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Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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(54) **Title:** FIRST TRIMESTER EPIGENETIC AND MICRORNA BIOMARKERS FOR PREECLAMPSIA

(57) **Abstract:** The subject invention pertains to biomarkers for identifying a subject as having high risk of the development PE. The biomarkers presented herein include miRNAs, post-translational modification of histone proteins, amount, expression and/or activity of histone or DNA modifying enzymes and methylation of sites in the genomic DNA. In certain embodiments, increased miR-17, increased acetylation of H4 histone protein, decreased amount, expression and/or activity of HDAC5 mRNA or protein or increased methylation of DNA at the genomic site CYP19A1 in the blood, serum or plasma of a subject compared to that of a control subject is used to predict the development of PE in the subject. The invention also provides kits and reagents to conduct assays to quantify biomarkers described herein. The invention further provides the methods of treating and/or managing PE in a subject identified as having a high risk of the development of PE.



DESCRIPTION

FIRST TRIMESTER EPIGENETIC AND MICRORNA BIOMARKERS FOR
PREECLAMPSIA

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CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 62/322,422, filed April 14, 2016, the disclosure of which is hereby incorporated by reference in its entirety, including all figures, tables and amino acid or nucleic acid sequences.

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The Sequence Listing for this application is labeled "Seq-List.txt" which was created on April 14, 2017 and is 32 KB. The entire content of the sequence listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

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Preeclampsia (PE) is responsible for 76,000 maternal and 500,000 infant deaths worldwide each year. Adverse maternal events include stroke, organ dysfunction and disseminated intravascular coagulation; whereas, adverse fetal complications include intrauterine growth restriction, premature birth, and stillbirth. PE is also associated with increased risk of chronic diseases in the mother and child later in life. The cause of PE is unknown; however, it is often diagnosed in the third trimester and there is no known prevention or cure.

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Most of the suggested biomarkers for PE focus on late gestation and lack sufficient sensitivity and specificity. Successful intervention of PE requires a better understanding of disease progression and development of accurate and early biomarkers that appear before the appearance of clinical symptoms. Placental DNA methylation and/or microRNA (miRNA) regulation, particularly, the presence of certain miRNAs in a mother's blood in the second or third trimester have been implicated in PE.

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BRIEF SUMMARY OF THE INVENTION

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The invention provides biomarkers which can be analyzed during the first trimester of pregnancy for identifying a subject as having high risk of the development PE later in the pregnancy. These noninvasive biomarkers presented herein include miRNAs, post-translational modification of histone proteins, amount, expression and/or activity of histone

or DNA modifying enzymes and methylation of certain sites in the genomic DNA of certain cells in the mother.

Accordingly, in one embodiment, the levels of certain miRNAs in a body fluid, for example, blood, serum or plasma, of a subject are used to predict the development of PE. In another embodiment, the levels post-translational modifications of histone proteins in the cells, for example, blood cells, of a subject are used to predict the development of PE. In a further embodiment, the amounts, expression and/or activities of certain enzymes capable of modifying histone proteins or sites in the genomic DNA of cells, for example, blood cells, of a subject are used to predict the development of PE. In an even further embodiment, the levels of methylation of certain sites in the genomic DNA of cells, for example, blood cells, of a subject are used to predict the development of PE.

In an embodiment, increased miR-17 (SEQ ID NO: 77) in blood, serum or plasma of a subject compared to a control subject is used to predict the development of PE in the subject. In another embodiment, increased acetylation of H4 histone protein on N-terminus, lysine 12 and/or lysine 16 and/or methylation/demethylation on lysine 20 or a combination thereof compared to that of a control subject is used to predict the development of PE in the subject. In a further embodiment, decreased amount, expression and/or activity of HDAC5 protein or mRNA compared to that of a control subject is used to predict the development of PE in the subject. In an even further embodiment, hypermethylation of DNA at the genomic site CYP19A1 (SEQ ID NO: 46) compared to that of a control subject is used to predict the development of PE in the subject. In certain embodiment, increased mirR-17; increased acetylation of histone H4 on N-terminal, lysine 12 and/or lysine 16 and/or methylation/demethylation of lysine 20 or a combination thereof; decreased amount, expression and/or activity of HDAC5 protein or mRNA; and increased methylation of DNA in the genomic site CYP19A1 compared to that of a control sample are used to predict development of PE in the subject.

The invention also provides the methods of treating and/or managing PE in a subject identified as having a high risk of the development of PE.

The invention further provides kits and reagents to conduct assays to quantify biomarkers described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication, with color drawing(s), will be provided by the Office upon request and payment of the necessary fee.

Figs. 1a-1d. MicroRNA profile in 1st trimester pregnant women who later developed severe PE compared to healthy pregnant women. Fig. 1a) Alterations in microRNA expression: The expression of 381 specific human microRNAs was profiled using TaqMan® Array Human MicroRNA Cards. The graph depicts the significant fold increase in 22 microRNAs after normalizing with U6 controls. All error bars, S.E.M. $*p \leq 0.03$ were determined by one sample one-way *t* test; C = 14, PE = 14. Fig. 1b) Disease and functions associated with the significant upregulated microRNAs: Ingenuity Pathway Analysis (IPA) was carried out to identify significant disease and functions associated with the altered microRNAs (Fig. 1a). Threshold bar (black line) indicates cut-off point of significance $p < 0.05$, using Fisher's exact test. Reproductive System Disease showed the most association with the significantly altered microRNAs. Other significant diseases and functions (*e.g.* inflammatory response, renal and urological disease, cardiovascular disease, cell death and survival) identified in the analysis are associated with PE pathogenesis. Fig. 1c) Screened microRNAs in the development of PE: 17 significant microRNAs (out of 22 significant microRNAs) are shown to be involved in the IPA gene database of pregnancy disorder, pregnancy induced hypertension, and PE (total 570 genes). The top three microRNAs (miR-296, miR-16-5p, and miR-17-5p) have the maximum number of targets. MiR-17 cluster (miR-17-5p and miR-19b) are shown (dark squares) to target highest number of genes (total 128). Fig. 1d) Expression of miR-17 and its cluster: Expression of miR-17 and its cluster miRNAs are shown. This selected group of miRNAs is involved in several pathways (angiogenesis, estrogen synthesis, invasion, *etc.*) which can lead to PE. miR-17 and its cluster show a consistent increase in all PE patients. All error bars, S.E.M $*p \leq 0.05$ was determined by one sample one-way *t* test; C = 14, PE = 14.

Figs. 2a-2b. Comparison of histone acetylation and histone deacetylase profile in 1st trimester pregnant women who later developed PE and healthy pregnant women. Fig. 2a) Hyperacetylated histone H4: The post-translational modifications of histone H4 were characterized by LC-MS. The representative spectrum shows the acetylation profile of H4 for one control (top) and one PE case (bottom) where mass 11307Da corresponds to dimethylated monoacetylated histone H4 and mass 11349Da corresponds to dimethylated

diacetylated histone H4. The larger peak ratio (abundance of mass 11349/abundance of mass 11307) observed in the PE case corresponds to increased histone H4 diacetylation. Fig. 2b) Histone deacetylase gene expression: Customized qPCR Taqman plate was used to detect the expression levels of 81 epigenetic genes encoding enzymes known or predicted to modify genomic DNA and histones to regulate chromatin accessibility and gene expression. The graph depicts the alterations in 11 histone deacetylase genes in PE patients after normalizing with housekeeping gene 18S. All error bars, S.E.M. $*p \leq 0.05$ determined by one sample one-way *t* test; C = 10, PE = 10.

Figs. 3a-3c. DNA methylation in 1st trimester pregnant women who later developed severe PE compared to healthy pregnant women. Fig. 3a) Overlapping disease and functions of differentially methylated genes identified in CHARM and Illumina analyses: Forty-three categories of disease and functions overlap in CHARM and Illumina analyses. Fifteen and nine specific diseases and functions are unique to the differentially methylated genes identified by CHARM or Illumina analysis, respectively (Left Venn diagram). Both hypo- and hyper-methylated genes are shown to be involved in 32 diseases and functions identified by Pathway analysis. Nine and twenty-nine diseases and functions represent only hypermethylated genes or only hypomethylated genes, respectively (Right Venn diagram). The inverted pyramid displays the common major biological functions and diseases in the CHARM/Illumina hypo-/hyper-methylated gene list. Disease/function associations with the methylated genes are calculated by IPA using Fisher's exact test, with $p < 0.05$ considered a significant association. Fig. 3b) Direct and indirect connections among differentially methylated genes and genes known to be involved in PE involvement of differentially methylated genes in IPA PE gene database are represented. Only interactions with zero or up to one intermediate were included and the rest were excluded. Upward diagonal bars signify connections with zero intermediates and the black line signifies connections with 1 intermediate. Hypermethylated CYP19A1 shows the highest number of relations with PE related members (preceded by hypomethylated genes). All are hypomethylated genes except three hypermethylated genes indicated by an oval symbol. Fig. 3c) Significant hypermethylated CYP19A1 gene: Methylation status of CYP19A1 was profiled in Infinium HumanMethylation450 assay. Scattered and bar graph (inside the box) shows the hypermethylated status of CYP19A1 in 12 PE cases and 24 controls. Symbol triangle (Δ) indicates PE patient's beta value and open circle (o) indicates control's beta value. Genome

studio analysis profiled the methylation level as a beta value. All error bars, S.E.M. *** $p < 0.001$ were determined by two-way t test. The p -value was corrected for multiplicity using false discovery rate method (p -value < 0.016).

Figs. 4a-4b. First trimester interdependent epigenetic and microRNA biomarkers for severe PE. Fig. 4a) Schematic representation of the epigenetic modifications of DNA methylation, histone acetylation, histone deacetylase expression, and microRNA expression that work in concert to deregulate genes leading to the development of severe PE. The schematic figure indicates that miRNAs work in concert with the methylation machinery and histone modification. Collectively, these mechanisms govern the expression of genes and phenotype of PE. The diagram shows the multifactorial mechanism for the development of PE. Epigenetic and microRNA regulators modulate the combination of several factors including angiogenesis, synthesis of estrogen, trophoblast invasion, hypoxia as well as oxidative stress, endothelial dysfunction and inflammatory response leading to PE. Key regulators located within the rectangular boxes are derived from the study and those markers can regulate genes (the oval boxes) leading to the development of PE. Fig. 4b) Interaction between miR-17-5p, CYP19A1, and HDAC5 (© 2000-2014 Ingenuity Systems, Inc.). Interaction between mir17, HDAC5 and CYP19A1 was analyzed using IPA path explorer (Ingenuity Systems, Inc., Redwood City, CA, USA) and is illustrated in Fig. 4b. miR-17, HDAC5, and CYP19A1 are shown here to be interconnected directly (miR17 targets HDAC5 and CYP19A1 directly) or via intermediate molecules which are known to regulate PE. These three epigenetic biomarkers work in concert regulating the signaling pathways, *e.g.*, 1) angiogenesis and endothelial cell development (*e.g.* BDNF, VEGFA, MAPK1), 2) hypoxia (*e.g.* CREB1, SLC2A4, HIF1A), 3) cell differentiation and movement (*e.g.* BMP2, RB1, STAT3), 4) oxidative stress (*e.g.* TNF), 5) cell death (*e.g.* PTEN, MYC, CDKN1A) and 6) inflammation (*e.g.* IL6, IL8)} in PE. Solid blue lines represent direct interactions between genes and dashed blue lines represent indirect interactions.

Fig. 5. miR-17 targets network genes and their functions: Known miR-17 targeted genes are shown to be primarily involved in angiogenesis. Genes known to be directly involved in PE are highlighted in red. Direct and indirect relationships are shown in solid and dashed lines, respectively. The figure (© 2000-2014 Ingenuity Systems, Inc.) was generated using software from Ingenuity Systems, Inc., Redwood City, CA, USA.

Fig. 6. miR-17 targeted gene network: Network analysis was carried out among the top 11 scored networks targeted by miR-17. These networks are associated with several

primary biological functions namely development of blood vessels, remodeling of blood vessels, cell migration and cell death (bar graph). The bar graph represents the correlation between this network of genes and specific biological function with negative log of p -values (p -values calculated by IPA using Fisher's exact test). The genes (indicated in grey boxes) are the direct high prediction targets of miR-17 or those that are experimentally observed. Solid lines represent direct interactions between members while dashed lines represent indirect interactions.

Figs. 7a-7b. Hierarchical heat map of biological functions and diseases regulated by Fig. 7a) hyper and Fig. 7b) hypo methylated genes. Heat map is displayed here to visualize the various significant diseases and functions related to methylated genes in 1st trimester patients who later developed PE. Classes of biological functions and diseases are represented by the large boxes with title (e.g. reproductive system disease). Each individual colored small box represents a particular biological function. Disease/function associations with the methylated genes are calculated by IPA. p value is designated to show the level of significance. Purple color indicates scores based on negative log of this p -value. Darker shades imply a higher score. The size of the rectangle also reflects increasing overlap significance among the genes involved in particular diseases and functions (using Fisher's exact test p -value).

Fig. 8. Role of CYP19A1 in the development of PE: Pathway analysis was carried out to analyze the relationship between CYP19A1 and genes associated with PE in IPA database. The 'Path Explorer' tool was used to generate the connections between CYP19A1 and the genes/molecules associated with PE. Downregulated CYP19A1 was overlaid in the predicted activity analysis using IPAs 'Molecule Activity Predictor' tool. Known genes in orange boxes depict upregulation and genes in blue boxes indicate downregulation. Rectangles represent genes and rounded rectangles are assigned for endogenous molecules.

Fig. 9. Upregulated or downregulated histones or DNA modifying enzymes in PE patients. $p = 0.03$ for all; $p = 0.04$ for hdac5.

Fig. 10 and Fig. 11. Upregulated miRNAs and specific combinations of mRNAs upregulated in PE patients.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1: Sequence of Histone H4 protein lacking the first methionine residue. The first methionine is removed from this sequence to correctly indicate the position for lysine residues, *e.g.*, lysine 12, lysine 16 and lysine 20.

SEQ ID NOs: 2-45: Sequences of genomic DNA sites that are hypomethylated in PE patients.

SEQ ID NOs: 46-55: Sequences of genomic DNA sites that are hypermethylated in PE patients.

SEQ ID NOs: 56 to 115 and 191: Sequences of pre-miRNAs and mature miRNAs that are differentially expressed in PE patients.

miRNA	SEQ ID NO:	Pre-miRNA	SEQ ID NO:	Mature miRNA
Hsa-miR-7c	56	GCAUCCGGGUUGAGGU AGUAGGUUGUAUGGU UUAGAGUUACACCCUG GGAGUUAACUGUACA ACCUUCUAGCUUCCU UGGAGC	57	UGAGGUAGUAGG UUGUAUGGUU
Hsa-miR-93	58	CUGGGGGCUCCAAAGU GCUGUUCGUGCAGGUA GUGUGAUUACCCAACC UACUGCUGAGCUAGCA CUUCCCGAGCCCCCGG	59	CAAAGUGCUGUU CGUGCAGGUAG
Hsa-miR-128a	60	UGAGCUGUUGGAUUC GGGGCCGUAGCACUGU CUGAGAGGUUUACAU UUCUCACAGUGAACCG GUCUCUUUUUCAGCUG CUUC	61 191	CGGGGCCGUAGC ACUGUCUGAGA OR UCACAGUGAACC GGUCUCUUU
Hsa-miR-140-3p	62	UGUGUCUCUCUCUGUG UCCUGCCAGUGGUUUU	63	UACCACAGGGUA GAACCACGG

		ACCCUAUGGUAGGUUA CGUCAUGCUGUUCUAC CACAGGGUAGAACCAC GGACAGGAUACCGGGG CACC		
Hsa-miR-142- 3p	64	GACAGUGCAGUCACCC AUAAAGUAGAAAGCA CUACUAAACAGCACUGG AGGGUGUAGUGUUUC CUACUUUAUGGAUGA GUGUACUGUG	65	UGUAGUGUUUCC UACUUUAUGGA
Hsa-miR-146b	66	CCUGGCACUGAGAACU GAAUUCCAUAGGCUGU GAGCUCUAGCAAUGCC CUGUGGACUCAGUUCU GGUGCCCGG	67	UGAGAACUGAAU UCCAUAGGCU
Hsa-miR-15a	68	CCUUGGAGUAAAGUA GCAGCACAUAAUGGUU UGUGGAUUUUGAAAA GGUGCAGGCCAUAUUG UGCUGCCUAAAAUA CAAGG	69	UAGCAGCACAU AUGGUUUGUG
Hsa-miR-196b	70	ACUGGUCGGUGAUUU AGGUAGUUUCCUGUU GUUGGGAUCCACCUUU CUCUCGACAGCACGAC ACUGCCUUCAUUACUU CAGUUG	71	UAGGUAGUUUCC UGUUGUUGGG
Hsa-miR-331- 5p	72	GAGUUUGGUUUUGUU UGGGUUUGUUCUAGG UAUGGUCCCAGGGAUC CCAGAUCAAACCAGGC	73	CUAGGUAUGGUC CCAGGGAUCC

		CCCUGGGCCUAUCCUA GAACCAACCUAAGCUC		
Hsa-miR-886- 5p	74	CACUCCUACCCGGGUC GGAGUUAGCUCAAGCG GUUACCUCCUCAUGCC GGACUUUCUAUCUGUC CAUCUCUGUGCUGGGG UUCGAGACCCGCGGGU GCUUACUGACCCUUUU AUGCAAUAA	75	CGGGUCGGAGUU AGCUCAAGCGG
Hsa-miR-17	76	GUCAGAAUAAUGUCA AAGUGCUUACAGUGCA GGUAGUGAU AUGUGC AUCUACUGCAGUGAAG GCACUUGUAGCAUUAU GGUGAC	77	CAAAGUGCUUAC AGUGCAGGUAG
Hsa-miR-26a- 5p	78	GUGGCCUCGUUCAAGU AAUCCAGGAUAGGCUG UGCAGGUCCCAAUGGG CCUAUUCUUGGUUACU UGCACGGGGACGC	79	UUCAAGUAAUCC AGGAUAGGCU
Hsa-miR-26a- 3p	80	GUGGCCUCGUUCAAGU AAUCCAGGAUAGGCUG UGCAGGUCCCAAUGGG CCUAUUCUUGGUUACU UGCACGGGGACGC	81	CCUAUUCUUGGU UACUUGCACG
Hsa-miR-130b	82	GGCCUGCCCGACACUC UUUCCCUGUUGCACUA CUAUAGGCCGCUGGGA AGCAGUGCAAUGAUG AAAGGGCAUCGGUCAG GUC	83	ACUCUUUCCCUGU UGCACUAC

Hsa-miR-7a	84	AGGUUGAGGUAGUAG GUUGUAUAGUUUAGA AUUACAUCAAGGGAG AUAACUGUACAGCCUC CUAGCUUUCCU	85	UGAGGUAGUAGG UUGUAUAGUU
Hsa-miR-29a	86	AUGACUGAUUUCUUU UGGUGUUCAGAGUCA AUAUAAUUUUCUAGC ACCAUCUGAAAUCGGU UAU	87	ACUGAUUUCUUU UGGUGUUCAG
Hsa-miR-517a	88	UCUCAGGCAGUGACCC UCUAGAUGGAAGCACU GUCUGUUGUAUAAAA GAAAAGAUCGUGCAUC CCUUUAGAGUGUUACU GUUUGAGA	89	CCUCUAGAUGGA AGCACUGUCU
Hsa-miR-191	90	CGGCUGGACAGCGGGC AACGGAAUCCCAAAG CAGCUGUUGUCUCCAG AGCAUUCCAGCUGCGC UUGGAUUUCGUCCCCU GCUCUCCUGCCU	91	CAACGGAAUCCCA AAAGCAGCUG
Hsa-miR-296	92	AGGACCCUUCAGAGG GCCCCCCCUCAAUCCU GUUGUGCCUAAUUCAG AGGGUUGGGUGGAGG CUCUCCUGAAGGGCUC U	93	AGGGCCCCCCCUC AAUCCUGU
Hsa-miR-18a	94	UGUUCUAAGGUGCAUC UAGUGCAGAUAGUGA AGUAGAUUAGCAUCU ACUGCCCUAAGUGCUC	95	UAAGGUGCAUCU AGUGCAGAUAG

		CUUCUGGCA		
Hsa-miR-19a	96	GCAGUCCUCUGUUAGU UUUGCAUAGUUGCACU ACAAGAAGAAUGUAG UUGUGCAAUUCUAUGC AAAACUGAUGGUGGCC UGC	97	AGUUUUGCAUAG UUGCACUACA
Hsa-miR-20a	98	GUAGCACUAAAGUGCU UAUAGUGCAGGUAGU GUUUAGUUAUCUACU GCAUUAUGAGCACUUA AAGUACUGC	99	UAAAGUGCUUAU AGUGCAGGUAG
Hsa-miR-19b-1	100	CACUGUUCUAUGGUUA GUUUUGCAGGUUUGC AUCCAGCUGUGUGAUA UUCUGCUGUGCAAUC CAUGCAAAACUGACUG UGGUAGUG	101	AGUUUUGCAGGU UUGCAUCCAGC
Hsa-miR-92a-1	102	CUUUCUACACAGGUUG GGAUCGGUUGCAAUGC UGUGUUUCUGUAUGG UAUUGCACUUGUCCCG GCCUGUUGAGUUUGG	103	AGGUUGGGAUCG GUUGCAAUGCU
Hsa-miR-106a	104	CCUUGGCCAUGUAAAA GUGCUUACAGUGCAGG UAGCUUUUUGAGAUC UACUGCAAUGUAAGCA CUUCUUACAUAACCAU GG	105	AAAAGUGCUUAC AGUGCAGGUAG
Hsa-miR-18b	106	UGUGUUAAGGUGCAU CUAGUGCAGUUAGUG AAGCAGCUUAGAAUCU	107	UAAGGUGCAUCU AGUGCAGUUAG

		ACUGCCCUAAAUGCCC CUUCUGGCA		
Hsa-miR-20b	108	AGUACCAAAGUGCUCA UAGUGCAGGUAGUUU UGGCAUGACUCUACUG UAGUAUGGGCACUCC AGUACU	109	CAAAGUGCUCAU AGUGCAGGUAG
Hsa-miR-19b-2	110	ACAUUGCUACUUACAA UUAGUUUUGCAGGUU UGCAUUUCAGCGUAUA UAUGUAUAUGUGGCU GUGCAAUCCAUGCAA AACUGAUUGUGAUAA UGU	111	AGUUUUGCAGGU UUGCAUUUCA
Hsa-miR-92a-2	112	UCAUCCCUGGGUGGGG AUUUGUUGCAUUACU UGUGUUCUAUAUAAA GUAUUGCACUUGUCCC GGCCUGUGGAAGA	113	GGGUGGGGAUUU GUUGCAUUAC
Hsa-miR-363	114	UGUUGUCGGGUGGAU CACGAUGCAAUUUUGA UGAGUAUCAUAGGAG AAAAAUUGCACGGUA UCCAUCUGUAAACC	115	CGGGUGGAUCAC GAUGCAAUUU

SEQ ID NOs: 116-190: Sequences of the probes for determining methylation of the genomic sites that are differentially methylated in PE patients.

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DETAILED DISCLOSURE OF THE INVENTION

As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are

used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising”. The transitional terms/phrases (and any grammatical variations thereof) “comprising”, “comprises”, “comprise”, “consisting essentially of”, “consists essentially of”, “consisting” and “consists” can be used interchangeably.

“Treatment”, “treating”, “palliating” and “ameliorating” (and grammatical variants of these terms), as used herein, are used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including but not limited to therapeutic benefit. A therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with PE such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with PE.

“Subject” refers to an animal, such as a mammal, for example a human. The methods described herein can be useful in both humans and non-human animals. In some embodiments, the subject is a mammal (such as an animal model of disease), and in some embodiments, the subject is human. The terms “subject” and “patient” can be used interchangeably.

Epigenetic dysregulation during early pregnancy may lead to PE. DNA methylation, histone modification, and miRNA are all inter-related and may work in concert to regulate gene expression leading to PE. The current invention provides that alterations in epigenetic features and miRNA could presage PE and be reflected in tissues of the pregnant mother, for example, during the first trimester in the blood of a pregnant mother who later developed PE.

To discover early noninvasive novel biomarkers of PE, epigenetic (DNA methylation, histone modification, and epigenetic modifying enzyme) and miRNA profiling was conducted in a case-controlled study in the first trimester in tissues of pregnant mothers, for example, in blood of pregnant mothers. Altered expressions of certain miRNA (*e.g.*, upregulated miR-17); altered post-translational modifications of certain histone proteins (*e.g.*, hyperacetylation of H4); altered expression, amount and/or activity of certain histone or DNA modifying enzymes (*e.g.*, decreased HDAC5 protein and/or mRNA); and/or altered methylation of certain genomic DNA sites (*e.g.*, hypermethylated CYP19A1) were found to be interrelated and associated with the development of PE.

Accordingly, an embodiment of the invention provides a method of predicting the development of PE in a subject, the method comprising:

- (a) determining the level of one or more miRNAs in:

i) a test sample obtained from the subject, and

ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to levels of one or miRNAs,

wherein the presence of the one or more miRNAs:

at different levels in the test sample as compared to the control sample, or

relative to the reference values indicates high risk of development of PE in the subject; and

(c) identifying the subject as having high risk of developing PE based on the level of one or more miRNAs in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as not having high risk of developing PE based on the level of one or more miRNAs in the test sample and withholding the therapy to the subject to treat and/or manage PE.

Various techniques are well known to a person of ordinary skill in the art to determine the level of miRNA in a sample. Non-limiting examples of such techniques include microarray analysis, real-time polymerase chain reaction (PCR), Northern blot, *in situ* hybridization, solution hybridization, or quantitative reverse transcription PCR (qRT-PCR). Methods of carrying out these techniques are routine in the art. Additional methods of determining the level of miRNA in a sample are also well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The reference values corresponding to levels of one or miRNAs indicate the level of miRNA associated with no risk or low risk of the development of PE or high risk of development of PE. As such, the reference values corresponding to levels of one or miRNAs may be indicative of the absence or presence of high risk of the development of PE. A reference value associated with no risk or low risk of the development of PE may be obtained based on samples obtained from subjects known to be free of PE. A reference value associated with high risk of the development of PE may be obtained based on samples obtained from subjects known to have PE.

For example, tissues from a group of pregnant women can be obtained during the first trimester and the levels of one or more miRNAs can be determined. The group of women can then be monitored for the development of PE. Reference values corresponding to levels of one or more miRNAs that are associated with low risk or no risk of the development of PE

or high risk of the development of PE can be determined based on the presence of absence of PE in various women whose samples were analyzed. Additional examples of determining references values associated with no risk or low risk or high risk of the development of PE are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The step of identifying the subject as having high risk or not having high risk of developing PE depends on the level of one or more miRNAs in the test sample. For example, if the levels of certain miRNAs in the test sample are significant higher or lower than the levels of corresponding miRNAs in the control sample, the subject is identified as having high risk of development of PE. For example, if the levels of one or more of miR-7a, miR-7c, miR-93, mir-106a, mir-126, miR-128a, miR-130b, miR-140-3p, miR-142-3p, miR-146b, miR-15a-5p, miR-17, miR-191, miR-196, miR-19b-1, miR-20a, miR-331-5p, miR-886-5p, miR-26a, miR-29a, miR-517a and miR-296 miRNAs are higher in the test sample compared to control sample, the subject is identified as having high risk of the development of PE (See Table 1). Thus, a woman is identified as having high risk of the development of PE if the levels of one or more of miR-7a, miR-7c, miR-93, mir-106a, mir-126, miR-128a, miR-130b, miR-140-3p, miR-142-3p, miR-146b, miR-15a-5p, miR-17, miR-191, miR-196, miR-19b-1, miR-20a, miR-331-5p, miR-886-5p, miR-26a, miR-29a, miR-517a and miR-296, are higher in a blood, serum or plasma sample of the woman compared to a control sample.

Table 1. miRNA significantly higher in a PE sample compared to a control sample.

mi-RNA	Mean	Std. Error of Mean	Median	Minimum	Maximum	Std. Deviation	<i>p</i> -value
mir7a	4.3634	1.3870	2.3007	0.0243	16.2470	5.1897	0.015
mir7c	3.7766	1.1799	1.6092	0.1085	12.4587	4.4148	0.018
mir93	2.8628	0.7776	2.3524	0.0349	9.6843	2.9093	0.016
mir106a	2.7417	0.7952	1.2418	0.0599	8.7076	2.9752	0.024
mir126	2.3437	0.6060	1.1842	0.0555	6.5371	2.2675	0.023

mir128a	6.6042	2.2748	3.6634	0.0162	30.0607	8.5116	0.014
mir130b	5.3420	1.5437	3.3804	0.0066	18.8090	5.7760	0.007
mir140-3p	5.2632	1.7166	2.9218	0.0858	20.6024	6.4230	0.014
mir142-3p	2.9965	0.8411	1.5552	0.0206	9.9850	3.1473	0.017
mir146b	3.6190	1.1652	0.9302	0.0472	12.3514	4.3598	0.021
mir15a	7.9395	3.1094	0.8989	0.0922	35.0204	11.6341	0.022
mir17	3.1044	0.9355	1.5041	0.0580	10.9931	3.5002	0.021
mir191	1.7720	0.3352	1.7303	0.0669	4.1845	1.2541	0.019
mir196b	6.3686	2.3113	2.5740	0.0979	28.4629	8.6479	0.019
mir19b	2.9521	0.9334	1.3627	0.0319	11.1013	3.4926	0.028
mir20a	3.2121	0.9201	1.2467	0.0328	10.1598	3.4427	0.016
mir26a	1.9812	0.3617	1.8041	0.0173	4.3605	1.3534	0.009
mir29a	5.9577	2.2675	2.2709	0.1725	28.0031	8.4840	0.024
mir296	3.1084	0.8898	1.7807	0.0478	9.6324	3.3293	0.017
mir331-5p	5.4130	1.6932	1.4936	0.0979	16.1418	6.3354	0.011
mir517a	3.5323	0.9610	2.2434	0.0048	10.2401	3.5957	0.01
mir886-5p	6.3978	2.0627	3.1252	0.0753	21.2943	7.7179	0.011

A further embodiment of the invention provides a kit comprising reagents to carry out the methods of the current invention. In one embodiment, the kit comprises primers or probes specific for miRNAs of interest. Reagents for treating the samples, for example, deproteinization, degradation of DNA, or removal of other impurities can also be provided in the kit.

An aspect of the invention provides a kit, for example, a point-of-care (POC) diagnostic device for assaying one or more miRNAs which can be used to identify the subject as having high risk of the development PE. In another embodiment, the kit comprises an oligonucleotide chip and reagents to conduct the assay to determine the levels of miRNAs corresponding to the oligonucleotides on the oligonucleotide chip. The oligonucleotide chip according to the invention contains oligonucleotides corresponding to a group of miRNAs that are present at different levels in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE.

In one embodiment, the oligonucleotide chip essentially consists of oligonucleotides corresponding to one or more miRNAs selected from miR-7c, miR-93, miR-128a, miR-140-3p, miR-142-3p, miR-146b, miR-15a, miR-196b, miR-331-5p, miR-886-5p, miR-17, miR-26a, miR-130b, miR-7a, miR-29a, miR-517a, miR-191, miR-296, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1, miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, and miR-363 and optionally, one or more control oligonucleotides.

For the purposes of the invention, the term “oligonucleotide chip essentially consists of oligonucleotides” indicates that the oligonucleotide chip contains oligonucleotides corresponding to only those miRNAs that present at different levels in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE and optionally, contain one or more control oligonucleotides.

The control oligonucleotides are oligonucleotides corresponding to an miRNA or messenger RNAs (mRNA) known to be present in the equal amount in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE. Non-limiting examples of control oligonucleotides include oligonucleotides corresponding to mRNAs of 18S, U6 form microRNA, β -actin, β -glucuronidase and Glyceraldehyde-3-phosphate

dehydrogenase (GAPDH). Additional examples of control miRNAs or mRNAs depend on the tissue under examination. A person of ordinary skill in the art can determine control oligonucleotides appropriate for a particular assay and such embodiments are within the purview of the invention.

5 Epigenetic biomarkers of PE according to the invention include post-translational modification of one or more histone proteins. Accordingly, an embodiment of the invention also provides a method of predicting the development PE in a subject, the method comprising:

10 (a) determining the levels of post-translational modifications of one or more histone proteins in:

- i) a test sample obtained from the subject, and
- ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to the levels of post-translational modifications of the one or more histone proteins,

15 wherein the presence of the post-translational modifications in the one or more histone proteins:

at different levels in the test sample as compared to the control sample, or
relative to the reference values indicates high risk of development of PE in the subject; and

20 (c) identifying the subject as having high risk of developing PE based the levels of post-translational modifications in the one or more histone proteins in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as having not having high risk of developing PE based on the levels of post-translational modifications in the one or more histone proteins in the test
25 sample and withholding the therapy to the subject to treat and/or manage PE.

Non-limiting examples of post-translational modifications of histone proteins include methylation, acetylation, ADP-ribosylation, ubiquitination, citrullination, and phosphorylation. The one or more histones can be selected from H1, H2A, H2B, H3, H4 and H5. In one embodiment, hyperacetylation of H3 is indicative of the development of PE.

30 Various techniques are well known to a person of ordinary skill in the art to determine the level of post-translational modifications of one or more histone proteins in a sample. Non-limiting examples of such techniques include protein mass-spectrometry and antibody based analysis.

Determination of post-translation modification of a histone protein by protein spectrometry in a sample involves analyzing protein lysates or purified histone protein from a sample and analyze them by mass spectrometry to identify specific peptides within the histone protein which have different spectrometric behavior based on the presence or absence of post-translational modifications, for example, acetylation, methylation, demethylation. Certain techniques of spectrometric analysis of post-translational modification of proteins are described in Harvey (2005), which is herein incorporated by reference in its entirety.

In a further embodiment, post-translational modification of a histone protein is determined in an antibody based assay using antibody specific for a post-translational modification. For example, acetylation of H4 histone protein on one or more of: N-terminus, Lysine 12, Lysine 16 and methylation/dimethylation on Lysine 20 is determined in an antibody based assay using antibody specific for the recited modification. In another embodiment, two or more antibodies specific for different post-translational modification are used to determine post-translational modification of a histone protein. Non-limiting examples of the antibody based assays include western blot analysis, enzyme immunoassay (EIA), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA) and antigen-antibody precipitation assay. Additional examples of antibody-based assays are well known to a person of ordinary skill in the art and such embodiments are within the purview of the current invention.

Methods of carrying out these techniques are routine in the art. Additional methods of determining the level post-translational modifications of histone proteins in a sample are also well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The reference value corresponding to levels of post-translational modifications of one or more histone proteins indicate the level of post-translational modifications associated with no risk or low risk of the development of PE or high risk of development of PE. As such, the reference values corresponding to levels of post-translational modifications of certain histone proteins may be indicative of the absence or presence of high risk of the development of PE. A reference value associated with no risk or low risk of the development of PE may be obtained based on samples obtained from subjects known to be free of PE. A reference value associated with high risk of the development of PE may be obtained based on samples obtained from subjects known to have PE. For example, tissues from a group of pregnant women can obtained during the first trimester and the levels of post-translational

modifications of one or more histone proteins can be determined. The group of women can then be monitored for the development of PE. Reference values corresponding to levels of post-translational modifications of one or more histone proteins that are associated with low risk or no risk of the development of PE or high risk of the development of PE can be determined based on the presence of absence of PE in various women whose samples were analyzed. Additional examples of determining references values associated with no risk or low risk or high risk of the development of PE are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The step of identifying the subject as having high risk or not having high risk of developing PE depends on the level of post-translational modifications of one or more histone proteins in the test sample. For example, if the levels of certain post-translational modifications of certain histone proteins in the test sample are significant higher or lower than the levels of corresponding post-translational modifications of certain histone proteins in the control sample, the subject is identified as having high risk of development of PE.

For example, a subject is identified as having high risk of the development of PE if H4 histone in a sample from the subject has one or more of:

- a) increased acetylation on N-terminus,
- b) increased acetylation on Lysine 12,
- c) increased acetylation on lysine 16, and
- d) increased methylation/demethylation on lysine 20.

In another example, a woman is identified as having high risk of the development of PE if H4 histone protein (SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG, SEQ ID NO: 1) in a buffy coat sample of blood from the woman has one or more of:

- a) increased acetylation on N-terminus,
- b) increased acetylation on Lysine 12,
- c) increased acetylation on lysine 16, and/or
- d) increased methylation/demethylation on lysine 20.

A further embodiment of the invention provides a kit comprising reagents to carry out the methods of the current invention, for example, identifying a subject as having high risk of the development of PE and optionally, administering therapy to treat and/or manage PE in the subject. The kit comprises reagents to conduct the assay to determine the levels of certain

post-translational modifications of certain histones, for example, an antibody chip containing specific antibodies.

An aspect of the invention provides a kit, for example, POC diagnostic device for assaying one or more post-translational modifications of histone proteins which can be used to identify the subject as having high risk of the development PE. The antibody chip according to the invention comprises or essentially consists of antibodies against histone proteins post-translationally modified on certain residues, wherein the histone proteins are post-translationally modified on certain residues at different levels in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE.

In one embodiment, the antibody chip essentially consists of one or more of antibodies against:

- a) human H4 histone protein acetylated on N-terminus,
- b) human H4 histone protein acetylation on Lysine 12,
- c) human H4 histone protein acetylation on lysine 16, and
- d) human H4 histone protein methylation/demethylation on lysine 20.

For the purposes of the invention, the term “antibody chip essentially consists of antibodies” indicates that the antibody chip contains antibodies against only those post-translationally modified histone proteins that are modified at different levels in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE and optionally, contain one or more control antibodies. The control antibodies can bind to histone proteins regardless of the post-translational modification. Thus, control antibodies can be used to determine the level of certain histone proteins; whereas, the post-translational modification specific antibodies can be used to determine the level of certain post-translational modifications in those histone proteins.

Epigenetic biomarkers of PE according to the invention also include expression, amount and/or activity of histone and DNA modifying enzymes, *i.e.*, one or more enzymes that mediate post-translational modification of histone proteins or modification of DNA, for example, methylation. Accordingly, an embodiment of the invention also provides a method of predicting the development of PE in a subject, the method comprising:

(a) determining the levels of expression, activity and/or amount of one or more histone or DNA modifying enzymes in:

i) a test sample obtained from the subject, and

ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to the levels of expression, activity and/or amount histone proteins or DNA modifying enzymes,

5 wherein the presence of expression, activity and/or amount of one or more histone or DNA modifying enzymes:

at different levels in the test sample as compared to the control sample, or

relative to the reference values indicates high risk of development of PE in the subject; and

10 (c) identifying the subject as having high risk of developing PE based on the levels of one or more histone or DNA modifying enzymes in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as not having high risk of developing PE based on the levels of one or more histone or DNA modifying enzymes in the test sample and withholding
15 the therapy to the subject to treat and/or manage PE if the subject.

Non-limiting examples of modifications of histone proteins include methylation, acetylation, ADP-ribosylation, ubiquitination, citrullination, and phosphorylation. Non-limiting examples of histone modifying enzymes include histone acetyl transferase (HAT), histone deacetylase (HDAC), histone methyltransferase (HMT) and histone demethylase.
20 Non-limiting examples of DNA modifying enzymes include DNA methyl transferase (DNMT). Additional examples of enzymes involved in modifying histone proteins or DNA are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The activity of histone or DNA modifying enzymes in the test sample and optionally,
25 the control sample can be determined by assays to determine the activity of histone or DNA modifying activity, expression and/or amount of histone or DNA modifying enzyme, expression and/or amount of mRNA encoding histone or DNA modifying enzyme. Various techniques are well known to a person of ordinary skill in the art to determine the level of expression, amount and/or activity of one or more histone or DNA modifying enzymes or the
30 corresponding mRNAs. Non-limiting examples of techniques used to determine the activity histone or DNA modifying enzymes include fluorometric and colorimetric assays; whereas, techniques used to determine histone or DNA modifying enzyme amount include mass spectrometry or antibody based assays. Example of techniques used to determine activity of

histone or DNA modifying enzymes are well known to a person of ordinary skill in the art and such methods are within the purview of the invention.

Determination of the amount of an enzyme by protein spectrometry in a sample involves analyzing protein lysates or purified enzymes of interest from a sample by mass spectrometry to identify the amounts of specific peptides within the histone protein. The amounts of enzymes within a sample can be determined based on the amount of peptides originating from the enzyme in the sample.

Non-limiting examples of the antibody based assays which can be used to determine the amount of histone or DNA modifying enzymes in a sample include western blot analysis, EIA, ELISA, RIA and antigen-antibody precipitation assay. Additional examples of antibody-based assays are well known to a person of ordinary skill in the art and such embodiments are within the purview of the current invention.

Methods of carrying out these techniques are routine in the art. Additional methods of determining the level amount or activity of histone or DNA modifying enzymes in a sample are also well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The reference value corresponding to levels of expression, amount and/or activity of histone or DNA modifying enzymes indicate the levels associated with no risk or low risk of the development of PE or high risk of development of PE. As such, the reference values corresponding to levels of expression, amount and/or activity of histone or DNA modifying enzymes may be indicative of the absence or presence of high risk of the development of PE. A reference value associated with no risk or low risk of the development of PE may be obtained based on samples obtained from subjects known to be free of PE. A reference value associated with high risk of the development of PE may be obtained based on samples obtained from subjects known to have PE. For example, tissues from a group of pregnant women can obtained during the first trimester and the levels of expression, amount and/or activity of histone or DNA modifying enzymes can be determined. The group of women can then be monitored for the development of PE. Reference values corresponding to levels of expression, amount and/or activity of histone or DNA modifying enzymes that are associated with low risk or no risk of the development of PE or high risk of the development of PE can be determined based on the presence of absence of PE in various women whose samples were analyzed. Additional examples of determining references values associated with no risk or

low risk or high risk of the development of PE are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The step of identifying the subject as having high risk or not having high risk of developing PE depends on the level of expression, amount and/or activity of histone or DNA modifying enzymes in the test sample. For example, if the levels of expression, amount and/or activity certain of histone or DNA modifying enzymes in the test sample are significant higher or lower than the levels of corresponding enzymes in the control sample, the subject is identified as having high risk of development of PE.

In one embodiment, a subject is identified as having high risk of the development of PE if histone deacetylase 1 (HDAC1) protein, mRNA or activity is increased in a test sample as compared to a control sample or histone deacetylase 5 (HDAC5) protein, mRNA or activity is decreased in a test sample as compared to a control sample. In another example, a woman is identified as having high risk of the development of PE if the HDAC1 protein, mRNA or activity is increased in a buffy coat sample of blood from the woman as compared to a control sample or HDAC5 protein, mRNA or activity is decreased in a buffy coat sample of blood from the woman as compared to a control sample.

Alternately, if the level of Aurora Kinase C (AURKC) protein, mRNA or activity is higher in the test sample compared to the control sample, the subject is identified as having high risk of developing PE (Figure 9).

Further, if the level of Aurora Kinase A (AURKA) or protein arginine N-methyltransferase 8 (PRMT8) protein, mRNA or activity is lower in the test sample compared to the control sample, the subject is identified as having high risk of developing PE (Figure 9).

A further embodiment of the invention provides a kit comprising reagents to carry out the methods of the current invention, for example, identifying a subject as having high risk of the development of PE and optionally, administering therapy to treat and/or manage PE in the subject. The kit comprises reagents to conduct the assay to determine the levels of expression, amount and/or activity of certain histone or DNA modifying enzymes, for example, an antibody chip containing antibodies against certain histone or DNA modifying enzymes or oligonucleotide chips containing mRNAs corresponding to certain histone or DNA modifying enzymes.

The antibody chip or oligonucleotide chip according to the invention contains antibodies or oligonucleotides corresponding to certain histone or DNA modifying enzymes,

wherein the histone or DNA modifying enzymes have different amount, expression and/or activity in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE and optionally, contain one or more control antibodies or control oligonucleotides.

5 In one embodiment, the antibody chip essentially consists of an antibody against HDAC1 and an antibody against HDAC5; whereas, the oligonucleotide chip essentially consists of an oligonucleotide corresponding to HDAC1 mRNA and an oligonucleotide corresponding to HDAC5 mRNA.

10 For the purposes of the invention, the term “chip essentially consists of antibodies or oligonucleotides” indicates that the antibody or oligonucleotide chip contains antibodies or oligonucleotides corresponding only those histone or DNA modifying enzymes that are present at different levels in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE and optionally, contains one or more control antibodies or
15 oligonucleotides. The control oligonucleotides or antibodies correspond to mRNA or proteins known to be present in the equal amount in a sample of an individual having a high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE. Non-limiting examples of control oligonucleotides or antibodies include oligonucleotides or antibodies corresponding to β -
20 actin, β -glucuronidase and GAPDH. Additional examples of control miRNAs or mRNAs depend on the tissue under examination. A person of ordinary skill in the art can determine control oligonucleotides appropriate for a particular assay and such embodiments are within the purview of the invention.

25 An aspect of the invention provides a kit, for example, a POC diagnostic device for assaying one or more histone or DNA modifying enzymes which can be used to identify the subject as having high risk of the development PE.

Epigenetic biomarkers of PE according to the invention also include the level of methylation of certain DNA loci in the genomic DNA of certain cells. Accordingly, an embodiment of the invention also provides a method of predicting the development of PE in a
30 subject, the method comprising:

(a) determining the levels of methylation of one or more sites in the genomic DNA in:

i) a test sample obtained from the subject, and

ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to levels of methylation of the one or more sites,

5 wherein the presence methylation of one or more sites in the genomic DNA:
at different levels in the test sample as compared to the control sample, or
relative to the reference values indicates high risk of development of PE in the
subject; and

(c) identifying the subject as having high risk of developing PE based the levels of
10 methylation of the one or more sites in the genomic DNA in the test sample and optionally,
administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as not having high risk of developing PE based the levels
methylation of the one or more sites in the genomic DNA in the test sample and withholding
the therapy to the subject to treat and/or manage PE.

15 As used herein, the term “level of methylation” as applied to a genomic site refers to
whether one or more cytosine residues present in a CpG context have or do not have a
methylation group. The level of methylation may also refer to the fraction of cells in a
sample that do or do not have a methylation group on such cytosines. These cytosines are
typically in the promoter region of the gene, though may also be found in the body of the
20 gene, including introns and exons. The Beta-value (Figure 5) is a ratio between methylated
probe intensity and total probe intensities (sum of methylated and demethylated probe
intensities). It is in the range of 0 and 1, which can also be interpreted as the percentage of
methylation.

Various techniques are well known to a person of ordinary skill in the art to determine
25 the level of methylation of one or more sites in the genomic DNA in a sample. Non-limiting
examples of such techniques include bisulfite conversion, digestion by restriction enzymes
followed by polymerase chain reaction (Combined Bisulfite Restriction Analysis, COBRA),
direct sequencing, cloning and sequencing, pyrosequencing, mass spectrometry analysis or
probe/microarray based assay. Certain techniques of determining methylation of genomic
30 sites are described in Eads *et al.*, Xiong *et al.*, Paul *et al.*, Warnecke *et al.*, Tost *et al.*, and
Ehrich *et al.*, the contents of which are herein incorporated in their entirety. Additional
techniques for determining DNA methylation of one or more sites in the genomic DNA of a

sample are well known to a person of ordinary skill in the art and such techniques are within the purview of the invention.

The reference value corresponding to levels methylation of one or more sites in the genomic DNA indicate the levels associated with no risk or low risk of the development of PE or high risk of the development of PE. As such, the reference values corresponding to levels of methylation of one or more sites in the genomic DNA may be indicative of the absence or presence of high risk of the development of PE. A reference value associated with no risk or low risk of the development of PE may be obtained based on samples obtained from women known to be free of PE. A reference value associated with high risk of the development of PE may be obtained based on samples obtained from women known to have PE. For example, tissues from a group of pregnant women can obtained during the first trimester and the levels methylation of one or more sites in the genomic DNA can be determined. The group of women can then be monitored for the development of PE. Reference values corresponding to the levels of methylation of one or more sites in the genomic DNA that are associated with low risk or no risk of the development of PE or high risk of the development of PE can be determined based on the presence of absence of PE in various women whose samples were analyzed. Additional examples of determining references values associated with no risk or low risk or high risk of the development of PE are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The step of identifying the subject as having high risk or not having high risk of developing PE depends on the levels methylation of one or more sites in the genomic DNA in the test sample. For example, if the levels of methylation of one or more sites in the genomic DNA in the test sample are significant higher or lower than corresponding levels in the control sample, the subject is identified as having high risk of development of PE. Table 2 provides the Illumina ID, the corresponding sequences of the genomic sites and the level of methylation of the genomic sites in PE patients.

In one embodiment, a subject is identified as having high risk of the development of PE if the methylation of one or more genomic sites selected from SEQ ID NOs: 46-55 is increased in the genomic DNA of a test sample as compared to a control sample. In a specific embodiment, a woman is identified as having high risk of the development of PE if methylation of genomic site CYP19A1 (SEQ ID NO: 46) is increased in a sample, for example, buffy coat sample of blood, from the woman as compared to a control sample.

In another embodiment, a subject is identified as having high risk of the development of PE the methylation of one or more genomic sites selected from SEQ ID NOs: 2-45 is decreased in the genomic DNA of a test sample as compared to a control sample. A further embodiment of the invention provides a kit comprising reagents to carry out the methods of the current invention. The kit comprises reagents to conduct the assay to determine the levels of methylation of certain sites in the genomic DNA in certain cells of a subject. The kit can include reagents for isolation of genomic DNA from a sample, reagents to treat the genomic DNA, for example, bisulfite treatment, specific primers to analyze the genomic sites of interests and reagents for PCR amplification of the sites of interest.

An aspect of the invention provides a kit, for example, POC diagnostic device for assaying methylation of one or more sites in the genomic DNA which can be used to identify the subject as having high risk of the development PE. PE arises from a complex interplay among several factors. Epigenetic mechanisms and miRNAs closely interact with each other, thereby creating reciprocal regulatory circuits which lead to gene regulation (Fig. 4a). The invention identifies novel interactive sets of epigenetic and miRNA biomarkers in the first trimester which can be used to predict the development of PE. Accordingly, one embodiment of the invention provides the methods of predicting, treating and/or managing PE in a subject; the method comprises determining two or more, for example, three, four, five, six, seven, eight, nine or ten biomarkers described herein to identify a subject as having a high risk of the development of PE. The multiple biomarkers can belong to the same class, for example, multiple miRNAs or multiple post-translational modifications of histone proteins; or the multiple biomarkers can be chosen from different classes, for example, a combination of miRNAs, post-translational modification of histones, histone or DNA modifying enzymes or methylation of certain genomic DNA sites. In an embodiment, the multiple biomarkers do not contain any biomarker from one or more classes described herein, *e.g.*, the multiple biomarkers may not contain an miRNA, a post-translational modification of histone, a histone or DNA modifying enzyme or a methylation of a genomic DNA site.

Accordingly, an embodiment of the invention also provides a method of predicting the development of PE in a subject, the method comprising:

(a) determining the levels of two or more biomarkers selected from one or more of miRNA, post-translational modification of histones, histone or DNA modifying enzymes, methylation of certain genomic DNA sites in:

i) a test sample obtained from the subject, and

ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to levels of one or more biomarkers,

wherein the presence of two or more biomarkers:

at different levels in the test sample as compared to the control sample, or
relative to the reference values indicates high risk of development of PE in the subject; and

(c) identifying the subject as having high risk of developing PE based on the levels of two or more biomarkers in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as not having high risk of developing PE based on the levels of two or more biomarkers in the test sample and withholding the therapy to the subject to treat and/or manage PE.

The combination of two or more biomarkers can be selected from the miRNAs, post-translational modification of histones, histone or DNA modifying enzymes, methylation of certain genomic DNA sites described earlier in this disclosure. In one embodiment, a subject is identified as having high risk of the development of PE if all of the analyzed biomarkers are significant different between the test sample and the control sample. In another embodiment, a subject is identified as having high risk of the development of PE if a pre-determined number of biomarkers out of the analyzed biomarkers are significant different between the test sample and the control sample. For example, if five biomarkers are analyzed, a subject can be identified as having high risk of the development of PE if more than three biomarkers are significant different between the test sample and the control sample.

In another embodiment, the comparison in the levels of two or more biomarkers between the test sample and the control sample is performed by as a combination of the two or more biomarkers, for example, by multivariable analysis. An example of multivariable analysis is multiple regression analysis. When the levels of two or more biomarkers are compared between the test sample and the control sample as a combination, the two or more biomarkers as a combination can be identified as significantly different between the two samples despite one or more of the multiple biomarkers not being different when considered individually.

In an embodiment, levels of four biomarkers, namely, miR-17, post-translational modification of H4 histone protein, amount of HDAC5 mRNA and/or protein and methylation of CYP19A1 site are determined. A subject is identified as having a high risk of the development of PE if the subject has increased miR-17, hyperacetylated H4 histone protein, decreased HDAC5 mRNA and/or protein and hypermethylated CYP19A1.

A further embodiment of the invention provides a kit, for example, POC diagnostic device, for identifying a subject as having high risk of PE based on the levels of two or more biomarkers. The POC device of the invention provides low-tech and cost-effective tool that still produces an accurate measurement, is portable, physically strong (compared to chip/sensing device), and simple to use. The kit can be used by virtually anyone, anywhere.

3-D printing technique can be used to manufacture the housing of the kit. Recycled materials, for example, recycled thermoplastic with added fibrous reinforcement, can be used to reduce the material cost and produce a light weight and unbreakable biomarker tool.

The invention provides a POC device capable of assaying miR-17, methylated CYP19A1, HDAC5 mRNA and acetylation of histone H4 in a sample, for example, a blood sample obtained from a subject. The sample can be treated before subject the sample to the analysis using the POC device.

The POC device can comprise of one or more locations for the introduction of the treated or untreated sample, which can be directed to two or more compartments, wherein each compartments is designed to assay different biomarker. For example, the POC device comprises of four compartments: one for assaying miR-17, one for assaying acetylation of H4 histone protein, one for assaying HDAC5 mRNA, and one for assaying methylation of CYP19A1.

Accordingly, a POC for reliable and rapid detection of biomarkers described herein is provided. In one embodiment, the POC utilizes an opto-fluidics-based platform for use as a biosensor.

In a certain embodiment, the POC incorporates functionalized colloidal nanoparticles trapped at the entrance to a nanofluidic channel providing a robust means for analyte detection at trace levels using surface enhanced Raman spectroscopy. The POC device can be used for sensitive detection of epigenetic modification in either blood or urine, is small and inexpensive, and can provide results in less than 15 minutes. Briefly, following the introduction of blood or urine, small molecules in the sample would compete with competing probes or aptamers depending on the molecule of interest. These probes will be already pre-

bound to small molecule derivatives, and Raman reporter molecules attached on nanoparticles. The competition releases the gold particles which then aggregate at a nanochannel constriction downstream. To demonstrate the diagnostic potential of the system, a “gate” can be imposed; *i.e.*, the lowest and highest value obtained from a healthy sample
5 considered the healthy range. All values beyond this range can be assumed to be indicative of a change from normal conditions. As such, a low-cost, rapid, sensitive epigenetic diagnostic and prognostic tool for early detection of pre-eclampsia is provided.

To practice the methods described herein for identifying a subject as having high risk of the development of PE, control samples can be obtained from one or more of the
10 following:

- a) an individual belonging to the same species as the subject and not having PE,
- b) an individual belonging to the same species as the subject and known to have a low risk or no risk of developing PE, or
- c) the subject prior to becoming pregnant.

15 Additional examples of control samples are well known to a person of ordinary skill in the art and such embodiments are within the purview of the current invention.

In certain embodiments, the control sample and the test sample are obtained from the same type of an organ or tissue. Non-limiting examples of the organ or tissue which can be used as samples are placenta, brain, eyes, pineal gland, pituitary gland, thyroid gland,
20 parathyroid glands, thorax, heart, lung, esophagus, thymus gland, pleura, adrenal glands, appendix, gall bladder, urinary bladder, large intestine, small intestine, kidneys, liver, pancreas, spleen, stoma, ovaries, uterus, skin, blood or buffy coat sample of blood. Additional examples of organs and tissues are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

25 In certain other embodiments, the control sample and the test sample are obtained from the same type of a body fluid. Non-limiting examples of the body fluids which can be used as samples include amniotic fluid, aqueous humor, vitreous humor, bile, blood, cerebrospinal fluid, chyle, endolymph, perilymph, female ejaculate, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum,
30 saliva, sputum, synovial fluid, vaginal secretion, blood, serum or plasma. Additional examples of body fluids are well known to a person of ordinary skill in the art and such embodiments are within the purview of the invention.

The methods described herein can be used to identify a subject as having high risk of the development of PE. In certain embodiments, the subject is a mammal. Non-limiting examples of mammals include human, ape, canine, pig, bovine, rodent, or feline.

In one embodiment, the methods described herein are used to identify a pregnant woman as having high risk of the development of PE. In another embodiment, the methods described herein are performed during the first trimester of pregnancy of a woman to identify the woman as having high risk of the development of PE.

Once a subject is identified as having high risk of the development of PE based on the methods described herein, the step of treating and/or managing PE includes one or more of:

- a) administering medications to lower blood pressure: these medications, called antihypertensives, are used to lower blood pressure,
- b) administering corticosteroids,
- c) administering anticonvulsant medications, for example, magnesium sulfate,
- d) bed rest for the patient,
- e) hospitalization to perform regular non-stress tests or biophysical profiles to monitor the fetus' well-being and measure the volume of amniotic fluid
- f) administering low-dose aspirin,
- g) administering calcium supplements
- h) inducing delivery before natural labor is initiated.

As such, the invention provides that epigenetics and miRNA regulation provides very early manifestation of PE pathogenesis – one that presages the clinical onset of PE by a few months, for example, four to five months. In addition, the invention indicates a new paradigm of discovering interactive epigenetic biomarkers for prediction of PE at a very early stage, for example, during the first trimester. This study also paves new avenues to look for biomarkers in a unique perspective for other diseases. These epigenetic changes happen prior to gene expression, and they are often reversible, making them good candidates for therapeutic interventions.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1 – ANALYSIS OF SAMPLES TO DETERMINE MIRNA BIOMARKERS OF PE

First trimester blood samples and uterine artery Doppler ultrasonography were obtained from 1007 women between 11 and 13 6/7 weeks of gestation. Epigenetic and miRNA profiling was performed on the serum or buffy coat samples from total of 51 controls and 17 severe PE cases. Cases and controls were closely matched with respect to age, sex, body mass index (BMI) and other relevant parameters (Table 3).

To compare PE women with controls, 381 miRNAs in 28 patients (C = 14, PE = 14) were examined. Expression profiles revealed 22 significantly upregulated miRNAs which are shown to be involved in reproductive system disease ($p < 0.02$) (Figs. 1a, 1b, 1c, Table 4). Out of these 22 miRNAs, namely, 7c, 93, 128a, 140-3p, 142-3p, 146b, 15a, 196b, 331-5p, 886-5p) are identified as novel biomarkers of PE.

Upregulation of certain miRNAs, namely, 17, 26a, 130b, 7a, 29a, 517a, 191 & 296 in the third trimester in the serum or in the placenta is reported. Conversely, Wang *et al.* (2008) and Hong *et al.* (2014) showed that miR-126 functions as a pro-angiogenic factor in rat placenta and is decreased in endothelial progenitor cells in term placenta of PE patients. However, evidence showed that miR-126 functions as a pro-angiogenic factor in rat placenta and is decreased in endothelial progenitor cells in term placenta of PE patients.

Several groups showed that the miR-17 cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1) and its paralog, the miR-106a cluster (miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, and miR-363) are significantly increased in term placentas of PE women. This is consistent with the results at 11-13 weeks (Fig. 1d) provided in the instant invention. In addition, the miR-17~92 cluster has been established as an anti-angiomiR and therefore can lead to inhibition of angiogenesis which is a hallmark of PE (Fig. 5).

EXAMPLE 2 – ANALYSIS OF SAMPLES TO DETERMINE POST-TRANSLATIONAL MODIFICATIONS OF HISTONES AS BIOMARKERS OF PE

Post-translationally modified (PTM) histones in buffy coat samples were characterized using reverse-phase liquid chromatography mass spectrometry. The profiles showed several species that corresponded in mass to core and linker histones variants and their PTM isoforms (data not shown, Su *et al.* (2007)). Multiple PTMs were measured; however, the core histones (H3, H2A, H2B) showed complicated spectra due to the presence

of multiple variants and a high degree of PTMs, in particular acetylation and methylation. ELISA study showed no changes in H2A in PE serum. Histone H4 showed unique spectra as H4 does not have sequence variants (uncomplicated by multiple variants, unlike other histones).

5 In H4 spectra, the most abundant species was observed at 11,306 Da which correspond in mass with dimethylation (DiMe) and N-terminal acetylation (N-Ac) of H4 (Su *et al.* (2007)). The next most abundant peaks, 11,348 Da and 11,390 Da, correspond to additional H4 acetylation (Su *et al.* (2007)). Ratio of relative abundance of 11349/11307 peak area demonstrated the H4 acetylation levels in patients with and without PE.

10 The statistical power was limited by small sample size (PE = 8, C = 8); however, more H4 acetylation was observed in PE patients ($p = 0.09$) compared to controls (Fig. 2a). Based on previous reports and peptide mass mapping and tandem mass spectrometry, the 11,306 Da peak was identified as N-Ac + K20DiMe; the 11,348/9 Da peak was identified as N-Ac + K16Ac + K20DiMe; and the 11390/1 Da peak was identified as N-Ac + K16Ac +
15 K12Ac + K20DiMe.

The evidence suggests that histone acetylation may mediate development of chronic inflammation by modulating the expression of pro-inflammatory cytokine TNF- α and interleukins, and activation of the transcription factor NF- κ B. These molecules are constitutively produced by a variety of cells under chronic inflammatory conditions, which in
20 turn leads to the development of major diseases such as PE. The invention provides that H4 acetylation is associated with PE.

EXAMPLE 3 – ANALYSIS OF SAMPLES TO DETERMINE HISTONE OR DNA MODIFYING ENZYMES AS BIOMARKERS OF PE

25 The expression levels of 81 epigenetic genes which are involved in histone modifications and DNA methylation was measured. Two of the eleven HDACs were significantly altered with no changes in HAT expression ($p < 0.05$) (Fig. 2b). Even though increased HDAC1 did not correspond with hyperacetylation, this may reflect specific actions of individual HDACs. The decrease in HDAC5 has been shown to increase H4 acetylation in
30 an unrelated study. This corresponds well to PE patients' hyperacetylated histone H4 profile. Thus, modulation of HDAC or histone acetylation levels may represent an underlying cause/consequence of cytokine dysregulation in PE.

In addition to the HDACs, aurora kinase A (AURKA), aurora kinase C (AURKC), and protein arginine methyltransferase 8 (PRMT8) were significantly altered ($p < 0.05$).

EXAMPLE 4 – ANALYSIS OF SAMPLES TO DETERMINE LEVEL OF METHYLATION OF GENOMIC DNA SITES AS BIOMARKERS OF PE

DNA methylation was determined using Comprehensive High-throughput Arrays for Relative Methylation (CHARM) [6 Control (C) and 6 PE patients]. Significant ($p \leq 0.05$) hypomethylation was associated with 81 genes in PE patients with an average methylation difference of 37.5% (min: 30%, max: 45%).

Using Infinium HumanMethylation450 assay in 36 samples (PE = 12, C = 24), 5904 significant CpG islands ($p < 0.05$) were identified to be associated with PE. Out of 5904 islands, 86 CpG islands were significantly methylated (adjusted p -values, corrected for multiplicity- $q = 0.01$). Of the 86 sites, 54 were associated with genes (10 hypermethylated & 44 hypomethylated, Table 2). Repetition of the same genes was not observed when the results of CHARM and Infinium assays were compared (Fig. 3a); however, repetitions were observed in the same families (*e.g.*, solute carrier (SLC) family, zinc finger protein (ZNF) family), related upstream and downstream regulators, and pathways (Table 5).

A large portion of these gene sets do not have a known function but several have been reported in the context of PE or a function related to PE (*e.g.* angiogenesis, invasion, migration *etc.*) (Fig. 3b). Using TARGETSCAN, mirBase and IPA, several of these genes are discovered as targets of the significantly upregulated miRNAs. In epigenetic gene expression assay changes in DNA methyl transferase were not observed. This lack of correlation may indicate that DNA methyltransferase (DNMT) activity might be the primary route, or frequency of DNA methylation may result from other mechanisms besides expression of DNMT since both hyper and hypo methylation are observed.

Hypermethylated CYP19A1 is provided as a first trimester PE biomarker (Fig. 3c). The synthesis of estrogens from C₁₉ steroids is catalyzed by aromatase P450 (P450arom, product of the *hCYP19A1* gene) and the ability of the human placenta to synthesize estrogens is vastly increased after the ninth week of gestation. Placental aromatase deficiency has been found in PE. In addition, biologically active estrogens and their metabolites formed by placental aromatase may also enhance angiogenesis and uteroplacental blood flow and reduce systemic vascular resistance. The scenario is mostly hampered in PE. Since the blood was collected after nine weeks of gestation, decreased CYP19A1 was expected in the patients

who develop PE. Epigenetic modifications presage any gene expression and pathophysiology (Fig. 4a). A hypermethylated gene leads to decreased gene expression which is expected to be evident at later gestation. Upregulated mir-17 and-106a cluster was associated with decreased expression of CYP19A1 in term placenta (correspondence of decreased estrogen levels with increasing severity of PE). These miRNA clusters inhibited trophoblast differentiation by repressing CYP19A1. miR-17 is also predicted to target HDAC5 and subsequently a decrease in HDAC5 has been shown to increase H4 acetylation. In addition, target analysis has shown that miR-17 and other miRNAs target several well-known PE markers, PAPP, VEGF, MMP, *etc.* Evidence also supports a role to these four markers in other PE pathologies (*e.g.* hypoxia, oxidative stress, inflammatory response, invasion, placental insufficiency) (Fig. 5). Therefore, these four interacting biomarkers underline the robustness of this analysis and also strengthen the previously published work and might serve as novel predictors of PE.

PE arises from a complex interplay among several factors. The invention shows that epigenetic mechanisms and miRNAs closely interact with each other, thereby creating reciprocal regulatory circuits which lead to gene regulation (Fig. 4a). The invention identifies novel interactive sets of noninvasive epigenetic and miRNA biomarkers in the first trimester which have a strong potential to predict the future development of PE.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

Table 2. Illumina ID, related genomic sequences and the level of methylation of the genomic sites in PE patients compared to control

Illumina ID*	Gene Name	Methylation status in PE Patients	Sequence of the genomic site	AlleleA_ProbeSeq**	AlleleB_ProbeSeq***	Ch. No.	Strand Orientation****	UCSC RefGene Accession
cg00073460	ZC3H12D	hypo	CGGTACTCACAG CTGGACACAAAC ATAGCTTGCAGG AGGAAGAGTGTC AG (SEQ ID NO: 2)	TCTAACACTCTTC CTCCTACAAACTA TATTATATCCAA CTATAAATACC (SEQ ID NO: 116)		6	F	NM_207360
cg00522231	ITGB1BP1	hypo	CGTCCGCCAGGG AAGCTGTCAGGG ATTATCTGCGGTT CCTGAGTAGCTG A (SEQ ID NO: 3)	TCAACTACTCAA AACCACAAATAA TCCCTAACAACTT CCCTAACAAACA (SEQ ID NO: 117)	TCAACTACTCA AAAACCGCAA ATAATCCCTAA CAACTTCCCTA ACGAACG (SEQ ID NO: 170)	2	R	NM_004763; NM_022334
cg00616135	LACTB	hypo	TTAGTTTGGATC CTCAACTTCTGGA ACAGCGCAGGGC ACACAGTAGACG (SEQ ID NO: 4)	CTTAATTTTAAAT CCTCAACTTCTAA AACAAACRCAAA CACACAATAAAC (SEQ ID NO: 118)		15	F	NM_032857; NM_171846
cg01844274	SYNE1	hypo	GTTACTCTTCCAG GGTGACACACAAG AGGCAATGAAGC CCAGGGAATTAC G (SEQ ID NO: 5)	TATTACTCTTCCA AAATACACACAA AAAACAATAAAA CCCCAAAAATTA C (SEQ ID NO: 119)		6	R	NM_182961; NM_033071

cg02203224	ARL6IP4; OGFOD2	hypo	CGCTGAAGCCCC ATTCCAGACCCCTG CTTCTGACAAACC TGAACCTAAGGCA (SEQ ID NO: 6)	CTACCTTAATTCA AATTTATCAAAA ACAAAATCTAAA ATAAACTTCAA C (SEQ ID NO: 120)		12	R	NM_018694; NM_00100225 1; NM_024623; NM_016638; NM_00100225 2
cg02313130	CAPN8	hypo	CGAGATGGGAAG ATTATCCTGACCC TAAATACACAAG TGTCCCTAAGAGG A (SEQ ID NO: 7)	TTCTCTTAAAAAC ACTTATATATTTA AAATCAAAATAA TCTTCCCATCTC (SEQ ID NO: 121)		1	F	NM_00114396 2
cg02452209	PTPRN2	hypo	CGGCCGCGGCTCT GATGCTTTTGCAG GCGGCATTGTGTC ACTGATTCAC (SEQ ID NO: 8)	AATAAATCAATA ACACAATACCAC CTACAAAACAT CAAAACCACAA CA (SEQ ID NO: 122)	AATAAATCAAT AACACAATACC GCCTACAAAAA CATCAAAACCG CGACCG (SEQ ID NO: 171)	7	R	NM_002847; NM_130842; NM_130843
cg02557110	SLC12A7	hypo	CGCTGGCTCTGCT TCCATTCCCTGAAG TCTCAGGCTCTCC CAGGGTGTCTCAG (SEQ ID NO: 9)	TCTAACACCCCTAA AAAAACCTAAAA CTTCAAAAATAA AAACAAAACCAA C (SEQ ID NO: 123)		5	R	NM_006598
cg03484267	KLF7	hypo	CGGCCCCCGCAG CCGTCACGGCTGC TGCAGCTGTTGCG ACCCCTCCCACC (SEQ ID NO: 10)	AATAAAAAAAT CACAACTACTAC AACAACTATAAC AACTACAAAAAC CA (SEQ ID NO: 124)	AATAAAAAA ATCGCAACAAC TACAACAACCG TAACGACTACG AAAAACCG (SEQ ID NO: 172)	2	F	NM_003709

cg04546999	SPRR1A	hypo	CAGTGCCAAAAA ATATCAGGTGGT GTTTCATCAAAA AGCTGAGCCAAC CG (SEQ ID NO: 11)	CCAATACCAAAA AATATCAAAATA TATTCATCAAAA AACTAAACCAAC C (SEQ ID NO: 125)		1	R	NM_005987
cg05337441	APOB	hypo	CGCCCCCATCCT GAGCCTGCAGGG GCCGCCAGCTGG TCCAATCCCCCA (SEQ ID NO: 12)	ATAAAAAAATTA AACCAACTAACR ACCCCTACAACT CAAAATAAAAAA C (SEQ ID NO: 126)		2	F	NM_000384
cg05747459	CNKSR2	hypo	TTGTCTCCAGCTA GAGGGCGCGGA GCGGCCAGAGAG CTAGAGGGCAGC G (SEQ ID NO: 13)	TTATCTCCAAC AAAAAACACAAA ACAACCAAAAAA CTAAAAAACCAAC A (SEQ ID NO: 127)	TTATCTCCAAC TAAAAAACGCG GAAACGACCA AAAAACTAAA AAACAACG (SEQ ID NO: 173)	X	R	NM_00116864 9; NM_00116864 8; NM_014927; NM_00116864 7
cg05775542	NAPG	hypo	GAACTGCCACAA AGTCATAGCTTCT TTTTTTTCTTGA GATAGGGTCTCG (SEQ ID NO: 14)	AAACTACCACA AAATCATAACTTC TTTTTTTCTTAA AATAAAATCTC (SEQ ID NO: 128)		18	F	NM_003826
cg06109379	IQSEC3	hypo	GTGGAGTCACCC GGCCACACTCGG GTGGGGCCCCAGG AATGGACGGGG CG (SEQ ID NO: 15)	ATAAAATCACCC AACCACACTCAA ATAAAACCCAAA AATAAACAAAAA CA (SEQ ID NO: 129)	ATAAAATCACCC CGACCACACTC GAATAAAACCC AAAAATAAAC GAAAACG (SEQ ID NO: 174)	12	F	NM_00117073 8; NM_015232

cg07532159	LAMA2	hypo	ATCTCATGGTTCA CCGTTTTTTAAGC CCGTCGGAAAG CGCAGTATTCCG (SEQ ID NO: 16)	ATCTCATAATTCA CCATTTTTTAAAC CCATCAAAAAAA CACAAATATCCA (SEQ ID NO: 130)	ATCTCATAATT CACCGTTTTT AAACCCGTCGA AAAAACGCAAT ATTCCG (SEQ ID NO: 175)	6	R	NM_00107982 3; NM_000426
cg08035151	LSM2	hypo	CGAGGAAACTGA GGCTTAGATCAG CTATACCACTGT TCAAGTCTACAA A (SEQ ID NO: 17)	TTTTATAAACTTA AACAAATAATAT AACTAATCTAAA CCTCAATTTCTC (SEQ ID NO: 131)		6	R	NM_021177
cg08944086	ADARB2	hypo	CGGTCCCATCCCT CCAGCGTCCCGCT CAGCTCCAGCAG CCAGGAGCCCGC (SEQ ID NO: 18)	ACAAACTCCTAA CTACTAAAACTA AACAAACACACTA AAAAAATAAAAC CA (SEQ ID NO: 132)	ACGAACTCCTA ACTACTAAAC TAAACGAAACG CTAAAAAATA AAACCG (SEQ ID NO: 176)	10	F	NM_018702
cg09268718	SCARF1	hypo	CGCCCCGCCCGCT CACAGGTCTCCGC GCAGCCTCGCTCA CCTGTGTCCGC (SEQ ID NO: 19)	ACAAACACAAAT AAACAAAACTAC ACAAAAACCTAT AAACAAACAAAA CA (SEQ ID NO: 133)	ACGAACACAA ATAAACGAAAC TACGCGAAAC CTATAAACGAA CGAAACG (SEQ ID NO: 177)	17	F	NM_145352; NR_028075; NM_003693; NR_028076; NM_145350
cg09276451	VASN; CORO7	hypo	CCTCATAGGCATC TGGGCTGTGACG CTTAGGATTCTTA AATAGTCTCTCG (SEQ ID NO: 20)	ACCTCATAAACAT CTAAACTATAACR CTTAAATTCCTA AATAATCTCTC (SEQ ID NO: 134)		16	R	NM_138440; NM_024535

cg12184421	CD247	hypo	CGCTTAGTGTCTCT GAGCATCTGTGG GAAGCTGACACA GCCTCACTCCTGC (SEQ ID NO: 21)	AACAAAAATAAA ACTATATCAACTT CCCACAAATACTC AAACACTAAAC (SEQ ID NO: 135)		1	F	NM_198053; NM_000734
cg12353636	PCDH21	hypo	TGTTACAGTTCTC ATTGGGAGGTTTC TCCTTGAGCATGA ACTTGGTAGCG (SEQ ID NO: 22)	TTATTACAATTCT CATTAATAAATTT CTCTTTAAACATA AACTTAATAAC (SEQ ID NO: 136)		10	F	NM_033100
cg13064046	SCAMP5	hypo	CGGCTCACTGCA AGTCCGCCTCGG GAAACACATGGG GTGGTTCCACCTC (SEQ ID NO: 23)	AAAAATAAAACC ACCCCATATTTT CCCRAAACRAAA CTTACAATAAAC (SEQ ID NO: 137)		15	R	NM_138967
cg13259177	RASA3	hypo	GGGGGCCCGGCT GATGGGACCCG GCTGATGGGGG CCGGAAGACAA CG (SEQ ID NO: 24)	AAAAACCCAACT AATAAAAAACCA ACTAATAAAAAA CCAAAAAACAA CA (SEQ ID NO: 138)	AAAAACCCGAC TAATAAAACC CGACTAATAAA AAACCGAAAA AACACG (SEQ ID NO: 178)	13	R	NM_007368
cg14741114	TTY15	hypo	CGCCGCGACCTG CGACCCCTCCAAG ACCCACCCCGC CAAGCCCGCCCC C (SEQ ID NO: 25)	AAACAAAAACTT AACAAAAATAAA ATCTTAAAAATC ACAAATCACAA A (SEQ ID NO: 139)	AAACGAAACT TAACGAAAAATA AAATCTTAAA AATCGCAATC GCGACG (SEQ ID NO: 179)	Y	R	NR_001545
cg15930811	C1orf151	hypo	CGCCATTTTATAT ATGGGACTTGAG CATCTGCATTT GGTAAGTGGAG	TCTCRCAATTACC AAAAACAAAAAT ACTCAAATCCCAT ATATAAAATAAC		1	R	NM_00103236 3

				(SEQ ID NO: 26)	(SEQ ID NO: 140)					
cg16027847	WDR27	hypo		CGGGACCTGCAG CCTGACATGCCCG AGCCCCACCCCTG CCACTCCCCTGA (SEQ ID NO: 27)	TCACAAAATAA CAAAAATAAAC TCAAAACATATCA AACTACAAATCC CA (SEQ ID NO: 141)	TCACGAAAATA ACAAAATAA AACTCGAACAT ATCAAACTACA AATCCCG (SEQ ID NO: 180)	6	F		NM_182552
cg16627211	AP3S1	hypo		CGCTACTGCAGC ATAAATTAGCTCA TCCTGACTGATAA CAAAAGGGATAT (SEQ ID NO: 28)	AAATATCCCTTTA TTATCAATCAAAA TAAACTAATTAT ACTACAATAAC (SEQ ID NO: 142)		5	R		NM_001284
cg16887334	OXT	hypo		CGCACTCGGCCTG ACCCACGGCGAC CCTCTGTGACCAA TCATACTACCAA (SEQ ID NO: 29)	TTAATAATATAAT TAATCACAAAAA ATCACCATAAATC AAACCAAAATACA (SEQ ID NO: 143)	TTAATAATATA ATTAATCACAA AAAATCGCCGT AAATCAAACCG AATACG (SEQ ID NO: 181)	20	F		NM_000915
cg17293719	ZNF645	hypo		AACCCATTATCAA CGTCATTAGGATC CAAGTTTCGGCTC ACAAGGGACCG (SEQ ID NO: 30)	TAACCCATTATCA ACRTCATTAAAT CCAAATTCRACT CACAAAAAACCC (SEQ ID NO: 144)		X	F		NM_152577
cg17568421	LOC10018 8947	hypo		CGGCCAGTTCCTT CTGGACACCTTGT CTGTCCTTGAGCT ATCATGTAATC (SEQ ID NO: 31)	AAATTACATAAT AACTCAAAAAACA AACAAAATATCC AAAAAAAACCTAA CC (SEQ ID NO: 145)		10	R		NR_024467

cg17695512	OR10AG1	hypo	TCCTGTAGTAATT GGGGAACATGC CAAAATTTTCCTT TGCCCTTTTGCG (SEQ ID NO: 32)	TTCTTATAATAAT TAAAAAAACATA CCAAATTTTCCTT TTACCCCTTTTAC (SEQ ID NO: 146)		11	F	NM_00100549 1
cg19394169	RPTOR	hypo	CGCCGCACCTCCA CTTCTGCCCATGC TTGTCCTGTGACC CTCGTGGTCAT (SEQ ID NO: 33)	ATAACCAACAAA ATCACAAAACAA ACATAAACAAAA ATAAAATACAA CA (SEQ ID NO: 147)	ATAACCAACGAA AATCACAAAC AAACATAAACA AAAAATAAAAT ACGACG (SEQ ID NO: 182)	17	F	NM_00116303 4; NM_020761
cg20765408	PARP4	hypo	TCCACCTACACCA ATGGTTTATGGAG CAGCCAAGAGTT TGTGAGGAGGCG (SEQ ID NO: 34)	TTCCACCTACACC AATAATTTATAAA ACAACCAAAAAT TTATAAAAAAAC (SEQ ID NO: 148)		13	F	NM_006437
cg22559596	INPP5A	hypo	CGGGGCTGTCTCT CACTGGCAGGGG CCACCTCTCCGTG GACCGACCTGAG (SEQ ID NO: 35)	CTCAAAATCAATCC ACAAAAAATAAA CCCCTACCAATAA AAAACAACCCCA (SEQ ID NO: 149)	CTCAAAATCGAT CCACGAAAAA ATAACCCCTAC CAATAAAAAAC AACCCCG (SEQ ID NO: 183)	10	R	NM_005539
cg26086288	SLC9A3	hypo	GACGCGGGGGCT GCAAGAACACGG GGAGACGTGTGC CCCTGGGTTCCC G (SEQ ID NO: 36)	AACACAAAAAAT ACAAAAACACAA AAAAACATATAC CCCTAAATTTCCC A (SEQ ID NO: 150)	AACGCGAAAA CTACAAAAACA CGAAAAAACGT ATACCCCTTAA ATTCCCG (SEQ ID NO: 184)	5	F	NM_004174
cg26993132	CDH15	hypo	CGGCTCCTGCCAC CCCCGACTCCCC ATCTGGAGACAG	CTCCCCCACCAC TATCTCCAAATAA AAAAATCAAAA	CTCCCCCACC ACTATCTCAA ATAAAAAATC	16	F	NM_004933

			TGGTGGGGGGGAG (SEQ ID NO: 37)	TAACAAAAACCA (SEQ ID NO: 151)	GAAAAATAACA AAAACCG (SEQ ID NO: 185)			
cg27554551	VASN; CORO7	hypo	GCCAGAGTCCA CCCCAGGCCCTCT GCGGCCCTGGAG AGGCAGGATGGC G (SEQ ID NO: 38)	ACCAAAAATCCA CCCCAAAACCTCT ACAACCCTAAAA AAACAAAATAAC A (SEQ ID NO: 152)	ACCAAAAATCC ACCCAAAACC TCTACGACCTT AAAAAACAA AATAACG (SEQ ID NO: 186)	16	F	NM_138440; NM_024535
cg00713642	IGBP1	hypo	TATTGCTTCTGCA CCAATATAAAGTT AAAAAATTCTAA GACAAGCCATCG (SEQ ID NO: 39)	ATATTACTTCTAC ACCAATATAAAA TTAAAAAATTCTA AAACAAACCATC (SEQ ID NO: 153)		X	F	NM_001551
cg02961385	CRTC1	hypo	AGGACGGAGCAG CAACGTGGGCCA GGCAGGGGTGC AGGAAAGCAACG CG (SEQ ID NO: 40)	AAACAAAACAA CAACATAAACCA AAACAAAATATC AAAAAACCAACA CA (SEQ ID NO: 154)	AAACGAAAC AACACGTAAA CCAAAACAA AATACAAA AACACGCG (SEQ ID NO: 187)	19	F	NM_015321; NM_00109848 2
cg21765032	BRUNOL5	hypo	TCTTGAAAGCATCA CCCCACCTGGGG AGGGTTTGGAGC ATGAAGTGGGCC G (SEQ ID NO: 41)	CTCTTAAACATC ACCCACCTAAA AAAAATTAAAA CATAAAATAAAC C (SEQ ID NO: 155)		19	F	NM_021938
cg25749512	ACVRL1	hypo	TCAGTGGGCCCTT CCTTCGGGCGGA CCCCAGAGTCAC CGCAGAGTGGTC G (SEQ ID NO: 42)	TCAATAAACCTT CCTTCAACAAA CCCCAAAATC CACAAAATAATC A (SEQ ID NO: 156)	TCAATAAACCC TTCCTTCGAAC GAACCCCAAAA TCACCGCAAAA TAATCG (SEQ ID NO: 188)	12	R	NM_000020

				(SEQ ID NO: 42)	(SEQ ID NO: 156)	(SEQ ID NO: 188)			
cg00295339	BANP	hypo		CGGCCCTGCATT TGGGCTCCCCAT GCTTCTCAGGGAT ACACTCAGCTC (SEQ ID NO: 43)	TAAACTAAATAT ATCCCTAAAAA CATAAAAAAACCC CAAATACAAAAA CC (SEQ ID NO: 157)		16	R	NM_017869; NM_079837
cg10818160	DMRTB1	hypo		GTAGCACTAAGC CTGGCATAAGTGC CTGTGCCCTGTAGC CCTAGCTACTCG (SEQ ID NO: 44)	TATAACACTAAA CCTAACATAATAT CCTATACCTATAA CCCTAACTACTC (SEQ ID NO: 158)		1	F	NM_033067
cg14161477	TMCO3	hypo		CGCCGCTCCGCCG TGCTGAGCCCTTG GCGCTGTCATCTG AGTCTTCCGTC (SEQ ID NO: 45)	AACAAAAAACTC AAATAACAACAC CAAAAACTCAAC ACAACAAAAACAA CA (SEQ ID NO: 159)	AACGAAAAACT CAAATAACAAC GCCAAAAACTC AACACGACGA AACGACG (SEQ ID NO: 189)	13	F	NM_017905
cg01916429	CYP19A1	hyper		CGAAGCTCATTG AAACAAAGAAAT CCAGAAACATTCT ACTGATCTTTGTG (SEQ ID NO: 46)	CCACAAAAATCA ATAAATAATTCT AAATTCTTTATT TCAATAAACTTC (SEQ ID NO: 160)		15	F	NM_031226; NM_000103
cg01933079	MAST4	hyper		AGGGTTTCACAG GGATTTTCTCAG GAGTGTGCCACA GTGCAAGCTGAC G (SEQ ID NO: 47)	AAAAATTTTCACA AAAAATTTTCTCA AAAAATATACCAC AATACAAACTAA C (SEQ ID NO: 161)		5	F	NM_00116466 4; NM_198828

cg04224092	VASH1	hyper	AAGAGATGGCTC ACCTTGGGAGGT GCCAGGCTGAAA CTAGGTCCTTTCC G (SEQ ID NO: 48)	AAAAAATAACT CACCTTAAAAAA TACCAAACTAAA ACTAAATCCTTTC C (SEQ ID NO: 162)		14	R	NM_014909
cg12440187	GNL1	hyper	CGGTGGTATGGCT GTAGACAACTGT CTCAGGAAACAG ACCCATGACCCA C (SEQ ID NO: 49)	AATAAATCATAA ATCTATTTCCCTAA AACAAATTATCTAC AACCATACCACC (SEQ ID NO: 163)		6	F	NM_005275
cg14613402	LHX8	hyper	ACCACAGGGCTTT TTGCAAGCCCATG GGAAAGACAGCC TGAGAGACTTCG (SEQ ID NO: 50)	AACCACAAAACCT TTTTACAAACCCA TAAAAAAACAA CCTAAAAAACTTC (SEQ ID NO: 164)		1	F	NM_00100193 3
cg15841167	MOG	hyper	GCAGCTAAGGGA CTTACATCTGAAG TCCCTCAAAGGA CTTTTATTGACG (SEQ ID NO: 51)	AACAACTAAAAA ACTTACATCTAAA ATCCCTCAAAAA ACTTTTATTAAAC (SEQ ID NO: 165)		6	F	NM_206813; NM_00117041 8; NM_206814; NM_206811; NM_206810; NM_206809; NM_00117041 7; NM_00100822 9; NM_00100822 8; NM_206812; NM_002433

cg15971010	SLC47A1	hyper	CGGACGCCAGGA CTCACCCCGGCTC TCCACCTCCGCTG GGGGTTTCAGGT (SEQ ID NO: 52)	ACCTAAAACCCC CAACAAAAATAA AAAACCAAAATA AATCCTAACATCC A (SEQ ID NO: 166)	ACCTAAAACCC CCAACGAAAT AAAAACCGA AATAATCCTA ACGTCCG (SEQ ID NO: 190)	17	R	NM_018242
cg17428744	UTP11L	hyper	CGGGTTGCTGTAT CTAGAAAGGTATG TTTAAAGCTAGA GCAGTACGGATT (SEQ ID NO: 53)	TAAATCCTACTA CTCTAACTTTAAA CATACCTTCTAAA TACAACAAACCC (SEQ ID NO: 167)		1	R	NM_016037
cg25434223	ELAVL3	hyper	CGCCCCCTAGG AGTGCACCAACC CCGGAGCCCCCT CAACACGGACCG C (SEQ ID NO: 54)	CRCRATCCRTATT AAAAAAACTCC RAAAATAATACA CTCCTAAAAAA C (SEQ ID NO: 168)		19	R	NM_001420; NM_032281; NM_001420; NM_032281
cg07349464	PDHX	hyper	TTACCTTCTTTCT TTCCTTCTATTTT TTAGATGGAGCCT TACTCTGTCG (SEQ ID NO: 55)	ATTACCTTCTTTC TTCTTTCTATTTT TTTAAATAAAACC TTACTCTATC (SEQ ID NO: 169)		11	R	NM_00113502 4; NM_00116615 8; NM_003477

*: Illumina ID indicates the ID number assigned to the sequence on Infinium HumanMethylation450 v1.2 BeadChip™ (Illumina Inc.).

** : Sequence of a probe used to determine the level of methylation in the genomic site associated with the Illumina ID.

***: Sequence of a probe used to determine the level of methylation in the genomic site associated with the Illumina ID.

****: F indicates forward and R indicates reverse strand sequence.

Table 3. Clinical Characteristics of the Study Groups. The characteristics of the women whose samples were used in the study are shown. Categorical data were studied with chi-square analyses. Means and medians of continuous data were studied using parametric and non-parametric tests as indicated. There were no significant between-group differences with respect to any of these characteristics (p-value <0.05 considered significant). The mean gestational age at delivery was 36.78 ± 2.19 weeks for the cases and 39.86 ± 1.15 weeks for the control group (p<0.0001). * Plus-minus values are means $\pm$ SD.

Maternal Characteristic	Cases (n = 16)	Controls (n = 28)
Maternal age (Years)	34 $\pm$ 5.25	32.29 $\pm$ 4.42
Race		
White (n, %)	14 (87.50)	24 (88.89)
Asian (n, %)	2 (12.50)	3 (11.11)
Parity	0.25 $\pm$ 0.45	0.32 $\pm$ 0.48
BMI	23.75 $\pm$ 4.28	22.43 $\pm$ 3.32
Gestational age at enrollment (weeks)	12.69 $\pm$ 0.71	12.47 $\pm$ 0.54

Table 4. Relevance of the significantly altered microRNAs to PE. Detail descriptions of 22 significant microRNAs are shown.

miRNA	Cluster Members	Pathways [Targets related to PE mechanisms]	Associated disorders	Epigenetic Targets	PMID related to PE
let-7a-5p	let-7a, let-7c	PI3K/AKT, PTEN, HGF, IL-8, [CCND, IGF2, (ADAMTS1,-14, -15, -5, -8), IGF2BP1-3, PAPPA]	Reproductive Disorders, Connective Tissue Disorders, Cancer	EZH2, UHRF1	21840305 (3rd trimester PE plasma)
miR-126a-3p	miR-126	PPARa/RXRa Activation, Angiogenesis, Inflammation, Cardiac Hypertrophy [IRS1, ADAM9, VEGFA]	Reproductive Disorders, Connective Tissue Disorders, Cancer	-	23553946 (PE placenta at term, EPC from placenta), 24811064 (1 st trimester pooled samples)
miR-128-3p	miR-128a	Epithelial Neoplasia, Mammary Neoplasm, Angiogenesis [VEGF, TGFBR1, WEE1, glucocorticoid, SERPINE1]	Cervical Cancer, Glioblastoma Cancer, Myelodysplastic Syndrome with 5q-syndrome	HDAC4, HDAC5, KMT2A, MBD1, SIRT1	21309633 (PE placenta)
miR-130a-3p	miR-130b	Endometriosis, Epithelial neoplasia, Breast Cancer [COL1A1, HOXB7,	Severe PE, Cancer	KMT2A, MBD4	22187671 (PE plasma 37-40 weeks)

miR-140-3p		SERPINE1]	Hematological Neoplasia, Cell Lymphoma, Breast Cancer [IGFBP1, HDAC4]	Cancer	HDAC4, HDAC5, MBD1, SIRT1, SIRT3	n/a
miR-142-3p	miR-142-3p	VEGF, Apoptosis, PI3K/AKT, PTEN, IL-8, Inflammation [BCL2L1]	Reproductive Disorders (fetal neural tube defect), Connective Tissue Disorders, Cancer		-	n/a
miR-146a-5p	miR-146b	IL-6 signaling, IL-10 signaling, PPAR signaling [RUNX1T1, INHBA, IL8]	Cell Death and Survival, Inflammatory response, Preeclampsia		UHRF1	n/a
miR-16-5p	miR-15a	TGF- β , STAT3 signaling, Angiogenesis, Inflammation [WNT3A, VEGF, IGF2]	Severe Late-onset PE, Reproductive Disorders, Connective Tissue Disorders, Cancer.		KMT2A, MBD1, SIRT4	19642860 (placenta), 22251611 (studied in plasma 12-16 weeks but no diff in PE), 23083510 (mesenchymal stem cells)
miR-17-5p	miR-17, miR-106a, miR-20a	Angiogenesis, NFAT Cardiac Hypertrophy,	PE, Reproductive Disorders,		HDAC4, HDAC5, KMT2A, SIRT7	23438603 (PE placenta), 22438230 (PE placenta)

miR-191-5p	miR-191	Glioblastoma Multiforme [VEGFA, RB1, TGFBR2, ADAM9, ADAMTS5]	Connective Tissue Disorders, Cancer		
	miR-191	IL-6 signaling, Glucocorticoid receptor signaling [IL6, HLTF, CEBPB]	Inflammatory response, Reproductive system disease	-	23830491 (PE placenta)
miR-196a-5p	miR-196b	Endothelial dysfunction, STAT3, PTEN signaling [IGF1, CDC25A, TGFBR3]	Cell Death and Survival, Cardiovascular System Development and Function	-	n/a
miR-19b-3p	miR-19b	Hypoxia, Endothelial Dysfunction, Oxidative Stress [LIF, IGF1, LDLR]	Cell Death and Survival, Inflammatory Disease, Preterm Birth, PE	HDAC4, MBD4, PCGF2, SIRT5	23438603 (PE placenta)
miR-26a-5p	miR-26a	TGF- β , NGF signaling, Endothelial dysfunction [IGF1, INHBB, SMAD4]	Late onset PE, Premature labor, Cellular movement	DNMT3B, EZH2	23830491 (PE placenta), 22187671 (PE plasma 37-40 weeks)

miR-296-5p	miR-296	Preeclampsia and Preterm Labor [ADAM17]	PE, Reproductive Disorders	DNMT3B, HDAC5, EHMT1, KMT2A, MBD4, PCGF2, SIRT5	23830491 (PE placenta), 19285651 (PE placenta)
miR-29b-3p	miR-29a	Angiogenesis, Systemic Inflammatory response, Hypoxia [VEGFA, STAT3, LIF]	Late onset PE, Disorder of Pregnancy, Cardiovascular System Development and Function	DNMT3A, DNMT3B, HDAC4, SIRT1	22716646 (PE placenta), 19642860 (PE placenta)
miR-331-5p	miR-331-5p	Apoptosis, PI3K, Leukocyte extravasation signaling [KRAS, LYN, CDH5]	Cell Death and Survival, Inflammatory response, Cellular Movement	-	n/a
miR-517a-3p	miR-517a	Hypopharyngeal Squamous Cell Carcinoma, Epithelial Neoplasia [IGF1, IL1A]	PE, IUGR.	-	22251611 (plasma 12-16 weeks), 24347821 (PE plasma)

Table 5. Canonical pathways, diseases and functions associated with hypermethylated and hypomethylated genes. IPA was carried out to analyze the involvement of methylated genes in several canonical pathways and disease and function. Pathways and the disease functions associated with the methylated genes are directly or indirectly related to PE.

HYPERMETHYLATED GENES	HYPOMETHYLATED GENES
Top Canonical Pathways	Top Canonical Pathways
FXR/RXR Activation	Guanine and Guanosine Salvage I
Bupropion Degradation	Adenine and Adenosine Salvage III
Acetone Degradation I (to Methylglyoxal)	OX40 Signaling Pathway
Estrogen Biosynthesis	Sphingomyelin Metabolism
Nicotine Degradation III	TREM1 Signaling
Associated network Diseases and Functions	Associated network Diseases and Functions
Immunological Disease, Inflammatory Disease, Inflammatory Response	Tissue Development, Cardiovascular System Development and Function, Organismal Development
Lipid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral Metabolism	Cancer, Cellular Development, Cellular Growth and Proliferation
Gene Expression, Cellular Development, Endocrine System Development and Function	Cell-To-Cell Signaling and Interaction, Cellular Function and Maintenance, Cellular Development
	Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism
	Cellular Development, Tissue Development, Cellular Growth and Proliferation

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CLAIMS

I claim:

1. A method of predicting the development of preeclampsia (PE) in a subject, the method comprising:
 - (a) determining the level of one or more microRNAs (miRNAs) in:
 - i) a test sample obtained from the subject, and
 - ii) optionally a control sample;
 - (b) optionally obtaining one or more reference values corresponding to levels of one or miRNAs, wherein the presence of the one or more miRNAs: at different levels in the test sample as compared to the control sample, or relative to the reference values indicates high risk of development of PE in the subject; and
 - (c) identifying the subject as having high risk of developing PE based on the level of one or more miRNAs in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or
 - (d) identifying the subject as not having high risk of developing PE based on the level of one or more miRNAs in the test sample and withholding the therapy to the subject to treat and/or manage PE.
2. The method of claim 1, wherein the control sample is obtained from one or more of the following: an individual belonging to the same species as the subject and not having PE, an individual belonging to the same species as the subject and known to have a low risk or no risk of developing PE; the subject prior to becoming pregnant.
3. The method of claim 2, wherein the control sample and the test sample are obtained from the same type of an organ or tissue.
4. The method of claim 3, wherein the organ or tissue is placenta, brain, eyes, pineal gland, pituitary gland, thyroid gland, parathyroid glands, thorax, heart, lung, esophagus, thymus gland, pleura, adrenal glands, appendix, gall bladder, urinary bladder,

large intestine, small intestine, kidneys, liver, pancreas, spleen, stoma, ovaries, uterus, skin, blood or buffy coat sample of blood.

5. The method of claim 2, wherein the control sample and the test sample are obtained from the same type of a body fluid.

6. The method of claim 5, wherein the body fluid is amniotic fluid, aqueous humor, vitreous humor, bile, blood, urine, cerebrospinal fluid, chyle, endolymph, perilymph, female ejaculate, lymph, mucus (including nasal drainage and phlegm), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sputum, synovial fluid, vaginal secretion, blood, serum or plasma.

7. The method of claim 1, wherein the levels of miRNAs in the test sample and optionally, the control sample, are determined by microarray analysis, real-time polymerase chain reaction (PCR), Northern blot, in situ hybridization, solution hybridization, or quantitative reverse transcription PCR (qRT-PCR).

8. The method of claim 1, wherein the one or more miRNAs are selected from miR-7c, miR-93, miR-128a, miR-140-3p, miR-142-3p, miR-146b, miR-15a, miR-196b, miR-331-5p, miR-886-5p, miR-17, miR-26a, miR-130b, miR-7a, miR-29a, miR-517a, miR-191, miR-296, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1, miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2 and miR-363.

9. The method of claim 1, wherein the subject is a mammal.

10. The method of claim 9, wherein the mammal is a human, ape, canine, pig, bovine, rodent, or feline.

11. An oligonucleotide chip consisting essentially of oligonucleotides corresponding to one or more miRNAs selected from miR-7c, miR-93, miR-128a, miR-140-3p, miR-142-3p, miR-146b, miR-15a, miR-196b, miR-331-5p, miR-886-5p, miR-17, miR-26a, miR-130b, miR-7a, miR-29a, miR-517a, miR-191, miR-296, miR-18a, miR-19a, miR-

20a, miR-19b-1, miR-92a-1, miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, and miR-363 and optionally, one or more control oligonucleotides.

12. A method of predicting the development of PE in a subject, the method comprising:
- (a) determining the levels of post-translational modifications of one or more histone proteins in:
 - i) a test sample obtained from the subject, and
 - ii) optionally a control sample;
 - (b) optionally obtaining one or more reference values corresponding to the levels of post-translational modifications of the one or more histone proteins, wherein the presence of the post-translational modifications in the one or more histone proteins:
 - at different levels in the test sample as compared to the control sample, or
 - relative to the reference values indicates high risk of development of PE in the subject; and
 - (c) identifying the subject as having high risk of developing PE based the levels of post-translational modifications in the one or more histone proteins in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or
 - (d) identifying the subject as having not having high risk of developing PE based on the levels of post-translational modifications in the one or more histone proteins in the test sample and withholding the therapy to the subject to treat and/or manage PE.

13. The method of claim 12, wherein the control sample is obtained from one or more of the following: an individual belonging to the same species as the subject and not having PE, an individual belonging to the same species as the subject and known to have a low risk or no risk of developing PE; the subject prior to becoming pregnant.

14. The method of claim 13, wherein the control sample and the test sample are obtained from the same type of an organ or tissue.

15. The method of claim 14, wherein the organ or tissue is placenta, brain, eyes, pineal gland, pituitary gland, thyroid gland, parathyroid glands, thorax, heart, lung,

esophagus, thymus gland, pleura, adrenal glands, appendix, gall bladder, urinary bladder, large intestine, small intestine, kidneys, liver, pancreas, spleen, stoma, ovaries, uterus, skin, blood or buffy coat sample of blood.

16. The method of claim 12, wherein the levels of post-translational modifications in the test sample and optionally, the control sample are determined by an antibody based assay or mass spectrometry.

17. The method of claim 16, wherein the antibody based assay is western blot analysis, enzyme immunoassay (EIA), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), antigen-antibody precipitation assay or antibody array analysis.

18. The method of claim 12, wherein the post-translation modification is methylation, acetylation, ADP-ribosylation, ubiquitination, citrullination, and phosphorylation.

19. The method of claim 12, wherein the one or more histones are selected from H1, H2A, H2B, H3, H4 and H5.

20. The method of claim 12, wherein the histone protein is H4 having the sequence of SEQ ID NO: 1 and increased level of acetylation of one or more of lysine 12, lysine 16, or N-terminus of H4 and/or increased level of methylation or dimethylation on lysine 20 in the test sample as compared to the control sample or the reference values indicates high risk of the development of PE in the subject.

21. The method of claim 12, wherein the subject is a mammal.

22. The method of claim 21, wherein the mammal is a human, ape, canine, pig, bovine, rodent, or feline.

23. An antibody array chip consisting essentially of antibodies against a group of post-translational modifications of histone H4 identified by SEQ ID NO: 1, wherein the group of post-translational modifications of histone H4 are present at different levels in a

sample of an individual having high risk of the development of PE as compared to the corresponding sample of an individual having no risk or low risk of the development of PE.

24. The antibody array of claim 23, the antibody array chip consisting essentially of antibodies against: Histone H4 acetylated at the N-terminal, Histone H4 acetylated on lysine 12, Histone H4 acetylated on lysine 16, Histone H4 methylated or dimethylated on lysine 20 and optionally, one or more control antibodies.

25. A method of predicting the development of PE in a subject, the method comprising:

(a) determining the levels of expression, activity and/or amount of one or more enzymes which post-translationally modify histone proteins or DNA (histone or DNA modifying enzymes) in:

i) a test sample obtained from the subject, and

ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to the levels of expression, activity and/or amount histone proteins and/or DNA modifying enzymes,

wherein the presence of expression, activity and/or amount of one or more histone or DNA modifying enzymes:

at different levels in the test sample as compared to the control sample, or

relative to the reference values indicates high risk of development of PE in the subject; and

(c) identifying the subject as having high risk of developing PE based on the levels of one or more histone and/or DNA modifying enzymes in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as not having high risk of developing PE based on the levels of one or more histone and/or DNA modifying enzymes in the test sample and withholding the therapy to the subject to treat and/or manage PE if the subject.

26. The method of claim 25, wherein the control sample is obtained from one or more of the following: an individual belonging to the same species as the subject and not having PE, an individual belonging to the same species as the subject and known to have a low risk or no risk of developing PE; the subject prior to becoming pregnant.

27. The method of claim 26, wherein the control sample and the test sample are obtained from the same type of an organ or tissue.

28. The method of claim 27, wherein the organ or tissue is placenta, brain, eyes, pineal gland, pituitary gland, thyroid gland, parathyroid glands, thorax, heart, lung, esophagus, thymus gland, pleura, adrenal glands, appendix, gall bladder, urinary bladder, large intestine, small intestine, kidneys, liver, pancreas, spleen, stoma, ovaries, uterus, skin, blood or buffy coat sample of blood.

29. The method of claim 25, wherein the amounts of histone or DNA modifying enzymes in the test sample and optionally, the control sample are determined by an antibody based assay or mass spectrometry.

30. The method of claim 29, wherein the antibody based assay is western blot analysis, enzyme immunoassay (EIA), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), antigen-antibody precipitation assay or antibody array analysis.

31. The method of claim 25, wherein the activity of histone or DNA modifying enzymes in the test sample and optionally, the control sample are determined by histone and/or DNA modifying activity assay.

32. The method of claim 25, wherein the expression of histone or DNA modifying enzymes in the test sample and optionally, the control sample are determined by expression of mRNAs corresponding to the histone and/or DNA modifying enzymes.

33. The method of claim 25, wherein the histone modifying enzyme is selected from histone acetyl transferase (HAT), histone deacetylase (HDAC), histone methyltransferase (HMT), histone demethylase and the DNA modifying enzyme is DNA methyl transferase (DNMT).

34. The method of claim 33, wherein the histone deacetylase is HDAC1 or HDAC5.

35. The method of claim 25, wherein the subject is a mammal.
36. The method of claim 35, wherein the mammal is a human, ape, canine, pig, bovine, rodent, or feline.
37. A method of predicting the development of PE in a subject, the method comprising:
- (a) determining the levels of methylation in one or more sites in the genomic DNA in:
 - i) a test sample obtained from the subject, and
 - ii) optionally a control sample;
 - (b) optionally obtaining one or more reference values corresponding to levels of methylation of the one or more sites, wherein the presence methylation of one or more sites in the genomic DNA: at different levels in the test sample as compared to the control sample, or relative to the reference values indicates high risk of development of PE in the subject; and
 - (c) identifying the subject as having high risk of developing PE based the levels of methylation of the one or more sites in the genomic DNA in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or
 - (d) identifying the subject as having high risk of developing PE based the levels methylation of the one or more sites in the genomic DNA in the test sample and withholding the therapy to the subject to treat and/or manage PE.
38. The method of claim 37, wherein the control sample is obtained from one or more of the following: an individual belonging to the same species as the subject and not having PE, an individual belonging to the same species as the subject and known to have a low risk or no risk of developing PE; the subject prior to becoming pregnant.
39. The method of claim 38, wherein the control sample and the test sample are obtained from the same type of an organ or tissue.

40. The method of claim 39, wherein the organ or tissue is placenta, brain, eyes, pineal gland, pituitary gland, thyroid gland, parathyroid glands, thorax, heart, lung, esophagus, thymus gland, pleura, adrenal glands, appendix, gall bladder, urinary bladder, large intestine, small intestine, kidneys, liver, pancreas, spleen, stoma, ovaries, uterus, skin, blood or buffy coat sample of blood.

41. The method of claim 37, wherein the levels of methylation of one or more sites in the genomic DNA in the test sample and optionally, the control sample are determined by bisulfite conversion, digestion by restriction enzymes followed by polymerase chain reaction (Combined Bisulfite Restriction Analysis, COBRA), direct sequencing, cloning and sequencing, pyrosequencing or mass spectrometry analysis.

42. The method of claim 37, wherein the one or more sites in the genomic DNA are selected from the sequence of SEQ ID NOs: 2-55.

43. The method of claim 37, wherein the subject is a mammal.

44. The method of claim 43, wherein the mammal is a human, ape, canine, pig, bovine, rodent, or feline.

45. A method of predicting the development of PE in a subject, the method comprising:

(a) determining the levels of two or more biomarkers selected from one or more of miRNA, post-translational modification of histones, histone or DNA modifying enzymes, methylation of certain genomic DNA sites in:

- i) a test sample obtained from the subject, and
- ii) optionally a control sample;

(b) optionally obtaining one or more reference values corresponding to levels of one or more biomarkers,

wherein the presence of two or more biomarkers:

at different levels in the test sample as compared to the control sample, or

relative to the reference values indicates high risk of development of PE in the subject; and

(c) identifying the subject as having high risk of developing PE based on the levels of the two or more biomarkers in the test sample and optionally, administering a therapy to the subject to treat and/or manage PE, or

(d) identifying the subject as not having high risk of developing PE based on the levels of the two or more biomarkers in the test sample and withholding the therapy to the subject to treat and/or manage PE.

46. The method of claim 45, wherein the subject is identified as having high risk of the development of PE if all of the analyzed biomarkers are significant different between the test sample and the control sample.

47. The method of claim 45, wherein the subject is identified as having high risk of the development of PE if a pre-determined number of biomarkers out of the analyzed biomarkers are significant different between the test sample and the control sample.

48. The method of claim 45, wherein the subject is identified as having high risk of the development of PE if the two or more biomarkers as a combination are significant different between the test sample and the control sample

49. The method of claim 45, wherein the two or more biomarkers comprise miR-17, post-translational modification of H4 histone protein, amount of HDAC5 mRNA and/or protein and methylation of CYP19A1, and the subject is identified as having a high risk of the development of PE if the subject has increased miR-17, hyperacetylated H4 histone protein, decreased HDAC5 mRNA and/or protein and hypermethylated CYP19A1.

50. A point-of-care (POC) diagnostic device for identifying a subject as having high risk of PE, the POC device capable of assaying miR-17, methylated CYP19A1, HDAC5 mRNA and/or protein and acetylation of histone H4 in a sample obtained from a subject.

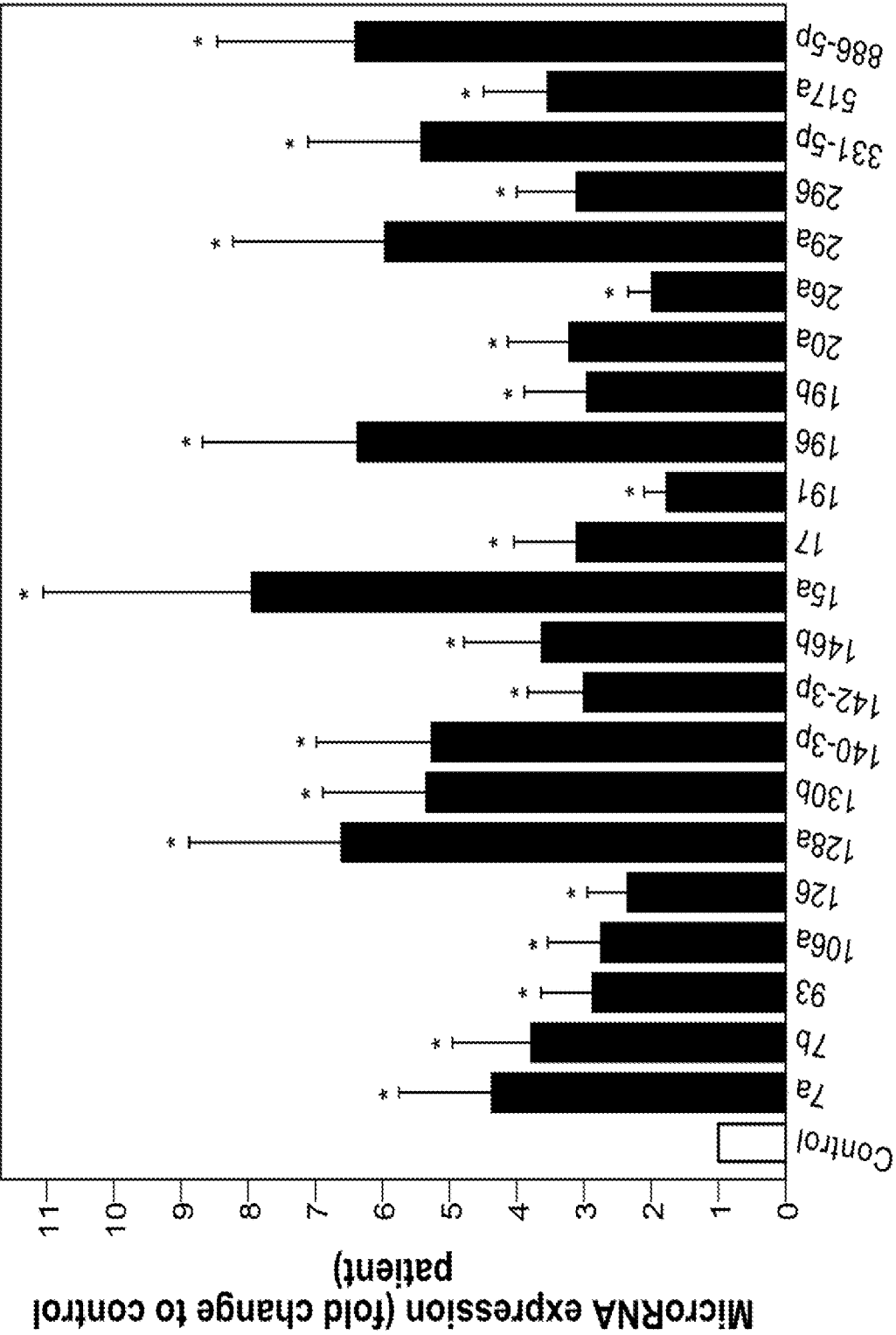


Fig. 1a

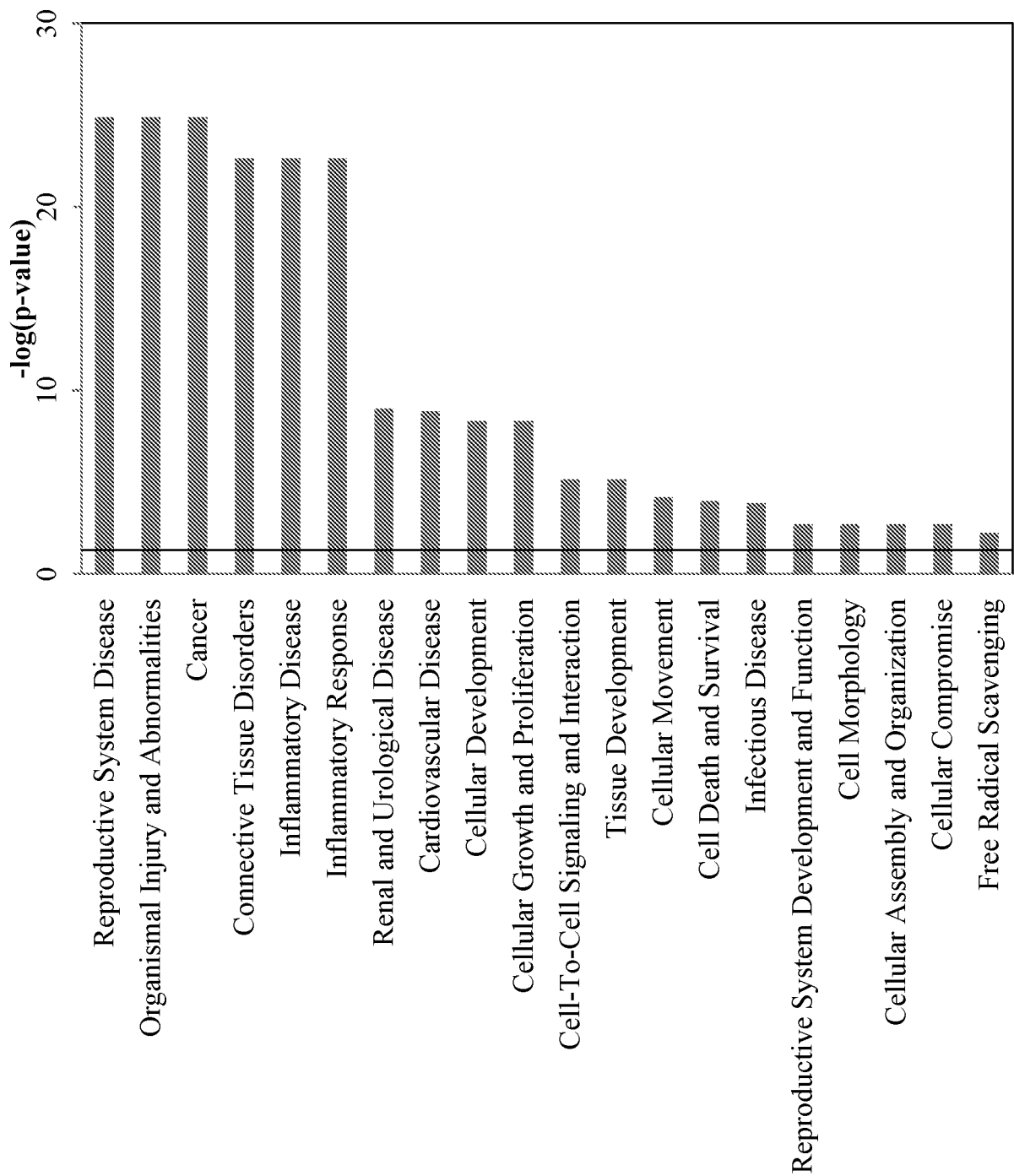


Fig. 1b

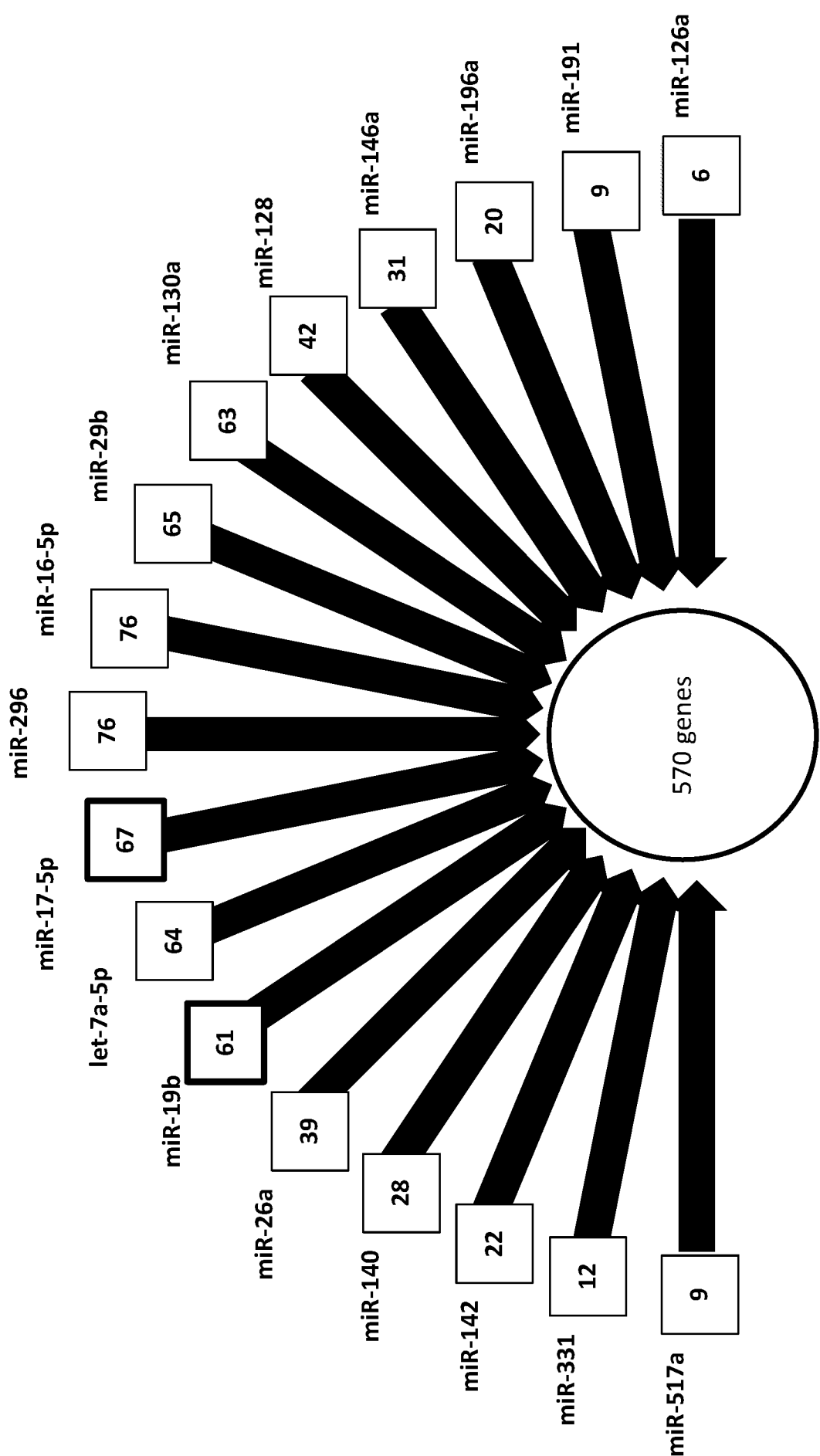


Fig. 1c

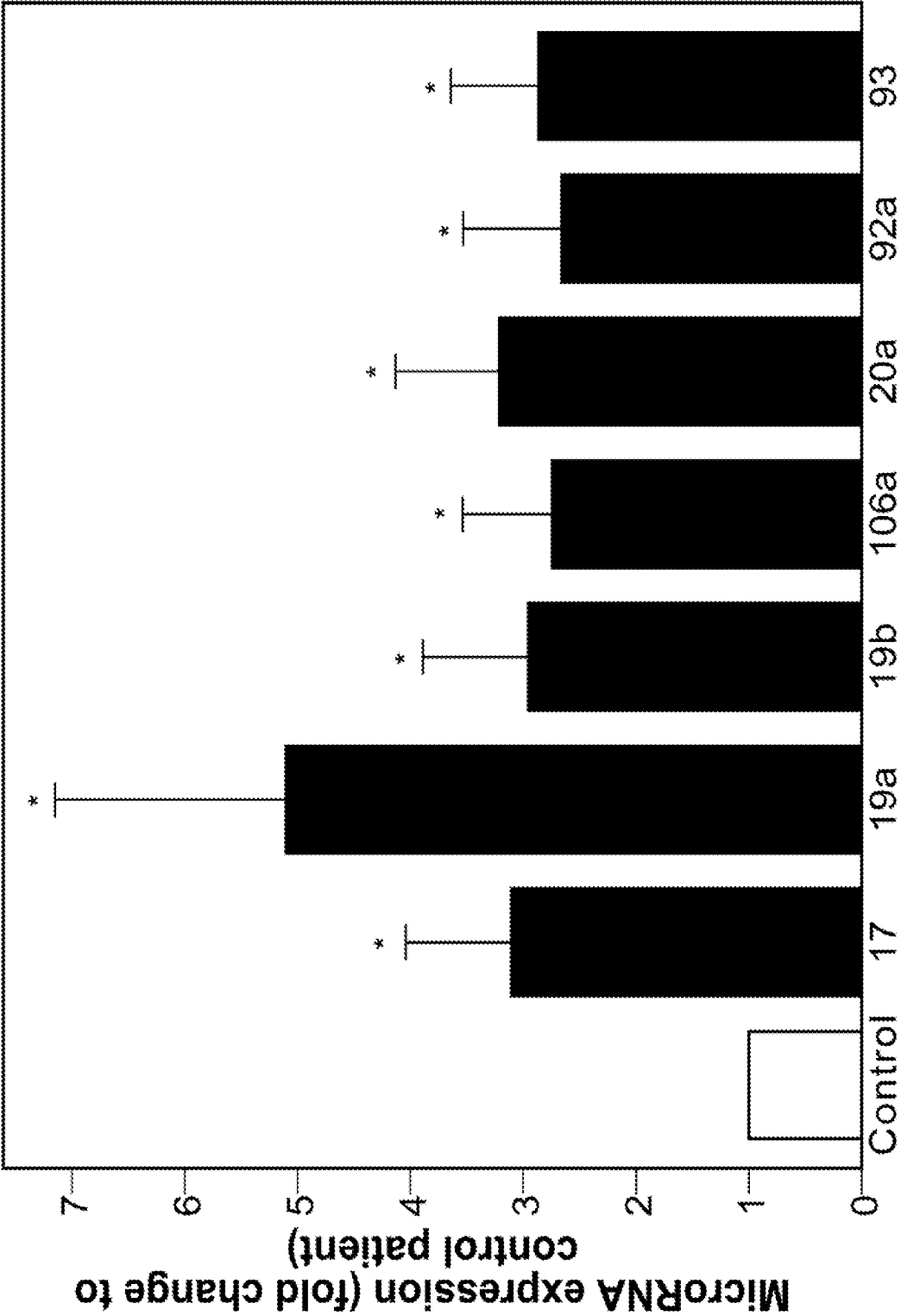


Fig. 1d

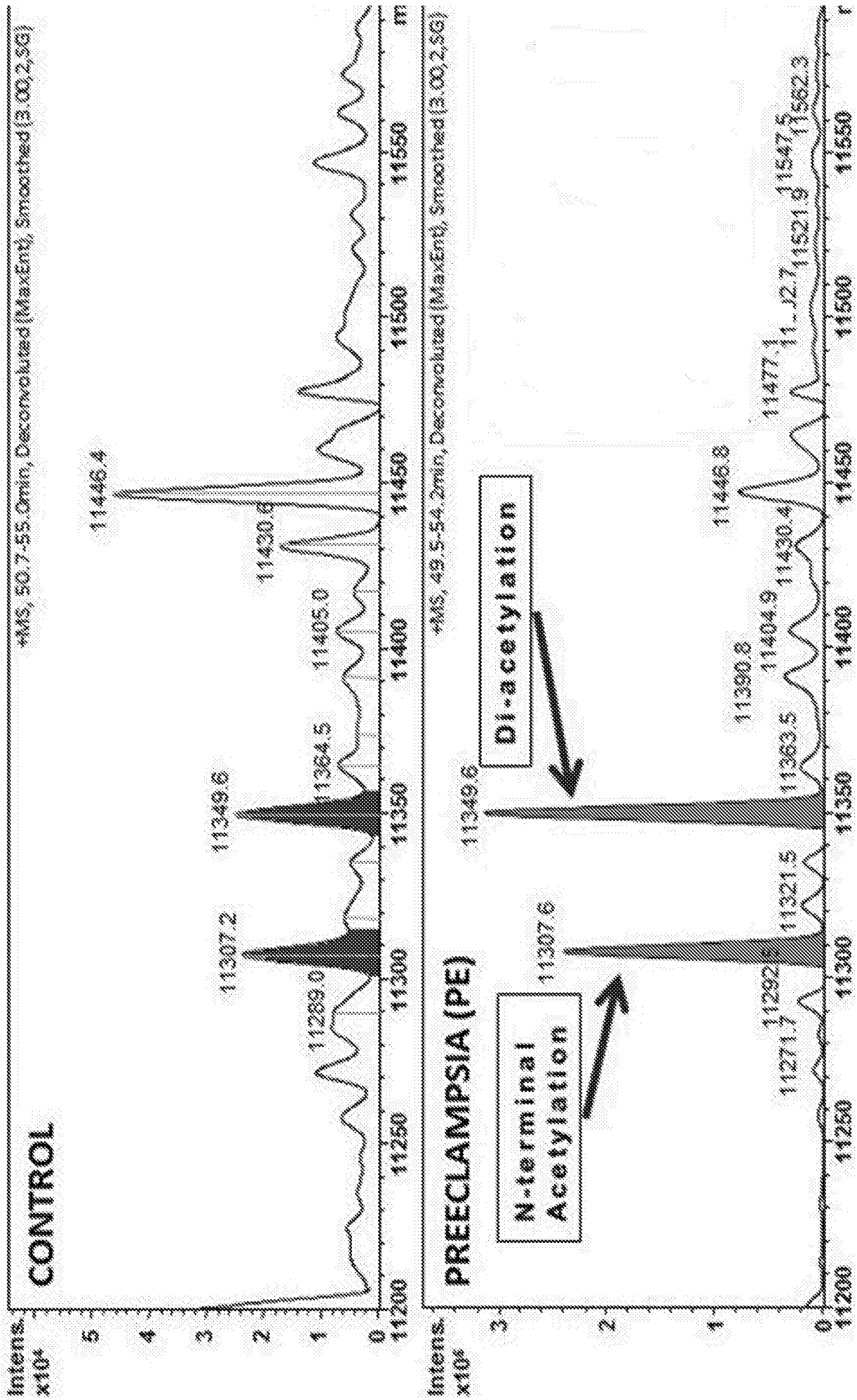


Fig. 2a

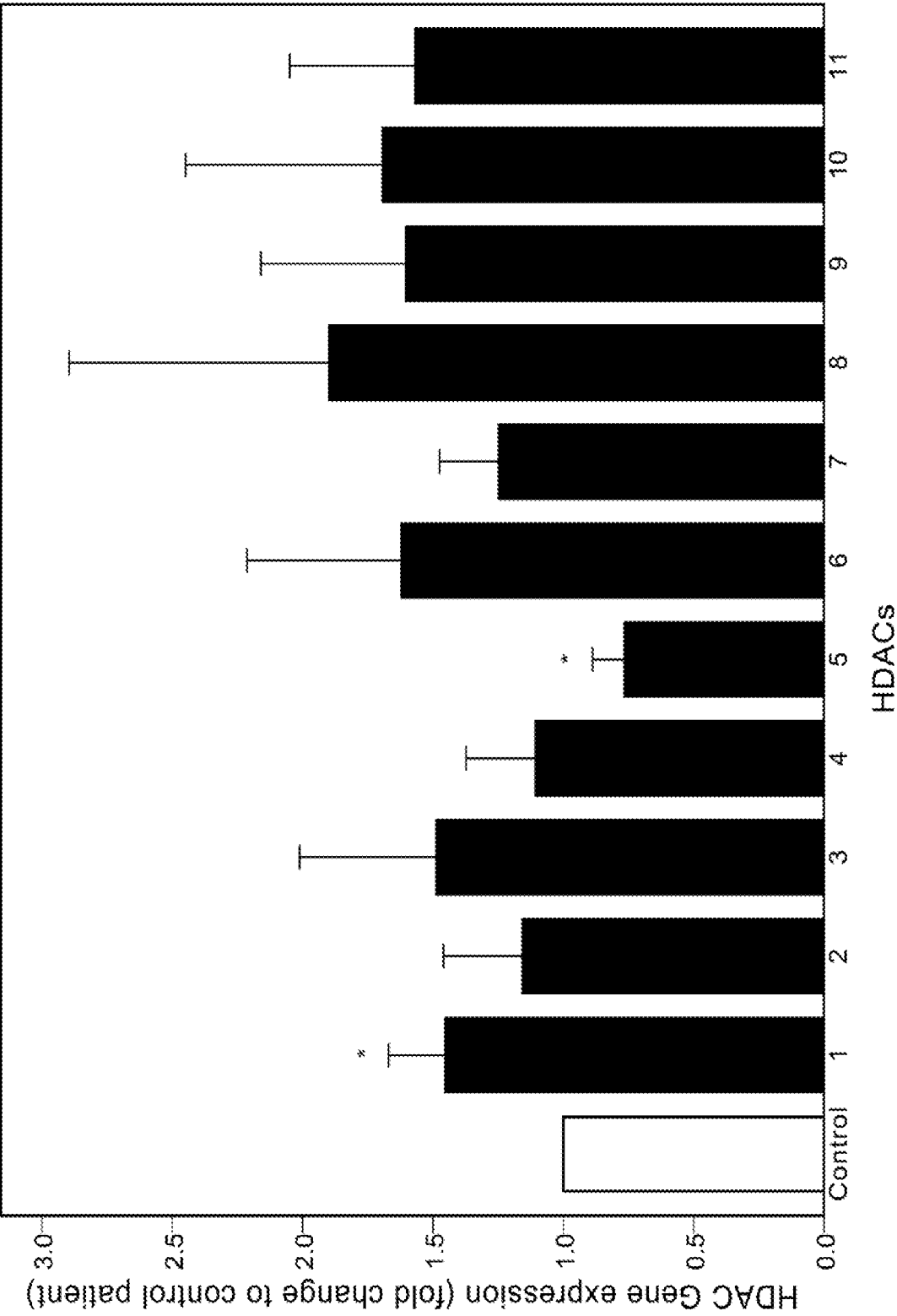


Fig. 2b

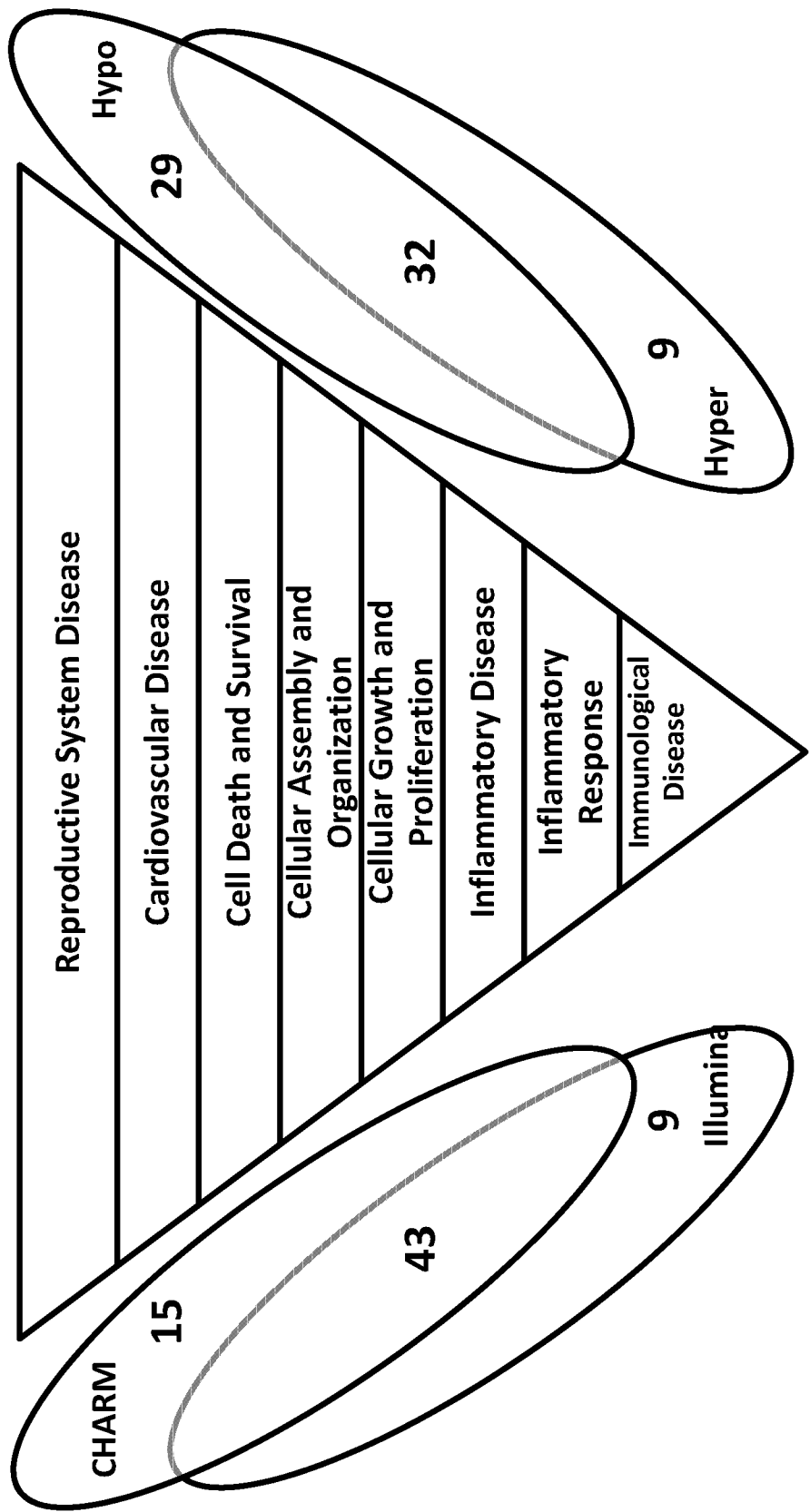


Fig. 3a

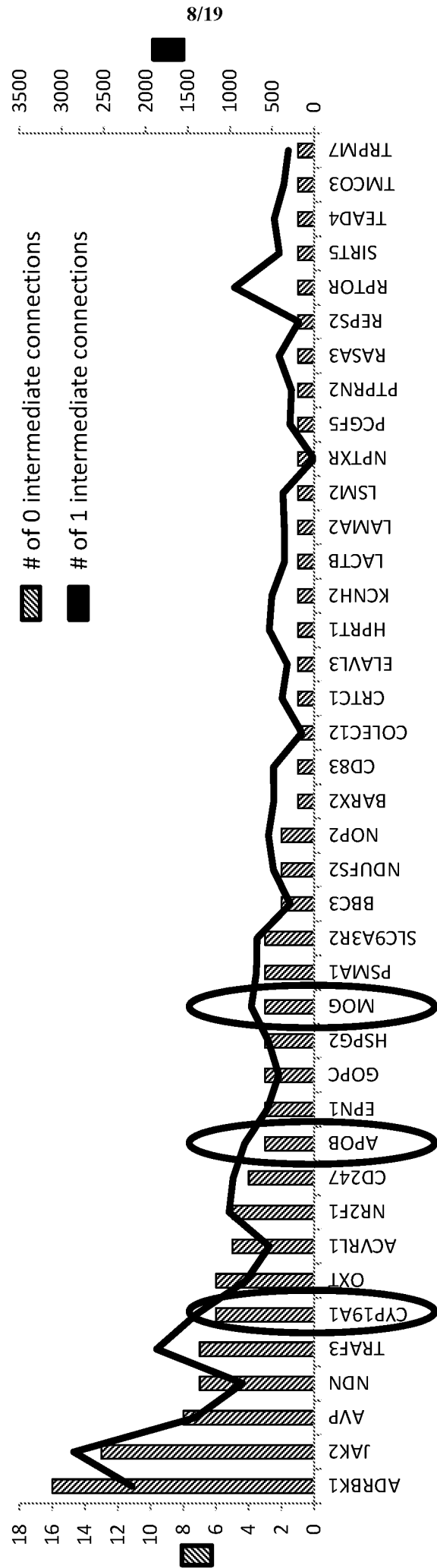


Fig. 3b

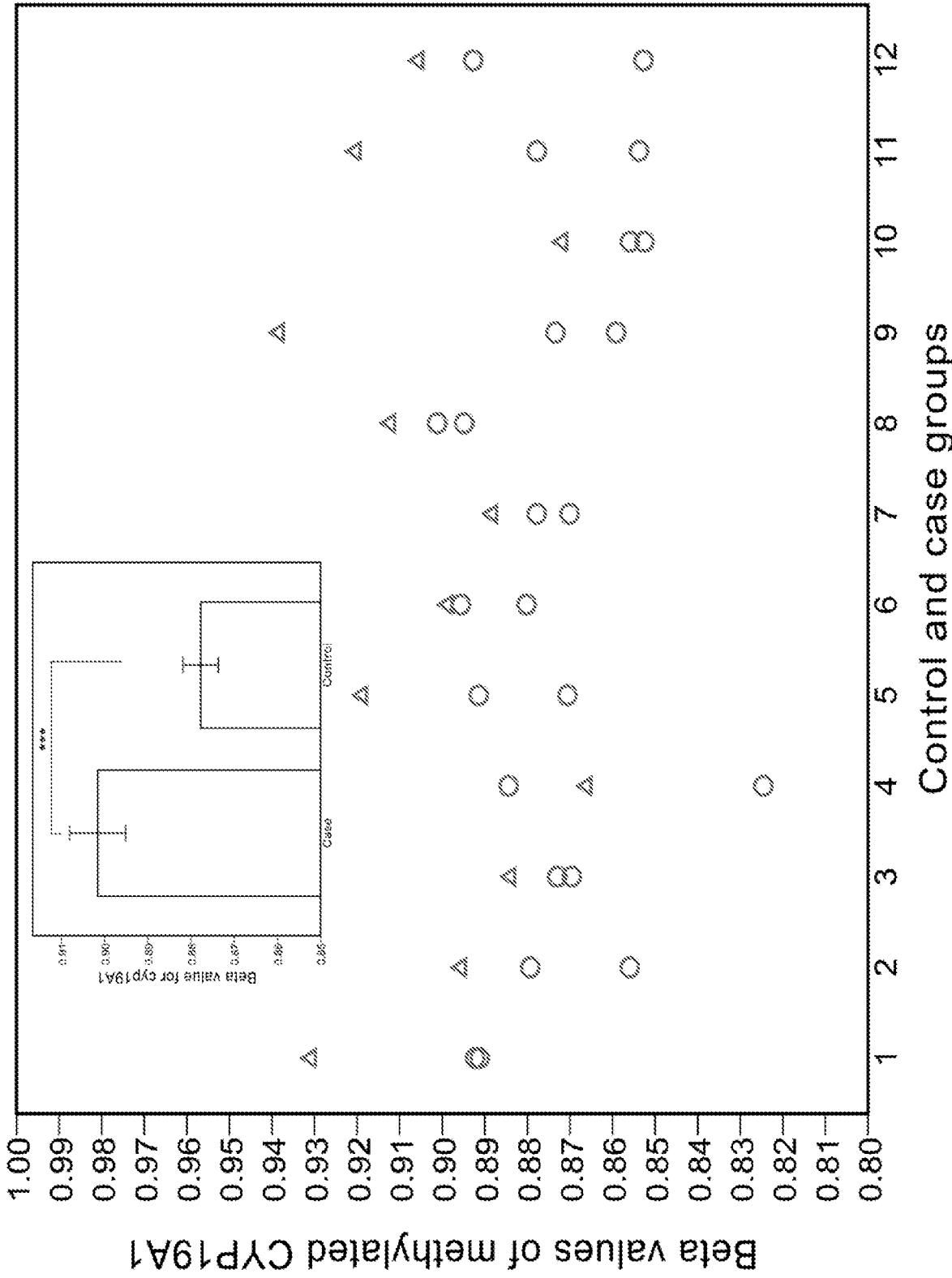
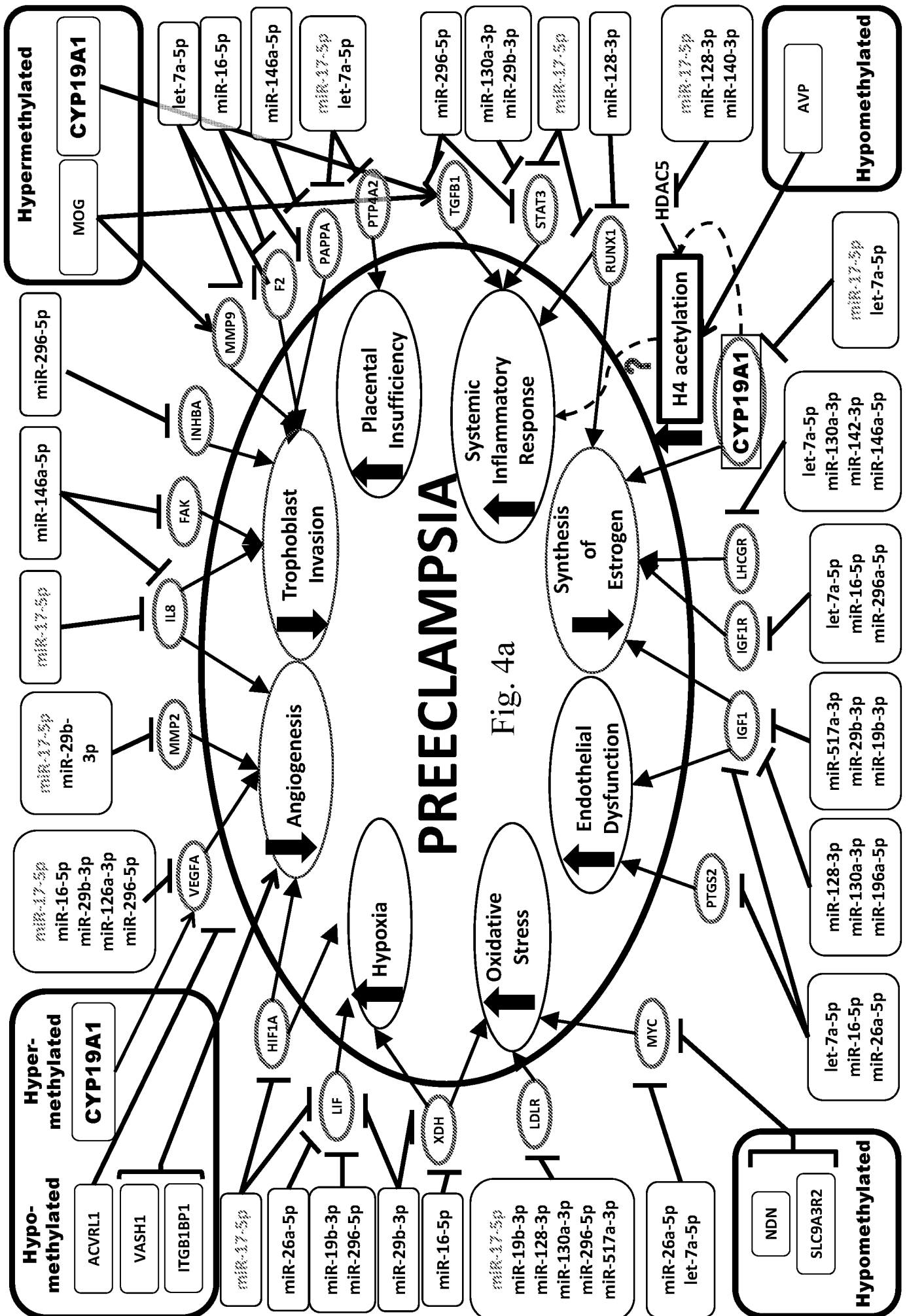


Fig. 3c



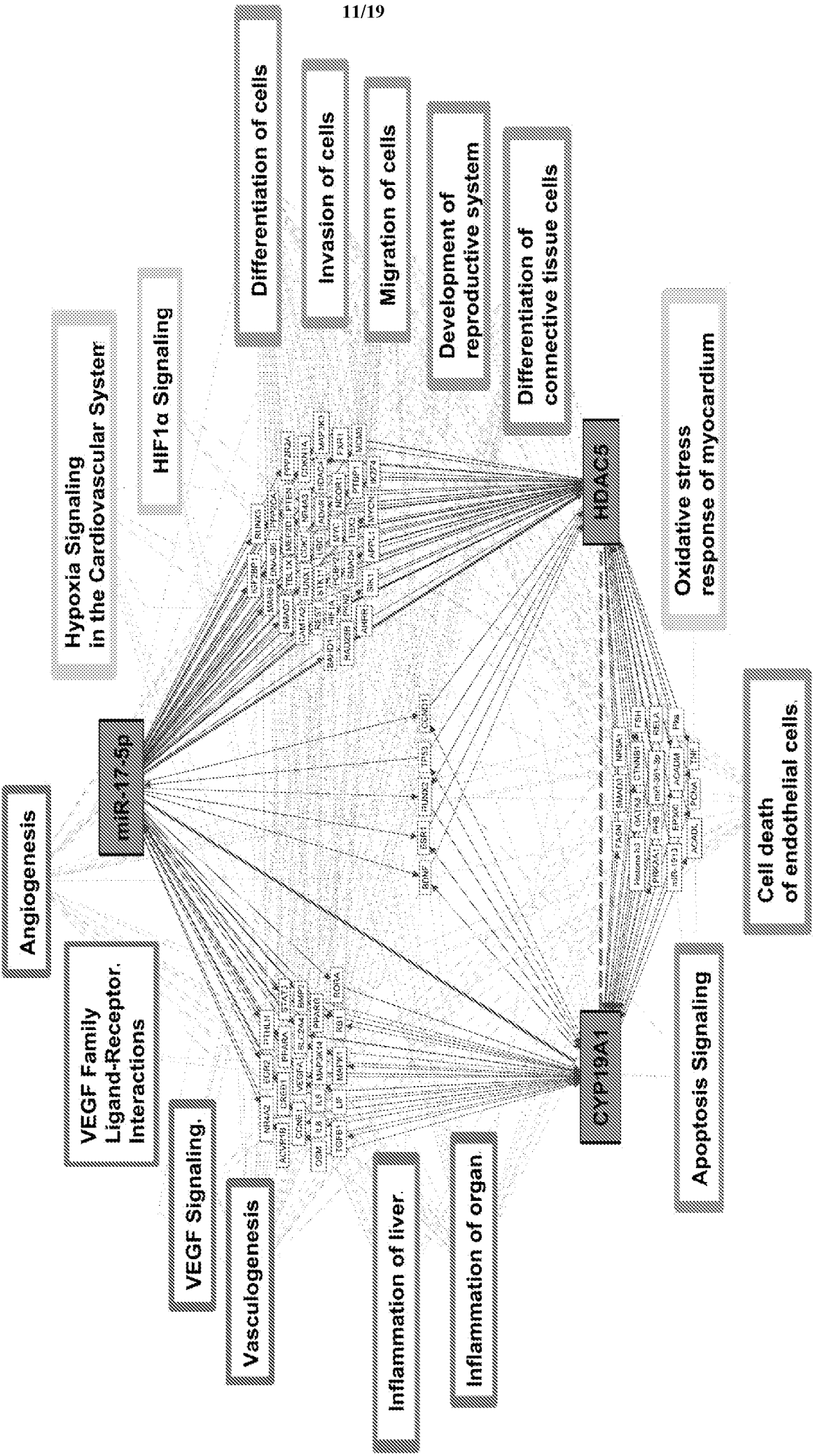


Fig. 4b

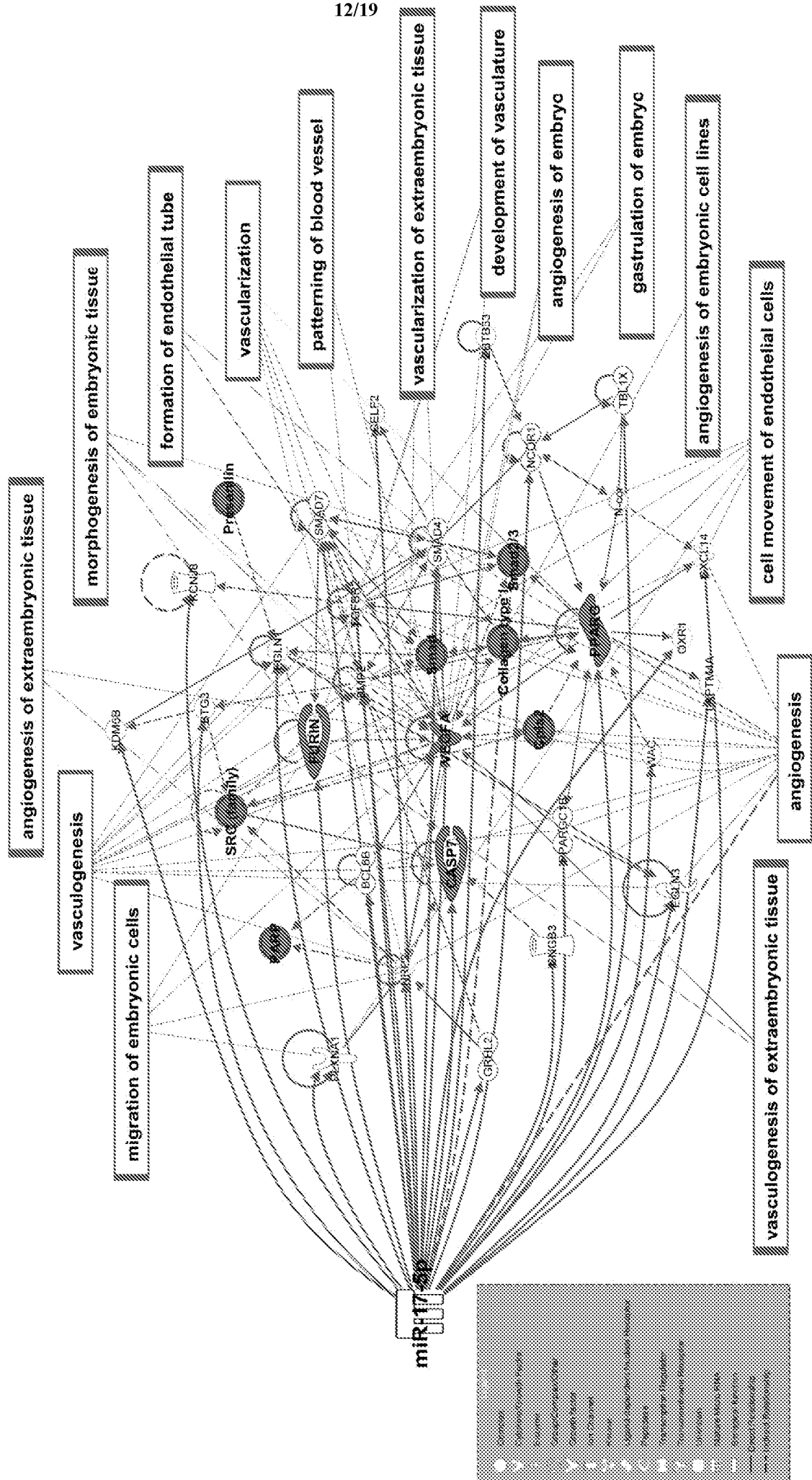


Fig. 5

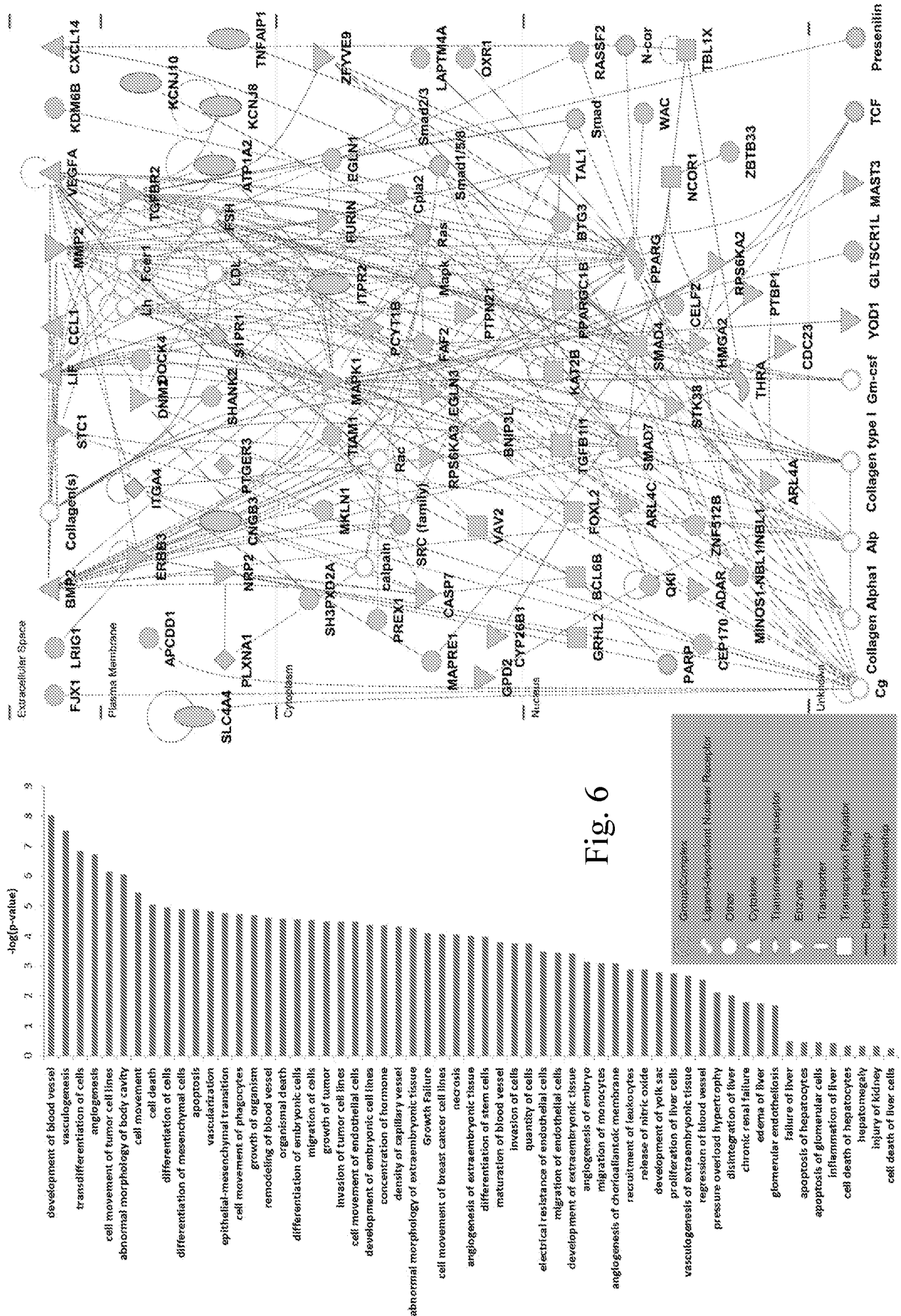




Fig. 7a

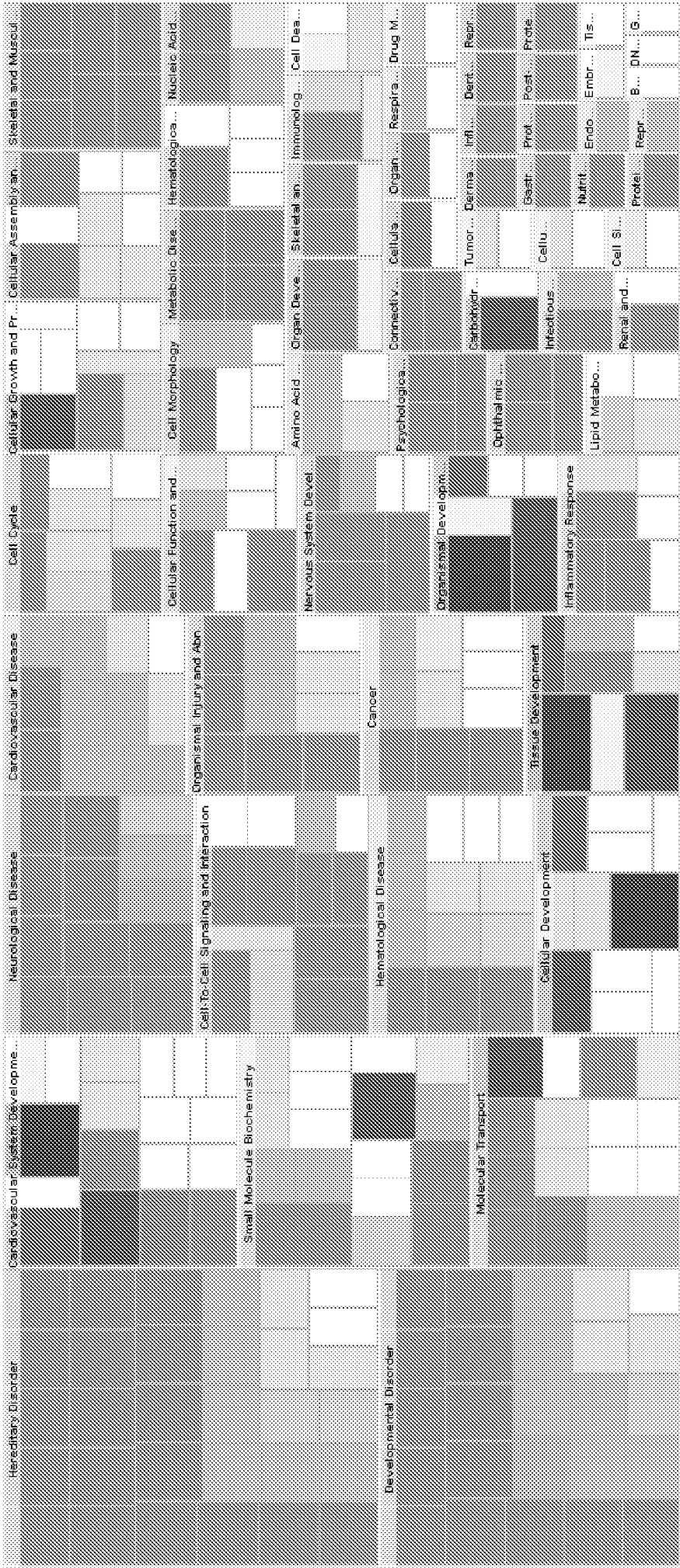
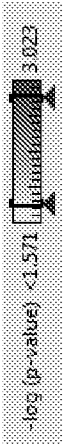
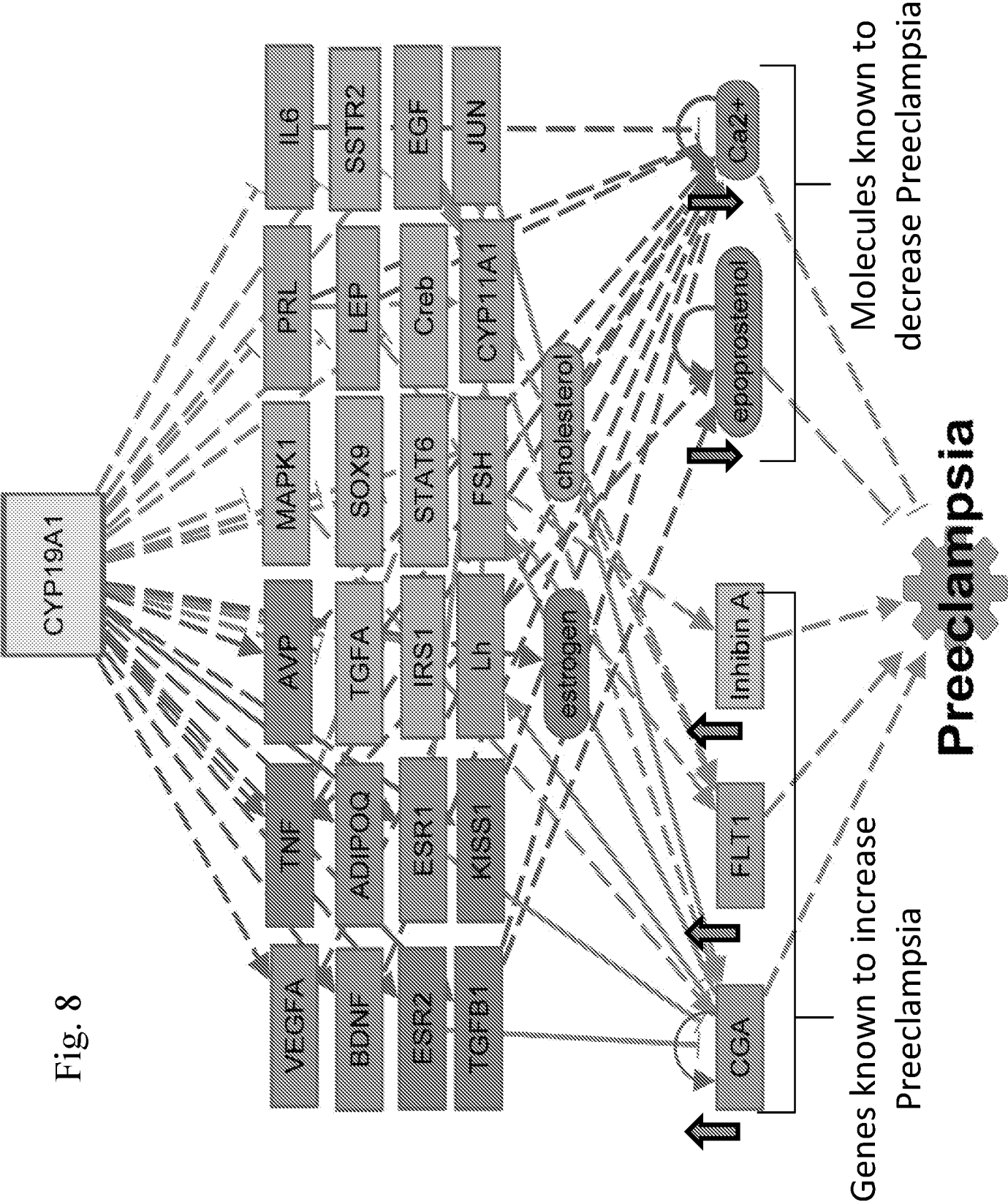


Fig. 7b



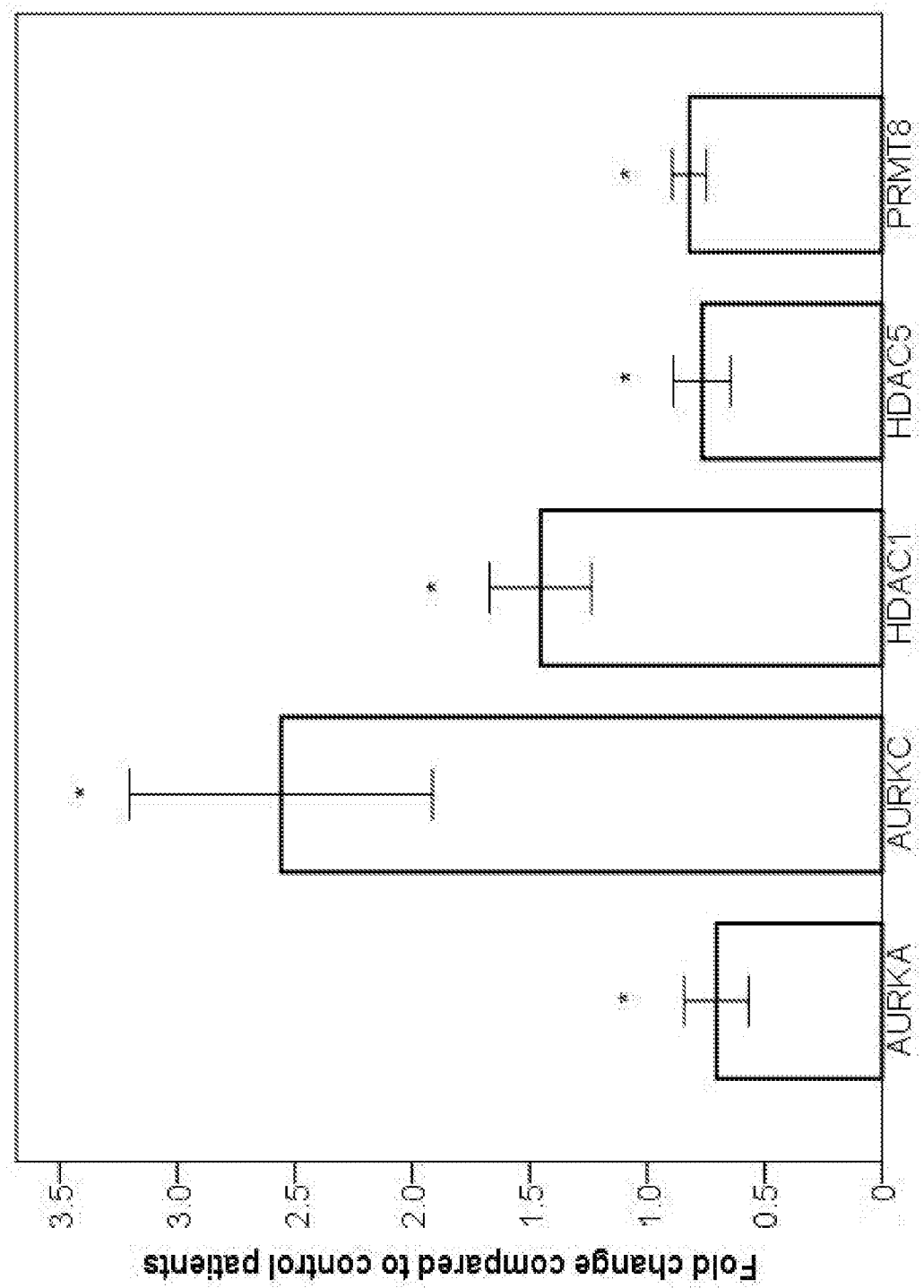


Fig. 9

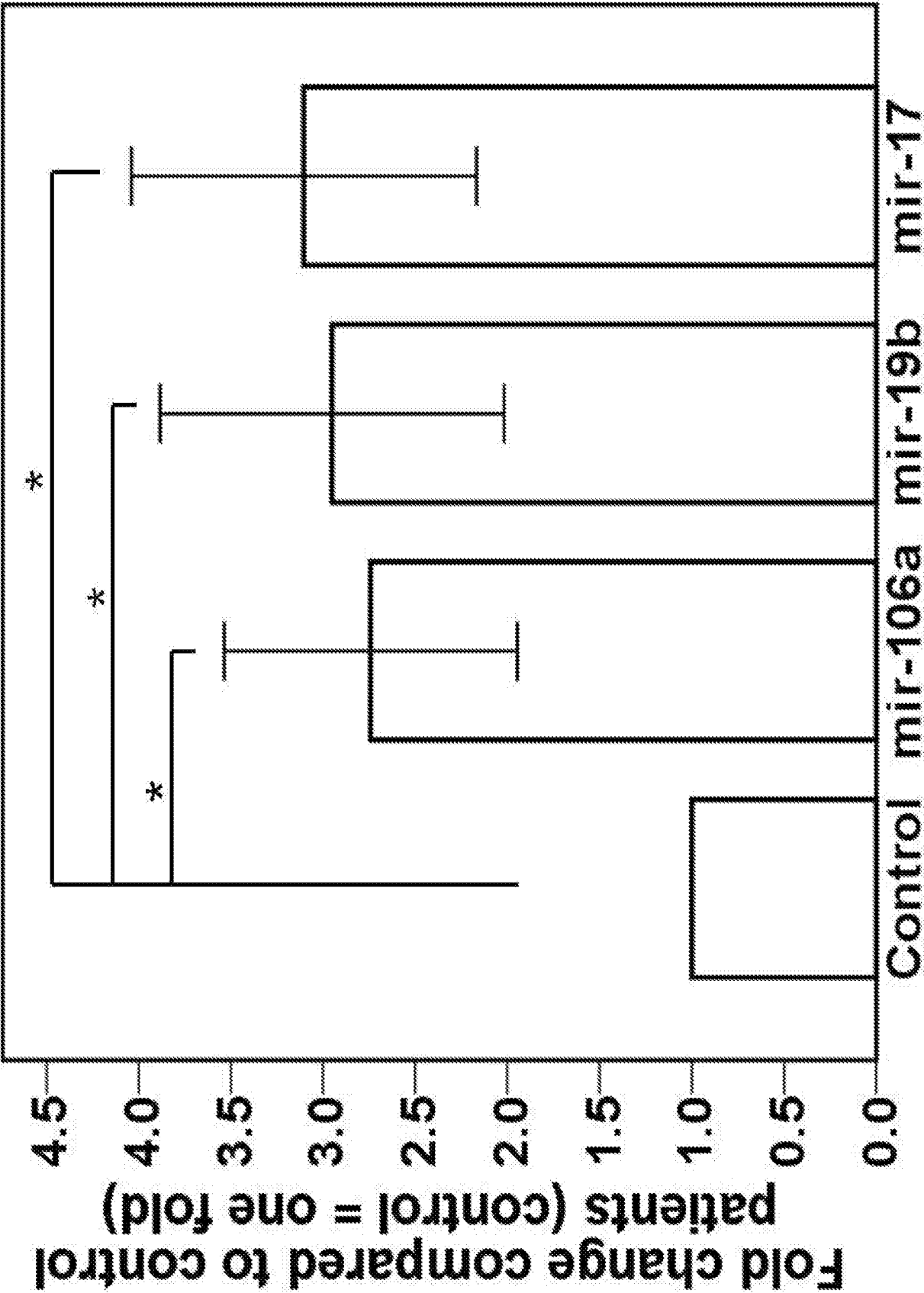


Fig. 10

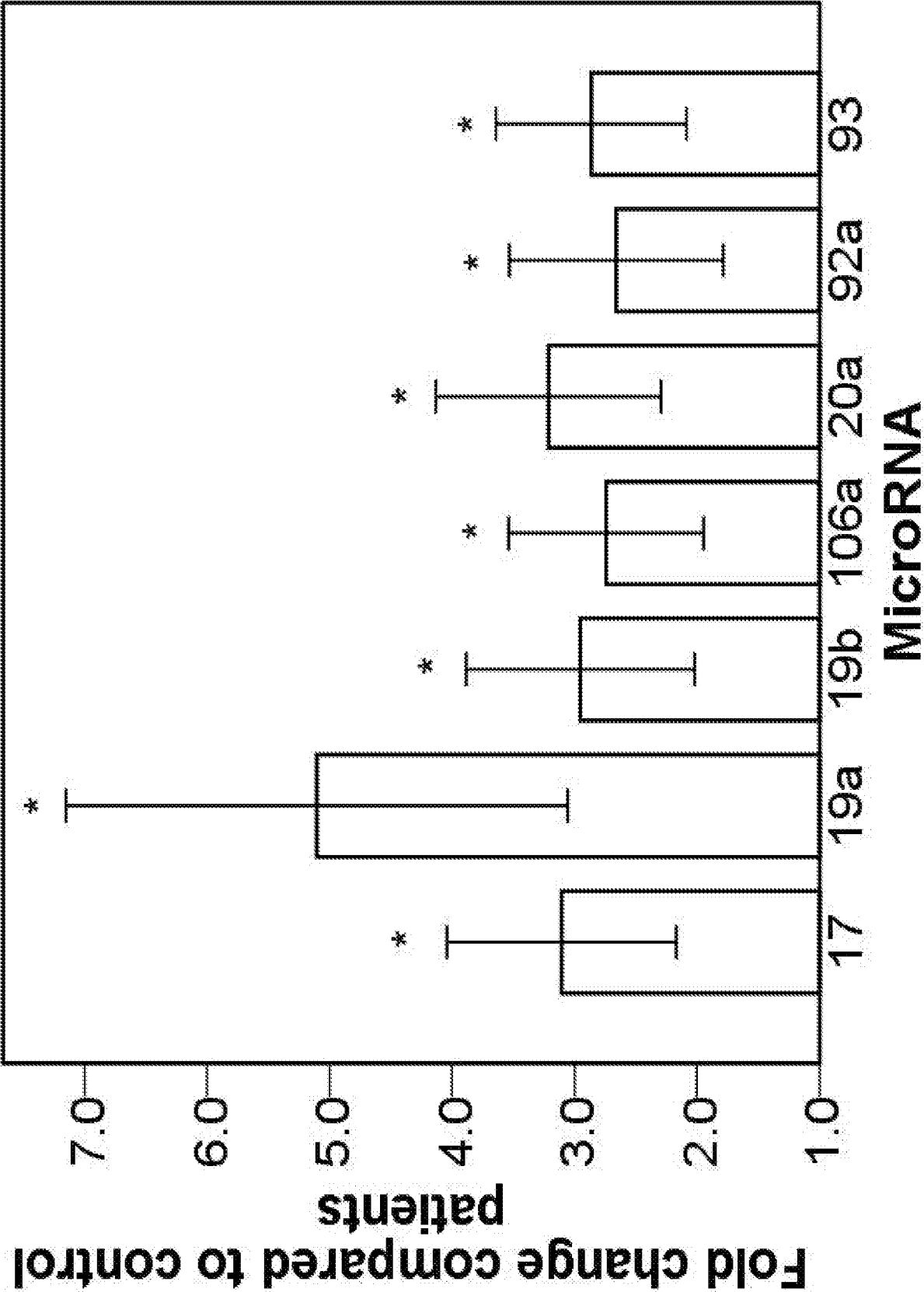


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/027593

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/113; C12Q 1/68; G01N 33/68 (2017.01)

CPC - C12Q 1/6837; C12Q 1/6883; C12Q 2600/118; C12Q 2600/154; C12Q 2600/178; G01N 2800/368 (2017.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/9.1; 435/6.11; 435/7.1; 436/503; 506/9 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2013/0245135 A1 (EDWARD E WINGER MD PROFESSIONAL CORP) 19 September 2013 (19.09.2013) entire document	1-11, 45-48 ----- 49
X -- Y	US 2016/0061824 A1 (UNIVERSITAETSSPITAL BASEL (USB) et al) 03 March 2016 (03.03.2016) entire document	12-19, 21, 22, 37-40, 43, 44 ----- 20, 23, 24, 41, 42, 50
X -- Y	WO 2014/143977 A2 (SERA PROGNOSTICS, INC.) 18 September 2014 (18.09.2014) entire document	25-30, 32, 33, 35, 36 ----- 23, 24, 31, 34
Y	WO 1999/019502 A1 (CHANDRA et al) 22 April 1999 (22.04.1999) entire document	20, 23, 24
Y	US 2013/0287772 A1 (HALBERT et al) 31 October 2013 (31.10.2013) entire document	31, 34
Y	WO 2015/002845 A1 (ANDERSON et al) 08 January 2015 (08.01.2015) entire document	41
Y	WO 2003/016497 A2 (BAUGHN et al) 27 February 2003 (27.02.2003) entire document	42
Y	KUMAR et al." The c-Myc-Regulated MicroRNA-17~92 (miR-17~92) and miR-106a~363 Clusters Target hCYP19A1 and hGCM1 To Inhibit Human Trophoblast Differentiation," Molecular and Cellular Biology, 31 May 2013 (31.05.2013), Vol. 33, No. 9, Pgs. 1782-1796. entire document	49

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 July 2017

Date of mailing of the international search report

26 JUL 2017

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/027593

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/0293115 A1 (YALE UNIVERSITY) 15 October 2015 (15.10.2015) entire document	50
A	WANG et al. "Preeclampsia Up-Regulates Angiogenesis-Associated MicroRNA (i.e., miR-17, -20a, and -20b) That Target Ephrin-B2 and EPHB4 in Human Placenta," Journal of Clinical Endocrinology and Metabolism, 30 June 2012 (30.06.2012), Vol. 97, No. 6, Pgs. E1051-E1059. entire document	1-50

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/027593

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1-55 were searched.