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Screening for preeclampsia pathogenesis related genes

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Abstract. – OBJECTIVES: Preeclampsia is a complication of pregnancy that severely threatens the health of the mother and infant, yet the mechanism of pathogenesis remains unclear. In this article, gene array technology was applied to identify the genes related to the pathogenesis of preeclampsia, and to explore the regulatory effect of epigenetic modification by on these genes.

PATIENTS AND METHODS: Placental tissue of preeclampsia patients was collected, and DNA methylation arrays and gene expression microarrays were used to identify the genes. The effect of methylation on the regulation of genes related to the pathogenesis of preeclampsia was also investigated.

RESULTS: The expression levels of more than ten genes were found to be significantly altered in the placental tissue of patients with preeclampsia as measured by gene expression microarray. This study also identified more than ten genes with notable changes in expression level as well as methylation level. The gene expression of CUEDC1 and DHX34 were verified in this study and the findings were consistent with previous reports.

CONCLUSIONS: Our research indicates that the occurrence of preeclampsia is correlates closely with differences in the expression of specific genes, which may be regulated through methylation.

Key Words:

Preeclampsia, Pathological mechanism, Hypertension in pregnancy, Gene chip, Methylation, Epigenetics.

Introduction

Preeclampsia is a systemic syndrome that affects approximately 3-5% of pregnancies globally. Characterized by the major symptoms hypertension and proteinuria, this complication arises primarily during the second half of pregnancy[1]. While several studies increasing our understanding of the pathophysiology of preeclampsia have been reported in recent years, the only definitive treatments for this condition are induced delivery or caesarean section. As of 2010, preeclampsia remains the leading cause of maternal mortality, preterm birth, and consequent neonatal morbidity and mortality[2]. Hypertensive disorders of pregnancy are estimated to account for 16% of maternal deaths in developed countries, and 9% of maternal deaths in Africa and Asia[3]. Preeclampsia is associated with a perinatal and neonatal mortality rate of 10% worldwide[4], and up to 25% of preeclampsia cases lead to fetal growth restriction[5,6].

The mechanism of pathogenesis involved in preeclampsia remains elusive, making it difficult to design effective treatments and preventative measures. It is generally acknowledged that decreased invasive ability of trophoblasts leads to shallow implantation and plays a central role in the pathogenesis of preeclampsia[7,8]. A variety of genetic and environmental factors could promote or inhibit the invasion of trophoblast cells[9].

Methylation is a fundamental epigenetic modification that, in addition to alterations in chromatin structure, regulates gene expression. Overall DNA methylation levels change in a tissue- and differentiation-dependent manner. Abnormal methylation patterns could, therefore, disturb cell differentiation and induce abnormal gene expression patterns. It has been shown that such anomalies play a major role in the development of cancer. Considering the relationship between methylation of invasion-suppressor genes and invasiveness of trophoblasts[10,11], genetic predisposition of preeclampsia[12-14] and the importance of DNA methylation in embryonic development, we hypothesized that abnormal DNA methylation in trophoblasts alters their invasive ability, and that preeclampsia develops as a consequence of DNA hypomethylation or demethylation of key invasion-suppressor genes. In this study, we used

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MeDIP-chip and gene expression microarray technology to identify genes related to the pathogenesis of preeclampsia. Additionally, we explored the methylation profile of specific genes and how this relates to modulation in expression levels in the context of preeclampsia.

Patients and Methods

Patients

Placental tissues were collected from 30 patients with preeclampsia who delivered in our Hospital from June 2009 to December 2010. The control group included 30 pregnant women who had operative delivery in our Hospital due to social factors or pelvic abnormalities. Patients of both groups were all in the third trimester of pregnancy. Any women with other pregnancy complications, multifestation, infectious diseases, drug addiction, congenital deformities of the fetus or a history of smoking were excluded. The gestational week of the patients was verified by B-ultrasound in the first trimester pregnancy.

Methods

Collection of Placental Tissue

The placentas of the patients, post-caesarean section were kept in sterile conditions, and a tissue sample (0.5 cm × 0.5 cm × 0.5 cm) was cut from the maternal surface of each placenta. The sections were rinsed several times in stroke-physiological saline solution and frozen immediately in liquid nitrogen for future use.

Extraction of Nucleic Acids

Extraction of RNA

The TRIzol method was used to extract RNA from 50 mg of frozen placental tissue. 100 µl of DEPC water was added to the sample, which was then centrifuged at maximum speed for 20-30 s, and then stored at –20°C.

Extraction and Purification of DNA

The proteinase K method was used to extract DNA from 30 mg of frozen placental tissue, The final DNA sample was suspended in TE pH 8.0 and stored at –20°C. Methylated DNA products were enriched, and amplified using the Sigma Aldrich (St Louis, MO, USA) WGA2 kit. The amplified DNA was processed with the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany). Samples were marked according to the Agilent protocol, and were hybridized by applying the SBC array. Qualification assessment was performed on the sample data.

Gene Selection with DNA Methylation Array and Gene Expression Microarray

Samples were obtained from 5 cases of severe preeclampsia and 5 control cases for gene selection. DNA methylation array and gene expression microarray were used to select the target genes. The gene expression array was obtained from Bohao Biotech Co., Ltd. (Shanghai, China). The methylation assay was done using Agilent Human CpG Island Microarray (Santa Clara, CA, USA) consisting of 27800 CpG islands.

Verification of Gene Expression

Quantitative PCR was used to verify test of gene expression in placental tissue. Primers used were: CUEDC1 Exon, CUEDC1mF GGCCTGCGAGAAGGACAGTAAGA, CUEDC1mR TCGGTTCTCAAGCACCATCTGTC; CUEDC1 mRNA, CUEDC1mF GGCCTGCGAGAAGGACAGTAAGA, CUEDC1mR TCGGTTCTCAAGCACCATCTGTC; DXH34 Exon, DHX34mF AGCTTCGTGTCCCTGCTGGA, DHX34mR AGGCGGGAGCAGTCACCATT; DXH34 mRNA, DHX34mF AGCTTCGTGTCCCTGCTGGA, DHX34mR AGGCGGGAGCAGTCACCATT; GAPDH mRNA, GAPDHmF TTTGGTATCGTGGAAGGACTCATGAC, GAPDHmR GGCAGGGATGATGTTCTGGAGAG.

Statistical Analysis

All data are expressed as the mean ± SEM and were analyzed by SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) using the Student's *t*-test for the comparison of mean values among groups. $p < 0.05$ was to be significant.

Results

Statistical Analysis of Patients' Clinical Index

Data on maternal age, days of pregnancy, systolic and diastolic blood pressure, sex and weight of infants were recorded for the preeclampsia and normal control pregnancy groups, with no statis-

tically significant differences in all parameters between the two groups except blood pressure and infant birth weight (Table I).

Genes Selected by Gene Expression Microarray

More than 1000 genes were screened for differential expression. In the experimental group, there were 15 genes which were found to be up-regulated by 3-fold (Table II), and 14 genes which were down-regulated by 3-fold in the preeclampsia group (Table III).

Genes Selected by DNA Methylation Array and Gene Expression Microarray

Altered methylation levels were detected in specific genes in the preeclampsia group (Table IV). The information related to each gene identified is shown in Tables V and VI.

Verification Test of Target Gene Expression

By using quantitative PCR, we verified the expression of CUEDC1 and DHX34 and found that the expression of CUEDC1 in the preeclampsia group was remarkably lower than in the normal pregnancy group (the relative expression in preeclampsia group is 0.616 ± 0.155, and 1.196 ± 0.294 in the control group, $p < 0.01$). The expression of DHX34 was higher in the preeclampsia group than in the normal pregnancy group (relative expression level in preeclampsia group is 1.805 ± 0.165, in control group is 1.004 ± 0.187, $p < 0.05$).

Discussion

Preeclampsia, is a hypertensive complication of pregnancy that appears to originate in the placenta and leads to widespread maternal endothelial dysfunction. To date, the molecular etiology of this condition had yet to be elucidated. Environmental toxins, impaired immunity, ischemia of the placenta, defective trophoblast activity, oxidative stress, and genetic factors are all considered to play a potential role in causing preeclampsia. Though all these factors may contribute to the pathogenesis of preeclampsia, evidence from various studies suggests that defective uterine spiral artery remodeling, resulting as a consequence of impaired trophoblast invasion, is the central mechanism involved in preeclampsia. Systemic vascular reaction is hypothesized to be secondary to placental reperfusion injury. In

Table I. Clinical index analysis between two groups.

Group	n	Age (years)	Days of pregnancy	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Sex of infant		Weight of infant (g)
						Male	Female	
Preeclampsia group	30	28. 5 ± 3.8	252. 9 ± 16. 3	151 ± 17. 5	95 ± 8. 6	17	13	2782.5 ± 715.3
Control group	30	27. 9 ± 3. 0	274. 4 ± 5. 8	102 ± 9. 4	72 ± 6. 2	16	14	3301.3 ± 305.0
p	—	0. 14	0. 15	< 0. 001	< 0. 001	0. 75	0. 006	

Table II. Genes up-regulated in preeclampsia.

Gene	Cytoband	Fold difference	*p*	Description
LAIR2	19q13.42	9.661357	0.014612	Homo sapiens leukocyte-associated immunoglobulin-like receptor 2 (LAIR2), transcript variant 1, mRNA [NM_002288]
CXCL1	4q13.3	8.316068	0.008167	Homo sapiens chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (CXCL1), mRNA [NM_001511]
LAMA3	18q11.2	6.5261064	0.02515	Homo sapiens laminin, alpha 3 (LAMA3), transcript variant 1, mRNA [NM_198129]
LYZL1	10p11.23	6.511507	0.003357	Homo sapiens lysozyme-like 1 (LYZL1), mRNA [NM_032517]
LY6K	8q24.3	5.8179126	0.018032	Homo sapiens lymphocyte antigen 6 complex, locus K (LY6K), transcript variant 1, mRNA [NM_017527]
MIG7	1p22.1	5.5469675	0.039932	Homo sapiens MIG7 (MIG7) mRNA, complete cds. [DQ080207]
CXCL2	4q13.3	4.992991	0.00449	Homo sapiens chemokine (C-X-C motif) ligand 2 (CXCL2), mRNA [NM_002089]
MADCAM1	19p13.3	4.546288	0.033039	Homo sapiens mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), transcript variant 1, mRNA [NM_130760]
INGX	Xq13.1	4.4690957	0.025899	Homo sapiens inhibitor of growth family, X-linked, pseudogene (INGX), non-coding RNA [NR_002226]
TXNDC6	3q22.3	4.3019214	0.007606	Homo sapiens thioredoxin domain containing 6 (TXNDC6), mRNA [NM_178130]
SGSM1	22q11.23	3.8367445	0.02992	Homo sapiens small G protein signaling modulator 1 (SGSM1), transcript variant 1, mRNA [NM_001039948]
OR51G1	11p15.4	3.8262496	0.039955	Homo sapiens olfactory receptor, family 51, subfamily G, member 1 (OR51G1), mRNA [NM_001005237]
RNASE11	14q11.2	3.758421	0.001691	Homo sapiens ribonuclease, RNase A family, 11 (non-active) (RNASE11), mRNA [NM_145250]
C6orf105	6p24.1	3.109747	0.013667	Homo sapiens chromosome 6 open reading frame 105 (C6orf105), transcript variant 2, mRNA [NM_032744]
DNAJC5G	2p23.3	3.0743237	0.016955	Homo sapiens DnaJ (Hsp40) homolog, subfamily C, member 5 gamma (DNAJC5G), mRNA [NM_173650]

preeclampsia, cytotrophoblast invasion of the arteries is restricted to the superficial decidua, and the arteries of the myometrial segments remain constricted and undilated.

In our research, we used gene expression microarray technology and found differentially expressed genes in the placentas of preeclampsia patients as compared to the placentas of normal women. Here we report what is currently known about these genes' functions in order to provide new directions for further study of preeclampsia pathogenesis.

One of the genes found to be overexpressed in preeclampsia placentas, LAIR-2 (leukocyte-associated immunoglobulin-like receptor 2), encodes a trophoblastic protein that may regulate trophoblast invasion by regulating interactions between the cell and the collagen matrix. LAIR-2 has been shown to localize specifically at the anterior border of extravillus trophoblast (EVT) anchoring cell columns in first trimester placentas, which is also the site of maximal trophoblast invasion[15]. It is possible that increased expression of this gene plays a role in the improper regulation of trophoblast invasion during preeclampsia. Another gene we found up-regulated in preeclampsia is DNAJC5G, which encodes a type of heat-shock protein known to have neuroprotective effects. Increased DNAJC5G expression can be induced by alcohol and isoflurane in the human body[16,17]. We observed increased expression of LAMA3, a gene encod-

Table III. Genes down-regulated in preeclampsia.

Gene	Cytoband	Fold difference	p	Description
SSTR1	14q21.1	7.732945	0.031792	Homo sapiens somatostatin receptor 1 (SSTR1), mRNA [NM_001049]
SYT6	1p13.2	6.788247	0.04211	Homo sapiens synaptotagmin VI (SYT6), mRNA [NM_205848]
LEMD1	1q32.1	4.818365	0.015775	Homo sapiens LEM domain containing 1 (LEMD1), mRNA [NM_001001552]
LOC641518	4q25	4.115658	0.002135	Homo sapiens hypothetical LOC641518 (LOC641518), transcript variant 1, non-coding RNA [NR_029373]
TPSAB1	16p13.3	3.787169	0.020223	Homo sapiens tryptase alpha/beta 1 (TPSAB1), mRNA [NM_003294]
ANKS1B	12q23.1	3.722327	0.023601	Homo sapiens ankyrin repeat and sterile alpha motif domain containing 1B (ANKS1B), transcript variant 2, mRNA [NM_181670]
HCG26	6p21.33	3.715303	0.03795	Homo sapiens HLA complex group 26 (non-protein coding) (HCG26), non-coding RNA [NR_002812]
MXRA5	Xp22.33	3.608944	0.034053	Homo sapiens matrix-remodelling associated 5 (MXRA5), mRNA [NM_015419]
TRIM31	6p22.1	3.561457	0.019691	Homo sapiens tripartite motif-containing 31 (TRIM31), mRNA [NM_007028]
PRIMA1	14q32.12	3.509333	0.036097	Homo sapiens proline rich membrane anchor 1 (PRIMA1), mRNA [NM_178013]
GTSF1	12q13.13	3.396331	0.011842	Homo sapiens gametocyte specific factor 1 (GTSF1), mRNA [NM_144594]
RIPPLY1	Xq22.3	3.333856	0.031402	Homo sapiens ripply1 homolog (zebrafish) (RIPPLY1), transcript variant 1, mRNA [NM_138382]
BTC	4q13.3	3.197099	0.027275	Homo sapiens betacellulin (BTC), mRNA [NM_001729]
SLAMF1	1q23.3	3.009183	0.027271	Homo sapiens signaling lymphocytic activation molecule family member 1 (SLAMF1), mRNA [NM_003037]

ing alpha 3 chain of laminin 5. Laminins are involved in regulating adhesion, motility, gene expression, and apoptosis. It was previously found that LAMA3 is important for repairing endothelial damage[18]. Ly6k, a member of the Ly-6 family, was also increased in expression. Ly6k was reported to be expressed in the testis[19,20], plasma cells, and in some types of cancer[21,22]. Augmented LY6K expression has been found to correlate with increased incidence of highly malignant forms of lung cancer and esophageal squamous cell carcinoma[20]. CXCL2, responsible for monocyte/ macrophage chemoattraction and activation, was increased in preeclampsia placental tissue as well. A recent study found that IL-1 significantly up-regulates mRNA and protein expression of CXCL2 in first trimester decidual cells[23]. Our study identified overexpression of lysozyme-like protein 1 (LYZL1), a member of the family of c-type lysozymes that is expressed in the human male reproductive tract[24].

Mucosal addressin cell adhesion molecule 1 (MADCAM-1) is predominantly expressed in endothelial venules of inflamed tissues where it assists with leucocyte extravasation. It has been reported that MADCAM-1 could be involved in the regulation of peripheral host responses, leukocyte migration across non-endothelial boundaries, and in homotypic alpha interactions in melanoma[25]. This gene was also increased in expression in our study. Increased OR51G1 and RNase 11 transcript levels were detected in the placentas or preeclampsia patients. OR51G1 is a member of the family of olfactory receptors (ORs) expressed in the nose. While ORs have several different protein sequences, they can be delegated to subfamilies based on certain sequence homologies and members of the same subfamily likely recognize structurally related odorants[26]. RNase 11 is a member of the RNase A superfamily. The functions of RNases 11-13 are still not clearly known. Human RNase 11 is most abundantly expressed

Table IV. Differentially expressed genes are identified in placental tissue of patients with preeclampsia by both gene microarray and methylation array.

Gene	Preeclampsia vs control fold difference	Preeclampsia vs control regulation	Control methylation	Preeclampsia methylation
TRIM2	1.4213927	Up	LOW	HIGH
ZNF512	1.1638403	Up	LOW	HIGH
ZNF512	1.1638403	Up	LOW	HIGH
PCYOX1L	1.3794929	Up	LOW	HIGH
LOC339524	1.6510698	Up	LOW	HIGH
TMEM185A	1.2519797	Up	LOW	HIGH
PPAP2B	1.3628085	Up	LOW	HIGH
TRIM2	1.3420411	Up	LOW	HIGH
ERICH1	1.347795	Up	LOW	HIGH
ACCN2	1.2265906	Up	LOW	HIGH
CUEDC1	1.6871873	Up	LOW	HIGH
VN1R1	1.378909	Up	LOW	HIGH
TINAG	1.614305	Down	HIGH	LOW
OR8B8	1.3904985	Down	HIGH	LOW
DMWD	1.6649054	Down	HIGH	LOW
ENHO	1.2280575	Down	HIGH	LOW
GPR37L1	1.6395358	Down	HIGH	LOW
NDST2	1.1029271	Down	HIGH	LOW
HMGCR	1.1891443	Down	HIGH	LOW
DHX34	1.6294718	Down	HIGH	LOW
3-Sep	2.3169706	Down	HIGH	LOW
MSI2	1.4369086	Down	HIGH	LOW
ALDH1B1	1.4111286	Down	HIGH	LOW

in the testis as compared to other tissues, suggesting a potential role in male reproductive organs[27].

SSTR1, found to be down-regulated in preeclampsia placentas in this study, is a somatostatin receptor that is activated by somatostatin and exerts downstream antiproliferative effects in many types of epithelial and endocrine cells[28]. Synaptotagmin VI, the product of the gene SYT6 down-regulated in preeclampsia, is a major component of the secretory apparatus involved in acrosomal exocytosis[29,30]. LAP2, Emerin, and MAN (LEM) domain-containing 1 (LEM-D1) is a nuclear lamina protein[31] thought to act as a bridging protein involved in nuclear organization[32]. TPSAB1, involved in both host defense and reproductive functions, was decreased in preeclampsia placentas[33]. Expression of the Ankyrin Repeat and Sterile Alpha Motif Domain-Containing Protein 1B (ANKS1B), a tyrosine kinase signal transduction gene mainly expressed in the brain, was also reduced compared to normal placental levels. Containing two SAM domains and one PTB (phosphotyrosine binding) domain, this protein has been demonstrated to regulate cell proliferation and differentiation[34].

From our data, three other genes whose expression was suppressed in preeclampsia include GTSF1, RIPPLY1 and BTC. GTSF1 is highly expressed in embryonic male and female gonads, and may play a critical role in germ cell development. Further investigation into the functions of GTSF1 may shed light on the pathophysiology of premature ovarian failure[35]. RIPPLY1 codes for a nuclear protein that binds to the transcriptional repressor Groucho and is necessary for proper transcriptional termination of genes involved in somite segmentation[36]. BTC is a member of the EGF family made by endothelial cells. This endothelial cell-derived factor promotes neural stem cell proliferation and neurogenesis[37]. BTC is known to play an important role in regulating the growth and differentiation of pancreatic beta cells, and can trigger proliferation of retinal pigment epithelial cells[38].

There is very little data on the functions of the genes C6orf105, INGX, SGSM 1, TXNDC6, LOC641518, HCG26, MXRA5, TRIM31, PRIMA1, and SLAMF1.

Many studies have shown that abnormal gene expression is related to various trophoblast invasion disorders. However, few studies have been done to determine the causes of differences in

Table V. Information on genes hypermethylated in preeclampsia.

Gene	Previous GC identifiers	RefSeq DNA sequence	Entrez gene cytogenetic band	Unigene	Preeclampsia vs control fold difference	Description
TRIM2 (tripartite motif containing 2)	23321	NM_001130067.1 NM_015271.3	4q31.3	NM_001130067	1.4213927	Member of the tripartite motif (TRIM) family, localizes to cytoplasmic filaments. Function unidentified, may contribute to modulation of neural cellular mechanisms.
ZNF512 (Zinc finger protein 512)	GC02P027780 GC02P027717 GC02P027659 GC02P027547	NC_000002.11 NT_022184.15	2p23	NM_032434	1.1638403	May be involved in transcriptional regulation
PCYOX1L (Prenylcysteine oxidase 1 like)	GC05P143883	NC_000005.9 NT_029289.11	5q32	NM_024028	1.3794929	Putative oxidoreductase.
LOC339524 (hypothetical LOC339524)	GC00U906948 GC01P086887 GC01U900453 GC01P087020 GC01P087309 GC01P085709	NC_000001.10 NT_032977.9	1p22.3	BC041867	1.6510698	_
TMEM185A (transmembrane protein 185A)	GC0XM148487 GC0XM137631	NC_000023.10 NT_011681.16	Xq28	AK128688	1.2519797	Predicted transmembrane protein. Shown to localize to the High CpG island of the fragile site FRAXF.
PPAP2 (phosphatidic acid phosphatase type 2B)	GC01M055877 GC01M056317 GC01M056329 GC01M056330 GC01M056671 GC01M056733 GC01M055072	NC_000001.10 NT_032977.9	1p32.2	NM_003713	1.3628085	Member of the phosphatidic acid phosphatase (PAP) family. May be involved in cell adhesion and cell-cell interactions
ERICH1 glutamate-rich 1	GC08M000605 GC08M000614 GC08M000454	NC_000008.10 NT_023736.17	8p23.3	BX647093	1.347795	—

Table continued

Table V *(Continued)*. Information on genes hypermethylated in preeclampsia.

Gene	Previous GC identifiers	RefSeq DNA sequence	Entrez gene cytogenetic band	Unigene	Preeclampsia vs control fold difference	Description
ACCN2 (amiloride-sensitive cation , channel 2 neuronal)	GC12P050746 GC12P050507 GC12P050168 GC12P048737 GC12P047485	NC_000012.11 NT_029419.12	12q12	NM_020039	1.2265906	Member of the degenerin/epithelial sodium channel (DEG/ENaC) superfamily. A cation channel with high affinity for Na+, also permeable to Ca2+, Li+ and K+. Functions as a postsynaptic proton receptor that influences intracellular Ca2+ concentration and calmodulin-dependent protein kinase II phosphorylation, thereby modulating dendritic spine density.
CUEDC1 (CUE domain containing 1)	GC17M056415 GC17M053295 GC17M051300	NC_000017.10 NT_010783.15	17q23.2	CR627470	1.6871873	–
VN1R1 (vomeronasal 1 receptor 1)	GC19M058408 GC19M062642 GC19M062658 GC19M054279	NC_000019.9 NT_011109.16	19q13.4	AK094452	1.378909	Similar to pheromone receptors and primarily localized to the olfactory mucosa.

gene expression. In recent years, the relationship between epigenetics and human disease has gained more attention and it has been found that epigenetic regulation plays an important role in tumor cell invasion. We propose that the regulatory mechanism of trophoblast invasion may be similarly be affected by epigenetic modulation. In this study, we used DNA methylation microarray combined with gene expression microarray technology to co-screen different genes and used the genes CUEDC1 and DHX34 to validate the findings. Both of these genes are functionally related to tumor invasiveness.

CUEDC1 (CUE domain-containing protein 1) may have a role in protein trafficking and degradation[39]. Defects in this gene are associated with early stage cervical cancer, implicating CUEDC1 as a putative tumor marker[40]. Li et al[41] reported that CUEDC1 is highly conserved across species and the human, mouse and rat sequences are similar and have the same CUE domain. Using a double-fluorescence reporting system, they found that over-expression of human-CUEDC1 could inhibit the TGF-β/Smad signaling pathway in mouse embryonic fibroblasts. TGF-β is the first factor, which has been demonstrated to regulate trophoblast invasion, and the TGF-β/Smad signaling pathway is one of major mechanisms regulating trophoblast invasion[40]. In our study, we found that CUEDC1 has a high methylation level in the placental tissue of preeclampsia patients in the third trimester. CUEDC1 RNA expression exhibited a downward trend, suggesting that its reduced expression in the placental tissue may be involved in the pathogenesis of preeclampsia, and that this differential expression may result from epigenetic regulation. Based on these findings, we hypothesize that CUEDC1 may regulate trophoblast invasion via the TGF-β/Smad signaling pathway. Further research is required to confirm this possibility.

DHX34 encodes an RNA helicase that is a kind of DEAD box protein. DEAD box proteins participate in a number of processes that alter the secondary structure of RNA, such as translation initiation, nuclear and mitochondrial DNA splicing, and ribosome and spliceosome assembly. Based on their localization patterns, some DEAD box proteins are predicted to regulate embryogenesis, spermatogenesis, cellular growth and division. Irregular cell morphology, growth, differentiation, metabolism, etc. could all be associated with abnormal expression of RNA helicase. DHX34 maps to the glioma suppressor region

Table VI. Information on genes hypomethylated in preeclampsia.

Gene type	Previous GC identifiers	RefSeq DNA sequence	Entrez gene cytogenetic band	Unigene	Preeclampsia vs control fold difference	Description
TINAG tubulointerstitial nephritis antigen)	GC06P054175 GC06P054281 GC06P054006	NC_000006.11 NT_007592.15	6p12.1	BC056235	1.614305	Basement membrane glycoprotein initially identified as a target of antibodies in some forms of immunologically mediated tubulointerstitial nephritis. Mediates adhesion of proximal tubule epithelial cells
OR8B8 (olfactory receptor, family 8, subfamily B, member 8)	GC11M126311 GC11M125823 GC11M123848 GC11M123163 GC11M123815	NC_000011.9 NT_033899.8	11q24.2	BC130419	1.3904985	Olfactory receptor; interacts with odorant molecules in the nose, responsible for recognition and G protein-mediated transduction of odorant signals
DMWD (dystrophia myotonica, WD repeat containing)	GC19M046917 GC19M046664 GC19M050962 GC19M050979 GC19M050978 GC19M042714	NC_000019.9 NT_011109.16	19q13.3	NM_004943	1.6649054	Putative regulatory function in meiosis
ENHO (energy associatedhomeostasis)	–	NC_000009.11 NT_008413.18	9p13.3	NM_198573	1.2280575	Involved in regulation of glucose homestasis and lipid metabolism (predicted by similarity)
GPR37L1 (G protein-coupled) receptor 37 like 1	GC01U990381 GC01P199381 GC01P198823 GC01P200358 GC01P173257	NC_000001.10 NT_004487.19	1q32.1	BC050334	1.6395358	Orphan receptor
NDST2 (N-deacetylase/ N-sulfotransferase (heparan) glucosaminyl) 2	GC10M074340 GC10M074665 GC10M075454 GC10M074906 GC10M075231 GC10M069557	NC_000010.10 NT_030059.13	10q22		1.1029271	Member of the N-deacetylase/ N-sulfotransferase subfamily of the sulfotransferase 1 proteins. Plays a role in determining the extent and pattern of sulfation of heparan sulfate
HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase)	GC05P073440 GC05P074869 GC05P074671 GC05P074717 GC05P069838	NC_000005.9 NT_006713.15	5q13.3-q14	NM_000859	1.1891443	Transmembrane glycoprotein l involved in contro of cholesterol biosynthesis
DHX34 (DEAH [Asp-Glu-Ala-His] box polypeptide 34)	GC19P052532 GC19P052547 GC19P052544 GC19P044277	NC_000019.9 NT_011109.16	19q13.3	NM_014681	1.6294718	DEAD box protein characterized by the conserved motif Asp-Glu-Ala-Asp (DEAD), and a putative RNA helicase. Maps to the glioma 19q tumor suppressor region and is a tumor suppressor candidate gene

Table continued

Table VI *(Continued)*. Information on genes hypomethylated in preeclampsia.

Gene type	Previous GC identifiers	RefSeq DNA sequence	Entrez gene cytogenetic band	Unigene	Preeclampsia vs control fold difference	Description
3-Sep (septin 3)	GC22P038987 GC22P040616 GC22P040697 GC22P025339	NC_000022.10 NT_011520.12	22q13.2	NM_019106	2.3169706	Member of the septin family of GTPases. May be a filament-forming cytoskeletal GTPase and play a role in cytokinesis
MSI2 (musashi homolog 2)	GC17P055073 GC17P057815 GC17P055676 GC17P055808 GC17P052688 GC17P050694	NC_000017.10 NT_010783.15	17q22	BX647939	1.4369086	RNA binding protein that regulates the expression of target mRNAs at the translational level. May play a role in proliferation and maintenance of stem cells in the central nervous system
ALDH1B1 (aldehyde dehydrogenase 1 family, member B1)	GC09P038704 GC09P038562 GC09P038382 GC09P038345	NC_000009.11 NT_008413.18	9p11.1	NM_000692	1.4111286	Member of the aldehyde dehydrogenase family of proteins. Plays a major role in detoxification ofalcohol-derived acetaldehyde and is involved in metabolism of corticosteroids, biogenic amines neurotransmitters, and lipid peroxidation

and is a tumor suppressor candidate gene. In our study, DHX34 showed low methylation levels in the placental tissue of preeclampsia patients in third trimester pregnancy as compared to normal placental tissue, while the RNA transcript levels of this gene were increased. Considering the role of DHX34 in tumor cell invasion, it is possible that DHX34 could regulate trophoblast invasion in early placental development, and its improper expression due to epigentic modulation may be responsible for abnormal trophoblast invasion.

Conclusions

Our research shows that the incidence of preeclampsia correlates strongly with specific genetic factors that may be regulated via DNA methylation. Further study on the regulatory mechanisms behind the modulation of these genes will allow exploration of more effective treatments, diagnostic strategies and preventative methods for preeclampsia.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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