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(71) **Applicant**: **THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY** [US/US]; 1705 El Camino Real, Palo Alto, California 94306-1106 (US).

(72) **Inventors**: **BUTTE, Atul J.**; 2020 Monterey Avenue, Menlo Park, California 94025 (US). **LING, Bruce Xuefeng**; 1400 Waverly Street, Palo Alto, California 94301 (US). **MILLER, Linda Liu**; 200 West Washington Square, Philadelphia, Pennsylvania 19106 (US). **MORGAN, Alexander A.**; Genome Technology Center, 855 South California Ave., Palo Alto, California 94304 (US). **CHEN, Gongxing**; 269 Campus Drive, CCSR, Stanford, California 94305 (US). **JI, Jun**; 269 Matadero Avenue, Palo Alto, California 94305 (US). **YANG, Ting**; 1711 Fumia Court, San Jose, California 95131 (US).

(74) **Agent**: **ALCAMO, Elizabeth A.**; Bozicevic, Field & Francis LLP., 1900 University Ave., Suite 200, East Palo Alto, California 94303 (US).

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(54) **Title**: METHODS AND COMPOSITIONS FOR PROVIDING A PREECLAMPSIA ASSESSMENT

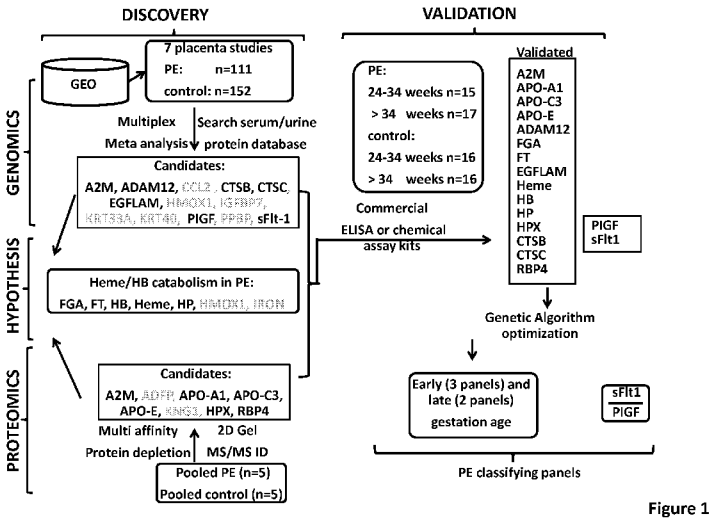

Figure 1

(57) **Abstract**: Preeclampsia markers, preeclampsia marker panels, and methods for obtaining a preeclampsia marker level representation for a sample are provided. These compositions and methods find use in a number of applications, including, for example, diagnosing preeclampsia, prognosing a preeclampsia, monitoring a subject with preeclampsia, and determining a treatment for preeclampsia. In addition, systems, devices and kits thereof that find use in practicing the subject methods are provided.

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METHODS AND COMPOSITIONS FOR PROVIDING A PREECLAMPSIA ASSESSMENT

CROSS REFERENCE TO RELATED APPLICATIONS

Pursuant to 35 U.S.C. § 119 (e), this application claims priority to the filing date of the United States Provisional Patent Application Serial No. 61/644,254, filed May 8, 2012; and United States Provisional Patent Application Serial No. 61/731,640, filed November 30, 2012; the disclosures of which are herein incorporated by reference.

FIELD OF THE INVENTION

This invention pertains to providing a preeclampsia assessment.

BACKGROUND OF THE INVENTION

Preeclampsia is a serious multisystem complication of pregnancy with adverse effects for mothers and babies. The incidence of the disorder is around 5-8% of all pregnancies in the U.S. and worldwide, and the disorder is responsible for 18% of all maternal deaths in the U.S. The causes and pathogenesis of preeclampsia remain uncertain, and the diagnosis relies on nonspecific laboratory and clinical signs and symptoms that occur late in the disease process, sometimes making the diagnosis and clinical management decisions difficult. Earlier and more reliable disease diagnosing, prognosing and monitoring will lead to more timely and personalized preeclampsia treatments and significantly advance our understanding of preeclampsia pathogenesis. The present invention addresses these issues.

SUMMARY OF THE INVENTION

Preeclampsia markers, preeclampsia marker panels, and methods for obtaining a preeclampsia marker level representation for a sample are provided. These compositions and methods find use in a number of applications, including, for example, diagnosing preeclampsia, prognosing a preeclampsia, monitoring a subject with preeclampsia, and determining a treatment for preeclampsia. In addition, systems, devices and kits thereof that find use in practicing the subject methods are provided.

In some aspects of the invention, a panel of preeclampsia markers is provided, the panel comprising one or more preeclampsia markers selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM

metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) and heme. In some embodiments, the panel comprises pikachurin and/or cathepsin C. In some embodiments, the panel comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4, and haptoglobin.

In some aspects of the invention, a method is provided for providing a preeclampsia marker level representation for a subject. In some embodiments, the method comprises evaluating a panel of preeclampsia markers in a blood sample from a subject to determine the level of each preeclampsia marker in the blood sample; and calculating the preeclampsia marker level representation based on the level of each preeclampsia marker in the panel. In some embodiments, the panel comprises one or more preeclampsia markers selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) and heme. In some embodiments, the panel comprises pikachurin and/or cathepsin C. In some embodiments, the panel comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4, and haptoglobin. In some embodiments, the method further comprises providing a report of the preeclampsia marker level representation. In certain embodiments, the preeclampsia marker representation is a preeclampsia score.

In some aspects of the invention, a method is provided for providing a preeclampsia assessment for a subject. In some embodiments, the preeclampsia assessment is a diagnosis of preeclampsia. In some embodiments, the method comprises obtaining a preeclampsia marker level representation for a sample from a subject, e.g. as described above or elsewhere herein, and providing a preeclampsia diagnosis for the subject based on the preeclampsia marker level representation. In some embodiments, the method further comprises comparing the preeclampsia marker level representation to a preeclampsia phenotype determination element, and providing a preeclampsia diagnosis for the subject based on the comparison. In some embodiments, the subject has symptoms of preeclampsia. In other embodiments, the subject is asymptomatic for preeclampsia. In some embodiments, the subject has one or more risk factors associated with preeclampsia. In other embodiments, the subject has no risk factors associated with preeclampsia. In some embodiments, the sample is collected at 20 or more weeks of gestation. In certain

embodiments, the sample is collected at 34 or more weeks of gestation.

In some aspects of the invention, a kit is provided for making a preeclampsia assessment for a sample. In some embodiments, the preeclampsia assessment is a preeclampsia diagnosis. In some embodiments, the kit comprises one or more detection elements for measuring the amount of marker in a sample for a panel of preeclampsia markers comprising one or more markers selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I ((ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) and heme; and a preeclampsia phenotype determination element. In some embodiments, the one or more detection elements detect the level of marker polypeptides in the sample. In some embodiments, the panel of preeclampsia markers comprises pikachurin and/or cathepsin C. In some embodiments, the panel of preeclampsia markers comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4 and haptoglobin.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying 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. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figure 1. Study outline of the multi-'omics' based discovery and validation of PE biomarkers. Candidate analytes, which failed subsequent validation, were greyed out.

Figure 2. Expression comparative analysis of PE biomarkers (PE versus controls). Forest plot summarizes the results of placenta mRNA expression meta analysis, and maternal serum analyte abundance quantification at different early and late gestational age weeks. Line plot represents 95% confidence interval.

Figure 3. Early or late onset biomarker panel scores were plotted as a function of the gestational weeks. *Different panel scores were scaled to the same scoring metric such that they can be directly compared. For either PE or control data points, a loess curve was fitted to represent the overall trend of biomarker scoring as a function of gestational age.

Figure 4. Composite overlay of different biomarker panels' loess fitted lines for both PE and control subjects as a function of gestational age weeks.

Figure 5. Boxplot display and scatter plot of biomarker distribution for sFlt-1 at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 6. Boxplot display and scatter plot of biomarker distribution for PlGF at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 7. Boxplot display and scatter plot of biomarker distribution for HPX at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 8. Boxplot display and scatter plot of biomarker distribution for FT at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 9. Boxplot display and scatter plot of biomarker distribution for ADAM12 at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 10. Boxplot display and scatter plot of biomarker distribution for HP at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 11. Boxplot display and scatter plot of biomarker distribution for A2M at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 12. Boxplot display and scatter plot of biomarker distribution for APO-E at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 13. Boxplot display and scatter plot of biomarker distribution for APO-CIII at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 14. Boxplot display and scatter plot of biomarker distribution for APO-AI at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 15. Boxplot display and scatter plot of biomarker distribution for RBP4 at different gestational age weeks in PE and control groups. Horizontal box boundaries and

midline denote sample quartiles.

Figure 16. Boxplot display and scatter plot of biomarker distribution for HB at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 17. Boxplot display and scatter plot of biomarker distribution for FGA at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 18. Boxplot display and scatter plot of biomarker distribution for Pikachurin at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 19. Boxplot display and scatter plot of biomarker distribution for CTSB at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 20. Boxplot display and scatter plot of biomarker distribution for CTSC at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 21. Boxplot display and scatter plot of biomarker distribution for Heme at different gestational age weeks in PE and control groups. Horizontal box boundaries and midline denote sample quartiles.

Figure 22 provides a summary of the validation by ELISA or biochemical methodology (for heme) of preeclampsia serological biomarkers that are predictive of preeclampsia when measured in combination with s-FLt-1 (soluble VEGF-R1), as compared to the current standard for prognosis ("sFlt-1/PlGF"). Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation. ROC curves of different analyte ratio combinations were analyzed to compute area under the curve (AUC) values.

Figure 23 provides a summary of the validation by ELISA or biochemical methodology (for heme) of preeclampsia serological biomarkers that are predictive of preeclampsia when measured in combination with s-FLt-1, as compared to the current standard for prognosis ("sFlt-1/PlGF"). Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation.

ROC curves of different analyte ratio combinations were analyzed to compute area under the curve (AUC) values.

Figure 24 provides a summary of the validation by ELISA or biochemical methodology (for heme) of preeclampsia serological biomarkers that are predictive of preeclampsia when measured in combination with HPX as compared to the current standard for prognosis ("s-FLt-1/PIGF"). Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation. ROC curves of different analyte ratio com combinations were analyzed to compute area under the curve (AUC) values.

Figure 25 provides a summary of the validation by ELISA or biochemical methodology (for heme) of preeclampsia serological biomarkers that are predictive of preeclampsia when measured in combination with CTSC, as compared to the current standard for prognosis ("s-FLt-1/PIGF"). Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation. ROC curves of different analyte ratio com combinations were analyzed to compute area under the curve (AUC) values.

Figure 26 provides a summary of the validation by ELISA of preeclampsia serological biomarkers that are predictive of preeclampsia when measured in combination with ADAM12, as compared to the current standard for prognosis ("s-FLt-1/PIGF"). Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation. ROC curves of different analyte ratio com combinations were analyzed to compute area under the curve (AUC) values.

Figure 27 demonstrates the improved accuracy in prognosing preeclampsia that is achieved by using the biomarker panel comprising hemopexin, ferritin, Cathepsin C, ADAM metallopeptidase domain 12, Keratin 33A, haptoglobin, alpha-2-macroglobulin, apolipoprotein E, apolipoprotein C-III, apolipoprotein A-I, retinol binding protein 4, hemoglobin, fibrinogen, pikachurin, sFlt-1 and PIGF ("panel") as compared to a panel consisting of sFlt-1/PIGF. Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation. ROC curves of the biomarker panel were analyzed to comptue area under the curve (AUC) values.

Figure 28 demonstrates the accuracy in prognosing preeclampsia that is achieved by using the biomarker panel comprising hemopexin, ferritin, Cathepsin C, ADAM metallopeptidase domain 12, Keratin 33A, haptoglobin, alpha-2-macroglobulin, apolipoprotein E, apolipoprotein C-III, apolipoprotein A-I, retinol binding protein 4, hemoglobin, fibrinogen, and pikachurin ("panel") (i.e. no sFlt-1 or PlGF measured) as compared to a panel consisting of sFlt-1/PlGF. Early stage (Normal N=16; PE N=16) predictions were made from samples collected at or before 34 weeks gestation. Late stage (Normal N=16; PE N=16) predictions were made from samples collected after 34 weeks gestation. ROC curves of the biomarker panel were analyzed to compute the area under the curve (AUC) values.

Figure 29 demonstrates different panels of biomarker combinations. +: the biomarker was chosen in the corresponding panel; -: the biomarker was not chosen in the panel.

Figure 30 demonstrates ROC curve AUC values with different combinations of biomarkers. The "biomarker" columns show the selection of sFlt-1, PlGF and Stanford validated biomarkers for each panel. The "number of SU biomarkers" columns show the number of Stanford validated biomarkers for early stage PE onset, late stage PE onset and overall summary, respectively. The "ROC curve AUC value" columns show the AUC value of ROC curve analyses for early stage PE onset, late stage PE onset and overall summary.

Figure 31 demonstrates sensitivity and specificity analyses for each biomarker panels in Figures 29 and 30. Upper panel: sensitivity of different panels with given specificity levels. Lower panel: specificity of different panels with given sensitivity levels.

Figure 32 depicts a scatter plot and ROC curve for Panel 1 and Panel 2 in Figure 27. Upper panels: logarithm combined biomarker value versus gestation age (weeks). Lower panels: ROC curve.

Figure 33 depicts a scatter plot and ROC curve for Panel 3 and Panel 4 in Figure 29. Upper panels: logarithm combined biomarker value versus gestation age (weeks). Lower panels: ROC curve.

Figure 34 depicts a scatter plot and ROC curve for Panel 5 and Panel 6 in Figure 29. Upper panels: logarithm combined biomarker value versus gestation age (weeks). Lower panels: ROC curve.

Figure 35 depicts a scatter plot and ROC curve for Panel 7 in Figure 29. Upper panel: logarithm combined biomarker value versus gestation age (weeks). Lower panel: ROC curve.

Figure 36 depicts the performance, gauged by ROC analyses, of PE serum protein biomarker panel 0, 1, and 2 in discriminating PE and control subjects.

DETAILED DESCRIPTION OF THE INVENTION

Preeclampsia markers, preeclampsia marker panels, and methods for obtaining a preeclampsia marker level representation for a sample are provided. These compositions and methods find use in a number of applications, including, for example, diagnosing preeclampsia, prognosing a preeclampsia, monitoring a subject with preeclampsia, and determining a treatment for preeclampsia. In addition, systems, devices and kits thereof that find use in practicing the subject methods are provided. These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the compositions and methods as more fully described below.

Before the present methods and compositions are described, it is to be understood that this invention is not limited to particular method or composition described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the

present disclosure supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

As summarized above, aspects of the subject invention include methods, compositions, systems and kits that find use in providing a preeclampsia assessment, e.g. diagnosing, prognosing, monitoring, and/or treating preeclampsia in a subject. By “preeclampsia” or “pre-eclampsia” it is meant a multisystem complication of pregnancy that may be accompanied by one or more of high blood pressure, proteinuria, swelling of the hands and face/eyes (edema), sudden weight gain, higher-than-normal liver enzymes, and thrombocytopenia. Preeclampsia typically occurs in the third trimester of pregnancy, but in severe cases, the disorder occur in the 2d trimester, e.g., after about the 22nd week of pregnancy. If unaddressed, preeclampsia can lead to eclampsia, i.e. seizures that are not related to a preexisting brain condition. By “diagnosing” a preeclampsia or “providing a preeclampsia diagnosis,” it is generally meant providing a preeclampsia determination, e.g. a determination as to whether a subject (e.g. a subject that has clinical symptoms of preeclampsia, a subject that is asymptomatic for preeclampsia but has risk factors associated with preeclampsia, a subject that is asymptomatic for preeclampsia and has no risk factors associated with preeclampsia) is presently affected by preeclampsia; a

classification of the subject's preeclampsia into a subtype of the disease or disorder; a determination of the severity of preeclampsia; and the like. By "prognosing" a preeclampsia, or "providing a preeclampsia prognosis," it is generally meant providing a preeclampsia prediction, e.g. a prediction of a subject's susceptibility, or risk, of developing preeclampsia; a prediction of the course of disease progression and/or disease outcome, e.g. expected onset of the preeclampsia, expected duration of the preeclampsia, expectations as to whether the preeclampsia will develop into eclampsia, etc.; a prediction of a subject's responsiveness to treatment for the preeclampsia, e.g., positive response, a negative response, no response at all; and the like. By "monitoring" a preeclampsia, it is generally meant monitoring a subject's condition, e.g. to inform a preeclampsia diagnosis, to inform a preeclampsia prognosis, to provide information as to the effect or efficacy of a preeclampsia treatment, and the like. By "treating" a preeclampsia it is meant prescribing or providing any treatment of a preeclampsia in a mammal, and includes: (a) preventing the preeclampsia from occurring in a subject which may be predisposed to preeclampsia but has not yet been diagnosed as having it; (b) inhibiting the preeclampsia, i.e., arresting its development; or (c) relieving the preeclampsia, i.e., causing regression of the preeclampsia.

In describing the subject invention, compositions useful for providing a preeclampsia assessment will be described first, followed by methods, systems and kits for their use.

PREECLAMPSIA MARKERS AND PANELS

In some aspects of the invention, preeclampsia markers and panels of preeclampsia markers are provided. By a "preeclampsia marker" it is meant a molecular entity whose representation in a sample is associated with a preeclampsia phenotype. For example, a preeclampsia marker may be differentially represented, i.e. represented at a different level, in a sample from an individual that will develop or has developed preeclampsia as compared to a healthy individual. In some instances, an elevated level of marker is associated with the preeclampsia phenotype. For example, the concentration of marker in a sample may be 1.5-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 7.5-fold, 10-fold, or greater in a sample associated with the preeclampsia phenotype than in a sample not associated with the preeclampsia phenotype. In other instances, a reduced level of marker is associated with the preeclampsia phenotype. For example, the concentration of marker in a sample may be 10% less, 20% less, 30% less, 40% less, 50% less or more in a sample associated with the preeclampsia phenotype than in a sample not associated with the preeclampsia phenotype.

Preeclampsia markers may include proteins associated with preeclampsia and their

corresponding genetic sequences, i.e. mRNA, DNA, etc. By a "gene" or "recombinant gene" it is meant a nucleic acid comprising an open reading frame that encodes for the protein. The boundaries of a coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A transcription termination sequence may be located 3' to the coding sequence. In addition, a gene may optionally include its natural promoter (i.e., the promoter with which the exons and introns of the gene are operably linked in a non-recombinant cell, i.e., a naturally occurring cell), and associated regulatory sequences, and may or may not have sequences upstream of the AUG start site, and may or may not include untranslated leader sequences, signal sequences, downstream untranslated sequences, transcriptional start and stop sequences, polyadenylation signals, translational start and stop sequences, ribosome binding sites, and the like.

As demonstrated in the examples of the present disclosure, the inventors have identified a number of molecular entities that are associated with preeclampsia and that find use either alone or in combination (i.e. as a panel) in providing a preeclampsia assessment, e.g. diagnosing preeclampsia, prognosing a preeclampsia, monitoring a subject with preeclampsia, determining a treatment for a subject affected with preeclampsia, and the like. These include, but are not limited to, hemopexin (HPX, GenBank Accession No. NM_000613.2); ferritin (FT, GenBank Accession Nos. NM_000146.3 (light polypeptide), NM_002032.2 (heavy polypeptide)); Cathepsin B (CTSB, Genbank Accession Nos. NM_001908.3 (variant 1), NM_147780.2 (variant 2), NM_147781.2 (variant 3), NM_147782.2 (variant 4), and NM_147783.2 (variant 5)); Cathepsin C (CTSC, Genbank Accession Nos. NM_001114173.1 (isoform a), NM_148170.3 (isoform b), NM_001114173.1 (isoform c)); ADAM metallopeptidase domain 12 (ADAM12, Genbank Accession Nos. NM_003474.4 (isoform 1), NM_021641.3 (isoform 2); Keratin 33A (KRT33A, Genbank Accession No. NM_004138.2); haptoglobin (HP, GenBank Accession Nos. NM_005143.3 (isoform 1), NM_001126102.1 (isoform 2)); alpha-2-macroglobulin (A2M, GenBank Accession No. NM_000014.4); apolipoprotein E (ApoE, GenBank Accession No. NM_000041.2); apolipoprotein C-III (ApoC3, GenBank Accession No. NM_000040.1); apolipoprotein A-I (ApoA1, GenBank Accession No. NM_000039.1); retinol binding protein 4, plasma (RBP4, GenBank Accession No. NM_006744.3); hemoglobin (GenBank Accession Nos. NM_000517.4 (alpha 2), NM_000518.4 (beta), NM_000559.2 (gamma A), NM_000184.2 (gamma G)); fibrinogen alpha (GenBank Accession No. NM_021871.2 (alpha chain); pikachurin (EGFLAM, GenBank Accession Nos. NM_152403.3 (isoform 1),

NM_182798.2 (isoform 2), NM_182801.2 (isoform 4), and NM_001205301.1 (isoform 5)), and the cofactor/prosthetic group heme. Of particular interest are the preeclampsia markers ADAM12, CTSC, and Pikachurin.

As mentioned above, also provided herein are preeclampsia panels. By a "panel" of preeclampsia markers it is meant two or more preeclampsia markers, e.g. 3 or more, 4 or more, or 5 or more markers, in some instances 6 or more, 7 or more, or 8 or more markers, sometimes 9 or more, or 10 or more markers, e.g. 12, 15, 17 or 20 markers, whose levels, when considered in combination, find use in providing a preeclampsia assessment, e.g. making a preeclampsia diagnosis, prognosis, monitoring, and/or treatment. Of particular interest are panels that comprise the preeclampsia markers ADAM12, CTSC, or Pikachurin. For example, in some embodiments, the preeclampsia panel may comprise Pikachurin and one or more of Hemopexin, ApoA1, ApoC3, RBP4, and/or Haptoglobin, e.g. it may comprise Pikachurin and Hemopexin; Pikachurin and ApoA1; Pikachurin and ApoC3; Pikachurin and RBP4; Pikachurin and Haptoglobin; Pikachurin, Hemopexin, and ApoA1; Pikachurin, Hemopexin, and ApoC3; Pikachurin, Hemopexin, and RBP4; Pikachurin, Hemopexin, and Haptoglobin; Pikachurin, ApoA1, and ApoC3; Pikachurin, ApoA1, and RBP4; Pikachurin, ApoA1, and Haptoglobin; Pikachurin, ApoC3, and RBP4; Pikachurin, ApoC3, and Haptoglobin; Pikachurin, RBP4, and Haptoglobin; Pikachurin, Hemopexin, ApoA1 and ApoC3; Pikachurin, Hemopexin, ApoA1 and RBP4; Pikachurin, Hemopexin, ApoA1, and Haptoglobin; Pikachurin, Hemopexin, ApoC3, and RBP4; Pikachurin, Hemopexin, ApoC3, and Haptoglobin; Pikachurin, Hemopexin, RBP4, and Haptoglobin; Pikachurin, ApoA1, ApoC3, RBP4; Pikachurin, ApoA1, ApoC3 and Haptoglobin; Pikachurin, ApoA1, RBP4, and Haptoglobin; Pikachurin, ApoC3, RBP4 and Haptoglobin; or Pikachurin, Hemopexin, ApoA1, ApoC3, RBP4, and haptoglobin.

In some instances, other preeclampsia markers known in the art may be included in the subject preeclampsia panels, e.g. soluble vascular endothelial growth factor/vascular permeability factor receptor (VEGF-R1, also known as FMS-like tyrosine kinase 1 or sFlt-1; Genbank Accession Nos. NM_001159920.1 (isoform 2), NM_001160030.1 (isoform 3), and NM_001160031.1 (isoform 4)); and placental growth factor (PlGF, Genbank Accession Nos.NM_002632.5 (isoform 1) and NM_001207012.1 (isoform 2)) (Verlohren et al. (2010) Amer Journal of Obstetrics and Gynecology 161: e1-e11). Thus, for example, the preeclampsia panel may comprise ADAM12 and one or more of PlGF, haptoglobin, ApoE, ApoA1, A2M, RBP4, hemoglobin, ApoC3, fibrinogen, and/or pikachurin. As another example, the preeclampsia panel may comprise CTSC and one or more of PlGF,

haptoglobin, ApoE, ApoA1, A2M, RBP4, hemoglobin, ApoC3, fibrinogen, Pikachurin, and/or heme. Other examples of preeclampsia panels of interest include HPX, PlGF, haptoglobin, ApoE, ApoA1, A2M, RBP4, hemoglobin, ApoC3, fibrinogen, Pikachurin, and/or heme; sFlt-1, haptoglobin, ApoE, ApoA1, A2M, RBP4, hemoglobin, ApoC3, fibrinogen, pikachurin, and/or heme; sFlt-1 and A2M; sFlt-1 and RBP4; sFlt-1 and hemoglobin; sFlt-1 and fibrinogen; sFlt-1 and pikachurin; sFlt1 and HPX; HPX and pikachurin; sFlt1, PlGF, and HPX; sFlt1, PlGF, HPX, CTSC, ADAM12, ApoE, ApoA1, RBP4, HB, and Pikachurin; sFlt1, HPX, ApoE, ApoA1, and Pikachurin; PlGF and Pikachurin; PlGF, HPX, CTSC, Adam12, HP, ApoE, RBP4, HB, Fibrinogen, and Pikachurin; and HPX, ApoA1, Pikachurin; HPX, CTSC, Adam12, HP, HB, Fibrinogen, and Pikachurin.

Other combinations of preeclampsia markers that find use as preeclampsia panels in the subject methods may be readily identified by the ordinarily skilled artisan using any convenient statistical methodology, e.g. as known in the art or described in the working examples herein. For example, the panel of analytes may be selected by combining genetic algorithm (GA) and all paired (AP) support vector machine (SVM) methods for preeclampsia classification analysis. Predictive features are automatically determined, e.g. through iterative GA/SVM, leading to very compact sets of non- redundant preeclampsia-relevant analytes with the optimal classification performance. While different classifier sets will typically harbor only modest overlapping gene features, they will have similar levels of accuracy in providing a preeclampsia assessment to those described above and in the working examples herein.

METHODS

In some aspects of the invention, methods are provided for obtaining a preeclampsia marker level representation for a subject. By a preeclampsia marker level representation, it is meant a representation of the levels of one or more of the subject preeclampsia marker(s), e.g. a panel of preeclampsia markers, in a biological sample from a subject. The term "biological sample" encompasses a variety of sample types obtained from an organism and can be used in a diagnostic, prognostic, or monitoring assay. The term encompasses blood and other liquid samples of biological origin or cells derived therefrom and the progeny thereof. The term encompasses samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components. The term encompasses a clinical sample, and also includes cell supernatants, cell lysates, serum, plasma, biological fluids, and tissue samples. Clinical samples for use in

the methods of the invention may be obtained from a variety of sources, particularly blood samples.

Sample sources of particular interest include blood samples or preparations thereof, e.g., whole blood, or serum or plasma, and urine. A sample volume of blood, serum, or urine between about 2µl to about 2,000µl is typically sufficient for determining the level of a preeclampsia gene product. Generally, the sample volume will range from about 10µl to about 1,750µl, from about 20µl to about 1,500µl, from about 40µl to about 1,250µl, from about 60µl to about 1,000µl, from about 100µl to about 900 µl, from about 200µl to about 800µl, from about 400µl to about 600µl. In many embodiments, a suitable initial source for the human sample is a blood sample. As such, the sample employed in the subject assays is generally a blood-derived sample. The blood derived sample may be derived from whole blood or a fraction thereof, e.g., serum, plasma, etc., where in some embodiments the sample is derived from blood, allowed to clot, and the serum separated and collected to be used to assay.

In some embodiments the sample is a serum or serum-derived sample. Any convenient methodology for producing a fluid serum sample may be employed. In many embodiments, the method employs drawing venous blood by skin puncture (e.g., finger stick, venipuncture) into a clotting or serum separator tube, allowing the blood to clot, and centrifuging the serum away from the clotted blood. The serum is then collected and stored until assayed. Once the patient derived sample is obtained, the sample is assayed to determine the level of preeclampsia marker(s).

The subject sample is typically obtained from the individual during the second or third trimester of gestation. By “gestation” it is meant the duration of pregnancy in a mammal, i.e. the time interval of development from fertilization until birth, plus two weeks, i.e. to the first day of the last menstrual period. By the second or third trimester, it is meant the second or third portions of gestation, each segment being 3 months long. Thus, for example, by the “first trimester” is meant from the first day of the last menstrual period through the 13th week of gestation; by the “second trimester” it is meant from the 14th through 27th week of gestation; and by the “third trimester” it is meant from the 28th week through birth, i.e. 38 - 42 weeks of gestation. Put another way, a subject sample may be obtained at about weeks 14 through 42 of gestation, at about weeks 18 through 42 of gestation, at about weeks 20 through 42 of gestation, at about weeks 24 through 42 of gestation, at about weeks 30 through 42 of gestation, at about weeks 34 through 42 of gestation, at about weeks 38 through 42 of gestation. Thus, in some embodiments, the subject sample may be obtained

early in gestation, e.g. at week 14 or more of gestation, e.g. at week 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 or more of gestation, more often at week 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or week 34 or more of gestation. In other embodiments, the subject sample may be obtained late in gestation, for example, after 34 weeks of gestation, e.g. at week 35, 36, 37, 38, 39, 40, or week 41 of gestation.

Once a sample is obtained, it can be used directly, frozen, or maintained in appropriate culture medium for short periods of time. Typically the samples will be from human patients, although animal models may find use, e.g. equine, bovine, porcine, canine, feline, rodent, *e.g.* mice, rats, hamster, primate, *etc.* Any convenient tissue sample that demonstrates the differential representation in a patient with preeclampsia of the one or more preeclampsia markers disclosed herein may be evaluated in the subject methods. Typically, a suitable sample source will be derived from fluids into which the molecular entity of interest, i.e. the RNA transcript or protein, has been released.

The subject sample may be treated in a variety of ways so as to enhance detection of the one or more preeclampsia markers. For example, where the sample is blood, the red blood cells may be removed from the sample (e.g., by centrifugation) prior to assaying. Such a treatment may serve to reduce the non-specific background levels of detecting the level of a preeclampsia marker using an affinity reagent. Detection of a preeclampsia marker may also be enhanced by concentrating the sample using procedures well known in the art (e.g. acid precipitation, alcohol precipitation, salt precipitation, hydrophobic precipitation, filtration (using a filter which is capable of retaining molecules greater than 30 kD, e.g. Centrim 30™), affinity purification). In some embodiments, the pH of the test and control samples will be adjusted to, and maintained at, a pH which approximates neutrality (i.e. pH 6.5-8.0). Such a pH adjustment will prevent complex formation, thereby providing a more accurate quantitation of the level of marker in the sample. In embodiments where the sample is urine, the pH of the sample is adjusted and the sample is concentrated in order to enhance the detection of the marker.

In practicing the subject methods, the level(s) of preeclampsia marker(s) in the biological sample from an individual are evaluated. The level of one or more preeclampsia markers in the subject sample may be evaluated by any convenient method. For example, preeclampsia gene expression levels may be detected by measuring the levels/amounts of one or more nucleic acid transcripts, e.g. mRNAs, of one or more preeclampsia genes. Protein markers may be detected by measuring the levels/amounts of one or more proteins/polypeptides. The terms “evaluating”, “assaying”, “measuring”, “assessing,” and

"determining" are used interchangeably to refer to any form of measurement, including determining if an element is present or not, and including both quantitative and qualitative determinations. Evaluating may be relative or absolute.

For example, the level of at least one preeclampsia marker may be evaluated by detecting in a sample the amount or level of one or more proteins/polypeptides or fragments thereof to arrive at a protein level representation. The terms "protein" and "polypeptide" as used in this application are interchangeable. "Polypeptide" refers to a polymer of amino acids (amino acid sequence) and does not refer to a specific length of the molecule. Thus peptides and oligopeptides are included within the definition of polypeptide. This term also refers to or includes post-translationally modified polypeptides, for example, glycosylated polypeptide, acetylated polypeptide, phosphorylated polypeptide and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

When protein levels are to be detected, any convenient protocol for evaluating protein levels may be employed wherein the level of one or more proteins in the assayed sample is determined. For example, one representative and convenient type of protocol for assaying protein levels is ELISA. In ELISA and ELISA-based assays, one or more antibodies specific for the proteins of interest may be immobilized onto a selected solid surface, preferably a surface exhibiting a protein affinity such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed material, the assay plate wells are coated with a non-specific "blocking" protein that is known to be antigenically neutral with regard to the test sample such as bovine serum albumin (BSA), casein or solutions of powdered milk. This allows for blocking of non-specific adsorption sites on the immobilizing surface, thereby reducing the background caused by non-specific binding of antigen onto the surface. After washing to remove unbound blocking protein, the immobilizing surface is contacted with the sample to be tested under conditions that are conducive to immune complex (antigen/antibody) formation. Such conditions include diluting the sample with diluents such as BSA or bovine gamma globulin (BGG) in phosphate buffered saline (PBS)/Tween or PBS/Triton-X 100, which also tend to assist in the reduction of nonspecific background, and allowing the sample to incubate for about 2-4 hrs at temperatures on the order of about 25°-27°C (although other temperatures may be used). Following incubation, the antisera-contacted surface is washed so as to remove non-immunocomplexed material. An exemplary washing procedure includes washing with a

solution such as PBS/Tween, PBS/Triton-X 100, or borate buffer. The occurrence and amount of immunocomplex formation may then be determined by subjecting the bound immunocomplexes to a second antibody having specificity for the target that differs from the first antibody and detecting binding of the second antibody. In certain embodiments, the second antibody will have an associated enzyme, e.g. urease, peroxidase, or alkaline phosphatase, which will generate a color precipitate upon incubating with an appropriate chromogenic substrate. For example, a urease or peroxidase-conjugated anti-human IgG may be employed, for a period of time and under conditions which favor the development of immunocomplex formation (e.g., incubation for 2 hr at room temperature in a PBS-containing solution such as PBS/Tween). After such incubation with the second antibody and washing to remove unbound material, the amount of label is quantified, for example by incubation with a chromogenic substrate such as urea and bromocresol purple in the case of a urease label or 2,2'-azino-di-(3-ethyl-benzthiazoline)-6-sulfonic acid (ABTS) and H_2O_2, in the case of a peroxidase label. Quantitation is then achieved by measuring the degree of color generation, e.g., using a visible spectrum spectrophotometer.

The preceding format may be altered by first binding the sample to the assay plate. Then, primary antibody is incubated with the assay plate, followed by detecting of bound primary antibody using a labeled second antibody with specificity for the primary antibody.

The solid substrate upon which the antibody or antibodies are immobilized can be made of a wide variety of materials and in a wide variety of shapes, e.g., microtiter plate, microbead, dipstick, resin particle, etc. The substrate may be chosen to maximize signal to noise ratios, to minimize background binding, as well as for ease of separation and cost. Washes may be effected in a manner most appropriate for the substrate being used, for example, by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, or rinsing a bead, particle, chromatograpic column or filter with a wash solution or solvent.

Alternatively, non-ELISA based-methods for measuring the levels of one or more proteins in a sample may be employed. Representative examples include but are not limited to mass spectrometry, proteomic arrays, xMAP™ microsphere technology, flow cytometry, western blotting, and immunohistochemistry.

As another example, the level of at least one preeclampsia marker may be evaluated by detecting in a patient sample the amount or level of one or more RNA transcripts or a fragment thereof encoded by the gene of interest to arrive at a nucleic acid marker representation. The level of nucleic acids in the sample may be detected using any

convenient protocol. While a variety of different manners of detecting nucleic acids are known, such as those employed in the field of differential gene expression analysis, one representative and convenient type of protocol for generating marker representations is array-based gene expression profiling protocols. Such applications are hybridization assays in which a nucleic acid that displays "probe" nucleic acids for each of the genes to be assayed/profiled in the marker representation to be generated is employed. In these assays, a sample of target nucleic acids is first prepared from the initial nucleic acid sample being assayed, where preparation may include labeling of the target nucleic acids with a label, e.g., a member of signal producing system. Following target nucleic acid sample preparation, the sample is contacted with the array under hybridization conditions, whereby complexes are formed between target nucleic acids that are complementary to probe sequences attached to the array surface. The presence of hybridized complexes is then detected, either qualitatively or quantitatively.

Specific hybridization technology which may be practiced to generate the marker representations employed in the subject methods includes the technology described in U.S. Patent Nos.: 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280. In these methods, an array of "probe" nucleic acids that includes a probe for each of the phenotype determinative genes whose expression is being assayed is contacted with target nucleic acids as described above. Contact is carried out under hybridization conditions, e.g., stringent hybridization conditions, and unbound nucleic acid is then removed. The term "stringent assay conditions" as used herein refers to conditions that are compatible to produce binding pairs of nucleic acids, e.g., surface bound and solution phase nucleic acids, of sufficient complementarity to provide for the desired level of specificity in the assay while being less compatible to the formation of binding pairs between binding members of insufficient complementarity to provide for the desired specificity. Stringent assay conditions are the summation or combination (totality) of both hybridization and wash conditions.

The resultant pattern of hybridized nucleic acid provides information regarding expression for each of the genes that have been probed, where the expression information is in terms of whether or not the gene is expressed and, typically, at what level, where the expression data, i.e., marker representation (e.g., in the form of a transcriptosome), may be both qualitative and quantitative.

Alternatively, non-array based methods for quantitating the level of one or more nucleic acids in a sample may be employed, including those based on amplification protocols, e.g., Polymerase Chain Reaction (PCR)-based assays, including quantitative PCR, reverse-transcription PCR (RT-PCR), real-time PCR, and the like.

General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., HaRBor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds., John Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference. Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, Sigma-Aldrich, and ClonTech.

The resultant data provides information regarding levels in the sample for each of the markers that have been probed, wherein the information is in terms of whether or not the marker is present and, typically, at what level, and wherein the data may be both qualitative and quantitative. As such, where detection is qualitative, the methods provide a reading or evaluation, e.g., assessment, of whether or not the target marker, e.g., nucleic acid or protein, is present in the sample being assayed. In yet other embodiments, the methods provide a quantitative detection of whether the target marker is present in the sample being assayed, i.e., an evaluation or assessment of the actual amount or relative abundance of the target analyte, e.g., nucleic acid or protein in the sample being assayed. In such embodiments, the quantitative detection may be absolute or, if the method is a method of detecting two or more different analytes, e.g., target nucleic acids or protein, in a sample, relative. As such, the term "quantifying" when used in the context of quantifying a target analyte, e.g., nucleic acid(s) or protein(s), in a sample can refer to absolute or to relative quantification. Absolute quantification may be accomplished by inclusion of known concentration(s) of one or more control analytes and referencing the detected level of the target analyte with the known control analytes (e.g., through generation of a standard curve). Alternatively, relative quantification can be accomplished by comparison of detected levels or amounts between two or more different target analytes to provide a relative quantification of each of the two or more different analytes, e.g., relative to each other.

Once the level of the one or more preeclampsia markers has been determined, the measurement(s) may be analyzed in any of a number of ways to obtain a preeclampsia marker level representation.

For example, the measurements of the one or more preeclampsia markers may be analyzed individually to develop a preeclampsia profile. As used herein, a "preeclampsia profile" is the normalized level of one or more preeclampsia markers in a patient sample, for example, the normalized level of serological protein concentrations in a patient sample. A profile may be generated by any of a number of methods known in the art. For example, the level of each marker may be $\log_2$ transformed and normalized relative to the expression of a selected housekeeping gene, e.g. ABL1, GAPDH, or PGK1, or relative to the signal across a whole panel, etc. Other methods of calculating a preeclampsia profile will be readily known to the ordinarily skilled artisan.

As another example, the measurements of a panel of preeclampsia markers may be analyzed collectively to arrive at a single preeclampsia score. By a "preeclampsia score" it is meant a single metric value that represents the weighted levels of each of the preeclampsia markers in the preeclampsia panel. As such, in some embodiments, the subject method comprises detecting the level of markers of a preeclampsia panel in the sample, and calculating a preeclampsia score based on the weighted levels of the preeclampsia markers. A preeclampsia score for a patient sample may be calculated by any of a number of methods and algorithms known in the art for calculating biomarker scores. For example, weighted marker levels, e.g. $\log_2$ transformed and normalized marker levels that have been weighted by, e.g., multiplying each normalized marker level to a weighting factor, may be totaled and in some cases averaged to arrive at a single value representative of the panel of preeclampsia markers analyzed.

In some instances, the weighting factor, or simply "weight" for each marker in a panel may be a reflection of the change in analyte level in the sample. For example, the analyte level of each preeclampsia marker may be $\log_2$ transformed and weighted either as 1 (for those markers that are increased in level in preeclampsia) or -1 (for those markers that are decreased in level in preeclampsia), and the ratio between the sum of increased markers as compared to decreased markers determined to arrive at a preeclampsia signature. In other instances, the weights may be reflective of the importance of each marker to the specificity, sensitivity and/or accuracy of the marker panel in making the diagnostic, prognostic, or monitoring assessment. Such weights may be determined by any convenient statistical machine learning methodology, e.g. Principle Component Analysis (PCA), linear regression,

support vector machines (SVMs), and/or random forests of the dataset from which the sample was obtained may be used. In some instances, weights for each marker are defined by the dataset from which the patient sample was obtained. In other instances, weights for each marker may be defined based on a reference dataset, or "training dataset".

For example, as disclosed in the examples here, in a preeclampsia panel comprising the markers Pikachurin, Hemopexin, ApoA1, ApoC3, RBP4, and Haptoglobin, Pikachurin levels are most significant, levels of Hemopexin, ApoA1 and ApoC3 are moderately important, and levels of RBP4 and haptoglobin are less significant.. As such, one example of an algorithm that may be used to arrive at a preeclampsia score would be an algorithm that considers Pikachurin levels most strongly, e.g. assigning Pikachurin measurements a weight of about 12-16, e.g. about 15; that considers hemopexin, ApoA1, and ApoC3 levels more modestly, e.g. assigning the measurements for these genes a weight of about 4-8, e.g. about 6; that considers RBP4 less still, e.g. assigning RBP4 measurements a weight of about 2, and that considers haptoglobin least, e.g. assigning haptoglobin measurements a weight of about 1 or less.

These methods of analysis may be readily performed by one of ordinary skill in the art by employing a computer-based system, e.g. using any hardware, software and data storage medium as is known in the art, and employing any algorithms convenient for such analysis. For example, data mining algorithms can be applied through "cloud computing", smartphone based or client-server based platforms, and the like.

In certain embodiments the expression, e.g. polypeptide level, of only one marker is evaluated to produce a marker level representation. In yet other embodiments, the levels of two or more, i.e. a panel, markers, e.g., 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 10 or more, or 15 or more markers is evaluated. Accordingly, in the subject methods, the expression of at least one marker in a sample is evaluated. In certain embodiments, the evaluation that is made may be viewed as an evaluation of the proteome, as that term is employed in the art.

In some instances, the subject methods of determining or obtaining a preeclampsia marker representation (e.g. preeclampsia profile or preeclampsia score) for a subject further comprise providing the preeclampsia marker representation as a report. Thus, in some instances, the subject methods may further include a step of generating or outputting a report providing the results of a preeclampsia marker evaluation in the sample, which report can be provided in the form of an electronic medium (e.g., an electronic display on a computer monitor), or in the form of a tangible medium (e.g., a report printed on paper or

other tangible medium). Any form of report may be provided, e.g. as known in the art or as described in greater detail below.

UTILITY

Preeclampsia marker level representations so obtained find many uses. For example, the marker level representation may be employed to diagnose a preeclampsia; that is, to provide a determination as to whether a subject is affected by preeclampsia, the type of preeclampsia, the severity of preeclampsia, etc. In some instances, the subject may present with clinical symptoms of preeclampsia, e.g. elevated blood pressure (e.g. 140/90 mm/Hg or higher), proteinuria, sudden weight gain (over 1-2 days or more than 2 pounds a week), water retention (edema), elevated liver enzymes, and/or thrombocytopenia (a depressed platelet count less than 100,000). In other instances, subject may be asymptomatic for preeclampsia but has risk factors associated with preeclampsia, e.g. a medical condition such as gestational diabetes, type I diabetes, obesity, chronic hypertension, renal disease, a thrombophilia; African-American or Filipino descent; age of greater than 35 years or less than 20 years; a family history of preeclampsia; nulliparity; preeclampsia in a previous pregnancy; and/or stress. In yet other instances, the subject may be asymptomatic for preeclampsia and have no risk factors associated with preeclampsia.

As another example, the preeclampsia marker level representation may be employed to prognose a preeclampsia; that is, to provide a preeclampsia prognosis. For example, the preeclampsia marker level representation may be used to predict a subject's susceptibility, or risk, of developing preeclampsia. By “predicting if the individual will develop preeclampsia”, it is meant determining the likelihood that an individual will develop preeclampsia in the next week, in the next 2 weeks, in the next 3 weeks, in the next 5 weeks, in the next 2 months, in the next 3 months, e.g. during the remainder of the pregnancy. The preeclampsia marker level representation may be used to predict the course of disease progression and/or disease outcome, e.g. expected onset of the preeclampsia, expected duration of the preeclampsia, expectations as to whether the preeclampsia will develop into eclampsia, etc. The preeclampsia marker level representation may be used to predict a subject’s responsiveness to treatment for the preeclampsia, e.g., positive response, a negative response, no response at all.

As another example, the preeclampsia marker level representation may be employed to monitor a preeclampsia. By “monitoring” a preeclampsia, it is generally meant monitoring

a subject's condition, e.g. to inform a preeclampsia diagnosis, to inform a preeclampsia prognosis, to provide information as to the effect or efficacy of a preeclampsia treatment, and the like.

As another example, the preeclampsia marker level representation may be employed to determine a treatment for a subject. The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. The subject therapy may be administered prior to the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease. The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Preeclampsia treatments are well known in the art, and may include bed rest, drinking extra water, a low salt diet, medicine to control blood pressure, corticosteroids, inducing pregnancy, and the like.

In some embodiments, the subject methods of providing a preeclampsia assessment, e.g. diagnosing a preeclampsia, prognosing a preeclampsia, monitoring the preeclampsia, treating the preeclampsia, and the like, may comprise comparing the obtained preeclampsia marker level representation to a preeclampsia phenotype determination element to identify similarities or differences with the phenotype determination element, where the similarities or differences that are identified are then employed to provide the preeclampsia assessment, e.g. diagnose the preeclampsia, prognose the preeclampsia, monitor the preeclampsia, determine a preeclampsia treatment, etc. By a “phenotype determination element” it is meant an element, e.g. a tissue sample, a marker profile, a value (e.g. score), a range of values, and the like that is representative of a phenotype (in this instance, a preeclampsia phenotype) and may be used to determine the phenotype of the subject, e.g. if the subject is healthy or is affected by preeclampsia, if the subject has a preeclampsia that is likely to

progress to eclampsia, if the subject has a preeclampsia that is responsive to therapy, etc.

For example, a preeclampsia phenotype determination element may be a sample from an individual that has or does not have preeclampsia, which may be used, for example, as a reference/control in the experimental determination of the marker level representation for a given subject. As another example, a preeclampsia phenotype determination element may be a marker level representation, e.g. marker profile or score, which is representative of a preeclampsia state and may be used as a reference/control to interpret the marker level representation of a given subject. The phenotype determination element may be a positive reference/control, e.g., a sample or marker level representation thereof from a pregnant woman that has preeclampsia, or that will develop preeclampsia, or that has preeclampsia that is manageable by known treatments, or that has preeclampsia that has been determined to be responsive only to the delivery of the baby. Alternatively, the phenotype determination element may be a negative reference/control, e.g. a sample or marker level representation thereof from a pregnant woman that has not developed preeclampsia, or an woman that is not pregnant. Phenotype determination elements are preferably the same type of sample or, if marker level representations, are obtained from the same type of sample as the sample that was employed to generate the marker level representation for the individual being monitored. For example, if the serum of an individual is being evaluated, the phenotype determination element would preferably be of serum.

In certain embodiments, the obtained marker level representation is compared to a single phenotype determination element to obtain information regarding the individual being tested for preeclampsia. In other embodiments, the obtained marker level representation is compared to two or more phenotype determination elements. For example, the obtained marker level representation may be compared to a negative reference and a positive reference to obtain confirmed information regarding if the individual will develop preeclampsia. As another example, the obtained marker level representation may be compared to a reference that is representative of a preeclampsia that is responsive to treatment and a reference that is representative of a preeclampsia that is not responsive to treatment to obtain information as to whether or not the patient will be responsive to treatment.

The comparison of the obtained marker level representation to the one or more phenotype determination elements may be performed using any convenient methodology, where a variety of methodologies are known to those of skill in the art. For example, those of skill in the art of ELISAs will know that ELISA data may be compared by, e.g. normalizing

to standard curves, comparing normalized values, etc. The comparison step results in information regarding how similar or dissimilar the obtained marker level profile is to the control/reference profile(s), which similarity/dissimilarity information is employed to, for example, predict the onset of a preeclampsia, diagnose preeclampsia, monitor a preeclampsia patient, etc. Similarly, those of skill in the art of arrays will know that array profiles may be compared by, e.g., comparing digital images of the expression profiles, by comparing databases of expression data, etc. Patents describing ways of comparing expression profiles include, but are not limited to, U.S. Patent Nos. 6,308,170 and 6,228,575, the disclosures of which are herein incorporated by reference. Methods of comparing marker level profiles are also described above. Similarity may be based on relative marker levels, absolute marker levels or a combination of both. In certain embodiments, a similarity determination is made using a computer having a program stored thereon that is designed to receive input for a marker level representation obtained from a subject, e.g., from a user, determine similarity to one or more reference profiles or reference scores, and return an preeclampsia prognosis, e.g., to a user (e.g., lab technician, physician, pregnant individual, etc.). Further descriptions of computer-implemented aspects of the invention are described below. In certain embodiments, a similarity determination may be based on a visual comparison of the marker level representation, e.g. preeclampsia score, to a range of phenotype determination elements, e.g. a range of preeclampsia scores, to determine the reference preeclampsia score that is most similar to that of the subject. Depending on the type and nature of the phenotype determination element to which the obtained marker level profile is compared, the above comparison step yields a variety of different types of information regarding the cell/bodily fluid that is assayed. As such, the above comparison step can yield a positive/negative prediction of the onset of preeclampsia, a positive/negative diagnosis of preeclampsia, a characterization of a preeclampsia, information on the responsiveness of a preeclampsia to treatment, and the like.

In other embodiments, the marker level representation is employed directly, i.e. without comparison to a phenotype determination element, to make a preeclampsia prognosis, preeclampsia diagnosis, or monitor a preeclampsia. For example, a patient may be predicted to develop preeclampsia if the concentration of ADAM12 in the patient's serum is about 950 pg/ml or greater; if the concentration of cathepsin C in the patient's serum is about 16 ng/ml or greater; or the concentration of pikachurin in the patient's serum is about 500 ng/ml or less. For other examples, see Tables 1 and 2 of the Examples below.

In some embodiments, the subject methods of providing a preeclampsia assessment,

e.g. diagnosing a preeclampsia, prognosing a preeclampsia, monitoring the preeclampsia, and the like, may comprise additional assessment(s) that are employed in conjunction with the subject marker level representation. For example, the subject methods may further comprise measuring one or more clinical parameters/factors associated with preeclampsia, e.g. blood pressure, urine protein, weight changes, water retention (edema), liver enzyme levels, and platelet count. For example, a subject maybe assessed for one or more clinical symptoms, e.g. hypertension, proteinuria, etc., at about week 14 or more of gestation, e.g. week 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or more of gestation, wherein a positive outcome of the clinical assessment (i.e. the detection of one or more symptoms associated with preeclampsia) is used in combination with the marker level representation to provide a preeclampsia diagnosis, a preeclampsia prognosis, to monitor the preeclampsia, etc. In some instances, the clinical parameters may be measured prior to obtaining the preeclampsia marker level representation, for example, to inform the artisan as to whether a preeclampsia marker level representation should be obtained, e.g. to make or confirm a preeclampsia diagnosis. In some instances, the clinical parameters may be measured after obtaining the preeclampsia marker level representation, e.g. to monitor a preeclampsia.

As another example, the subject methods of providing a preeclampsia assessment may further comprise assessing one or more factors associated with the risk of developing preeclampsia. Non-limiting examples of preeclampsia risk factors include, for example, a medical condition such as gestational diabetes, type I diabetes, obesity, chronic hypertension, renal disease, a thrombophilia; African-American or Filipino descent; age of greater than 35 years or less than 20 years; a family history of preeclampsia; nulliparity; preeclampsia in a previous pregnancy; and stress. For example, a subject maybe assessed for one or more risk factors, e.g. medical condition, family history, etc., when pregnancy is first confirmed or thereafter, wherein a positive outcome of the risk assessment (i.e. the determination of one or more risk factors associated with preeclampsia) is used in combination with the marker level representation to provide a preeclampsia diagnosis, a preeclampsia prognosis, to monitor the preeclampsia, etc.

The subject methods may be employed for a variety of different types of subjects. In many embodiments, the subjects are within the class mammalian, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), lagomorpha (e.g. rabbits) and primates (e.g., humans, chimpanzees, and monkeys). In certain embodiments, the animals or hosts, i.e., subjects (also referred to herein as patients), are humans.

In some embodiments, the subject methods of providing a preeclampsia assessment include providing a diagnosis, prognosis, or result of the monitoring. In some embodiments, the preeclampsia assessment of the present disclosure is provided by providing, i.e. generating, a written report that includes the artisan's assessment, for example, the artisan's determination of whether the patient is currently affected by preeclampsia, of the type, stage, or severity of the subject's preeclampsia, etc. (a "preeclampsia diagnosis"); the artisan's prediction of the patient's susceptibility to developing preeclampsia, of the course of disease progression, of the patient's responsiveness to treatment, etc. (i.e., the artisan's "preeclampsia prognosis"); or the results of the artisan's monitoring of the preeclampsia. Thus, the subject methods may further include a step of generating or outputting a report providing the results of an artisan's assessment, which report can be provided in the form of an electronic medium (e.g., an electronic display on a computer monitor), or in the form of a tangible medium (e.g., a report printed on paper or other tangible medium). Any form of report may be provided, e.g. as known in the art or as described in greater detail below.

REPORTS

A "report," as described herein, is an electronic or tangible document which includes report elements that provide information of interest relating to the assessment of a subject and its results. In some embodiments, a subject report includes at least a preeclampsia marker representation, e.g. a preeclampsia profile or a preeclampsia score, as discussed in greater detail above. In some embodiments, a subject report includes at least an artisan's preeclampsia assessment, e.g. preeclampsia diagnosis, preeclampsia prognosis, an analysis of a preeclampsia monitoring, a treatment recommendation, etc. A subject report can be completely or partially electronically generated. A subject report can further include one or more of: 1) information regarding the testing facility; 2) service provider information; 3) patient data; 4) sample data; 5) an assessment report, which can include various information including: a) reference values employed, and b) test data, where test data can include, e.g., a protein level determination; 6) other features.

The report may include information about the testing facility, which information is relevant to the hospital, clinic, or laboratory in which sample gathering and/or data generation was conducted. Sample gathering can include obtaining a fluid sample, e.g. blood, saliva, urine etc.; a tissue sample, e.g. a tissue biopsy, etc. from a subject. Data generation can include measuring the marker concentration in preeclampsia patients versus healthy individuals, i.e. individuals that do not have and/or do not develop preeclampsia.

This information can include one or more details relating to, for example, the name and location of the testing facility, the identity of the lab technician who conducted the assay and/or who entered the input data, the date and time the assay was conducted and/or analyzed, the location where the sample and/or result data is stored, the lot number of the reagents (e.g., kit, etc.) used in the assay, and the like. Report fields with this information can generally be populated using information provided by the user.

The report may include information about the service provider, which may be located outside the healthcare facility at which the user is located, or within the healthcare facility. Examples of such information can include the name and location of the service provider, the name of the reviewer, and where necessary or desired the name of the individual who conducted sample gathering and/or data generation. Report fields with this information can generally be populated using data entered by the user, which can be selected from among pre-scripted selections (e.g., using a drop-down menu). Other service provider information in the report can include contact information for technical information about the result and/or about the interpretive report.

The report may include a patient data section, including patient medical history (which can include, e.g., age, race, serotype, prior preeclampsia episodes, and any other characteristics of the pregnancy), as well as administrative patient data such as information to identify the patient (e.g., name, patient date of birth (DOB), gender, mailing and/or residence address, medical record number (MRN), room and/or bed number in a healthcare facility), insurance information, and the like), the name of the patient's physician or other health professional who ordered the monitoring assessment and, if different from the ordering physician, the name of a staff physician who is responsible for the patient's care (e.g., primary care physician).

The report may include a sample data section, which may provide information about the biological sample analyzed in the monitoring assessment, such as the source of biological sample obtained from the patient (e.g. blood, saliva, or type of tissue, etc.), how the sample was handled (e.g. storage temperature, preparatory protocols) and the date and time collected. Report fields with this information can generally be populated using data entered by the user, some of which may be provided as pre-scripted selections (e.g., using a drop-down menu). The report may include a results section. For example, the report may include a section reporting the results of a protein level determination assay (e.g., “1.5 nmol/liter ADAM12 in serum”), or a calculated preeclampsia score.

The report may include an assessment report section, which may include information

generated after processing of the data as described herein. The interpretive report can include a prediction of the likelihood that the subject will develop preeclampsia. The interpretive report can include a diagnosis of preeclampsia. The interpretive report can include a characterization of preeclampsia. The assessment portion of the report can optionally also include a recommendation(s). For example, where the results indicate that preeclampsia is likely, the recommendation can include a recommendation that diet be altered, blood pressure medicines administered, etc., as recommended in the art.

It will also be readily appreciated that the reports can include additional elements or modified elements. For example, where electronic, the report can contain hyperlinks which point to internal or external databases which provide more detailed information about selected elements of the report. For example, the patient data element of the report can include a hyperlink to an electronic patient record, or a site for accessing such a patient record, which patient record is maintained in a confidential database. This latter embodiment may be of interest in an in-hospital system or in-clinic setting. When in electronic format, the report is recorded on a suitable physical medium, such as a computer readable medium, e.g., in a computer memory, zip drive, CD, DVD, etc.

It will be readily appreciated that the report can include all or some of the elements above, with the proviso that the report generally includes at least the elements sufficient to provide the analysis requested by the user (e.g. a calculated preeclampsia marker level representation; a prediction, diagnosis or characterization of preeclampsia).

REAGENTS, SYSTEMS AND KITS

Also provided are reagents, systems and kits thereof for practicing one or more of the above-described methods. The subject reagents, systems and kits thereof may vary greatly. Reagents of interest include reagents specifically designed for use in producing the above-described marker level representations of preeclampsia markers from a sample, for example, one or more detection elements, e.g. antibodies or peptides for the detection of protein, oligonucleotides for the detection of nucleic acids, etc. In some instances, the detection element comprises a reagent to detect the expression of a single preeclampsia marker, for example, the detection element may be a dipstick, a plate, an array, or cocktail that comprises one or more detection elements, e.g. one or more antibodies, one or more oligonucleotides, one or more sets of PCR primers, etc. which may be used to detect the expression of one or more preeclampsia marker simultaneously,

One type of reagent that is specifically tailored for generating marker level

representations, e.g. preeclampsia marker level representations, is a collection of antibodies that bind specifically to the protein markers, e.g. in an ELISA format, in an xMAP™ microsphere format, on a proteomic array, in suspension for analysis by flow cytometry, by western blotting, by dot blotting, or by immunohistochemistry. Methods for using the same are well understood in the art. These antibodies can be provided in solution. Alternatively, they may be provided pre-bound to a solid matrix, for example, the wells of a multi-well dish or the surfaces of xMAP microspheres.

Another type of such reagent is an array of probe nucleic acids in which the genes of interest are represented. A variety of different array formats are known in the art, with a wide variety of different probe structures, substrate compositions and attachment technologies (e.g., dot blot arrays, microarrays, etc.). Representative array structures of interest include those described in U.S. Patent Nos.: 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280.

Another type of reagent that is specifically tailored for generating marker level representations of genes, e.g. preeclampsia genes, is a collection of gene specific primers that is designed to selectively amplify such genes (e.g., using a PCR-based technique, e.g., real-time RT-PCR). Gene specific primers and methods for using the same are described in U.S. Patent No. 5,994,076, the disclosure of which is herein incorporated by reference.

Of particular interest are arrays of probes, collections of primers, or collections of antibodies that include probes, primers or antibodies (also called reagents) that are specific for at least 1 gene/protein selected from the group consisting of hemopexin, ferritin, Cathepsin B, Cathepsin C, ADAM metallopeptidase domain 12, Keratin 33A, Haptoglobin, alpha-2-macroglobulin, apolipoprotein E, apolipoprotein C-III, apolipoprotein A-I, retinol binding protein 4, hemoglobin, fibrinogen, and pikachurin, or a biochemical substrate specific for the cofactor/prosthetic group heme, in some instances for a plurality of these genes/polypeptides, e.g., at least 2, 3, 4, 5, 6, 7, 8 or more genes/polypeptides. In certain embodiments, the collection of probes, primers or antibodies include reagents specific for one or more of Cathepsin C and Pikachurin. In certain embodiments, the collection of probes, primers, or antibodies includes reagents specific for Pikachurin and one or more of Hemopexin, ApoA1, ApoC3, RBP4, and/or Haptoglobin. In certain embodiments, the collection of probes, primers, or antibodies includes reagents specific for Pikachurin, Hemopexin, ApoA1, ApoC3, RBP4, and Haptoglobin. In certain embodiments, the

collection of probes, primers, or antibodies includes reagents specific for hemopexin, ferritin, Cathepsin B, Cathepsin C, ADAM metallopeptidase domain 12, Keratin 33A, Haptoglobin, alpha-2-macroglobulin, apolipoprotein E, apolipoprotein C-III, apolipoprotein A-I, retinol binding protein 4, hemoglobin, fibrinogen, and pikachurin as well as a biochemical substrate specific for heme. The subject probe, primer, or antibody collections or reagents may include reagents that are specific only for the genes/proteins/cofactors that are listed above, or they may include reagents specific for additional genes/proteins/cofactors that are not listed above, such as probes, primers, or antibodies specific for genes/proteins/cofactors whose expression pattern are known in the art to be associated with preeclampsia, e.g. sFLT-1 (VEGF-R1) and PlGF.

In some instances, a system may be provided. As used herein, the term “system” refers to a collection of reagents, however compiled, e.g., by purchasing the collection of reagents from the same or different sources. In some instances, a kit may be provided. As used herein, the term “kit” refers to a collection of reagents provided, e.g., sold, together. For example, the nucleic acid- or antibody-based detection of the sample nucleic acid or protein, respectively, may be coupled with an electrochemical biosensor platform that will allow multiplex determination of these biomarkers for personalized preeclampsia care.

The systems and kits of the subject invention may include the above-described arrays, gene-specific primer collections, or protein-specific antibody collections. The systems and kits may further include one or more additional reagents employed in the various methods, such as primers for generating target nucleic acids, dNTPs and/or rNTPs, which may be either premixed or separate, one or more uniquely labeled dNTPs and/or rNTPs, such as biotinylated or Cy3 or Cy5 tagged dNTPs, gold or silver particles with different scattering spectra, or other post synthesis labeling reagent, such as chemically active derivatives of fluorescent dyes, enzymes, such as reverse transcriptases, DNA polymerases, RNA polymerases, and the like, various buffer mediums, *e.g.* hybridization and washing buffers, prefabricated probe arrays, labeled probe purification reagents and components, like spin columns, etc., signal generation and detection reagents, *e.g.* labeled secondary antibodies, streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like.

The subject systems and kits may also include one or more preeclampsia phenotype determination elements, which element is, in many embodiments, a reference or control sample or marker representation that can be employed, e.g., by a suitable experimental or computing means, to make a preeclampsia prognosis based on an "input" marker level

profile, e.g., that has been determined with the above described marker determination element. Representative preeclampsia phenotype determination elements include samples from an individual known to have or not have preeclampsia, databases of marker level representations, e.g., reference or control profiles or scores, and the like, as described above.

In addition to the above components, the subject kits will further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, CD, etc., on which the information has been recorded. Yet another means that may be present is a website address which may be used via the internet to access the information at a removed site. Any convenient means may be present in the kits.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

As the leading cause of maternal morbidity and mortality, preeclampsia (PE) is a pregnancy-related vascular disorder affecting 5%-8% of all pregnancies (Berg et al. Overview of maternal morbidity during hospitalization for labor and delivery in the United States: 1993-1997 and 2001-2005. Obstetrics and gynecology 2009;113:1075-81; Mackay et al. Pregnancy-related mortality from preeclampsia and eclampsia. Obstetrics and gynecology 2001;97:533-8). PE, which often causes fetal growth restriction and pre-term

delivery as well as fetal mortality and morbidity, can be remedied by delivery of the placenta and fetus (Powe et al. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation 2011;123:2856-69). The etiology of PE is incompletely understood. Current diagnosis of PE is based on the signs of hypertension and proteinuria (Gynecologists ACOOA ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Obstetrics and gynecology 2002;99:159-67), but lacks sensitivity and specificity and carries a poor prognosis for adverse maternal and fetal outcomes (Zhang et al. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstetrics and gynecology 2001;97:261-7). Thus, there is a need to identify PE biomarkers that can provide a definitive diagnosis with the opportunity for better monitoring of the condition's progression, and thus improved outcomes and economic benefits.

Although the pathophysiology remains largely elusive, PE is a multisystem disorder of pregnancy with the placenta playing a pivotal role. Investigators have used genetic, genomic and proteomic approaches to compare PE and control placental tissues. Transcriptional profiling of case-control samples has identified disease-specific expression patterns, canonical pathways and gene-gene networks (Lapaire et al. Microarray screening for novel preeclampsia biomarker candidates. Fetal diagnosis and therapy 2012;31:147-53; Nishizawa et al. Microarray analysis of differentially expressed fetal genes in placenta tissue derived from early and late onset severe preeclampsia. Placenta 2007;28:487-97; Loset et al. transcriptional profile of the decidua in preeclampsia. American journal of obstetrics and gynecology 2011;204:84 e1-27; Johansson et al. Partial correlation network analyses to detect altered gene interactions in human disease: using preeclampsia as a model. Human genetics 2011;129:25-34; Sitras et al. Differential placental gene expression in severe preeclampsia. Placenta 2009;30:424-33; Tsai et al. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signaling pathways. Placenta 2011;32:175-82; Winn et al. Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2. Endocrinology 2009;150:452-62). Proteomics-based biomarker studies (Kolla et al. Quantitative proteomic (iTRAQ) analysis of 1st trimester maternal plasma samples in pregnancies at risk for preeclampsia. Journal of biomedicine & biotechnology 2012;2012:305964; Mary et al. Dynamic proteome in enigmatic preeclampsia: an account of molecular mechanisms and biomarker discovery. Proteomics Clinical applications 2012;6:79-90; Carty et al. Urinary

proteomics for prediction of preeclampsia. Hypertension 2011;57:561-9) have also revealed candidate biomarkers for future testing. Placental angiogenic and anti-angiogenic factor imbalance, elevated soluble fms-like tyrosine kinase (sFlt-1) and decreased placental growth factor (PlGF) levels, are suggested in the pathogenesis of PE (Shibata et al. Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. The Journal of clinical endocrinology and metabolism 2005;90:4895-903; Maynard et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. The Journal of clinical investigation 2003;111:649-58; Wolf et al. Circulating levels of the antiangiogenic marker sFLT-1 are increased in first versus second pregnancies. American journal of obstetrics and gynecology 2005;193:16-22; Rajakumar et al. Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. Placenta 2005;26:563-73; Taylor et al. Altered tumor vessel maturation and proliferation in placenta growth factor-producing tumors: potential relationship to post-therapy tumor angiogenesis and recurrence. International journal of cancer Journal international du cancer 2003;105:158-64; Tidewell et al. Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. American journal of obstetrics and gynecology 2001;184:1267-72; Torry et al. Preeclampsia is associated with reduced serum levels of placenta growth factor. American journal of obstetrics and gynecology 1998;179:1539-44), and the sFlt-1/PlGF ratio has been proposed as a useful index in the diagnosis and management of PE (Stepan et al. [use of angiogenic factors (sflt-1/plgf ratio) to confirm the diagnosis of preeclampsia in clinical routine: First experience]. Zeitschrift fur Geburtshilfe und Neonatologie. 2010;214:234-238; Verlohren et al. An automated method for the determination of the sflt-1/pigf ratio in the assessment of preeclampsia. Am. J. Obst. And Gyn. 2010;202:161 e161-161 e111). However, no widely applicable, sensitive and specific molecular PE test in routine clinical practice is currently available.

In light of these considerations, there is a strong rationale and need to discover diagnostic and prognostic biomarkers for PE. We employed a comprehensive unbiased multi-'omics' approach, integrating results from microarray multiplex meta-analysis, and proteomic identification by two-dimensional (2D) gel analysis. Our applied parametric method (Morgan et al. Comparison of multiplex meta analysis techniques for understanding the acute rejection of solid organ transplants. BMC bioinformatics 2010;11 Suppl 9:S6; Chen

et al. Differentially expressed RNA from public microarray data identifies serum protein biomarkers for cross-organ transplant rejection and other conditions. PLoS computational biology 2010;6) in meta-analysis allowed us to identify consistent and significant differential gene expression across experiments to develop biomarkers for downstream experimental validation. Serum proteins are routinely used to diagnose diseases, but sensitive and specific biomarkers are hard to find and may be due to their low serological abundance, which can easily be masked by highly abundant proteins. Our serum protein marker discovery method (Ling et al. Plasma profiles in active systemic juvenile idiopathic arthritis: Biomarkers and biological implications. Proteomics 2010) combines antibody-based serum abundant protein depletion and 2D gel comparative profiling to discover differential protein gel spots between PE and control sera for subsequent protein mass spectrometric identification. We hypothesized that there would be differential serological signatures allowing PE diagnosis. To validate our discovery findings, we tested all the candidates with available ELISA assays, a higher-throughput method. To construct and optimize a sensitive and specific biomarker panel with the least number of protein analytes, a genetic algorithm was used. Close examination of the biomarkers from comparative transcriptomics and proteomics, and their associated pathways led to new hypothesis about their role in PE pathophysiology.

The presented results validated our hypothesis that sensitive and specific serological biomarker panels can be constructed to diagnose PE. To our knowledge, this represents the first study to employ a muti-'omics'-based biomarker approach to uncover novel PE biomarkers superior to sFlt-1, PlGF, and sFlt-1/PlGF ratio in PE discrimination. We believe that the functional significance of these PE biomarkers and their associated pathways will provide new insights into the disease pathogenesis and lead to effective novel therapeutics.

MATERIALS AND METHODS

Study design. The overall sample allocation, PE biomarker discovery, validation, and predictive panel construction steps are illustrated in Figure 1. Our study was conducted in two phases: (1) the discovery phase, which included both the *in silico* expression analysis (n=111 PE and n=152 control placenta samples) and the proteomics 2D gel profiling (pooled n=5 PE and pooled n=5 control serum proteomes); and (2) the validation phase, which was comprised of the analysis of independent PE (n=32) and control (n=32) cohorts. All the serum samples were purchased from ProMedDX Inc. (Norton, MA 02766, http://www.promeddx.com). All serum samples were collected after informed consent was

obtained, and included detailed case report forms. Excluded from this study were patients who were current smokers, had a history of substance abuse, used in vitro fertilization assistance, had chronic hypertension, and pregnancies complicated by intrauterine growth restriction. Case (PE) and control (normal pregnant) cohorts were matched for gestational age, ethnicity, and parity.

Multiplex meta-analysis of expression comparing PE and control placentas. As shown in Table 1 below, seven PE placenta expression studies (Nishizawa et al. Microarray analysis of differentially expressed fetal genes in placenta tissue derived from early and late onset severe preeclampsia. Placenta 2007;28:487-97; Sitras et al. Differential placental gene expression in severe preeclampsia. Placenta 2009;30:424-33; Tsai et al. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. Placenta 2011;32:175-82; Winn et al. Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2. Endocrinology 2009;150:452-62; Founds et al. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. Placenta 2009;30:15-24; Nishizawa et al. Comparative gene expression profiing of placentas from patients with severe preeclampsia and unexplained fetal growth restriction. Reproductive biology and endocrinology 2011;9:107) were combined and subjected to multiplex meta-analysis with the method we previously developed (Morgan et al. Comparison of multiplex meta analysis techniques for understanding the acute rejection of solid organ transplants. BMC bioinformatics 2010;11 Suppl 9:S6; Chen et al. Differentially expressed RNA from public microarray data identifies serum protein biomarkers for cross-organ transplant rejection and other conditions. PLoS computational biology 2010;6). For each of the 22,394 genes tested, we calculated the meta-fold change across all studies. Significant genes were selected if they were measured in 5 or more studies and the meta effect *p* value was less than 4.5×10^{-5}. We then filtered the gene sets through a list of 3,638 proteins with known detectable abundances in sera, plasma, or urine (Dudley and Butte. Disease signatures are robust across tissues and experiments. Pacific Symposium on Biocomputing Pacific Symposium on Biocomputing 2009:27-38).

Table 1. Expression data sets used for multiplex meta analysis based PE marker discovery.

Dataset	Title	Tissue	Cases	Controls
Nishizawa et al	Differentially Expressed Genes in	placenta	10	4

Placenta 2007	Placental Tissue of Severe Preeclampsia			
Tsai et al Placenta 2011	Transcriptional Profiling of Human Placentas from Pregnancies Complicated by Preeclampsia	placenta	23	37
Nishizawa et al 2011	Gene expression profiling for placentas from pre-eclamptic, unexplained FGR and normal pregnancies	placenta	8	8
Winn et al Endocrinology 2009	Severe Preeclampsia-Related Changes in Gene Expression at the Maternal-Fetal Interface	placenta	12	11
Sitras et al Placenta 2009	Placental gene expression in severe preeclampsia	placenta	17	26
Founds et al Placenta 2009	Chorionic villus sampling (CVS) microarray in preeclampsia	CVS	4	8
Roten et al MolHumRep 2011	Transcription profiling of human decidua basalis to identify pre-eclampsia susceptibility genes	Decidua basalis	37	58
		Total	**111**	**152**

2D gel analysis comparing pooled PE and control patient serum samples. To enrich samples for lower abundance serum proteins, serum samples were depleted of the top fourteen serum-abundant proteins (albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein A-I, apolipoprotein A-II, complement C-III and transthyretin) using the Agilent Multiple Affinity Removal System (Agilent, Santa Clara, CA). Specifically, the depletion enabled the increased loading of the remaining proteins by fifteen-fold (Ling et al. Plasma profiles in active systemic juvenile idiopathic arthritis: Biomarkers and biological implications. Proteomics 2010). Further sample processing, 2D gel electrophoresis, comparative analysis, and differential gel spot protein identification via mass spectrometry was performed as previously described (Ling et al, *supra*).

ELISA assays validating PE marker candidates. All assays were ELISA assays, and performed using commercial kits following vendors' instructions. All assays were performed to measure serum levels of selected analytes: alpha-2-macroglobin (A2M), Abnova Inc. (Taipei, Taiwan); disintegrin and metalloproteinase domain-containing protein 12 (ADAM12), Mybiosource (SD, US); adipophilin (ADRP), Biotang Inc. (MA, US); apolipoprotein (APO) A-I, Abcam Inc. (MA, US); apolipoprotein (APO) C-III, Abnova Inc. (Taipei, Taiwan); apolipoprotein (APO)-E, Abcam Inc. (MA, US); cathepsin B (CTSB), Abcam. (MA, US); cathepsin C (CTSC), USCN Life Science (Wuhan, China); chemokine (C-C motif) ligand 2 (CCL2), Abnova (Taipei, Taiwan); haptoglobin (HP), Abcam Inc. (MA, US);

hemopexin (HPX), Abcam Inc. (MA, US); PlGF, R&D system Inc. (MN, US); heme oxygenase 1 (HMOX1), Biotang Inc. (MA, US); insulin-like growth factor binding protein 7 (IGFBP7), USCN Life Science (Wuhan, China); total iron, Abnova Inc. (Taipei, Taiwan); hemoglobin (HB), Bethyl laboratory (TX, US); hemoxygenase 1 (HMOX1), Biotang Inc. (MA, US); keratin 33A (KRT33A), USCN Life Science (Wuhan, China); keratin 40 (KRT40), USCN Life Science (Wuhan, China); kininogen 1 (KNG1), Abcam Inc. (MA, US); pikachurin (EGFLAM), EIAab Science (Wuhan, China); pro-platelet basic protein (PPBP), Abnova Inc. (Taipei, Taiwan); retinol-binding protein 4 (RBP4), Abcam Inc. (MA, US); and soluble fms-like tyrosine kinase (sFlt-1, R&D system Inc. (MN, US).

Statistical analyses. Patient demographic data was analyzed using the "Epidemiological calculator" (R epicalc package). Student's t test was performed to calculate *p* values for continuous variables, and Fisher exact test was used for comparative analysis of categorical variables. Forest plotting with R rmeta package was used both to represent the placental expression meta analysis and to graphically summarize the serum protein ELISA results. Case (PE) and control samples are not paired; thus the initial serum protein forest plot analysis should be interpreted with caution. Bootstrapping method was used to create "paired" samples from case and control groups for the subsequent forest plotting analysis of the ELISA results. Therefore, serum protein forest plot analysis provides an overall effect estimation of each analyte's capability in discriminating PE and normal pregnant control subjects. Hypothesis testing was performed using Student's t-test (two tailed) and Mann-Whitney U-test (two tailed), and local FDR (Efron et al. Empirical bayes analysis of microarray experiment. J Am Stat Assoc 2001;96:1151-60) to correct for multiple hypothesis testing issues. Biomarker feature selection and panel optimization was performed using a genetic algorithm (R genalg package). The predictive performance of each biomarker panel analysis was evaluated by ROC curve analysis (Zweig et al. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical chemistry 1993;39:561-77; Sing et al. ROCR: visualizing classifier performance in R. Bioinformatics 2005;21:3940-1). The biomarker panel score was defined as the ratio between the geometric means of the respective up- and down-regulated protein biomarkers in the maternal circulation.

RESULTS

Multi-'Omics'-based discovery revealing PE marker candidates. As shown in Figure 1, previous placental expression studies were combined for a multiplex meta-analysis to discover biomarker candidates diagnosing PE from normal controls. This effort identified

A2M, ADAM12, CCL2, CTSB, CTSC, EGFLAM, HOMX1, IGFBP7, KRT33A, KRT40, PIGF, PPBP, and sFlt-1 as differential placental biomarkers for PE. In parallel, 2D gel analysis was performed to compare serological PE and control pooled proteomes, revealing highly discriminating protein spots that were later sequenced. The 2D gel profiling led to the identification of A2M, ADFP, APO A-I, APO C-III, APO-E, KNG1, HP, HPX, and RBP4 marker candidates.

Close examination of the combined PE biomarker list found A2M, HMOX-1 and HPX can be involved in heme/hemoglobin catabolism pathway. Extracellular heme can cause undesirable organ, tissue and cellular injury and there are receptor pathways for endocytosis of extracellular heme and hemoglobin (HB) in complex with HPX and HP, respectively (Hvidberg et al. Identification of the receptor scavenging hemopexin-heme complexes. Blood 2005;106:2572-9). Heme are ultimately broken down of the porphyrin ring into bilirubin, carbon monoxide, and iron, whereas iron is bound to ferritin (FT). A2M is an acute phase protein and heme was proposed to be a new regulatory element in controlling liver A2M expression during inflammation (Lyoumi et al. Heme and acute inflammation role in vivo of heme in the hepatic expression of positive acute-phase reactants in rats. European journal of biochemistry / FEBS 1999;261:190-6). HPX, with the highest affinity for heme of any known protein, serves as scavenger to remove free heme from circulation as free heme can cause oxidant stress due to its catalytic activity (Delanghe et al. Hemopexin: a review of biological aspects and the role in laboratory medicine. Clinica chimica acta; international journal of clinical chemistry 2001;312:13-23; Tolosano et al. Heme scavenging and the other facets of hemopexin. Antioxidants & redox signaling 2010;12:305-20). Plasma HPX was found as a potential regulator of vascular responsiveness to angiotensin II in PE patients (Bakker et al. Hemopexin as a Potential Regulator of Vascular Responsiveness to Angiotensin II. Reprod Sci 2012). Fibrinogen (FGA) was recently proposed to be a heme-associated, carbon monoxide sensing molecule (Nielsen et al. Fibrinogen is a heme-associated, carbon monoxide sensing molecule: a preliminary report. Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis 2011;22:443-7). Preeclampsia involves an acute-phase reaction as well as systemic oxidative stress. Increased levels of cell-free hemoglobin, oxidation markers, and the antioxidative heme scavengers were found in PE (Olsson et al. Increased levels of cell-free hemoglobin, oxidation markers, and the antioxidative heme scavenger alpha(1)-microglobulin in preeclampsia. Free radical biology & medicine 2010;48:284-91). Induction of HMOX-1 has been shown to down regulate hypoxia-induced reactive oxygen species and sFlt-1 (Olsson

et al, supra), and many of the pathological factors of placental ischemia experimentally (George et al. Induction of heme oxygenase 1 attenuates placental ischemia-induced hypertension. Hypertension 2011;57:941-8). This suggests that PE placenta ischemia and resulted dysfunctional heme/hemoglobin catabolism is part of the PE pathophysiology.

Sample characteristics. The PE and control subjects used for serological protein biomarker validation can be divided into early (PE, n=15; control, n=16) and late (PE, n=17; control, n=16) gestation groups. As summarized in Table 2 and Table 3 below, no significant differences in age (*p* value, early 0.89, late 0.857, overall 0.6), gestational age (*p* value, early 0.851, late 0.895, overall 0.824) at enrollment, ethnicity (*p* value, early 0.57, late 0.123, overall 0.289), or subjects' concurrent medical conditions and other clinical features (*p* value, overall 0.35) were observed.

The PE patients were diagnosed with preeclampsia characterized by both hypertension and proteinuria. As shown in Table 4, all of the 32 PE patients had both hypertension and proteinuria; 43.8% of them had headache; 21.9% of them had edema; and 25.0% of them had other additional symptoms. Other characteristics, including body mass index (BMI, prior to pregnancy), blood pressure (BP), protein/creatinine ratio (PCR), and pregnancy history were also shown in Table 5.

Table 2. Ethnicity, age and week of gestation.

	Early stage			**Late stage**			**Overall**
Characteristic	**Control n = 15 (48.4%)**	**PE n = 16 (51.6%)**	***p* value**	**Control n = 17 (51.5%)**	**PE n = 16 (48.5%)**	***p* value**	***p* value**
Ethnicity			**0.57**			**0.123**	**0.289**
African American	5 (33.3%)	5 (31.2%)		2 (11.8%)	4 (25%)		
Asian	2 (13.3%)	0 (0)		0 (0%)	0 (0)		
Hispanic	8 (53.3%)	10 (62.5%)		11 (64.7%)	12 (75%)		
Other	0 (0%)	1 (6.2%)		4 (23.5%)	0 (0%)		
Age (year)							
mean (SD)	24.3 (4.5)	24.1 (6.1)	**0.89**	27.9 (9.0)	26.6 (7.7)	**0.857**	**0.6**
Week of gestation							
mean (SD)	30.3 (3.2)	30.1 (2.9)	**0.851**	37.1 (1.4)	37.2 (1.6)	**0.895**	**0.824**

Table 3. Concurrent medical conditions and clinical features.

Characteristic	**Control n = 32 (50%)**	**PE n = 32 (50%)**	***p* value**
Concurrent Medical Conditions / Clinical Features			**0.35**
Anemia	0 (0)	2 (6.2%)	
Asthma , Other: Chlamydia (2009)	1 (3.1%)	0 (0)	
Asthma , Other: Group B Streptococcus carrier, Maternal deficiency anemia, Thrombocytopenia	1 (3.1%)	0 (0)	
Crohn's Disease	0 (0)	1 (3.1%)	
Diabetes - Type II	2 (6.2%)	1 (3.1%)	
Diabetes - Type II , Morbid Obesity , Other: History of depression	1 (3.1%)	0 (0)	
Diabetes - Type II , Other: Left breast lump	1 (3.1%)	0 (0)	
Diabetes (Gestational)	1 (3.1%)	3 (9.4%)	
Diabetes (Gestational) , Obesity	1 (3.1%)	0 (0)	
Fatty Liver	1 (3.1%)	0 (0)	
Hyperthyroidism	1 (3.1%)	0 (0)	
Migraines , Urinary Tract Infection (UTI)	1 (3.1%)	0 (0)	
NONE	19 (59.4%)	24 (75%)	
Other: Borderline gestational diabetes	1 (3.1%)	0 (0)	
Other: Hepatitis C Antibody = Reactive	0 (0)	1 (3.1%)	
Other: History of cardiac surgery at birth, Marginal cord insertion	1 (3.1%)	0 (0)	

Table 4. PE patients' presenting signs and symptoms.

Presenting Signs and Symptoms	**Number (percentage)**
Hypertension	32 (100%)
Proteinuria	32 (100%)
Headache	14 (43.8%)
Edema	7 (21.9%)
Others	8 (25.0%)

Table 5. PE patients' clinical information.

Characteristics	Statistics
BMI (prior to pregnancy) (kg/m^2)	29.1 (23.0, 33.9)
Systolic blood pressure	146.0 (134.0, 157.5)
Diastolicv blood pressure	85.5 (77.0, 94.5)
Protein/creatinine ratio (PCR) test results (mg/g)	803.5 (449.5, 1492.0)
Prior history of preeclampsia	

Yes	3 (9.4%)
No	28 (87.5%)
Multiple gestation	
Yes	3 (9.4%)
No	29 (90.6%)
Number of abortions (induced or spontaneous)	0 (0,1)
Number of full term pregnancies	0 (0,1.25)
Number of premature pregnancies	0 (0,0)
Smoking history	
Never	32 (100%)
Total number of pregnancies	2 (1,4)
Vitro fertilization (IVF) utilized for this pregnancy	
No	32 (100%)

Biomarker validation using PE and control maternal serum samples. To identify whether the PE serological protein panel could enable development of an immediate practical clinical tool, based on available ELISA assays, biomarker candidates, from expression meta-analysis and 2D gel profiling, were validated with available serum assays using PE (n=32) and gestation age-matched control samples (n=32). Detailed with whisker box and scatter plots in Figures 5-21, total of 11 proteins were validated by ELISA assays (Mann-Whitney tests p value < 0.05). Each validated biomarker's median, mean and standard deviation of maternal serum abundance, in PE and control samples, are summarized in Table 6.

Table 6. Maternal serum levels of the validated PE biomarkers.

Analyte	PE trend		unit	Early Stage				Late Stage			
	early	late		Normal		PE		Normal		PE	
				Median (IRQ)	Mean (SD)	Median (IRQ)	Mean (SD)	Median (IRQ)	Mean (SD)	Median (IRQ)	Mean (SD)
PIGF	↓	↓	pg/ml	413.775 (224.915, 685.23)	529.3831 (432.0385)	97.517 (51.5845, 190.7)	115.5138 (82.96284)	222.279 (163.592, 289.860)	238.1095 (111.4536)	184.488 (113.236, 223.832)	202.6929 (132.7476)
sFlt-1	↑	↑	pg/ml	1697.860 (1128.18, 4273.93)	3034.023 (2578.738)	19841.33 (15728.35, 21608.61)	18646.25 (3582.492)	5610.460 (4191.8, 6735.835)	5531.241 (1811.915)	14216.20 (12347.56, 19749.3)	14414.28 (5575.346)
HPX	↑	↑	µg/ml	1071.2 (692.4, 1301.0)	984.05 (388.333)	1382.8 (1173.6, 1787.0)	1580.72 (546.4721)	954.4 (538.0, 1131.6)	894.15 (331.4866)	1482.0 (1013.6, 1654.4)	1347.624 (585.2598)
FT	↑	↑	ng/ml	60.1820 (45.2425, 77.196)	70.83125 (42.72209)	92.604 (61.286, 131.1405)	118.9008 (100.8934)	73.296 (60.568, 82.6475)	76.26706 (29.61479)	78.743 (59.956, 126.565)	101.1071 (77.08354)
ADAM12	↑	↑	pg/ml	511.312 (437.654, 642.321)	584.0489 (275.761)	774.993 (637.229, 1150.178)	920.1977 (416.3522)	666.4185 (594.874, 791.842)	703.6862 (217.2496)	883.889 (626.676, 1367.639)	1345.369 (1472.54)
ApoCIII	↑	↑	ng/ml	341.347 (249.478, 422.359)	364.7076 (153.4417)	419.171 (357.329, 575.544)	486.2566 (187.4748)	291.58 (240.72, 345.74)	321.8587 (126.7332)	453.789 (308.93, 725.843)	585.7512 (413.1066)
HP	↓	↓	µg/ml	1624.092 (1215.95,	1718.014 (764.1215)	1181.584 (684.6,	1482.707 (1284.595)	1806.74 (1190.09,	1750.72 (684.0882)	985.616 (592.04,	1510.514 (1514.988)

				2274.07)		1794.1)		2163.1)		1880.785)	
A2M	↓	↓	µg/ml	5796.424 (3501.2, 7737.565)	5729.148 (3064.134)	3365.067 (2648.269, 5958.964)	4259.341 (2175.836)	8141.38 (5300.6, 10234.086)	7754.764 (3265.09)	3435.427 (2343.675, 6752.9)	4340.768 (2862.513)
ApoE	↓	↓	µg/ml	290.6 (104.2, 519.0)	364.425 (301.4971)	138.8 (63.0, 210.4)	215.8933 (257.5736)	398.0 (125.0, 478.4)	377.9 (236.3411)	147.2 (60.4, 190.0)	150.0235 (107.6536)
ApoA1	↓	↓	ng/ml	7980.084 (5775.72, 11076.6)	8337.692 (3158.728)	4945.356 (3892.8, 5824.573)	4708.506 (1707.14)	6253.298 (5624.062, 7881.77)	6748.614 (2287.602)	4724.142 (3138.58, 7075.28)	5483.643 (3794.902)
RBP4	↑	↓	ng/ml	38255.0 (29018.5, 40955.5)	35180.38 (7031.125)	38899.0 (33460.5, 39895.0)	36931.67 (7307.52)	41616.5 (38830.5, 44429.5)	49253.5 (38081.63)	33179 (29558, 37386)	33897.47 (8499.767)
Pikachurin	↓	↓	ng/ml	601.751 (563.772, 792.09)	659.1049 (152.046)	293.261 (267.39, 367.83)	327.7657 (117.4519)	536.551 (459.173, 626.57)	536.551 (952.295)	317.657 (266.816, 409.67)	317.657 (623.497)
HB	↓	↓	ng/ml	10348.769 (8865.08, 11407.7)	10047.15 (2067.523)	9477.79 (8039.066, 10572.16)	9290.402 (2319.195)	10739.081 (8743, 11415.2)	10427.59 (1828.554)	9396.6 (7735.557, 11153.9)	9556.862 (2697.67)
FGA	↓	↓	µg/ml	287.9755 (263.725, 318.32)	294.3416 (38.95516)	262.177 (244.575, 284.35)	262.0381 (27.15886)	292.8455 (282.528, 322.95)	300.2975 (33.7449)	257.37 (236.425, 287.503)	261.2202 (35.3109)
CTSB	↓	↑	ng/ml	123.26 (81.67, 169.64)	142.581 (91.086)	96.44 (76.94, 145.35)	109.19 (40.752)	109.21 (87.32, 121.48)	108.371 (36.376)	152.20 (104.88, 190.06)	165.385 (75.224)
CTSC	↓	↑	ng/ml	12.891 (11.372,13. 874)	13.966 (4.1775)	12.519 (9.9765,16. 6975)	14.6674 (6.0903)	13.649 (12.107, 17.017)	15.335 (5.0667)	18.179 (12.781,22 .402)	20.029 (9.484)

Heme	↓	↓	ng/ml	36.35769 (28.18, 65.419)	47.69588 (28.23451)	29.1868 (18.619, 53.09)	38.9996 (27.22612)	51.58 (30.57, 65.29)	59.19132 (42.67749)	31.7 (20.38, 44.79)	47.28054 (60.14049)

Forest plots (Figure 2) summarize the PE to control ratios of all 11 validated PE markers across placental expression meta-analyses, and early and late gestation maternal serum analyses. The biomarkers derived from the proteomic and expression meta-analyses consistently shared the same trend of up- or down-regulation between PE and control samples.

PE biomarker panel construction. Using data from the ELISA assays, we constructed different panels with various subsets of the assays. We sought to identify biomarker panels of optimal feature number, balancing the need for small panel size, accuracy of classification, goodness of class separation (PE versus control), and sufficient sensitivity and specificity. With the aim to develop a multiplexed antibody-based assay for PE diagnosis, we used a genetic algorithm method to construct biomarker panels from the 9 validated PE protein biomarkers for early and late gestational age PE, comparing to the sFlt-1/PIGF ratio in assessing PE. The algorithm guided panel construction processes, leading to early and late gestational age biomarker panels, which had complete separation between PE and control subjects (Table 7 below, and in Figures 22-28). These chosen biomarker panels are non-redundant, indicating non-inclusive relationships. The sFlt-1/PIGF ratio's PE assessment utility (panel 0: early onset, receiver operating characteristics curve ROC area under the curve 1.00, *p* value $4.35X10^{-4}$; late onset, ROC AUC 0.86, *p* value $2.94X10^{-4}$; Figure 35), previously through the multicenter trial validation (Verlohren et al. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of preeclampsia. American journal of obstetrics and gynecology 2010;202:161 e1-61 e11), was confirmed in this study and used as a benchmark for our newly derived biomarker panels. Panel 2 of Table 7 (early onset, ROC AUC 1.00, *p* value $1.43X10^{-4}$) has three proteins, HPX, APO A-I, and pikachurin. Panel 5 (late onset, ROC AUC 1.00, *p* value $3.65X10^{-5}$) has six proteins, HPX, HP, APO C-III, APO A-I, RBP4, and pikachurin.

Table 7. Biomarker panels integrating maternal serum levels of the validated PE biomarkers. Panel 0 is the benchmark panel sFlt-1/PIGF ratio. Biomarkers marked with an * are up-regulated in PE. (+), included in panel; (-), not included.

	PE onset: Early				PE onset: Late		
Panel	**0**	**1**	**2**	**3**	**0**	**4**	**5**
sFlt-1*	+	-	-	-	+	-	-
PIGF	+	-	-	-	+	-	-
HPX	-	-	+	+	-	+	+
FT	-	-	-	-	-	-	-
ADAM12*	-	-	-	+	-	+	-
HP	-	-	-	-	-	+	+

A2M	-	-	-	-	-	-	-
APO-E	-	-	-	-	-	-	-
APO-CIII*	-	-	-	-	-	+	+
APO-AI	-	+	+	-	-	+	+
RBP4	-	-	-	-	-	-	+
HB	-	-	-	+	-	+	-
FGA	-	+	-	-	-	-	-
CTSC*	-	-	-	-	-	-	-
CTSB*	-	-	-	-	-	-	-
Pikachurin*	-	+	+	+	-	+	+
Panel size	2	3	3	4	2	7	6
ROC AUC	1.00	1.00	1.00	1.00	0.86	1.00	1.00
***p* value**	4.35E-04	3.18E-04	1.43E-04	4.17E-04	2.94E-04	1.69E-04	3.65E-04

To demonstrate the efficacy of the biomarker panel as a classifier for PE disease activity according to disease onset, the biomarker panel scores were plotted as a function of time of the gestational age (details shown in Figure 3, composite summary in Figure 4). According to the scatter plot analysis, our early-onset PE biomarker panel's performance was comparable to the sFlt-1/PIGF ratio. For gestational age > 34 weeks samples, our biomarker panel's performance is better than the sFlt-1/PIGF ratio that has several errors of diagnosis around week 36. Among the early and late gestational age biomarker panels, HPX, APO A-I, and pikachurin are present in both panels, suggesting their critical role in the diagnosis and perhaps pathophysiology of PE.

Pathway analysis of PE biomarkers. We analyzed the validated biomarkers that are significantly differentially expressed in PE as a composite, using Ingenuity Pathway Analysis software (IPA version 7.6, Ingenuity Systems, Inc., Redwood City, CA). In addition to the heme/hemoglobin degradation pathway revealed during our multi-'omic' discovery effort, our pathway analysis led to the identification of the following statistically significant canonical pathways which may play important roles in PE pathophysiology: liver X receptor (LXR)/retinoid X receptor (RXR) activation, *p* value $5.13X10^{-9}$; atherosclerosis signaling, *p* value $5.01X10^{-7}$; IL-12 signaling and production in macrophages, *p* value $8.51X10^{-7}$; acute phase response signaling, *p* value $1.91X10^{-6}$; production of nitric oxide and reactive oxygen species in macrophages, *p* value $2.82X10^{-6}$; clathrin-mediated endocytosis signaling, *p* value $2.88X10^{-6}$; farnesoid X receptor (FXR)/RXR activation, *p* value $2.04X10^{-5}$; hepatic fibrosis / hepatic stellate cell activation, *p* value $2.88X10^{-3}$; phosphatidylethanolamine biosynthesis II, *p* value $1.05X10^{-2}$; coagulation system, *p* value $2.04X10^{-2}$; growth hormone signaling, *p* value $4.27X10^{-2}$; reelin signaling in neurons, *p* value $4.57X10^{-2}$; and VEGF family ligand-receptor interactions, *p* value $4.79X10^{-2}$.

DISCUSSION

We have applied a multi-'omics' approach to develop validated PE biomarkers, integrating discoveries from placental mRNA expression meta-analysis and depleted serological proteome 2D gel comparative profiling. Comparing PE and control sera with commercially available ELISA assays, we have validated 11 protein markers, including sFlt-1 and PIGF, and found that our identified PE biomarkers were superior over sFlt-1/PIGF ratio in predicting PE. The concept of combining a transcriptomic approach in placenta tissue with a proteomic approach in serum is novel. It combines the advantages of a study in tissue which is closer to the focus of the pathophysiology with those of a study in serum which is more appropriate for clinical use. Taking proteins that have been discovered/predicted from the discovery phase to an ELISA-based validation phase makes the findings of this study translatable into clinical practice.

When comparing the discoveries from expression meta-analysis and 2D gel serum proteomics, only A2M showed up in both analyses. This could be due to the following reasons: (1) the discordant expression of protein and mRNA as previously characterized (Griffin et al. Complementary profiling of gene expression at the transcriptome and proteome levels in Saccharomyces cerevisiae. MCP 2002;1:323-33; Ideker et al. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 2001;292:929-34; Baliga et al. Coordinate regulation of energy transduction modules in Halobacterium sp. analyzed by a global systems approach. Proceedings of the National Academy of Sciences of the United States of America 2002;99:14913-8; Chen et al. Discordant protein and mRNA expression in lung adenocarcinomas. Molecular & cellular proteomics : MCP 2002;1:304-13); (2) the lack of translation of the placental expression into circulation protein level abundance; (3) 2D gel technology detection limit of 0.5–5 ng. Optimized 2D gel technique has a dynamic range of ~5 orders of magnitude in protein concentration (Gibson et al. Comparative analysis of synovial fluid and plasma proteomes in juvenile arthritis--proteomic patterns of joint inflammation in early stage disease. J Proteomics 2009;72:656-76), whereas serological protein concentrations vary over ~10 orders of magnitude, with the highest concentrations reaching mg/ml (Anderson, N. The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics 2002;1:845-67). Even with the depletion step, protein detection by our 2D gel is limited to proteins whose serological concentrations are >10 ug/mL, clearly influencing the composition of the protein biomarkers we detected. In addition, potentially informative low

molecular weight proteins may bind to albumin and thus be removed at the depletion step (Tirumalai et al. Characterization of the low molecular weight human serum proteome. Mol Cell Proteomics 2003;2:1096-103), which could be of potential disadvantages. Thus, candidates with pg/mL concentration, e.g. sFlt-1 and PlGF, would not be found applying the 2D gel serum proteomics based approach. Thus, candidates with pg/ml concentration, e.g. sFlt-1 and PlGF, would not be found applying 2D gel serum proteomics based approach. Publically available genome-wide gene expression data on disease tissues can be effectively mined to provide significant synergies to complement our 2D serum proteomics efforts to unveil differential PE biomarker candidates of low serum abundance (pg/mL). Notably, our productive PE discovery efforts support the notion that the multi-'omics' approach for biomarker analyses are comprehensive, complementary, and effective in identifying candidates of a broad dynamic range of serological protein expression, varying from pg/mL to ug/mL.

From the initial expression meta analysis and 2D gel discovered biomarker candidates, we hypothesized that PE placenta ischemia and resulted dysfunctional heme/hemoglobin catabolism pathway is part of the PE pathophysiology. Validation of five (FGA, FT, HB, heme and HP) of the seven hypothesis generated candidates to separate PE and control sera, in conjunction with other validated biomarkers (HP, HPX and HB), provides compelling evidence for the role of heme/hemoglobin catabolism pathway in PE pathophysiology. Close examination of the heme/hemoglobin metabolism pathway may not only support placental ischemia as a central factor in PE development but also may lead to the identification of novel targets for PE therapeutics (Cudmore et al. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. Circulation 2007;115:1789-97).

Additional pathway analyses of the protein markers corroborate growing evidence implicating roles of lipid homeostasis, IL-12, and coagulation canonical pathways in PE pathophysiology. LXR/RXR activation pathway was identified as the most significant pathway. This supports recent findings (Weedpon-Fekjaer et al. Expression of liver X receptors in pregnancies complicated by preeclampsia. Placenta 2010;31:818-24) that PE is associated with hyperlipidemia and that the regulators of lipid homeostasis are important in the PE pathophysiology. The previous evidence of IL-12 (Bachmayer et al. Aberrant uterine natural killer (NK)-cell expression and altered placental and serum levels of the NK-cell promoting cytokine interleukin-12 in pre-eclampsia. Am J Reprod Immunol 2006;56:292-301; Daniel et al. Plasma interleukin-12 is elevated in patients with preeclampsia. Am J Reprod

Immunol 1998;39:376-80; Sakai et al. The ratio of interleukin (IL)-18 to IL-12 secreted by peripheral blood mononuclear cells is increased in normal pregnant subjects and decreased in pre-eclamptic patients. Journal of reproductive immunology 2004;61:133-43), in PE patients, with less activity in placenta and more abundance in sera was reflected as in line with our PE biomarker panel pattern pathway analysis.

A previous multicenter case-control study (Verlohren et al. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of preeclampsia. American journal of obstetrics and gynecology 2010;202:161 e1-61 e11) with an automated assay, demonstrating the utilities of sFlt-1 and PIGF for PE assessment, reported serum abundance of sFlt-1 (PE: 12,981±965 vs control: 2641±100.5 pg/mL) and PIGF (PE: 76.06±10.71 vs control: 341.5±13.57 pg/mL). Although with greater variation, possibly due to different sample cohorts or assay platforms, the trend of alteration reflected in our results, sFlt-1 (PE: 16,398.02±5142.32 vs control: 4,282.63±2,532.90 pg/mL) and PIGF (PE: 161.83±118.98 vs control: 383.75±343.84 pg/mL) was in line with their report. As shown in Figures 5-21 and summarized in Table 8 (below), in contrast to sFlt-1 and PIGF where protein abundance differs significantly (p value<0.05) between early and late gestational age samples in both normal and PE groups respectively, our biomarkers (Table 8), except RBP4, ADAM12 and pikachurin, were not significantly (p value>0.05) different between early and late gestation sera. Our results here indicate that sFlt-1 and PIGF are regulated during placental development as a function of gestation, and differential expression between PE and control might be due to placental adaptation during PE. The PE biomarkers found in this study are not significantly different between early and late gestation in either PE or control sera. Therefore, their differential expression in PE might directly gauge the pathogenesis of PE and disease development or reflect features that are present at fairly advanced stages of the pathogenesis, e.g. proteinuria and high blood pressure, which are not necessarily related to its pathophysiology.

Table 8. Comparison of biomarker's abundances at early and late gestational age time points. *Fold was calculated by the ratio of the medians of early and late gestational age samples' assayed biomarker abundances. ** p value: Mann-Whitney U test

Analyte	**Control**		**PE**	
	Fold*	***p* value****	**Fold***	***p* value****
PIGF	0.449787	0.020445	1.754707	0.021946
sFlt-1	1.823071	0.002984	0.773039	0.017316

HPX	0.908643	0.509422	0.852538	0.433073
FT	1.076743	0.235105	0.850348	0.550803
ADAM12	1.204841	0.034792	1.462044	0.776988
APO-CIII	0.882512	0.445036	1.204613	1
HP	1.019037	0.780443	1.018754	0.940656
A2M	1.353563	0.079568	1.019117	0.852335
APO-E	1.036976	0.668931	0.694897	0.820737
APO-AI	0.80941	0.146736	1.164625	0.911083
RBP4	1.400028	0.028797	0.917843	0.176456
HB	1.037866	0.589581	1.028681	0.852335
FGA	1.020235	0.5095	0.996879	0.794372
pikachurin	0.832876	0.047833	1.070773	0.501947
CTSC	1.058762	0.2351	1.452113	0.05324
CTSB	0.886013	0.3608	1.578183	0.02849
Heme	0.867821	0.365668	0.736443	0.909777

Our genetic algorithm-based biomarker panel construction led to final early and late gestational age biomarker panels for PE assessment. Compared to the benchmark sFlt-1/PlGF ratio in PE assessment, our biomarker panels clearly outperform at later gestational weeks. Although the sFlt-1 and PlGF imbalance used for PE diagnosis has been demonstrated, there is mounting evidence to support the notion that normal sFlt-1 and PlGF expression actually characterizes healthy pregnancies (Daponte et al. Soluble fms-like tyrosine kinase-1 (sflt-1) and serum placental growth factor (plgf) as biomarkers for ectopic pregnancy and missed abortion. The Journal of clinical endocrinology and metabolism. 2011;96:E1444-1451). Therefore, sFlt-1 and PlGF may really be general markers for failed pregnancies, e.g. ectopic pregnancies, missed abortions, rather than specific to PE. Our multi-'omics' approach discovered panels of multiple biomarkers, reflecting the multifaceted aspects of PE pathophysiology, and have the potential to provide a definitive diagnosis of PE patients, to identify patients at risk, and to be used to monitor disease progression.

Example 2

The protein levels of additional panels of preeclampsia markers described in Example 1 and 2 were assayed in serum of preeclampsia patients to determine the accuracy of these additional panels in diagnosing early onset preeclampsia (e.g. onset of

preeclampsia prior to 34-35 weeks of gestation) or late onset preeclampsia (i.e. onset of preeclampsia at 34-35 weeks of gestation or later). Panels of particular interest were the following (see Figure 29):

- Panel 1: sFlt1, PIGF
- Panel 2-early: sFlt1, PIGF, HPX
- Panel 2-late: sFlt1, PIGF, HPX, CTSC, ADAM12, ApoE, ApoA1, RBP4, HB, Pikachurin
- Panel 3: sFlt1
- Panel 4-early: sFlt1, HPX
- Panel 4-late: sFlt1, HPX, ApoE, ApoA1, Pikachurin
- Panel 5: PIGF
- Panel 6-early: PIGF, Pikachurin
- Panel 6-late: PIGF, HPX, CTSC, Adam12, HP, ApoE, RBP4, HB, Fibrinogen, Pikachurin
- Panel 7-early: HPX, ApoA1, Pikachurin
- Panel 7-late: HPX, CTSC, Adam12, HP, HB, Fibrinogen, Pikachurin.

Panels 1, 3, and 5 comprise markers that form the current standard for diagnosing preeclampsia. Panel 2-early and Panel 2-late comprise panel 1 and additional preeclampsia markers disclosed herein. Panel 4-early and Panel 4-late comprise panel 3 and additional preeclampsia markers disclosed herein. Panel 6-early and Panel 6-late comprise panel 5 and additional preeclampsia markers disclosed herein. Panel 7-early and Panel 7-late comprise no additional preeclampsia markers disclosed herein.

As illustrated in Figures 30-35, all of the panels that include the preeclampsia markers disclosed herein (the “Stanford Biomarkers”, panels 2, 4 and 7) performed more accurately than the current standard for diagnosing preeclampsia at the designated time (i.e. early: onset of preeclampsia prior to 34-35 weeks of gestation; late: onset of preeclampsia at 34-35 weeks of gestation or later). Indeed, many of the panels that include the preeclampsia markers disclosed herein (panel 2 early, panel 2 late, panel 4 early, panel 4 late, panel 6 early , panel 7 early and panel 7 late) provide 100% accuracy in diagnosing preeclampsia at their designated time (AUC=1).

Example 3

The protein levels of a panel of preeclampsia markers (Pikachurin, Hemopexin, ApoA1, ApoC3, RBP4, Haptoglobin) was statistically assessed to determine how to weigh

the contribution of each polypeptide to a preeclampsia score for a sample based on this panel.

Using the random forest algorithm, haptoglobin levels were determined to be least significant; RBP4 levels were determined to be about 2-fold more significant than haptoglobin; hemopexin, ApoA1 and ApoC3 levels were determined to be about 6-fold more significant that haptoglobin and about 3-fold more signification than RBP4; and Pikachurin levels were determined to be most significant, i.e. about 15-fold more significant than haptoglobin, about 7.5-fold more significant than RBP4, and about 2.5-fold more significant than hemopexin, ApoA1 and ApoC3 (see table 9, below).

Table 9.

Protein	Importance
Pikachurin	14.81
Hemopexin	6.15
ApoA1	5.97
ApoC3	5.89
RBP4	2.07
Haptoglobin	0.89

Thus, to arrive at a preeclampsia score using the Pikachurin/Hemopexin/ApoA1/ ApoC3/RBP4/Haptoglobin panel, Pikachurin levels may be assigned a weight of about 12-16, e.g. about 15; hemopexin, ApoA1, and ApoC3 levels may be assigned a weight of about 4-8, e.g. about 6; RBP4 levels may be assigned a weight of about 2; and haptoglobin levels may be assigned a weight of 1 or less.

The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural

and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present invention is embodied by the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of providing a preeclampsia marker level representation for a subject, the method comprising:

evaluating a panel of preeclampsia markers in a blood sample from a subject to determine the level of each preeclampsia marker in the blood sample; and

calculating the preeclampsia marker level representation based on the level of each preeclampsia marker in the panel.

2. The method according to claim 1, wherein the one or more preeclampsia markers is selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) and heme.

3. The method according to claim 2, wherein the panel of preeclampsia markers comprises pikachurin and/or cathepsin C.

4. The method according to claim 2, wherein the panel of preeclampsia markers comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4 and haptoglobin.

5. The method according to claim 1, further comprising providing a report of the preeclampsia marker level representation.

6. The method according to claim 1, wherein the preeclampsia marker representation is a preeclampsia score.

7. A panel of preeclampsia markers comprising one or more preeclampsia markers selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen

alpha (FGA), pikachurin (EGFLAM) and heme.

8. The panel according to claim 7, wherein the panel comprises pikachurin and/or cathepsin C.

9. The panel according to claim 7, wherein the panel comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4, and haptoglobin.

10. A method for providing a preeclampsia diagnosis for a subject, the method comprising:

obtaining a preeclampsia marker level representation for a sample from a subject, and

providing a preeclampsia diagnosis for the subject based on the preeclampsia marker level representation.

11. The method according to claim 10, wherein the preeclampsia marker level representation is based on the level of preeclampsia markers in a panel of preeclampsia markers comprising one or more markers selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), and heme.

12. The method according to claim 11, wherein the panel of preeclampsia markers comprises pikachurin and/or cathepsin C.

13. The method according to claim 11, wherein the panel of preeclampsia markers comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4 and haptoglobin.

14. The method according to claim 10, wherein the subject has symptoms of preeclampsia.

15. The method according to claim 10, wherein the subject is asymptomatic for preeclampsia.

16. The method according to claim 10, wherein the subject has risk factors associated with preeclampsia.

17. The method according to claim 10, wherein the sample is collected at 20 or more weeks of gestation.

18. The method according to claim 10, wherein the sample is collected at 34 or more weeks of gestation.

19. The method according to claim 10, wherein the method further comprises comparing the preeclampsia marker level representation to a preeclampsia phenotype determination element, and providing a preeclampsia diagnosis for the subject based on the comparison.

20. A kit for making a preeclampsia diagnosis, the kit comprising:

(a) one or more detection elements for measuring the amount of marker in a sample for a panel of preeclampsia markers comprising one or more markers selected from the group consisting of hemopexin (HPX), ferritin (FT), Cathepsin B (CTSB), Cathepsin C (CTSC), ADAM metallopeptidase domain 12 (ADAM12), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (ApoC3), apolipoprotein A-I ((ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), and pikachurin (EGFLAM) and heme; and

(b) a preeclampsia phenotype determination element.

21. The kit according to claim 20, wherein the panel of preeclampsia markers comprises pikachurin and/or cathepsin C.

22. The kit according to claim 20, wherein the panel of preeclampsia markers comprises pikachurin, hemopexin, ApoA1, ApoC3, RBP4 and haptoglobin

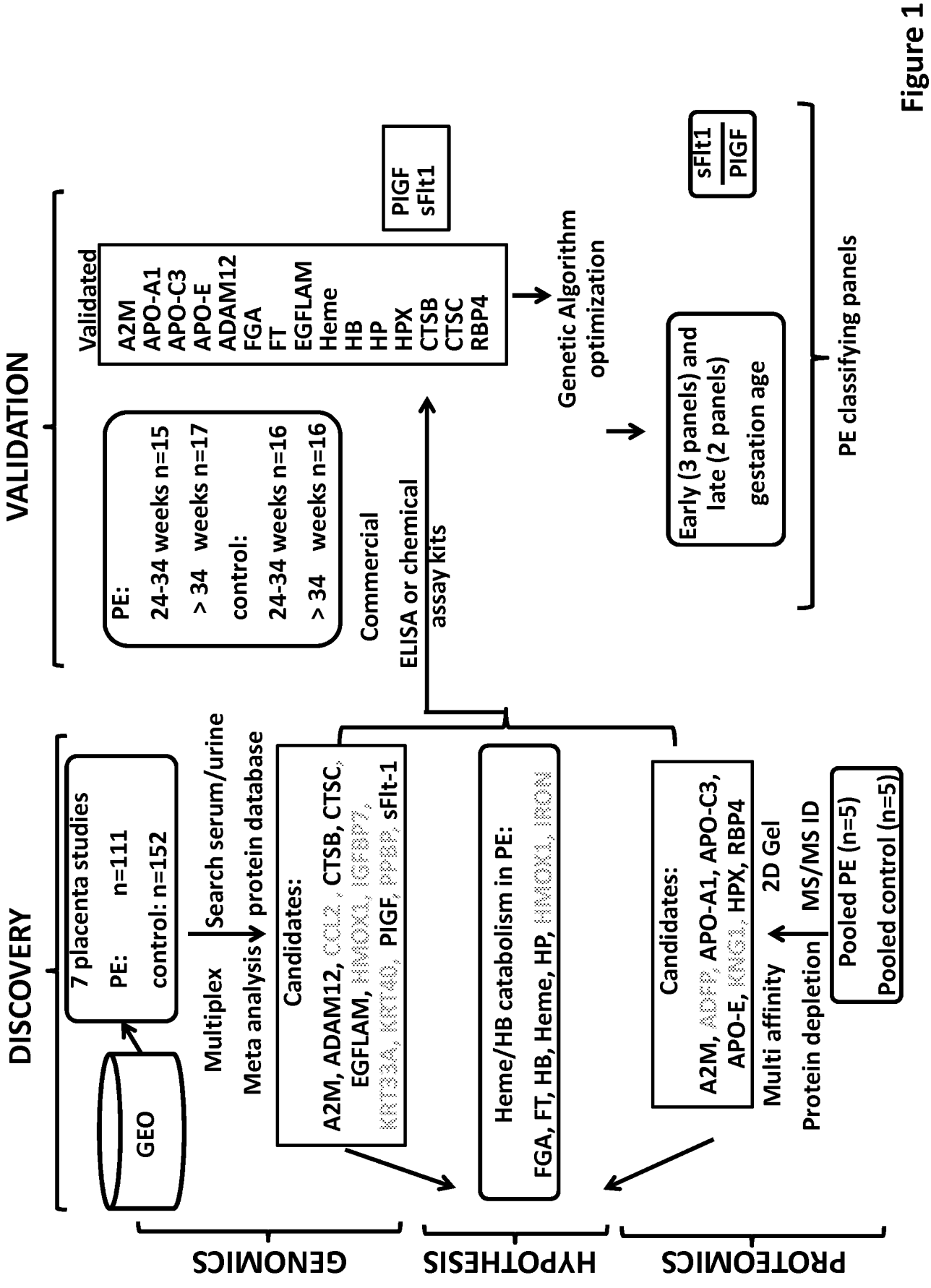
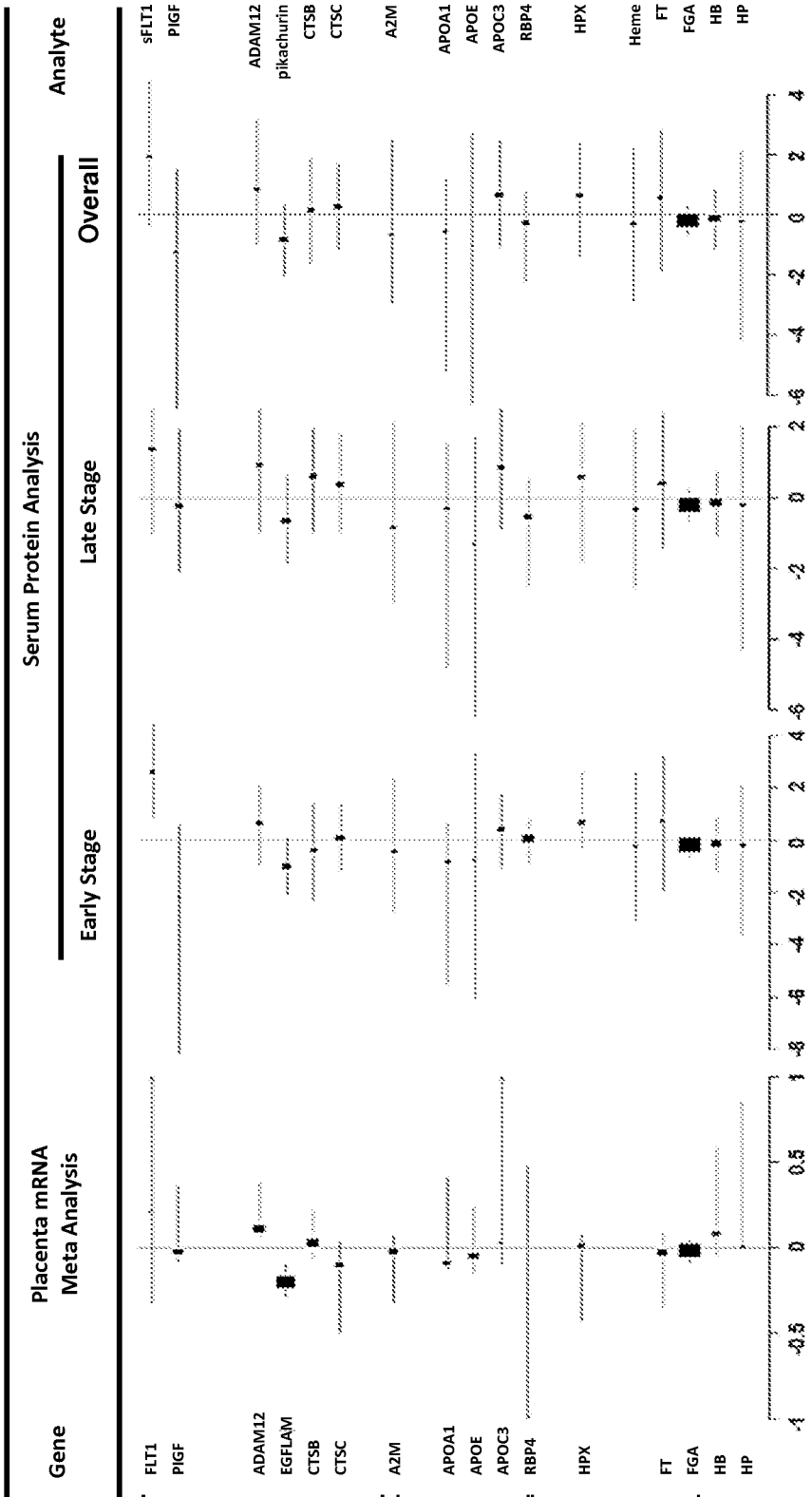

DISCOVERY
VALIDATION
GEO
7 placenta studies
PE: n=111
control: n=152
Multiplex
Meta analysis
Search serum/urine protein database
Candidates:
A2M, ADAM12, CCL2, CTSB, CTSC, EGFLAM, HMOX1, IGFBP7, KRT33A, KRT40, PIGF, PPBP, sFlt-1
Heme/HB catabolism in PE:
FGA, FT, HB, Heme, HP, HMOX1, IRON
Candidates:
A2M, ADIPP, APO-A1, APO-C3, APO-E, KNG1, HPX, RBP4
Multi affinity
2D Gel
Protein depletion
MS/MS ID
Pooled PE (n=5)
Pooled control (n=5)
PE:
24-34 weeks n=15
> 34 weeks n=17
control:
24-34 weeks n=16
> 34 weeks n=16
Commercial ELISA or chemical assay kits
Validated
A2M
APO-A1
APO-C3
APO-E
ADAM12
FGA
FT
EGFLAM
Heme
HB
HP
HPX
CTSB
CTSC
RBP4
PIGF
sFlt1
Genetic Algorithm optimization
Early (3 panels) and late (2 panels) gestation age
sFlt1/PIGF
PE classifying panels
GENOMICS
HYPOTHESIS
PROTEOMICS

Figure 1


Literature
mRNA Meta analysis
2D Gel Proteomics
Heme/HB catabolism Hypothesis
Gene
Placenta mRNA Meta Analysis
Serum Protein Analysis
Early Stage
Late Stage
Overall
Analyte
FLT1
PIGF
ADAM12
EGFLAM
CTSB
CTSC
A2M
APOA1
APOE
APOC3
RBP4
HPX
FT
FGA
HB
HP
sFLT1
PIGF
ADAM12
pikachurin
CTSB
CTSC
A2M
APOA1
APOE
APOC3
RBP4
HPX
Heme
FT
FGA
HB
HP
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0
0.5
1
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-4
-2
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4
Log fold change

Figure 2

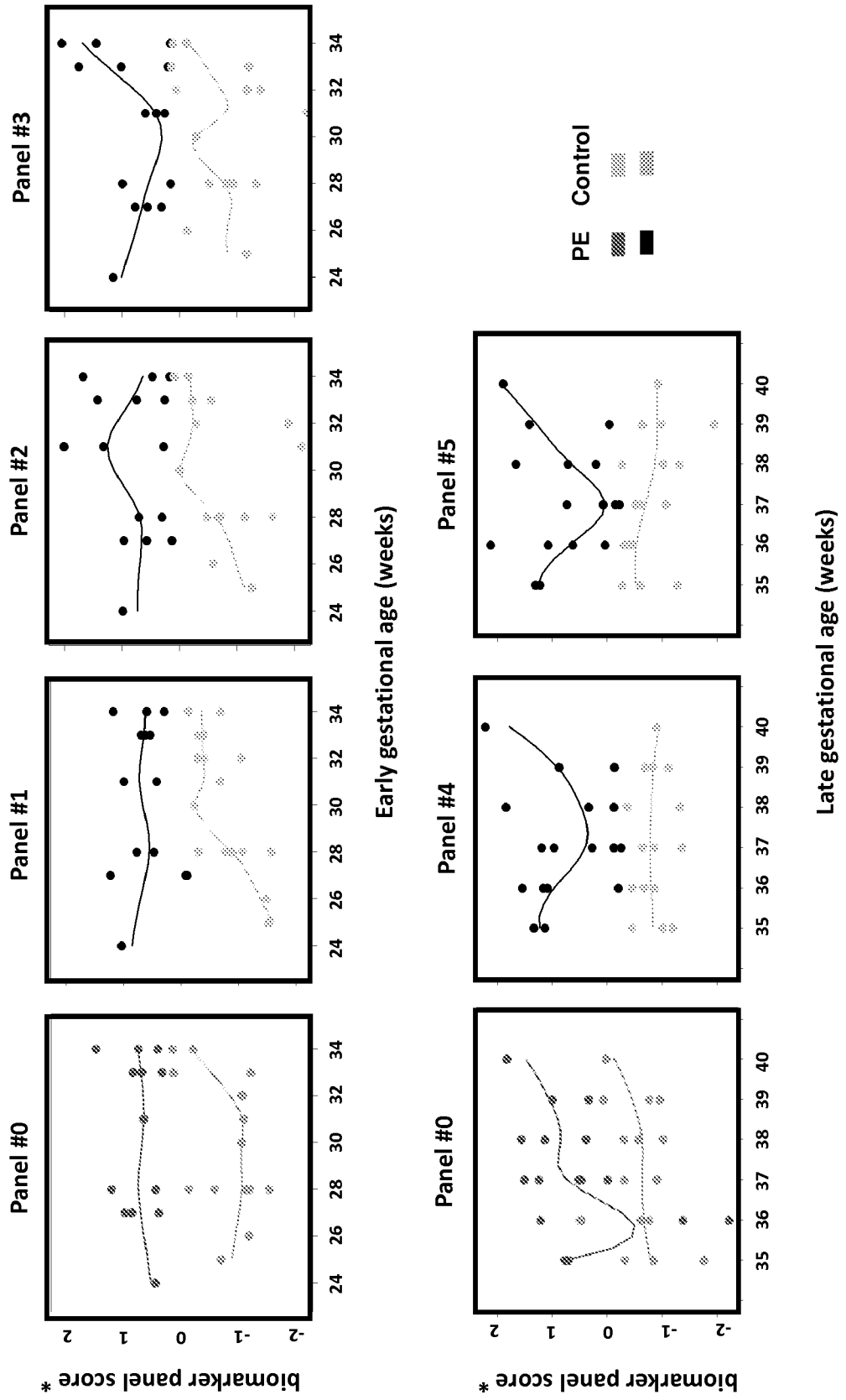

Panel #0
Panel #1
Panel #2
Panel #3
Panel #4
Panel #5
biomarker panel score *
Early gestational age (weeks)
Late gestational age (weeks)
PE
Control

Figure 3

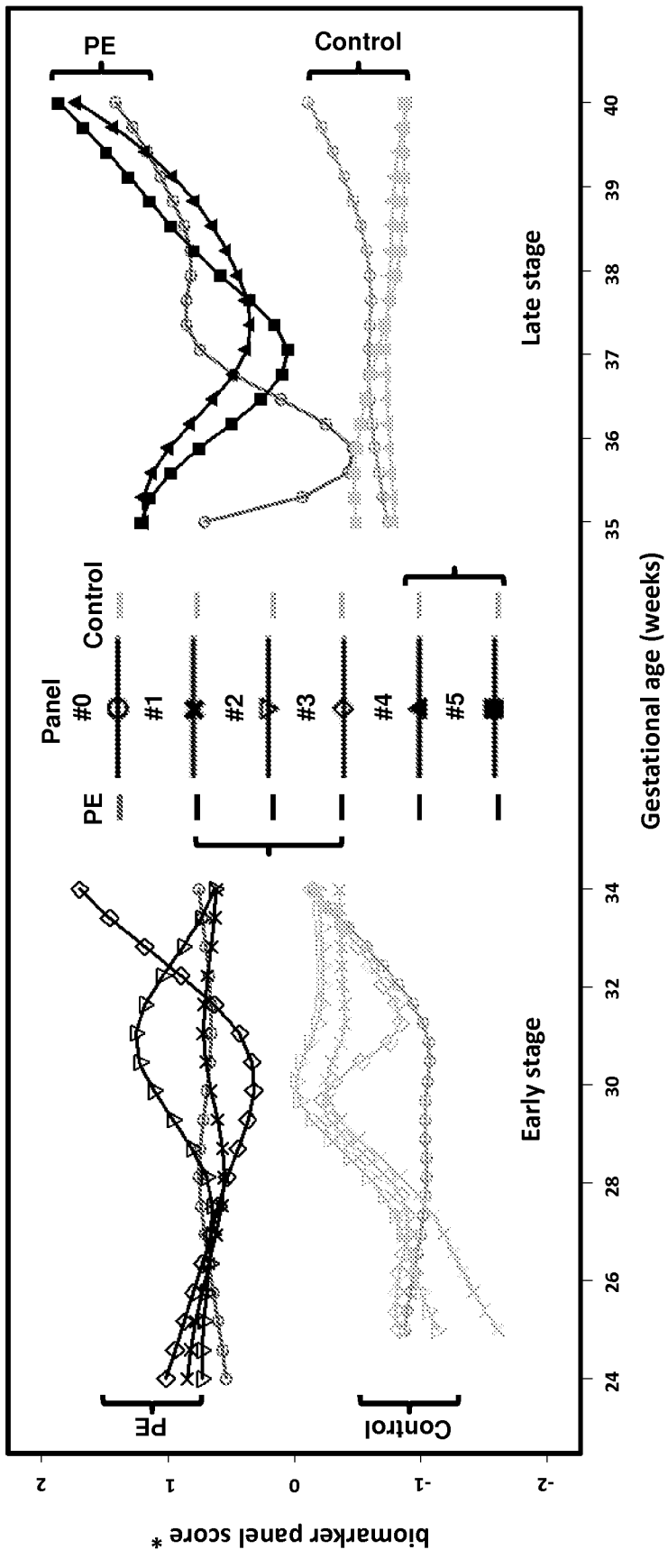
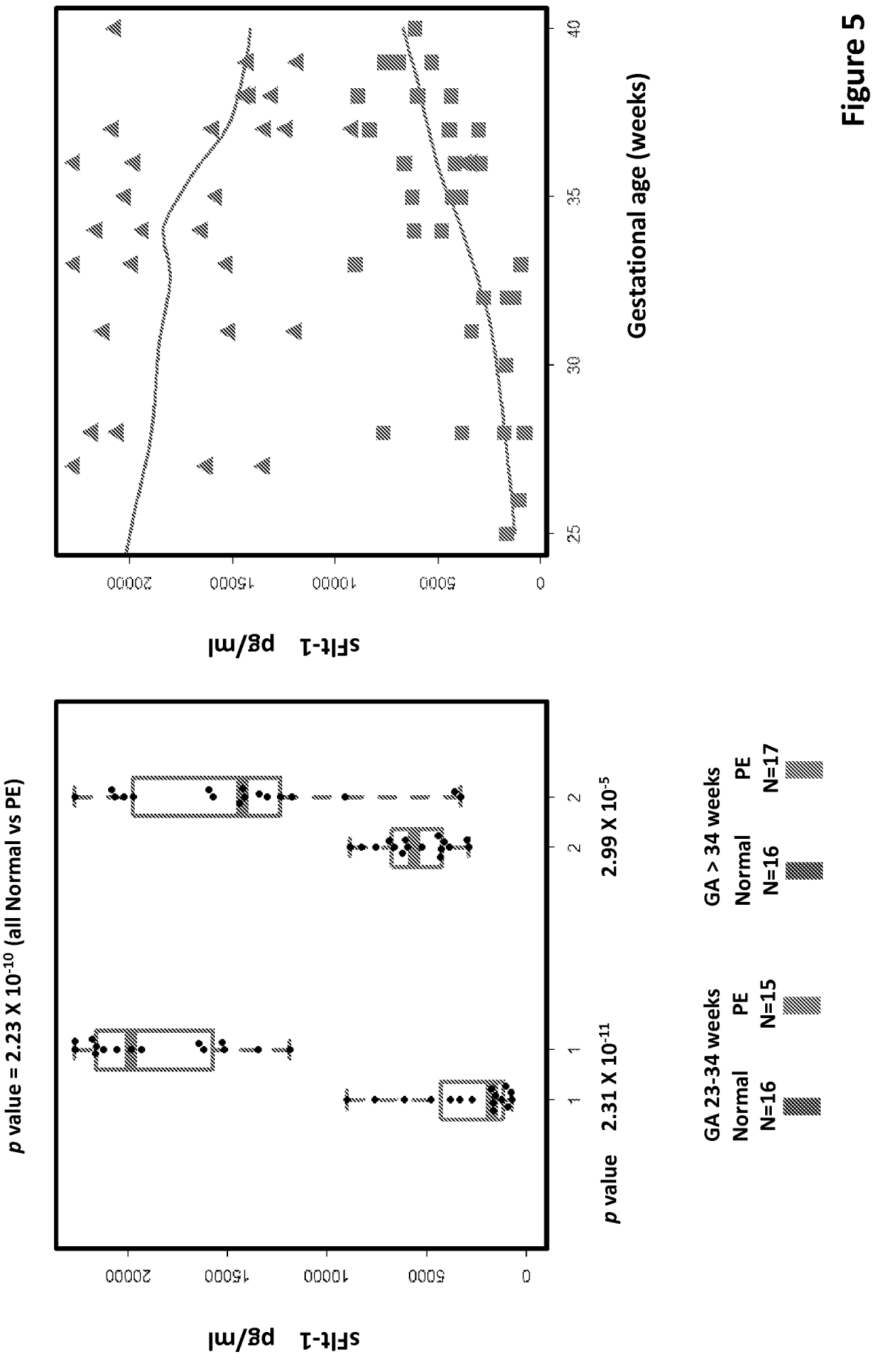

biomarker panel score *
2
1
0
-1
-2
PE
Control
Early stage
Late stage
24
26
28
30
32
34
35
36
37
38
39
40
Gestational age (weeks)
Panel
PE
Control
#0
#1
#2
#3
#4
#5

Figure 4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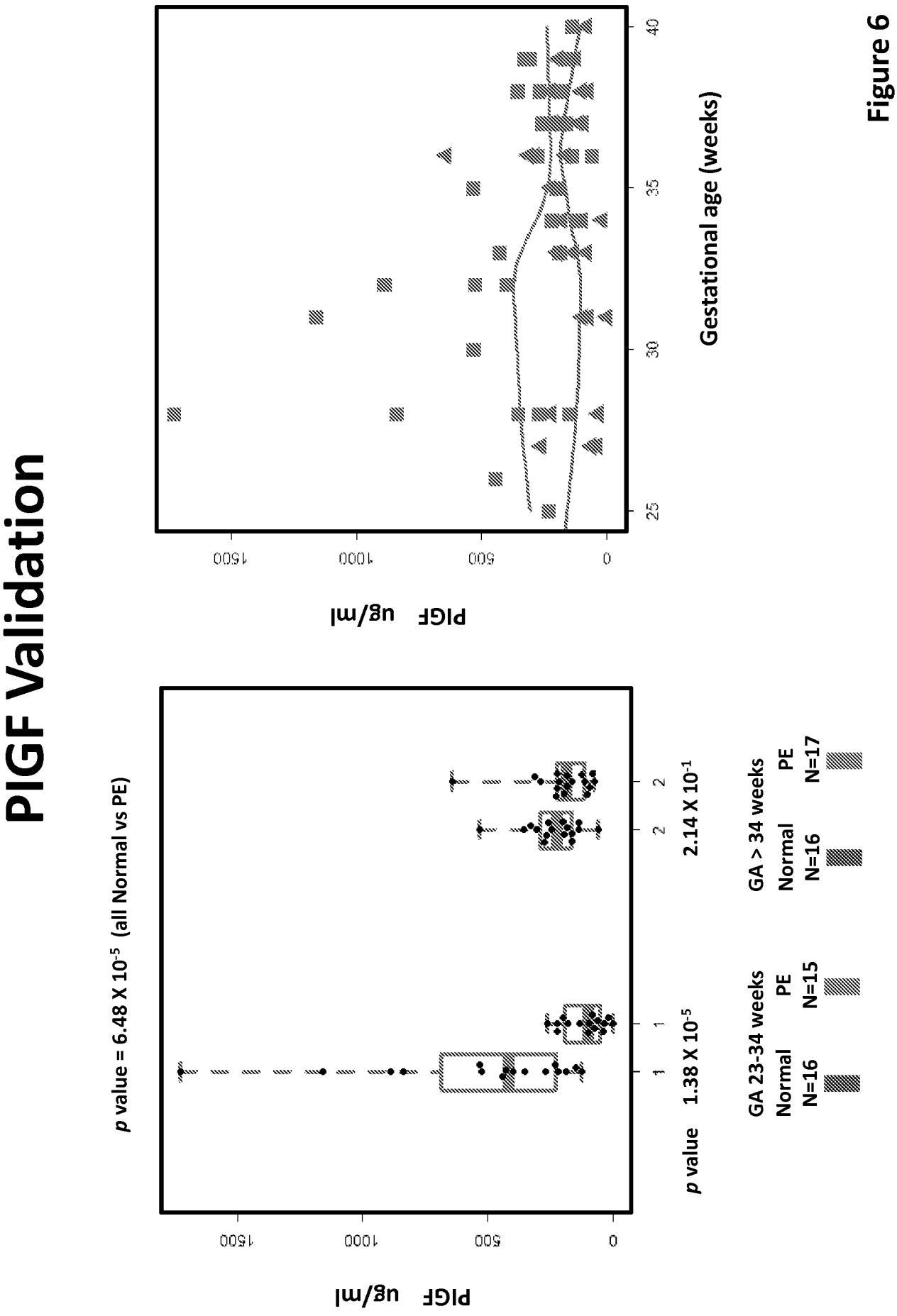
p value = 2.23 X 10-10 (all Normal vs PE)
sFlt-1 pg/ml
0
5000
10000
15000
20000
1
1
2
2
p value 2.31 X 10-11
2.99 X 10-5
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17
sFlt-1 pg/ml
0
5000
10000
15000
20000
25
30
35
40
Gestational age (weeks)

Figure 5


PIGF Validation
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PIGF ug/ml
0
500
1000
1500
1 1 1 1
2 2 2 2
p value 1.38 X 10-5 2.14 X 10-1
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
PIGF ug/ml
0
500
1000
1500
25
30
35
40
Gestational age (weeks)

Figure 6

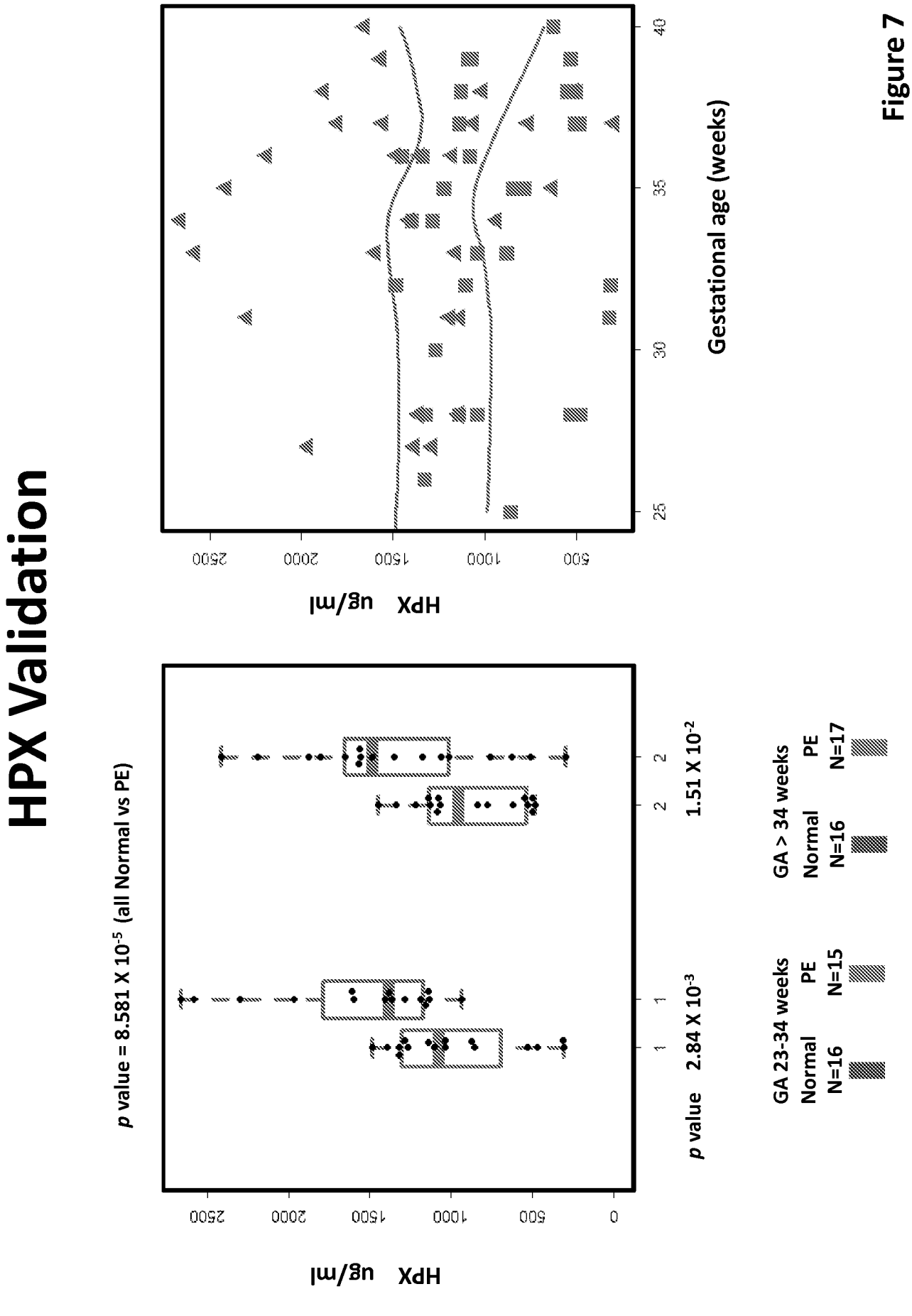
HPX Validation
p value = 8.581 X 10-5 (all Normal vs PE)
HPX ug/ml
0
500
1000
1500
2000
2500
1
1
2
2
p value 2.84 X 10-3
1.51 X 10-2
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
HPX ug/ml
500
1000
1500
2000
2500
25
30
35
40
Gestational age (weeks)

Figure 7

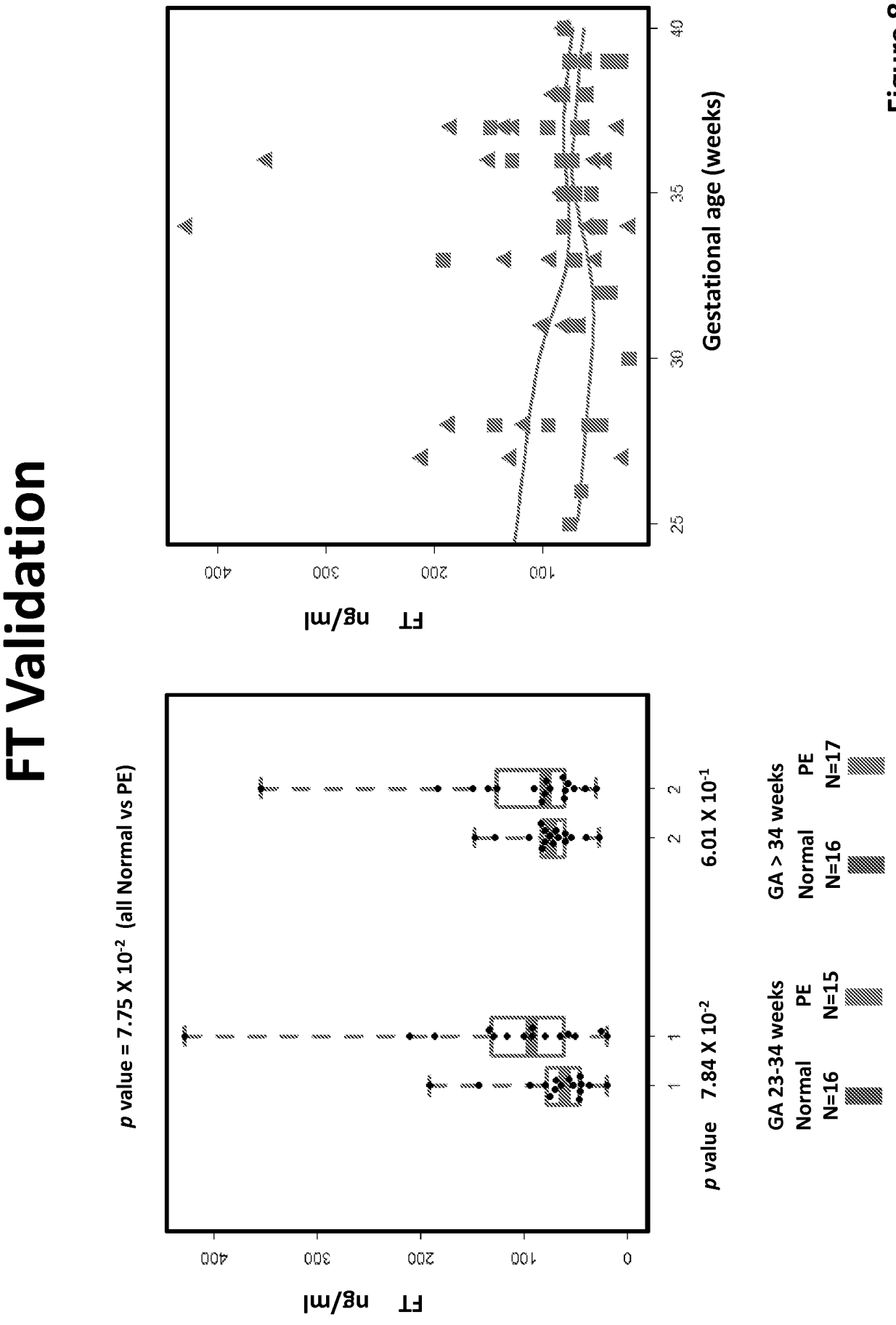
FT Validation
p value = 7.75 X 10-2 (all Normal vs PE)
FT ng/ml
0
100
200
300
400
1
1
2
2
p value 7.84 X 10-2
6.01 X 10-1
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
FT ng/ml
100
200
300
400
25
30
35
40
Gestational age (weeks)

Figure 8

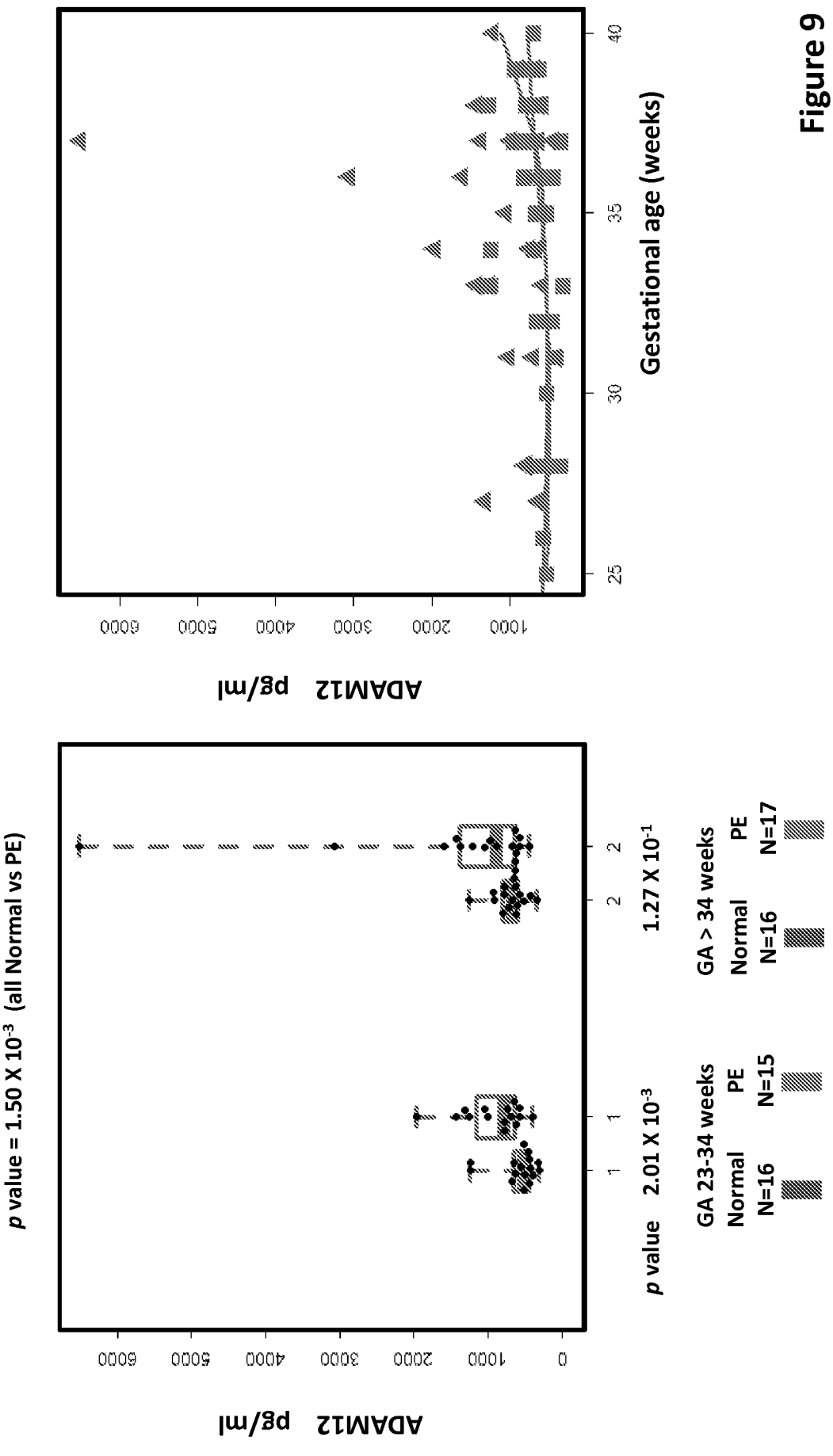
ADAM12 Validation
p value = 1.50 X 10-3 (all Normal vs PE)
ADAM12 pg/ml
0
1000
2000
3000
4000
5000
6000
1
1
2
2
p value 2.01 X 10-3
1.27 X 10-1
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17
ADAM12 pg/ml
1000
2000
3000
4000
5000
6000
25
30
35
40
Gestational age (weeks)

Figure 9

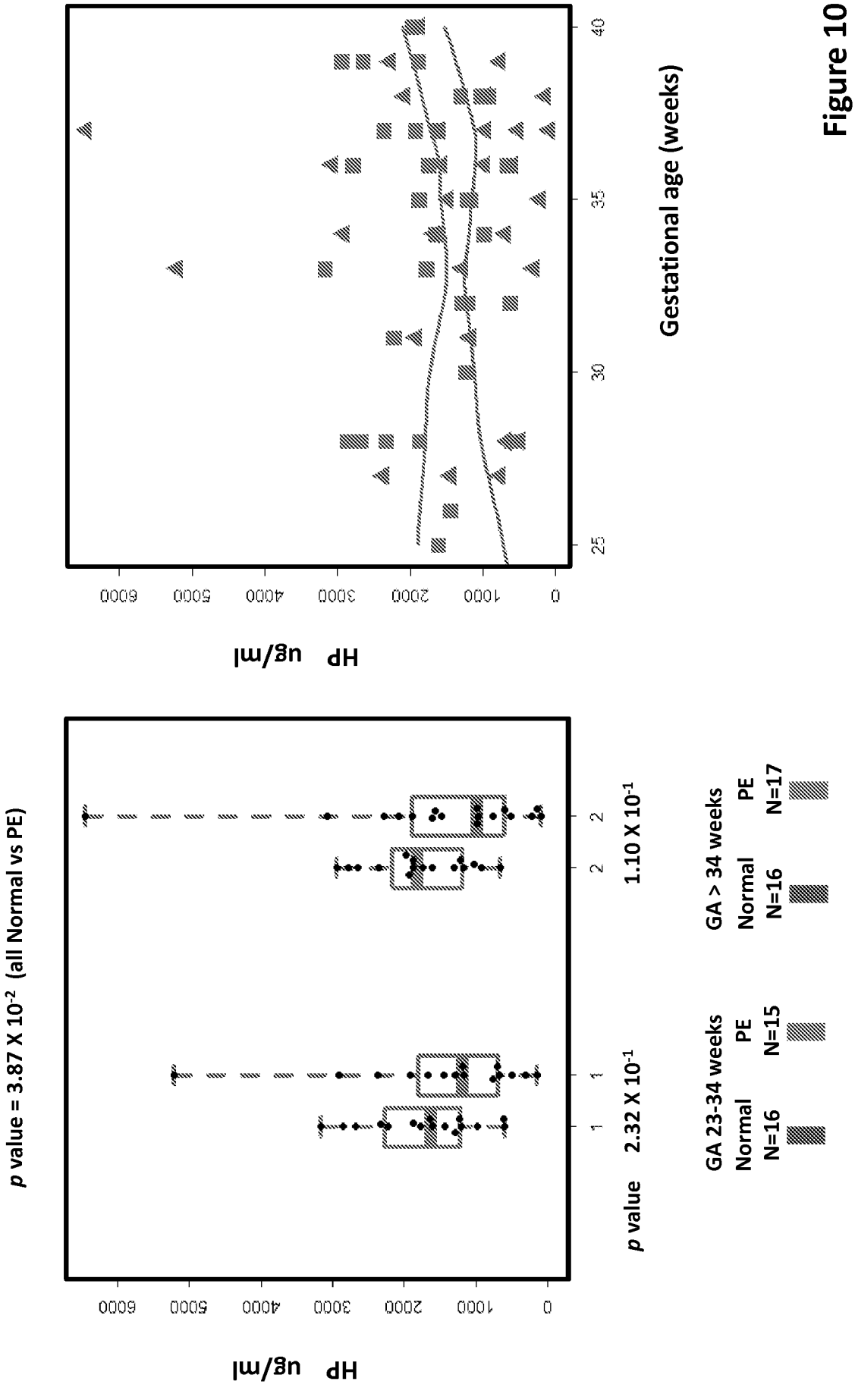
HP Validation
HP ug/ml
0
1000
2000
3000
4000
5000
6000
25
30
35
40
Gestational age (weeks)
p value = 3.87 X 10-2 (all Normal vs PE)
p value 2.32 X 10-1
1.10 X 10-1
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17

Figure 10

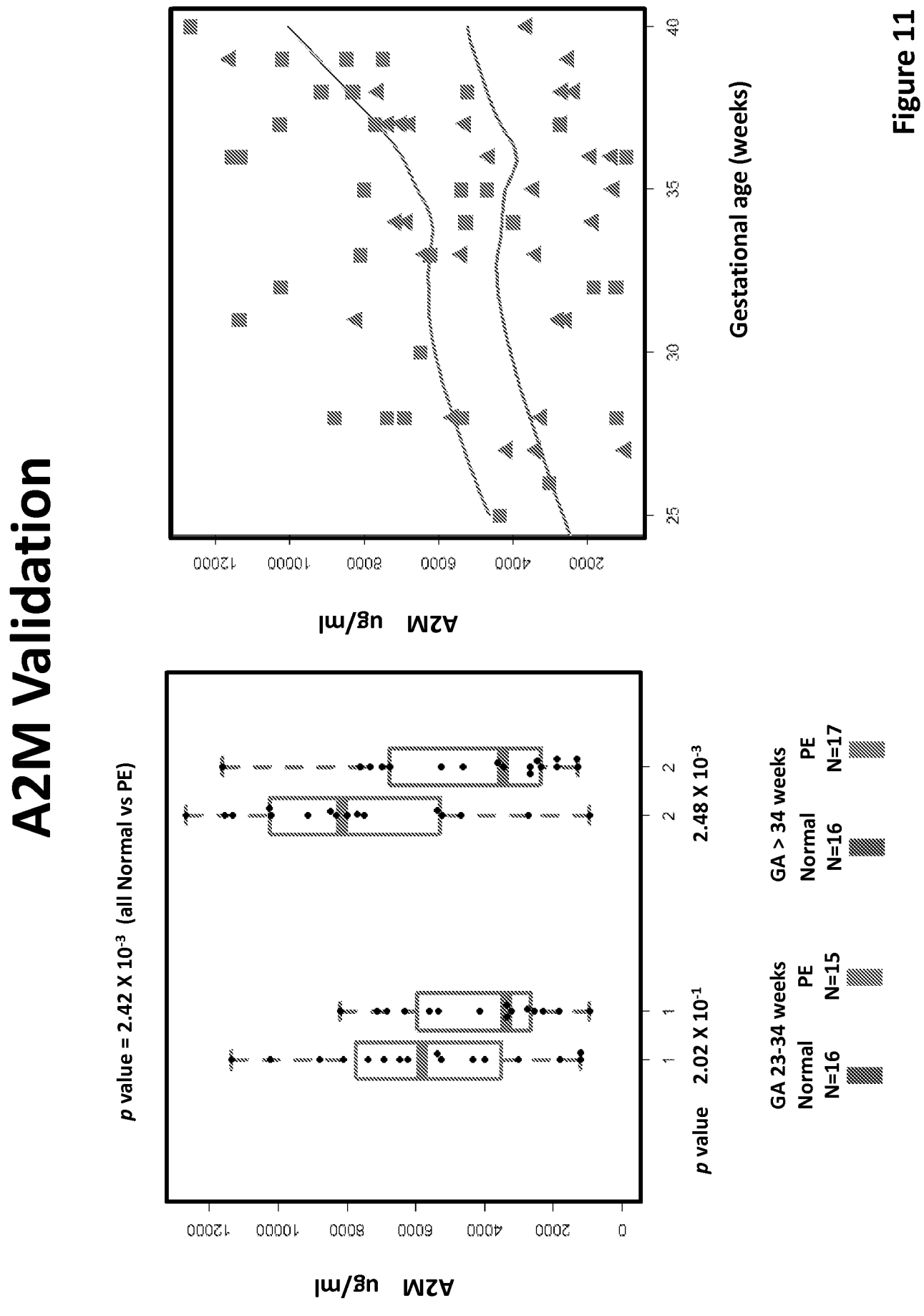
A2M Validation
A2M ug/ml
Gestational age (weeks)
p value = 2.42 X 10-3 (all Normal vs PE)
A2M ug/ml
p value 2.02 X 10-1
2.48 X 10-3
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17

Figure 11

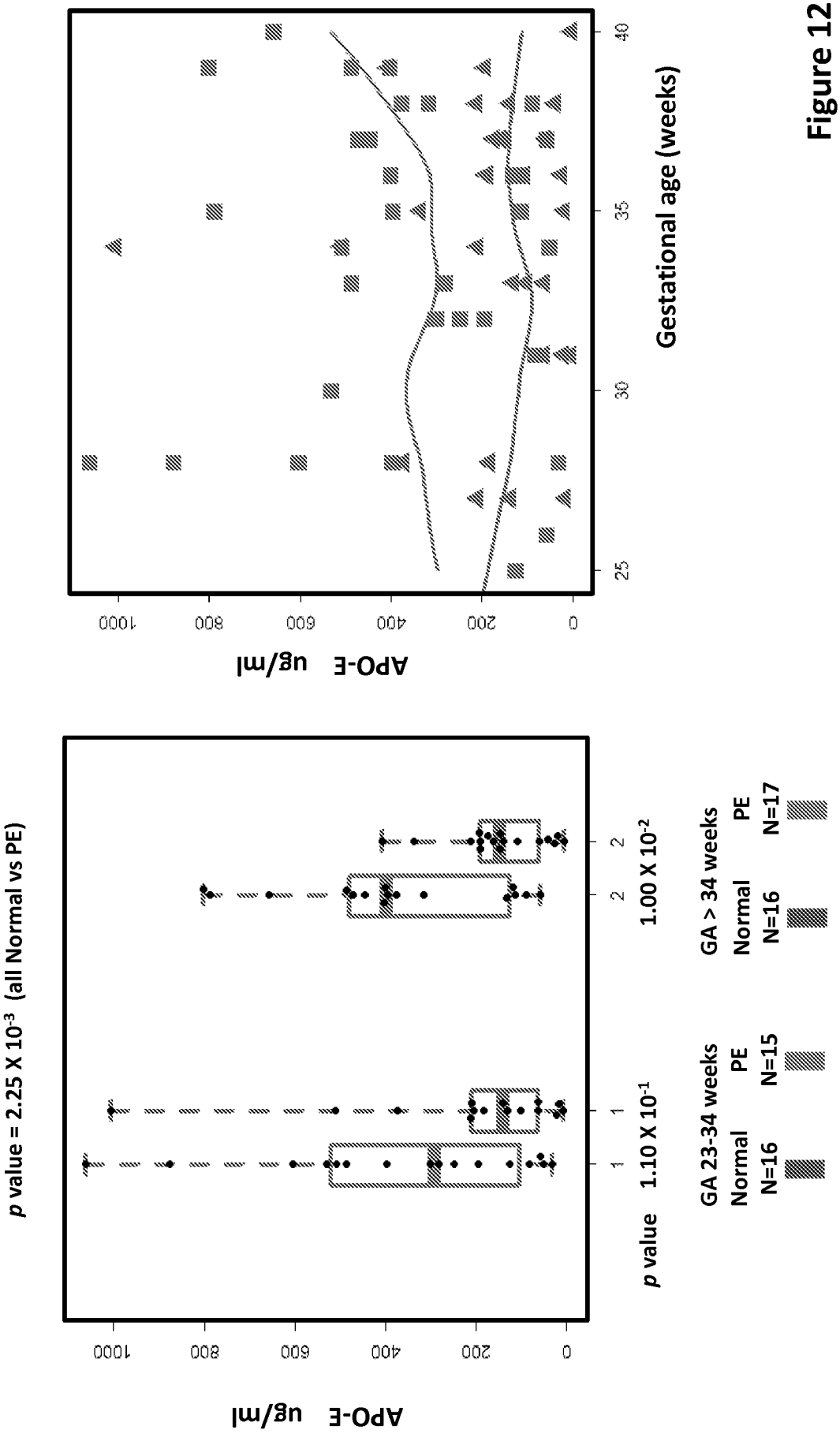
APO-E Validation
p value = 2.25 X 10-3 (all Normal vs PE)
APO-E ug/ml
0
200
400
600
800
1000
1 1 1
1 1 2
1.10 X 10-1
p value
GA 23-34 weeks
Normal N=16
PE N=15
2 2 2
2 2 3
1.00 X 10-2
GA > 34 weeks
Normal N=16
PE N=17
APO-E ug/ml
0
200
400
600
800
1000
25
30
35
40
Gestational age (weeks)

Figure 12

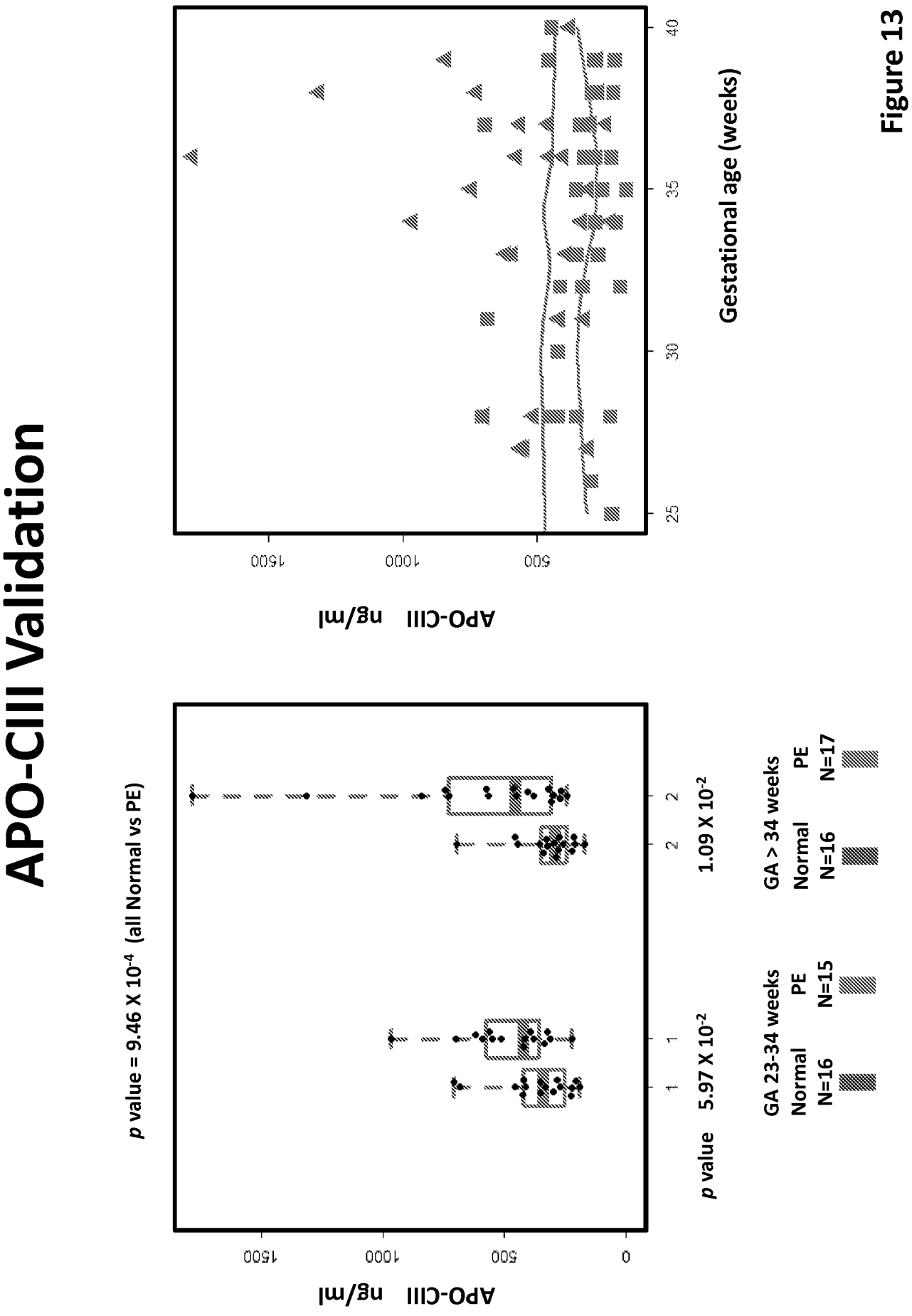
APO-CIII Validation
APO-CIII ng/ml
1500
1000
500
0
1 1 2 2
p value = 9.46 X 10-4 (all Normal vs PE)
p value 5.97 X 10-2
1.09 X 10-2
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17
APO-CIII ng/ml
1500
1000
500
25 30 35 40
Gestational age (weeks)

Figure 13

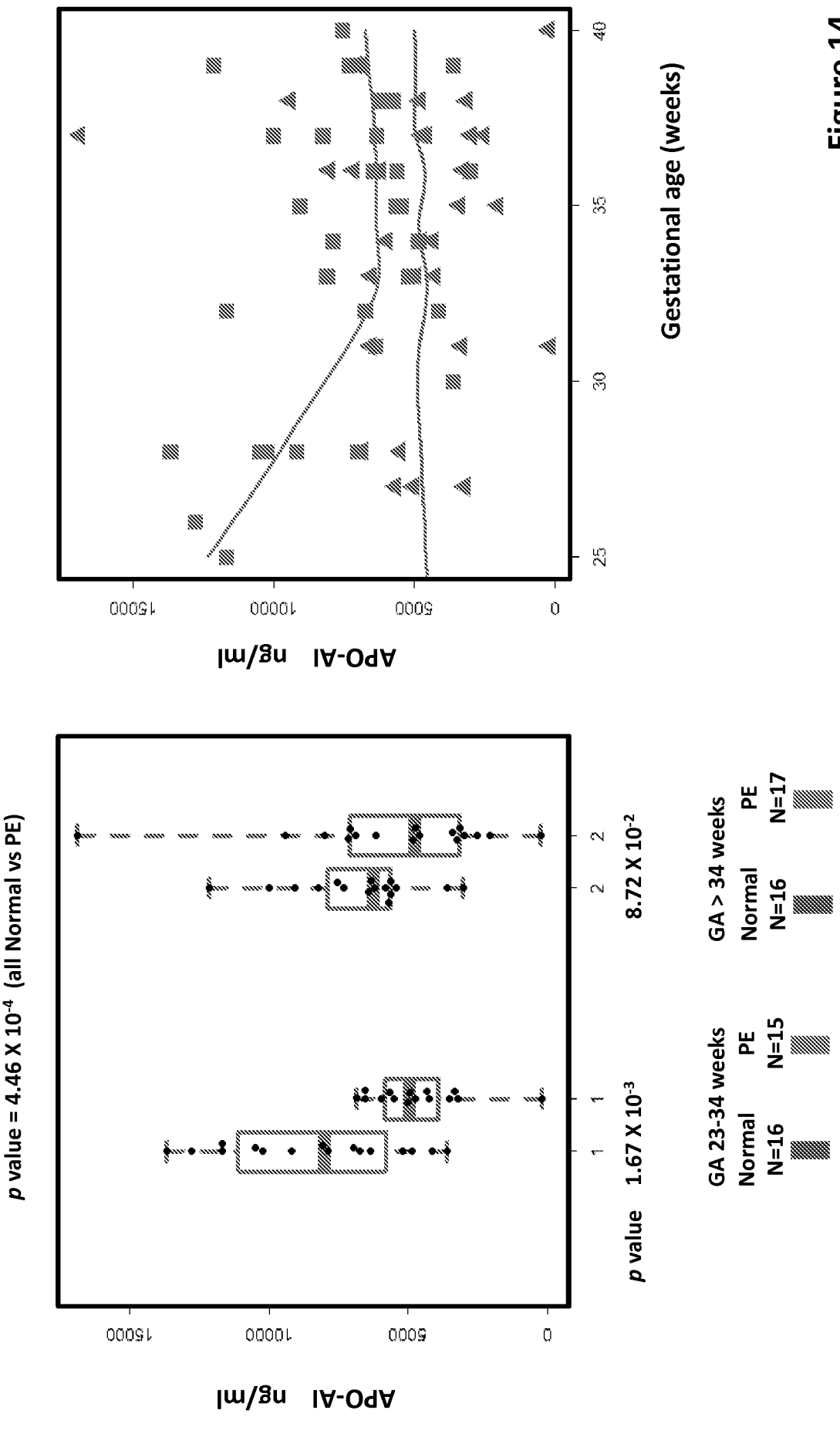
APO-AI Validation
p value = 4.46 X 10-4 (all Normal vs PE)
APO-AI ng/ml
0
5000
10000
15000
1
1
2
2
p value 1.67 X 10-3
8.72 X 10-2
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
APO-AI ng/ml
0
5000
10000
15000
25
30
35
40
Gestational age (weeks)

Figure 14

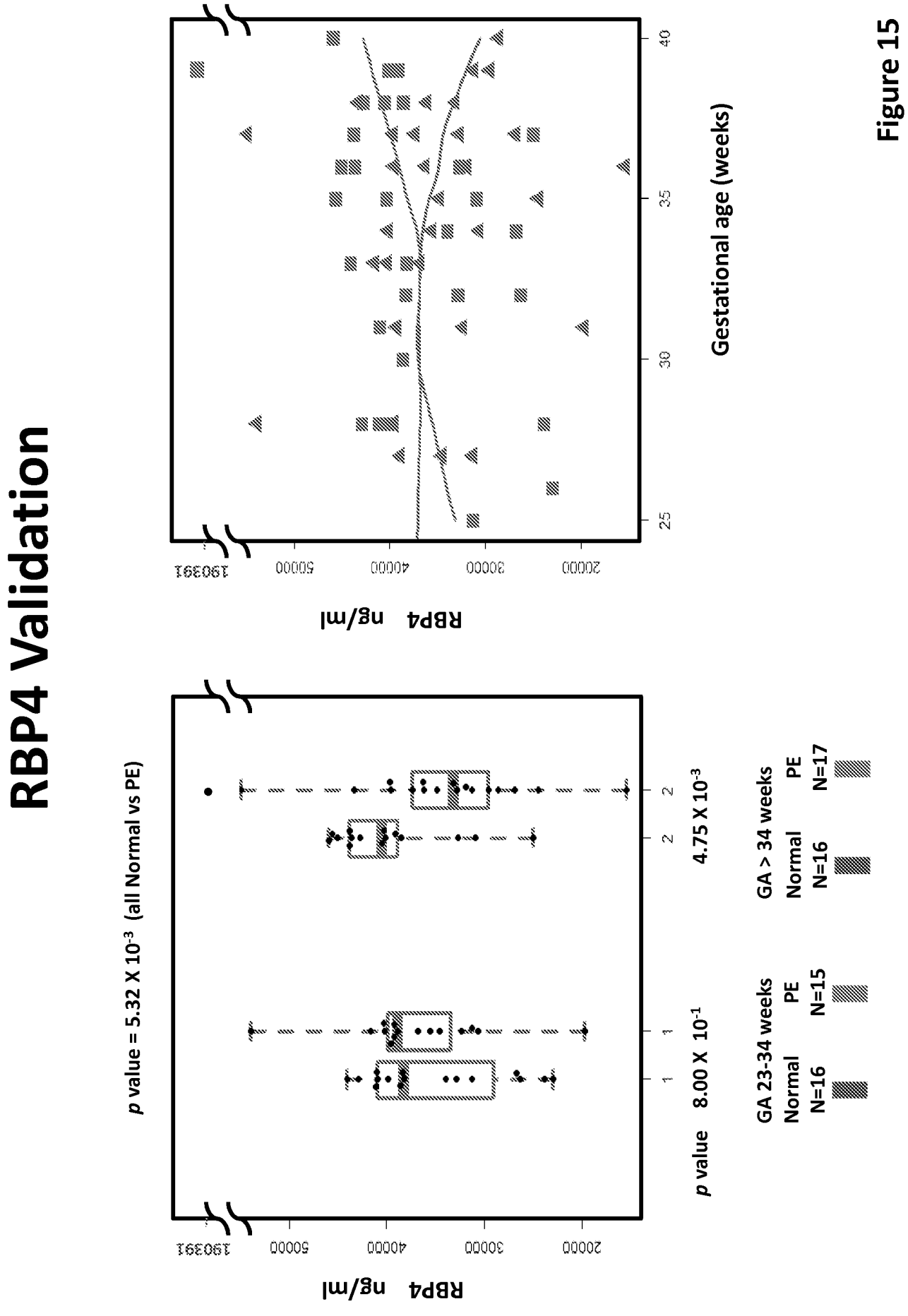
RBP4 Validation
p value = 5.32 X 10-3 (all Normal vs PE)
RBP4 ng/ml
190391
50000
40000
30000
20000
1
2
p value 8.00 X 10-1
4.75 X 10-3
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
RBP4 ng/ml
Gestational age (weeks)
25
30
35
40

Figure 15

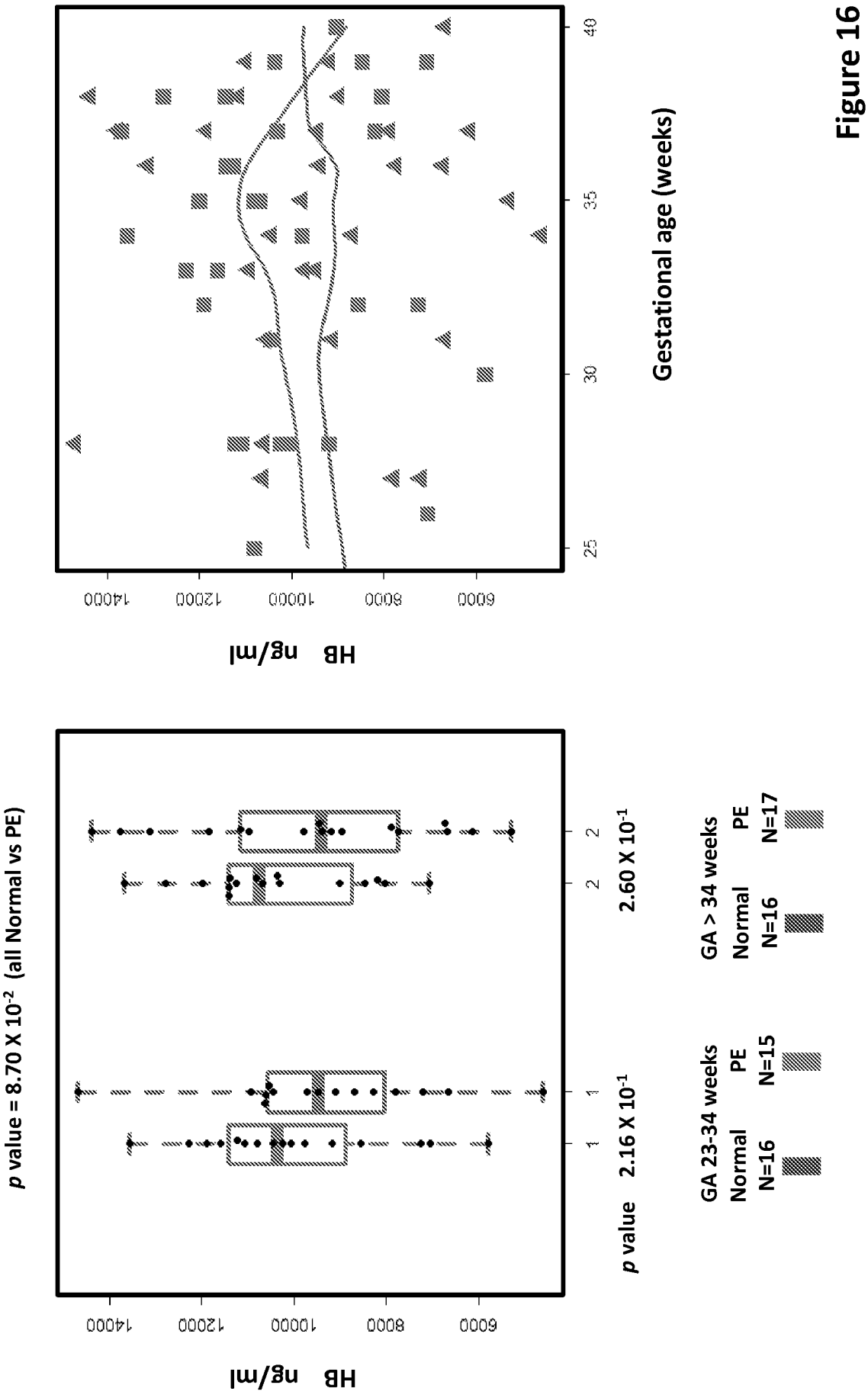
HB Validation
p value = 8.70 X 10-2 (all Normal vs PE)
HB ng/ml
14000
12000
10000
8000
6000
1
1
2
2
p value 2.16 X 10-1
2.60 X 10-1
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17
HB ng/ml
14000
12000
10000
8000
6000
25
30
35
40
Gestational age (weeks)

Figure 16

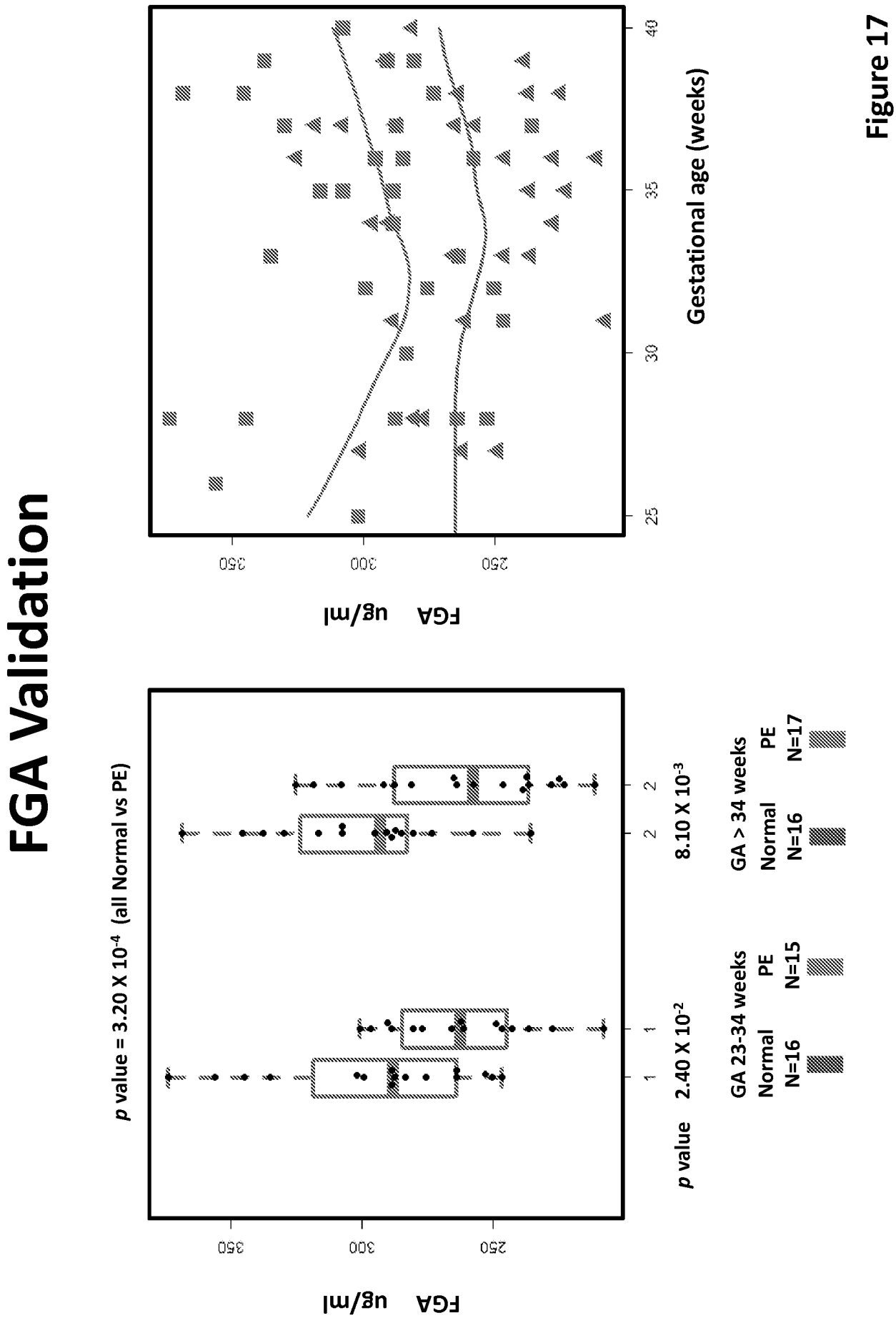
FGA Validation
FGA ug/ml
350
300
250
25
30
35
40
Gestational age (weeks)
FGA ug/ml
350
300
250
p value = 3.20 X 10-4 (all Normal vs PE)
1
1
2
2
p value 2.40 X 10-2
8.10 X 10-3
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17

Figure 17

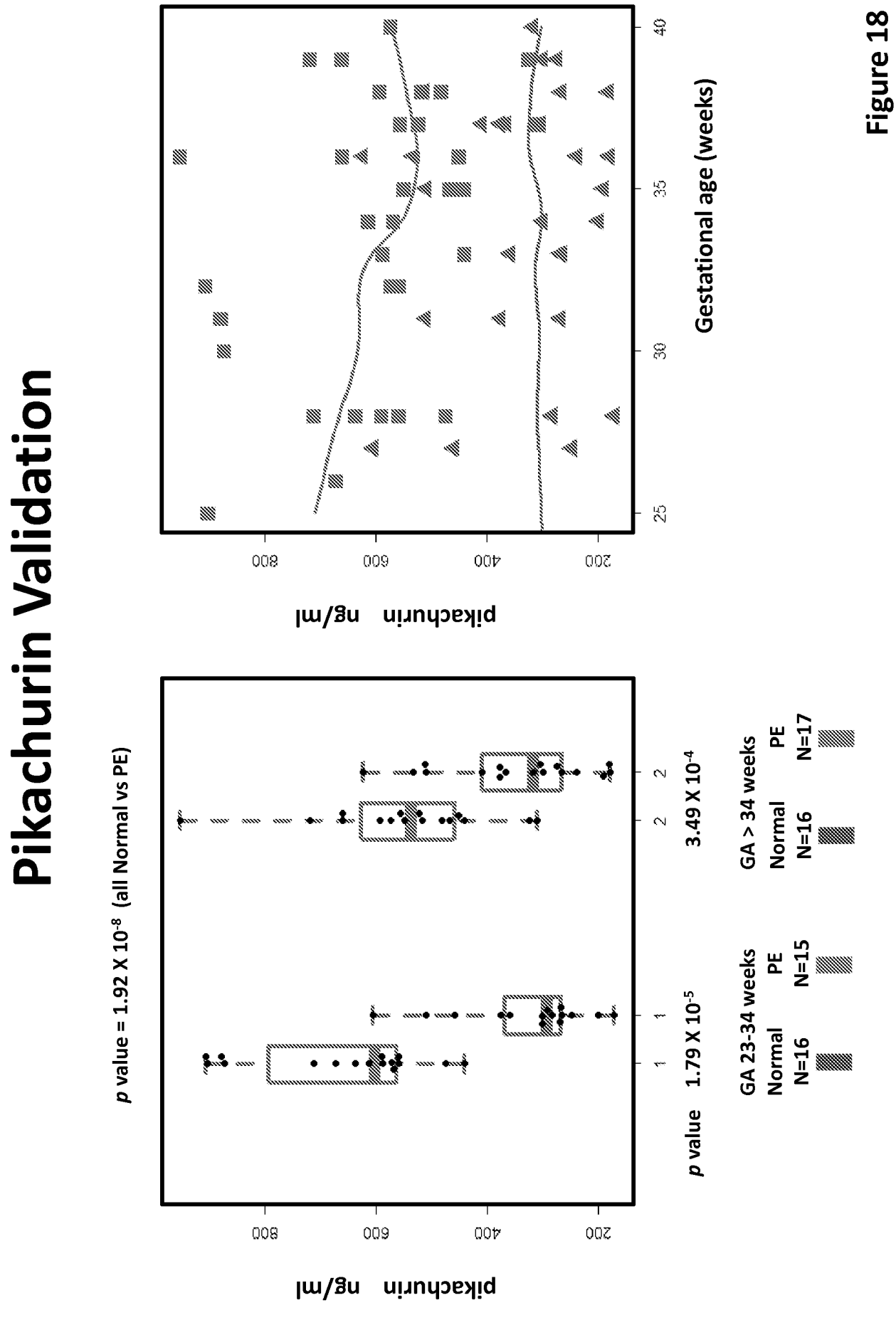
Pikachurin Validation
p value = 1.92 X 10-8 (all Normal vs PE)
pikachurin ng/ml
800
600
400
200
1
1
2
2
p value 1.79 X 10-5
3.49 X 10-4
GA 23-34 weeks
Normal PE
N=16 N=15
GA > 34 weeks
Normal PE
N=16 N=17
pikachurin ng/ml
800
600
400
200
25
30
35
40
Gestational age (weeks)

Figure 18

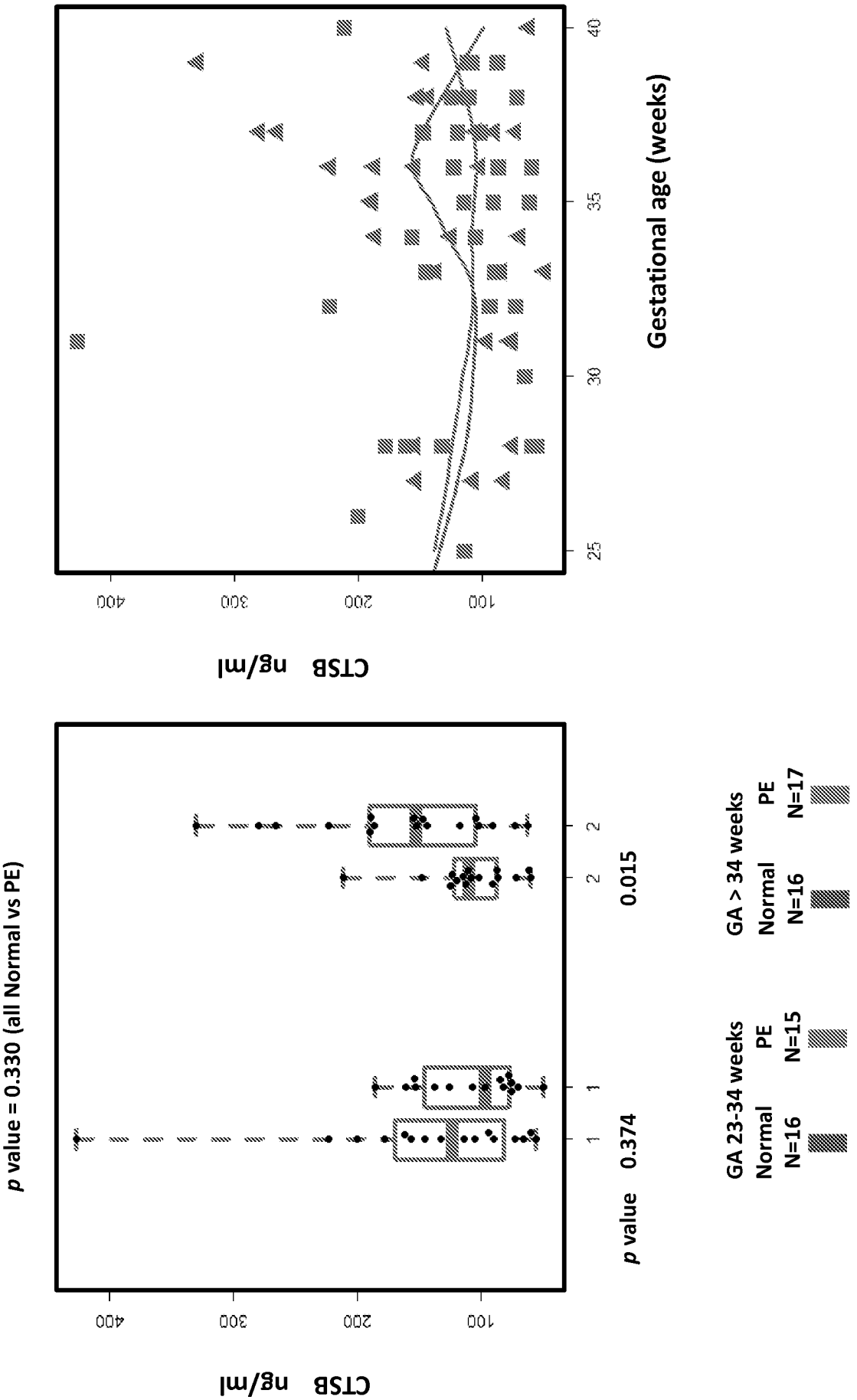
CTSB Validation
p value = 0.330 (all Normal vs PE)
CTSB ng/ml
400
300
200
100
1
1
2
2
p value 0.374
0.015
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
CTSB ng/ml
400
300
200
100
25
30
35
40
Gestational age (weeks)

Figure 19

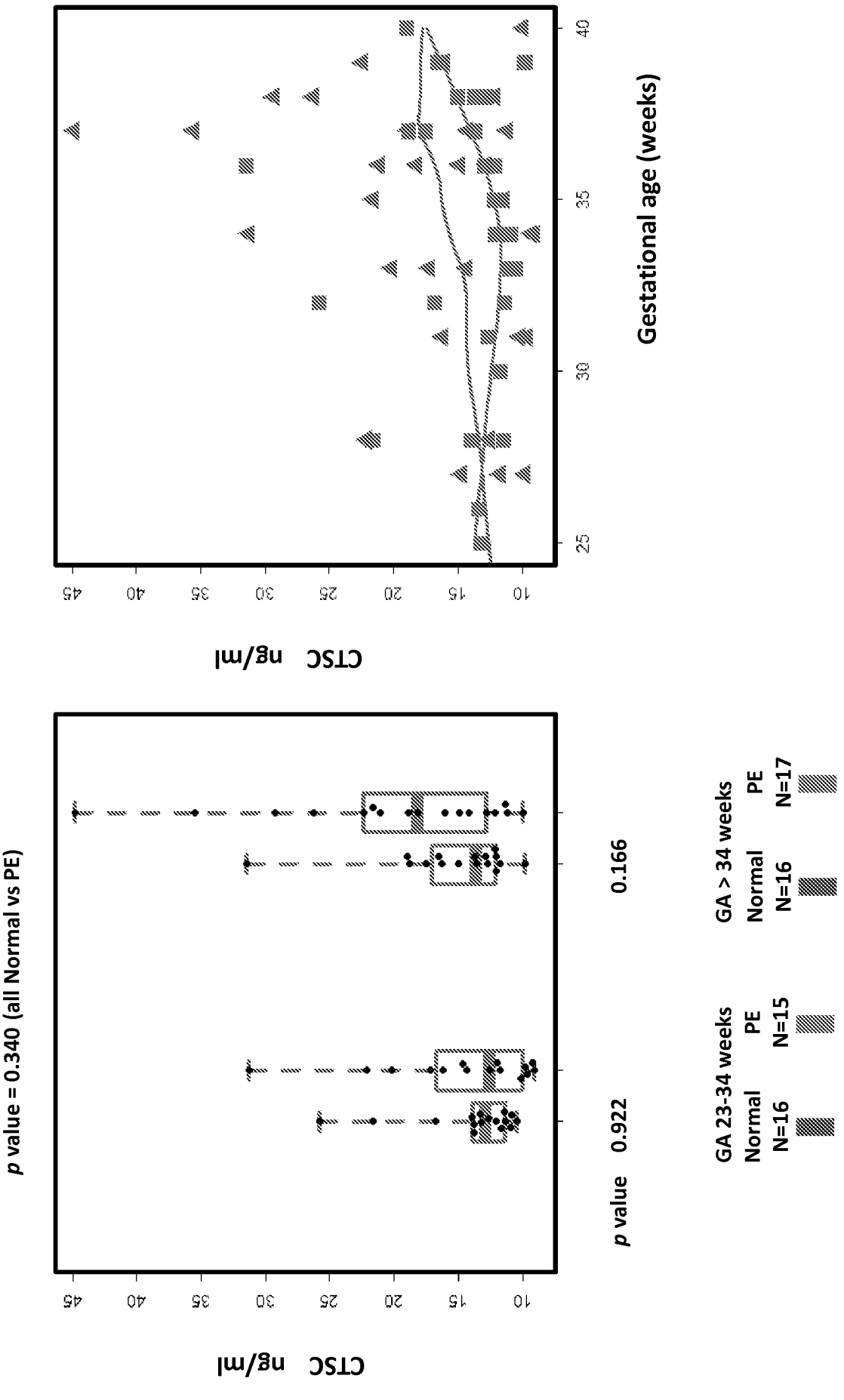
CTSC Validation
p value = 0.340 (all Normal vs PE)
CTSC ng/ml
45
40
35
30
25
20
15
10
p value 0.922 0.166
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
CTSC ng/ml
Gestational age (weeks)
25
30
35
40

Figure 20

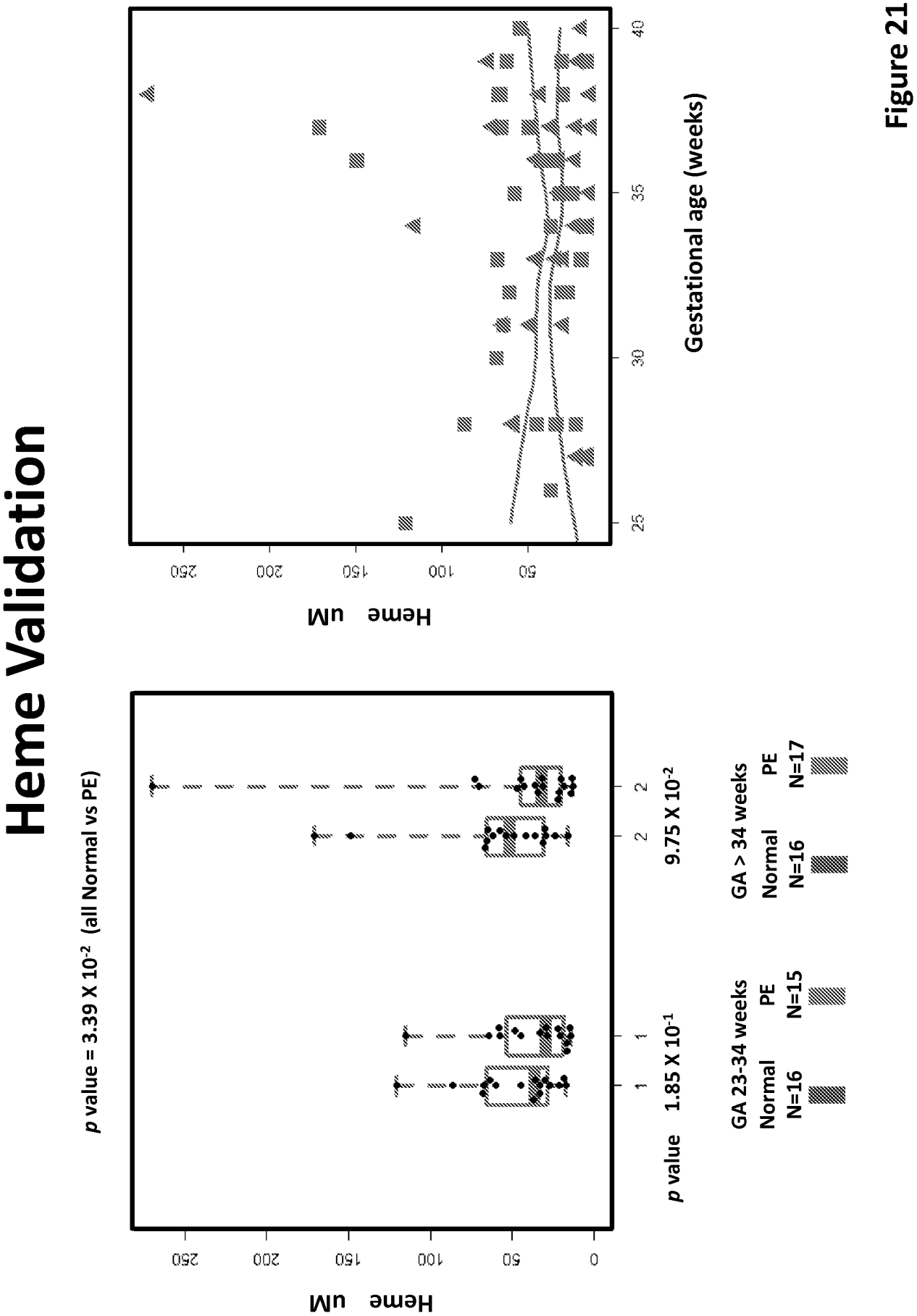
Heme Validation
p value = 3.39 X 10-2 (all Normal vs PE)
Heme uM
0
50
100
150
200
250
1
1
2
2
p value 1.85 X 10-1
9.75 X 10-2
GA 23-34 weeks
Normal N=16
PE N=15
GA > 34 weeks
Normal N=16
PE N=17
Heme uM
50
100
150
200
250
25
30
35
40
Gestational age (weeks)

Figure 21

Biomarker	Early stage ROC AUC	Late stage ROC AUC	Overall ROC AUC
sFlt-1 /PlGF **	0.992	0.894	0.951
sFlt-1/A2M	0.996	0.934	0.969
sFlt-1/RBP4	1.000	0.941	0.976
sFlt-1/Hemoglobin	1.000	0.930	0.971
sFlt-1/Fibrinogen	1.000	0.930	0.974
sFlt-1/Pikachurin	1.000	0.910	0.964
HPX/Pikachurin	0.895	0.977	0.930

Figure 22

Biomarker	Early stage ROC AUC	Late stage ROC AUC	Overall ROC AUC
sFlt-1/PlGF **	0.992	0.894	0.951
sFlt-1/Heme	0.960	0.883	0.924
sFlt-1/Haptoglobin	0.965	0.844	0.910
sFlt-1/APOE	0.977	0.883	0.934
sFlt-1/APO A1	0.984	0.941	0.963
sFlt-1/A2M	0.996	0.934	0.969
sFlt-1/RBP4	1.000	0.941	0.976
sFlt-1/Hemoglobin	1.000	0.930	0.971
sFlt-1/ApoC3	0.910	0.812	0.865
sFlt-1/Fibrinogen	1.000	0.930	0.974
sFlt-1/Pikachurin	1.000	0.910	0.964

Figure 23

Biomarker	Early stage ROC AUC	Late stage ROC AUC	Overall ROC AUC
sFlt-1/PlGF	0.992	0.894	0.951
HPX/PIGF	0.859	0.820	0.841
HPX/Heme	0.766	0.777	0.772
HPX/Haptoglobin	0.832	0.762	0.798
HPX/APOE	0.813	0.801	0.805
HPX/APO A1	0.816	0.863	0.826
HPX/A2M	0.777	0.879	0.824
HPX/RBP4	0.727	0.797	0.773
HPX/Hemoglobin	0.684	0.887	0.799
HPX/ApoC3	0.508	0.605	0.557
HPX/Fibrinogen	0.734	0.648	0.694
HPX/Pikachurin	0.895	0.977	0.930

Figure 24

Biomarker	Early stage ROC AUC	Late stage ROC AUC	Overall ROC AUC
sFlt-1/PlGF	0.992	0.894	0.951
CTSC/PlGF	0.898	0.730	0.814
CTSC/Heme	0.672	0.715	0.689
CTSC/Haptoglobin	0.734	0.645	0.679
CTSC/APOE	0.777	0.738	0.725
CTSC/APO A1	0.797	0.742	0.771
CTSC/A2M	0.719	0.742	0.725
CTSC/RBP4	0.613	0.660	0.630
CTSC/Hemoglobin	0.613	0.715	0.664
CTSC/ApoC3	0.645	0.688	0.663
CTSC/Fibrinogen	0.629	0.695	0.660
CTSC/Pikachurin	0.816	0.883	0.848

Figure 25

Biomarker	Early stage ROC AUC	Late stage ROC AUC	Overall ROC AUC
sFlt-1/PlGF	0.992	0.894	0.951
Adam12/PlGF	0.848	0.844	0.834
Adam12/Haptoglobin	0.832	0.695	0.750
Adam12/APOE	0.801	0.832	0.803
Adam12/APO A1	0.773	0.871	0.816
Adam12/A2M	0.762	0.836	0.808
Adam12/RBP4	0.684	0.824	0.759
Adam12/Hemoglobin	0.703	0.848	0.771
Adam12/ApoC3	0.523	0.555	0.516
Adam12/Fibrinogen	0.793	0.863	0.827
Adam12/Pikachurin	0.836	0.883	0.869

Figure 26

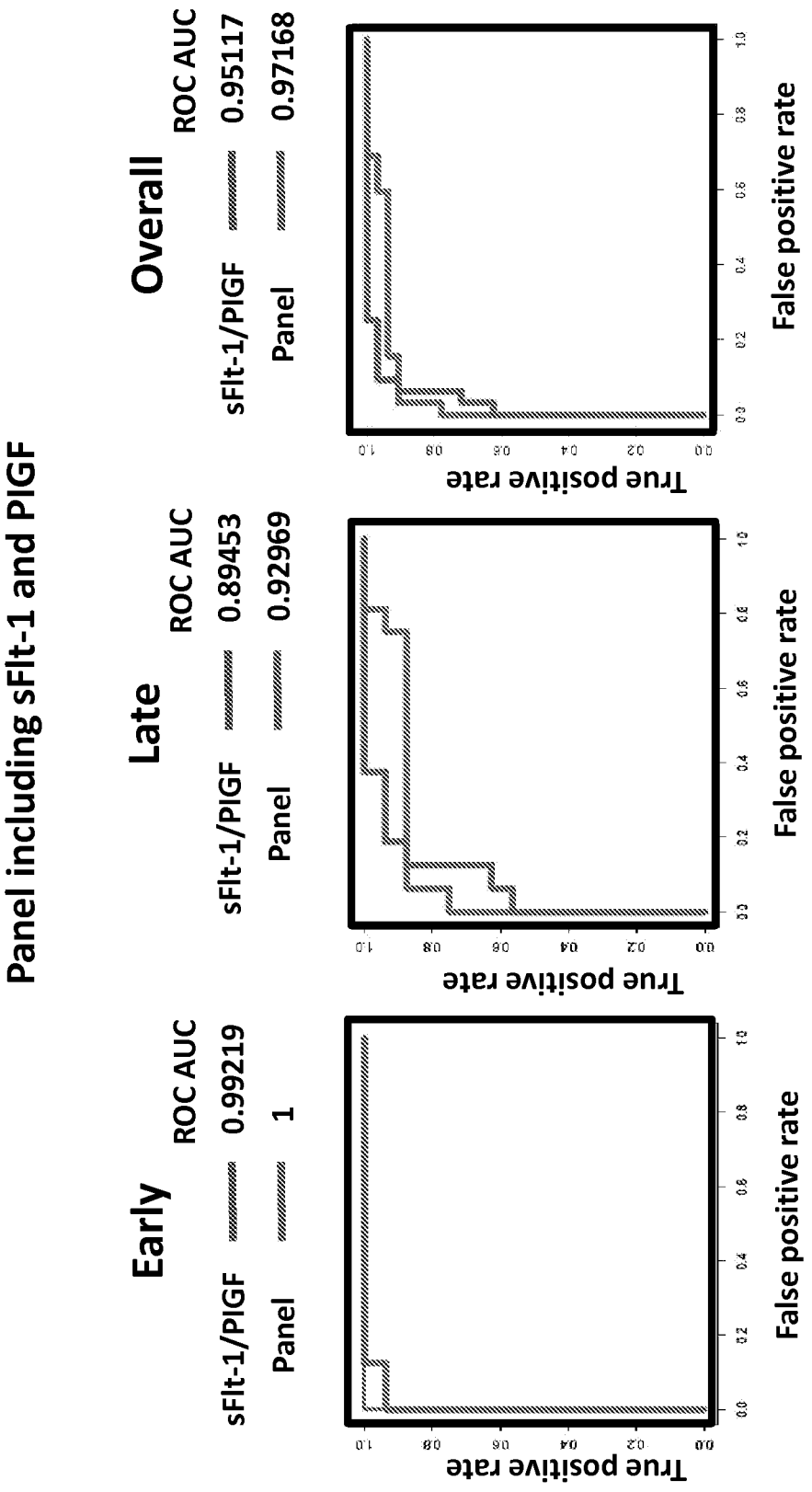
Panel including sFlt-1 and PIGF
Early
ROC AUC
sFlt-1/PIGF 0.99219
Panel 1
Late
ROC AUC
sFlt-1/PIGF 0.89453
Panel 0.92969
Overall
ROC AUC
sFlt-1/PIGF 0.95117
Panel 0.97168
True positive rate
False positive rate

Figure 27

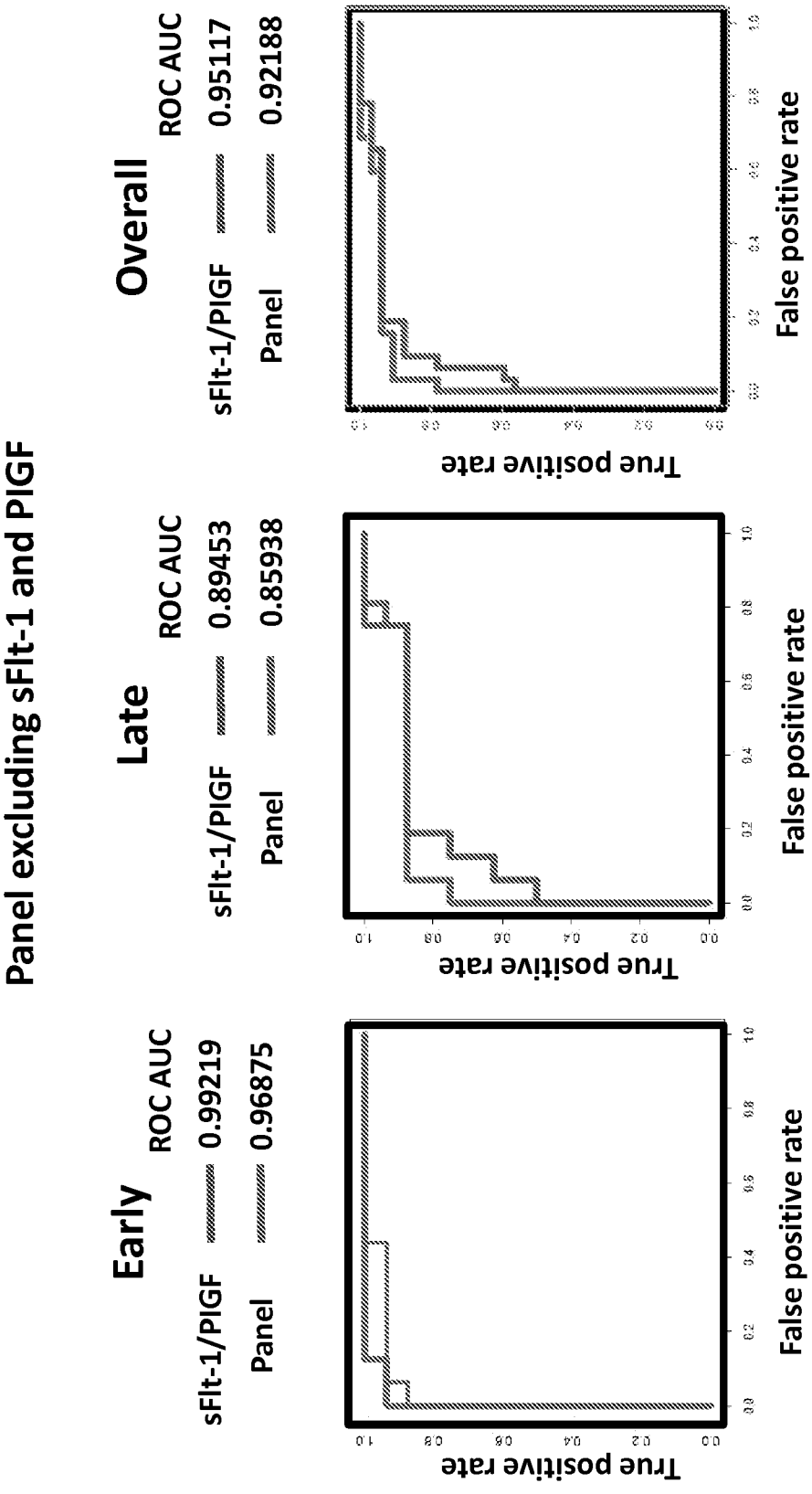
Panel excluding sFlt-1 and PIGF
Early
ROC AUC
sFlt-1/PIGF 0.99219
Panel 0.96875
Late
ROC AUC
sFlt-1/PIGF 0.89453
Panel 0.85938
Overall
ROC AUC
sFlt-1/PIGF 0.95117
Panel 0.92188
True positive rate
False positive rate

Figure 28

Different panels of biomarker combinations

biomarkers	Panel 1	Panel 2		Panel 3	Panel 4		Panel 5	Panel 6		Panel 7	
		early	late		early	late		early	late	early	late
sFlt-1	+	+	+	+	+	+	-	-	-	-	-
PIGF	+	+	+	-	-	-	+	+	+	-	-
HPX	-	+	+	-	+	+	-	-	+	+	+
Ferritin	-	-	-	-	-	-	-	-	-	-	-
CTSC	-	-	+	-	-	-	-	-	+	-	+
Adam12	-	-	+	-	-	-	-	-	+	-	+
HP	-	-	-	-	-	-	-	-	+	-	+
A2M	-	-	-	-	-	-	-	-	-	-	-
ApoE	-	-	+	-	-	+	-	-	+	-	-
ApoC3	-	-	-	-	-	-	-	-	-	-	-
ApoA1	-	-	+	-	-	+	-	-	-	+	-
RBP4	-	-	+	-	-	-	-	-	+	-	-
HB	-	-	+	-	-	-	-	-	+	-	+
Fibrinogen	-	-	-	-	-	-	-	-	+	-	+
Pikachurin	-	-	+	-	-	+	-	+	+	+	+

\+ Biomarkers chosen
\- Biomarkers not chosen

Figure 29

ROC curve AUC values with different combinations of biomarkers

	biomarkers			number of SU biomarkers			ROC curve AUC value		
Panel	**sFlt-1**	**PIGF**	**Stanford biomarkers**	**early**	**late**	**overall**	**early stage**	**late stage**	**overall stage**
1	+	+	-	0	0	0	1	0.8602941	0.9511719
2	+	+	+	1	8	8	1	1	1
3	+	-	-	0	0	0	1	0.8970588	0.9619141
4	+	-	+	1	4	4	1	1	1
5	-	+	-	0	0	0	0.9208333	0.6286765	0.7910156
6	-	+	+	1	9	9	1	0.9852941	0.9814453
7	-	-	+	3	7	8	1	1	1

\+ Biomarkers chosen
\- Biomarkers not chosen

Figure 30

Sensitivity with given specificity level

specificity level	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7
100%	78.13%	100%	93.75%	100%	12.50%	75.00%	100%
95%	90.63%	100%	93.75%	100%	43.75%	90.63%	100%
90%	90.63%	100%	93.75%	100%	50.00%	90.63%	100%
85%	90.63%	100%	93.75%	100%	50.00%	93.75%	100%
80%	93.75%	100%	93.75%	100%	50.00%	100%	100%

Specificity with given sensitivity level

sensitivity level	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7
100%	31.25%	100%	37.50%	100%	12.50%	84.37%	100%
95%	40.62%	100%	40.62%	100%	40.62%	84.37%	100%
90%	96.87%	100%	100%	100%	53.12%	96.87%	100%
85%	96.87%	100%	100%	100%	62.50%	96.87%	100%
80%	96.87%	100%	100%	100%	62.50%	96.87%	100%

Figure 31

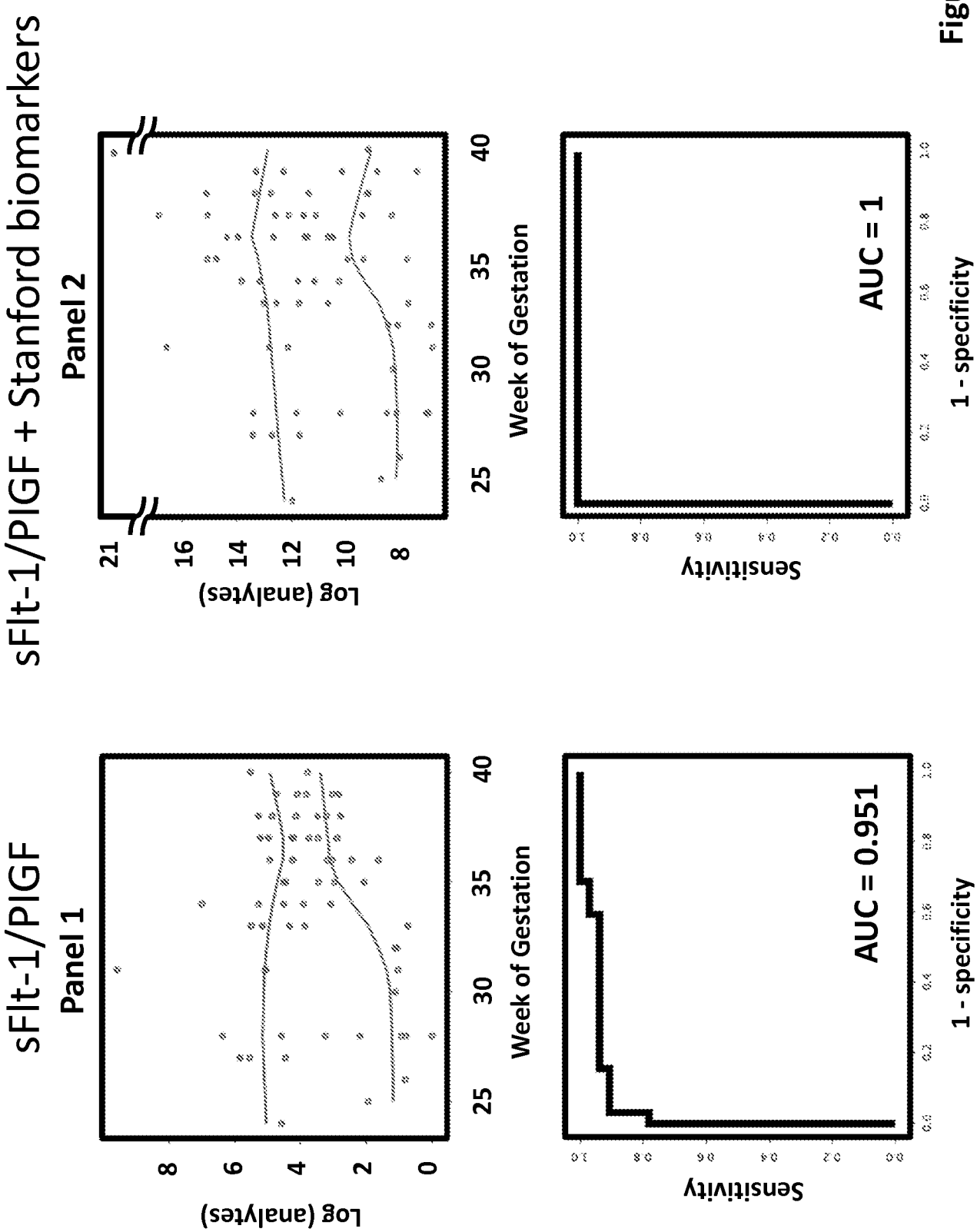
sFlt-1/PlGF
Panel 1
Log (analytes)
Week of Gestation
Sensitivity
1 - specificity
AUC = 0.951
sFlt-1/PlGF + Stanford biomarkers
Panel 2
Log (analytes)
Week of Gestation
Sensitivity
1 - specificity
AUC = 1

Figure 32

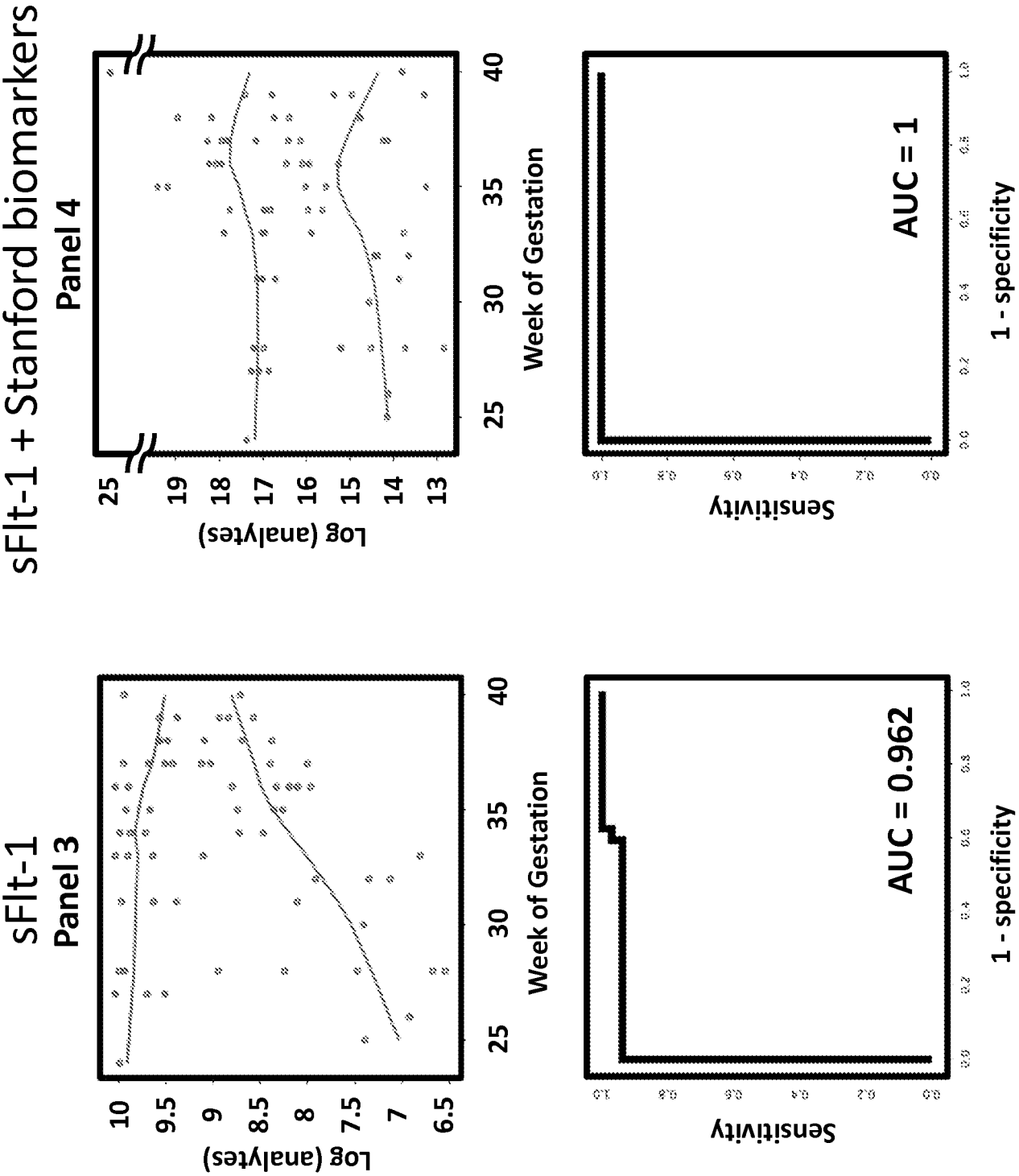
sFlt-1
Panel 3
Log (analytes)
Week of Gestation
Sensitivity
1 - specificity
AUC = 0.962
sFlt-1 + Stanford biomarkers
Panel 4
Log (analytes)
Week of Gestation
Sensitivity
1 - specificity
AUC = 1

Figure 33

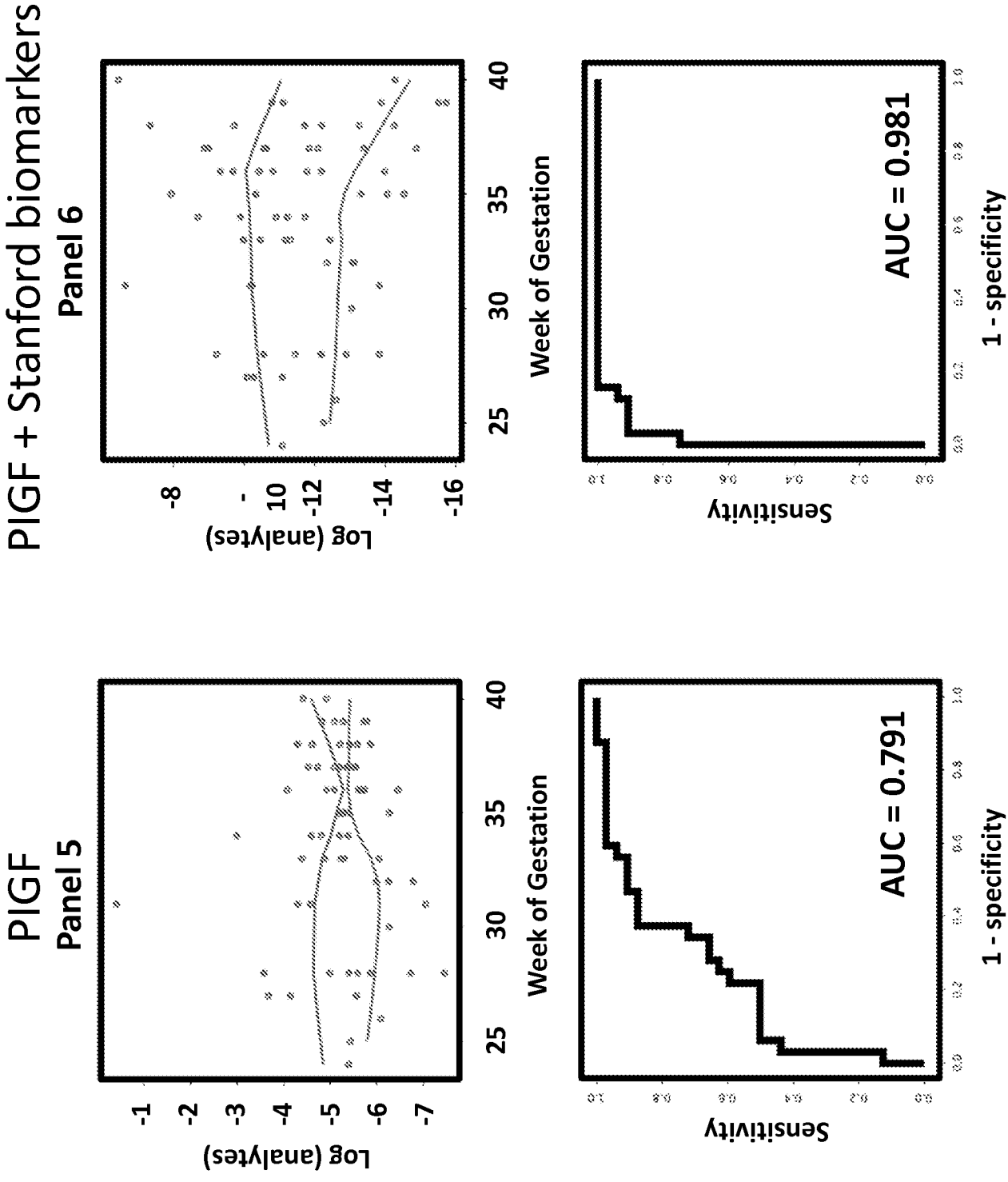
PIGF
Panel 5
Log (analytes)
-1
-2
-3
-4
-5
-6
-7
25
30
35
40
Week of Gestation
Sensitivity
1 - specificity
AUC = 0.791
PIGF + Stanford biomarkers
Panel 6
Log (analytes)
-8
-10
-12
-14
-16
25
30
35
40
Week of Gestation
Sensitivity
1 - specificity
AUC = 0.981

Figure 34

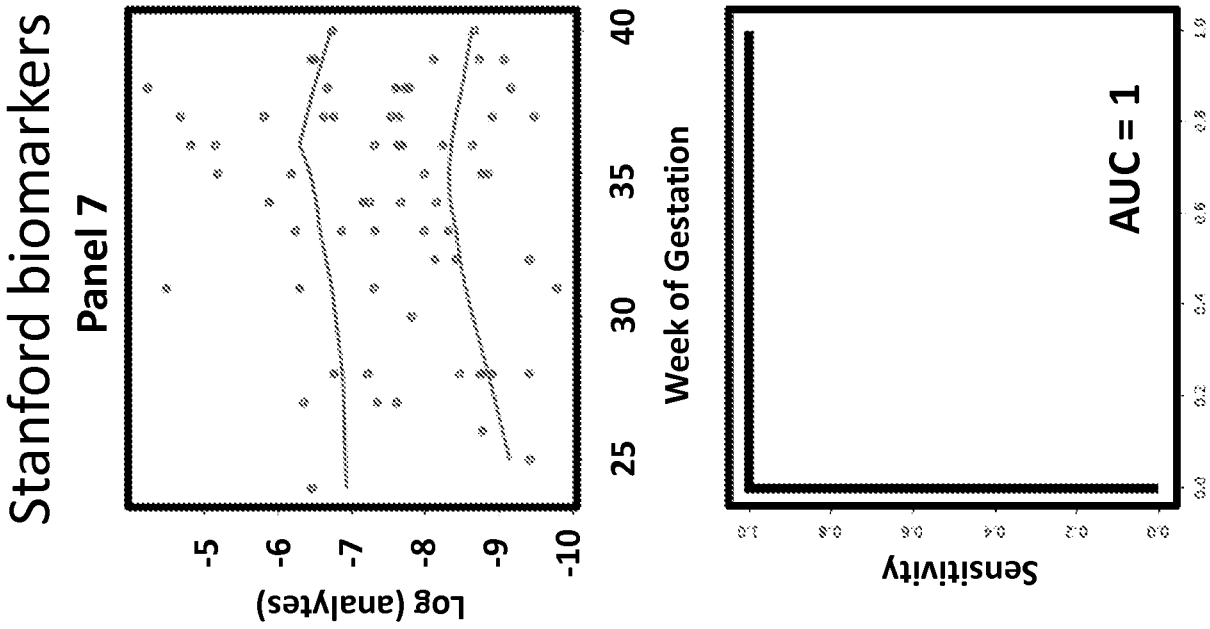
Stanford biomarkers
Panel 7
Log (analytes)
-5
-6
-7
-8
-9
-10
25
30
35
40
Week of Gestation
Sensitivity
1 - specificity
AUC = 1

Figure 35

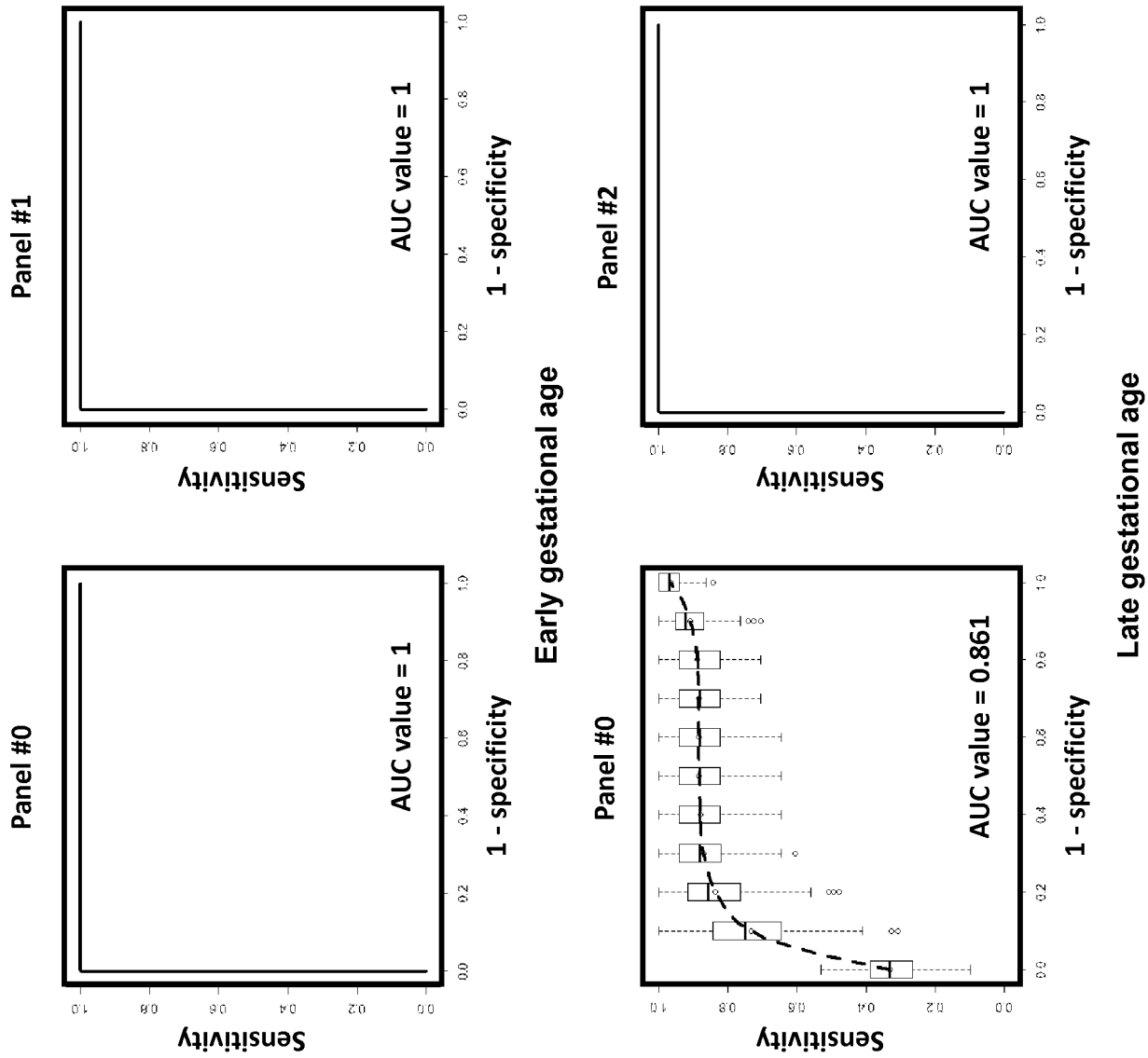
Panel #1
AUC value = 1
Sensitivity
1 - specificity
Panel #0
AUC value = 1
Sensitivity
1 - specificity
Early gestational age
Panel #2
AUC value = 1
Sensitivity
1 - specificity
Panel #0
AUC value = 0.861
Sensitivity
1 - specificity
Late gestational age

Figure 36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/039918

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.1
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)
IPC(8) - C12Q 1/68; C40B 40/10; G01N 33/50, 33/566 (2013.01)
USPC - 435/4, 6.1, 7.93, 26; 436/501; 506/7, 15; 514/9.8; 530/351

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

CPC - A61K 38/1709; G01N 33/689, 2800/50, 2800/60, 2800/70 (2013.01)

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

Patbase, Google Patents, Google, Pubmed

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 2010/0267034 A1 (LO et al) 21 October 2010 (21.10.2010) entire document	1, 2, 5-7, 10, 11, 14, 16-20 --- 3, 4, 8, 9, 12, 13, 15, 21, 22
Y	SCHUTZER et al. 'Distinct Cerebrospinal Fluid Proteomes Differentiate Post-Treatment Lyme Disease from Chronic Fatigue Syndrome,' PLoS One, 23 February 2011 (23.02.2011), Vol. 6, Iss. 2, Pgs. 1-8. entire document	3, 4, 8, 9, 12, 13, 21, 22
Y	WO 2011/127219 A1 (KUSLICH et al) 13 October 2011 (13.10.2011) entire document This document can be viewed by entering the doc number at the following url: http://worldwide.espacenet.com/numberSearch?locale=en_EP	4, 9, 13, 22
Y	US 2008/0233583 A1 (FISHER et al) 25 September 2008 (25.09.2008) entire document	15
P, X	US 2012/0142559 A1 (TUYTTEN et al) 07 June 2012 (07.06.2012) entire document	1-22

☐ Further documents are listed in the continuation of Box C. ☐

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Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2013	08 OCT 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

摘要

本发明提供了先兆子痫标志物、先兆子痫标志物小组，以及用于获得样本的先兆子痫标志物水平表现的方法。这些组合物和方法可用于多种应用，包括，例如诊断先兆子痫、预测先兆子痫、监测患有先兆子痫的患者，以及确定先兆子痫的治疗过程。另外，本发明提供了可用于实施主题方法的系统、装置和试剂盒。