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Original Article

The expression of imprinted genes IGF2 and PHLDA2 in mid-pregnancy have predictive values for the development of pre-eclampsia

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Abstract: Objective: Insulin-like growth factor-2 (IGF-2) and pleckstrin homology-like domain, family A, member 2 (PHLDA2) appear to play an important role in paracrine interactions at the maternal-fetal interface in human pregnancy. But the potential predictive value of serum IGF-2 and PHLDA2 concentrations in pre-eclampsia remains to be established. The goal of the present study was to explore the expression of IGF2 and PHLDA2 in mid-pregnancy with pre-eclampsia (PE). Methods: Samples of serum were collected from women with normal mid-pregnancy women (control group, n=21), mild pre-eclampsia (M-PE group, n=19) and severe pre-eclampsia women (S-PE, n=19). The expressions of IGF2 and PHLDA2 in the serum were determined by ELISA and western blot. After delivery, the fresh placental tissues were collected. The expression levels of IGF2 and PHLDA2 in placenta were detected by real-time PCR, western blot and immunohistochemistry. Results: It was found that the concentrations of IGF2 and PHLDA2 were remarkably dropped in comparison with control group. And there was significant difference in PHLDA2 expression between mild pre-eclampsia and severe pre-eclampsia in serum. After delivery, the differences in imprinted genes IGF2 and PHLDA2 were found in placental tissues between PE and normal pregnancy. mRNA and protein expression levels were all obviously decreased in PE groups. Conclusion: These data are the first to demonstrate imprinted genes IGF2 and PHLDA2 associated with PE in mid-pregnancy. It illustrates that IGF2 and PHLDA2 may participate in PE pathogenesis.

Keywords: Pre-eclampsia, imprinted genes, IGF2, PHLDA2

Introduction

Preeclampsia (PE), identified by the presence of hypertension and proteinuria after 20 weeks of pregnancy [1], is a systemic disease that involved many organs such as the brain, eyes, liver and kidneys [2]. It's one of the most common hypertensive disorders and is a leading cause of morbidity and mortality for pregnant women and perinatal babies [3, 4]. Furthermore, PE is strongly associated with intrauterine growth restriction, iatrogenic prematurity, placental abruption, and stillbirth of the child [5-7]. Despite great efforts, the exact pathophysiology of PE remains unknown. Because of the serious health consequences of PE, risk assessment and identification of women at risk early in the pregnancy remain a major challenge in prenatal care.

Imprinted genes are monoallelic ally expressed by virtue of an epigenetic process initiated in the germline [8], and they are considered to be crucial for the regulation of embryonic growth and placental development [9]. Imprinted genes are typically found clustered in chromosomal domains, where they are coordinately regulated by discreet DNA elements called 'imprinting centers'. Among the ~30 imprinted genes that have known biological functions, a large percentage are expressed in trophoblast and control placental growth and/or development [10, 11]. The paternally expressed IGF2 is located about 100 KB from the maternally expressed non-coding gene H19 on human chromosome 11. IGF2 plays major role in embryonic and placental growth. There are reports in the literature that the paternally expressed IGF2 encodes insulin-like growth

factor II, which is a mitogen involved in embryonic growth. IGF2 may beimplicated in the placental supply of maternal nutrients. Studies have shown that PHLDA2 protein appropriately restricted to villous and intermediate cytotrophoblast in the intrauterine growth restriction (IUGR) placentae [12]. The domain containing pleckstrin homology-like domain, family A, member 2 (PHLDA2), located on distal chromosome 7 in mice and chromosome band 11p15.5 in humans, is controlled by the Kvdmr1 DNA element [13, 14]. Imprinted gene PHLDA2 is upregulated in placentas from intrauterine growth restricted human pregnancies. Elevated expression of the maternally expressed imprinted gene PHLDA2, has been reported in the human placenta of growth restricted pregnancies. However, the expression of IFG2 and PHLDA2 in mid-pregnancy or pre-eclampsia has not yet been elucidated.

To address this lack of information, this study examined expression of the maternally expressed imprinted genes PHLDA2 and IFG2 in mid-pregnancy or pre-eclampsia.

Materials and methods

Study participants

The study was approved by Nanfang Hospital, Southern Medical University of China Ethics Committee and informed consent was obtained from patients and control women. 19 patients with mild pre-eclampsia (M-PE), 19 patients with severe pre-eclampsia (S-PE) and 21 normal control pregnant women were recruited from Southern Medical University of China between October 2015 and September 2016. Exclusion criteria were cardiac and multi-foetus pregnancies, systemic lupus erythematosus, renal disease and structural or chromosomal anomalies. None of the PE patients and healthy controls had history of smoking or hypertension. The diagnose of PE was based on modified American Congress of Obstetricians and Gynaecologists criteria.

Sample collection

Placentas were excised immediately after delivery (elective caesarean section or vaginal delivery). Small pieces of placenta tissues (1*1*1 cm^3) in the central region avoiding calcification and infarct areas were obtained. Tissues were immediately frozen and stored at -80°C.

RT-PCR analysis

Total RNA was extracted from the placental tissue samples using GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, Dorset, UK). 5 μg of RNA was reverse transcribed using M-MuLV reverse transcriptase (Promega, Southampton, UK) with random hexamers, according to manufacturer's instructions. Quantitative RT-PCR was performed using Chromo Four Colour Real Time Detector (MJ Research) in a 20 ml reaction containing 5 ml of cDNA (diluted 1 in 50), 1X Buffer 2 mM $MgCl_2$, 2 mM dNTPs, 0.65 Units Taq (Fermentas (Thermo), Loughborough, UK), 1 mM of each primer (SigmaeAldrich, Dorset, UK) and 0.12X Sybr Green (Invitrogen, Paisley, UK). Conditions for amplification were 15 min at 94°C, and then subjected to 30 thermal cycles at 94°C (30 sec), 65°C (1 min) and 72°C (1 min), followed by a final extension at 72°C (30 sec) using a thermal sequencer (Zymoreactor, Atto, Tokyo, Japan). The internal control was b-actin. Primer sequences were as follows: IGF2 forward: 5'-GGACTTGAGTCCCTGAACCA-3', reverse: 5'-TGAAAATTCCCGTGGAAGG-3'. PHLDA2 forward: 5'-CCATCCTCAAGGTGGACTGC-3', reverse: 5'-TTCCTGGCGGCTGCGAAAGT-3'. GAPDH forward: 5'-CCATCGTCCACCGCAAA T-3', and reverse: 5'-GCTGTCACCTTCACCGTTC-3'.

Western blotting analysis

NP-40 lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) containing the protease inhibitor (PMSF) was used and total proteins were isolated. Western blots were performed as previously described [15]. Samples containing 60 μg protein were electrophoresed on 12% (w/v) SDS polyacrylamide gel and transferred onto polyvinylidene difluoride (PVDF) membranes. Then the PVDF membranes were blocked with 5% nonfat milk for 1 h followed by incubation with the following primary antibodies: PHLDA2 antibody and IGF2 antibody (1:400 dilution, BIOSS, Beijing, China) at 4°C overnight. After washing with Tween-20 in Tris-buffered saline (TTBS), the membrane was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:5000 dilution, Beyotime Institute of Biotechnology) at 37°C for 1 hr. The optical density of each protein was normalized to the corresponding β-actin signal using ImageJ software (NIH).

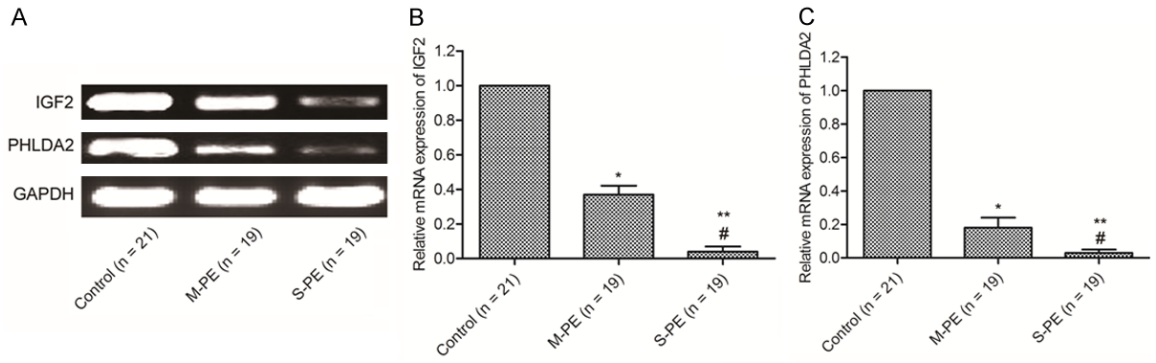

Figure 1. mRNA levels of IGF2 and PHLDA2 in placenta. RT-PCR analysis was used to determine the relative mRNA expression levels of IGF2 and PHLDA2 in placenta. Expression levels were normalized against GAPDH expression. Data values and error bars show the means and standard devations. Each experiment was repeated at least three times and typical results are shown. $^{*}P < 0.05$ and $^{**}P < 0.01$ verusus control group, $^{\#}P < 0.05$ versus M-PE group.

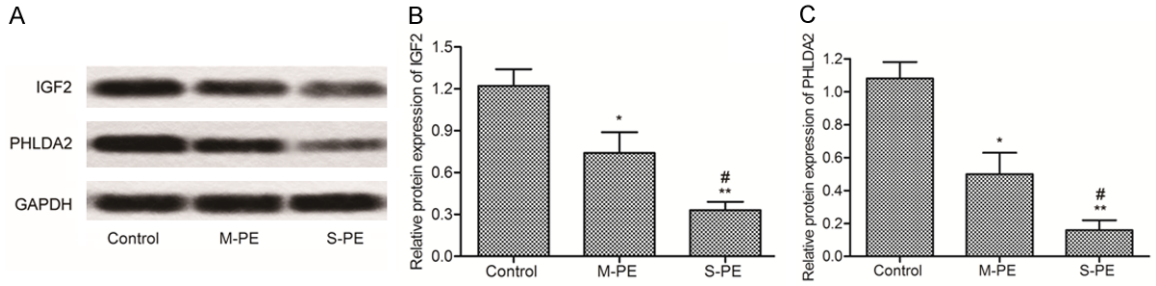

Figure 2. Pretein levels of IGF2 and PHLDA2 in placenta. (A) The relative expression levels of IGF2 and PHLDA2 protein were analyzed by western blot. Relative protein expression of IGF2. (B) and PHLDA2 (C) was quantified using Image-Pro Plus 6.0 software and normalized to GAPDH; Data are represented as the mean ± SD of three experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ verusus control group, $^{\#}P < 0.05$ versus M-PE group.

Immunohistochemistry analysis

Staining of the paraffin-embedded sections was performed with the CSA II kit (Dako, Hamburg, Germany). Briefly, antigen retrieval was performed by microwaving in citrate buffer, pH 6.0, for 15 min. The tissues were then blocked for endogenous peroxidase, incubated with IGF2 or PHLDA2 antibody (1:3000 dilution, BIOSS, Beijing, China), washed in PBS (0.1% Tween 20), and followed by incubation with anti-rabbit HRP polymer (1:200, Envision, Dako) for 30 minutes. Normal healthy pregnant women were used as positive controls in all experiments.

Enzyme-linked immunosorbent assay (ELISA)

Concentrations of IGF2 and PHLDA2 in serum were measured using ELISA kits (Boster, Wuhan, China) according to the manufacturer's instructions.

Statistical analysis

Data with normal distribution were also evaluated by one-way ANOVA with the Bonferroni's post hoc test or repeated measures ANOVA. All results were presented as mean ± SD from a minimum of three replicates. $P < 0.05$ was considered statistically significant.

Results

The mRNA level of IGF2 and PHLDA2 in placenta

The IGF2 and PHLDA2 expression levels in the placenta tissues of the control women and PE patients were analyzed by real-time polymerase chain reaction (PCR). We found that the mRNA expression of IGF2 and PHLDA2 was notably reduced in mild pre-eclampsia (M-PE group), compared with conreol group (**Figure 1**, $P < 0.05$). In Severe preeclampsia (S-PE group),

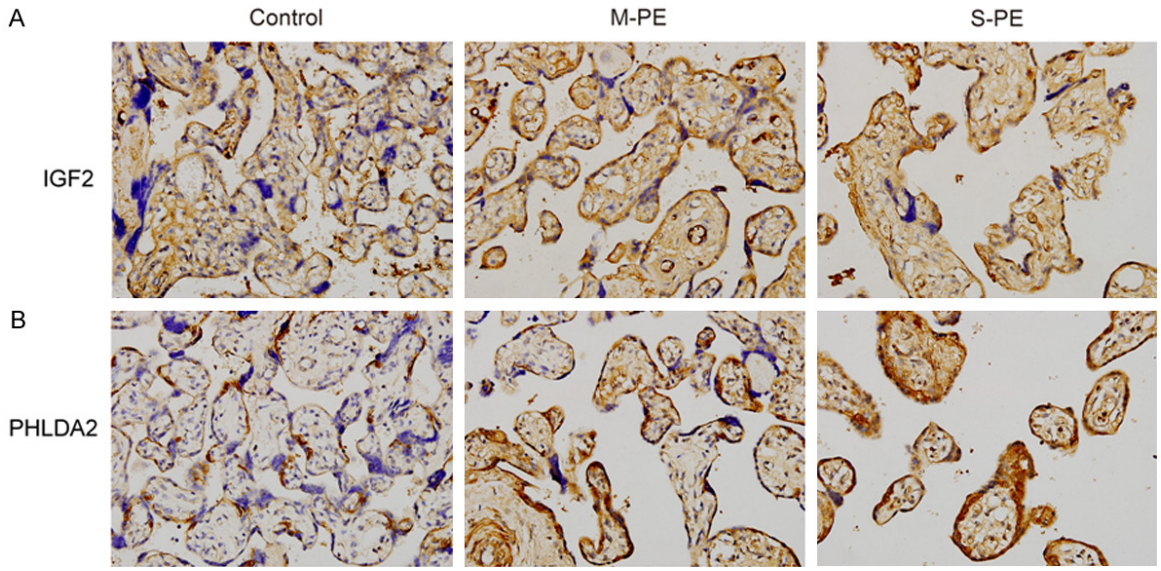

Figure 3. The level of IGF2 and PHLDA2 in placenta was measured by immunohistochemistry analysis. Immunohistochemical detection shows IGF2 (A) and PHLDA2 (B) protein level in placenta. The staining is both nuclear and cytoplasmic.

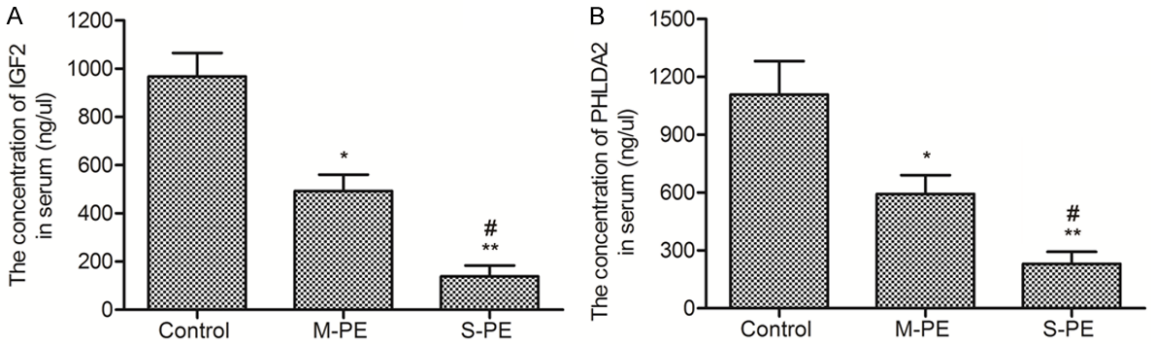

Figure 4. Levels of IGF2 and PHLDA2 in serum. The concentration levels of IGF2 (A) and PHLDA2 (B) in serum were detected by ELISA. Each experiment was repeated at least three times and typical results are shown. $^{*}P < 0.05$ and $^{**}P < 0.01$ verusus control group, $^{\#}P < 0.05$ versus M-PE group.

imprinted genes IGF2 and PHLDA2 were reduced greatly in comparison with control group or M-PE group (**Figure 1**, $P < 0.05$ or $P < 0.01$).

Protein expression level of IGF2 and PHLDA2 in placenta

Using western blotting, we further compared the protein expression of IGF2 and PHLDA2 in placenta. The results showed that the PHLDA2 protein (**Figure 2B**, $P < 0.05$) and the IGF2 protein (**Figure 2C**, $P < 0.05$) levels in PE group were significant lower than those in the control group. As compared with the M-PE group, the expression of two imprinted genes was less in S-PE group. The results showed that the expression of IGF2 and PHLDA2 was inversely proportional to the severity of the PE.

In order to further explore, we detected IGF2 and PHLDA2 expression by immunohistochemistry. Immunohistochemistry was performed on histologic sections. As shown in **Figure 3**, we could obviously see that those two imprinted genes were all evidently depressed decreased in PE groups. There was almost no protein expression of IGF2 and PHLDA2 in S-PE group, compared with the control/M-PE group. The results were similar to western blotting analysis.

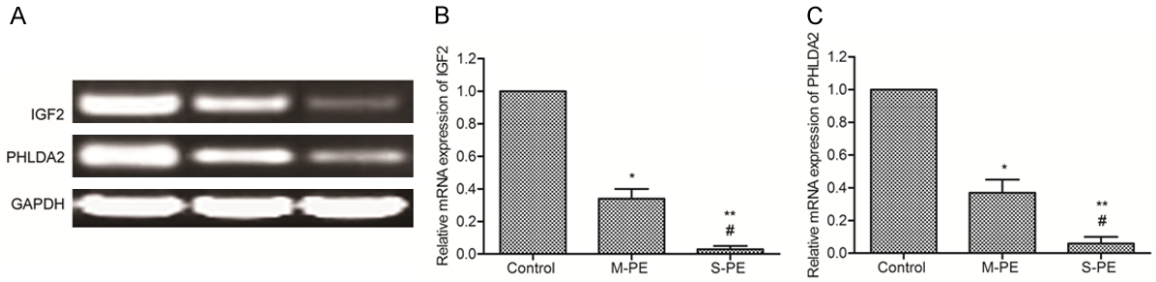

Figure 5. mRNA levels of IGF2 and PHLDA2 in serum. A: mRNA Expression of IGF2 and PHLDA2 was analyzed by RT-PCR. B: Graphs showing the mRNA level of IGF2. C: Graphs showing the mRNA level of PHLDA2. Each experiment was repeated at least three times and typical results are shown. $^{*}P < 0.05$ and $^{**}P < 0.01$ verusus control group, $^{\#}P < 0.05$ versus M-PE group.

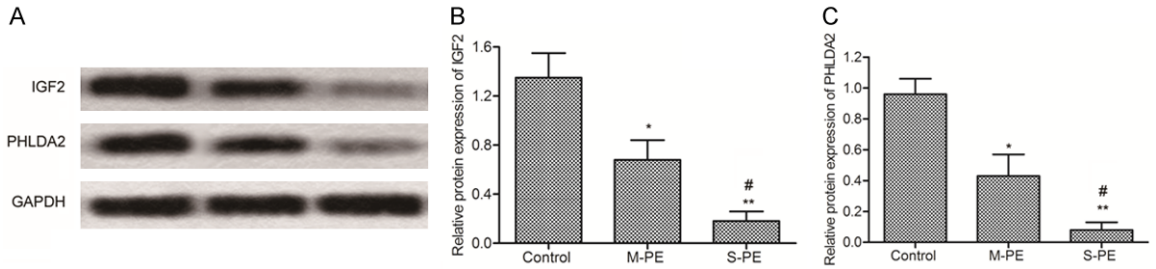

Figure 6. Protein expression levels of IGF2 and PHLDA2 in serum. (A) Expression of IGF2 and PHLDA2 was analyzed by western blotting. Relative protein expression of IGF2 (B) and PHLDA2 (C) was quantified using Image-Pro Plus 6.0 software and normalized to GAPDH; Data are represented as the mean ± SD of three experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ verusus control group, $^{\#}P < 0.05$ versus M-PE group.

Level of IGF2 and PHLDA2 in serum

To investigate the expression level of IGF2 and PHLDA2 in serum, ELISA was performed to assess the concentration of those in serum. The M-PE and S-PE patients showed a decrease of concentration levels of IGF2 and PHLDA2 ($P < 0.05$), as shown in **Figure 4**.

The mRNA level of IGF2 and PHLDA2 in serum

The PHLDA2 expression levels in the serum of the control women and PE patients were analyzed by RT-PCR. Clearly, the level of IGF2 and PHLDA2 were obviously down-regulated in PE patients, compared with that in the control group (**Figure 5**, $P < 0.05$). Furthermore, the two imprinted genes, IGF2 and PHLDA2, had the least mRNA expression in S-PE group (**Figure 5**, $P < 0.01$).

Protein expression level of IGF2 and PHLDA2 in serum

At the same time, protein was extracted from the serum. Similar results were obtained for protein expression. These results were confirmed using western analysis (**Figure 6**). It showed that proteins of IGF2 and PHLDA2 were significantly reduced in M-PE and S-PE groups, compared with the control group ($P < 0.05$). We found that when compared to M-PE group, protein expression of IGF2 and PHLDA was much fewer in S-PE. Those results were similar with RT-PCR analysis in **Figure 5**.

Discussion

PE is the most common gestational complication and the patients show several typical clinical symptoms. It affects approximately 2% of pregnant women worldwide and responsible for more than 50,000 maternal deaths annually [16, 17]. PE is the leading cause of maternal and perinatal morbidity and mortality, particularly when it occurs before the 34th week of gestation [18, 19]. In addition, it expressly initiates intrauterine placental abruption, iatrogenic prematurity, growth restriction, and stillbirth of the child. Because we unable to predict PE by previous obstetric history and risk factors,

more and more research has focused on the identification of women at high risk of developing PE [20, 21]. This maybe allow more intensive monitoring of this high risk group as well as targeted prophylactic intervention, timely diagnosis and treatment [22]. The identification of PE biomarkers in early pregnancy would capacitate appropriate stratification of a pregnancy into high and low risk, such that a positive predictive test would allow specific therapeutic interventions [22].

This study was set up to identify novel potential PE markers, to subsequently be tested in biomarker discovery approaches. Many researchers have found some candidate biomarkers [23, 24]. The finding that these markers are also identified by their approach demonstrates that the approach has the potential to identify other promising biomarkers as well.

Imprinted genes have been demonstrated to regulate the endocrine lineage of the mouse placenta, in particular expression of placental lactogens [25-27]. IGF2, an imprinted gene and peptide hormones, mediates a variety of metabolic and mitogenic effects on the surface of target tissues and cells. Several studies have shown that IGF2 is expressed in many tissues and regulates the proliferation and survival of a variety of tissues [28]. In addition, IGF2 stimulates various processes that are involved in the proliferation and apoptosis of first trimester trophoblasts. IGF2 appears to play an important role in EVCT proliferation, and facilitates the nutrient and oxygen supply through placental exchange. Compelling evidence in recent years has also suggested that IGF2 contributes to the regulation of placentation and prenatal and postnatal growth. In our experiments, we focus on the detection of IGF2 mRNA and protein expression in serum and placenta, which were collected from PE patients and healthy pregnant women. As described in the results section, placental IGF2 expression level was remarkably fell in PE patients, at RNA level and protein level. We subsequently analysed imprinted gene IGF2 in serum. It is obvious that IGF2 from PE women were also inhibited, compared with control group. The IGF2 expression was inversely proportional to the severity of PE.

PHLDA2 is another maternally expressed imprinted gene that participates in the early growth and development of the placenta [14]. In this study, we explored PHLDA2 expression levels in serum and in placenta from PE patients. There was a significant anomaly. As shown in **Figures 1-3**, placental PHLDA2 expression level was all markably fell in M-PE and S-PE patients, at RNA level and protein level. And these results were similar to serum results. We subsequently analysed imprinted gene PHLDA2 in serum. It is obvious that expression of PHLDA2 from PE women also were inhibited, compared with healthy pregnant women. The imprinted gene PHLDA2 expression was inversely proportional to the severity of PE. A number of studies have demonstrated abnormally changed placental PHLDA2 in pregnancies. As reported that it's upregulated expression inhibits placental growth and increases the risk of low birth weights in infants [14, 29]. There was a significant change of PHLDA2 expression and maternal serum PHLDA2 level in our study. PHLDA2 expression was significantly associated with placental weight. This is of clinical interest as RFM is thought to represent a fetal adaptation to prolonged placental insufficiency which, if undetected, may result in still birth [30]. So detecting PHLDA2 expression is significative for pregnant women.

To summarize, we have used integrative data to identify two changed imprinted genes in serum from PE. It's just a preliminary investigation for IGF2 and PHLDA2 in serum of PE. These data are the first to demonstrate imprinted genes IGF2 and PHLDA2 associated with PE in mid-pregnancy. It illustrates that IGF2 and PHLDA2 may participate in PE pathogenesis and indicate its potential application in the early diagnosis of PE. The mechanism of these two imprinted genes and whether they can be as potential markers on PE need further study in the future. These are necessary to further determine how these markers interrelate and whether sufficiently reliable prediction accuracy can be obtained before a large-scale PE screening program can be introduced.

Disclosure of conflict of interest

None.

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References

[1] Redman CW and Sargent IL. Latest advances in understanding preeclampsia. Science 2005; 308: 1592-1594.

[2] Williamson RD, O'Keeffe GW and Kenny LC. Activin signalling and pre-eclampsia: from genetic risk to pre-symptomatic biomarker. Cytokine 2015; 71: 360-365.

[3] Mary S, Kulkarni MJ, Malakar D, Joshi SR, Mehendale SS and Giri AP. Placental proteomics provides insights into pathophysiology of pre-eclampsia and predicts possible markers in plasma. J Proteome Res 2017; 16: 1050-1060.

[4] Herzog EM, Eggink AJ, Reijnierse A, Kerkhof MA, de Krijger RR, Roks AJ, Reiss IK, Nigg AL, Eilers PH, Steegers EA and Steegers-Theunissen RP. Impact of early- and late-onset pre-eclampsia on features of placental and newborn vascular health. Placenta 2017; 49: 72-79.

[5] Kwiatkowski S, Dolegowska B, Kwiatkowska E, Rzepka R, Marczuk N, Loj B and Torbe A. Maternal endothelial damage as a disorder shared by early preeclampsia, late preeclampsia and intrauterine growth restriction. J Perinat Med 2016; [Epub ahead of print].

[6] Tallarek AC and Stepan H. [Preeclampsia and HELLP syndrome as an obstetric emergency]. Med Klin Intensivmed Notfmed 2012; 107: 96-100.

[7] Silasi M, Cohen B, Karumanchi SA and Rana S. Abnormal placentation, angiogenic factors, and the pathogenesis of preeclampsia. Obstet Gynecol Clin North Am 2010; 37: 239-253.

[8] Moore GE, Ishida M, Demetriou C, Al-Olabi L, Leon LJ, Thomas AC, Abu-Amero S, Frost JM, Stafford JL, Chaoqun Y, Duncan AJ, Baigel R, Brimioulle M, Iglesias-Platas I, Apostolidou S, Aggarwal R, Whittaker JC, Syngelaki A, Nicolaides KH, Regan L, Monk D, Stanier P. The role and interaction of imprinted genes in human fetal growth. Philos Trans R Soc Lond B Biol Sci 2015; 370: 20140074.

[9] Kusinski L C CWN, Sandovici I, Constância M. Contribution of placental genomic imprinting and identification of imprinted genes. The Guide to Investigation of Mouse Pregnancy 2013; 275.

[10] Weksberg R, Smith AC, Squire J and Sadowski P. Beckwith-Wiedemann syndrome demonstrates a role for epigenetic control of normal development. Hum Mol Genet 2003; 12: R61-68.

[11] Angiolini E, Fowden A, Coan P, Sandovici I, Smith P, Dean W, Burton G, Tycko B, Reik W, Sibley C and Constancia M. Regulation of placental efficiency for nutrient transport by imprinted genes. Placenta 2006; 27 Suppl A: S98-102.

[12] McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, Weksberg R, Thaker HM and Tycko B. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 2006; 27: 540-549.

[13] Salas M, John R, Saxena A, Barton S, Frank D, Fitzpatrick G, Higgins MJ and Tycko B. Placental growth retardation due to loss of imprinting of Phlda2. Mech Dev 2004; 121: 1199-1210.

[14] Tunster SJ, Creeth HD and John RM. The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. Dev Biol 2016; 409: 251-260.

[15] Tybl E, Shi FD, Kessler SM, Tierling S, Walter J, Bohle RM, Wieland S, Zhang J, Tan EM and Kiemer AK. Overexpression of the IGF2-mRNA binding protein p62 in transgenic mice induces a steatotic phenotype. J Hepatol 2011; 54: 994-1001.

[16] Abalos E, Cuesta C, Grosso AL, Chou D and Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol 2013; 170: 1-7.

[17] Bhorat I, Naidoo DP and Moodley J. Maternal cardiac haemodynamics in severe pre-eclampsia complicated by acute pulmonary oedema: a review. J Matern Fetal Neonatal Med 2016; 1-9. [Epub ahead of print].

[18] Hogberg U. The World Health Report 2005: "make every mother and child count"-including Africans. Scand J Public Health 2005; 33: 409-411.

[19] Eastwood KA, Patterson C, Hunter AJ, McCance DR, Young IS and Holmes VA. Evaluation of the predictive value of placental vascularisation indices derived from 3-Dimensional power Doppler whole placental volume scanning for prediction of pre-eclampsia: a systematic review and meta-analysis. Placenta 2017; 51: 89-97.

[20] Kell DB and Kenny LC. A dormant microbial component in the development of preeclampsia. Front Med (Lausanne) 2016; 3: 60.

[21] Norwitz ER BMA, Saade GR, Miller H. Obstetric clinical algorithms: management and evidence. John Wiley & Sons; 2011.

[22] Phipps E, Prasanna D, Brima W and Jim B. Preeclampsia: updates in pathogenesis, definitions, and guidelines. Clin J Am Soc Nephrol 2016; 11: 1102-1113.

[23] Pennings JL, Kuc S, Rodenburg W, Koster MP, Schielen PC and de Vries A. Integrative data mining to identify novel candidate serum biomarkers for pre-eclampsia screening. Prenat Diagn 2011; 31: 1153-1159.

[24] Blankley RT, Fisher C, Westwood M, North R, Baker PN, Walker MJ, Williamson A, Whetton AD, Lin W, McCowan L, Roberts CT, Cooper GJ, Unwin RD and Myers JE. A label-free selected reaction monitoring workflow identifies a subset of pregnancy specific glycoproteins as potential predictive markers of early-onset preeclampsia. Mol Cell Proteomics 2013; 12: 3148-3159.

[25] Koukoura O, Sifakis S, Spandidos DA. DNA methylation in the human placenta and fetal growth (review). Mol Med Rep 2012; 5: 883-889.

[26] Tunster S, Jensen AB, John RM. Imprinted genes in mouse placental development and the regulation of fetal energy stores. Reproduction 2013; 145: R117-R137.

[27] Patten MM, Cowley M, Oakey RJ, Feil R. Regulatory links between imprinted genes: evolutionary predictions and consequences. Proc Biol Sci 2016; 283.

[28] De Nunzio C, Aronson W, Freedland SJ, Giovannucci E and Parsons JK. The correlation between metabolic syndrome and prostatic diseases. Eur Urol 2012; 61: 560-570.

[29] Janssen AB, Tunster SJ, Heazell AE and John RM. Placental PHLDA2 expression is increased in cases of fetal growth restriction following reduced fetal movements. BMC Med Genet 2016; 17: 17.

[30] Warrander LK and Heazell AE. Identifying placental dysfunction in women with reduced fetal movements can be used to predict patients at increased risk of pregnancy complications. Med Hypotheses 2011; 76: 17-20.