnormal placentation. The main cause of the fetal compromise is disturbance in uteroplacental perfusion. There are a number of hypotheses regarding the main cause of this disorder, including abnormal placentation, immunological background, and abnormal inflammatory response. Impaired trophoblastic invasion, placental hypoxic degeneration, and apoptosis have been shown by many studies, defining preeclampsia as a placental pathology [28].

miRNAs are expressed abundantly in the human placenta [29]. Several studies have reported that specific miRNAs are over-expressed and under-expressed in PE [30]. Pineless et al. [5] examined the expression levels of miRNAs in placentas of preeclamptic patients. They screened 157 miRNAs, of which 153 were detected in the placental tissue. They found that the expression of two miRNAs (miR-210 and miR-182) was significantly higher in PE compared to the control group. The expression of mir-210 was localized to the syncytiotrophoblast, and the target gene was found to be responsible for increased immune response, apoptosis and lipid metabolism. However, Zhu et al. [30] have demonstrated that up-regulation of miR-210 in sPE pregnancies but down-regulation of miR-210 in mild PE placentas. Interestingly, miR-210 targets *HIF-1α* (a potential marker of hypoxia) [31], which might lead to hypoxic conditions in PE. Mayor-Lynn et al. [19] did not find any significant difference of miR-210 expression between PE and control placentas (elective cesarean section without labor). Nevertheless, miR-210 was significantly down-regulated in preterm patients. Whitehead et al. [32] found that miR-424 was significantly up-regulated in pregnancies complicated by severe preterm fetal growth restriction compared to gestation-matched controls. Alteration of the miRNA expression in PE suggests the down- or up-regulation of potential target genes which may contribute to the pathology of PE. The association between PE and the altered miRNA expression suggests the possibility of a functional role for miRNA in this disease.

Our study presents an analysis of miR-424 expression in preeclamptic placental samples with the use of miRNA PCR technology. Our results indicate that aberrant lower expression of miR-424 is associated with sPE. Mouillet et al. [23] have found that miR-424 is uniquely downregulated in primary human trophoblasts by hypoxia and the expression of miR-424 is directly correlated with the differentiation of trophoblasts. miRNAs have strong effects on the expression of different target genes and protein transcription. They are post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts, usually resulting in mRNA degradation and/or translational inhibition [33]. Changed expression of miR-424 in case of pregnancy-related hypertensive disorders, may affect the Wnt signaling pathway and pathways in cancer (Fig. 2). These factors have a strong correlation with the development of PE [34,35]. Our findings may provide novel targets for further investigation of the pathogenesis of PE and miR-424 may be a potential biomarker for the diagnosis of PE. However, follow-up studies with larger sample size are required to confirm the significant down-regulation of miR-424.

## Declaration of Competing Interest

No conflicts of interest are disclosed.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.05.017.

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