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Gene expression profiling of maternal blood in early onset severe preeclampsia: identification of novel biomarkers

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Abstract

Aims: To investigate candidate genes in peripheral blood mononuclear cell (PBMC) that are associated with early onset severe preeclampsia (ES-PE) and to describe candidate genes function using microarrays and real-time polymerase chain reaction (PCR).

Methods: PBMC RNA was extracted from six patients with ES-PE and five uncomplicated pregnancies. The HG_U133 plus 2.0 Affymetrix GeneChips that represented 47,000 genes were used to measure gene expression in each sample. Significance analysis of microarray identified potential signature genes characterizing ES-PE vs. uncomplicated pregnancies. Eight genes were selected for confirmation by real-time PCR of 32 patients with ES-PE and 24 uncomplicated pregnancies, matched for maternal age, parity, race and gestational weeks.

Results: Using a whole-genome approach to study the molecular determinants of ES-PE, 72 genes were found to be differentially expressed between cases and controls, including 38 up-regulated genes and 34 down-regulated genes in the group of ES-PE. Killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2 (KIR3DL2), aldo-keto reductase family 1, member C3 (AKR1C3), churchill domain containing 1 (CHURC1), and solute carrier family 25, member 13 (SLC25A13) were validated to be down-regulated in the patients with ES-PE by real-time PCR.

Conclusions: Expression of genes with diverse function is associated with ES-PE risk, providing opportunities for the development of non-invasive diagnosis.

Keywords: Affymetrix; biomarker; blood; early onset preeclampsia; gene expression profiling; leukemia; microarray; severe preeclampsia.

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Introduction

Preeclampsia (PE) is a common pregnancy-specific syndrome (3–5%) of first pregnancies, and ranks among the leading causes of maternal and perinatal morbidity and mortality (12%) worldwide [7]. According to whether the clinical manifestations occur before or after 32 weeks of gestation, PE was classified into early and late onset type [15]. Large epidemiological studies have demonstrated that women with a history of early onset severe preeclampsia (ES-PE) show an increase risk of cardiovascular disease [2]. The likelihood that PE will recur is almost 50% for ES-PE [4], whereas it is 10%–20% for PE near term [3]. The ES-PE is regarded as more dangerous than late onset severe PE and of a different etiology. Despite the urgent need to diagnose PE early and to have a non-invasive tool for prognosis and treatment monitoring, no predictive test is available to identify pregnant women who will subsequently develop PE and which of those may develop the early or late type of the syndrome. Thus a standardized blood-based test capable of detecting individuals at risk for ES-PE would represent a major advance in clinical care.

Microarray studies of disease have been based on RNA derived from biopsy/tissue samples. The systematic study of the gene expression patterns of placenta revealed differences in gene expression patterns associated with preeclampsia [12]. However, blood samples are less invasive, allow for a larger sample size, and make feasible repeated sampling to monitor disease progression. Several studies have shown that total RNA derived from circulating blood can distinguish between control subjects and patients with various disease types [6]. Fetal DNA or RNA testing of preeclampsia is being investigated however, and their clinical utility is still unclear. In this study, we analyzed blood cell-derived RNA for patterns of differential gene expression of Chinese Han population that could serve as indicators of normal and abnormal biological processes and provide information on mechanisms of ES-PE.

Materials and methods

Subjects and sample acquisition

Informed consent was obtained from each subject in accordance with the protocol approved by the Human Studies Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University. Maternal blood samples from 67 singleton pregnancies (38 ES-PE and 29 uncomplicated pregnancies controls) were collected between September 2006 and October 2008. We randomly selected 6 ES-PE and 5 controls to perform oligonucleotide array. After that, the remaining 32 ES-PE and 24 controls were used as samples to validate gene expression using real-time PCR. ES-PE was defined as new onset hypertension (systolic blood pressure $\geq$160 mm Hg or diastolic blood pressure $\geq$110 mm Hg at least twice 6 h apart) and proteinuria (2 g per 24-h period or 2–4+ on random urine dipstick in the acute setting) after 20 weeks of gestation with severe criteria according to guidelines published by the American College of Obstetricians and Gynecologists in women who required delivery between 24 and 32 gestation weeks because of PE [1]. The control group comprised healthy pregnant women in gestational weeks 24–32 recruited during a routine visit to an antenatal clinic. Only those whose pregnancy continued normally and resulted in a full-term delivery of a healthy child with normal weight were included in the study. Only women with the aforementioned criteria within their first pregnancies were included. Women with a concurrent diagnosis of an upper urinary tract infection, chronic hypertension (hypertension before the 20th week of gestation), diabetes mellitus, or pre-exiting renal disease were not included. None of the control had a family history of PE.

RNA extraction and microarray procedures

Blood samples (8 mL) were drawn into EDTA Vacutainer tubes (Becton Dickinson) by routine venipuncture, put immediately on 4°C) and transferred to the laboratory within 2 h for blood processing. Total RNA was isolated from lymphocytes with Trizol (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The RNA preparations were then DNase treated and purified with the RNeasy Mini Kit (Qiagen, Valencia, CA). RNA quality was assessed on an Agilent 2100 Bioanalyzer RNA 6000 NanoChip (Agilent, Palo Alto, CA), and quantity was determined by absorbance at 260 nm in a Beckman Coulter DU640 spectrophotometer. With 2.5 μg of purified total RNA, samples were prepared for cRNA amplification and hybridization, as previously described [18]. Microarray analysis was performed overnight by using high-density human genome U133 plus 2.0 GeneChips (HG-U133 plus 2.0; Affymetrix, Santa Clara, CA). HG-U133 plus 2.0 GeneChip contains 54,646 25-mer probe sets, represents about 47,000 genes. Subsequently, the chips were scanned with an Affymetrix GeneChip Scanner 3000, and the data were extracted by GeneChip Operating software Version 1.4(GCOS1.4) (Affymetrix Inc.). Signal intensities were centered to the 50th percentile of each chip, and for each individual probe set, to the median intensity of each specific subset first, to minimize possible technical bias, and then to the whole sample set. Only genes identified by the GCOS software as "present" or "marginal" in all samples were analyzed.

Microarray data analysis

Raw data without normalization were analyzed by significance analysis of microarrays (SAM). Normalized data were first filtered to eliminate the genes which were absent in all experimental conditions and replicates. Then, parametric analysis assuming unequal variance was applied to test statistical significance. Fold-ratios were derived by comparing normalized data between controls and preeclampsia. Only genes that are up- or down-regulated more than two-fold were filtered. Genes were deemed statistically different between groups if they had an adjusted $P < 0.05$ and an average fold-change difference of >2. Average-linkage hierarchical clustering analysis using centered correlation analysis and visualization was performed using the CLUSTER and TREEVIEW programs.

Go classification

Gene ontology analysis was performed using an online data base known as the Molecular Analysis System (MAS, http://bioinfo.capitalbio.com/mas/). Molecular functions, biological processes and biological pathways up/down-regulated by "signature" genes of the ES-PE were identified and the statistical significance of up/down-regulation was quantified by a random overlapping P-value using the binomial test with all the genes represented by the Affymetrix®HG-U133 plus 2.0 microarrays as the reference list. From the 38 blood genes over-expressed and 34 blood genes under-expressed in ES-PE, we selected a set of 8 genes to be evaluated by quantitative real-time RT-PCR. We used the following criteria to make our selection: P, fold change, discrimination power form ROC curve analysis between ES-PE patients and the control group, and the function of the gene.

Design of specific primers

Oligonucleotide primers specific for down-regulated genes killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2 (KIR3DL2), aldo-keto reductase family 1, member C3 (AKR1C3), churchill domain containing 1 (CHURC1), solute carrier family 25, member 13 (SLC25A13) and up-regulated genes calponin2 (CNN2), V-set and immunoglobulin domain containing 4 (VSIG4), matrix metallopeptidase 8 (MMP8), proteasome 26S subunit ATPase 5 (PSMC5) isoforms were designed using Primer Express™ Version 2.0 software (PE Applied Biosystems, Inc., Foster City, CA). Beta-actin is chosen as an endogenous control/reference. Sequences of the primer sets used for real-time PCR verification are listed in Table 1.

Validation of gene expression data by quantitative RT-PCR

The remaining 32 ES-PE and 24 controls were enough to perform SYBR Green I real-time PCR assay validation. First-strand cDNA was synthesized from 8 μg of total RNA using the ABI High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) on a Perkin-Elmer (Wellesley, MA) DNA Thermal Cycler according to the manufacturer's protocol. The resulting cDNA was subjected to a 40-cycle PCR amplification followed by real-time PCR reaction using the Power SYBR® Green PCR Master Mix Kit protocol (Applied Biosystems, P/N4367659). Four replicates were run for each gene for each sample in a final volume of 20 μL, and multiple negative water blanks were included in every analysis. Amplification efficiency and specificity of the primer pairs were determined using serial dilution of reference cDNA generated from a normal blood RNA pool with confirmation on agarose gel to ensure that the values

Table 1 Primer pairs used for real-time PCR validation.

Gene	Forward primer	Reverse primer	Amplicon (bp)
AKR1C3	TGTGCCTTGGCAAAAAAGC	GGTAGCGCAGGGCAATCA	54
CHURC1	TGCCTGGAGAATGGATCTTTCT	AAAATCCCGCTTACTGCACACT	65
KIR3DL2	AGGGCCCCTGCTGAAATC	GCTCAAACATGACATCTGACCAA	64
SLC25A13	GGGATCTACAAGGGTGCCAAA	GGAAAGTAGATGGCCGAGAAAG	64
β-actin	TACGCCAACACAGTGCTGTCT	TGCATCCTGTCGGCAATG	70
VSIG4	TGGATGACCGGAGCCACTAC	ACTTGGTTGCCATCAGGAGTCT	60
CNN2	GAAGGGCCTGAAGGATGGA	GAGCCCGGCTGTAGCTTGT	59
MMP8	CCCAACTATGCTTTCAGGGAAA	GCCTGAATGCCATCGATGT	67
PSMC5	CGAGAACGGCGAGTCCAT	TGACCTTGGCTACTGCCATCT	57

were within linear range and the amplification efficiency was approximately equal for each of the target gene tested. SYBR Green PCR assays were performed on cDNA samples in 96-well optical plates in an ABI PRISM 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). Analysis of relative gene expression data was performed using the comparative CT method (ΔΔCT) with β-actin (ACTB) as an endogenous control/reference assay. Cycling parameters for a two-step PCR were 95°C for 10 min, 1 cycle, then 60°C for 1 min, 95°C for 15 s, 40 cycles. The confirmation of specific amplification and lack of primer dimmer formation were determined by calculated melting dissociation curve. The real-time PCR products were examined in 2% agarose gel.

Statistical analysis

All values are expressed as mean ± SD. Statistical significance (defined as $P < 0.05$) of clinical characteristics of ES-PE and normal control subjects was evaluated using one-way analysis of variance. P-values of different gestational weeks (<28 gestation weeks and 28–32 gestation weeks) were calculated by Fisher's Exact test. All statistical analyses were performed with SPSS 12.0 (SPSS Inc, Chicago, IL).

Results

Gene expression signature of early onset severe preeclampsia in peripheral leukocytes

Patients were recruited from among those who presented with ES-PE of Beijing Obstetrics and Gynecology Hospital. To explore whether we can identify a gene expression signature of ES-PE from peripheral blood samples, 38 patients and 29 controls were analyzed in this study. Six patients and five controls were analyzed using the Affymetrix® HG-U133 plus 2.0 chips representing 47,000 individual human genes, which showed significant differences in the expression levels of 72 genes. The demographic and pregnancy characteristics of each group are summarized in Table 2. Patients with ES-PE and controls were similar in maternal age, pre-pregnancy body mass index, parity, and race. All subjects were of Chinese Han origin. According to different gestational weeks, we classified ES-PE into two groups: <28 gestation weeks (n = 10, 26.4%) and 28–32 gestation weeks (n = 28, 73.6%). The high risk of hypoproteinemia, high blood viscosity, proteinuria >5 g, abnormal renal function, thrombocytopenia, placental abruption, peritoneal fluid, fetal growth restriction, neonatal asphyxia and neonatal death were observed in the group of <28 gestation weeks ($P < 0.05$, data not shown).

Using SAM analysis, 72 genes were differentially expressed between case and control groups based on the following criteria: (1) false discovery rate (FDR) <5.4%; (2) average fold change >2.0. Thirty-eight genes were over-expressed and 34 genes were under-expressed in the patient group (Table 3). We also did cluster analysis on the 72 genes to arrange the samples according to similarities in gene expression patterns as described previously. Hierarchical cluster analysis of the

Table 2 Clinical characteristics of early onset severe preeclampsia and normal control subjects.

	Normal pregnancies (n = 29)	Early onset severe pre-eclampsia (n = 38)	P-value
Maternal age (years)	28.2 ± 2.58	30.0 ± 2.83	0.304
Body height (m)	1.62 ± 0.06	1.60 ± 0.05	0.481
Prepregnancy body weight (kg)	58.3 ± 3.47	60.7 ± 2.67	0.421
Prepregnancy BMI (kg/m^2)	22.0 ± 1.38	23.4 ± 1.20	0.094
Gestational age at onset of preeclampsia (weeks)	–	27 ± 3.12	
Gestational age at delivery (weeks)	38.6 ± 1.14	29.5 ± 1.87	0.000
Systolic blood pressure (mm Hg)	–	173 ± 12	
Diastolic blood pressure (mm Hg)	–	112 ± 8	
Infant birth weight (g)	3750 ± 199	1650 ± 385	0.000

Values are expressed as mean ± SD and were analyzed with one-way analysis of variance; significant at $P < 0.05$. BMI = body mass index.

Table 3 List of genes, up- or down-regulated by a factor of at least 2.0 between ES-PE and controls.

Original source file data		
Probe set ID	Gene name	Describe
Down-regulation, <0.5		
206529_x_at	SLC26A4	solute carrier family 26, member 4
205573_s_at	SNX7	sorting nexin 7
230720_at	RNF182	ring finger protein 182
206632_s_at	APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
206881_s_at	LILRA3	leukocyte immunoglobulin-like receptor, subfamily A
218638_s_at	SPON2	spondin 2, extracellular matrix protein
204101_at	MTM1	myotubularin 1
219534_x_at	RMND5B;CDKN1C	required for meiotic nuclear division 5 homolog B
1559680_at	TTL	tubulin tyrosine ligase
207509_s_at	LAIR2	leukocyte-associated immunoglobulin-like receptor 2
210196_s_at	PSG1	pregnancy specific beta-1-glycoprotein 1
228568_at	Gcom1	GRINL1A combined protein
241525_at	LOC200772	hypothetical protein LOC200772
1554273_a_at	LRAP	leukocyte-derived arginine aminopeptidase
1562371_s_at	MGC26733	hypothetical protein MGC26733
220646_s_at	KLRF1	killer cell lectin-like receptor subfamily F, member 1
203780_at	EVA1	epithelial V-like antigen 1
226736_at	CHURC1	churchill domain containing
209160_at	AKR1C3	aldo-keto reductase family 1, member C3
202458_at	PRSS23	protease, serine, 23
207314_x_at	KIR3DL2	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2
221670_s_at	LHX3	LIM homeobox 3
1557158_s_at	MLL3	myeloid/lymphoid or mixed-lineage leukemia 3
203775_at	SLC25A13	solute carrier family 25, member 13
207840_at	CD160	CD160 molecule
230464_at	EDG8	endothelial differentiation, sphingolipid G-protein-coupled receptor, 8
224036_s_at	LMBR1	limb region 1 homolog
207796_x_at	KLRD1	killer cell lectin-like receptor subfamily D, member 1
223836_at	KSP37	Ksp37 protein
211397_x_at	KIR2DL2	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 2
228601_at	LOC401022	hypothetical LOC401022
1553177_at	SH2D1B	SH2 domain containing 1B
210354_at	IFNG	interferon, gamma
207583_at	ABCD2	ATP-binding cassette, sub-family D (ALD), member 2
Up-regulation, >2		
213096_at	TMCC2	transmembrane and coiled-coil domain family 2
205239_at	AREG	amphiregulin (schwannoma-derived growth factor)
209503_s_at	PSMC5	proteasome 26S subunit, ATPase, 5
201605_x_at	CNN2	calponin 2
203936_s_at	MMP9	Matrixmetallo peptidase 9
211372_s_at	IL1R2	interleukin 1 receptor, type II
1555423_at	SSH2	Protein phosphatase Slingshot homolog 2
1567287_at	OR5K2	Olfactory receptor 5K2
210432_s_at	SCN3A	Sodium channel, voltage-gated, type III, alpha
206676_at	CEACAM8	carcinoembryonic antigen-related cell adhesion molecule 8
229961_x_at	FLJ44968	FLJ44968
1569481_s_at	SNX22	Sorting nexin 22
204438_at	MRC1L1;MRC1	Mannose receptor, C type 1-like 1
221152_at	COL8A1	Collagen, type VII, alpha 1
213650_at	GOLGA8B	golgi autoantigen, golgin subfamily a, 8B
205513_at	TCN1	transcobalamin I (vitamin B12 binding protein, R binder family)
223821_s_at	SUSD4	Sushi domain containing 4
215342_s_at	RABGAP1L	RAB GTPase activating protein 1-like
216054_x_at	MYL4	myosin, light polypeptide 4
219890_at	CLEC5A	C-type lectin domain family 5, member A
223565_at	PACAP	Proapoptotic caspase adaptor protein

(Table 3 continued)

Original source file data		
Probe set ID	Gene name	Describe
223660_at	ADORA3	Adenosine A3 receptor
1555199_at	GOSR1	Golgi SNAP receptor complex member 1
202286_s_at	TACSTD2;CCND1	Tumor-associated calcium signal transducer
210004_at	OLR1	Oxidized low density lipoprotein receptor 1
207329_at	MMP8	matrix metallopeptidase 8
219478_at	WFDC1	WAP four-disulfide core domain 1
207802_at	CRISP3	cysteine-rich secretory protein 3
208937_s_at	ID1	Inhibitor of DNA binding 1
206177_s_at	ARG1	Arginase
207014_at	GABRA2	Gamma-aminobutyric acid A receptor, alpha 2
211430_s_at	F7;LOC652848	coagulation factor VII
205403_at	IL1R2	Interleukin 1 receptor, type II
231790_at	DMGDH	dimethylglycine dehydrogenase
201324_at	EMP1	epithelial membrane protein 1
210712_at	LDHAL6B	lactate dehydrogenase A-like 6B
1553781_at	MGC14289	similar to RIKEN cDNA 1200014N16 gene
204787_at	VSIG4	V-set and immunoglobulin domain containing 4

above-mentioned, differentially expressed genes resulted in a clear separation of the ES-PE from the healthy controls.

MAS in GenMAPP showed that after correction for multiple hypothesis testing there were statistically significant enrichments of transcripts within the 38 genes that are up-regulated in the ES-PE, displaying extracellular space (P = 9.0E-6), cell proliferation (P = 0.0016), smooth muscle contraction (P = 0.01). The over-expressed genes included CNN2, VSIG4, PSMC5 and MMP8. On the other hand, statistically significant association of the 34 down-regulated genes in ES-PE was observed with pathways such as natural killer cell mediated cytotoxicity (P = 0.00), antigen processing and presentation (P = 6.3E-5) and arachidonic acid metabolism (P = 0.05), suggesting immune response associated with the circulating cells of ES-PE. The significant down-regulated genes included KIR3DL2, AKR1C3, CHURC1, SLC25A13.

SYBR green I real-time PCR validation

To further validate the preeclampsia-status classifier genes, we determined expression levels of the 8 preeclampsia-status classifier genes using the SYBR Green I real-time PCR methodology on 56 samples for which enough RNA was available. The initially identified candidate genes were assayed by real-time PCR on a sample set of 32 ES-PE and 24 controls. To control for technical variation, we performed the comparative Ct method between a candidate gene and a housekeeping gene (ΔC_T = Ct of target gene–Ct of housekeeping gene, $\Delta\Delta C_T = \Delta C_T$ of target gene–ΔC_T of housekeeping gene) to assess differences between the tested experimental groups. We found statistically significant differences in the expression of AKR1C3, KIR3DL2, SLC25A13, CHURC1 in ES-PE, which selected from the array results. For CNN2, VSIG4, MMP8 and PSMC5, the findings were inconsistent with microarray (Table 4). We stress here that the true specificity of the assay will require the inclusion of patients with ES-PE pathology other than the disease.

Discussion

It is increasingly evident that a gene plays an important role in the development of preeclampsia. We report here

Table 4 Selected markers characterized for their up/down-regulation between different groups.

Marker name	Factor	Affymetrix HG-U133 plus 2.0		Result from microarray	Location
Down-regulated	Fold down	Probe ID	Array fold		
AKR1C3	–83.66	209160_at	0.46	consistent	10p15-p14
KIR3DL2	–23.25	207314_x_at	0.47	consistent	19q13.4
SLC25A13	–20.69	203775_at	0.47	consistent	7q21.3
CHURC1	–11.08	226736_at	0.45	consistent	14q23.3
VSIG4	–11.78	204787_at	3.84	inconsistent	Xq12-q13.3
MMP8	–11.80	207329_at	2.31	inconsistent	11q22.3
CNN2	–5.99	201605_x_at	2.03	inconsistent	19p13.3
PSMC5	–20.69	209503_s_at	2.02	inconsistent	17q23-q25

Compared with early onset severe preeclampsia, women with normotensive pregnancies have an 83.7-fold increase in AKR1C3, an 11.1-fold increase in CHURC1, a 23.2-fold increase in KIR3DL2 and a 20.7-fold increase in SLC25A13.

the feasibility of using gene expression profiling of circulating blood cells to identify novel blood biomarker in ES-PE patients. Our study suggests several points. First, through a whole-genome gene expression profiling analysis of cases and controls, we have identified a 4-gene signature in peripheral blood cells that distinguishes early onset severe preeclamptic patients from controls. Significant down-regulation of KIR3DL2, AKR1C3, CHURC1 and SLC25A13 were observed from microarray analysis and further validated by SYBR Green I realtime RT-PCR assays. Moreover, this investigation also suggested that many of the biological pathways such as natural killer cell mediated cytotoxicity, antigen processing and presentation and arachidonic acid metabolism have been associated with development of preeclampsia. Although our primary interest was ES-PE, we believe that the approach shown in our study can be used to identify biomarkers in other diseases.

Many clinical studies correlated alterations in expression of individual genes with preeclampsia, often with contradictory results. Han et al. have report that the expression of caspase-10 and death receptor 3 (DR-3) was significantly increased, whereas insulin-like growth factor binding protein-3 (IGFBP-3) was strongly down-regulated [5]. Okazaki et al. showed that the mRNA expression of pregnancy-specific beta1 glycoprotein and trophoblast glycoprotein is up-regulated in cells circulating in blood of women with preeclampsia [11]. Surprisingly, none of these genes are present in our set of 72 genes. This could be due to the fact that we determine gene expression at the period of early onset of the disease, whereas most previous studies did not confine the time of onset and the origin of samples. Killer cell immunoglobulin-like receptors (KIRs) are of particular interest in the context of preeclampsia. They have been divided into distinct groups, depending on the number of external immunoglobulin domains (2D or 3D). The presence of a long cytoplasmatic tail with two immune tyrosine-based inhibitory motifs (ITIMs) allows the transduction of inhibitory signals and characterizes the inhibitory KIRs (2DL, 3DL), whereas the presence of short cytoplasmatic tails corresponds to the activating KIR receptors (2DS, 3DS) [19]. KIRs are glycoproteins expressed on the cell surface of natural killer (NK) and subsets of T cells. A well-described way of NK cells to recognize target cells is surveillance for cells with reduced levels of MHC class I molecules, a phenotypic change that frequently occurs in tumor variants and virally infected cells. Different inhibitory KIRs are specific for HLA-A (KIR3DL2), HLA-B (KIR3DL1), and different subgroups of HLA-C (KIR2DL1–3). Defective placentation is the likely pathogenesis of preeclampsia [10]. This involves reduced trophoblast invasion into both the decidua and spiral arteries. The loose plugs of endovascular trophoblast that normally limit the maternal circulation to the placenta may be incomplete [8]. The correlation between the development of ES-PE and primigravidae is suggestive of a defect in fetal–maternal immune recognition. Our work provides molecular evidence that this immune problem may be mediated by defective KIR3DL2 expression. We observed reduced KIR3DL2 transcription levels in ES-PE and propose that lacking killer-inhibitory receptors for self-HLA class I molecules results in abnormal activation of NK/T cells, weaken inhibitory signaling, powering NK cells recognition. Trophoblasts lacking KIR3DL2 are vulnerable to attack by the maternal immune system, thereby developing vessels which cannot adequately nourish the developing placenta.

Aldo-Keto Reductases (AKRs) are a superfamily of NAD(P)H linked oxidoreductases that catalyze the NADP(H)-dependent reduction of a wide variety of substrates. AKR1C3 possesses 3α-HSD, 17β-HSD, 20α-HSD and prostaglandin (PG) F synthase activities and catalyzes androgen, estrogen, progesterone and PG metabolism [13]. AKR1C3, therefore, has the capability of regulating ligand access to multiple nuclear receptors including the androgen receptor (AR), estrogen receptor, progesterone receptor and peroxisome proliferators activated receptor gamma (PPARγ) in endocrine peripheral tissues. AKR1C3 may prevent PPARγ signaling by preventing the formation of its ligand 15d-PGJ_2 and by stimulating the phosphorylation of the receptor, leading to a pro-proliferative response. AKR1C3 is uniquely positioned to reduce PGH_2 and PGD_2 to yield PGF epimers leading to the inactivation of PPARγ upon exposure to oxidative stress. Moreover, since the PGF epimers bind to the F-series-prostanoid (FP) receptor, this could lead to activation of NFκB and propagation of an inflammatory response involving induction of COX-2. Severe Deoxycorticosterone (DOC) excess as is seen in 17α- and 11β-hydroxylase deficiencies causes hypertension, and moderate DOC overproduction in late pregnancy is associated with hypertension. Excess DOC will bind to the mineralocorticocoid receptor causing hypertension in pregnancy when AKR1C3 decreases [16]. AKR1C3 protects the mineralocorticoid receptor from activation by DOC in mineralocorticoid target cells of the kidney and colon. Oxidative stress may mediate endothelial cell dysfunction and contribute to the pathophysiology of preeclampsia as there is evidence of increased prooxidant activity formation along with decreased antioxidant protection preeclampsia. Higher placental NADPH oxidase activity has been reported in women with early-onset preeclampsia as compared with those with late-onset of disease which is consistent with the concept that early-onset preeclampsia is more dependent on placental dysfunction than the later-onset disease. Down-regulated AKR1C3 expression in maternal blood may result in lacking of anti-oxidant response elements, therefore, reactive oxygen species produced under conditions of oxidative stress, attack polyunsaturated fatty acids to form a series of lipid peroxides which are implicated in the patho-

genesis of atherosclerotic plaques. The lipid peroxides further damage the structure and function of vascular endothelial cell.

No biological function of Churchill (ChCh) in relation to preeclampsia is known to date. ChCh is a zinc-containing protein involved in neural induction during embryogenesis. ChCh was discovered in a differential screen for neural inducing factors present in chick embryos [9]. ChCh was identified as a late FGF response gene that is up-regulated within 4–5 h of signaling from both the organizer and FGF and shows no indication of down-regulation in the presence of BMP. It is difficult to speculate on a preeclampsia-promoting function of CHURC1. Zinc finger proteins are known to participate in a variety of cellular activities including development, differentiation, and tumor suppression and though originally determined to be DNA binding proteins, there is increasing evidence that zinc finger proteins also recognize RNA and participate in protein-protein and protein-lipid interactions.

The SLC25A13 gene consists of 18 exons and encodes a liver type mitochondrial aspartate-glutamate carrier named citrin, which plays an important role in malate-aspartate NADH shuttling, urea synthesis, and gluconeogenesis. In humans, loss-of-function mutations in the SLC25A13 gene encoding citrin cause both adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis (NICCD), collectively referred to as human citrin deficiency [17]. The urea cycle processes excess nitrogen to make urea, which is excreted in urine. An adequate supply of aspartate must be transported out of mitochondria to participate in a process called the urea cycle which is a sequence of chemical reactions that takes place in liver cells. These reactions process excess nitrogen that is generated as the body uses proteins. Research reveals that loss of citrin first causes deficiency of aspartate in the cytosol, which results in an increase in cytosolic NADH/NAD(+) ratio and then activation of fatty acid synthesis pathway to compensate the aberrant ratio [14]. SLC25A13 expression is down-regulated in the ES-PE. As a result of citrin deficiency, arginine cannot be supplied from the urea cycle which is the enzyme-substrate complex of endothelia nitric oxide synthase (eNOS), therefore, nitrogen monoxidum cannot generate enough from eNOS.

The VSIG4, CNN2, MMP8 and PSMC5 yielded inconsistent results. For VSIG4, discordant observations between the array hybridization (up 3.84-fold) and real-time PCR (significantly down 12-fold, $P=0.003$) were made for ES-PE. We have also observed, in the microarray, 2-fold up-regulations were for CNN2, 2.3-fold for MMP8 and 2-fold for PSMC5. In quantitative PCR, however, we found a significantly decreased expression level.

From these data, we have identified AKR1C3, CHURC1, SLC25A13 and KIR3DL2 which might be involved in the molecular mechanisms underlying the progression of ES-PE, and highlight potential targets for therapeutic intervention.

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