Screening for differential methylation status in human placenta in preeclampsia using a CpG island plus promoter microarray

RUI-ZHE JIA[1], XIANG ZHANG[1], PING HU[2], XIAO-MEI LIU[1], XIANG-DONG HUA[1], XIN WANG[1] and HONG-JUAN DING[1]

[1]Department of Obstetrics and [2]Center of Prenatal Diagnosis, Nanjing Maternity and Child Health Hospital of Nanjing Medical University, Nanjing, P.R. China

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Abstract. The development of preeclampsia (PE) seriously affects the health of the mother and the child, but the precise pathogenesis of PE remains elusive. The placenta is considered to play a key role and DNA methylation may be associated with altered placental development and function. The aim of this study was to perform a genome-wide analysis of the DNA methylation profile in placentas from pregnancies with severe preeclampsia. The authors analyzed normal and placental tissues with PE for aberrant DNA methylation using methylated DNA immunoprecipitation (MeDIP) and a human CpG island plus promoter microarray. The methylation status of identified candidate genes were validated by bisulfite sequencing PCR (BSP). Microarray analysis identified 296 genes that showed significantly aberrant DNA methylation in preeclampsia (PE). These genes were located more frequently in chromosome 1 (10.5%, P=0.005), chromosome 12 (8.1%, P=0.062) and chromosome 19 (7.4%, P=0.117). Functional analysis divided these genes into different functional networks. In addition, the methylation profile of six of these genes (*CAPN2*, *EPHX2*, *ADORA2B*, *SOX7*, *CXCL1* and *CDX1*) in nine patients with PE was validated by BSP. This study demonstrated aberrant patterns of DNA methylation in PE, which may be involved in the pathophysiology of PE. Future work will assess the potential prognostic and therapeutic value for these findings in PE.

Correspondence to: Dr Xiang Zhang, Department of Obstetrics, Nanjing Maternal and Child Health Hospital of Nanjing Medical University, Nanjing 210004, P.R. China
E-mail: xzhang579@yahoo.com.cn

Dr Hong-Juan Ding, Department of Obstetrics, Nanjing Maternal and Child Health Hospital of Nanjing Medical University, Nanjing 210004, P.R. China
E-mail: hjding987@yahoo.com.cn

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Introduction

Preeclampsia (PE) is a pregnancy-related disorder defined by the onset of maternal hypertension and proteinuria and characterized by widespread endothelial dysfunction. The development of hypertensive disorders during pregnancy seriously affects the health of the mother and the child, and accounts for a large proportion of maternal and perinatal mortality and morbidity. PE is the second leading cause of maternal death in China.

The precise origin of PE remains elusive. It is a multifactorial disorder, and its onset and progression have been attributed to the mother's genetic background, immune maladaptations, abnormal placental trophoblastic infiltration of the uterine spiral arteries, vascular endothelial cell dysfunction, dyslipidemia and insulin resistance, amongst others (1). Although the molecular mechanisms that contribute to the development of PE are not well understood, the placenta is considered to play a key role in the disease (2). Disruption of placental development in the first trimester of pregnancy has been suggested to be involved in the etiology of disorders such as PE and intrauterine growth restriction (3).

Epigenetic regulation of the placenta evolves during preimplantation development and further gestation. Epigenetic marks, such as DNA methylation, histone modifications and non-coding RNAs, affect gene expression patterns (4). Recent research shows that tissues in the placenta have a high degree of variability in terms of the overall DNA methylation profile and the promoter methylation of certain genes showed evidence for association with PE, such as the *TUSC3* gene (5). Therefore, it is necessary to screen a comprehensive list of human DNA methylation variations in the placenta with PE. The development of the DNA methylation chip provides a powerful method for research in epigenomics.

In the present study, we performed a genome-wide analysis of the DNA methylation profile in placental tissues from pregnancies with severe PE using methylated DNA immunoprecipitation (MeDIP) and the NimbleGen HG18 CpG Promoter Chip. Comparing gene expression data from the DNA methylation chip between placental samples with PE and normal placental tissue, we identified related genes and specific methylation alterations with potential functional and clinical relevance in PE, which could result in the development

Table I. Demographic characteristics of the study populations.

Parameters	PE n=9	Control n=9	P-value
Maternal age (years)	29.0±2.9	28.0±2.6	NS
Maternal height (cm)	157.4±2.7	162.0±1.7	NS
Maternal weight (kg)	70.8±3.5	76.2±7.7	NS
Body mass index (kg/m^2)	28.7±2.0	29.0±2.6	NS
Systolic blood pressure (mmHg)	151.6±14.1	116.7±5.8	<0.05
Diastolic blood pressure (mmHg)	95.4±5.1	78.7±2.3	<0.05
Proteinuria (g/24 h)	5.3±2.5	not detected	<0.05
Gestation at delivery (weeks)	35.0±2.6	39.4±0.2	<0.05
Birth weight (g)	2110.0±484.0	3616.7±28.9	<0.05

PE, preeclampsia; NS, non-significant.

of the disease. These findings may have both prognostic and therapeutic potential for PE.

Materials and methods

Patients and tissue samples. Placental tissue was obtained from women who were hospitalized in the Department of Obstetrics of the Nanjing Maternal and Child Health Hospital (China). All samples were collected with the approval of the appropriate institutional ethics committee and written consent was given by each pregnant woman. All women were delivered by elective cesarean delivery in the absence of labor and the placental tissues were obtained at the time of cesarean section. We selected nine pregnancies complicated by severe PE with delivery occurring after 33 weeks. Also, nine pregnant women with a normal term pregnancy were recruited as the control group. The relevant clinical details for the patients are shown in Table I. Specific exclusion criteria at the time of recruitment included a history of cardiac disease or suspected chronic hypertension, chronic illness, or long-term use of medications, multiple pregnancies, premature rupture of membranes and fetal anomalies.

PE was defined according to the International Society for the Study of Hypertension in Pregnancy (6,7). PE was defined as the increase in blood pressure to ≥140/90 mmHg at 20 weeks after gestation, accompanied by urinary protein excretion (300 mg protein in a 24-h urine specimen). Severe PE was defined as either severe hypertension (systolic blood pressure of ≥160 mmHg and/or diastolic blood pressure of ≥110 mmHg on ≥2 occasions 6 h apart) plus mild proteinuria or mild hypertension plus severe proteinuria (>2 g/24 h).

At least two sites were sampled from each placenta. Contamination with the maternal decidua and amniotic membranes was excluded by morphological observation. Tissues were washed with normal saline and then immediately snap-frozen in liquid nitrogen and stored at -80˚C until analysis.

Genomic DNA preparation. Genomic DNA was extracted from frozen placenta tissue using QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Briefly, placental tissue was homogenized using a hand-held homogenizer, digested with Proteinase K (Merck KGaA, Darmstadt, Germany) and thereafter treated with RNase (Roche Diagnostics, Switzerland) for 30 min at 37˚C, then precipitated and washed. The DNA quality and quantity was assessed using a NanoDrop™ spectrophotometer (Thermo Scientific, USA) with A260/A280 ratio within 1.7-2.0 considered to be a criterion for quality control.

Sonication of genomic DNA. Approximately 40 μg of genomic DNA from each placenta tissue were sheared to 300-1,000 bp using a sonifier (Bioruptor model 200; Diagenode, Sparta, NJ). The sonication conditions were carefully maintained as follows: the sonicator was turned on for 30 sec and then off for 30 sec 20 times; ice-cold water was replaced around the tube on every 4th cycle to maintain the chilled temperature of each sample and all procedures were performed on ice. Finally, 4 μl of each sample was loaded onto a 2% agarose gel to verify the fragment size of DNA (mean size, 200-800 bp; average, 400 bp).

Methylated DNA immunoprecipitation (MeDIP) and microarray analysis. We used MBD2b-sepharose-4B (ShanghaiBio Corporation, China) to capture and enrich methylated DNA. For microarray analysis, the experimental and control samples were purified with a Qiagen PCR purification kit (Qiagen) and amplified with a whole genome amplification kit (WGA4; Sigma).

Six samples from three normal placentas (three placentas were pooled to form a control group) and three placentas from women with PE (three placental samples were pooled to form an experimental group) were assayed using a NimbleGen human CpG island plus promoter microarray (385K). This NimbleGen array chip comprises of 385,000 isothermal probes which cover all reported Human Refseq gene promoters (24,659) that range from -800 bp to +200 bp relative to the transcription start sites (TSS). All reported CpG islands (28,226) were annotated on the UCSC genome browser. We undertook DNA methylation expression analysis according to their instructions.

After the quality of each sample was verified, genome-amplified products were labeled using a NimbleGen Dual-Color

Table II. The primers of bisulfite sequencing PCR for *CAPN2*, *EPHX2*, *ADORA2B*, *SOX7*, *CXCL1* and *CDX1*.

Genes	Primers	Product size (bp)
CAPN2	S 5'-AGTTTAGGGTAGTTGGTTTAGTTT-3' R 5'-AAATATAACCTCAACCRCTC-3'	318
EPHX2	S 5'-GTTGTTTAGGTAAGGGGGTTTAG-3' R 5'-ACTCCATAAATAACTCCTCTCCC-3'	273
ADORA2B	S 5'-AGGTTGTTAGGGTTGAAGTAAG-3' R 5'-ACTCACTCCAAACTAACCCTAC-3'	442
SOX7	S 5'-AGTTAGAAGTGAGGGAGTTGGA-3' R 5'-AACATTAACCATAAACCCCTCA-3'	319
CXCL1	S 5'-TTTAGGGATTTGGGGTAGAA-3' R 5'-TTCCCTACAAAATCTACAAACAC-3'	588
CDX1	S 5'-TGGTTATTGTGTGAAGTTGGTT-3' R 5'-CACCCAAACCTTTTATAACTCC-3'	380

DNA labeling kit (Roche NimbleGen, USA). Briefly, 1 μg of each genome-amplified product was mixed with either Cy5 (experimental amplicon) or Cy3 (control amplicon), random primer buffer and nuclease-free water to a final volume of 80 μl. The mixture was incubated at 95˚C for 10 min and immediately transferred to an ice-water bath for 2 min. After that, the dNTP nucleotide mix and Klenow enzyme were added to the mixture and incubated at 37˚C for 2 h while protected from light. The reaction was terminated using a stop solution. After the samples were washed and centrifuged, 6 μg each of Cy5- and Cy3-labeled samples were mixed in 5 μl diluted water. After that, 0.4 μl Alignment Oligo, 3.6 μl Hybridization Component A and 9.0 μl 2X hybridization buffer were added in the mixture. The mixture was incubated at 95˚C for 3 min, 42˚C for 5 min and then hybridized to NimbleGen human CpG island plus promoter microarray slides (385K) from Roche NimbleGen. The hybridization reaction was performed at 42˚C for 16-20 h. Slides were subsequently washed with NimbleGen wash buffers and then dried in a dark desiccator. The two-color NimbleGen array was scanned with a GenePix 4000B Scanner. Array data were extracted and analyzed using NimbleScan and SignalMap software. We reported only those genes with significant (P<0.05) differential expression of ≥2.0-fold changes. We also used the KEGG Orthology Based Annotation System 2.0 (KOBAS; http://kobas.cbi.pku.edu.cn) to perform interaction pathway and functional analysis of these candidate genes.

Bisulfite sequencing PCR (BSP). We further investigated samples from 18 placentas by bisulfite sequencing PCR (BSP), including 9 normal placentas and 9 placentas from women with PE. BSP was performed as previously described (8). The primers used for BSP are shown in Table II. One microgram of normal and patient genomic DNA was treated using the EpiTect bisulfite kit (Qiagen) according to the manufacturer's instructions, respectively. Amplification products were visualized by UV illumination on 1% agarose gel (Bio-west, China) that contained ethidium bromide. For BSP clone sequence analysis, the PCR products were subcloned into a pMD-19-T vector (Takara Bio, Inc., Japan). Ten to twenty clones were selected for sequencing to identify methylation differences between each pair of normal and patient tissues.

Statistical analysis. Data aere expressed as the mean ± SD. The Student's t-test was used to analyze microarray for methylated DNA expression profiles and only expression changes that were statistically significant and at least 2 times higher or lower than the expression levels in controls were considered. A P-value <0.05 was considered statistically significant. For the distribution of gene expression (9), we compared the actual positive/negative genes with the predicated results on each chromosome with the Chi-square test. Again, a P-value <0.05 was considered to be statistically significant.

Results

DNA methylation microarray expression data. Using the human CpG island plus promoter microarray, we focused on those genes that had a normalized $\log_2$ ratio of >+2.0 and <-2.0 in the preeclampsia group when compared to the normal group as shown by the microarray analysis. Then, we identified 3,280 genes that were differentially hypermethylated or hypomethylated in patients with PE. The methylation status of CpG islands in gene promoters, which were defined as regions from 800 bp upstream to 200 bp downstream in this study, were examined based on results from the Human (*Homo sapiens*) Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway). From our results, comparing to normal placental tissues, 102 genes in total showed significant hypermethylation in the promoter-associated CpG islands in PE tissue samples, while 194 genes showed significant hypomethylation (Fig. 1). Those differentially methylated genes might be involved in the development of PE.

The 296 candidate genes were distributed across all chromosomes (Fig. 2), but showed a significant regularity that was statistically significant: there were 31 on chromosome 1 (10.5%, P=0.005), 24 on chromosome 12 (8.1%, P=0.062) and 22 on chromosome 19 (7.4%, P=0.117).

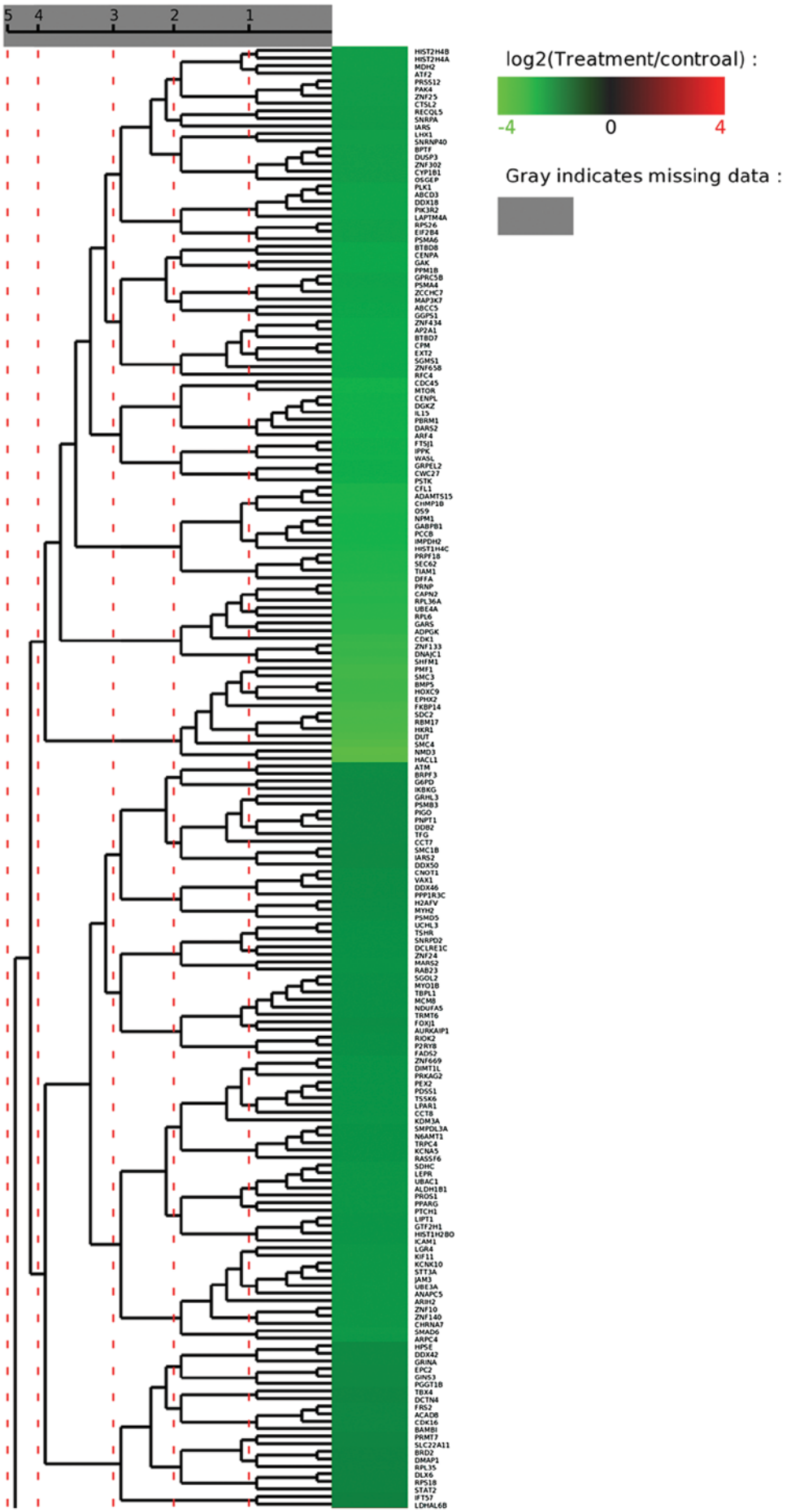

Figure 1. Part I. Complete-Linkage Cluster Analysis of DNA methylation candidates in preeclampsia.

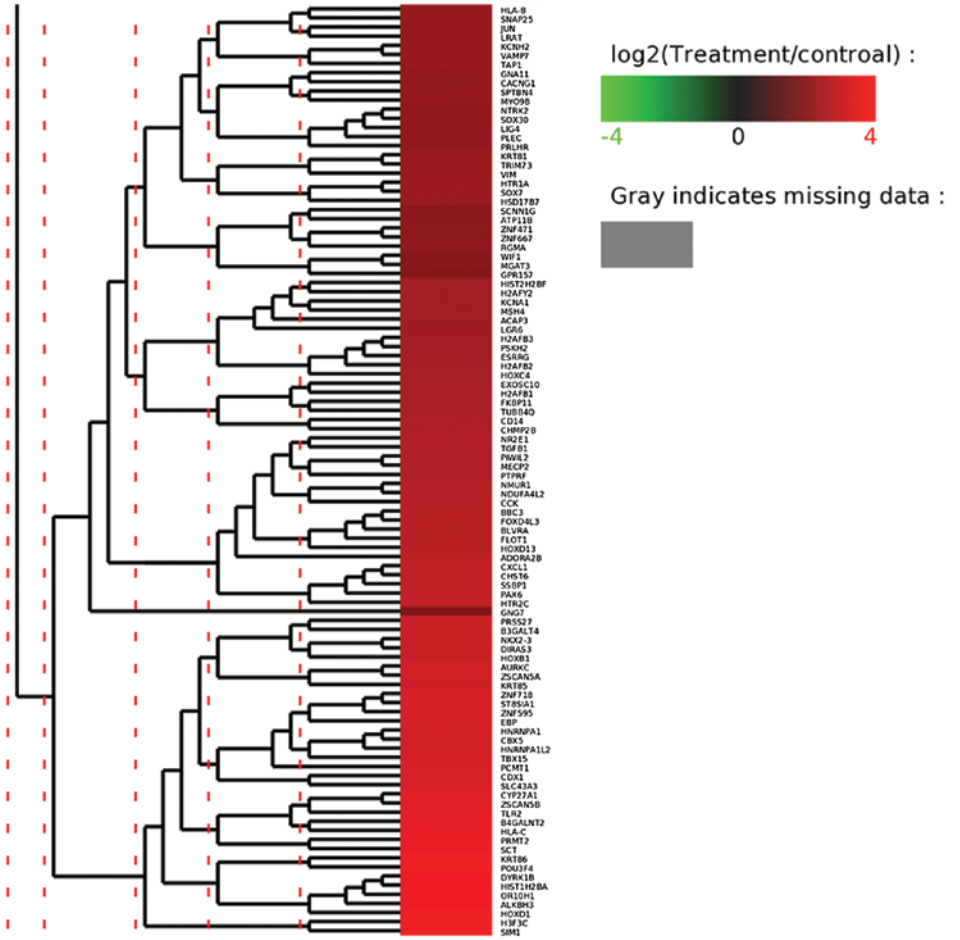

Figure 1. Part II. Complete-Linkage Cluster Analysis of DNA methylation candidates in preeclampsia. DNA methylation analysis was performed by the human CpG island plus promoter microarray. Data are mean-centered $\log_2$ expression values across the samples. Red indicates ≥2-fold upregulation; green indicates ≤2-fold downregulation.

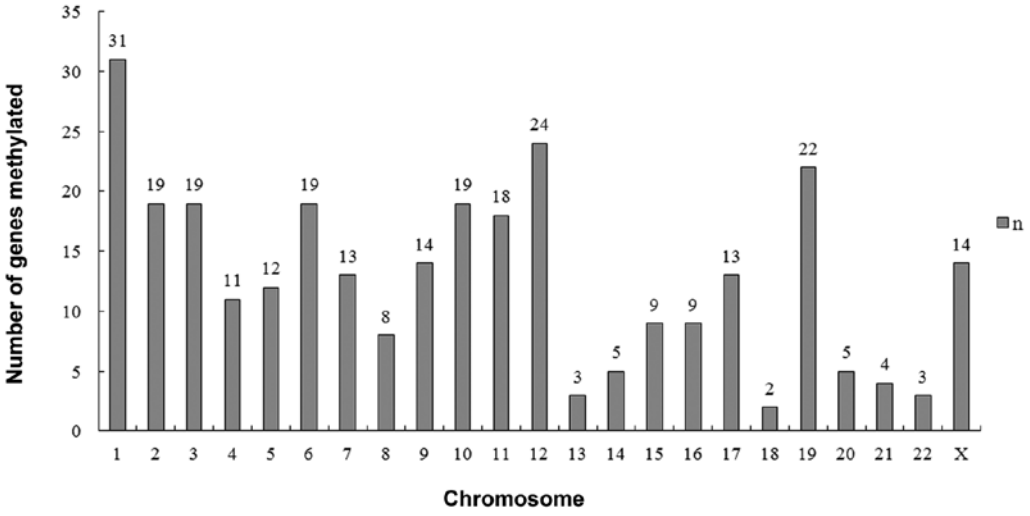

Figure 2. The number of methylated genes in each chromosome.

We also used the KEGG Orthology Based Annotation System 2.0 (KOBAS; http://kobas.cbi.pku.edu.cn) to perform interaction pathway and functional analysis of these 296 candidate genes. The analysis divided the 296 candidate genes into 182 functional subclasses, with a majority of genes falling into the top 8 functional networks after classification (Table III). The major functions of the genes involved in these networks included metabolism, transcription, translation, replication and repair, cell motility, cell growth and death, signal transduction, membrane transport, receptors and channels, and protein

Table III. Functional networks of 296 genes identified by microarray.

Gene name	Focus genes	Biological function
IARS; IARS2; PDSS1; GGPS1; PCCB; LIPT1; EBP; HSD17B7; CYP27A1; DGKZ; ALDH1B1; FADS2; SGMS1; EPHX2; ST8SIA1; B3GALT4; MDH2; ACAD8; SDHC; CYP1B1; HPSE; CHST6; EXT2; MGAT3; B4GALNT2; STT3A; PIGO; MARS2; G6PD; LDHAL6B; BLVRA; NDUFA5; NDUFA4L2; IPPK; OS9; LRAT; IMPDH2; DUT; PNPT1	39	Lipid metabolism; carbohydrate metabolism; energy metabolism; nucleotide metabolism; amino acid metabolism; glycan biosynthesis and metabolism; metabolism of cofactors and vitamins
CENPL; PRMT7; CBX5; KDM3A; RFC4; MECP2; PRMT2; CENPA; PMF1; NPM1; DMAP1; PBRM1; BPTF; PIWIL2; EPC2; BRPF3; SGOL2; GARS; PSTK; DARS2; FOXJ1; ZNF718; SOX7; ZNF667; ZNF25; ZNF140; TBX4; GRHL3; HKR1; HOXD1; HOXB1; SIM1; HOXD13; ZSCAN5A; POU3F4; NKX2-3; FOXD4L3; LHX1; ZNF10; DLX6; ZNF133; ZNF471; ZNF669; ZNF24; TBX15; ZNF302; GABPB1; VAX1; CDX1; HOXC9; ZNF658; SOX30; PAX6; HOXC4; ZSCAN5B; TBPL1; ZNF434; ZNF595; DDX42; HNRNPA1L2; RBM17; SNRNP40; HNRNPA1; DDX46; SNRPA; PRPF18; GTF2H1; LIG4; RECQL5; SHFM1; ALKBH3; SSBP1; MSH4; GINS3; MCM8; SNRPD2; CNOT1; ZCCHC7; EXOSC10; RPS18; RPL35; RPS26; RPL6; RPL36A; DIMT1L; NMD3; FTSJ1; GRPEL2; FKBP11; CWC27;FKBP14; DNAJC1; CCT7; CCT8; SNAP25; VAMP7; N6AMT1; PCMT1; TRMT6; EIF2B4; UBE3A; BTBD8; BTBD7; UBE4A; PSMD5; UBAC1; TRIM73; ARIH2	108	Transcription; translation; folding, sorting and degradation; replication and repair
RAB23; BMP5; PTCH1; LGR4; SCT; CCK; P2RY8; TSHR; LEPR; NMUR1; HTR1A; PRLHR; PIK3R2; JUN; MTOR; ABCC5; TAP1; SLC43A3; ABCD3; SLC22A11; NTRK2; DUSP3; PPM1B; ATF2; SEC62; SMAD6; TGFB1; DIRAS3; GNG7; ARF4; STAT2; PTPRF; CHRNA7; GNA11; HTR2C; RASSF6; AURKAIP1; WIF1; IL15; RGMA; PRNP; BAMBI; SDC2	43	Membrane transport; signal transduction; signaling molecules and interaction
SMC3; CDK1; ANAPC5; ATM; CDC45; SMC1B; ARPC4; KRT81; DCTN4; KIF11; CFL1; MYO9B; SPTBN4; MYH2; PLEC; TUBB4Q; MYO1B; KRT86; KRT85; WASL; VIM; DFFA; BBC3; LPAR1; PLK1; CHMP2B; ACAP3; CHMP1B; AP2A1; JAM3; CTSL2; LAPTM4A; PAK4; SMC4; DDB2	35	Cell motility; cell growth and death; cell communication
ADORA2B; KCNH2; SCNN1G; TRPC4; OR10H1; KCNA1; GPR157; KCNK10; KCNA5; LGR6; GPRC5B; NR2E1; ESRRG	13	Receptors and channels
PSKH2; DYRK1B; TSSK6; GAK; CDK16; RIOK2; ADAMTS15; CAPN2; PRSS12; OSGEP; PRSS27;CPM; BRD2; AURKC; PSMB3; UCHL3; PSMA4; PSMA6	18	Protein kinases; peptidases
H2AFY2; H2AFB1; H2AFB2; HIST1H4C; H2AFV; H2AFB3; HIST1H2BA; HIST1H2BO; HIST2H4A; H3F3C; HIST2H4B; IKBKG; CXCL1; TLR2; CD14; MAP3K7; HLA-B; HLA-C; TIAM1; ICAM1; PROS1; HIST2H2BF; DCLRE1C	23	Immune system and immune system diseases
PRKAG2; FLOT1; PPP1R3C; CACNG1; PPARG	5	Endocrine system disorder; circulatory system and cardiovascular diseases
IFT57; PEX2; GRINA; FRS2; ATP11B; PGGT1B; ADPGK; HACL1; DDX50; DDX18; SMPDL3A; TFG	12	Miscellaneous functions

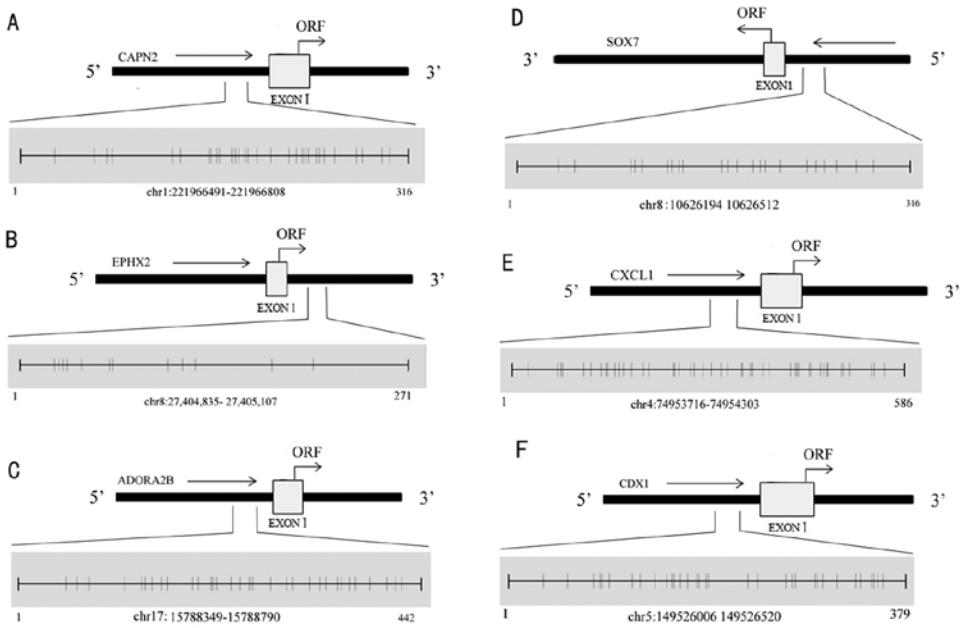

Figure 3. Schemes of promoter region of genes. Distribution of CpG dinucleotides in a subsection of the genes. (A) *CAPN2*, (B) *EPHX2*, (C) *ADORA2B*, (D) *SOX7*, (E) *CXCL1* and (F) *CDX1*.

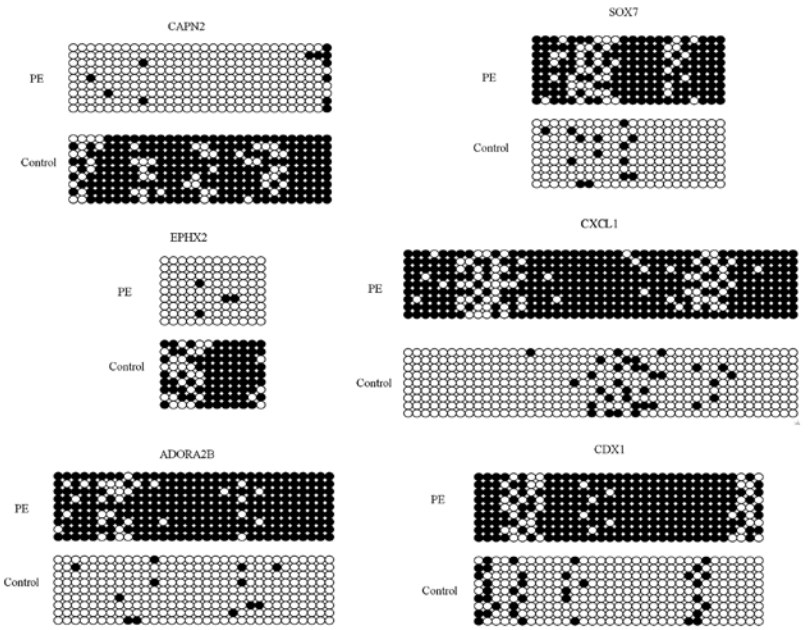

Figure 4. Bisulfite sequencing analysis of methylation status of promoter CpG islands of genes. The circles show the CpG sites of amplified DNA by bisulfite sequencing primers. Filled circles are methylated and open circles are unmethylated cytosines at CpG sites.

kinases. They were also involved in several immune, endocrine and cardiovascular system disorder-related pathways.

Validation of methylation profile of six candidate genes in patients with PE. Due to the impracticability of validating all genes identified in the microarray, we focused on 6 genes to confirm their methylation status in 9 patients with PE and 9 healthy pregnancies, including *CAPN2*, *EPHX2*, *ADORA2B*, *SOX7*, *CXCL1* and *CDX1*. These six genes were selected because they have higher $\log_2$ ratio and their potential functions were related to the molecular mechanisms postulated to underlie PE.

The methylation pattern for these genes was confirmed and quantified more accurately by BSP for these PE and control samples. We investigated the methylation status of 30 CpG sites in the *CAPN2* promoter, 12 CpG sites in the *EPHX2* promoter, 32 CpG sites in the *ADORA2B* promoter, 22 the CpG sites in *SOX7* promoter, 46 CpG sites in the *CXCL1* promoter and 33 CpG sites in the *CDX1* promoter, respectively (Fig. 3).

The methylation status of these promoter CpG dinucleotides are shown in Fig. 4. The promoter CpG dinucleotides of these genes (*ADORA2B*, *SOX7*, *CXCL1*, *CDX1*) in PE cases were mostly methylated, while they were mostly unmethylated

in control cases. Hypermethylation of CpG islands was 60-75% in the PE cases and 5-9% in the controls. The promoter CpG dinucleotides of genes *CAPN2* and *EPHX2* in PE cases were mostly unmethylated, while they were mostly methylated in control cases. Hypermethylation of CpG islands was 1-2% in the PE cases and 67-87% in the controls.

Discussion

In this study, we performed a genome-wide methylation analysis of the placenta in patients with PE and identified 296 genes with aberrant levels of DNA methylation compared to normal control placental tissues. We mapped these genes to their chromosomal locations and observed that there was a bias towards chromosomes 1, 12 and 19. Thirty-one genes mapped to chromosome 1 and at least 3 of them [*DIRAS3* (10), *JUN* (11), *MTOR* (12)] have been reported to interact functionally with vasoconstrictors, which is a critical factor in the development of PE (13). Therefore, alterations of the methylation of genes on chromosome 1, 12 and 19 may be associated with the pathogenesis of PE. We validated the DNA methylation status of 6 candidate genes using BSP, including *CAPN2*, *EPHX2*, *ADORA2B*, *SOX7*, *CXCL1* and *CDX1*.

Cross (13) reviewed various interpretations of the genetics of PE and concluded that there were 3 independent mechanisms: pre-existing borderline maternal hypertension that is exacerbated by pregnancy, elevated levels of the vasoconstrictor, angiotensin II, in the maternal circulation as a result of the placental overproduction of renin, and placental pathology. Also, recent research has shown that angiogenic factors are involved in blood pressure modulation in pregnancy (14). Furthermore, Kulkarni *et al* (15) recently reported dysregulation of angiogenic factors (vascular endothelial growth factor and placental growth factor) in PE. In our study, we found that certain genes related to angiogenic and anti-angiogenic functions had aberrant DNA methylation profiles, including *HOXD1* (16), *DIRAS3* (10) and *CXCL1* (17). Placental pathology, especially abnormal placental trophoblastic infiltration of the uterine spiral arteries, is also considered to be an important mechanism underlying PE.

EPHB4(18) and WNT2(19) were found to be responsible for the vascularization of the placenta, which is associated with the invasion of the spiral arteries. We found that *CDX1* showed significant hypermethylation in PE placentas. While the function of *CDX1* in the placenta is unknown, its homolog, CDX2, is believed to be associated with embryonic implantation (20). This suggests that CDX1 may be important in the development of PE and it is possible that the DNA hypermethylation in the gene promoter causes a subtle difference in the degree of trophoblast invasiveness among individual human placentas. Further studies are needed to identify the intrinsic function of CDX1 in the human placenta and its relation to the development of PE.

Many of the other genes detected in our initial screen may play an important role in the development of PE. For example, KCNH2 (21), SCNN1G (22), EPHX2 (23) and CAPN2 (24), a Ca^{2+}-dependent cysteine protease, have been implicated in the pathogenesis of hypertension, but the role of these genes in PE is currently unknown.

In our study, we observed 296 genes with aberrant DNA methylation in PE. These 296 genes can be clustered into specific functional networks. During gestation, the epigenome is particularly susceptible to dysregulation because the DNA synthesis rate is high and the elaborate DNA methylation patterns and chromatin modifications required for normal tissue development are being established during this time (25). Among the 296 genes, 108 gene functions are related to transcription, translation, folding, sorting and degradation, replication and repair and 35 gene functions are related to cell motility, cell growth and death, cell communication. The observed abnormal DNA methylation in PE may reflect alterations in the normal temporal regulation of gene expression patterns in the placenta.

It is believed that PE is a syndrome of exaggerated innate inflammatory response. Xie *et al* (26) indicated that SNPs of the proinflammatory mediator, TLR2, appeared to alter susceptibility to developing the maternal preeclamptic syndrome. In our study, we have also found that 23 genes with aberrant methylation profiles in the PE placental samples were related to the immune system, including TLR2. Furthermore, the presence of metabolic abnormalities, such as insulin resistance (27) and abnormal lipid metabolism (28), contribute to the development of PE and 39 related genes were identified on our array data.

Several groups have already presented data of the analysis of DNA methylation in PE. Using the Illumina GoldenGate Methylation panel and bisulfite pyrosequencing, Yuen *et al* identified 1,505 CpG sites of 807 genes, including *TUSC3* and *TIMP3* (5,29). Using mass spectrometry on a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass array, Bellido *et al* (30) compared the quantitative methylation changes of *RASSF1* and *SERPINB5* in placenta and plasma samples. Kulkarni *et al* (31) measured the global DNA methylation level in placentas of women with PE using the Methylamp™ Global DNA methylation quantification kit. Using 5-methylcytosine immunohistochemistry and Alu and LINE-1 repeat pyrosequencing, Gao *et al* (32) investigated global DNA methylation as well as DNA methylation of the paternally imprinted H19 gene in preeclamptic placentas. There is relatively little concordance between our findings and these other reports. One likely explanation is the technical differences between the assays used in these studies.

In conclusion, the NimbleGen human CpG island plus promoter microarray technique is a useful tool to study the genome-wide DNA methylation profile of placental samples from women with PE. We explored the aberrant patterns of DNA methylation in PE and provided evidence for altered patterns of placental DNA methylation during the pathophysiology of PE. The identification of placental gene-specific methylation profile may provide valuable insights into pathways that are likely to be epigenetically regulated. The future study of DNA methylation is important for the understanding of PE or other complex diseases. This might be of value as prognostic biomarkers, predictors of response to therapy and constitute future therapeutic targets.

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