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FIG. 1A

Table 1: Median 24 week samples. Markers are ranked using Lasso regression (alpha = 1).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp.Q6HBL7:ISM2	Q6HBL7	ISM2_HUMAN	IstHmin-2	ISM2, TAIL1, THSD9, PSECD137	Homo sapiens (human)	ISM2	TAIL1, THSD3
2	sp.Q00891:QSOX1	Q00891	QSOX1_HUMAN	Sulfhydryl oxidase 1	QSOX1, QSOX6, URQ2F520/FRQ5613	Homo sapiens (human)	QSOX1	QSOX6
3	sp.P11142:HSP70	P11142	HSP70_HUMAN	Heat shock cognate 71 kDa protein	HSPA8, HSC70, HSP73, HSPA10	Homo sapiens (human)	HSPA8	HSC70, HSP73, HSPA10
4	sp.P62805:H4	P62805	H4_HUMAN	Histone H4	H4C1, H4A, H4FA, HIST3H4A, H4C2, H4J, H4FI, HIST3H4B, H4C3, H4G, H4FG, HIST1H4C, H4C4, H4J, H4FB, HIST1H4D, H4C5, H4J, H4FJ, HIST1H4E, H4C6, H4J, H4FI, HIST1H4F, H4C9, H4J, H4FP, HIST1H4G, H4C11, H4JE, H4FE, HIST1H4J, H4C12, H4J, H4FD, HIST2H4E, H4C13, H4J, H4FK, HIST1H4I, H4C14, H4J, H4F2, H4FN, HIST2H4, HIST2H4A, H4C15, H4J, H4FO, HIST2H4B, H4-15, HIST4B-1	Homo sapiens (human)	H4C1, H4C2, H4C3, H4C4, H4C5, H4C6, H4C9, H4C11, H4C12, H4C13, H4C14, H4C15, H4-15	H4A, H4FA, HIST3H4A, H4J, H4FI, HIST3H4B, H4G, H4FG, HIST1H4C, H4J, H4FB, HIST1H4D, H4J, H4FJ, HIST1H4E, H4J, H4FI, HIST1H4F, H4J, H4FP, HIST1H4G, H4J, H4FE, HIST1H4J, H4J, H4FD, HIST2H4E, H4J, H4FK, HIST2H4, H4J, H4F2, H4FN, HIST2H4A, HIST2H4B, HIST4B-1

(57) Abstract: Disclosed herein are methods of preparing and analyzing samples for biomarkers associated with placenta accreta in pregnant subjects. Also disclosed are method of assessing the risk of placenta accreta in pregnant subjects.

[Continued on next page]

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METHODS OF PREPARING AND ANALYZING SAMPLES FOR BIOMARKERS ASSOCIATED WITH PLACENTA ACCRETA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application number 63/278,456, filed on November 11, 2021, the contents of which are incorporated by reference herein in their entirety.

BACKGROUND

[0002] Placenta accreta is a serious pregnancy condition that occurs when the placenta grows too deeply into the uterine wall. Typically, the placenta detaches from the uterine wall after childbirth. With placenta accreta, part or all of the placenta remains attached. This can cause severe blood loss after delivery. It is also possible for the placenta to invade the muscles of the uterus (placenta increta) or grow through the uterine wall (placenta percreta).

[0003] Placenta accreta is considered a high-risk pregnancy complication. If the condition is diagnosed during pregnancy, there is increases likelihood that a woman will require cesarian delivery followed by hysterectomy.

[0004] The incidence of placenta accreta spectrum has increased by a factor of approximately 8 since the 1970s, probably owing to increases in cesarean delivery. The incidence of cesarean birth is much higher in women with placenta accreta than those without. For example, in a first pregnancy, the incidence of cesarian birth in women without placenta accreta is 0.03%, while in women with placenta accreta, the incidence is 100-fold higher, or 3%.

[0005] Women with major risk factors, such as placenta previa, previous cesarean delivery, endometrial ablation, or other uterine surgery, should undergo obstetrical sonography in the middle-to-late second trimester to assess for possible placenta accreta spectrum. Patients with suspected placenta accreta spectrum should be referred to a center with multidisciplinary expertise and experience.

[0006] Present diagnosis of placenta accreta involves ultrasound, the specificity and sensitivity of which relies on knowledge of patient's clinical status (e.g., clinical suspicion for accreta, prior knowledge of risk factors). Needed are methods of more sensitive ways of predicting and detecting placenta accreta in a pregnant woman. Provided herein are methods and compositions that address this need.

SUMMARY

[0007] Disclosed herein are circulating microparticle (CMP)-associated proteins, useful for the prediction and detection of plasma accreta. In some embodiments, the CMP-associated proteins are collected from around about 20 weeks to around about 37 weeks of gestation, that can be used to assess risk of placenta accreta. The biomarkers are presented in Tables 1-6, and Table 9, the tables of FIGS. 1A-1D, FIGS. 2A-2C, FIGS. 3A-3K, FIGS. 4A-4J, FIGS. 5A-5C, FIGS. 6A-6F, as well as tables in Example 1. Also provided are surrogates useful for the detection of the biomarkers, presented in FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B. Panels of biomarkers are also presented.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The accompanying drawings, which are incorporated herein and form a part of the specification, illustrate exemplary embodiments and, together with the description, further serve to enable a person skilled in the pertinent art to make and use these embodiments and others that will be apparent to those skilled in the art. The invention will be more particularly described in conjunction with the following drawings wherein:

[0009] **FIGS. 1A-1D** depict Table 1 comprising median 24-week samples. The markers are ranked using Lasso regression ($\alpha = 1$). The top 20 markers are displayed. This technique optimizes proteins that may interact and "collaborate" in panels rather than isolated markers. Individual markers are ranked by frequency of utility in multimarker panels.

[0010] **FIGS. 2A-2C** depict Table 2 comprising median 34-week samples. The markers are ranked using Lasso regression ($\alpha = 1$). The top 20 markers are displayed and ranked by frequency of utility in multimarker panels.

[0011] **FIGS. 3A-3K.** depict Table 3 comprising the top 50 protein markers at median 24 weeks using an ensemble feature selection routine that selects the top individual markers that distinguish case from control. The protein markers that overlap with the top 20 of the Lasso regression of Table 1 are indicated with asterisk. Individual markers are ranked by performance in distinguishing case/control.

[0012] **FIGS. 4A-4J** depict Table 4 comprising the top 50 protein markers at median 34 weeks using an ensemble feature selection routine that selects the top individual markers that distinguish case from control. The protein markers that overlap with the top 20 of the Lasso regression (Table 2) are indicated with an asterisk. Individual markers are ranked by performance in distinguishing case/control.

[0013] **FIGS. 5A-5C** depict Table 5 comprising 24-week markers. Individual markers ranked by performance in distinguishing case/control.

[0014] **FIGS. 6A-6F** depict Table 6 comprising 34-week markers. Individual markers ranked by performance in distinguishing case/control.

[0015] **FIG. 7** depicts Table 7 comprising 24-week markers. Top performing multiplex panels are ranked by average AUC of an iterative cross-validation procedure.

[0016] **FIG. 8** depicts Table 8 comprising 34-week markers. Top performing multiplex panels are ranked by average AUC of an iterative cross-validation procedure.

[0017] **FIG. 9** illustrates a schematic of a protocol for identifying predictive circulating microparticle protein panels for placenta accreta.

[0018] **FIGS. 10A-10C** provide surrogate peptides, useful for the detection of the biomarkers of Table 1.

[0019] **FIGS. 11A-11C** provide surrogate peptides, useful for the detection of the biomarkers of Table 2.

[0020] **FIGS. 12A-12H** provide surrogate peptides, useful for the detection of the biomarkers of Table 3.

[0021] **FIGS. 13A-13H** provide surrogate peptides, useful for the detection of the biomarkers of Table 4.

[0022] **FIGS. 14A-14D** provide surrogate peptides, useful for the detection of the biomarkers of Table 5.

[0023] **FIGS. 15A-15B** provide surrogate peptides, useful for the detection of the biomarkers of Table 6.

[0024] **FIG. 16A** is a density plot of protein versus permuted with a first shaded area represents actual protein AUC and a second shaded area represents AUC from randomly permuting the sample labels (placenta accreta spectrum vs. control) for the second trimester (e.g., 24 weeks).

[0025] **FIG. 16B** is a density plot of protein versus permuted with a first shaded area represents actual protein AUC and a second shaded area represents AUC from randomly permuting the sample labels (placenta accreta spectrum vs. control) for the third trimester (e.g., 37 weeks).

[0026] **FIG. 17** depicts a schematic of exemplary canonical pathways, upstream regulators and molecular and cellular function analyses proposed in the second and third trimester leading to morbid placental adherence.

DETAILED DESCRIPTION

I. Introduction

[0027] Disclosed herein are methods, compositions, systems and articles of manufacture useful in preparing a sample for the detection of biomarkers useful in determining risk of developing, and for treating, placenta accreta. In some embodiments, determination involves detection of placenta accreta biomarkers found in microparticle-enriched fractions from the blood of pregnant women. Exemplary biomarkers useful for the detection of placenta accreta in either or both the second and third trimester are presented in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, and Table 9. As discussed below, Table 7 and Table 8 present biomarker panels for placenta accreta. Additional marker sets are also presented herein.

II. Subjects

[0028] Subjects for providing samples for prediction and treatment of placenta accreta are pregnant human females. The stage of pregnancy can be calculated from the first day of the last normal menstrual period of the pregnant subject.

[0029] In some embodiments, the pregnant women may be about 20 weeks to about 37 weeks of pregnancy. In some embodiments, the pregnant woman may be about 20 weeks of pregnancy, about 21 weeks of pregnancy, about 22 weeks of pregnancy, about 23 weeks of pregnancy, about 24 weeks of pregnancy, about 25 weeks of pregnancy, about 26 weeks of pregnancy, about 27 weeks of pregnancy, about 28 weeks of pregnancy, about 29 weeks of pregnancy, about 30 weeks of pregnancy, about 31 weeks of pregnancy, about 32 weeks of pregnancy, about 33 weeks of pregnancy, about 34 weeks of pregnancy, about 35 weeks of pregnancy, about 36 weeks of pregnancy, or about 37 weeks of pregnancy.

[0030] Pregnant subjects of the methods described herein can belong to one or more classes including primiparous (no previous child brought to delivery, interchangeably referred to herein as nulliparous or parity=0) or multiparous (at least one previous child brought to at least 20 weeks of gestation, referred to interchangeably herein as parity >0, parity ≥ 1), primigravida (first pregnancy) or multigravida (more than one pregnancy).

[0031] In some embodiments, the pregnant human subject is asymptomatic. In some embodiments, the subject may have a risk factor of placenta accreta. The most common risk factor is a previous cesarean delivery, with the incidence of placenta accreta spectrum increasing with the number of prior cesarean deliveries. In a systematic review, the rate of placenta accreta spectrum increased from 0.3% in women with one previous cesarean delivery to 6.74% for women with five or more cesarean deliveries. Additional risk factors include advanced maternal age, multiparity, prior uterine surgeries or curettage, and Asherman syndrome. Placenta previa is another significant risk factor.

III. Sample Preparation

[0032] A sample for use in the methods of the present disclosure is a biological sample obtained from a pregnant subject. In certain embodiments, the sample is collected during a stage

of pregnancy described in the preceding section. In some embodiments, the sample is a blood, saliva, tears, sweat, nasal secretions, urine, amniotic fluid or cervicovaginal fluid sample. In some embodiments, the sample is a blood sample. In some embodiments, the sample is a blood plasma sample. In some embodiments, the sample is a blood serum sample. In some embodiments, the sample is a blood product in a different matrix (e.g. Citrate buffer, or Streck tube). In some embodiments, the sample has been stored frozen (e.g., -20°C or -80°C).

[0033] The term “microparticle” refers to an extracellular microvesicle or lipid raft protein aggregate having a hydrodynamic diameter of about 50 to about 5000 nm. As such, the term microparticle encompasses exosomes (about 50 to about 100 nm), microvesicles (about 100 to about 300 nm), ectosomes (about 50 to about 1000 nm), apoptotic bodies (about 50 to about 5000 nm) and lipid-protein aggregates of the same dimensions.

[0034] The term “microparticle-associated protein” refers to a protein or fragment thereof that is detectable in a microparticle-enriched sample from a mammalian (e.g., human) subject. As such the term “microparticle-associated protein” is not restricted to proteins or fragments thereof that are physically associated with microparticles at the time of detection. The term “microparticle-associated peptide” refers to a protein fragment that is detectable in such a sample.

[0035] The term “polypeptide” as used herein refers to a polymer of amino acids. This includes oligopeptides (which typically have fewer than 10 amino acids), peptides (which typically have between about 10 and about 50 amino acids), and proteins (which include polypeptides assuming secondary, tertiary, or quaternary structures). Depending on context, the term “protein” may refer to a polypeptide lacking secondary structure.

[0036] Biomarkers for placenta accreta can be derived from microparticles. Microparticles can be isolated from blood (e.g., serum or plasma) or other biological samples, by size exclusion chromatography. The mobile phase/elution buffer can be, for example, a buffered solution such as PBS, or a non-buffered solution. Water, as a mobile phase, refers to non-buffered water, e.g., distilled, deionized, or distilled de-ionized water (“ddH₂O”). The high molecular weight fraction can be collected to obtain a microparticle-enriched sample. Proteins within the microparticle-enriched sample are then extracted before digestion with a proteolytic enzyme such as trypsin to

obtain a digested sample comprising a plurality of peptides. The digested sample is then subjected to a peptide purification, concentration, and/or a fractionation step before analysis to obtain a proteomic profile of the sample, e.g., by liquid chromatography and mass spectrometry. In some embodiments, the purification/concentration step comprises reverse phase chromatography (e.g., ZipTip[®] pipette tip with 0.2 μ L C18 resin, from Millipore Corporation, Billerica, MA) or ultrafiltration. In some embodiments, the fractionation step may involve the fractionation into 96 fractions with a high pH reverse phase offline HPLC fractionator be with a Mobile phase A is DI H₂O with 20 mM Formic Acetate, pH 9.3; mobile phase B is Acetonitrile (Optima[™], LC/MS grade, Fisher Chemical[™]) with 20mM Formic Acetate, pH 9.3.

[0037] In some embodiments, for example, detection of proteins by mass spectrometry, a method of sample preparation can include fragmenting proteins in a sample. Fragmentation can be accomplished using proteases, such as trypsin. Tryptic fragments can usefully serve as surrogate biomarkers because their unique mass can be associated with the parent protein.

[0038] In certain embodiments, the microparticles are placental-derived exosomes or endothelial-derived exosomes. Such exosomes can be isolated using capture agents, such as antibodies, against surface markers for these cells of origin. For example, placental-derived exosomes can be isolated using antibodies directed to PLAP (placental alkaline phosphatase), Klotho, CD34, CD44 or leukemia inhibitory factor (LIF). Endothelial-derived exosomes can be isolated using antibodies directed to ICAM or VCAM.

IV. Methods of Detection

[0039] Biomarkers can be detected and quantified by any method known in the art. This includes, without limitation, immunoassay, chromatography, mass spectrometry, electrophoresis and surface plasmon resonance.

[0040] Detection of a biomarker includes detection of an intact protein, or detection of surrogate for the protein, such as a fragment. Exemplary fragments are provided in FIG. 10A to FIG. 15B.

[0041] Immunoassay methods include, for example, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), sandwich assays and Western blot, immunoprecipitation, immunohistochemistry, immunofluorescence, antibody microarray, dot blotting, and FACS.

[0042] Chromatographic methods include, for example, affinity chromatography, ion exchange chromatography, size exclusion chromatography/gel filtration chromatography, hydrophobic interaction chromatography and reverse phase chromatography, including, e.g., HPLC.

1. Mass Spectrometry

[0043] In some embodiments, detecting the level (*e.g.*, including detecting the presence) of a microparticle-associated protein is accomplished using a liquid chromatography/mass spectrometry (LC/MS)-based proteomic analysis. In an exemplary embodiment, the method involves subjecting a sample to size exclusion chromatography and collecting the high molecular weight fraction (*e.g.*, by size-exclusion chromatography) to obtain a microparticle-enriched sample. In some embodiments, the size exclusion chromatography includes a size-exclusion column comprising an agarose solid phase and an aqueous liquid phase. The microparticle-enriched sample is then disrupted (using, for example, chaotropic agents, denaturing agents, reducing agents and/or alkylating agents) and the released contents subjected to proteolysis. The disrupted microsome preparation, containing a plurality of peptides, is then processed using the tandem column system described herein prior to peptide analysis by mass spectrometry, to provide a proteomic profile of the sample. The methods disclosed herein avoid the necessity of protein concentration/purification, buffer exchange and liquid chromatography steps associated with previous methods.

[0044] Proteins in a sample can be detected by mass spectrometry. Mass spectrometers typically include an ion source to ionize analytes, and one or more mass analyzers to determine mass. Mass analyzers can be used together in tandem mass spectrometers. Ionization methods include, among others, electrospray or laser desorption methods. Mass analyzers include quadrupoles, ion traps, time-of-flight instruments and magnetic or electric sector instruments. In certain embodiments, the mass spectrometer is a tandem mass spectrometer (*e.g.*, “MS-MS”) that uses a first mass analyzer to select ions of a certain mass and a second mass analyzer to

analyze the selected ions. One example of a tandem mass spectrometer is a triple quadrupole instrument, the first and third quadrupoles act as mass filters, and an intermediate quadrupole functions as a collision cell. Mass spectrometry also can be coupled with up-stream separation techniques, such as liquid chromatography or gas chromatography. So, for example, liquid chromatography coupled with tandem mass spectrometry can be referred to as “LC-MS-MS”.

[0045] Mass spectrometers useful for the analyses described herein include, without limitation, Altis™ quadrupole, Quantis™ quadrupole, Quantiva™ or Fortis™ triple quadrupole from ThermoFisher Scientific, the 8050 or 8060 triple quadrupoles from Shimadzu, the Xevo TQ-XS™ triple quadrupole from Waters, QSight™ Triple Quad LC/MS/MS from Perkin Elmer, Thermo Orbitrap Mass Spectrometer Tribrid Eclipse with a Thermo Fisher Scientific Nanospray Flex™ Ion Source, and others.

[0046] Generally, any mass spectrometric (MS) technique that can provide precise information on the mass of peptides, and preferably also on fragmentation and/or (partial) amino acid sequence of selected peptides (*e.g.*, in tandem mass spectrometry, MS/MS; or in post source decay, TOF MS), can be used in the methods and compositions disclosed herein. In some embodiments, any MS technique can provide process information on the mass of peptides wherein the mass comprises more than 100, more than 1000, more than 10,000, more than 100,000 peptides from a biological sample. Suitable peptide MS and MS/MS techniques and systems are known in the art (see, *e.g.*, *Methods in Molecular Biology*, vol. 146: “Mass Spectrometry of Proteins and Peptides”, by Chapman, ed., Humana Press 2000; Kassel & Biemann (1990) *Anal. Chem.* 62:1691-1695; *Methods Enzymol* 193: 455-79; or *Methods in Enzymology*, vol. 402: “Biological Mass Spectrometry”, by Burlingame, ed., Academic Press 2005) and can be used in practicing the methods disclosed herein. Accordingly, in some embodiments, the disclosed methods comprise performing quantitative MS to measure one or more peptides. Such quantitative methods can be performed in an automated (Villanueva, et al., *Nature Protocols* (2006) 1(2):880-891) or semi-automated format. In particular embodiments, MS can be operably linked to a liquid chromatography device (LC-MS/MS or LC-MS) or gas chromatography device (GC-MS or GC-MS/MS).

[0047] Selected reaction monitoring is a mass spectrometry method in which a first mass analyzer selects a protein of interest (precursor), a collision cell fragments the protein into

product fragments and one or more of the fragments is detected in a second mass analyzer. The precursor and product ion pair is called an SRM "transition". The method is typically performed in a triple quadrupole instrument. When multiple fragments of a protein are analyzed, the method is referred to as Multiple Reaction Monitoring Mass Spectrometry ("MRM-MS").

[0048] Typically, protein samples are digested with a proteolytic enzyme, such as trypsin, to produce peptide fragments. Heavy isotope labeled analogues of certain of these peptides are synthesized as standards. These standards are referred to as Stable Isotopic Standards or "SIS". SIS peptides are mixed with a protease-treated sample. The mixture is subjected to triple quadrupole mass spectrometry. Peptides corresponding to the daughter ions of the SIS standards and the target peptides are detected with high accuracy, in either the time domain or the mass domain. Usually, a plurality of the daughter ions is used to unambiguously identify the presence of a parent ion, and one of the daughter ions, usually the most abundant, is used for quantification. SIS peptides can be synthesized to order or can be available as commercial kits from vendors such as, for example, e.g., Thermo Fisher Scientific (Waltham, MA) or Biognosys AG (Zurich, Switzerland).

[0049] As used herein, the terms "multiple reaction monitoring (MRM)" or "selected reaction monitoring (SRM)" refer to a MS-based quantification method that is particularly useful for quantifying analytes that are in low abundance. In an SRM experiment, a predefined precursor ion and one or more of its fragments are selected by the two mass filters of a triple quadrupole instrument and monitored over time for precise quantification. Multiple SRM precursor and fragment ion pairs can be measured within the same experiment on the chromatographic time scale by rapidly toggling between the different precursor/fragment pairs to perform an MRM experiment. A series of transitions (precursor/fragment ion pairs) in combination with the retention time of the targeted analyte (*e.g.*, peptide or small molecule such as chemical entity, steroid, hormone) can constitute a definitive assay. A large number of analytes can be quantified during a single LC-MS experiment. The term "scheduled," or "dynamic" in reference to MRM or SRM, refers to a variation of the assay wherein the transitions for a particular analyte are only acquired in a time window around the expected retention time, significantly increasing the number of analytes that can be detected and quantified in a single LC-MS experiment and contributing to the selectivity of the test, as retention time is a property dependent on the

physical nature of the analyte. A single analyte can also be monitored with more than one transition. Finally, the assay can include standards that correspond to the analytes of interest (*e.g.*, peptides having the same amino acid sequence as that of analyte peptides), but differ by the inclusion of stable isotopes. Stable isotopic standards (SIS) can be incorporated into the assay at precise levels and used to quantify the corresponding unknown analyte. Additional levels of specificity are contributed by the co-elution of the unknown analyte and its corresponding SIS, and by the properties of their transitions (*e.g.*, the similarity in the ratio of the level of two transitions of the analyte and the ratio of the two transitions of its corresponding SIS).

[0050] Accordingly, detection of a protein target by MRM-MS involves detection of one or more peptide fragments of the protein, typically through detection of a stable isotope standard peptide against which the peptide fragment is compared. Typically, an SIS will, itself, be fragmented in a collision cell as the original digested fragment, and one or more of these fragments is detected by the mass spectrometer.

[0051] Mass spectrometry assays, instruments and systems suitable for biomarker peptide analysis can include, without limitation, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS; MALDI-TOF post-source-decay (PSD); MALDI-TOF/TOF; surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) MS; electrospray ionization mass spectrometry (ESI-MS); ESI-MS/MS; ESI-MS/(MS)_n (*n* is an integer greater than zero); ESI 3D or linear (2D) ion trap MS; ESI triple quadrupole MS; ESI quadrupole orthogonal TOF (Q-TOF); ESI Fourier transform MS systems; desorption/ionization on silicon (DIOS); secondary ion mass spectrometry (SIMS); atmospheric pressure chemical ionization mass spectrometry (APCI-MS); APCI-MS/MS; APCI-(MS)_n; ion mobility spectrometry (IMS); inductively coupled plasma mass spectrometry (ICP-MS) atmospheric pressure photoionization mass spectrometry (APPI-MS); APPI-MS/MS; and APPI-(MS)_n. Peptide ion fragmentation in tandem MS (MS/MS) arrangements can be achieved using techniques known in the art, such as, *e.g.*, collision induced dissociation (CID). As described herein, detection and quantification of biomarkers by mass spectrometry can involve multiple reaction monitoring (MRM), such as described, *inter alia*, by Kuhn *et al.* (2004) *Proteomics* **4**:1175-1186. Scheduled multiple-reaction-monitoring (Scheduled MRM) mode acquisition

during LC-MS/MS analysis enhances the sensitivity and accuracy of peptide quantitation. Anderson and Hunter (2006) *Mol. Cell. Proteomics* **5(4)**:573-588. Mass spectrometry-based assays can be advantageously combined with upstream peptide or protein separation or fractionation methods, such as, for example, with the tandem column system described herein.

[0052] In another embodiment, useful in biomarker discovery, fractionated samples are analyzed by nano flow HPLC (e.g., Ultimate 3000, Thermo Fisher Scientific) followed by Thermo Orbitrap Mass Spectrometer (Tribid Eclipse). The ion source can be a Nanospray Flex™ Ion Source (Thermo Fisher Scientific) equipped with Column Oven (PRSO-V2, Sonation) to heat up the nano column (PicoFrit, 100 µm x 250 mm x 15 µm tip, New Objective) for peptide separation. Peptides can be engaged on a trap column and then were delivered to the separation nano column by the mobile phase.

V. Biomarkers

[0053] As used herein, the term “biomarker” refers to a biological molecule, the presence, form or amount of which exhibits a statistically significant difference between two states. Accordingly, biomarkers are useful, alone or in combination, for classifying a subject into one of a plurality of groups. Biomarkers may be naturally occurring or non-naturally occurring. For example, a biomarker may be a naturally occurring protein or a non-naturally occurring fragment of a protein. Fragments of a protein can function as a proxy or surrogate peptide for the protein or as stand-alone biomarkers.

[0054] Provided herein are compositions of matter comprising one or a plurality of placenta accreta biomarkers in substantially pure form. The biomarkers can be mixed in a container, or can be physically separated, for example, through attachment to solid supports at different addressable locations. As used herein, a chemical entity, such as a polynucleotide or polypeptide, is “substantially pure” if it is the predominant chemical entity of its kind in a composition. This includes the chemical entity representing more than 50%, more than 80%, more than 90% or more than 95% or of the chemical entities of its kind in the composition. A chemical entity is “essentially pure” if it represents more than 98%, more than 99%, more than 99.5%, more than 99.9%, or more than 99.99% of the chemical entities of its kind in the composition. Chemical entities which are essentially pure are also substantially pure.

1. Protein Biomarkers

[0055] Provided herein are one or more protein biomarkers associated with increased risk of placenta accreta. Exemplary Biomarkers for inferring placenta accreta in the second trimester are presented in Table 1 (24 weeks +/- 2 weeks), Table 3 (24 weeks +/- 2 weeks), and Table 5 (24 weeks +/- 2 weeks). Biomarkers for inferring placenta accreta in the third trimester are presented in Table 2 (35 weeks +/- 2 weeks), Table 4 (35 weeks +/- 2 weeks), and Table 6 (35 weeks +/- 2 weeks). Although the data collected for the biomarkers of these tables are from pregnant subject at 24 weeks +/- 2 weeks gestation or 35 weeks +/- 2 weeks gestation, the biomarkers may be relevant for assessment at gestational ages that fall outside this range. As discussed below, Table 7 and Table 8 present panels of biomarker for inferring placenta accreta at around 24 and around 35 weeks, respectively. In some embodiments, the one or more protein biomarkers associated with increased risk of placenta accreta include a plurality of protein biomarkers.

[0056] The tables in the drawings provide the UniProt entry number and entry name, protein names, gene names, organism (all homo sapiens), primary gene names and gene name synonyms. The contents of Tables 1, 2, 3, 4, 5, 6, 7, and 8 of FIGS. 1A-1D, FIGS. 2A-2C, FIGS. 3A-3K, FIGS. 4A-4J, FIGS. 5A-5C, FIGS. 6A-6F, and FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, FIGS. 15A-15B including their protein name, gene name (primary and synonym), and/or peptide sequence are hereby incorporated in their entirety into the detailed description as if they were provided herein. For the purpose of inclusion into the detailed description. Table 9 provides exemplary biomarkers of the disclosure, useful for the prediction of plasma accreta (protein name and synonyms provided).

Table 9: Exemplary biomarkers

Isthmin-2
Sulfhydryl oxidase 1
Heat shock cognate 71 kDA protein
Histone H4
Complement component C6
B cell receptor heavy chain variable region
MS-CI heavy chain variable region
Hemoglobin subunit gamma-2
Fibrinogen-like protein 1

Cartilage acidic protein 1
ITIH4 protein, Inter-alpha-trypsin inhibitor heavy chain H4
Hemoglobin subunit gamma-1 (Gamma-1-globin) (Hb F Agamma) (Hemoglobin gamma-1 chain) (Hemoglobin gamma-A chain)
Uncharacterized protein DKFZp686A0568
Ceruloplasmin, Ferroxidase
Pregnancy-specific beta-1-glycoprotein 4, PS-beta-G-4, PSBG-4, Pregnancy-specific glycoprotein 4 (Pregnancy-specific beta-1 glycoprotein 9, PS-beta-G-9, PSBG-9, Pregnancy specific glycoprotein 9)
C3/CS convertase, Complement factor B, Complement factor B Ba fragment, Complement factor B Bb fragment
Ig heavy chain variable region
Immunoglobulin heavy variable 3-20
Myosin-reactive immunoglobulin kappa chain variable region
Cofilin-1 (18 kDa phosphoprotein, p18) (Cofilin, non-muscle isoform)
Ubiquitin carboxylterminal hydrolase 1, EC 3.4.19.12 (Deubiquitinating enzyme 1, hUBP) Ubiquitin thioesterase 1) (Ubiquitin-specific processing protease 1)
Isthmin- 2 (Thrombospondin and AMOP domain-containing isthmin-like protein 1) (Thrombospondin type-1 domain-containing protein 3)
Tenascin C (Hexabrachion), isoform CRA a
Ig-like domain-containing protein
Immunoglobulin lambda variable 8-61
Immunoglobulin lambda variable variable 10-54
Immunoglobulin lambda variable 7-46
Immunoglobulin lambda variable 2-18
Immunoglobulin lambda variable 3-9
Immunoglobulin heavy variable variable 3-64
Immunoglobulin heavy variable 4-4
Immunoglobulin kappa variable 2D-29
Immunoglobulin kappa variable 1D-8
Immunoglobulin kappa variable 3D-11
Immunoglobulin heavy variable 1-45
Immunoglobulin kappa variable 6D-21
Immunoglobulin heavy variable 6-1
Immunoglobulin heavy variable 2-26
Immunoglobulin heavy variable 3-73
Immunoglobulin heavy variable 3-74
Isthmin-2 (Thrombospondin and AMOP domain-containing isthmin-like protein 1) (Thrombospondin type-1 domain-containing protein 3)
Zinc finger protein 655 (Vav-interacting Krueppel-like protein)
Sulfhydryl oxidase 1 hQSOX, EC 1.8.3.2 (Quiescin Q6)
Lymphatic vessel endothelial hyaluronic acid receptor 1, LYVE-1 (Cell surface retention sequence-binding protein 1, CRSBP-1) (Extracellular link domain-containing protein 1) (Hyaluronic acid receptor)

Heat shock cognate 71 kDa protein, EC 3.6.4.10 (Heat shock 70 kDa protein 8) (Lipopolysaccharide-associated protein 1, LAP-1, LPS-associated protein 1)
Bone marrow proteoglycan, BMPG (Proteoglycan 2) [Cleaved into: Eosinophil granule major basic protein, EMBP, MBP (Pregnancy associated major basic protein)]
Ceruloplasmin, Ferroxidase
Amiloride-sensitive amine oxidase [copper containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amiloride-binding protein 1) (Amine oxidase copper domain containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)
Histone H4
C3/CS convertase, Complement factor B, Complement factor B Ba fragment, Complement factor B Bb fragment
Lumican (Keratan sulfate proteoglycan lumican, KSPG lumican)
Cad herin-5 (7B4 antigen) (Vascular endothelial cadherin, VE-cadherin) {CD antigen CD144}
B cell receptor heavy chain variable region
Uncharacterized protein DKFZp686A0568
Structural maintenance of chromosomes protein 4 SMC protein 4, SMC 4 (Chromosome-associated polypeptide C, hCAP-C) (XCAP-C homolog)
Alpha-2-macroglobulin, Alpha-2-M (C3 and PZP like alpha-2-macroglobulin domain-containing protein 5)
Fructose-bisphosphate aldolase A, EC 4.1.2.13 (Lung cancer antigen NY-LU-1) (Muscle-type aldolase)
Afamin, Alpha-albumin Alpha-Alb
Serum paraoxonase/arylesterase 1, PON 1, EC 3.1.1.2, EC 3.1.1.81, EC 3.1.8.1 (Aromatic esterase 1, A-esterase 1) (K-45) (Serum aryldialkylphosphatase 1)
Elongation factor 1-alpha 1, EF-1-alpha-1 (Elongation factor Tu, EF-Tu) (Eukaryotic elongation factor 1 A-1, eEF1A-1) (Leukocyte receptor cluster member 7)
Plasma serine protease inhibitor, Acrosomal serine protease inhibitor Plasminogen activator inhibitor 3 Short name: PAI-3 Short name: PAI3 Protein C inhibitor Short name: PCI Serpin AS
Complement component C6
Complement receptor type 2, Cr2 (Complement C3d receptor) (Epstein-Barr virus receptor, EBV receptor) (CD antigen CD21)
Alpha-mannosidase 2, EC 3.2.1.114 (Golgi alphanmannosidase II, AMan II, Man II) (Mannosidase alpha class 2A member 1) (Mannosyl-o ligosaccharide 1,3-1,6-alphanmannosidase)
Angiotensin 1-10
Hemoglobin subunit gamma-2 (Gamma-2-globin) (Hb F Ggamma) (Hemoglobin gamma-2 chain) (Hemoglobin gamma-G chain)
Alpha-1B glycoprotein (Alpha-1-B glycoprotein)
cDNA FU51742, highly similar to Inter-alpha-trypsin inhibitor heavy chain H4
Uncharacterized protein
Phosphoglycerate kinase 1, EC 2.7.2.3 (Cell migration-inducing gene 10 protein) (Primer recognition protein 2, PRP 2)
Myosin-reactive immunoglobulin heavy chain variable region
C3/CS convertase

Endothelial protein C receptor, Activated protein C receptor, APC receptor Endothelial cell protein C receptor, CD antigen: CD201
MS-CI heavy chain variable region
Inter-alpha (Globulin) inhibitor H4 (Plasma Kallikrein-sensitive glycoprotein)
Tenascin-X, Hexabrachion like protein
Neogenin (Immunoglobulin superfamily DCC subclass member 2)
Ge-globulin, Group-Specific Component Vitamin D-binding protein
Basement membrane-specific heparan sulfate proteoglycan core protein Perlecan
Serpin All
cDNA FLJ26301 fis, clone DMC07540
60 kDa heat shock protein, mitochondrial, EC 5.6.1.7 (60 kDa chaperonin) (Chaperonin 60, CPN60) (Heatshock protein 60, HSP-60, Hsp60 (HuCHA60) (Mitochondrial matrix protein P1) (P60 lymphocyte protein)
Uncharacterized protein DKFZp686115196
Ig heavy chain variable region
EGF-containing fibulin-like extracellular matrix protein 1 (Extracellular protein S1-5) (Fibrillin-like protein) (Fibulin-3, FIBL-3)
Putative fatty acid-binding protein 5-like protein 3 (Fatty acid-binding protein 5 pseudogene 3)
Histone H2B type 1-K, H2B K (HIRA-interacting protein 1)
Insulin-like growth factor-binding protein 3, IBP-3, IGF-binding protein 3, IGFBP-3
Fibulin-1, FIBL-1
ICOS ligand, 87 homolog 2, B7-H2, 87-like protein GIS0, 87-related protein 1, B7RP-1 CD antigen: CD275
Neogenin (Immunoglobulin superfamily DCC subclass member 2)
IgG L chain
Ig-like domain-containing protein
Sulfhydryl oxidase 1, hQSOX EC 1.8.3.2 (Quiescin Q6)
Isthmin-2 (Thrombospondin and AMOP domain-containing isthmin-like protein 1) (Thrombospondin type-I domain-containing protein 3)
Cryocrystalglobulin CC 1 kappa light chain variable region
Heparin cofactor 2 (Heparin cofactor II, HC-11) (Protease inhibitor leuserpin-2, HLS2) (Serpin D1)
Probable non-functional immunoglobulin kappa variable 1-37
Tenascin C (Hexabrachion), isoform CRA a isoform CRA a
cDNA, FLJ92374, highly similar to Homo sapiens C-type lectin domain family 3, member B (CLEC3B), mRNA
Ubiquitin carboxy I-terminal hydrolase 1, EC 3.4.19.12 (Deubiquitinating enzyme 1, hUBP) (Ubiquitin thioesterase 1) (Ubiquitin-specific processing protease 1)
Zinc finger protein 655 (Vav-interacting Krueppel-like protein)
Myosin-reactive immunoglobulin heavy chain variable region
Albumin, Albumin, isoform CRA k

Prolow-density lipoprotein receptor-related protein 1, LRP-1 (Alpha-2-macroglobulin receptor, A2MR) (Apolipoprotein E receptor, APOER) (CD antigen CD91) [Cleaved into: Low-density lipoprotein receptor-related protein 1 85 kDa subunit, LRP-85; Low-density lipoprotein receptor-related protein 1 515kDa subunit, LRP-515; Low density lipoprotein receptor related protein 1 intracellular domain, LRPICD]
Ig heavy chain variable region
Ficolin-2 (37 kDa elastin-binding protein) (Collagen/fibrinogen domain containing protein 2) (EBP-37) (Ficolin-B) (Ficolin-beta) (Hucolin) (L-ficolin) (Serum lectin p35)
Hemoglobin subunit theta-1 (Hemoglobin theta-I chain) (Theta-1-globin)
Apolipoprotein A-II, Apo-AII, ApoA-11 (Apolipoprotein A2) [Cleaved into: Proapolipoprotein A-II, ProapoA-11; Truncated apolipoprotein A-II (Apolipoprotein A-11(1-76))]
Plexin domain-containing protein 2 (Tumor endothelial marker 7-related protein)
V 4-4 protein
Ig heavy chain variable region
Alpha-2-HS-glycoprotein, Alpha-2-Z-globulin Ba-alpha-2-glycoprotein Fetuin-A
Immunoglobulin epsilon heavy chain, Immunoglobulin epsilon heavy chain ND
Amiloride-sensitive amine oxidase [copper-containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amiloride-binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)
Out at first protein homolog, HCV NS5A-transactivated protein 13 target protein 2
Keratin, type I cytoskeletal 17 (39.1) (Cytokeratin-17, CK-17) (Keratin-I7, K17)
Immunoglobulin lambda variable 10-54
MS-C2 light chain variable region, MS-C4 light chain variable region
Heat shock 70 kDa protein 1A (Heat shock 70 kDa protein 1, HSP70-1, HSP70.1)
Basement membrane-specific heparan sulfate proteoglycan core protein Perlecan
Complement subcomponent C1r
Extracellular superoxide dismutase [Cu-Zn]
MS-C1 heavy chain variable region
Alpha globin chain
Alpha-2-macroglobulin, Alpha-2-M (C3 and PZP-like alpha-2-macroglobulin domain containing protein 5)
Lactotransferrin, Lactoferrin, Growth-inhibiting protein 12, Talalactoferrin
Apolipoprotein(a), Apo(a), Lp(a)
Angiotensinogen, Serpin A8
Myosin light polypeptide 6 (17 kDa myosin light chain, LCI 7) (Myosin light chain 3, MLC-3) (Myosin light chain alkali 3, Myosin light chain A3) (Smooth muscle and nonmuscle myosin light chain alkali 6)
E3 SUMO-protein ligase ZBED1, EC 2.3.2.- (DNA replication-related element-binding factor) (Putative Ac-like transposable element) (Zinc finger BED domain-containing protein 1) (dREF homo log)
Immunoglobulin kappa variable 2D-29
Myosin-reactive immunoglobulin light chain variable region

Endoplasmic reticulum chaperone BiP, Heat shock protein 70 family protein 5, HSP70 family protein 5. 78 kDa glucose-regulated protein, GRP-78, Bindingimmunoglobulin protein, BiP
Ceruloplasmin
Pantetheinase, EC 3. 5 .1. 92 (Pantetheine hydrolase) (Tiff66) (Vascular non-inflammatory molecule 1, Vanin-1)
Immunoglobulin kappa variable 5-2 (Ig kappa chain V region EV15)
Roundabout homolog 4 (Magic roundabout)
Lymphatic vessel endothelial hyaluronic acid receptor 1, LYVE-1 (Cell surface retention sequence-binding protein 1, CRSBP-1) (Extracellular link domain-containing protein 1) (Hyaluronic acid receptor)
L-selectin (CD62 antigen-like family member L) (Leukocyte adhesion molecule 1, LAM-1) (Leukocyte surface antigen Leu-8) (Leukocyte-endothelial cell adhesion molecule 1, LECAM 1) (Lymph node homing receptor) (TQ 1) (gp90- MEL) (CD antigen CD62L)
Actin-related protein 2/3 complex subunit 5 (Arp2/3 complex 16 kDa subunit, p16-ARC)
Annexin A2 (Annexin II) (Annexin-2((Calpactin I heavy chain) (Calpactin-1 hevay chain) (Chromobindin-8) (Lipocortin II) (Placental anticoagulant protein IV, PAP-IV) (Protein I) (p36)
Myosin-6 (Myosin heavy chain 6) (Myosin heavy chain, cardiac muscle alpha isoform, MyHC-alpha)
Vinculin (Metavinculin, MV)
Complement receptor type 2, Cr2 (Complement C3d receptor) (Epstein-Barr virus receptor, EBV receptor) (CD antigen CD21)
Zinc finger protein 655 (Vav-interacting Krueppel-like protein)
Ceruloplasmin
Complement receptor type 2, Cr2 (Complement C3d receptor) (Epstein-Barr virus receptor, EBV receptor) (CD antigen CD21)
Actin-related protein 2/3 complex subunit 5 (Arp2/3 complex 16 kDa subunit, p16-ARC)
Vinculin (Metavinculin, MV)
Ceruloplasmin
Annexin A2 (Annexin II) (Annexin-2((Calpactin I heavy chain) (Calpactin-1 hevay chain) (Chromobindin-8) (Lipocortin II) (Placental anticoagulant protein IV, PAP-IV) (Protein I) (p36)
Immunoglobulin lambda variable 10-54
Myeloperoxidase
Amiloride-sensitive amine oxidase [copper-containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amiloride-binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)
Insulin-like growth factor-binding protein 5, IBP-5, IGF-binding protein 5, IGFBP-5
Myosin regulatory light polypeptide 9 (20 kDa myosin light chain, LC20) (MLC-2C) (Myosin RLC) (Myosin regulatory light chain 2, smooth muscle isoform) (Myosin regularoty light chain 9) (myosin regulatory light chain MRLC1)
Tubulin beta-1 chain
Beta-parvin, Affixin

Bridging integrator 2 (Breast cancer-associated protein 1)
MHC class I antigen
Epididymis secretory sperm binding protein
V4-3 protein
Complement component 8 subunit beta, complement component c8 beta chain
V5-4 protein
Tubulin beta-1 chain
Immunoglobulin lambda variable 10-54
Epididymis secretory sperm binding protein
Myeloperoxidase
Insulin-like growth factor binding protein 5, IBP-5, IGF-binding protein 5, IGFBP-5
Amiloride-sensitive amine oxidase [copper-containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amiloride-binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)
Complement component 8 subunit beta, complement component c8 beta chain
MHC class I antigen
Beta-parvin, Affixin
V5-4 protein
V4-3 protein
Myosin regulatory light polypeptide 9 (20 kDA myosin light chain, LC20) (MLC-2C) (Myosin RLC) (Myosin regulatory light chain 2, smooth muscle isoform) (Myosin regulatory light chain 9) (myosin regulatory light chain MRLC1)
Bridging integrator 2 (Breast cancer-associated protein 1)

[0057] Also provided for each biomarker is one or more peptide fragments from the protein that function as surrogate markers. A surrogate marker can be used as a measure of the protein for purposes of the models described herein. Accordingly, in some embodiments, the detection of one or more peptide fragments of a protein biomarker serves to detect the protein biomarker. Peptides useful as surrogates for biomarkers are presented in FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

[0058] The biomarkers can be detected using *de novo* sequencing of proteins from microparticles isolated from a sample (e.g., blood) taken from a pregnant woman. Proteins can be sequenced by mass spectrometry, e.g., single or double (MS/MS) mass spectrometry. Both parent proteins (such as those provided in Tables 1-6, and Table 9; or the panels of Tables 7 and 8) and peptide fragments of the parent proteins (such as those described above in FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B) are useful as biomarkers of placenta accreta. Accordingly, in some embodiments, detection of a

named protein biomarker encompasses detection by a surrogate, e.g., one or more fragments of the protein.

[0059] Proteins, e.g., peptides, detected by mass spectrometry are analyzed to identify those that are up-regulated (increased in amounts) or down-regulated (decreased in amounts) compared with controls. Proteins showing statistically significant differential expression are further analyzed to identify the parent protein. Such proteins can be identified in a protein database such as SwissProt.

[0060] In certain embodiments, biomarkers are in a composition in which the peptide biomarker is paired with a stable isotopic standard of the peptide. In some embodiments, the composition may include one pair of a peptide biomarker and stable isotopic standard of the peptide or a plurality of pairs with each pair comprising a peptide biomarker and a stable isotopic standard of the peptide. The peptide biomarker may include a surrogate biomarker as is described in more detail herein. Such compositions are useful for detection in multiple reaction monitoring mass spectrometry.

[0061] For purposes of mass spectrometry, proteins can be detected intact, or through fragmentation, e.g., LCMS or in multiple reaction monitoring (MRM). In such cases, proteins can be fragmented proteolytically before analysis. Proteolytic fragmentation includes both chemical and enzymatic fragmentation. Chemical fragmentation includes, for example, treatment with cyanogen bromide. Enzymatic fragmentation includes, for example, digestion with proteases such as trypsin, chymotrypsin, LysC, ArgC, GluC, LysN and AspN. Detection of these protein fragments, or fragmented forms of them produced in mass spectrometry, can function as surrogates for the full protein.

2. Biomarker Panels

[0062] In certain embodiments, biomarkers are analyzed as a panel. A panel is a plurality of biomarkers used in an algorithm to make a prediction or inference. As used herein, a panel comprising a group of identified biomarkers includes at least those identified biomarkers. A panel consisting of a group of identified biomarkers includes only the identified biomarkers. A panel consisting essentially of a group of identified biomarkers includes the identified biomarkers and no more than one or two other biomarkers. For example, a panel consisting

essentially of four identified biomarkers can include up to six total biomarkers. A panel can exist as a conceptual grouping, as a composition of matter (e.g., comprising purified biomarkers, or as an article, such as solid support attached to a capture reagent such as an antibody, further bound to the biomarker. The solid support can be, for example, one or more solid particles, such as beads, or a chip in which biomarkers are attached in an array format.

[0063] Exemplary panels of biomarkers for assessing placenta accreta at around 24 weeks are presented in Table 7.

[0064] Exemplary panels of biomarkers for assessing placenta accreta at around 34 weeks are presented in Table 8.

VI. Data Analysis

[0065] As used herein, the term “analysis” refers to any algorithm that transforms inputs into outputs. Analyses include, without limitation, statistical analyses, machine learning analyses and neural net analyses. The term “data” may include data received from various data sources, metadata associated with the data, and/or a combination of both data and metadata.

A. Measurements

[0066] A measurement of a variable, such as sequencing reads mapping to a position, can be any combination of numbers and words. A measure can be any scale, including nominal (e.g., name or category), ordinal (e.g., hierarchical order of categories), interval (distance between members of an order), ratio (interval compared to a meaningful “0”), or a cardinal number measurement that counts the number of things in a set. Measurements of a variable on a nominal scale indicate a name or category, e.g., category into which the sequencing read is classified. Measurements of a variable on an ordinal scale produce a ranking, such as “first”, “second”, “third”. Measurements on a ratio scale include, for example, any measure on a pre-defined scale, absolute number of reads, normalized or estimated numbers, as well as statistical measurements such as frequency, mean, median, standard deviation, or quantile. Measurements that involve quantification are typically determined at the ratio scale level.

B. Analysis

[0067] In some embodiments, analysis statistical analysis of a sufficiently large number of samples to provide statistically meaningful results. Any statistical method known in the art can be used for this purpose. Exemplary methods, or tools, include, without limitation, correlational, Pearson correlation, Spearman correlation, chi-square, comparison of means (e.g., paired T-test, independent T-test, ANOVA) regression analysis (e.g., simple regression, multiple regression, linear regression, non-linear regression, logistic regression, polynomial regression, stepwise regression, ridge regression, lasso regression, elastic net regression) or non-parametric analysis (e.g., Wilcoxon rank-sum test, Wilcoxon sign-rank test, sign test). Such tools are included in commercially available statistical packages such as MATLAB, JMP Statistical Software and SAS. Such methods produce models or classifiers which one can use to classify a particular biomarker profile into a particular state.

[0068] In statistics and machine learning, lasso (least absolute shrinkage and selection operator; also Lasso or LASSO) is a regression analysis method that performs both variable selection and regularization in order to enhance the prediction accuracy and interpretability of the resulting statistical model.

[0069] Statistical analysis can be operator implemented or implemented by machine learning.

[0070] Certain classifiers, such as cut-offs, can be executed by human inspection. Other classifiers, such as multivariate classifiers, can require a computer to execute the classification algorithm.

1. Machine Learning

[0071] In some variations, analysis may involve implementing machine learning techniques including linear and non-linear models, e.g., processes such as CART - classification and regression trees), artificial neural networks such as back propagation networks, discriminant analyses (e.g., Bayesian classifier or Fischer analysis), logistic classifiers, and support vector classifiers (e.g., support vector machines).

[0072] Classification rules, algorithms, also referred to as models, can be generated by mathematical analysis, including by machine learning techniques that perform analysis of datasets of biomarker measurements derived from subjects classed into one or another group. In some embodiments, one or more classification rules or algorithms may employ one or more of the following: cut-off, linear regression including multiple linear regression, partial least squares regression, principal components regression, binary decision trees including recursive partitioning processes further including classification and regression trees, artificial neural networks including back propagation networks, discriminant analyses further including Bayesian classifier or Fischer analysis, logistic classifiers, and support vector classifiers including support vector machines. In some variations, these datasets of biomarker measurements may comprise more than 100, more than 1000, more than 10,000, or more than 100,000 data entries. In some variations, machine learning techniques that perform analysis of datasets of biomarker measurements derived from subjects classed into one or another group may access test data from the subjects and execute the one or more classification rules on the test data.

[0073] Diagnostic tests are characterized by sensitivity (percentage classified as positive that are true positives) and specificity (percentage classified as negative that are true negatives). The relative sensitivity and specificity of a diagnostic test can involve a trade-off – higher sensitivity can mean lower specificity, while higher specificity can mean lower sensitivity. These relative values can be displayed on a receiver operating characteristic (ROC) curve. The diagnostic power of a set of variables, such as biomarkers, is reflected by the area under the curve (AUC) of an ROC curve.

[0074] In some embodiments, the classifiers of this disclosure have a sensitivity value, a specificity value, a positive predictive value, or a negative predictive value of at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%. Classifiers of this disclosure have an AUC of at least 0.6, at least 0.7, at least 0.8, at least 0.9 or at least 0.95.

[0075] Classification can be based on a measurement of a biomarker being above or below a selected cutoff level or value or threshold level or value. In certain embodiments, a cutoff value is obtained by measuring biomarker levels in a plurality of positive and negative reference samples, e.g., at least 10, 20, 50, 100 or 200 samples of each type (e.g., samples from control subjects and test subjects). A cutoff value can be established with respect to a measure of

central tendency, such as mean, median or mode in the negative samples. A measure of deviation from this measure of central tendency can be used to set the cutoff. For example, the cutoff can be set based on variance or standard deviation. For example, the cutoff can be based on Z score, that is, a number of standard deviations above a mean of normal samples, for example one standard deviation, two standard deviations, three standard deviations or four standard deviations. For example, cutoff values can be selected so that the diagnostic test has at least an 80%, a 90%, a 95%, a 98%, a 99%, a 99.5%, or a 99.9% sensitivity value, specificity value and/or positive predictive value.

[0076] Numerically, an increased risk is associated with an odds ratio of over 1.0, preferably over 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.0 for placenta accreta.

VII. Methods of Assessing Risk of Placenta Accreta

[0077] The phrase “increased risk” of a condition, as used herein, indicates that a subject has a greater likelihood of developing the condition than a general population of subjects. So, for example, a subject who is at “increased risk of placenta accreta” has a greater likelihood of developing placenta accreta than a general population of subjects at the same stage of pregnancy, optionally compared with a population sharing one or more demographic or risk factors. These may include, for example, age, placenta previa, previous cesarean delivery, endometrial ablation, in vitro fertilization, prior uterine infection, or other uterine surgery. For example, a test may indicate that a woman at around 24 weeks or around 34 weeks of pregnancy has a higher risk of developing placenta accreta than a general or control population of woman at around 24 weeks or around 34 weeks pregnancy.

[0078] Classifying can employ a classification rule, algorithm or model determined by statistical analysis and/or machine learning. In some embodiments, the classification rule may be based on one or more values. The one or more values may include one or more demographic or risk factors of a subject compared to the general population of subjects. The one or more values may also include measured values of one or more protein biomarkers.

A. Assessing Placenta Accreta

[0079] Provided herein are methods of assessing risk for placenta accreta at anywhere between 20 weeks of pregnancy to about 37 weeks of pregnancy, for example, classifying a pregnant human female as being at an increased risk of placenta accreta. The methods can involve determining a measure of one or a plurality of the biomarkers in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, or Table 9, and associating the measure to risk of placenta accreta. For example, one can use a panel that includes 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more, or, no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 biomarkers from any one or more of Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, or Table 9. in the determination. In general, an amount of a biomarker that shows a difference compared to a control amount of the biomarker (e.g., a healthy pregnant female or a non-placenta accreta pregnant female), which difference is statistically significant, is associated with increased risk of placenta accreta. The difference can be an up-regulation or a down-regulation, which can be easily determined by the practitioner. Alternatively, determination may be based on a classification algorithm that may employ non-linear and/or hyperdimensional methods.

[0080] In some embodiments, pathways can be interrogated to aid the determination of risk of plasma accreta. For example, in some embodiments, one or more canonical pathways may be over-represented by differentially expressed proteins in second trimester placenta accreta cases. Such pathways may be one or more of the erythropoietin signaling pathway; and the iron homeostasis signaling pathway (making reference to Table 1.7 in Example 1).

[0081] In some embodiments, certain targets may be activated or inhibited, and may be interrogated to aid the determination of risk of plasma accreta. Exemplary targets are provided in Table 1.8 in Example 1.

[0082] In some embodiments, cellular and molecular functions around iron handling and erythrocyte function are over-represented and may be interrogated to aid the determination of risk of plasma accreta. Exemplary targets are provided in Table 1.9 in Example 1.

[0083] In some embodiments, one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1) are useful to distinguish plasma accreta from controls. In some embodiments, a

biomarker panel of the disclosure comprises one, two, three, four, or all five of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1). In some embodiments one or more of these markers are useful to distinguish plasma accreta from controls in the second trimester.

[0084] In some embodiments, one or more of ISM2, ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein, are useful to distinguish plasma accreta from controls. In some embodiments, a biomarker panel of the disclosure comprises one, two, three, or all four of ISM2, ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein. In some embodiments one or more of these markers are useful to distinguish plasma accreta from controls in the third trimester.

[0085] In other embodiments a determination is based on the use of a panel of biomarkers, for example those that are provided in Tables 7 or 8.

B. Assessing Placenta Accreta – Second Trimester

[0086] Provided herein are methods of assessing risk for placenta accreta during the second trimester (e.g. at around 24 weeks of pregnancy), for example, classifying a pregnant human female as being at an increased risk of placenta accreta. The methods can involve determining a measure of one or a plurality of the biomarkers in Table 1, Table 3, Table 5, or Table 9 and associating the measure to risk of placenta accreta. For example, one can use a panel that includes 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more, or, no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 biomarkers from the tables in the determination. In general, an amount of a biomarker that shows a difference compared to a control amount of the biomarker (e.g., a healthy pregnant female or a non-placenta accreta pregnant female), which difference is statistically significant, is associated with increased risk of placenta accreta. The difference can be an up-regulation or a down-regulation, which can be easily determined by the practitioner. Alternatively, determination may be based on a classification algorithm that may employ non-linear and/or hyperdimensional methods.

[0087] A biomarker panel can comprise of any of the biomarker panels presented in Table 7. A biomarker panel can consist essentially of any of the biomarker panels presented in Table 7. A

biomarker panel can consist of any of the biomarker panels presented in Table 7. In some embodiments, one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1) are useful to distinguish placenta accreta from controls.

C. Assessing Placenta Accreta – Third Trimester

[0088] Provided herein are methods of assessing risk for placenta accreta during the third trimester (e.g. at around 35 weeks of pregnancy), for example, classifying a pregnant human female as being at increased risk of placenta accreta. The methods can involve determining a measure of one or a plurality of the biomarkers in Table 2, Table 4, Table 6, or Table 9 and associating the measure to risk of placenta accreta. For example, one can use a panel that includes 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more, or, no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 biomarkers from the tables in the determination. In general, an amount of a biomarker that shows a difference compared to a control amount of the biomarker (e.g., a healthy pregnant female or a non-placenta accreta pregnant female), which difference is statistically significant, is associated with increased risk of placenta accreta. The difference can be an up-regulation or a down-regulation, which can be easily determined by the practitioner. Alternatively, determination may be based on a classification algorithm that may employ non-linear and/or hyperdimensional methods.

[0089] A biomarker panel can comprise of any of the biomarker panels presented in Table 8. A biomarker panel can consist essentially of any of the biomarker panels presented in Table 8. A biomarker panel can consist of any of the biomarker panels presented in Table 8.

[0090] In some embodiments, one or more of ISM2, ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein, are useful to distinguish placenta accreta from controls.

VIII. Methods of Treating Subjects at Increased Risk of Placenta Accreta

[0091] Methods of treating pregnant subjects suffering from or at increased risk of placenta accreta include assessing the risk of placenta accreta in a pregnant subject and administering one or more therapeutic interventions useful in treating placenta accreta, reducing the risk or

placenta accreta and/or reducing neonatal complications of placenta accreta. In some embodiments, administering one or more therapeutic interventions includes administering an effective amount of one or more treatments designed to reduce the risk of placenta accreta. In some embodiments, one or more treatments may include performing a Cesarean hysterectomy, recommending bed rest to the subject to prevent preterm labor, performing a prophylactic embolization, leaving a portion of the placenta left in-situ, inserting a uterine balloon tamponade, administering methotrexate, inserting one or more temporal internal iliac occlusion balloon catheters, inserting one or more ureteral stents, or the like.

[0092] Patients with suspected placenta accreta spectrum should be referred to a center having advanced multidisciplinary surgical expertise and experience in any of the therapeutic interventions listed above or standard therapeutic interventions in the field.

[0093] Surgical planning for uterine conservation would be planned if future fertility was desired or planning for or performing a hysterectomy would be undertaken if fertility was not desired.

[0094] This can allow for appropriate planning to ensure access to specialists/surgeons and properly equipped facilities (i.e., blood bank support).

IX. Kits and other Articles of Manufacture

[0095] In another embodiment, provided herein are articles of manufacture, e.g., kits of reagents useful in detecting in a sample biomarkers, for increased risk of placenta accreta, in particular, placenta accreta. Reagents capable of detecting protein biomarkers include but are not limited to antibodies. Antibodies capable of detecting protein biomarkers are also typically directly or indirectly linked to a molecule such as a fluorophore or an enzyme, which can catalyze a detectable reaction to indicate the binding of the reagents to their respective targets.

[0096] In some embodiments, the kits further comprise sample processing materials comprising a high molecular weight gel filtration composition (e.g., agarose such as SEPHAROSE) in a low volume (e.g., 1ml, 3ml, 5ml, 10ml) vertical column for rapid preparation of a microparticle-enriched sample from plasma. For instance, the microparticle-

enriched sample can be prepared at the point of care before freezing and shipping to an analytical laboratory for further processing.

[0097] In some embodiments, the kits further comprise instructions for assessing risk of placenta accreta, in particular, placenta accreta. As used herein, the term “instructions” refers to directions for using the reagents contained in the kit for detecting the presence (including determining the expression level) of a protein(s) of interest in a sample from a subject. The proteins of interest may comprise one or more biomarkers of placenta accreta.

[0098] In another embodiment, a kit comprises one or more containers wherein each container containing one or a plurality of stable isotope standard (SIS) peptides corresponding to peptide biomarkers, e.g., peptides produced from protease (e.g., trypsin) digestion of biomarker proteins. In another embodiment, a majority or all of the SIS peptides correspond to the biomarker peptides. In another embodiment, the kit further comprises the biomarker peptides which the SIS peptides correspond.

[0099] In another embodiment, provided is a composition of matter that includes protein biomarkers of placenta accreta and, for a plurality of those biomarkers, a corresponding stable isotope standard peptide. This can be prepared by combining a sample comprising proteins isolated from microparticles, with stable isotope standard peptides.

X. Systems

[0100] Provided herein also are systems comprising a computer comprising a processor and memory. The computer can be configured to receive into memory one or more measurements of one or more biomarkers provided herein that are measured from a sample. The memory can include computer readable instructions which, when executed, classify the sample as at risk of placenta accreta or not at risk of placenta accreta. The computer system can be operatively coupled to a computer network with the aid of a communications interface. The network can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network in some cases is a telecommunication and/or data network. The network can include one or more computer servers, which can enable distributed computing, such as cloud computing. The system can include a first computer connected with a second computer through a communications network, such as, a high-speed transmission network

including, without limitation, Digital Subscriber Line (DSL), Cable Modem, Fiber, Wireless, Satellite and, Broadband over Powerlines (BPL). Accordingly, results providing classification of a sample as at increased risk or as not at increased risk of placenta accreta can be transmitted from a transmitting computer to a remote receiving computer, such as located at the office of a healthcare provider or to a mobile device, such as a smart phone.

EXAMPLES

Example 1:

[0101] Maternal EDTA plasma samples were collected from patients who were > 18 years of age, receiving prenatal care and planning on delivery at a hospital in the second and third trimesters at a median of about 26 (+/- 2) and about 35 (+/- 2) weeks' gestation, respectively. Pregnancy dating was confirmed by ultrasound at ≤ 12 weeks gestation. The samples were aliquoted and stored at -80 degrees centigrade. 35 Placenta accreta spectrum, referred to herein as "PAS", cases and 70 controls were analyzed including 27 cases of grade 1 PAS, 7 cases of grade 2 PAS, and 1 case of grade 3 PAS as defined by the International Federation of Gynaecology and Obstetrics ("FIGO") (Table 1.1).

Table 1.1. FIGO clinical and histologic criteria for PAS

	Clinical Criteria	Histologic Criteria
Grade 1	Attempts at manual removal of adherent placenta results in heavy bleeding requiring mechanical or surgical measures	Hysterectomy specimen with extended areas of absent decidua between villous tissue and myometrium
Grade 2	Macroscopic findings: hypervascularity, placental bulge, "dimple sign"	Hysterectomy specimen shows placental villi within muscular fibers
Grade 3	Macroscopic findings of Grade 2 AND placental villi seen to be invading through uterine surface +/- invasion into other pelvic structures	Hysterectomy specimen showing villous tissue breaching the uterine serosa

[0102] Cases were defined as subjects with clinical or histologic grade 1 to 3 PAS consistent with the 2019 FIGO PAS classification (Table 1.1), delivery >23 weeks gestation and inclusion

in the LIFECODES biobank. Prospective cases were first identified in the electronic medical record using the following word searches for records between 2007 and 2020: “adhere-” in operative reports and discharge summaries as well as “-creta,” “hyst-” or “previa” in pathology reports. The medical record for each prospective case was independently reviewed by two obstetricians within the institution’s multidisciplinary PAS team. The higher grade between the clinical and histologic grades was designated as the assigned grade. Disagreement in either inclusion status or assigned PAS grade were adjudicated by a review committee. Subjects identified to have a PAS diagnosis were cross-referenced with the LIFECODES biobank for inclusion. Controls were defined as subjects without a diagnosis of PAS and randomly matched 2:1. Cases and controls were matched by gestational age of sampling (+1 week) and number of fetuses. Exclusion criteria were defined as current cancer diagnosis, use of immunomodulating medication or documented fetal chromosomal abnormality. Univariate analyses were conducted with chi-square tests and continuous variables were compared with Wilcoxon tests using SAS 9.4. All tests were two-tailed; $P < 0.05$ was used to define statistical significance.

[0103] Compared to controls, cases were more likely to be of older maternal age and have placenta previa at delivery. Twenty (57.1%) of the PAS cases did not have a history of cesarean section nor placenta previa at delivery. Twenty-three (65.7%) of the PAS cases were determined by clinical criteria rather than histologic criteria. Notably, only 11% of grade 1 PAS cases were detected by ultrasound prior to delivery, compared to 57% and 100% of grade 2 and 3 PAS cases, respectively (Table 1.2).

Table 1.2 Characteristics and grading determination of PAS cases. +Data displayed as median (+IQR) or n (%)

	Cases⁺ n = 35	Controls⁺ n = 70	p-value
Maternal age (yrs)	36.9 (+ 2.9)	32.9 (+ 2.7)	<0.01
# prior cesarean sections			0.33
0	23 (65.7)	55 (78.6)	
1	7 (20.0)	10 (14.3)	
>2	5 (14/3)	5 (7.1)	

	Cases⁺ n = 35	Controls⁺ n = 70	p-value
Placenta previa at delivery	8 (22.9)	6 (8.6)	<0.01
PAS diagnosed by ultrasound		N/A	
Grade 1 (n=27)	3 (11.1)		
Grade 2 (n=7)	4 (57.1)		
Grade 3 (n=1)	1 (100.0)		
PAS grade and determination			
Grade 1	27 (77.1)		
Clinical	21		
Histologic	0		
Both concordant	6		
Grade 2	7 (20.0)		
Clinical	2		
Histologic	1		
Both concordant	4		
Grade 3	1 (2.9)		
Clinical	0		
Histologic	0		
Both concordant	1		

[0104] To enrich each sample for circulating microparticles (“CMP”), Size Exclusion Chromatography (SEC) was used for CMP isolation. Anonymized EDTA plasma samples identified only by a study number that was agnostic to case or control status were randomly assorted and shipped on dry ice to NX Prenatal, Inc. (Houston, TX) where CMP protein enrichment was carried out by SEC and isocratically eluted using the NeXosome Elution Reagent. This involved NeXosome Isolation Columns manufactured by AmericanBio, Inc. (Canton, MA). Briefly, these columns were packed by AmericanBio with Sepharose 4B-CL (4% agarose, particle size 45-165 µm) from Cytiva (Marlborough, MA) to a total packed volume of 10mL and delivered to NX Prenatal. Once received by NX Prenatal, the columns were stored at 2–8 °C until use. Prior to using the columns for CMP isolation, the columns were allowed to equilibrate to room temperature (overnight) and subsequently washed with NeXosome Elution Reagent. EDTA plasma samples were thawed and 0.5mL of plasma was applied and allowed to

incorporate into the NeXosome Isolation Column. The plasma samples were not filtered, diluted, or pretreated prior to application to the columns. Following the incorporation of the sample into the column, the NeXosome Elution Reagent was added and 0.5mL column fractions were collected. The eluted fractions yielded two peaks. The CMPs were captured in the column void volume and resolved from the high abundant soluble protein peak. Samples were processed according to a randomization scheme. Each CMP-containing fraction (0.5 mL aliquots of each fraction) was pooled within each individual sample and a total protein measurement was performed, using the Pierce BCA Protein Assay Kit (ThermoFisher Scientific). An aliquot containing a total protein of 200 µg from each individual CMP isolate pool was then transferred to 2-mL microcentrifuge tubes (VWR, Radnor, PA) and stored at – 80 °C pending completion of all CMP isolate processing. All CMP isolates were then shipped on dry ice to BGI Americas Corporation (Cambridge, MA) for proteomic analysis.

[0105] A total of 158 Plasma enriched exosome samples were individually processed for LC-MS/MS analysis. 100 µL of enriched exosomes were mixed with 700 µL of lysis buffer that contained 9M urea, at pH 8.5, and 0.5% Rapigest (SKU: 186001861, Waters™). Samples were water-bath sonicated for 30 minutes followed by spinning at high-speed (14,000 rpm) in a centrifuge for 10 minutes. The protein concentration of samples was measured by the BCA assay (Cat No: A53225, ThermoFisher Scientific) post sample lysis.

[0106] 50 µg of each sample was taken from the lysate and normalized to the same volume with lysis buffer. Samples were reduced in 10 mM DTT for 25 minutes at 60 °C, then the reduced samples were alkylated in 20 mM IAM (iodoacetamide) in a dark environment for 20 minutes at room temperature. Excess IAM in the samples was quenched by adding 100 mM DTT. DI water and HEPE buffer at pH 8.5 were added to each sample so that the final urea concentration was diluted to 1.6 M, and a final pH of 8, for enzymatic digestion. 1 µg of Tryp/LysC (Cat No: A41007, ThermoFisher Scientific) was added to each sample. The samples were incubated overnight at 37 °C for 12 hours. An additional 1 µg of Tryp/LysC was added to each sample the next day and they were incubated for another 4 hours to complete the enzymatic digestion.

[0107] 10% TFA was added into the digested samples (peptides) to produce a final concentration of 1% TFA—the pH was tested and the samples were acidic. Then, acidified

samples were passed through a 10 mg SEK PAK column (Cat No: 60108-302, ThermoFisher Scientific) for desalting. 20% of the desalted peptides of each sample was taken and pooled together to create a composite “library” of peptides. The library samples were then fractionated into 96 fractions with a high pH, reverse-phase, offline HPLC fractionator (Vanquish™, ThermoFisher Scientific). The mobile phase A was made up of DI H₂O with 20 mM formic acetate, pH 9.3; the mobile phase B was made up of acetonitrile (Optima™, LC/MS grade, Fisher Chemical™) with 20mM formic acetate, pH 9.3. The gradient of separation is displayed in Table 1.3. 96 fractions were then combined into 24 fractions and readied for liquid chromatography mass spectrometry (LC/MS) analysis.

Table 1.3 High-pH, Reverse-Phase HPLC Fractionation Gradient Information

Time[min]	Flow[ml/min]	%B
0.00	0.500	2.0
1.00	0.500	6.0
12.00	0.500	20.0
30.00	0.500	28.0
50.00	0.500	65.0
53.00	0.500	98.0
57.00	0.500	98.0
59.00	0.500	2.0
60.00	0.500	2.0

[0108] All fractionated samples were analyzed by nanoflow HPLC (Ultimate 3000, Thermo Fisher Scientific) followed by Thermo Orbitrap mass spectrometer (Tribid Eclipse) analysis. A Nanospray Flex™ Ion Source (Thermo Fisher Scientific) was equipped with Column Oven (PRSO-V2, Sonation) to heat up the nanocolumn (PicoFrit, 100 µm x 250 mm x 15 µm tip, New Objective) for peptide separation. The nanoLC method is water acetonitrile based that was 150 minutes long with a 0.300 µL/min flowrate. For each sample injection, all peptides were first engaged on a trap column (Cat. No: 160454, Thermo Fisher) and then were delivered to the separation nanocolumn by the mobile phase. The specifics of the gradient used are provided in Table 1.4.

Table 1.4. High pH Reverse Phase HPLC Fractionation Gradient Information

Time[min]	Flow[ml/min]	%B
0.00	0.500	2.0
1.00	0.500	6.0
12.00	0.500	20.0
30.00	0.500	28.0
50.00	0.500	65.0
53.00	0.500	98.0
57.00	0.500	98.0
59.00	0.500	2.0
60.00	0.500	2.0

[0109] For the DDA library construction, a DIA library-specific DDA, MS2-based mass spectrometry method on Eclipse was used to sequence fractionated peptides that were eluted from the nanocolumn. For the full MS spectrum, a resolution of 120,000 was used with a scan range of 375 m/z – 1500 m/z. For the dd-MS(MS2), a resolution of 15,000 was used, and the isolation window is 1.6 Da. ‘Standard’ AGC target and ‘Auto’ Max Ion injection times (Max IT) were selected for both MS1 and MS2 acquisition. The collision energy (NCE) was set to 35%, and the total cycle time is 1 second. For DIA analytical samples, a high-resolution, full MS scan, followed by two segment DIA methods, was used for the DIA data acquisition. For the full MS scan, a resolution of 120,000 was used for the range of 400 m/z – 1200 m/z with a ‘Standard’ AGC target and 50 ms Max IT. For both DIA segments, the details of the isolation windows (IW) and the precursor mass ranges are shown in Table 1.5 and Table 1.6. For the DIA fragments scan, a resolution of 30,000 was used for the range of 110 m/z – 1,800 m/z with a ‘Standard’ AGC target and ‘Auto’ Max IT.

Table 1.5. DIA segment 1 Precursor Scan Range Information.

Segment 1 (400 – 800 m/z, IW 15 m/z, Overlap 1 m/z)	
399.5 – 415.5	609.5 – 625.5
414.5 – 430.5	624.5 – 640.5

Segment 1 (400 – 800 m/z, IW 15 m/z, Overlap 1 m/z)	
429.5 – 445.5	639.5 – 655.5
444.5 – 460.5	654.5 – 670.5
459.5 – 475.5	669.5 – 685.5
474.5 – 490.5	684.5 – 700.5
489.5 – 505.5	699.5 – 715.5
504.5 – 520.5	714.5 – 730.5
519.5 – 535.5	729.5 – 745.5
534.5 – 550.5	744.5 – 760.5
549.5 – 565.5	759.5 – 775.5
564.5 – 580.5	774.5 – 790.5
579.5 – 595.5	789.5 – 800.5
594.5 – 610.5	

Table 1.6. DIA segment 2 Precursor Scan Range Information.

Segment 2 (800 – 1200 m/z, IW 25 m/z, Overlap 1 m/z)	
799.5 – 825.5	999.5 – 1025.5
824.5 – 850.5	1024.5 – 1050.5
849.5 – 875.5	1049.5 – 1075.5
874.5 – 900.5	1074.5 – 1100.5
899.5 – 925.5	1099.5 – 1125.5
924.5 – 950.5	1024.5 – 1150.5
949.5 – 975.5	1049.5 – 1175.5
974.5 – 1000.5	1074.5 – 1200.5

[0110] The initial process is based on the sample data generated from a high-resolution mass spectrometer. The DDA data was identified by the Andromeda search engine within MaxQuant, and Spectronaut™ was used for the identification of results for spectral library construction. MaxQuant was used for the identification of DDA data, which served as a spectrum library for

the subsequent DIA analysis. The analysis pipeline used raw data as input files and set corresponding parameters and human databases (UP000005640), then the identification and quantitative analysis was performed. The identified peptides satisfied a FDR of $\leq 1\%$ to construct the final spectral library. For this DIA dataset, Spectronaut™ was employed to construct spectral library information to complete deconvolution and extraction, then the mProphet algorithm was used to complete an analytical quality control (1% FDR) to obtain reliable quantitative results. GO, COG, and Pathway functional annotation analysis and time series analysis were also performed in the pipeline described above. MStats, the core algorithm of which is a linear mixed effect model, was used to process the DIA quantification results data according to the predefined comparison group, and then a significance test was performed based on the model. Thereafter, differential protein screening was executed and a fold change of ≥ 2 and an adj P-value of < 0.05 was defined as a significant difference. Based on the quantitative comparison results, the differential proteins between comparison groups were identified; finally a function enrichment analysis, a protein-protein interaction (PPI) examination, and a subcellular localization analysis of the differential proteins were carried out. The sample classification analyses were then implemented as described below.

[0111] PAS was classified using regularized (L1) regression to define a restricted set of candidate CMP proteins from the superset of all identified proteins. To select the putative panel from the restricted set of candidate CMP proteins, a cross-validation procedure using logistic regression was chosen. The sample was randomly divided into a training and validation set (80% vs. 20%). The proteins in the training set were then ranked by their Akaike information criterion (AIC) using an ensemble feature selection procedure. The top 10 proteins were then passed to the glmulti package in R version 3.6.3 where the training set was subjected to fivefold cross-validation. To avoid overfitting, given the limited sample size, the model was restricted to no more than 5 predictors. The model with the greatest area under the curve (AUC) and the lowest standard deviation of the AUC was then tested against the set-aside, external validation set. The AUC and standard deviation of the AUC of this external validation set was then recorded and the workflow re-iterated for a total of 1000 iterations (Fig. 9). The models were then ranked by their mean AUC and mean standard deviation of the AUC. The workflow was then repeated with randomly permuted sample labels. Predictive statistics for the observed versus permuted data were then compared.

[0112] To establish a panel of CMP proteins that would serve as a classifier for the risk of PAS in the second and third trimesters, a two-step iterative workflow using regularized (L1) regression followed by a cross-validation procedure using logistic regression was used. The two-step workflow was also repeated with randomly permuted sample labels to simulate random chance. In second trimester samples, the mean of all area under the curves (AUCs) for the observed (e.g., first shaded area) versus permuted (e.g., second shaded area) panels was significantly different (0.72 vs. 0.45; $p < 2.20 \times 10^{-16}$; FIG. 16A). The top performing panel of markers distinguished PAS from controls with a mean AUC of 0.83. CMP proteins of this panel included: isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1). Within third trimester samples, the mean of all AUCs for the observed (e.g., first shaded area) versus permuted (e.g., second shaded area) panels were also significantly different (0.60 vs 0.52; $p = 2.79 \times 10^{-5}$; FIG. 16B). The top performing panel distinguished PAS from controls with a mean AUC of 0.78. CMP proteins in this panel included ISM2, ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein. Additionally, the mean of all AUC in the second trimester was significantly greater than that in the third trimester ($p = 1.59 \times 10^{-12}$).

[0113] To determine the biological function of those proteins identified as differentially expressed by PAS status in the differential expression analysis, an Ingenuity Pathway Analysis was also applied. Core analysis with the Ingenuity Knowledge Base reference set was used. Direct and indirect relationships were considered for network and regulatory analyses. IPA is a curated bioinformatic repository of functionally annotated analytes which allows for functional annotation, canonical pathway and network analyses, and upstream regulator analysis. Significantly ($P < 0.05$) over-represented canonical pathways, upstream regulators, and molecular and cellular functions were identified. Only relevant pathways and biological functions containing two or more overlapping hits were included, while only upstream regulators with a predicted activation state (activated, inhibited) were included. Overlap ratios were calculated as the percent of overlap between differentially expressed proteins and the target pathway.

[0114] To determine the biological function of those proteins identified as differentially expressed by PAS status in the differential expression analysis, an Ingenuity Pathway Analysis

was also applied. This revealed significant over-representation of canonical pathways, upstream regulators, and molecular and cellular functions. In the second trimester, proteomic changes in PAS yielded significant over-representation of several canonical pathways including iron homeostasis signaling and erythropoietin signaling (Table 1.7).

Table 1.7. Canonical pathways significantly over-represented by differentially expressed proteins in second trimester placenta accreta cases.

Ingenuity Canonical Pathways	-log(p-value)	Overlap ratio	Molecules
Erythropoietin Signaling Pathway	4.33	0.0169	HBA1/HBA2, HBB, HBG1
Iron homeostasis signaling pathway	4.64	0.0216	HBA1/HBA2, HBB, HBG1

[0115] Master upstream regulators included seven targets predicted to be significantly activated and six predicted to be significantly inhibited (Table 1.8).

Table 1.8. Select master upstream regulators of target molecules in second trimester placenta accreta dataset.

Master Regulator	Molecule Type	Predicted Activation State	p-value of overlap	Target molecules in dataset
signal peptidase	complex	Activated	0.0000173	ALB, ANGPTL2, GRN, HBA1/HBA2, HBB, NID1, VNN1
GNRHR	G-protein coupled receptor	Activated	0.000143	GRN, HBA1/HBA2, HBB, VNN1
DRD3	G-protein coupled receptor	Activated	0.000148	GRN, HBA1/HBA2, HBB, VNN1
ARAF	kinase	Activated	0.000408	GRN, HBA1/HBA2, HBB, VNN1
G protein alpha	group	Activated	0.000471	GRN, HBA1/HBA2, HBB, VNN1
F10	peptidase	Activated	0.000649	ANGPTL2, HBA1/HBA2, HBB, VNN1
BDKRB2	G-protein coupled receptor	Activated	0.000826	GRN, HBA1/HBA2, HBB, VNN1

Master Regulator	Molecule Type	Predicted Activation State	p-value of overlap	Target molecules in dataset
MAP2K7	kinase	Inhibited	0.000139	ALB, ANGPTL2, HBA1/HBA2, HBB, HBG1
CDK5R1	kinase	Inhibited	0.000269	ANGPTL2, GRN, HBA1/HBA2, HBB, VNN1
SERPINB1	other	Inhibited	0.000691	ANGPTL2, GRN, HBA1/HBA2, HBB, NID1, VNN1

[0116] IPA molecular and cellular functions analyses revealed 43 select annotated functions which were significantly over-represented based on differentially-expressed molecular hits from second trimester placenta accreta analyses (Table 1.9). Cellular and molecular functions around iron handling and erythrocyte function agreed with canonical iron homeostasis and erythropoietin signaling pathways.

Table 1.9: Select over-represented molecular and cellular functions of proteins altered in second trimester placenta accreta

Diseases or Functions Annotation	p-value	Molecules
Heinz body anemia	1.59E-07	HBA1/HBA2, HBB
Mineral deficiency	2.90E-07	ALB, HBA1/HBA2, HBB
Iron deficiency anemia of pregnancy	4.77E-07	HBA1/HBA2, HBB
Refractory iron deficiency	4.77E-07	HBA1/HBA2, HBB
Hereditary persistence of fetal hemoglobin	1.59E-06	HBB, HBG1
Transport of oxygen	2.38E-06	HBA1/HBA2, HBB
Biosynthesis of hydrogen peroxide	1.99E-05	ALB, HBA1/HBA2, HBB
Catabolism of hydrogen peroxide	3.01E-05	HBA1/HBA2, HBB
Hemolysis	3.59E-05	ALB, HBA1/HBA2, HBB
Degranulation of phagocytes	9.34E-05	ALB, GRN, HBB, VNN1
Degranulation of myeloid cells	9.86E-05	ALB, GRN, HBB, VNN1
Familial hemolytic anemia	1.29E-04	HBA1/HBA2, HBB
Peripheral vascular disease	1.49E-04	ALB, GRN, HBA1/HBA2, HBB
Aggregation of blood platelets	1.60E-04	ALB, ANGPTL2, HBB
Polycythemia	1.90E-04	GRN, HBA1/HBA2, HBB
Receptor-mediated endocytosis	2.10E-04	ALB, HBA1/HBA2, HBB
Synthesis of reactive oxygen species	2.30E-04	ALB, GRN, HBA1/HBA2, HBB
Quantity of reticulocytes	3.26E-04	HBA1/HBA2, HBB
Endocytosis	4.02E-04	ALB, GRN, HBA1/HBA2, HBB
Degranulation of neutrophils	9.46E-04	GRN, HBB, VNN1

Diseases or Functions Annotation	p-value	Molecules
Formation of epithelial tissue	9.64E-04	GRN, NID1
Hypertension	1.02E-03	ALB, ANGPTL2, HBA1/HBA2, HBG1
Ischemic stroke	1.11E-03	ALB, GRN
Production of reactive oxygen species	1.54E-03	GRN, HBA1/HBA2, HBB
Production of superoxide	1.95E-03	HBA1/HBA2, HBB
Quantity of myeloid cells	5.34E-03	GRN, HBA1/HBA2, HBB
Consumption of oxygen	5.88E-03	HBA1/HBA2, HBB
Cell movement of myeloid cells	5.88E-03	ALB, ANGPTL2, GRN
Generation of reactive oxygen species	6.20E-03	ALB, HBA1/HBA2
Innate immune response	7.24E-03	GRN, VNN1
Coagulation of blood	1.01E-02	HBB, HBG1
Severe inflammatory disorder	1.05E-02	GRN, HBG1
Synthesis of nitric oxide	1.18E-02	ALB, ANGPTL2
Inflammatory response	1.48E-02	ANGPTL2, GRN, VNN1
Cell movement of neutrophils	1.73E-02	ALB, GRN
Apoptosis	2.49E-02	ALB, GRN, HBA1/HBA2, HBB, VNN1
Quantity of metal ion	2.73E-02	GRN, HBA1/HBA2
Abnormal morphology of epithelial tissue	2.75E-02	GRN, NID1
Bleeding	2.95E-02	ALB, GRN
Development of vasculature	3.62E-02	ANGPTL2, GRN, NID1
Preeclampsia	3.73E-02	ANGPTL2, HBG1
Chronic inflammatory disorder	3.77E-02	ALB, GRN, HBA1/HBA2
Development of epithelial tissue	4.45E-02	GRN, NID1

[0117] As with second trimester data, IPA Core Analysis revealed significant over-representation of canonical pathways, upstream regulators, and molecular and cellular functions in the third trimester in PAS. Canonical pathway analysis of third trimester proteomic changes in PAS revealed significant over-representation of pathways including immune and extracellular signaling pathways, specifically involving IL-15 (Table 1.10).

Table 1.10 Canonical Pathway analysis of Third Trimester Changes

Ingenuity Canonical Pathways	-log(p-value)	Overlap Ratio	Molecules
Communication between Innate and Adaptive Immune Cells	2.9	0.00431	IGHV3-20, IGKV3D-11, IGKV4-1, IGLV2-18

Ingenuity Canonical Pathways	-log(p-value)	Overlap Ratio	Molecules
B Cell Receptor Signaling	3.54	0.00634	IGHV3-20, IGKV3D-11, IGKV4-1, IGLV2-18
Integrin Signaling	3.72	0.0141	ARPC5, ILK, PARVB
IL-15 Signaling	3.84	0.00758	IGHV3-20, IGKV3D-11, IGKV4-1, IGLV2-18

[0118] Master upstream regulators included three predicted to be significantly activated and two significantly inhibited (Table 1.11).

Table 1.11. Select master upstream regulators of target molecules in third trimester placenta accreta dataset.

Master Regulator	Molecule Type	Predicted Activation State	p-value of overlap	Target molecules in dataset
DVL1	other	Activated	0.000188	GAPDH, HSPG2, ILK, MPO, PARVB
Histone h3	group	Activated	0.00121	ARPC5, GAPDH, HSPG2, ILK, MPO
ADCY	group	Activated	0.00168	ARPC5, GAPDH, HSPG2, PARVB
RGS (2,4,7,16,18)	group	Inhibited	0.0000907	GAPDH, HSPG2, ILK, MPO, SPP2

[0119] Molecular and cellular functional analyses revealed 24 select annotated functions which were significantly over-represented based on differentially expressed molecular hits from third trimester analyses in PAS (Table 1.12).

Table 1.12. Select over-represented molecular and cellular functions of proteins altered in third trimester placenta accreta

Diseases or Functions Annotation	p-Value	Molecules
Classical complement pathway	0.0000102	IGKV4-1, C1QB, IGKV3D-11
Receptor-mediated endocytosis	0.000487	IGKV4-1, HSPG2, IGKV3D-11
Cell movement	0.00278	IGKV4-1, MPO, HSPG2, GAPDH, IGKV3D-11, PARVB, ILK
Reorganization of actin cytoskeleton	0.00342	PARVB, ILK

Diseases or Functions Annotation	p-Value	Molecules
Attachment of cells	0.0035	HSPG2, ILK
Apoptosis of cardiomyocytes	0.00764	MPO, GAPDH
Cellular infiltration	0.0079	MPO, GAPDH, ILK
Leukocyte migration	0.0087	IGKV4-1, MPO, IGKV3D-11, ILK
Migration of connective tissue cells	0.00895	HSPG2, ILK
Aggregation of blood platelets	0.00908	MPO, ILK
Degranulation of cells	0.00916	SPP2, ARPC5, MPO
DNA damage	0.0159	MPO, GAPDH
Organization of cellular membrane	0.0165	ARPC5, ILK
Cell death of epithelial cell lines	0.0213	MPO, GAPDH
Cell death of kidney cell lines	0.0213	GAPDH, ILK
Familial heart disease	0.0242	HSPG2, ILK
Acute respiratory disorder	0.0258	MPO, GAPDH
Immune mediated inflammatory disease	0.0267	MPO, C1QB, GAPDH, ILK
Degranulation of neutrophils	0.0286	ARPC5, MPO
Cell movement of neutrophils	0.0288	MPO, ILK
Adhesion of immune cells	0.0305	MPO, ILK
Organization of cytoskeleton	0.0322	ARPC5, GAPDH, PARVB, ILK
Disorder of pregnancy	0.0337	MPO, GAPDH, ILK
Migration of cells	0.0376	IGKV4-1, MPO, HSPG2, IGKV3D-11, ILK

[0120] There was some agreement, particularly around immune signaling and cytoskeletal and cell growth functions, between these cellular and molecular functions and the canonical pathways revealed by IPA.

EXEMPLARY EMBODIMENTS

[0121] The following exemplary embodiments are provided herein.

Set I:

[0122] Embodiment I-1. A method of preparing a peptide sample comprising:

(a) providing a blood, serum or plasma sample from a pregnant subject at around 24 weeks or 34 weeks of pregnancy;

(b) enriching the sample for microparticles by loading the sample on a size-exclusion column and eluting the microparticles from the column using water as the mobile phase, to produce a microparticle-enriched fraction;

(c) preparing a microparticle-associated peptide fraction from the microparticle-enriched fraction by contacting with a protease;

(d) separating the microparticle-associated peptides by mass spectrometry; and

(e) measuring, based on a mass spectrometry signal, one or more peptides corresponding to each of one or more protein biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3 or Table 5, wherein the blood sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4 or Table 6, wherein the blood sample is collected at about 34 weeks of pregnancy.

[0123] Embodiment I-2. The method of embodiment I-1, wherein the one or more protein biomarkers is a plurality of protein biomarkers.

[0124] Embodiment I-3. The method of embodiment I-1, wherein the biomarkers comprise a panel of no more than any of 10, 9, 8, 7, 6, 5, 4 or 3 protein biomarkers.

[0125] Embodiment I-4. The method of embodiment I-1, wherein the biomarkers comprise, consist essentially of or consist of a panel of biomarkers selected from:

(i) a biomarker panel of Table 7, wherein the blood sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 8, wherein the blood sample is collected at about 34 weeks of pregnancy.

[0126] Embodiment I-5. The method of embodiment I-1, wherein measuring the one or more peptides comprises measuring a surrogate biomarker of any of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, FIGS. 15A-15B.

[0127] Embodiment I-6. The method of embodiment I-1, wherein the pregnant subject has one or more risk factors for placenta accreta.

[0128] Embodiment I-7. The method of embodiment I-1, wherein the pregnant subject is primigravida, multigravida, primiparous or multiparous.

[0129] Embodiment I-8. The method of embodiment I-1, wherein the blood sample is plasma or serum.

[0130] Embodiment I-9. The method of embodiment I-1, wherein the water is deionized distilled water ("ddH₂O").

[0131] Embodiment I-10. The method of embodiment I-1, wherein the size-exclusion chromatography is performed with an agarose solid phase and an aqueous liquid phase.

[0132] Embodiment I-11. The method of embodiment I-1, wherein the preparing step further comprises using ultrafiltration or reverse-phase chromatography.

[0133] Embodiment I-12. The method of embodiment I-1, wherein the preparing step further comprises denaturation using urea, reduction using dithiothreitol, alkylation using iodoacetamine, and digestion using trypsin after the size exclusion chromatography.

[0134] Embodiment I-13. The method of embodiment I-1, wherein the microparticles are further purified to enrich for placental-derived exosomes or vascular endothelial-derived exosomes.

[0135] Embodiment I-14. The method of embodiment I-1, wherein mass spectrometry comprises liquid chromatography/mass spectrometry (LC/MS), e.g., liquid chromatography/triple quadrupole mass spectrometry.

[0136] Embodiment I-15. The method of embodiment I-1, wherein the mass spectrometry comprises multiple reaction monitoring.

[0137] Embodiment I-16. The method of embodiment I-1, wherein the peptide(s) are selected from:

(i) a biomarker panel of Table 7, wherein the blood sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 8, wherein the blood sample is collected at about 34 weeks of pregnancy.

[0138] Embodiment I-17. A panel comprising a plurality of substantially pure protein biomarkers or surrogate biomarkers selected from:

- (i) a protein biomarker of Table 1, Table 3 or Table 5;
- (ii) a protein biomarker of Table 2, Table 4 or Table 6;
- (iii) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C or FIGS. 12A-12H; and
- (iv) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D or FIGS. 15A-15B.

[0139] Embodiment I-18. The panel of embodiment I-17, further comprising a stable isotope standard peptide paired with each of the surrogate biomarkers.

[0140] Embodiment I-19. A kit comprising one or a plurality of containers, wherein each container comprises one or more of each of a plurality of Stable Isotopic Standards, each stable isotopic standard corresponding to a surrogate peptide for a biomarker from a panel of biomarkers selected from:

- (i) a protein biomarker of Table 1, Table 3, or Table 5;
- (ii) a protein biomarker of Table 2, Table 4, or Table 6
- (iii) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C or FIGS. 12A-12H; and
- (iii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D or FIGS. 15A-15B.

[0141] Embodiment I-20. A composition comprising one or a plurality of pairs of polypeptides, each pair comprising a protein biomarkers or surrogate biomarkers selected from:

- (i) a protein biomarker of Table 1, Table 3, or Table 5;
- (ii) a protein biomarker of Table 2, Table 4, or Table 6
- (iii) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C or FIGS. 12A-12H; and

(iii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D or FIGS. 15A-15B.

[0142] Embodiment I-21. A computer readable medium in tangible, non-transitory form comprising code to implement a classification rule generated by a method as described herein.

[0143] Embodiment I-22. A system comprising:

(a) a computer comprising:

(i) a processor; and

(ii) a memory, coupled to the processor, the memory storing a module comprising:

(1) test data for a sample from a subject including values indicating a measure of one or more protein biomarkers in the fraction, wherein the protein biomarkers are selected from

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the sample is collected at about 20 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the sample is collected at about 37 weeks of pregnancy;

(2) a classification rule which, based on values including the measurements, classifies the subject as being at increased risk of placenta accreta, wherein the classification rule is configured to have a sensitivity of at least 75%, at least 85% or at least 95%; and

(3) computer executable instructions for implementing the classification rule on the test data.

[0144] Embodiment I-23. The system of embodiment I-22, wherein the protein biomarker is a surrogate biomarker selected from:

(iii) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C or FIGS. 12A-12H; and

(iii) a surrogate biomarker of FIGS. 13A-13H, FIG. 14A-14D or FIG 15A-15B.

[0145] Embodiment I-24. A method comprising:

(a) at a computer system comprising a processor and a memory, coupled to the processor, the memory storing a module comprising:

(1) test data for a sample from a subject including values indicating a measure of one or more protein biomarkers in the fraction, wherein the protein biomarkers are selected from

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the sample is collected at about 34 weeks of pregnancy;

(2) a classification rule to be executed by the processor, which, based on values including the measurements, classifies the subject as being at increased risk of placenta accreta, wherein the classification rule is configured to have a sensitivity of at least 75%, at least 85% or at least 95%;

(b) accessing the text data; and

(c) executing the classification rule on the test data.

[0146] Embodiment I-25. A method of assessing risk of placenta accreta in a pregnant subject, the method comprising:

(a) preparing a microparticle-enriched fraction from a blood sample from a pregnant subject;

(b) determining a measure of one or more microparticle-associated protein biomarkers in the fraction, wherein protein biomarkers are selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 34 weeks of pregnancy;

(c) assessing risk of placenta accreta based on the one or more measures.

[0147] Embodiment I-26. The method of embodiment I-25, wherein the protein biomarker is a surrogate biomarker selected from:

(iii) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C or FIGS. 12A-12H; and

(iii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D or FIGS. 15A-15B.

[0148] Embodiment I-27. The method of embodiment I-25, wherein determining a quantitative measure comprises contacting the sample with one or more capture reagents, each capture reagent specifically binding one of the protein biomarkers, and detecting binding between the capture reagent in the protein biomarker.

[0149] Embodiment I-28. The method of embodiment I-27, comprising performing an immunoassay.

[0150] Embodiment I-29. The method of embodiment I-28, wherein the immunoassay is selected from the group consisting of enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA).

[0151] Embodiment I-30. The method of embodiment I-25, wherein the assessing comprises executing a classification rule, which rule classifies the subject at being at risk of placenta accreta, and wherein execution of the classification rule produces a correlation between placenta accreta or term birth with a p value of less than at least 0.05.

[0152] Embodiment I-31. The method of embodiment I-25, wherein the assessing comprises executing a classification rule, which rule classifies the subject at being at risk of placenta accreta, and wherein execution of the classification rule produces a receiver operating

characteristic (ROC) curve, wherein the ROC curve has an area under the curve (AUC) of at least 0.6, at least 0.7, at least 0.8 or at least 0.9.

[0153] Embodiment I-32. The method of embodiment I-25, wherein values on which the classification rule classifies a subject further include at least one of: placenta previa, previous cesarean delivery, endometrial ablation, in vitro fertilization, prior uterine infection, or previous uterine surgery.

[0154] Embodiment I-33. The method of any of the preceding embodiments, wherein the classification rule employs cut-off, linear regression (e.g., multiple linear regression (MLR), partial least squares (PLS) regression, principal components regression (PCR)), binary decision trees (e.g., recursive partitioning processes such as CART - classification and regression trees), artificial neural networks such as back propagation networks, discriminant analyses (e.g., Bayesian classifier or Fischer analysis), logistic classifiers, and support vector classifiers (e.g., support vector machines).

[0155] Embodiment I-34. The method of embodiment I-25, wherein the classification rule is configured to have a sensitivity, specificity, positive predictive value, or negative predictive value of at least 70%, least 80%, at least 90% or at least 95%.

[0156] Embodiment I-35. The method of embodiment I-25, wherein assessing an increased risk of placenta accreta comprises determining that the protein biomarker (if upregulated) is above or (if down regulated) is below a threshold level.

[0157] Embodiment I-36. The method of embodiment I-35, wherein the threshold level represents a level at least one, at least two or at least three z scores from a measure of central tendency (e.g., mean, median or mode) for the protein determined from at least 50, at least 100 or at least 200 control subjects.

[0158] Embodiment I-37. The method of embodiment I-25, wherein the assessing comprises comparing the measure of each protein in the panel to a reference standard.

[0159] Embodiment I-38. The method of embodiment I-25, further comprising communicating the risk of placenta accreta for a pregnant subject to a health care provider.

[0160] Embodiment I-39. A method of treating placenta accreta in a pregnant subject, the method comprising:

(a) assessing risk of placenta accreta for a pregnant subject according to the method of any one of embodiments I-25 to I-38; and

(b) administering a therapeutic intervention to the subject effective to decrease the risk of placenta accreta and/or reduce neonatal complications of placenta accreta.

[0161] Embodiment I-40. The method of embodiment I-39, wherein treating comprises a therapeutic intervention selected from the group consisting of:

(i) referring the subject to a medical center with advanced multidisciplinary surgical expertise and experience;

(ii) surgical uterine conservation; and

(iii) performing a Cesarean hysterectomy, performing a prophylactic embolization, inserting a uterine balloon tamponade, a temporal internal iliac occlusion balloon catheter, a ureteral stents, administering methotrexate, leaving a portion of the placenta in-situ, and referring the subject to bed rest to prevent preterm labor..

[0162] Embodiment I-41. A method comprising administering to a pregnant subject determined to have an increased risk of placenta accreta by a method as described herein, a therapeutic intervention effective to reduce the risk of placenta accreta.

[0163] Embodiment I-42. A method of administering to a pregnant subject an effective amount of a treatment designed to reduce the risk of placenta accreta, wherein the subject has an altered quantitative measure as compared to a reference standard of any one of the panels of protein biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3 or Table 5, wherein the blood sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4 or Table 6, wherein the blood sample is collected at about 34 weeks of pregnancy.

[0164] Embodiment I-43. The method of embodiment I-42, wherein the protein biomarker is a surrogate biomarker selected from:

- (i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C or FIGS. 12A-12H; and
- (ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D or FIGS. 15A-15B.

[0165] Embodiment I-44. A method comprising:

a) measuring, via mass spectrometry, masses of more than 100, more than 1000, more than 10,000 or more than 100,000 peptides from a biological sample comprising peptide fragments of proteins, to produce a dataset comprising more than 100, more than 1000, more than 10,000 or more than 100,000 data entries;

b) at a computer system comprising one or more processors and memory storing programs for execution by the one or more processors;

(1) identifying from among the data entries, based on the masses, peptides corresponding to:

(i) a protein biomarker of Table 1, Table 3 or Table 5, wherein the biological sample is collected at about 24 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4 or Table 6, wherein the biological sample is collected at about 34 weeks of pregnancy;

(2) executing a classification rule on the measures of the identified peptides, which classification rule classifies the sample as being from subject at increased risk of placenta accreta.

Set II:

[0166] Embodiment II-1. A method of assessing the risk of placenta accreta in a pregnant subject, comprising:

- (a) providing a sample from a pregnant subject between about 20 weeks of pregnancy to about 37 weeks of pregnancy;
- (b) preparing a microparticle-associated peptide fraction from the sample;
- (c) measuring a plurality of protein biomarkers in the fraction; and
- (d) executing a classification rule on one or more measurement values of (c), wherein the classification rule classifies the sample as being from a subject at increased risk of placenta accreta.

[0167] Embodiment II-2. The method of embodiment II-1, wherein the protein biomarkers comprise a panel of no more than any of 10, 9, 8, 7, 6, 5, 4 or 3 protein biomarkers.

[0168] Embodiment II-3. The method of embodiment II-1, wherein the protein biomarkers comprise, consist essentially of or consist of a panel of biomarkers selected from:

- (i) a biomarker panel of Table 7; and
- (ii) a protein biomarker of Table 8.

[0169] Embodiment II-4. The method of embodiment II-1, wherein the plurality of protein biomarkers comprise:

- (i) a plurality of protein biomarkers from Table 1, Table 3, and Table 5;
- (ii) a plurality of protein biomarkers from Table 2, Table 4, and Table 6;
- (iii) a plurality of protein biomarkers from Table 9;
- (iv) two or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); or
- (v) two or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

[0170] Embodiment II-5. The method of embodiment II-1, wherein measuring the plurality of biomarkers comprises measuring the relevant surrogate biomarkers of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

[0171] Embodiment II-6. The method of embodiment II-1, wherein the pregnant subject has one or more risk factors for placenta accreta.

[0172] Embodiment II-7. The method of embodiment II-1, wherein the pregnant subject is primigravida, multigravida, primiparous or multiparous.

[0173] Embodiment II-8. The method of embodiment II-1, wherein the sample is a blood sample.

[0174] Embodiment II-9. The method of embodiment II-1, wherein the sample is plasma or serum.

[0175] Embodiment II-10. A panel comprising a plurality of substantially pure protein biomarkers or surrogate biomarkers selected from:

- (i) a protein biomarker of Table 1, Table 3, or Table 5;
- (ii) a protein biomarker of Table 2, Table 4, or Table 6
- (iii) a protein biomarker of Table 9;
- (iv) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;
- (v) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;
- (vi) isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); and
- (vii) isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

[0176] Embodiment II-11. The panel of embodiment II-10, further comprising a stable isotope standard peptide paired with each of the surrogate biomarkers of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

[0177] Embodiment II-12. A method of preparing a peptide sample, comprising:

(a) providing a sample from a pregnant subject between about 20 weeks of pregnancy to about 37 weeks of pregnancy;

(b) enriching the sample for microparticles by loading the sample on a size-exclusion column and eluting the microparticles from the column using water as a mobile phase, to produce a microparticle-enriched fraction;

(c) preparing a microparticle-associated peptide fraction from the microparticle-enriched fraction by contacting the microparticle-enriched fraction with a protease;

(d) separating the microparticle-associated peptides by mass spectrometry; and

(e) measuring, based on a mass spectrometry signal, one or more peptides corresponding to one or more protein biomarkers.

[0178] Embodiment II-13. The method of embodiment II-12, wherein the one or more protein biomarkers includes a plurality of protein biomarkers.

[0179] Embodiment II-14. The method of embodiment II-12, wherein the protein biomarkers comprise a panel of no more than any of 10, 9, 8, 7, 6, 5, 4 or 3 protein biomarkers.

[0180] Embodiment II-15. The method of embodiment II-12, wherein the protein biomarkers comprise, consist essentially of or consist of a panel of biomarkers selected from:

(i) a biomarker panel of Table 7; and

(ii) a protein biomarker of Table 8.

[0181] Embodiment II-16. The method of embodiment II-12, wherein the protein biomarkers comprise:

(i) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); or

(ii) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

[0182] Embodiment II-17. The method of embodiment II-12, wherein measuring the one or more peptides comprises measuring a surrogate biomarker of any of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

[0183] Embodiment II-18. The method of embodiment II-12, wherein the pregnant subject has one or more risk factors for placenta accreta.

[0184] Embodiment II-19. The method of embodiment II-12, wherein the pregnant subject is primigravida, multigravida, primiparous or multiparous.

[0185] Embodiment II-20. The method of embodiment II-12, wherein the blood sample is plasma or serum.

[0186] Embodiment II-21. The method of embodiment II-12, wherein the water is deionized distilled water.

[0187] Embodiment II-22. The method of embodiment II-12, wherein the size-exclusion column comprises an agarose solid phase and an aqueous liquid phase.

[0188] Embodiment II-23. The method of embodiment II-12, wherein preparing the microparticle-associated peptide fraction further comprises using ultrafiltration or reverse-phase chromatography.

[0189] Embodiment II-24. The method of embodiment II-12, wherein preparing the microparticle-associated peptide fraction further comprises denaturation of the microparticle-enriched fraction using urea, reduction of the microparticle-enriched fraction using dithiothreitol, alkylation of the microparticle-enriched fraction using iodoacetamine, and digestion of the microparticle-enriched fraction using trypsin.

[0190] Embodiment II-25. The method of embodiment II-12, wherein enriching the sample for microparticles includes further purifying the microparticles to enrich for placental-derived exosomes or vascular endothelial-derived exosomes.

[0191] Embodiment II-26. The method of embodiment II-12, wherein separating the microparticle-associated peptides by mass spectrometry comprises separating the microparticle-associated peptides by liquid chromatography/mass spectrometry (LC/MS) including liquid chromatography/triple quadrupole mass spectrometry.

[0192] Embodiment II-27. The method of embodiment II-12, wherein separating the microparticle-associated peptides by mass spectrometry includes the mass spectrometry comprising multiple reaction monitoring.

[0193] Embodiment II-28. The method of embodiment II-12, wherein the one or more peptides are selected from:

(i) a biomarker panel of Table 7, wherein the blood sample is collected at about 20 weeks of pregnancy; and

(ii) a protein biomarker of Table 8, wherein the blood sample is collected at about 37 weeks of pregnancy.

[0194] Embodiment II-29. A kit comprising one or a plurality of containers, wherein each container comprises one or more of each of a plurality of Stable Isotopic Standards, wherein each stable isotopic standard corresponds to a surrogate peptide for a biomarker from a panel of biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5;

(ii) a protein biomarker of Table 2, Table 4, or Table 6;

(iii) a protein biomarker of Table 9;

(iv) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;

(v) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;

(vi) isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); and

(vi) isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

[0195] Embodiment II-30. A composition comprising one or a plurality of pairs of polypeptides, wherein each pair of polypeptides comprise one or more protein biomarkers or one or more surrogate biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5;

(ii) a protein biomarker of Table 2, Table 4, or Table 6

(iii) a protein biomarker of Table 9;

(iv) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;

(v) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;

(vi) isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); and

(vii) isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

[0196] Embodiment II-31. A computer readable medium in tangible, non-transitory form comprising code implementing one or more classification rules generated by analysis of one or more datasets of biomarker measurements derived from one or more pregnant subjects classified into a first group at risk for placenta accreta or a second group not at risk of placenta accreta.

[0197] Embodiment II-32. A system comprising:

(a) a computer comprising:

(i) a processor; and

(ii) a memory, coupled to the processor, the memory storing a module comprising:

(1) test data for a sample from a subject including one or more values, wherein each value indicates a measurement of one or more protein biomarkers in a fraction of microparticle-associated peptides, wherein the one or more protein biomarkers are selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 20 weeks of pregnancy;

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 37 weeks of pregnancy;

(iii) a protein biomarker of Table 9;

(iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein; and

(2) a classification rule which, based on the one or more values wherein each value indicates the measurement, classifies the subject as being at increased risk of placenta accreta, wherein the classification rule is configured to have a sensitivity value of at least 75%, at least 85% or at least 95%; and

(3) computer executable instructions for implementing the classification rule on the test data.

[0198] Embodiment II-33. The system of embodiment II-32, wherein the protein biomarker is a surrogate biomarker selected from:

(i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H; and

(ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B.

[0199] Embodiment II-34. A method comprising:

(a) using a computer system to compile test data, the computer system comprising a processor and a memory, coupled to the processor, the memory storing a module comprising:

(1) test data for a sample from a subject including values indicating one or more measurement values of one or more protein biomarkers of the disclosure in a fraction of microparticle-associated peptides;

(2) a classification rule to be executed by the processor, which, based on values including the one or more measurement values, classifies the subject as being at increased risk of placenta accreta, wherein the classification rule is configured to have a sensitivity of at least 75%, at least 85% or at least 95%;

(b) accessing the test data; and

(c) executing the classification rule on the test data.

[0200] Embodiment II-35. A method of assessing risk of placenta accreta in a pregnant subject, the method comprising:

(a) preparing a microparticle-enriched fraction from a blood sample from a pregnant subject;

(b) determining a quantitative measure of one or more microparticle-associated protein biomarkers in the microparticle-enriched fraction, wherein the one or more microparticle-associated protein biomarkers are selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 20 weeks of pregnancy;

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 37 weeks of pregnancy;

(iii) a protein biomarker of Table 9;

(iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein; and

(c) assessing risk of placenta accreta based on the one or more quantitative measures.

[0201] Embodiment II-36. The method of embodiment II-35, wherein the protein biomarker is a surrogate biomarker selected from:

(i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;

(ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;

(iii) a surrogate biomarker of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(iv) a surrogate biomarker of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

[0202] Embodiment II-37. The method of embodiment II-35, wherein determining the quantitative measure of one or more microparticle-associated protein biomarkers comprises contacting the sample with one or more capture reagents, each capture reagent specifically binding one of the protein biomarkers, and detecting binding between the capture reagent and the protein biomarker.

[0203] Embodiment II-38. The method of embodiment II-37, wherein determining the quantitative measure of one or more microparticle-associated protein biomarkers comprises performing an immunoassay.

[0204] Embodiment II-39. The method of embodiment II-38, wherein the immunoassay is selected from the group consisting of an enzyme immunoassay (EIA), an enzyme-linked immunosorbent assay (ELISA), and a radioimmunoassay (RIA).

[0205] Embodiment II-40. The method of embodiment II-35, wherein the assessing risk of placenta accreta comprises executing a classification rule, wherein the classification rule classifies the subject at being at risk of placenta accreta, and wherein execution of the classification rule produces a correlation between placenta accreta or term birth with a p value of less than at least 0.05.

[0206] Embodiment II-41. The method of embodiment II-35, wherein the assessing risk of placenta accreta comprises executing a classification rule, wherein the classification rule classifies the subject at being at risk of placenta accreta, and wherein execution of the classification rule produces a receiver operating characteristic (ROC) curve, wherein the ROC curve has an area under the curve (AUC) of at least 0.6, at least 0.7, at least 0.8 or at least 0.9.

[0207] Embodiment II-42. The method of embodiment II-35, wherein the classification rule classifies a subject based on one or more values wherein the one or more values further include at least one of: placenta previa, previous cesarean delivery, endometrial ablation, in vitro fertilization, prior uterine infection, or previous uterine surgery.

[0208] Embodiment II-43. The method of embodiments II-25 to II-32, wherein the classification rule employs cut-off, linear regression including multiple linear regression, partial least squares regression, principal components regression, binary decision trees including recursive partitioning processes further including classification and regression trees, artificial neural networks including back propagation networks, discriminant analyses further including Bayesian classifier or Fischer analysis, logistic classifiers, and support vector classifiers including support vector machines.

[0209] Embodiment II-44. The method of embodiment II-35, wherein the classification rule is configured to have a sensitivity value, a specificity value, a positive predictive value, or a negative predictive value of at least 70%, least 80%, at least 90% or at least 95%.

[0210] Embodiment II-45. The method of embodiment II-35, wherein assessing risk of placenta accreta comprises determining that the protein biomarker, if upregulated, is above a threshold level or if down regulated, is below the threshold level.

[0211] Embodiment II-46. The method of embodiment II-45, wherein the threshold level represents a level at least one, at least two or at least three z scores from a measure of central tendency including a mean, a median or a mode for the protein biomarker determined from at least 50, at least 100 or at least 200 control subjects.

[0212] Embodiment II-47. The method of embodiment II-35, wherein the assessing risk of placenta accreta comprises comparing the one or more quantitative measures of each protein biomarker in the panel to a reference standard.

[0213] Embodiment II-48. The method of embodiment II-35, further comprising communicating the risk of placenta accreta for a pregnant subject to a health care provider.

[0214] Embodiment II-49. A method of treating placenta accreta in a pregnant subject, the method comprising:

(a) assessing risk of placenta accreta for a pregnant subject according to the method of any one of embodiments II-1 to II-9, and II-35 to II-48; and

(b) administering a therapeutic intervention to the subject effective to decrease the risk of placenta accreta and/or reduce neonatal complications of placenta accreta.

[0215] Embodiment II-50. The method of embodiment II-49, wherein administering the therapeutic intervention comprises a therapeutic intervention selected from the group consisting of:

(i) referring the subject to a medical center having advanced multidisciplinary surgical expertise and experience;

(ii) planning surgical uterine conservation; and

(iii) performing a Cesarean hysterectomy, performing a prophylactic embolization, inserting a uterine balloon tamponade, a temporal internal iliac occlusion balloon catheter, a

ureteral stents, administering methotrexate, leaving a portion of the placenta in-situ, and referring the subject to bed rest to prevent preterm labor.

[0216] Embodiment II-51. A method comprising administering to a pregnant subject determined to have an increased risk of placenta accreta by a method according to any one of embodiments II-1 to II-9, and II-35 to II-48, a therapeutic intervention effective to reduce the risk of placenta accreta.

[0217] Embodiment II-52. A method of administering to a pregnant subject an effective amount of a treatment designed to reduce the risk of placenta accreta, wherein the subject has an altered quantitative measure as compared to a reference standard of any one of a panel of protein biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 20 weeks of pregnancy;

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 37 weeks of pregnancy;

(iii) a protein biomarker of Table 9;

(iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

[0218] Embodiment II-53. The method of embodiment II-52, wherein the protein biomarker is a surrogate biomarker selected from:

(i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS 12A-12H;

(ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;

(iii) a surrogate biomarker of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(iv) a surrogate biomarker of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

[0219] Embodiment II-54. A method comprising:

a) measuring, via mass spectrometry, masses of more than 100, more than 1000, more than 10,000 or more than 100,000 peptides from a biological sample comprising peptide fragments of proteins, to produce a dataset comprising more than 100, more than 1000, more than 10,000 or more than 100,000 data entries;

b) using a computer system comprising one or more processors and memory storing programs for execution by the one or more processors in

(1) identifying from among the data entries, based on the masses, peptides corresponding to:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the biological sample is collected at about 20 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the biological sample is collected at about 37 weeks of pregnancy;

(iii) a protein biomarker of Table 9;

(iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

(2) executing a classification rule on one or more measurement values of the identified peptides, wherein the classification rule classifies the sample as being from a subject at increased risk of placenta accreta.

[0220] As used herein, the following meanings apply unless otherwise specified. The word “may” is used in a permissive sense (i.e., meaning having the potential to), rather than the mandatory sense (i.e., meaning must). The words “include”, “including”, and “includes” and the like mean including, but not limited to. The singular forms “a,” “an,” and “the” include plural referents. Thus, for example, reference to “an element” includes a combination of two or more elements, notwithstanding use of other terms and phrases for one or more elements, such as “one or more.” The phrase “at least one” includes “one”, “one or more”, “one or a plurality” and “a plurality”. The term “or” is, unless indicated otherwise, non-exclusive, i.e., encompassing both “and” and “or.” The term “any of” between a modifier and a sequence means that the modifier modifies each member of the sequence. So, for example, the phrase “at least any of 1, 2 or 3” means “at least 1, at least 2 or at least 3”. The term "consisting essentially of" refers to the inclusion of recited elements and other elements that do not materially affect the basic and novel characteristics of a claimed combination. Unless otherwise specified, the term “about” in reference to a value refers to 90% to 110% of that value or 95% to 105% of that value.

[0221] It should be understood that the description and the drawings are not intended to limit the invention to the particular form disclosed, but to the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present invention as defined by the appended claims. Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description and the drawings are to be construed as illustrative only and are for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as examples of embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed or omitted, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims. Headings used herein are for organizational purposes only and are not meant to be used to limit the scope of the description.

CLAIMS

What is claimed is:

1. A method of assessing the risk of placenta accreta in a pregnant subject, comprising:
 - (a) providing a sample from a pregnant subject between about 20 weeks of pregnancy to about 37 weeks of pregnancy;
 - (b) preparing a microparticle-associated peptide fraction from the sample;
 - (c) measuring a plurality of protein biomarkers in the fraction; and
 - (d) executing a classification rule on one or more measurement values of (c), wherein the classification rule classifies the sample as being from a subject at increased risk of placenta accreta.
2. The method of claim 1, wherein the protein biomarkers comprise a panel of no more than any of 10, 9, 8, 7, 6, 5, 4 or 3 protein biomarkers.
3. The method of claim 1, wherein the protein biomarkers comprise, consist essentially of or consist of a panel of biomarkers selected from:
 - (i) a biomarker panel of Table 7; and
 - (ii) a protein biomarker of Table 8.
4. The method of claim 1, wherein the plurality of protein biomarkers comprise:
 - (i) a plurality of protein biomarkers from Table 1, Table 3, and Table 5;
 - (ii) a plurality of protein biomarkers from Table 2, Table 4, and Table 6;
 - (iii) a plurality of protein biomarkers from Table 9;
 - (iv) two or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); or

(v) two or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

5. The method of claim 1, wherein measuring the plurality of biomarkers comprises measuring the relevant surrogate biomarkers of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

6. The method of claim 1, wherein the pregnant subject has one or more risk factors for placenta accreta.

7. The method of claim 1, wherein the pregnant subject is primigravida, multigravida, primiparous or multiparous.

8. The method of claim 1, wherein the sample is a blood sample.

9. The method of claim 1, wherein the sample is plasma or serum.

10. A panel comprising a plurality of substantially pure protein biomarkers or surrogate biomarkers selected from:

- (i) a protein biomarker of Table 1, Table 3, or Table 5;
- (ii) a protein biomarker of Table 2, Table 4, or Table 6;
- (iii) a protein biomarker of Table 9;
- (iv) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;
- (v) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS 15A-15B;
- (vi) isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); and
- (vii) isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

11. The panel of claim 10, further comprising a stable isotope standard peptide paired with each of the surrogate biomarkers of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

12. A method of preparing a peptide sample, comprising:

(a) providing a sample from a pregnant subject between about 20 weeks of pregnancy to about 37 weeks of pregnancy;

(b) enriching the sample for microparticles by loading the sample on a size-exclusion column and eluting the microparticles from the column using water as a mobile phase, to produce a microparticle-enriched fraction;

(c) preparing a microparticle-associated peptide fraction from the microparticle-enriched fraction by contacting the microparticle-enriched fraction with a protease;

(d) separating the microparticle-associated peptides by mass spectrometry; and

(e) measuring, based on a mass spectrometry signal, one or more peptides corresponding to one or more protein biomarkers.

13. The method of claim 12, wherein the one or more protein biomarkers includes a plurality of protein biomarkers.

14. The method of claim 12, wherein the protein biomarkers comprise a panel of no more than any of 10, 9, 8, 7, 6, 5, 4 or 3 protein biomarkers.

15. The method of claim 12, wherein the protein biomarkers comprise, consist essentially of or consist of a panel of biomarkers selected from:

(i) a biomarker panel of Table 7; and

(ii) a protein biomarker of Table 8.

16. The method of claim 12, wherein the protein biomarkers comprise:

(i) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); or

(ii) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

17. The method of claim 12, wherein measuring the one or more peptides comprises measuring a surrogate biomarker of any of FIGS. 10A-10C, FIGS. 11A-11C, FIGS. 12A-12H, FIGS. 13A-13H, FIGS. 14A-14D, and FIGS. 15A-15B.

18. The method of claim 12, wherein the pregnant subject has one or more risk factors for placenta accreta.

19. The method of claim 12, wherein the pregnant subject is primigravida, multigravida, primiparous or multiparous.

20. The method of claim 12, wherein the blood sample is plasma or serum.

21. The method of claim 12, wherein the water is deionized distilled water.

22. The method of claim 12, wherein the size-exclusion column comprises an agarose solid phase and an aqueous liquid phase.

23. The method of claim 12, wherein preparing the microparticle-associated peptide fraction further comprises using ultrafiltration or reverse-phase chromatography.

24. The method of claim 12, wherein preparing the microparticle-associated peptide fraction further comprises denaturation of the microparticle-enriched fraction using urea, reduction of the microparticle-enriched fraction using dithiothreitol, alkylation of the microparticle-enriched fraction using iodoacetamine, and digestion of the microparticle-enriched fraction using trypsin.

25. The method of claim 12, wherein enriching the sample for microparticles includes further purifying the microparticles to enrich for placental-derived exosomes or vascular endothelial-derived exosomes.

26. The method of claim 12, wherein separating the microparticle-associated peptides by mass spectrometry comprises separating the microparticle-associated peptides by liquid

chromatography/mass spectrometry (LC/MS) including liquid chromatography/triple quadrupole mass spectrometry.

27. The method of claim 12, wherein separating the microparticle-associated peptides by mass spectrometry includes the mass spectrometry comprising multiple reaction monitoring.

28. The method of claim 12, wherein the one or more peptides are selected from:

(i) a biomarker panel of Table 7, wherein the blood sample is collected at about 20 weeks of pregnancy; and

(ii) a protein biomarker of Table 8, wherein the blood sample is collected at about 37 weeks of pregnancy.

29. A kit comprising one or a plurality of containers, wherein each container comprises one or more of each of a plurality of Stable Isotopic Standards, wherein each stable isotopic standard corresponds to a surrogate peptide for a biomarker from a panel of biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5;

(ii) a protein biomarker of Table 2, Table 4, or Table 6;

(iii) a protein biomarker of Table 9;

(iv) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;

(v) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;

(vi) isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); and

(vii) isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

30. A composition comprising one or a plurality of pairs of polypeptides, wherein each pair of polypeptides comprise one or more protein biomarkers or one or more surrogate biomarkers selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5;

(ii) a protein biomarker of Table 2, Table 4, or Table 6;

- (iii) a protein biomarker of Table 9;
- (iv) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;
- (v) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;
- (vi) isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and cartilage acidic protein 1 (CRAC1); and
- (vii) isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and Ig-like domain-containing protein.

31. A computer readable medium in tangible, non-transitory form comprising code implementing one or more classification rules generated by analysis of one or more datasets of biomarker measurements derived from one or more pregnant subjects classified into a first group at risk for placenta accreta or a second group not at risk of placenta accreta.

32. A system comprising:

(a) a computer comprising:

(i) a processor; and

(II) a memory, coupled to the processor, the memory storing a module comprising:

(1) test data for a sample from a subject including one or more values, wherein each value indicates a measurement of one or more protein biomarkers in a fraction of microparticle-associated peptides, wherein the one or more protein biomarkers are selected from:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 20 weeks of pregnancy;

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 37 weeks of pregnancy;

(iii) a protein biomarker of Table 9;

(iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein; and

(2) a classification rule which, based on the one or more values wherein each value indicates the measurement, classifies the subject as being at increased risk of placenta accreta, wherein the classification rule is configured to have a sensitivity value of at least 75%, at least 85% or at least 95%; and

(3) computer executable instructions for implementing the classification rule on the test data.

33. The system of claim 32, wherein the protein biomarker is a surrogate biomarker selected from:

(i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H; and

(ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B.

34. A method comprising:

(a) using a computer system to compile test data, the computer system comprising a processor and a memory, coupled to the processor, the memory storing a module comprising:

(1) test data for a sample from a subject including values indicating one or more measurement values of one or more protein biomarkers of the disclosure in a fraction of microparticle-associated peptides;

(2) a classification rule to be executed by the processor, which, based on values including the one or more measurement values, classifies the subject as being at increased risk of placenta accreta, wherein the classification rule is configured to have a sensitivity of at least 75%, at least 85% or at least 95%;

(b) accessing the test data; and

(c) executing the classification rule on the test data.

35. A method of assessing risk of placenta accreta in a pregnant subject, the method comprising:

- (a) preparing a microparticle-enriched fraction from a blood sample from a pregnant subject;
- (b) determining a quantitative measure of one or more microparticle-associated protein biomarkers in the microparticle-enriched fraction, wherein the one or more microparticle-associated protein biomarkers are selected from:
 - (i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 20 weeks of pregnancy;
 - (ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 37 weeks of pregnancy;
 - (iii) a protein biomarker of Table 9;
 - (iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and
 - (v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein; and
- (c) assessing risk of placenta accreta based on the one or more quantitative measures.

36. The method of claim 35, wherein the protein biomarker is a surrogate biomarker selected from:

- (i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS. 12A-12H;
- (ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;
- (iii) a surrogate biomarker of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and
- (iv) a surrogate biomarker of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

37. The method of claim 35, wherein determining the quantitative measure of one or more microparticle-associated protein biomarkers comprises contacting the sample with one or more capture reagents, each capture reagent specifically binding one of the protein biomarkers, and detecting binding between the capture reagent and the protein biomarker.

38. The method of claim 37, wherein determining the quantitative measure of one or more microparticle-associated protein biomarkers comprises performing an immunoassay.

39. The method of claim 38, wherein the immunoassay is selected from the group consisting of an enzyme immunoassay (EIA), an enzyme-linked immunosorbent assay (ELISA), and a radioimmunoassay (RIA).

40. The method of claim 35, wherein the assessing risk of placenta accreta comprises executing a classification rule, wherein the classification rule classifies the subject at being at risk of placenta accreta, and wherein execution of the classification rule produces a correlation between placenta accreta or term birth with a p value of less than at least 0.05.

41. The method of claim 35, wherein the assessing risk of placenta accreta comprises executing a classification rule, wherein the classification rule classifies the subject at being at risk of placenta accreta, and wherein execution of the classification rule produces a receiver operating characteristic (ROC) curve, wherein the ROC curve has an area under the curve (AUC) of at least 0.6, at least 0.7, at least 0.8 or at least 0.9.

42. The method of claim 35, wherein the classification rule classifies a subject based on one or more values wherein the one or more values further include at least one of: placenta previa, previous cesarean delivery, endometrial ablation, in vitro fertilization, prior uterine infection, or previous uterine surgery.

43. The method of any claim of claims 25-32, wherein the classification rule employs cut-off, linear regression including multiple linear regression, partial least squares regression, principal components regression, binary decision trees including recursive partitioning processes further including classification and regression trees, artificial neural networks including back propagation networks, discriminant analyses further including Bayesian classifier

or Fischer analysis, logistic classifiers, and support vector classifiers including support vector machines.

44. The method of claim 35, wherein the classification rule is configured to have a sensitivity value, a specificity value, a positive predictive value, or a negative predictive value of at least 70%, least 80%, at least 90% or at least 95%.

45. The method of claim 35, wherein assessing risk of placenta accreta comprises determining that the protein biomarker, if upregulated, is above a threshold level or if down regulated, is below the threshold level.

46. The method of claim 45, wherein the threshold level represents a level at least one, at least two or at least three z scores from a measure of central tendency including a mean, a median or a mode for the protein biomarker determined from at least 50, at least 100 or at least 200 control subjects.

47. The method of claim 35, wherein the assessing risk of placenta accreta comprises comparing the one or more quantitative measures of each protein biomarker in the panel to a reference standard.

48. The method of claim 35, further comprising communicating the risk of placenta accreta for a pregnant subject to a health care provider.

49. A method of treating placenta accreta in a pregnant subject, the method comprising:

(a) assessing risk of placenta accreta for a pregnant subject according to the method of any one of claims 1 to 9, and 35 to 48; and

(b) administering a therapeutic intervention to the subject effective to decrease the risk of placenta accreta and/or reduce neonatal complications of placenta accreta.

50. The method of claim 49, wherein administering the therapeutic intervention comprises a therapeutic intervention selected from the group consisting of:

(i) referring the subject to a medical center having advanced multidisciplinary surgical expertise and experience;

- (ii) planning surgical uterine conservation; and
- (iii) performing a Cesarean hysterectomy, performing a prophylactic embolization, inserting a uterine balloon tamponade, a temporal internal iliac occlusion balloon catheter, a ureteral stents, administering methotrexate, leaving a portion of the placenta in-situ, and referring the subject to bed rest to prevent preterm labor.

51. A method comprising administering to a pregnant subject determined to have an increased risk of placenta accreta by a method according to any one of claims 1 to 9, and 35 to 48, a therapeutic intervention effective to reduce the risk of placenta accreta.

52. A method of administering to a pregnant subject an effective amount of a treatment designed to reduce the risk of placenta accreta, wherein the subject has an altered quantitative measure as compared to a reference standard of any one of a panel of protein biomarkers selected from:

- (i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the blood sample is collected at about 20 weeks of pregnancy;
- (ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the blood sample is collected at about 37 weeks of pregnancy;
- (iii) a protein biomarker of Table 9;
- (iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and
- (v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

53. The method of claim 52, wherein the protein biomarker is a surrogate biomarker selected from:

- (i) a surrogate biomarker of FIGS. 10A-10C, FIGS. 11A-11C, or FIGS 12A-12H;
- (ii) a surrogate biomarker of FIGS. 13A-13H, FIGS. 14A-14D, or FIGS. 15A-15B;

(iii) a surrogate biomarker of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(iv) a surrogate biomarker of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

54. A method comprising:

a) measuring, via mass spectrometry, masses of more than 100, more than 1000, more than 10,000 or more than 100,000 peptides from a biological sample comprising peptide fragments of proteins, to produce a dataset comprising more than 100, more than 1000, more than 10,000 or more than 100,000 data entries;

b) using a computer system comprising one or more processors and memory storing programs for execution by the one or more processors in

(1) identifying from among the data entries, based on the masses, peptides corresponding to:

(i) a protein biomarker of Table 1, Table 3, or Table 5, wherein the biological sample is collected at about 20 weeks of pregnancy; and

(ii) a protein biomarker of Table 2, Table 4, or Table 6, wherein the biological sample is collected at about 37 weeks of pregnancy;

(iii) a protein biomarker of Table 9;

(iv) one or more of isthmin-2 (ISM2), sulfhydryl oxidase 1 (QSOX1), histone H4 (H4), hemoglobin subunit gamma-2 (HBG2) and/or cartilage acidic protein 1 (CRAC1); and

(v) one or more of isthmin-2 (ISM2), ubiquitin carboxyl-terminal hydrolase 1 (UBP1), immunoglobulin lambda variables 10-54 (LVX54) and/or Ig-like domain-containing protein.

(2) executing a classification rule on one or more measurement values of the identified peptides, wherein the classification rule classifies the sample as being from a subject at increased risk of placenta accreta.

FIG. 1A

Table 1: Median 24 week samples. Markers are ranked using Lasso regression (alpha = 1).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp.Q6H6L7.ISM2	Q6H6L7	ISM2_HUMAN	Isthmin-2	ISM2 TAIL1, THSD3, PSEC0137	Homo sapiens (Human)	ISM2	TAIL1, THSD3
2	sp.O00391.QSOX1	Q00391	QSOX1_HUMAN	Sulfinhydryl oxidase 1	QSOX1 QSOX5, UNQ2520/PRO6013	Homo sapiens (Human)	QSOX1	QSOX6
3	sp.P11142.HSP7C	P11142	HSP7C_HUMAN	Heat shock cognate 71 kDa protein	HSPA8 HSC70, HSP73, HSPA10	Homo sapiens (Human)	HSPA8	HSC70, HSP73, HSPA10
4	sp.P62805.H4	P62805	H4_HUMAN	Histone H4	H4C1 H4/A H4FA HIST1H4A; H4C2 H4/I H4FI HIST1H4B; H4C3 H4/G H4FG HIST1H4C; H4C4 H4/B H4FB HIST1H4D; H4C5 H4/J H4FJ HIST1H4E; H4C6 H4/C H4FC HIST1H4F; H4C8 H4/H H4FH HIST1H4H; H4C9 H4/M H4FM HIST1H4I; H4C11 H4/E H4FE HIST1H4J; H4C12 H4/D H4FD HIST1H4K; H4C13 H4/K H4FK HIST1H4L; H4/N H4F2 H4FN HIST2H4; H4C15 H4/O HIST2H4A; H4C15 H4/O H4FO HIST2H4B; H4-16 HIST4H4	Homo sapiens (Human)	H4C1; H4C2; H4C3; H4C4; H4C5; H4C6; H4C8; H4C9; H4C11; H4C12; H4C13; H4C14; H4C15; H4-16	H4/A H4FA HIST1H4A; H4/I H4FI HIST1H4B; H4/G H4FG HIST1H4C; H4/B H4FB HIST1H4D; H4/J H4FJ HIST1H4E; H4/C H4FC HIST1H4F; H4/H H4FH HIST1H4H; H4/M H4FM HIST1H4I; H4/E H4FE HIST1H4J; H4/D H4FD HIST1H4K; H4/K H4FK HIST1H4L; H4/N H4F2 H4FN HIST2H4; H4/O HIST2H4A; H4/O H4FO HIST2H4B; HIST4H4

FIG. 1B

Table 1. Median 24 week samples. Markers are ranked using Lasso regression (alpha = 1).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
5	U.A3K374.A3K374			Complement component C6		Homo sapiens (Human)		
6	U.A0A1C945T1.A0A1C945T1			B cell receptor heavy chain variable region		Homo sapiens (Human)		
7	U.A0A1Z5J3U7.A0A1Z5J3U7			MS-C1 heavy chain variable region		Homo sapiens (Human)		
8	sp.P69892.HBG2	P69892	HBG2_HUMAN	Hemoglobin subunit gamma-2	HBG2	Homo sapiens (Human)	HBG2	
9	sp.Q08830.FGL1	Q08830	FGL1_HUMAN	Fibrinogen-like protein 1	FGL1 HFREP1	Homo sapiens (Human)	FGL1	HFREP1
10	sp.Q9NQ79.CRAC1	Q9NQ79	CRAC1_HUMAN	Cartilage acidic protein 1	CRTAC1 ASPIC1, CEP68	Homo sapiens (Human)	CRTAC1	ASPIC1, CEP68
11	U.B7Z6C8.B7Z6C8			ITIH4 protein, Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4	Homo sapiens (Human)		

FIG. 1C

Table 1: Median 24 week samples. Markers are ranked using Lasso regression ($\alpha = 1$).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
12	sp:P69891.HBG1	P69891	HBG1_HUMAN	Hemoglobin subunit gamma-1 (Gamma-1-globin) (Hb F Agamma) (Hemoglobin gamma-1 chain) (Hemoglobin gamma-A chain)	HBG1 PRO2979	Homo sapiens (Human)	HBG1	
13	tr:Q5K793.Q5K793			Uncharacterized protein DKFZp686A0568	DKFZp686A0568	Homo sapiens (Human)		
14	sp:P03259.CEB1			Ceruloplasmin, Ferroxidase	CP	Homo sapiens (Human)		
15	sp:Q00888.PSG4	Q00888	PSG4_HUMAN	Pregnancy-specific beta-1-glycoprotein 4, PS-beta-G-4, PSG-4, Pregnancy-specific glycoprotein 4 (Pregnancy-specific beta-1-glycoprotein 9, PS-beta-G-9, PSG-9, Pregnancy-specific glycoprotein 9)	PSG4 CGM4, PSG9	Homo sapiens (Human)	PSG4	CGM4, PSG9

FIG. 1D

Table 1: Median 24 week samples. Markers are ranked using Lasso regression (alpha = 1).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
16	E0448174.044124			C3/C5 convertase, Complement factor B, Complement factor B Ba fragment, Complement factor B Bb fragment		Homo sapiens (Human)		
17	E04218279.04218279			Ig heavy chain variable region	IgH	Homo sapiens (Human)		
18	sp.A0A0C4DH32.HV32_0	A0A0C4DH32	HV320_HUMAN	Immunoglobulin heavy variable 3-20	IGHV3-20	Homo sapiens (Human)	IGHV3-20	
19	E024065.024065			Myosin-reactive immunoglobulin kappa chain variable region		Homo sapiens (Human)		
20	sp.P23528.COF1	P23528	COF1_HUMAN	Cofilin-1 (18 kDa phosphoprotein, p18) (Cofilin, non-muscle isoform)	COF1 CFL	Homo sapiens (Human)	CFL1	CFL

FIG. 2A

Table 1: Median 34 week samples. Markers are ranked using Lasso regression (alpha = 1).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp.Q947S2.URP1	Q947S2	URP1_HUMAN	Ubiquitin carboxyl-terminal hydrolase 1, EC 3.4.19.12 (Deubiquitinase 1) (Ubiquitin thioesterase 1) (Ubiquitin-specific-processing protease 1)	USP1	Homo sapiens (Human)	USP1	
2	sp.Q6H9L7.ISM2	Q6H9L7	ISM2_HUMAN	Isthmin-2 (Thrombospondin and ANGIO domain-containing isthmin-like protein 1) (Thrombospondin type-1 domain-containing protein 3)	ISM2, TAIL1, THSD3, PSEC0137	Homo sapiens (Human)	ISM2	TAIL1, THSD3
3	sp.A0A075B6I9.LV861	A0A075B6I9		Tenascin C (Hexabrachion), isoform CRA_a	TNC, hc6_29852	Homo sapiens (Human)		
4	sp.A0A075B6I9.LV861	A0A075B6I9		Ig-like domain-containing protein		Homo sapiens (Human)		
5	sp.A0A075B6I9.LV861	A0A075B6I9	LV861_HUMAN	Immunoglobulin lambda variable 8-61	IGLV8-61	Homo sapiens (Human)	IGLV8-61	
6	sp.A0A075B6I9.LV861	A0A075B6I9	LV861_HUMAN	Immunoglobulin lambda variable 10-54	IGLV10-54	Homo sapiens (Human)	IGLV10-54	
7	sp.A0A075B6I9.LV745	A0A075B6I9	LV745_HUMAN	Immunoglobulin lambda variable 7-46	IGLV7-46	Homo sapiens (Human)	IGLV7-46	
8	sp.A0A075B6I9.LV218	A0A075B6I9	LV218_HUMAN	Immunoglobulin lambda variable 2-18	IGLV2-18	Homo sapiens (Human)	IGLV2-18	

FIG. 2B

Table 2: Median 34 week samples. Markers are ranked using Lasso regression (alpha = 1).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
9	sp.A0A075B6K5.LV39	A0A075B6K5	LV39_HUMAN	immunoglobulin lambda variable 3-9	IGLV3-9	Homo sapiens (Human)	IGLV3-9	
10	sp.A0A075B6Q5.HV26	A0A075B6Q5	HV364_HUMAN	immunoglobulin heavy variable 3-54	IGHV3-64	Homo sapiens (Human)	IGHV3-54	
11	sp.A0A075B6R2.HV40	A0A075B6R2	HV404_HUMAN	immunoglobulin heavy variable 4-4	IGHV4-4	Homo sapiens (Human)	IGHV4-4	
12	sp.A0A075B6S2.KVD29	A0A075B6S2	KVD29_HUMAN	immunoglobulin kappa variable 2D-29	IGKV2D-29	Homo sapiens (Human)	IGKV2D-29	
13	sp.A0A087WS20.KVD0	A0A087WS20	KVD02_HUMAN	immunoglobulin kappa variable 1D-8	IGKV1D-8	Homo sapiens (Human)	IGKV1D-8	
14	sp.A0A0A0MPZ8.KVD1	A0A0A0MPZ8	KVD11_HUMAN	immunoglobulin kappa variable 3D-11	IGKV3D-11	Homo sapiens (Human)	IGKV3D-11	
15	sp.A0A0A0MS14.HV14	A0A0A0MS14	HV145_HUMAN	immunoglobulin heavy variable 1-45	IGHV1-45	Homo sapiens (Human)	IGHV1-45	
16	sp.A0A0A0MT36.KVD2	A0A0A0MT36	KVD21_HUMAN	immunoglobulin kappa variable 6D-21	IGKV6D-21	Homo sapiens (Human)	IGKV6D-21	
17	sp.A0A0B4U1U7.HV601	A0A0B4U1U7	HV601_HUMAN	immunoglobulin heavy variable 6-1	IGHV6-1	Homo sapiens (Human)	IGHV6-1	
18	sp.A0A0B4U1V2.HV225	A0A0B4U1V2	HV225_HUMAN	immunoglobulin heavy variable 2-26	IGHV2-26	Homo sapiens (Human)	IGHV2-26	

FIG. 2C

Table 2: Median 34 week samples. Markers are ranked using Lasso regression ($\alpha = 1$).		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
19	sp.A0A0B4I1V6.HV373	A0A0B4I1V6	HV373_HUMAN	immunoglobulin heavy variable 3-73	IGHV3-73	Homo sapiens (Human)	IGHV3-73	
20	sp.A0A0B4I1X5.HV374	A0A0B4I1X5	HV374_HUMAN	immunoglobulin heavy variable 3-74	IGHV3-74	Homo sapiens (Human)	IGHV3-74	

FIG. 3A

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp.Q6P988 NOTUM	Q6P988	ISM2_HUMAN	Isthmin-2 (Thrombospondin and AMCP domain-containing isthmin-like protein 1) (Thrombospondin type-1 domain-containing protein 2)	ISM2, TAIL1, THSD3, PSEC0137	Homo sapiens (Human)	ISM2	TAIL1, THSD3
2	sp.Q6P988 NOTUM	Q6P988	ZNF655_HUMAN	Zinc finger protein 655 (Vav-interacting Krueppel-like protein)	ZNF655 VIK	Homo sapiens (Human)	ZNF655	VIK
3	sp.Q6P988 NOTUM	Q6P988	QSOX1_HUMAN	Sulphydryl oxidase 1, HOSOX, EC 1.9.3.2 (Quiescin Q6)	QSOX1, QSOX6, UNQ2520/PRO6013	Homo sapiens (Human)	QSOX1	QSOX6
4	sp.Q9YSY7 LYVE1	Q9YSY7	LYVE1_HUMAN	Lymphatic vessel endothelial hyaluronate receptor 1, LYVE-1 (Cell surface retention sequence-binding protein 1, CRSBP-1) (Extracellular link domain-containing protein 1) (Hyaluronic acid receptor)	LYVE1, CRSBP1, HAR, XLKEL1, UNQ230/PRO263	Homo sapiens (Human)	LYVE1	CRSBP1, HAR, XLKEL1

* Marker also found in Table 1

FIG. 3B

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
5	sp P11147 HSP7C *	P11147	HSP7C_HUMAN	Heat shock cognate 71 kDa protein, EC 3.6.4.10 (Heat shock 70 kDa protein 8) (Lipopolyaccharide-associated protein 1, LAP-1, LPS-associated protein 1)	HSPA8 HSC70, HSP73, HSPA10	Homo sapiens (Human)	HSPA8	HSC70, HSP73, HSPA10
6	sp.P13727.PRG2	P13727	PRG2_HUMAN	Bone marrow proteoglycan, BMPG (Proteoglycan 2) [Cleaved into: Eosinophil granule major basic protein, EMBP, MBP (Pregnancy-associated major basic protein)]	PRG2 MBP	Homo sapiens (Human)	PRG2	MBP
7	EN.P19801.CER1 *			Ceruloplasmin, Ferroxidase	CP	Homo sapiens (Human)		
8	sp P19801.AOC1	P19801	AOC1_HUMAN	Aminoxide-sensitive amine oxidase [copper-containing; DAO, Diamine oxidase, EC 1.4.3.22 (Aminoxide-binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)]	AOC1 ABPL, DAO1	Homo sapiens (Human)	AOC1	ABPL, DAO1

* Marker also found in Table 1

FIG. 3C

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names {primary }	Gene names (synonym)
9	sp.P52865.H4	P52865	H4_HUMAN	Histone H4	H4C1 H4/A H4FA HIST1H4A; H4C2 H4/I H4FI HIST1H4B; H4C3 H4/G H4FG HIST1H4C; H4C4 H4/B H4FB HIST1H4D; H4C5 H4/J H4FJ HIST1H4E; H4C6 H4/K H4FK HIST1H4F; H4C8 H4/N H4FN HIST1H4H; H4C9 H4/M H4FM HIST1H4I; H4C11 H4/E H4FE HIST1H4J; H4C12 H4/O H4FO HIST1H4L; H4C13 H4/Q H4FQ HIST1H4K; H4C14 H4/R H4FR HIST1H4M; H4C15 H4/S H4FS HIST1H4N; H4C16 H4/T H4FT HIST1H4P	Homo sapiens (Human)	H4C1; H4C2; H4C3; H4C4; H4C5; H4C6; H4C8; H4C9; H4C11; H4C12; H4C13; H4C14; H4C15; H4C16	H4/A H4FA HIST1H4A; H4/I H4FI HIST1H4B; H4/G H4FG HIST1H4C; H4/B H4FB HIST1H4D; H4/J H4FJ HIST1H4E; H4/K H4FK HIST1H4F; H4/N H4FN HIST1H4H; H4/M H4FM HIST1H4I; H4/E H4FE HIST1H4J; H4/O H4FO HIST1H4L; H4/R H4FR HIST1H4M; H4/S H4FS HIST1H4N; H4/T H4FT HIST1H4P
10	tr.R22123.046124			C3/C5 convertase, Complement factor B Ba fragment, Complement factor B 8b fragment		Homo sapiens (Human)		
11	sp.P51884.LUM	P51884	LUM_HUMAN	Lumican (Keratan sulfate proteoglycan lumican, KSPS lumican)	LUM LOC, SLRR2D	Homo sapiens (Human)	LUM	LEC, SLRR2D
12	sp.P33151.CADH5	P33151	CADH5_HUMAN	Cadherin-5 (784 antigen) [Vascular endothelial cadherin, VE-cadherin] (CD antigen CD133)	CADH5	Homo sapiens (Human)	CADH5	

* Marker also found in Table 1

FIG. 3D

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
13	P04010 P04010			B cell receptor heavy chain variable region		Homo sapiens (Human)		
14	P04010 P04010			Uncharacterized protein DKFZp686A0568	DKFZp686A0568	Homo sapiens (Human)		
15	sp.Q9NT13.SMC4	Q9NT13	SMC4_HUMAN	Structural maintenance of chromosomes protein 4, SMC protein 4, SMC-4 (Chromosome-associated polypeptide C, hCAP-C) (XCAP-C homolog)	SMC4 CAPC, SMC4L1	Homo sapiens (Human)	SMC4	CAPC, SMC4L1
16	sp.P01023.A2MG	P01023	A2MG_HUMAN	Alpha-2-macroglobulin, Alpha 2-M (C3 and F2p-like alpha-2-macroglobulin domain-containing protein 5)	A2M CPAMD5, FWP007	Homo sapiens (Human)	A2M	CPAMD5
17	sp.P04075.ALDOA	P04075	ALDOA_HUMAN	Fructose-bisphosphate aldolase A, EC 4.1.2.13 (lung cancer antigen NY-LU-1) (Muscle-type aldolase)	ALDOA ALDA	Homo sapiens (Human)	ALDOA	ALDA

FIG. 3E

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
18	sp.P43562.AFAM			Afamin, Alpha-albumin, Alpha-Aib	AFM, ALB2, ALBA	Homo sapiens (Human)		
19	sp.P27169.PON1	P27169	PON1_HUMAN	Serum paraoxonase/arylesterase 1, PON 1, EC 3.1.1.2, EC 3.1.1.81, EC 3.1.8.1 (Aromatic esterase 1, A-esterase 1) (K-45) (Serum arylalkylphosphatase 1)	PON1 PON	Homo sapiens (Human)	PON1	PON
20	sp.P68104.EF1A1	P68104	EF1A1_HUMAN	Elongation factor 1- α 1, EF-1- α -1 (Elongation factor Tu, EF-Tu) (Eukaryotic elongation factor 1 A-2, eEF1A-1) (Leukocyte receptor cluster member 7)	EEF1A1 EEF1A, EF1A, LENG7	Homo sapiens (Human)	EEF1A1	EEF1A, EF1A, LENG7

FIG. 3F

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
21	sp.P20023.CR2			Plasma serine protease inhibitor, Acrosomal serine protease inhibitor Plasminogen activator inhibitor 3 Short name: PAI-3 Short name: PAI3 Protein C inhibitor Short name: PCI Serpins A5	SERPINA5, PCI, PLANH3, PROCI	Homo sapiens (Human)		
22	MAN2A1			Complement component C6		Homo sapiens (Human)		
23	sp.P20023.CR2	P20023	CR2_HUMAN	Complement receptor type 2, CR2 (Complement C3d receptor) (Epstein-Barr virus receptor, EBV receptor) (CD antigen CD21)	CR2, C3DR	Homo sapiens (Human)	CR2	C3DR
24	sp.Q16706.MA2A1	Q16706	MA2A1_HUMAN	Alpha-mannosidase 2, EC 3.2.1.114 (Golgi alpha-mannosidase II, AMan II, Man II) (Mannosidase alpha class 2A member 1) (Mannosyl-oligosaccharide 1,3-1,6-alpha-mannosidase)	MAN2A1, MAN2	Homo sapiens (Human)	MAN2A1	MAN2

FIG. 3G

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names {primary }	Gene names {synonym }
25	M05133.1.052333.1			Angiotensin 1-10	AGT	Homo sapiens (Human)		
26	sp.P09992.HBG2	P09992	HBG2_HUMAN N	Hemoglobin subunit gamma-2 (gamma-2-globin) (Hb F; Ggamma) (Hemoglobin gamma-2 chain) (Hemoglobin gamma-G chain)	HBG2	Homo sapiens (Human)	HBG2	
27	sp.P04217.A1BG	P04217	A1BG_HUMAN N	Alpha-1B-glycoprotein (Alpha-1-B glycoprotein)	A1BG	Homo sapiens (Human)	A1BG	
28	U67384.1.07244.1			cDNA FLJ51742, highly similar to Inter-alpha-trypsin inhibitor heavy chain H4		Homo sapiens (Human)		
29	U140936.1.0831069			Uncharacterized protein		Homo sapiens (Human)		
30	sp.P00558.PGK1	P00558	PGK1_HUMAN N	Phosphoglycerate kinase 1, EC 2.7.2.3 (Cell migration-inducing gene 10 protein) (Primer recognition protein 2, PRP 2)	PGK1 PGKA, MG10, OK/SW-6110	Homo sapiens (Human)	PGK1	PGKA

FIG. 3H

Table 3: Top 50 protein markers at median 28 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
31	E0081153.0001.003			Myosin-reactive immunoglobulin heavy chain variable region		Homo sapiens (Human)		
32	E0081153.0002.002			I3/C5 convertase		Homo sapiens (Human)		
33	E0081153.0003.003			Endothelial protein C receptor, Activated protein C receptor, APC receptor, Endothelial cell protein C receptor, CD_antigen: CD201	PROCR, EPCR	Homo sapiens (Human)		
34	E0081153.0017.004.12 E0081153.0017.004.17			M5-C1 heavy chain variable region		Homo sapiens (Human)		
35	E0081153.0020.002			Inter-alpha (Globulin) inhibitor H4 (Plasma Kallikrein-sensitive glycoprotein)	ITIH4	Homo sapiens (Human)		

FIG. 3I

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
36	sp.P22105.TENX			Tenascin-X, Hexabrachion-like protein	TNXB, HXBL, TNX, TNXB1, TNXB2, XB	Homo sapiens (Human)		
37	sp.Q92859.NEO1	Q92859	NEO1_HUMAN	Neogenin (immunoglobulin superfamily DCC subclass member 2)	NEO1 IGDCC2, NGN	Homo sapiens (Human)	NEO1	IGDCC2, NGN
38	tr.V9HNV6.V9HNV6			Gc-globulin, Group-Specific Component, Vitamin D-binding protein	HEL-S-51	Homo sapiens (Human)		
39	sp.P93160.PGBM			Basement membrane-specific heparan sulfate proteoglycan core protein, Perlecan	HSPG2	Homo sapiens (Human)		
40	sp.Q86U17.SPA11	Q86U17	SPA11_HUMAN	Serpins A11	SERPINA11	Homo sapiens (Human)	SERPINA11	
41	tr.Q62P85.Q62P85			cDNA FLJ26301 fis, clone DMC07540		Homo sapiens (Human)		

FIG. 3J

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
42	sp.P10809.CH60	P10809	CH60_HUMA N	60 kDa heat shock protein, mitochondrial, EC 5.6.1.7 (60 kDa chaperonin) (Chaperonin 60, Cpn60) (Heat shock protein 60, Hsp-60, Hsp60) (HuChA60) (Mitochondrial matrix protein P1) (P60 lymphocyte protein)	HSPD1 HSP60	Homo sapiens (Human)	HSPD1	HSP60
43	tr.Q66026.Q66026			Uncharacterized protein DKFZp686I5196	DKFZp686I5196	Homo sapiens (Human)		
44	tr.AQ4264.AQ4264			Ig heavy chain variable region	IgH	Homo sapiens (Human)		
45	sp.Q12805.FBLN3	Q12805	FBLN3_HUMA N	ECF-containing fibulin-like extracellular matrix protein 3 (Extracellular protein 51-5) (Fibulin-like protein) (Fibulin-3, FBL-3)	EFEMP1 FBLN3, FBNL	Homo sapiens (Human)	EFEMP1	FBLN3, FBNL
46	sp.ASM101.FB5L3	ASB101	FB5L3_HUMA N	Putative fatty acid-binding protein 5-like protein 3 (Fatty acid-binding protein 5 pseudogene 3)	FABP5P3 FABP5L3	Homo sapiens (Human)	FABP5P3	FABP5L3

FIG. 3K

Table 3: Top 50 protein markers at median 24 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
47	sp.O60814.H2BK	Q60814	H2BK_HUMAN	Histone H2B type 1-K, H2BK (HIRA-interacting protein 1)	H2BC12 H2BFT, HIRIP1, HIST1H2BK	Homo sapiens (Human)	H2BC12	H2BFT, HIRIP1, HIST1H2BK
48	sp.P17935.IBP3	P17935	IBP3_HUMAN	Insulin-like growth factor-binding protein 3, IBP-3, IGF-binding protein 3, IGFBP-3	IGFBP3 IBP3	Homo sapiens (Human)	IGFBP3	IBP3
49	sp.P23142.FBLN1	P23142	FBLN1_HUMAN	Fibulin-1, FBL-1	FBLN1 PP213	Homo sapiens (Human)	FBLN1	
50	sp.Q75144.ICOSL			ICOS ligand, B7 homolog 2, B7-H2, B7-like protein G150, B7-related protein 1, B7RP-1 CD_antigen: CD275	ICOSLG, B7H2, B7RP1, ICOSL, KIAA0653	Homo sapiens (Human)		

FIG. 4A

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp.Q92859.NEO1	Q92859	NEO1_HUMAN	Neogenin (Immunoglobulin superfamily DCC subclass member 2)	NEO1 IGDCC2, NGN	Homo sapiens (Human)	NEO1	IGDCC2, NGN
2	tr.S6C4Q9.S6C4Q9			IgG L chain		Homo sapiens (Human)		
3	tr.A0A0G2JRQ6.A0A0G2JRQ6**			Ig-like domain-containing protein		Homo sapiens (Human)		
4	sp.O00391.QSOX1	O00391	QSOX1_HUMAN	Sulfhydryl oxidase 1, hQSOX, EC 1.8.3.2 (Quiescin Q6)	QSOX1 QSCN6, UNQ2520/PRO6013	Homo sapiens (Human)	QSOX1	QSCN6
5	sp.Q6H9L7.ISM2*	Q6H9L7	ISM2_HUMAN	Isthmin-2 (Thrombospondin and AMOP domain-containing isthmin-like protein 1) (Thrombospondin type-1 domain-containing protein 3)	ISM2 TAIL1, THSD3, PSEC0137	Homo sapiens (Human)	ISM2	TAIL1, THSD3
6	tr.B1N7B8.B1N7B8			Cryocrystalglobulin CCI kappa light chain variable region		Homo sapiens (Human)		

* Marker also found in Table 1

** Marker also found in Table 2

FIG. 4B

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
7	sp.P05546.HEP2	P05546	HEP2_HUMAN	Heparin cofactor 2 (Heparin cofactor II, HC-II) (Protease inhibitor leuserpin-2, HLS2) (Serpine D1)	SERPIND1 HC F2	Homo sapiens (Human)	SERPIND1	HCF2
8	tr.A0A075B6S9.A0A075B6S9			Probable non-functional immunoglobulin kappa variable 1-37	IGKV1-37	Homo sapiens (Human)		
9	tr.A0A024R884.A0A024R884**			Tenascin C (Hexabrachion), isoform CRA_a	TNC	Homo sapiens (Human)		
10	tr.B2R582.B2R582			cDNA, FLJ92374, highly similar to Homo sapiens C-type lectin domain family 3, member B (CLEC3B), mRNA		Homo sapiens (Human)		
11	sp.Q94782.UBP1	Q94782	UBP1_HUMAN	Ubiquitin carboxyl-terminal hydrolase 1, EC 3.4.19.12 (Deubiquitinating enzyme 1, hUBP) (Ubiquitin-thioesterase 1) (Ubiquitin-specific-processing protease 1)	USP1	Homo sapiens (Human)	USP1	
12	sp.Q6P988.NOTUM	Q8N720	ZN655_HUMAN	Zinc finger protein 655 (Vav-interacting Krueppel-like protein)	ZN655 VIK	Homo sapiens (Human)	ZN655	VIK

* Marker also found in Table 1

** Marker also found in Table 2

FIG. 4C

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
13	tr.Q9UL93.Q9UL93			Myosin-reactive immunoglobulin heavy chain variable region		Homo sapiens (Human)		
14	tr.C9JKR2.C9JKR2			Albumin, Albumin, isoform CRA_k	ALB, ORF Names: hCG_14_967	Homo sapiens (Human)		
15	sp.Q07954.LRP1	Q07954	LRP1_HUMAN	Pro-low-density lipoprotein receptor-related protein 1, LRP-1 (Alpha-2-macroglobulin receptor, A2MR) (Apolipoprotein E receptor, APOER) (CD antigen CD91) [Cleaved into: Low-density lipoprotein receptor-related protein 1 85 kDa subunit, LRP-85; Low-density lipoprotein receptor-related protein 1 515 kDa subunit, LRP-515; Low-density lipoprotein receptor-related protein 1 intracellular domain, LRPICD]	LRP1 A2MR, APR	Homo sapiens (Human)	LRP1	A2MR, APR
16	tr.A0A2U8J975.A0A2U8J975			Ig heavy chain variable region	IgH	Homo sapiens (Human)		

FIG. 4D

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
17	sp.Q15485.FCN2	Q15485	FCN2_HUMAN	Ficolin-2 (37 kDa elastin-binding protein) (Collagen/fibrinogen domain-containing protein 2) (EBP-37) (Ficolin-B) (Ficolin-beta) (Hucolin) (L-ficolin) (Serum lectin p35)	FCN2 FCNL	Homo sapiens (Human)	FCN2	FCNL
18	sp.P02042.HBD	P09105	HBAT_HUMAN	Hemoglobin subunit theta-1 (Hemoglobin theta-1 chain) (Theta-1-globin)	HBQ1	Homo sapiens (Human)	HBQ1	
19	sp.P02652.APOA2	P02652	APOA2_HUMAN	Apolipoprotein A-II, Apo-AII, ApoA-II (Apolipoprotein A2) [Cleaved into: Proapolipoprotein A-II, ProapoA-II; Truncated apolipoprotein A-II (Apolipoprotein A-II(1-76))]]	APOA2	Homo sapiens (Human)	APOA2	
20	sp.Q6UX71.PXDC2	Q6UX71	PXDC2_HUMAN	Plexin domain-containing protein 2 (Tumor endothelial marker 7-related protein)	PLXDC2 TEM7 R, UNQ2514/PRO6003	Homo sapiens (Human)	PLXDC2	TEM7R

FIG. 4E

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
21	tr.A2MYD1.A2 MYD1			V4-4 protein	V4-4	Homo sapiens (Human)		
22	tr.A0A068LKQ2 .A0A068LKQ2			Ig heavy chain variable region		Homo sapiens (Human)		
23	sp.P02765.FETU A			Alpha-2-HS-glycoprotein, Alpha-2-Z-globulin Ba-alpha-2-glycoprotein Fetuin-A	AHSG, FETUA	Homo sapiens (Human)		
24	sp.P0DOX4.IGE			Immunoglobulin epsilon heavy chain, Immunoglobulin epsilon heavy chain ND		Homo sapiens (Human)		
25	sp.P19801.AOC 1	P19801	AOC1_HUMAN	Amiloride-sensitive amine oxidase [copper-containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amiloride-binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)	AOC1 ABP1, DAO1	Homo sapiens (Human)	AOC1	ABP1, DAO1
26	sp.Q86UD1.OA F			Out at first protein homolog, HCV NS5A-transactivated protein 13 target protein 2	OAF, NS5A TP13 TP2	Homo sapiens (Human)		

FIG. 4F

Table 4: Top 50 protein markers at median 34 weeks.	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
27	sp.Q04695.K1C17	K1C17_HUMAN	Keratin, type I cytoskeletal 17 (39.1) (Cytokeratin-17, CK-17) (Keratin-17, K17)	KRT17	Homo sapiens (Human)	KRT17	
28	sp.A0A075B6I4.LVX54	LVX54_HUMAN	Immunoglobulin lambda variable 10-54	IGLV10-54	Homo sapiens (Human)	IGLV10-54	
29	tr.A0A109PP82.A0A109PP82		MS-C2 light chain variable region, MS-C4 light chain variable regionImp		Homo sapiens (Human)		
30	sp.P0DMV8.HS71A	HS71A_HUMAN	Heat shock 70 kDa protein 1A (Heat shock 70 kDa protein 1, HSP70-1, HSP70.1)	HSPA1A HSP72, HSPA1, HSX70	Homo sapiens (Human)	HSPA1A	HSP72, HSPA1, HSX70
31	sp.P98160.PGBM		Basement membrane-specific heparan sulfate proteoglycan core protein, Perlecan	HSPG2	Homo sapiens (Human)		
32	tr.A0A3B3ISR2.A0A3B3ISR2		Complement subcomponent C1r	C1R	Homo sapiens (Human)		
33	sp.P08294.SODE		Extracellular superoxide dismutase [Cu-Zn]	SOD3	Homo sapiens (Human)		
34	tr.A0A125U0U7.A0A125U0U7		MS-C1 heavy chain variable region		Homo sapiens (Human)		
35	tr.U3PXP0.U3PXP0		Alpha globin chain	HBA2	Homo sapiens (Human)		

FIG. 4G

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
36	sp.P01023.A2M G	P01023	A2MG_HUMAN	Alpha-2-macroglobulin, Alpha-2-M (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 5)	A2M CPAMD5, FWP007	Homo sapiens (Human)	A2M	CPAMD5
37	sp.P02788.IRFL			Lactotransferrin, Lactoferrin, Growth-inhibiting protein 12, Talactoferrin	LTF, GIG12, LF	Homo sapiens (Human)		
38	sp.P08519.APO A			Apolipoprotein(a), Apo(a), Lp(a)	LPA	Homo sapiens (Human)		
39	sp.P01019.ANG T			Angiotensinogen, Serpin A8	AGT, SERPINA8	Homo sapiens (Human)		
40	sp.P60660.MYL 6	P60660	MYL6_HUMAN	Myosin light polypeptide 6 (17 kDa myosin light chain, LC17) (Myosin light chain 3, MLC-3) (Myosin light chain alkali 3, Myosin light chain A3) (Smooth muscle and nonmuscle myosin light chain alkali 6)	MYL6	Homo sapiens (Human)	MYL6	

FIG. 4H

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
41	sp.O96006.ZBE D1	O96006	ZBED1_HUMAN	E3 SUMO-protein ligase ZBED1, EC 2.3.2.- (DNA replication-related element-binding factor) (Putative Ac-like transposable element) (Zinc finger BED domain-containing protein 1) (dREF homolog)	ZBED1 ALTE, DREF, hDREF, KIAA0785, TRAMP	Homo sapiens (Human)	ZBED1	ALTE, DREF, hDREF, KIAA0785, TRAMP
42	sp.A0A075B6S2.KVD29	A0A075B6S2	KVD29_HUMAN	Immunoglobulin kappa variable 2D-29	IGKV2D-29	Homo sapiens (Human)	IGKV2D-29	
43	tr.Q9UL77.Q9U L77			Myosin-reactive immunoglobulin light chain variable region		Homo sapiens (Human)		
44	sp.P11021.BIP			Endoplasmic reticulum chaperone BiP, Heat shock protein 70 family protein 5, HSP70 family protein 5.78 kDa glucose-regulated protein, GRP-78, Binding-immunoglobulin protein, BiP	HSPA5, GRP78	Homo sapiens (Human)		
45	tr.H7C5R1.H7C 5R1			Ceruloplasmin	CP	Homo sapiens (Human)		

FIG. 4I

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
46	sp.O95497.VNN1	O95497	VNN1_HUMAN	Pantetheinase, EC 3.5.1.92 (Pantetheinase) (Tiff66) (Vascular non-inflammatory molecule 1, Vanin-1)	VNN1	Homo sapiens (Human)	VNN1	
47	sp.P06315.KV50	P06315	KV502_HUMAN	Immunoglobulin kappa variable 5-2 (Ig kappa chain V region EV15)	IGKV5-2	Homo sapiens (Human)	IGKV5-2	
48	sp.Q8WZ75.BO4	Q8WZ75	ROBO4_HUMAN	Roundabout homolog 4 (Magic roundabout)	ROBO4 UNQ421/PRO3674	Homo sapiens (Human)	ROBO4	
49	sp.Q9Y5Y7.VE1	Q9Y5Y7	LYVE1_HUMAN	Lymphatic vessel endothelial hyaluronate receptor 1, LYVE-1 (Cell surface retention sequence-binding protein 1, CRSBP-1) (Extracellular link domain-containing protein 1) (Hyaluronic acid receptor)	LYVE1 CRSBP1, HAR, XLKDI, UNQ230/PRO263	Homo sapiens (Human)	LYVE1	CRSBP1, HAR, XLKDI

FIG. 4J

Table 4: Top 50 protein markers at median 34 weeks.		Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
50	spP14151.LYA M1	P14151	LYAM1_HUMAN	L-selectin (CD62 antigen-like family member L) (Leukocyte adhesion molecule 1, LAM-1) (Leukocyte surface antigen Leu-8) (Leukocyte-endothelial cell adhesion molecule 1, LECAM1) (Lymph node homing receptor) (TQ1) (gp90-MEL) (CD antigen CD62L)	SELL LNHR, LYAM1	Homo sapiens (Human)	SELL	LNHR, LYAM1

FIG. 5A

Table 5: 24-week markers.		Subcellular location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp O15511 ARPC5_HUMAN	cyto	O15511	ARPC5_HUMAN	Actin-related protein 2/3 complex subunit 5 (Arp2/3 complex 16 kDa subunit, p16-ARC)	ARPC5 ARC16	Homo sapiens (Human)	ARPC5	ARC16
2	sp P07355 ANXA2_HUMAN	cyto	P07355	ANXA2_HUMAN	Annexin A2 (Annexin II) (Annexin-2) (Calpactin I heavy chain) (Calpactin-1 heavy chain) (Chromobindin-8) (Lipocortin II) (Placental anticoagulant protein IV, PAP-IV) (Protein I) (p36)	ANXA2 ANX2, ANX2L4, CAL1H, LPC2D	Homo sapiens (Human)	ANXA2	ANX2, ANX2L4, CAL1H, LPC2D
3	sp P13533 MYH6_HUMAN	nucl	P13533	MYH6_HUMAN	Myosin-6 (Myosin heavy chain 6) (Myosin heavy chain, cardiac muscle alpha isoform, MyHC-alpha)	MYH6 MYHCA	Homo sapiens (Human)	MYH6	MYHCA
4	sp P18206 VINC_HUMAN	cyto	P18206	VINC_HUMAN	Vinculin (Metavinculin, MV)	VCL	Homo sapiens (Human)	VCL	

FIG. 5B

Table 5: 24-week markers.		Subcellular location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
5	sp P20023 CR2_HUMAN	E.R.	P20023	CR2_HUMAN	Complement receptor type 2, Cr2 (Complement C3d receptor) (Epstein-Barr virus receptor, EBV receptor) (CD antigen CD21)	CR2 C3DR	Homo sapiens (Human)	CR2	C3DR
6	sp Q6P988 NOTUM_HUMAN	extr	Q8N720	ZN655_HUMAN	Zinc finger protein 655 (Vav-interacting Kruessel-like protein)	ZN655 VIK	Homo sapiens (Human)	ZN655	VIK
7	tr H7C5R1 H7C5R1_HUMAN	cyto			Ceruloplasmin	CP	Homo sapiens (Human)		
8	sp P20023 CR2_HUMAN	E.R.	P20023	CR2_HUMAN	Complement receptor type 2, Cr2 (Complement C3d receptor) (Epstein-Barr virus receptor, EBV receptor) (CD antigen CD21)	CR2 C3DR	Homo sapiens (Human)	CR2	C3DR
9	sp O15511 ARPC5_HUMAN	cyto	O15511	ARPC5_HUMAN	Actin-related protein 2/3 complex subunit 5 (Arp2/3 complex 16 kDa subunit, p16-ARC)	ARPC5 ARC16	Homo sapiens (Human)	ARPC5	ARC16
10	sp P18206 VIN_C_HUMAN	cyto			Vinculin, Metavinculin, MV	VCL	Homo sapiens (Human)		

FIG. 5C

Table 5: 24-week markers.		Subcellular location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
11	tr H7C5R1 H7C5R1_HUMAN	cyto			Ceruloplasmin	CP	Homo sapiens (Human)		
12	sp P07355 ANXA2_HUMAN	cyto	P07355	ANXA2_HUMAN	Annexin A2 (Annexin II) (Annexin-2) (Calpactin I heavy chain) (Calpactin-1 heavy chain) (Chromobindin-8) (Lipocortin II) (Placental anticoagulant protein IV, PAP-IV) (Protein I) (p36)	ANXA2 ANX2, ANX2L4, CALIH, LPC2D	Homo sapiens (Human)	ANXA2	ANX2, ANX2L4, CALIH, LPC2D

FIG. 6A

Table 6: 34-week markers.									
		Subcellular_location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
1	sp A0A075B614 LVX54_HUMAN	extr	A0A075B614	LVX54_HUMAN	Immunoglobulin lambda variable 10-54	IGLV10-54	Homo sapiens (Human)	IGLV10-54	
2	sp P05164 PERM1_HUMAN	extr			Myeloperoxidase	MPO	Homo sapiens (Human)		
3	sp P19801 AOC1_HUMAN	extr	P19801	AOC1_HUMAN	Amloride-sensitive amine oxidase [copper-containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amloride-binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)	AOC1, ABP1, DAO1	Homo sapiens (Human)	AOC1	ABP1, DAO1
4	sp P24593 IBP5_HUMAN	extr	P24593	IBP5_HUMAN	Insulin-like growth factor-binding protein 5, IBP-5, IGF-binding protein 5, IGFBP-5	IGFBP5, IBP5	Homo sapiens (Human)	IGFBP5	IBP5

FIG. 6B

Table 6: 34-week markers.		Subcellular _location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
5	sp P24844 MYL9_HUMAN	mito	P24844	MYL9_HUMAN	Myosin regulatory light polypeptide 9 (20 kDa myosin light chain, LC20) (MLC-2C) (Myosin RLC) (Myosin regulatory light chain 2, smooth muscle isoform) (Myosin regulatory light chain 9) (Myosin regulatory light chain MRLC1)	MYL9, MLC2, MRLC1, MYRL2	Homo sapiens (Human)	MYL9	MLC2, MRLC1, MYRL2
6	sp Q9H4B7 TBB1_HUMAN	cyto	Q9H4B7	TBB1_HUMAN	Tubulin beta-1 chain	TUBB1	Homo sapiens (Human)	TUBB1	
7	sp Q9HBI1 PARVB_HUMAN	mito	Q9HBI1	PARVB_HUMAN	Beta-parvin, Affixin	PARVB, C GI-56	Homo sapiens (Human)	PARVB	
8	sp Q9UBW5 BIN2_HUMAN	nucl	Q9UBW5	BIN2_HUMAN	Bridging integrator 2 (Breast cancer-associated protein 1)	BIN2, BRAP1	Homo sapiens (Human)	BIN2	BRAP1

FIG. 6C

Table 6: 34-week markers.		Subcellular location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
9	tr A0A1L2BM35 A0A1L2BM35_HUMAN	mito			MHC class I antigen	HLA-A	Homo sapiens (Human)		
10	tr A0A384MDQ7 A0A384MDQ7_HUMAN	extr			Epididymis secretory sperm binding protein		Homo sapiens (Human)		
11	tr A2MYC9 A2MYC9_HUMAN	mito			V4-3 protein	V4-3	Homo sapiens (Human)		
12	tr F5GY80 F5GY80_HUMAN	extr			Complement component 8 subunit beta, Complement component C8 beta chain	C8B	Homo sapiens (Human)		
13	tr Q5NV79 Q5NV79_HUMAN	mito			V5-4 protein	V5-4	Homo sapiens (Human)		
14	sp Q9H4B7 TBB1_HUMAN	cyto	Q9H4B7	TBB1_HUMAN	Tubulin beta-1 chain	TUBB1	Homo sapiens (Human)	TUBB1	
15	sp A0A075B61 L VX54_HUMAN	extr	A0A075B614	L VX54_HUMAN	Immunoglobulin lambda variable 10-54	IGLV10-54	Homo sapiens (Human)	IGLV10-54	

FIG. 6D

Table 6: 34-week markers.		Subcellular _location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
16	tr A0A384MDQ7 A0A384MDQ7_HU MAN	extr			Epididymis secretory sperm binding protein		Homo sapiens (Human)		
17	sp P05164 PERM_ HUMAN	extr			Myeloperoxidase	MPO	Homo sapiens (Human)		
18	sp P24593 IBP5_ HUMAN	extr	P24593	IBP5_HUMAN	Insulin-like growth factor-binding protein 5, IBP-5, IGF-binding protein 5, IGFBP-5	IGFBP5 IB P5	Homo sapiens (Human)	IGFBP5	IBP5
19	sp P19801 AOC1 _HUMAN	extr	P19801	AOC1_HUMAN	Amiloride-sensitive amine oxidase [copper- containing], DAO, Diamine oxidase, EC 1.4.3.22 (Amiloride- binding protein 1) (Amine oxidase copper domain-containing protein 1) (Histaminase) (Kidney amine oxidase, KAO)	AOC1, AB P1, DAO1	Homo sapiens (Human)	AOC1	ABP1, DAO1

FIG. 6E

Table 6: 34-week markers.		Subcellular _location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
20	tr F5GY80 F5GY80_HUMAN	extr			Complement component 8 subunit beta, Complement component C8 beta chain	C8B	Homo sapiens (Human)		
21	tr A0A1L2BM35 A0A1L2BM35_HUMAN	mito			MHC class I antigen	HLA-A	Homo sapiens (Human)		
22	sp Q9HBI1 PARVB_HUMAN	mito	Q9HBI1	PARVB_HUMAN	Beta-parvin, Affixin	PARVB, C GI-56	Homo sapiens (Human)	PARVB	
23	tr Q5NV79 Q5NV79_HUMAN	mito			V5-4 protein	V5-4	Homo sapiens (Human)		
24	tr A2MYC9 A2MYC9_HUMAN	mito			V4-3 protein	V4-3	Homo sapiens (Human)		

FIG. 6F

Table 6: 34-week markers.		Subcellular _location	Entry	Entry name	Protein names	Gene names	Organism	Gene names (primary)	Gene names (synonym)
25	sp P24844 MYL9_HUMAN	mito	P24844	MYL9_HUMAN	Myosin regulatory light polypeptide 9 (20 kDa myosin light chain, LC20) (MLC-2C) (Myosin RLC) (Myosin regulatory light chain 2, smooth muscle isoform) (Myosin regulatory light chain 9) (Myosin regulatory light chain regulatory light chain MRLC1)	MYL9, MLC2, MRLC1, MYRL2	Homo sapiens (Human)	MYL9	MLC2, MRLC1, MYRL2
26	sp Q9UBW5 BIN2_HUMAN	nucl	Q9UBW5	BIN2_HUMAN	Bridging integrator 2 (Breast cancer-associated protein 1)	BIN2, BRAP1	Homo sapiens (Human)	BIN2	BRAP1

FIG. 7

Table 7 – Median 24-week samples. Biomarker panels

Var.1	Var.2	Var.3	Var.4	Var.5	nAUCmean
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.8323
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.8163
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.8119
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7950
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7823
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7820
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7747
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q08530.FGL1_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7696
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7689
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7662
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q6H9L7.ISM2_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7653
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P60892.HB52_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7577
sp.O00391.QS0X1_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7500
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7490
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7460
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	0.7440
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7429
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7425
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7405
sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7398
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	0.7347
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7267
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7232
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7225
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7210
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7143
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q6H9L7.ISM2_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7127
sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q6H9L7.ISM2_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7113
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q9NQ79.CRAC1_HUMAN	0.7081
sp.O00391.QS0X1_HUMAN	sp.P62805.H4_HUMAN	sp.P60892.HB52_HUMAN	sp.Q08530.FGL1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.7063
sp.O00391.QS0X1_HUMAN	sp.P11142.HSP7C_HUMAN	sp.P62805.H4_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	tr.ADA125U0U7.ADA125U0U7_HUMAN	0.7024

FIG. 8

Table 8 – Median 34-week samples. Biomarker panels

Var. 1	Var. 2	Var. 3	Var. 4	Var. 5	oAUCmean
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	0.7792
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.7206
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6918
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	0.6875
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.6875
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6813
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6792
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6750
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6686
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6604
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6563
sp.A0A0758614.LVX54_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	--	0.6466
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6288
sp.A0A0758614.LVX54_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	0.6205
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	0.6125
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	0.6125
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	0.6125
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6125
sp.A0A0758614.LVX54_HUMAN	sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	sp.Q6H9L7.ISM2_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	0.6080
sp.A0A0758614.LVX54_HUMAN	sp.O94782.URP1_HUMAN	tr.A0A0G2IR06.A0A0G2IR06_HUMAN	--	--	0.6000

FIG. 9

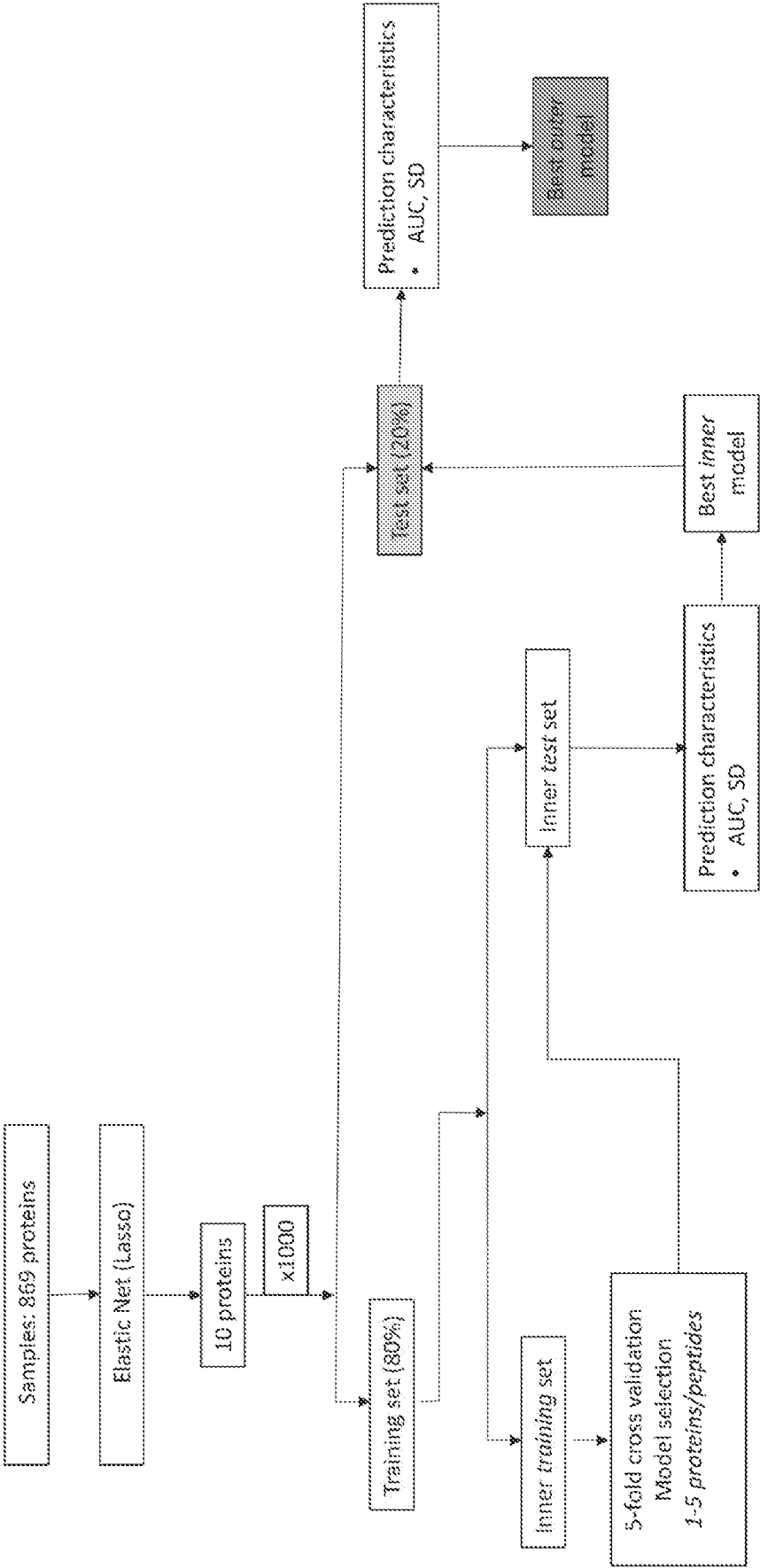


Table 1: Median 24
hour average
noise levels
regression
analysis

[illegible]

Table 1: Median 24-week samples, markers are ranked using Lasso regression (abs) = 1.

[illegible]

Fig. 10C

[illegible]

Fig. 1

[illegible]

[illegible]

*Marker also found in Table 1

Fig. 12B

Table 2: Top 40 protein markers at median 24 weeks	Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
7	sp P0450 CERU {Gln->pyro-Glu}QSED STFTLGE R	{Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	AEEHLG ILGFQLH ADVGDK	AETGDK VYVELK	AGLQAF QVQCIC arbitrarily ethyl (C)NK	ALYLOD arbitrarily (NQ)YTD ETFR	ALYLOY TDEYFR TIEK	ALYLOY TDEYFR TIEK	DDDEEES NK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK
8	sp P19801 AOC1 ADVFSDL SNQELK	{Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	AEEHLG ILGFQLH ADVGDK	AETGDK VYVELK	AGLQAF QVQCIC arbitrarily ethyl (C)NK	ALYLOD arbitrarily (NQ)YTD ETFR	ALYLOY TDEYFR TIEK	ALYLOY TDEYFR TIEK	DDDEEES NK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK
9	sp P62805 H4 TVTAMD VYVALK R	{Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	AEEHLG ILGFQLH ADVGDK	AETGDK VYVELK	AGLQAF QVQCIC arbitrarily ethyl (C)NK	ALYLOD arbitrarily (NQ)YTD ETFR	ALYLOY TDEYFR TIEK	ALYLOY TDEYFR TIEK	DDDEEES NK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK
10	tr B4E124 B4E124 {Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	AEEHLG ILGFQLH ADVGDK	AETGDK VYVELK	AGLQAF QVQCIC arbitrarily ethyl (C)NK	ALYLOD arbitrarily (NQ)YTD ETFR	ALYLOY TDEYFR TIEK	ALYLOY TDEYFR TIEK	DDDEEES NK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK
11	sp P51584 LUM EDAVSA AFK	{Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	AEEHLG ILGFQLH ADVGDK	AETGDK VYVELK	AGLQAF QVQCIC arbitrarily ethyl (C)NK	ALYLOD arbitrarily (NQ)YTD ETFR	ALYLOY TDEYFR TIEK	ALYLOY TDEYFR TIEK	DDDEEES NK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK
12	sp P93151 CADH5 DTGENLE TPSSFTK	{Gln->pyro-Glu}QYTD STFRVY ER	{Gln->pyro-Glu}QYTD STFRVY ER	AEEHLG ILGFQLH ADVGDK	AETGDK VYVELK	AGLQAF QVQCIC arbitrarily ethyl (C)NK	ALYLOD arbitrarily (NQ)YTD ETFR	ALYLOY TDEYFR TIEK	ALYLOY TDEYFR TIEK	DDDEEES NK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIASGLG PLIC[Carb amidometh yl (C)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK	DIPTGLG FM[Oxidation ion (M)JK

*Marker also found in Table 1

[illegible]

[illegible]

Table 3: Top 50 protein markers at median 24 weeks	Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
26	sp P6892 HBG2	MTGVAA SALSSR																		
27	sp P04217 A1BG	ATWSGA VLASGR AVLR	ATWSGA VLASGR AVLR	C[Carbamidomethyl] (C)EGGPI DVTPELL R	C[Carbamidomethyl] (C)EGGPI DVTPELL R	PAALVRED R	GVLRGV ITELLRR	GVTFLLR R	GVTFLLR R	HQPLLTG DTQGR	(NQ)HAVA LGDGGR YTC[Carbamidomethyl] (C)JR	IFFELNA VALDGG GRYTC[Carbamidomethyl] ethyl (C)JR	LELHVD GPPRRPQ LR	LLELTGPP K	LRC[Carbamidomethyl] (C)HAPLE GAR	NIDeamidation (NQ)HGA QEFVHLD SPAIR	NGVAQE FVHLDSP AIK	HGHEKELL VPR	SGSLTCGW TQLSK	SLPAPWL SMQDAIDAI on (M)APVS WITPGLK
28	tr B7Z544 B7Z544		NRNVHS AGAAAGS R	NVHSAG AAGSR																
29	tr HGVW93 HGVW93		PGQVDA (NQ)IVDA AMAGR																	
30	sp P03558 PGK1	AKSSMV GVNLPQ K	GC[Carbamidomethyl] (C)HTGG GDTATCI Carbamidomethyl (C)C[Carbamidomethyl] yl (C)AK	VSHVSTG GGASLEL LEGK																
31	tr Q9UL93 Q9UL93	VQLVESG GGVYQF GR																		
32	tr Q33GZ8 Q33GZ8	[Glu-pyro-Glu]QC[Carbamidomethyl] (C)IRPNG MWDGET amidomethyl (C)IDNGA GHCI[Carbamidomethyl] yl (C)IRPNG SLGAVR	[Glu-pyro-Glu]QHLG DVLNPLP L	C[Carbamidomethyl] (C)PAPVS FENDeamidation (NQ)GT TPR	C[Carbamidomethyl] (C)PAPVS FENGAYT PR	DMTEVIS SLENANY K	DMTEVIS SLENANY K	DMTEVIS SLENANY R	ESASLMV DR	GESGGA VFLER	FRFQVYG LVSWGL YNPQ[Carbamidomethyl] amidomethyl (C)JLQSA DK	GESGGA VFLER	QC[Carbamidomethyl] (C)IRPNG amidomethyl (NQ)JEM WDGETA VC[Carbamidomethyl] (C)IDNGA GHCI[Carbamidomethyl] yl (C)IRPNG SLGAVR	QC[Carbamidomethyl] (C)IRPNG amidomethyl (NQ)JEM WDGETA VC[Carbamidomethyl] (C)IDNGA GHCI[Carbamidomethyl] yl (C)IRPNG SLGAVR	QC[Carbamidomethyl] (C)IRPNG amidomethyl (NQ)JEM WDGETA VC[Carbamidomethyl] (C)IDNGA GHCI[Carbamidomethyl] yl (C)IRPNG SLGAVR	QHLGDV ENFLPL	SKVPPPR DFEINL R	VLM[Oxidation] (M)SVLN DNSR	VLM[Oxidation] (M)SVLN DNSR	

[illegible]

Fig. 12G

Table 3: Top 50 protein markers at median 24 weeks.		Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
40	sp-Q86U17 SPA11	GNALAL LVLDPDG K	HQLSEPA PAYHR	IPPTINF ALR	QESHFVD ER	QVEAAL QPQLHR	SLRHILA LPSEK	WGQALL PSLLDLH LPR													
41	tr-Q6ZP85 Q6ZP85	NSVFLQ Deamidation (NQ)MNS LR	NSVFLQ MNIDeamidation (NQ)MNSLR																		
42	sp-P10809 CHI60	ALM[Oxidation] (M)LLQGV DILLADA VAVTMOxidation (M)GPK	LVQDVA NNTNEE AGDQTT TATVLAR																		
43	tr-Q6N096 Q6N096	NTLFLQL SSLR																			
44	tr-A0A068 1.E.02.A0 A068LKQ 2	[Glu->pyro- Glu]QLQL Q[Deamidation] (NQ)ESG VK PGLVK	[Glu->pyro- Glu]QLQL C[Carbamidomethylation] QESGPGL VK	LTSVTAA DTAVYY C[Carbamidomethylation] (C)AR	Q[Deamidation] (NQ)QLQL QESGPGL VK	QLQLQES SVTISV GFLVK DR															
45	sp-Q12805 FBLN3	ADQVCIC acetylation (C)INLR	C[Carbamidomethylation] (C)IVC[Carbamidomethylation] byl (C)IPVSN AMC[Carbamidomethylation] byl (C)JR	C[Carbamidomethylation] (C)IVNH GGYLCIC acetylation (C)ILPK	DIDEC[Ca thiuronat byl (C)HIVED AC[Carbamidomethylation] (C)IK	EHIVDLE MLTVSSI GTFR	ELPQSV YK	FSC[Carbamidomethylation] (C)M[C[Ca thiuronat byl (C)PCGY QYVR	IPSNPSHR	IQ[C[Carbamidomethylation] (C)JAAGY EQSEHNY C[Carbamidomethylation] (C)QDDDE C[Carbamidomethylation] (C)IAGT RNC[Carbamidomethylation] M(C)JR	LNC[Carbamidomethylation] (C)IEDIDE C[Carbamidomethylation] (C)IK	IPSNPSHR	NPC[Carbamidomethylation] (C)QDFYI LTPENR	RGEQCIC acetylation (C)VIDIDH C[Carbamidomethylation] (C)TIPPY C[Carbamidomethylation] (C)HQR	RNPADPQ R	RNPADPQ R	SGNENID acetylation (NQ)KEE YLR	SGNENGE FYLR	SVPSDFQ HOATTV ANTINTF R		

121

[illegible]

FIG. 13A

Table 4: Top 30 protein markers at median 34 weeks. Using an ensemble feature selection routine		Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
1	sp. Q92859 NBD1	IVVASL VSTR	EHNQV LGLVK	LIVAGLP R	NEEALDT ESSER	TYTHYF LVPEVDI LSLVR	VLDFEV ISDLVFL K														
2	tr. S6C4Q9 S6C4Q9	YAASSVL SLTFEQW ESHR																			
3	tr. A6AKG2 IR06.A0A GG2IRQ6	ASQGSN SLAWYQ R	LESGVPS R	ILLYAAS R																	
4	sp. Q00391 QSOX1	[Glu->Pro] GHIQND HSQEAA K	AAPGQEP PEHMAEL GSAAR QR	AHESPSNI ILDEPAA R	AVRPAL VLAALD C[Carbamidomethyl] (C)IAEET NSAVCIC arabidomethyl (C)IR	DQNVVA AAPELA MGALEL ESR	ELC[Carbamidomethyl] (C)ISACIC arabidomethyl (C)YHNER	RGVTDFP SC[Carbamidomethyl] (C)YLLF R	GYVHYF FGC[Carbamidomethyl] (C)IR	REVGRFP VLEGQR	IVNADLE SALHYL R	KFGVTDFP PSC[Carbamidomethyl] (C)YLLF R	KVNWIG C[Carbamidomethyl] (C)RGSE PHEE	LAGAPSE DPQPPK	LDVPVW DYEAEL NFLK	LEEDKRR AR	LDALLES HHDTWP PAC[Carbamidomethyl] (C)PPEEP AKLEED GFAR	NGSGAV (NQ)GSG FVPA DVQTLR			
5	sp. Q649L7 ISM2	AC[Carbamidomethyl] (C)IDNP LEBEYLA QLQEAQ	DTLGLPS BEWK	EEEEAPL LPR	ERLDYQ PTAR	EDTTPWI LC[Carbamidomethyl] (C)IK	GAGMPN LISTDFSP K	LDTYQPT AR		SMISGES STLAAQH C[Carbamidomethyl] (C)DLFSC C[Carbamidomethyl] (C)PGTE DK SR	TPEVTPL R	TPEVTPL RLELQK	TRPC[Carbamidomethyl] W	(C)GYGC YLSQML R							
6	tr. BIN788 BIN788	ASQSVSN VLAWYQ OK	ASQSVSN VLAWYQ PR	ASQSVSN YLAWYQ PR	SNWPTF GGQTR	SNWPTF GGQTR															
7	sp. P05546 HEP2	[Glu->Pro] GHIQFEL LDFK	[Glu->Pro] GHIQFEL LDFK	DALENID PATQMMI LNC[Carbamidomethyl] (C)IYFK	DALENID PATQMMI LNC[Carbamidomethyl] (C)IYFK	DOVNTF DMFAPV GISTAMG MISLGLK	ENVITN DWPEGE EDDDYL DLEK	EYYPALAA QIADPSD PAPISK	FAFNLYR	PKLEKNY NLVESLK NFR	FPVEMIO addition (N)ITHN NFR	FPVEMTH NHNFR	FTYDRFP LFLVTEH R	GETHBQ VHSLHF K	GGETAQS ADPQWE ADPQWE QLNNE	GGETAQS ADPQWE ADPQWE QLNNE	GSWVAK FPVEMTH NHNFR	HQQTIV NEEGTQ ATTVTY GFPELST QVR	MLFDNM Deamidation (NQ)GNM GNMAGIS DQR	MLFDEN GNMAGIS DQR	MLFDEN (NQ)GNM GNMAGIS DQR

*Marker also found in Table 2

Fig. 13B

Table 4: Top 50 protein markers at weeks 34 weeks, using an ensemble feature selection routine		Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
8	α A0A075 B6S9 A0A075B6S9	LLIYSAS NIDseamide NLSGVP (N)QLQS GVPER																			
	α A0A075 R884 A0A075R884	AGTPVY TLHGVR SIR	DIAPSE PSESFDE HTVDGE NQVFDH R	DTDSAIT NCI[Carb amidomethyl] (C)A1SV K	ELEPGVE YTHR	ENPATI NAATEL DTTK	EFTTGL DAPR	FTTDLDS PR	GHEHSIQ FADMK	GLEPGQE YNNVLT AEK	IQALNGP LR	EAQGQY ELR	QTGLAPG QEVESL HIVK	SQTVSAI ATTAMG SPK	TAHISGL PPSIDFIV YLSGLAP SIR	VATYLPV VEGLK R					
9	α B2R582 B2R582	GGILSTP QTGSEND ALVEYLR																			
	α B2R582 B2R582	GGILSTP QTGSEND ALVEYLR																			
10	α B2R582 B2R582	GGILSTP QTGSEND ALVEYLR																			
	α B2R582 B2R582	GGILSTP QTGSEND ALVEYLR																			
11	α B2R582 B2R582	GGILSTP QTGSEND ALVEYLR																			
	α B2R582 B2R582	GGILSTP QTGSEND ALVEYLR																			
12	α Q9UL53 Q9UL53	VQIVESG GGVYQP GR																			
	α Q9UL53 Q9UL53	VQIVESG GGVYQP GR																			
13	α Q9UL53 Q9UL53	VQIVESG GGVYQP GR																			
	α Q9UL53 Q9UL53	VQIVESG GGVYQP GR																			
14	α C9JHR2 C9JHR2	DLGEENF KALAVV R																			
	α C9JHR2 C9JHR2	DLGEENF KALAVV R																			

*Marker also found in Table 2

Table 4: Top 50

[illegible]

4913

Table 4: Top 50 protein markers at median 34 years. Using an ensemble feature selection routine	Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
29	tr A0A109 P082.A0A 109P082	IARNLLTY AASTLQGL GVFPR	DIRMTQSL PSSLAS VGDR	M(Oxidation (M)H(QSP SSLSASV GDR	MTQSPSS LSASVGD R	SINPDEA VATGAA VQAALAM GDK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		
30	sp P082MV 8.H1571A	IAQRHLY EVCGSTR	ATAGDT HLCGEDF DNR	NOVALN PQNTVFD AK	NOVALN PQNTVFD AK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		
31	sp P08160 P08160 P08160	IALEVEEC IAQHEDLY EVCGSTR	DIRMTQSL PSSLAS VGDR	M(Oxidation (M)H(QSP SSLSASV GDR	MTQSPSS LSASVGD R	SINPDEA VATGAA VQAALAM GDK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		
32	tr A0A109 P082.A0A 109P082	IARNLLTY AASTLQGL GVFPR	DIRMTQSL PSSLAS VGDR	M(Oxidation (M)H(QSP SSLSASV GDR	MTQSPSS LSASVGD R	SINPDEA VATGAA VQAALAM GDK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		
33	sp P082MV 8.H1571A	IAQRHLY EVCGSTR	ATAGDT HLCGEDF DNR	NOVALN PQNTVFD AK	NOVALN PQNTVFD AK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		
34	tr A0A109 P082.A0A 109P082	IARNLLTY AASTLQGL GVFPR	DIRMTQSL PSSLAS VGDR	M(Oxidation (M)H(QSP SSLSASV GDR	MTQSPSS LSASVGD R	SINPDEA VATGAA VQAALAM GDK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		
35	tr U3PXP0 U3PXP0 U3PXP0	IALEVEEC IAQHEDLY EVCGSTR	DIRMTQSL PSSLAS VGDR	M(Oxidation (M)H(QSP SSLSASV GDR	MTQSPSS LSASVGD R	SINPDEA VATGAA VQAALAM GDK	EHLLMA LADLDEL LIR	EHLLMA LADLDEL LIR	GHTPTQAP GALNQR	HLSTHFP APGDRF QR	RAHYELA DAGQYR	QVNVLS ASDASFP PVK	LC(Carbamidomethyl (C)NEC(Carbamidomethyl (C)ADGS FELSTR	LHQMSV ADSGV VC(Carbamidomethyl (C)JR	LPAVEPT DQAQYL VC(Carbamidomethyl (C)JR	LYTRQAS PADAQ VC(Carbamidomethyl (C)JR	SPAYTLV WTR	VQVSPER		

Fig. 13G

Table 4: Top 50 protein markers at median 34 weeks. Using an unbiased feature selection routine		Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15	Peptide Sequence - 16	Peptide Sequence - 17	Peptide Sequence - 18	Peptide Sequence - 19	Peptide Sequence - 20
41	sp.096006: ZBED1	[Glu->pro]-GluK[Daa mutation] (NQ)R[Deamidation] (NQ)NVA- HC[Carbamidomethyl] (C)IM[Oxidation] (M)ILVSN[Deamidation] (NQ)R																			
42	sp.A6A775: B6S2.KYD	PGQFPQL R																			
43	sp.Q9UL77: Q9UL77	ASQSISSY LNWTQQ KPGK																			
44	sp.P11021: BP	AKPEELN MDLPR	ADAGTIAG LNVMR	DNHLLG TFDELGGP PAPP	ELEEVQ PUSK	FEELNMD LFR	HNPTAA ALAYGLD NR	ITITNDQ NR	ITPSYVA FTPEGER	KSDDEIV LVGGSTR	KVTHAV VTVPAFV NDAQR	INDeamidation (NQ)GRV EELNDQ GNR	NELESYA YSLK	NGRVHII ANDQGR R	NQLTSNP ENTVFDA K	NQLTSNP ENTVFDA KR	SDIDEHL VGGSTR	TEAPEEIS AMWLTK	TKPVTV DIGGGQT R	TWNPDES VQQDK	VTHAVV TVPAFV DAQR

Fig. 14A

Table 5 24-Week Markers		Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15
1	sp1A0A0751B64JL_VX51 4_HUMAN	NNN[Deamidation] (NQ)NNR PSGISER	NNNRPSG ISER	IPCI[Carbamidomethyl] (C)FLAG DTR	QALAQIS LPR	VVLEGGH DPLR										
2	sp1P051641 PERM_HUMAN	FPTDQLT PDQER	IANYFTN AFR	DNGPNY VQR	DTVIWPF R	FYGSPEE LAR	GDFPSPH VSGPR	GHDSTEE PPLFSSH KPR	KYADGE VDVVVL EDPLPGG K	LQPSSTT TMAK	PGYQSS WASRPIS TAEYALL YHTLQE ATK	VDLDVA GTK	VVQPTLE QTQYSW ER	YADGEV DVVVLE DPLPGGK		
3	sp1P1980J1 AOC1_HUMAN	AGVFSDL SNQELK	C[Carbamidomethyl] (C)LAFT DVAPR	DNGPNY VQR	DTVIWPF R	FYGSPEE LAR	GDFPSPH VSGPR	GHDSTEE PPLFSSH KPR	KYADGE VDVVVL EDPLPGG K	LQPSSTT TMAK	PGYQSS WASRPIS TAEYALL YHTLQE ATK	VDLDVA GTK	VVQPTLE QTQYSW ER	YADGEV DVVVLE DPLPGGK		
4	sp1P24593J1 BP5_HUMAN	ALSMC[C arabamidon ethyl (C)]PPSPL GC[Carbamidomethyl] (C)ELVK	AVYLPN C[Carbamidomethyl] (C)DR	AVYLPN C[Carbamidomethyl] (C)DRK	FVGGAE NTAHPR	HSAPEMR										
5	sp1P2484J1 MYL9_HUMAN	EAFNMID QNR	EDLHDM LASLGK	GNFNYY EFTR	LNGTDPE DVIR	NAFAC[C arabamidon ethyl (C)]FDEE ASGFIHE DHLR										
6	sp1Q9H4B7 TEB1_HUMAN	ALSV AEL TQQMED AR	AVLVDL EPGTMDS IR	EVDQQL LSVQTR	FWEMIGE EHGIDLA GSDR	GASALQL ER	GHIYTEG AELIENV LEVVR	IMNSFSV MPSPK	ISVYVNE AYGR	LGALFQP DSFVHG NSGAGN NWAK	LHFFMPG FAPLTAQ GSQQYR	LITPTYG DINHLVS ELTMSGIT TSLR				

24

[illegible]

[illegible]

Fig. 14

[illegible]

Fig. 15A

Table 6 34-Week Markers		Peptide Sequence - 1	Peptide Sequence - 2	Peptide Sequence - 3	Peptide Sequence - 4	Peptide Sequence - 5	Peptide Sequence - 6	Peptide Sequence - 7	Peptide Sequence - 8	Peptide Sequence - 9	Peptide Sequence - 10	Peptide Sequence - 11	Peptide Sequence - 12	Peptide Sequence - 13	Peptide Sequence - 14	Peptide Sequence - 15
1	sp O1551 ARPC5_H UMAN	ALAAAG VGSTVR	GFESPSD NSSAMLL QWHEK													
2	sp P0735 ANXA2_H UMAN	AEDGSVI DYELIDQ DAR	DALNIET VNILNTR AIK	GVDEVTH VNILNTR KQPR	LSLEGDH STPPSAY GSVK	RAEDGS VIDYELI DQDAR	TNQELQE INR									
3	sp P1353 MYH6_H UMAN	DIDDLEL TLAK	DLEEATL QHEATA AALR	INATLET KQPR	LAEQELI ETSER	LASADIE TYLLEK	LELQSAL EEAEASL EHEEGK R	RVIQYFA SIAAIGD R								
4	sp P18206 VINC_HU MAN	AIPDLTA PVAAVQ AAVSNL VR	AVAGNIS DPGLQK	DPSASPG DAGEQAI R	IDYLDGS R	ELTPQVV SAAR	ETVQTTE DQILK	GLVAEG HR	LVQAAQ MLQSDP YSVPAR	MLGQMT DQVADL R	MSAEINEI IR	MTGLVD EADTK	NQIEEA LK	SLLDASE EAIKK	TISPMVM DAK	VMLVNS MNTVK
5	sp P20023 CR2_HUM AN	AFIGC[Car bamidomet hyl (C)]PPPPK	QLLTKPQ HQFVR	QSLQELP AGSR												
6	sp Q6P988 NOTUM_ HUMAN	DVPASFA PAC[Carba midometh yl (C)]JPWPH IR	GC[Carba midomethyl (C)]JPVHL VDSC[Car bamidomet hyl (C)]JPWPH IR	GLADSG WFLDNK	HTDC[Car bamidomet hyl (C)]VDIT C[Carbami donethyl (C)]APTE AIR	LGYPAIQ VR	LYQNLG R	QFQEGEE WNC[Carb amidometh yl (C)]FFGY K	SLAQSLY PC[Carbam idomethyl (C)]SAQQ LNEDLR	YWNGVV PER						

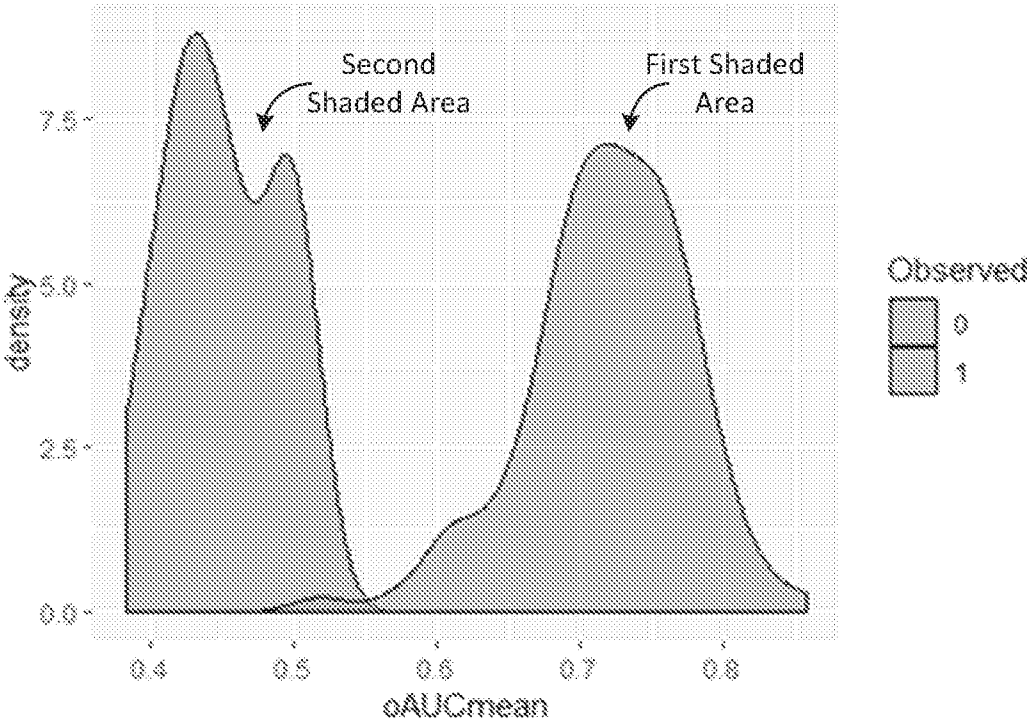


FIG. 16A

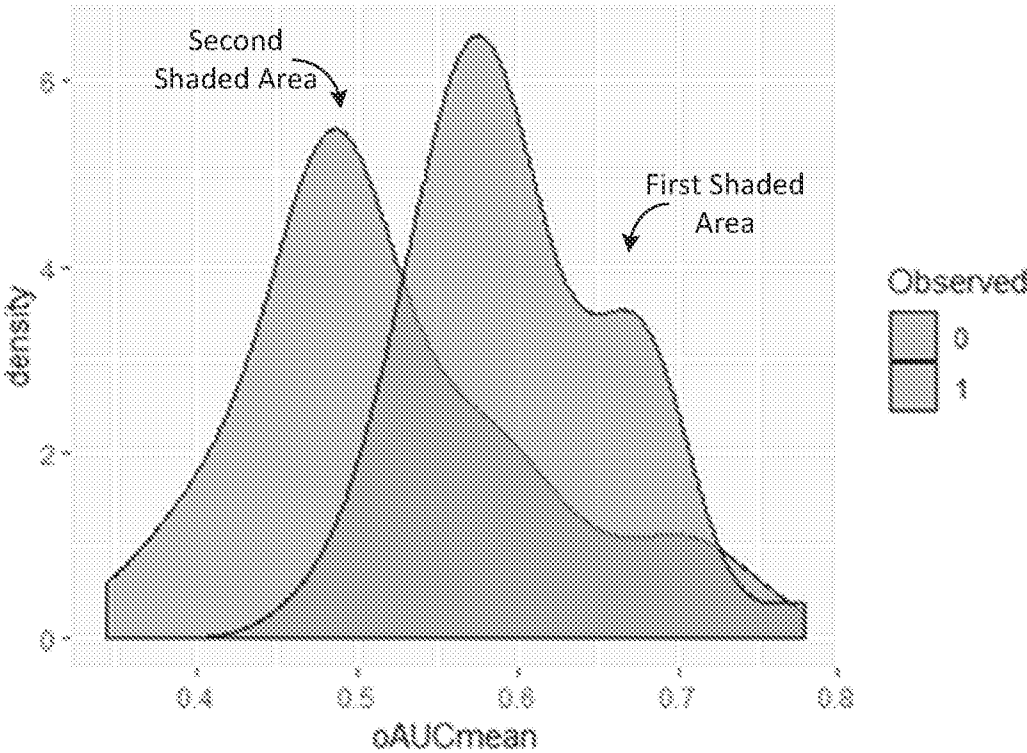


FIG. 16B

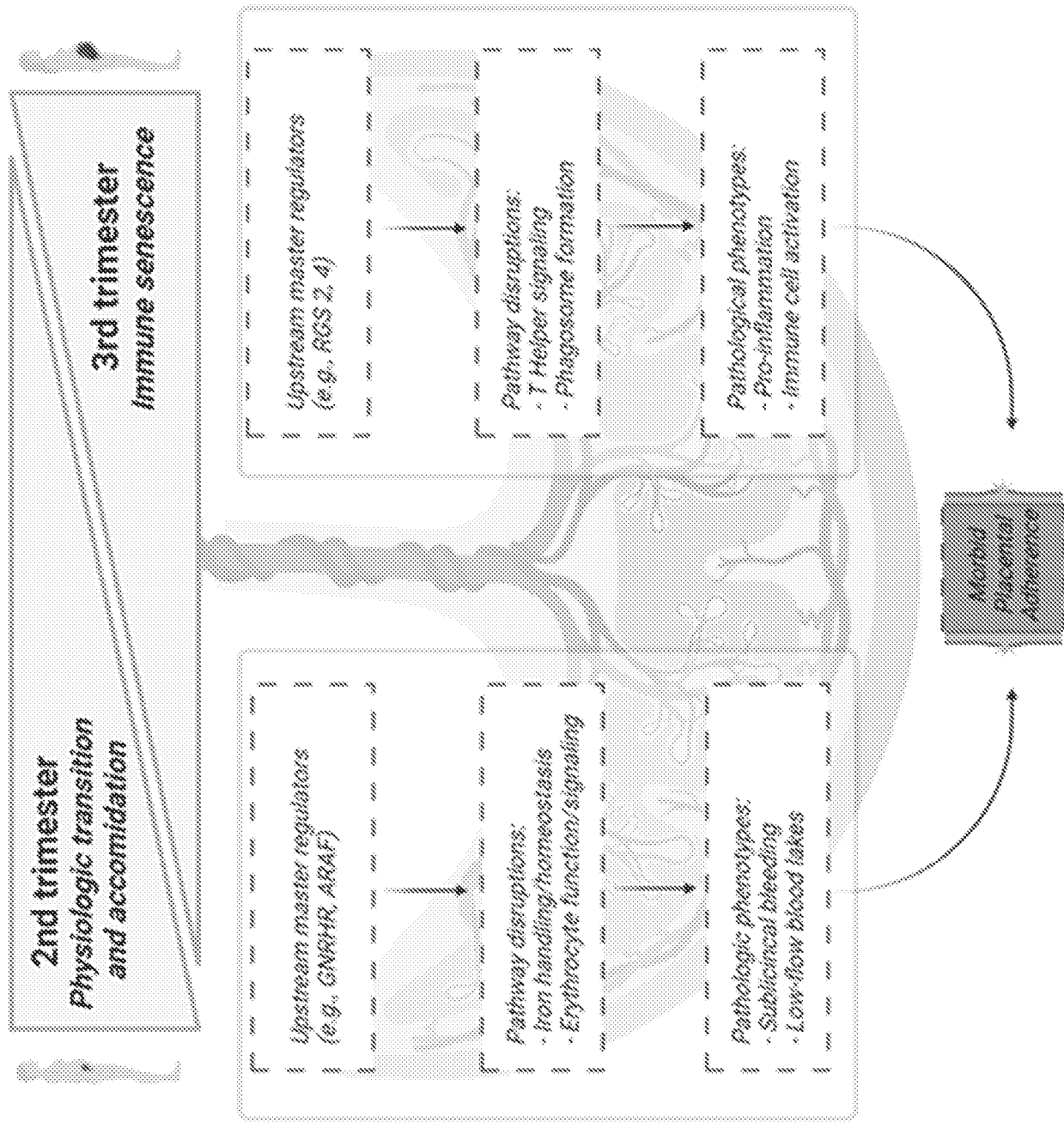


FIG. 17