METHODS AND SYSTEMS FOR DETERMINING A PREGNANCY-RELATED STATE OF A SUBJECT

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Patent Application No. 62/805,515, filed February 14, 2019, U.S. Patent Application No. 62/890,248, filed August 22, 2019, and U.S. Patent Application No. 62/926,786, filed October 28, 2019, each of which is entirely incorporated by reference herein.

BACKGROUND

[0002] Every year, about 15 million pre-term births are reported globally. Pre-term birth may affect as many as about 10% of pregnancies, of which the majority are spontaneous pre-term births. Currently, there may be no meaningful, clinically actionable diagnostic screenings or tests available for many pregnancy-related complications such as pre-term birth. However, pregnancy-related complications such as pre-term birth are a leading cause of neonatal death and of complications later in life. Further, such pregnancy-related complications can cause negative health effects on maternal health. Thus, to make pregnancy as safe as possible, there exists a need for rapid, accurate methods for identifying and monitoring pregnancy-related states that are non-invasive and cost-effective, toward improving maternal and fetal health.

SUMMARY

[0002a] The present disclosure provides a use of a drug in the manufacture of a medicament for treating a pregnant subject for a pregnancy-related hypertensive disorder, wherein: (a) a cell-free blood sample is to be obtained from said pregnant subject; (b) nucleic acid molecules obtained or derived from said cell-free blood sample are to be assayed to determine at least one ribonucleic acid (RNA) level of at least one pregnancy-associated genomic locus, wherein said at least one pregnancy-associated genomic locus is differentially expressed in a first population of subjects with said pregnancy-related hypertensive disorder as compared to a second population of subjects without said pregnancy-related hypertensive disorder, wherein said assaying comprises selectively enriching said nucleic acid molecules for said at least one pregnancy-associated genomic locus, wherein said selectively enriching comprises use of nucleic acids having sequence complementarity with a nucleic acid sequence of said at least one pregnancy-associated genomic locus; (c) said at least one RNA level of said at least one pregnancy-associated genomic locus determined in (b) is to be computer processed (i) against at least one reference RNA level of said at least one pregnancy-associated genomic locus or (ii) using a trained machine learning algorithm; wherein (d) said pregnant subject is determined to have or be at an elevated risk of having said pregnancy-related hypertensive disorder based at

least in part on said computer processing in (c); and wherein (e) the drug is to be administered to said pregnant subject to treat said pregnancy-related hypertensive disorder, based on said determination in (d).

[0003] The present disclosure provides methods, systems, and kits for identifying or monitoring pregnancy-related states by processing cell-free biological samples obtained from or derived from subjects. Cell-free biological samples (e.g., plasma samples) obtained from subjects may be analyzed to identify the pregnancy-related state (which may include, e.g., measuring a presence, absence, or relative assessment of the pregnancy-related state). Such subjects may include subjects with one or more pregnancy-related states and subjects without pregnancy-related states. Pregnancy-related states may include, for example, pre-term birth, full-term birth, gestational age, due date (e.g., due date for an unborn baby or fetus of a subject), onset of labor, pregnancy-related hypertensive disorders (e.g., preeclampsia), eclampsia, gestational diabetes, a congenital disorder of a fetus of the subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications (e.g., post-partum depression, hemorrhage or excessive bleeding, pulmonary embolism, cardiomyopathy, diabetes, anemia, and hypertensive disorders), hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions (e.g., anemia, apnea, bradycardia and other heart defects, bronchopulmonary dysplasia or chronic lung disease, diabetes, gastroschisis, hydrocephaly, hyperbilirubinemia, hypocalcemia, hypoglycemia, intraventricular hemorrhage, jaundice, necrotizing enterocolitis, patent ductus arteriosis, periventricular leukomalacia, persistent pulmonary hypertension, polycythemia, respiratory distress syndrome, retinopathy of prematurity, and transient tachypnea), and fetal development stages or states (e.g., normal fetal organ function or development, and abnormal fetal organ function or development).

[0004] In an example, the present disclosure provides a method for identifying a presence or susceptibility of a pregnancy-related state of a subject, comprising assaying transcripts and/or metabolites in a cell-free biological sample derived from the subject to detect a set of biomarkers, and analyzing the set of biomarkers with a trained algorithm to determine the presence or susceptibility of the pregnancy-related state. In some examples, the method comprises assaying the transcripts in the cell-free biological sample derived from the subject to detect the set of biomarkers. In some examples, the transcripts are assayed with nucleic acid sequencing. In some examples, the method comprises assaying the metabolites in the cell-free biological sample derived from the subject to detect the set of biomarkers. In some examples, the metabolites are assayed with a metabolomics assay.

[0005] In another example, the present disclosure provides a method for identifying a presence or susceptibility of a pregnancy-related state of a subject, comprising assaying a cell-free biological sample derived from the subject to detect a set of biomarkers, and analyzing the set of biomarkers with a trained algorithm to determine the presence or susceptibility of the pregnancy-related state among a set of at least three distinct pregnancy-related states at an accuracy of at least about 80%. In some examples, the pregnancy-related state is selected from the group consisting of pre-term birth, full-term birth, gestational age, due date, onset of labor, pregnancy-related hypertensive disorders (e.g., preeclampsia), eclampsia, gestational diabetes, a congenital disorder of a fetus of the subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications (e.g., post-partum depression, hemorrhage or excessive bleeding, pulmonary embolism, cardiomyopathy, diabetes, anemia, and hypertensive disorders), hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions (e.g., anemia, apnea, bradycardia and other heart defects, bronchopulmonary dysplasia or chronic lung disease, diabetes, gastroschisis, hydrocephaly, hyperbilirubinemia, hypocalcemia, hypoglycemia, intraventricular hemorrhage, jaundice, necrotizing enterocolitis, patent ductus arteriosis, periventricular leukomalacia, persistent pulmonary hypertension, polycythemia, respiratory distress syndrome, retinopathy of prematurity, and transient tachypnea), and fetal development stages or states (e.g., normal fetal organ function or development, and abnormal fetal organ function or development). In some examples, the pregnancy-related state is a sub-type of pre-term birth, and the at least three distinct pregnancy-related states include at least two distinct sub-types of pre-term birth. In some examples, the sub-type of pre-term birth is a molecular sub-type of pre-term birth, and the at least two distinct sub-types of pre-term birth include at least two distinct molecular sub-types of pre-term birth. In some examples, the distinct molecular subtypes of pre-term birth comprise a molecular subtype of pre-term birth selected from the group consisting of history of prior pre-term birth, spontaneous pre-term birth, ethnicity specific pre-term birth risk (e.g., among an African-American population), and pre-term premature rupture of membrane (PPROM). In some examples, the method further comprises identifying a clinical intervention for the subject based at least in part on the presence or susceptibility of the pregnancy-related state. In some examples, the clinical intervention is selected from a plurality of clinical interventions. In some examples, the set of biomarkers comprises a genomic locus associated with due date, wherein the genomic locus is selected from the group consisting of genes listed in **Table** 1 and **Table 7**. In some examples, the set of biomarkers comprises a genomic locus associated with gestational

age, wherein the genomic locus is selected from the group consisting of genes listed in **Table 2**. In some examples, the set of biomarkers comprises a genomic locus associated with pre-term birth, wherein the genomic locus is selected from the group consisting of genes listed in **Table 5**, genes listed in **Table 6**, genes listed in **Table 8**, RAB27B, RGS18, CLCN3, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, the set of biomarkers comprises at least 5 distinct genomic loci. In some examples, the set of biomarkers comprises at least 10 distinct genomic loci. In some examples, the set of biomarkers comprises at least 25 distinct genomic loci. In some examples, the set of biomarkers comprises at least 50 distinct genomic loci. In some examples, the set of biomarkers comprises at least 100 distinct genomic loci. In some examples, the set of biomarkers comprises at least 150 distinct genomic loci.

[0006] In another example, the present disclosure provides a method for identifying or monitoring a presence or susceptibility of a pregnancy-related state of a subject, comprising: (a) using a first assay to process a cell-free biological sample derived from said subject to generate a first dataset; (b) using a second assay to process a vaginal or cervical biological sample derived from said subject to generate a second dataset comprising a microbiome profile of said vaginal or cervical biological sample; (c) using an algorithm (e.g., a trained algorithm) to process at least said first dataset and said second dataset to determine said presence or susceptibility of said pregnancy-related state, which trained algorithm has an accuracy of at least about 80% over 50 independent samples; and (d) electronically outputting a report indicative of said presence or susceptibility of the pregnancy-related state of said subject.

[0007] In some examples, said first assay comprises using cell-free ribonucleic acid (cfRNA) molecules derived from said cell-free biological sample to generate transcriptomic data, using cell-free deoxyribonucleic acid (cfDNA) molecules derived from said cell-free biological sample to generate genomic data, using proteins derived from said cell-free biological sample to generate proteomic data, or using metabolites derived from said cell-free biological sample to generate metabolomic data. In some examples, said cell-free biological sample is from a blood of said subject. In some examples, said cell-free biological sample is from a urine of said subject.

[0008] In some examples, said first dataset comprises a first set of biomarkers associated with said pregnancy-related state. In some examples, said second dataset comprises a second set of biomarkers associated with said pregnancy-related state. In some examples, said second set of biomarkers is different from said first set of biomarkers.

[0009] In some examples, said pregnancy-related state is selected from the group consisting of pre-term birth, full-term birth, gestational age, due date, onset of labor, pregnancy-related hypertensive disorders, eclampsia, gestational diabetes, a congenital disorder of a fetus of the

subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications, hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions, and fetal development stages or states.

[0010] In some examples, said pregnancy-related state comprises pre-term birth. In some examples, said pregnancy-related state comprises gestational age.

[0011] In some examples, said cell-free biological sample is selected from the group consisting of cell-free ribonucleic acid (cfRNA), cell-free deoxyribonucleic acid (cfDNA), cell-free fetal DNA (cffDNA), plasma, serum, urine, saliva, amniotic fluid, and derivatives thereof. In some examples, said cell-free biological sample is obtained or derived from said subject using an ethylenediaminetetraacetic acid (EDTA) collection tube, a cell-free RNA collection tube, or a cell-free DNA collection tube. In some examples, the method further comprises fractionating a whole blood sample of said subject to obtain said cell-free biological sample.

[0012] In some examples, said first assay comprises a cfRNA assay or a metabolomics assay. In some examples, said metabolomics assay comprises targeted mass spectroscopy (MS) or an immune assay. In some examples, said cell-free biological sample comprises cfRNA or urine. In some examples, said first assay or said second assay comprises quantitative polymerase chain reaction (qPCR). In some examples, said first assay or said second assay comprises a home use test configured to be performed in a home setting.

[0013] In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject at a sensitivity of at least about 80%. In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject at a sensitivity of at least about 90%. In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject at a sensitivity of at least about 95%.

[0014] In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject at a positive predictive value (PPV) of at least about 70%. In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject at a positive predictive value (PPV) of at least about 80%. In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state thereof of said subject at a positive predictive value (PPV) of at least about 90%.

[0015] In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject with an Area Under Curve (AUC) of at least about

0.90. In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject with an Area Under Curve (AUC) of at least about 0.95. In some examples, said trained algorithm determines said presence or susceptibility of said pregnancy-related state of said subject with an Area Under Curve (AUC) of at least about 0.99.

[0016] In some examples, said subject is asymptomatic for one or more of: pre-term birth, onset of labor, pregnancy-related hypertensive disorders, eclampsia, gestational diabetes, a congenital disorder of a fetus of the subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications, hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions, and abnormal fetal development stages or states.

[0017] In some examples, said trained algorithm is trained using at least about 10 independent training samples associated with said presence or susceptibility of said pregnancy-related state. In some examples, said trained algorithm is trained using no more than about 100 independent training samples associated with said presence or susceptibility of said pregnancy-related state. In some examples, said trained algorithm is trained using a first set of independent training samples associated with a presence or susceptibility of said pregnancy-related state and a second set of independent training samples associated with an absence or no susceptibility of said pregnancy-related state. In some examples, the method further comprises using said trained algorithm to process a set of clinical health data of said subject to determine said presence or susceptibility of said pregnancy-related state.

[0018] In some examples, (a) comprises (i) subjecting said cell-free biological sample to conditions that are sufficient to isolate, enrich, or extract a set of ribonucleic (RNA) molecules, deoxyribonucleic acid (DNA) molecules, proteins, or metabolites, and (ii) analyzing said set of RNA molecules, DNA molecules, proteins, or metabolites using said first assay to generate said first dataset. In some examples, the method further comprises extracting a set of nucleic acid molecules from said cell-free biological sample, and subjecting said set of nucleic acid molecules to sequencing to generate a set of sequencing reads, wherein said first dataset comprises said set of sequencing reads. In some examples, (b) comprises (i) subjecting said vaginal or cervical biological sample to conditions that are sufficient to isolate, enrich, or extract a population of microbes, and (ii) analyzing said population of microbes using said second assay to generate said second dataset.

[0019] In some examples, said sequencing is massively parallel sequencing. In some examples, said sequencing comprises nucleic acid amplification. In some examples, said nucleic

acid amplification comprises polymerase chain reaction (PCR). In some examples, said sequencing comprises use of simultaneous reverse transcription (RT) and polymerase chain reaction (PCR). In some examples, the method further comprises using probes configured to selectively enrich said set of nucleic acid molecules corresponding to a panel of one or more genomic loci. In some examples, said probes are nucleic acid primers. In some examples, said probes have sequence complementarity with nucleic acid sequences of said panel of said one or more genomic loci.

[0020] In some examples, said panel of said one or more genomic loci comprises at least one genomic locus selected from the group consisting of ACTB, ADAM12, ALPP, ANXA3, APLF, ARG1, AVPR1A, CAMP, CAPN6, CD180, CGA, CGB, CLCN3,CPVL, CSH1, CSH2, CSHL1, CYP3A7, DAPP1, DCX, DEFA4, DGCR14, ELANE, ENAH, EPB42, FABP1, FAM212B-AS1, FGA, FGB, FRMD4B, FRZB, FSTL3, GH2, GNAZ, HAL, HSD17B1, HSD3B1, HSPB8, Immune, ITIH2, KLF9, KNG1, KRT8, LGALS14, LTF, LYPLAL1, MAP3K7CL, MEF2C, MMD, MMP8, MOB1B, NFATC2, OTC, P2RY12, PAPPA, PGLYRP1, PKHD1L1, PKHD1L1, PLAC1, PLAC4, POLE2, PPBP, PSG1, PSG4, PSG7, PTGER3, RAB11A, RAB27B, RAP1GAP, RGS18, RPL23AP7, S100A8, S100A9, S100P, SERPINA7, SLC2A2, SLC38A4, SLC4A1, TBC1D15, VCAN, VGLL1, B3GNT2, COL24A1, CXCL8, and PTGS2.

[0021] In some examples, said panel of said one or more genomic loci comprises at least 5 distinct genomic loci. In some examples, said panel of said one or more genomic loci comprises at least 10 distinct genomic loci.

[0022] In some examples, said panel of said one or more genomic loci comprises a genomic locus associated with pre-term birth, wherein said genomic locus is selected from the group consisting of ADAM12, ANXA3, APLF, AVPR1A, CAMP, CAPN6, CD180, CGA, CGB, CLCN3,CPVL, CSH2, CSHL1, CYP3A7, DAPP1, DGCR14, ELANE, ENAH, FAM212B-AS1, FRMD4B, GH2, HSPB8, Immune, KLF9, KRT8, LGALS14, LTF, LYPLAL1, MAP3K7CL, MMD, MOB1B, NFATC2, P2RY12, PAPPA, PGLYRP1, PKHD1L1, PKHD1L1, PLAC1, PLAC4, POLE2, PPBP, PSG1, PSG4, PSG7, RAB11A, RAB27B, RAP1GAP, RGS18, RPL23AP7, TBC1D15, VCAN, VGLL1, B3GNT2, COL24A1, CXCL8, and PTGS2.

[0023] In some examples, said panel of said one or more genomic loci comprises a genomic locus associated with gestational age, wherein said genomic locus is selected from the group consisting of ACTB, ADAM12, ALPP, ANXA3, ARG1, CAMP, CAPN6, CGA, CGB, CSH1, CSH2, CSHL1, CYP3A7, DCX, DEFA4, EPB42, FABP1, FGA, FGB, FRZB, FSTL3, GH2, GNAZ, HAL, HSD17B1, HSD3B1, HSPB8, ITIH2, KNG1, LGALS14, LTF, MEF2C, MMP8, OTC, PAPPA, PGLYRP1, PLAC1, PLAC4, PSG1, PSG4, PSG7, PTGER3, S100A8, S100A9,

S100P, SERPINA7, SLC2A2, SLC38A4, SLC4A1, VGLL1, RAB27B, RGS18, CLCN3, B3GNT2, COL24A1, CXCL8, and PTGS2.

[0024] In some examples, the panel of said one or more genomic loci comprises a genomic locus associated with due date, wherein the genomic locus is selected from the group consisting of genes listed in **Table** 1 and **Table 7**. In some examples, the panel of said one or more genomic loci comprises a genomic locus associated with gestational age, wherein the genomic locus is selected from the group of genes listed in **Table 2**. In some examples, the panel of said one or more genomic loci comprises a genomic locus associated with pre-term birth, wherein the genomic locus is selected from the group consisting of genes listed in **Table 5**, genes listed in **Table 6**, genes listed in **Table 8**, RAB27B, RGS18, CLCN3, B3GNT2, COL24A1, CXCL8, and PTGS2.

[0025] In some examples, the panel of the one or more genomic loci comprises at least 5 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 10 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 25 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 50 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 100 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 150 distinct genomic loci.

[0026] In some examples, said cell-free biological sample is processed without nucleic acid isolation, enrichment, or extraction.

[0027] In some examples, said report is presented on a graphical user interface of an electronic device of a user. In some examples, said user is said subject.

[0028] In some examples, the method further comprises determining a likelihood of said determination of said presence or susceptibility of said pregnancy-related state of said subject.

[0029] In some examples, said trained algorithm comprises a supervised machine learning algorithm. In some examples, said supervised machine learning algorithm comprises a deep learning algorithm, a support vector machine (SVM), a neural network, or a Random Forest.

[0030] In some examples, the method further comprises providing said subject with a therapeutic intervention for said presence or susceptibility of said pregnancy-related state. In some examples, said therapeutic intervention comprises hydroxyprogesterone caproate, a vaginal progesterone, a natural progesterone IVR product, an prostaglandin F2 alpha receptor antagonist, or a beta2-adrenergic receptor agonist.

[0031] In some examples, the method further comprises monitoring said presence or susceptibility of said pregnancy-related state, wherein said monitoring comprises assessing said presence or susceptibility of said pregnancy-related state of said subject at a plurality of time

points, wherein said assessing is based at least on said presence or susceptibility of said pregnancy-related state determined in (d) at each of said plurality of time points.

[0032] In some examples, a difference in said assessment of said presence or susceptibility of said pregnancy-related state of said subject among said plurality of time points is indicative of one or more clinical indications selected from the group consisting of: (i) a diagnosis of said presence or susceptibility of said pregnancy-related state of said subject, (ii) a prognosis of said presence or susceptibility of said pregnancy-related state of said subject, and (iii) an efficacy or non-efficacy of a course of treatment for treating said presence or susceptibility of said pregnancy-related state of said subject.

[0033] In some examples, the method further comprises stratifying said pre-term birth by using said trained algorithm to determine a molecular sub-type of said pre-term birth from among a plurality of distinct molecular subtypes of pre-term birth. In some examples, the plurality of distinct molecular subtypes of pre-term birth comprises a molecular subtype of pre-term birth selected from the group consisting of history of prior pre-term birth, spontaneous pre-term birth, ethnicity specific pre-term birth risk (e.g., among an African-American population), and pre-term premature rupture of membrane (PPROM).

[0034] In another example, the present disclosure provides a computer-implemented method for predicting a risk of pre-term birth of a subject, comprising: (a) receiving clinical health data of said subject, wherein said clinical health data comprises a plurality of quantitative or categorical measures of said subject; (b) using an algorithm (e.g., a trained algorithm) to process said clinical health data of said subject to determine a risk score indicative of said risk of pre-term birth of said subject; and (c) electronically outputting a report indicative of said risk score indicative of said risk of pre-term birth of said subject.

[0035] In some examples, said clinical health data comprises one or more quantitative measures selected from the group consisting of age, weight, height, body mass index (BMI), blood pressure, heart rate, glucose levels, number of previous pregnancies, and number of previous births. In some examples, said clinical health data comprises one or more categorical measures selected from the group consisting of race, ethnicity, history of medication or other clinical treatment, history of tobacco use, history of alcohol consumption, daily activity or fitness level, genetic test results, blood test results, imaging results, and fetal screening results.

[0036] In some examples, said trained algorithm determines said risk of pre-term birth of said subject at a sensitivity of at least about 80%. In some examples, said trained algorithm determines said risk of pre-term birth of said subject at a specificity of at least about 80%. In some examples, said trained algorithm determines said risk of pre-term birth of said subject at a positive predictive value (PPV) of at least about 80%. In some examples, said trained algorithm

determines said risk of pre-term birth of said subject at a negative predictive value (NPV) of at least about 80%. In some examples, said trained algorithm determines said risk of pre-term birth of said subject with an Area Under Curve (AUC) of at least about 0.9.

[0037] In some examples, said subject is asymptomatic for one or more of: pre-term birth, onset of labor, pregnancy-related hypertensive disorders, eclampsia, gestational diabetes, a congenital disorder of a fetus of said subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications, hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions, and abnormal fetal development stages or states.

[0038] In some examples, said trained algorithm is trained using at least about 10 independent training samples associated with pre-term birth. In some examples, said trained algorithm is trained using no more than about 100 independent training samples associated with pre-term birth. In some examples, said trained algorithm is trained using a first set of independent training samples associated with a presence of pre-term birth and a second set of independent training samples associated with an absence of pre-term birth.

[0039] In some examples, said report is presented on a graphical user interface of an electronic device of a user. In some examples, said user is said subject.

[0040] In some examples, said trained algorithm comprises a supervised machine learning algorithm. In some examples, said supervised machine learning algorithm comprises a deep learning algorithm, a support vector machine (SVM), a neural network, or a Random Forest.

[0041] In some examples, the method further comprises providing said subject with a therapeutic intervention based at least in part on said risk score indicative of said risk of pre-term birth. In some examples, said therapeutic intervention comprises hydroxyprogesterone caproate, a vaginal progesterone, a natural progesterone IVR product, an prostaglandin F2 alpha receptor antagonist, or a beta2-adrenergic receptor agonist.

[0042] In some examples, the method further comprises monitoring said risk of pre-term birth, wherein said monitoring comprises assessing said risk of pre-term birth of said subject at a plurality of time points, wherein said assessing is based at least on said risk score indicative of said risk of pre-term birth determined in (b) at each of said plurality of time points.

[0043] In some examples, the method further comprises refining said risk score indicative of said risk of pre-term birth of said subject by performing one or more subsequent clinical tests for said subject, and processing results from said one or more subsequent clinical tests using a trained algorithm to determine an updated risk score indicative of said risk of pre-term birth of

said subject. In some examples, said one or more subsequent clinical tests comprise an ultrasound imaging or a blood test. In some examples, said risk score comprises a likelihood of said subject having a pre-term birth within a pre-determined duration of time.

[0044] In some examples, said pre-determined duration of time is about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 1.5 days, about 2 days, about 2.5 days, about 3 days, about 3.5 days, about 4 days, about 4.5 days, about 5 days, about 5.5 days, about 6 days, about 6.5 days, about 7 days, about 8 days, about 9 days, about 10 days, about 12 days, about 14 days, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, or more than about 13 weeks.

[0045] In another example, the present disclosure provides a computer system for predicting a risk of pre-term birth of a subject, comprising: a database that is configured to store clinical health data of said subject, wherein said clinical health data comprises a plurality of quantitative or categorical measures of said subject; and one or more computer processors operatively coupled to said database, wherein said one or more computer processors are individually collectively programmed to: (i) use an algorithm (e.g., a trained algorithm) to process said clinical health data of said subject to determine a risk score indicative of said risk of pre-term birth of said subject; and (ii) electronically output a report indicative of said risk score indicative of said risk of pre-term birth of said subject.

[0046] In some examples, the computer system further comprises an electronic display operatively coupled to said one or more computer processors, wherein said electronic display comprises a graphical user interface that is configured to display said report.

[0047] In another example, the present disclosure provides a non-transitory computer readable medium comprising machine-executable code that, upon execution by one or more computer processors, implements a method for predicting a risk of pre-term birth of a subject, said method comprising: (a) receiving clinical health data of said subject, wherein said clinical health data comprises a plurality of quantitative or categorical measures of said subject; (b) using an algorithm (e.g., a trained algorithm) to process said clinical health data of said subject to determine a risk score indicative of said risk of pre-term birth of said subject; and (c) electronically outputting a report indicative of said risk score indicative of said risk of pre-term birth of said subject.

[0048] In another example, the present disclosure provides a method for determining a due date, due date range, or gestational age of a fetus of a pregnant subject, comprising assaying a cell-free biological sample derived from said pregnant subject to detect a set of biomarkers, and

analyzing said set of biomarkers with a trained algorithm to determine said due date, due date range, or gestational age of said fetus.

[0049] In some examples, the method further comprises analyzing an estimated due date of said fetus of said pregnant subject using said trained algorithm, wherein said estimated due date is generated from ultrasound measurements of said fetus. In some examples, said set of biomarkers comprises a genomic locus associated with due date, wherein said genomic locus is selected from the group of genes listed in **Table 1** and **Table 7**.

[0050] In some examples, said set of biomarkers comprises at least 5 distinct genomic loci. In some examples, said set of biomarkers comprises at least 10 distinct genomic loci. In some examples, said set of biomarkers comprises at least 25 distinct genomic loci. In some examples, said set of biomarkers comprises at least 50 distinct genomic loci. In some examples, said set of biomarkers comprises at least 100 distinct genomic loci. In some examples, said set of biomarkers comprises at least 150 distinct genomic loci.

[0051] In some examples, the method further comprises identifying a clinical intervention for said pregnant subject based at least in part on said determined due date. In some examples, said clinical intervention is selected from a plurality of clinical interventions.

[0052] In some examples, said time-to-delivery is less than 7.5 weeks. In some examples, said genomic locus is selected from ACKR2, AKAP3, ANO5, C1orf21, C2orf42, CARNS1, CASC15, CCDC102B, CDC45, CDIPT, CMTM1, COPS8, CTD-2267D19.3, CTD-2349P21.9, CXorf65, DDX11L1, DGUOK, DPAGT1, EIF4A1P2, FANK1, FERMT1, FKRP, GAMT, GOLGA6L4, KLLN, LINC01347, LTA, MAPK12, METRN, MKRN4P, MPC2, MYL12BP1, NME4, NPM1P30, PCLO, PIF1, PTP4A3, RIMKLB, RP13-88F20.1, S100B, SIGLEC14, SLAIN1, SPATA33, TFAP2C, TMSB4XP8, TRGV10, and ZNF124.

[0053] In some examples, said time-to-delivery is less than 5 weeks. In some examples, said genomic locus is selected from C2orf68, CACNB3, CD40, CDKL5, CTBS, CTD-2272G21.2, CXCL8, DHRS7B, EIF5A2, IFITM3, MIR24-2, MTSS1, MYSM1, NCK1-AS1, NR1H4, PDE1C, PEMT, PEX7, PIF1, PPP2R3A, RABIF, SIGLEC14, SLC25A53, SPANXN4, SUPT3H, ZC2HC1C, ZMYM1, and ZNF124.

[0054] In some examples, said time-to-delivery is less than 7.5 weeks. In some examples, said genomic locus is selected from ACKR2, AKAP3, ANO5, C1orf21, C2orf42, CARNS1, CASC15, CCDC102B, CDC45, CDIPT, CMTM1, collectionga, COPS8, CTD-2267D19.3, CTD-2349P21.9, DDX11L1, DGUOK, DPAGT1, EIF4A1P2, FANK1, FERMT1, FKRP, GAMT, GOLGA6L4, KLLN, LINC01347, LTA, MAPK12, METRN, MPC2, MYL12BP1, NME4, NPM1P30, PCLO, PIF1, PTP4A3, RIMKLB, RP13-88F20.1, S100B, SIGLEC14,

SLAIN1, SPATA33, STAT1, TFAP2C, TMEM94, TMSB4XP8, TRGV10, ZNF124, and ZNF713.

[0055] In some examples, said time-to-delivery is less than 5 weeks. In some examples, said genomic locus is selected from ATP6V1E1P1, ATP8A2, C2orf68, CACNB3, CD40, CDKL4, CDKL5, CEP152, CLEC4D, COL18A1, collectionga, COX16, CTBS, CTD-2272G21.2, CXCL2, CXCL8, DHRS7B, DPPA4, EIF5A2, FERMT1, GNB1L, IFITM3, KATNAL1, LRCH4, MBD6, MIR24-2, MTSS1, MYSM1, NCK1-AS1, NPIPB4, NR1H4, PDE1C, PEMT, PEX7, PIF1, PPP2R3A, PXDN, RABIF, SERTAD3, SIGLEC14, SLC25A53, SPANXN4, SSH3, SUPT3H, TMEM150C, TNFAIP6, UPP1, XKR8, ZC2HC1C, ZMYM1, and ZNF124.

[0056] In some examples, said trained algorithm comprises a linear regression model or an ANOVA model. In some examples, said ANOVA model determines a maximum-likelihood time window corresponding to said due date from among a plurality of time windows. In some examples, said maximum-likelihood time window corresponds to a time-to-delivery of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, or 20 weeks. In some examples, said ANOVA model determines a probability or likelihood of a time window corresponding to said due date from among a plurality of time windows. In some examples, said ANOVA model calculates a probability-weighted average across said plurality of time windows to determine an average or expected time window distance.

[0057] In another example, the present disclosure provides a method for identifying or monitoring a presence or susceptibility of a pregnancy-related state of a subject, comprising: (a) using a first assay to process a first cell-free biological sample derived from the subject to generate a first dataset; (b) based at least in part on the first dataset generated in (a), using a second assay different from the first assay to process a second cell-free biological sample derived from the subject to generate a second dataset indicative of the presence or susceptibility of the pregnancy-related state at a specificity greater than the first dataset; (c) using a trained algorithm to process at least the second dataset to determine the presence or susceptibility of the pregnancy-related state, which trained algorithm has an accuracy of at least about 80% over 50 independent samples; and (d) electronically outputting a report indicative of the presence or susceptibility of the pregnancy-related state of the subject.

[0058] In some examples, the first assay comprises using cell-free ribonucleic acid (cfRNA) molecules derived from the first cell-free biological sample to generate transcriptomic data, using cell-free deoxyribonucleic acid (cfDNA) molecules derived from the first cell-free biological sample to generate genomic data, using proteins derived from the first cell-free biological sample to generate proteomic data, or using metabolites derived from the first cell-

free biological sample to generate metabolomic data. In some examples, the first cell-free biological sample is from a blood of the subject. In some examples, the first cell-free biological sample is from a urine of the subject. In some examples, the first dataset comprises a first set of biomarkers associated with the pregnancy-related state. In some examples, the second dataset comprises a second set of biomarkers associated with the pregnancy-related state. In some examples, the second set of biomarkers is different from the first set of biomarkers.

[0059] In some examples, the pregnancy-related state is selected from the group consisting of pre-term birth, full-term birth, gestational age, due date, onset of labor, pregnancy-related hypertensive disorders (e.g., preeclampsia), eclampsia, gestational diabetes, a congenital disorder of a fetus of the subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications (e.g., post-partum depression, hemorrhage or excessive bleeding, pulmonary embolism, cardiomyopathy, diabetes, anemia, and hypertensive disorders), hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions (e.g., anemia, apnea, bradycardia and other heart defects, bronchopulmonary dysplasia or chronic lung disease, diabetes, gastroschisis, hydrocephaly, hyperbilirubinemia, hypocalcemia, hypoglycemia, intraventricular hemorrhage, jaundice, necrotizing enterocolitis, patent ductus arteriosis, periventricular leukomalacia, persistent pulmonary hypertension, polycythemia, respiratory distress syndrome, retinopathy of prematurity, and transient tachypnea), and fetal development stages or states (e.g., normal fetal organ function or development, and abnormal fetal organ function or development). In some examples, the pregnancy-related state comprises pre-term birth. In some examples, the pregnancy-related state comprises gestational age. In some examples, the cell-free biological sample is selected from the group consisting of cell-free ribonucleic acid (cfRNA), cell-free deoxyribonucleic acid (cfDNA), cell-free fetal DNA (cffDNA), plasma, serum, urine, saliva, amniotic fluid, and derivatives thereof. In some examples, the first cell-free biological sample or the second cell-free biological sample is obtained or derived from the subject using an ethylenediaminetetraacetic acid (EDTA) collection tube, a cell-free RNA collection tube, or a cell-free DNA collection tube. In some examples, the method further comprises fractionating a whole blood sample of the subject to obtain the first cell-free biological sample or the second cell-free biological sample. In some examples, (i) the first assay comprises a cfRNA assay and the second assay comprises a metabolomics assay, or (ii) the first assay comprises a metabolomics assay and the second assay comprises a cfRNA assay. In some examples, (i) the first cell-free biological sample comprises cfRNA and the second cell-free biological sample

comprises urine, or (ii) the first cell-free biological sample comprises urine and the second cell-free biological sample comprises cfRNA. In some examples, the first assay or the second assay comprises quantitative polymerase chain reaction (qPCR). In some examples, the first assay or the second assay comprises a home use test configured to be performed in a home setting. In some examples, the first assay or the second assay comprises a metabolomics assay. In some examples, the metabolomics assay comprises targeted mass spectroscopy (MS) or an immune assay.

[0060] In some examples, the first dataset is indicative of the presence or susceptibility of the pregnancy-related state at a sensitivity of at least about 80%. In some examples, the first dataset is indicative of the presence or susceptibility of the pregnancy-related state at a sensitivity of at least about 90%. In some examples, the first dataset is indicative of the presence or susceptibility of the pregnancy-related state at a sensitivity of at least about 95%. In some examples, the first dataset is indicative of the presence or susceptibility of the pregnancy-related state at a positive predictive value (PPV) of at least about 70%. In some examples, the first dataset is indicative of the presence or susceptibility of the pregnancy-related state at a positive predictive value (PPV) of at least about 80%. In some examples, the first dataset is indicative of the presence or susceptibility of the pregnancy-related state at a positive predictive value (PPV) of at least about 90%. In some examples, the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a specificity of at least about 90%. In some examples, the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a specificity of at least about 95%. In some examples, the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a specificity of at least about 99%. In some examples, the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a negative predictive value (NPV) of at least about 90%. In some examples, the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a negative predictive value (NPV) of at least about 95%. In some examples, the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a negative predictive value (NPV) of at least about 99%. In some examples, the trained algorithm determines the presence or susceptibility of the pregnancy-related state of the subject with an Area Under Curve (AUC) of at least about 0.90. In some examples, the trained algorithm determines the presence or susceptibility of the pregnancy-related state of the subject with an Area Under Curve (AUC) of at least about 0.95. In some examples, the trained algorithm determines the presence or susceptibility of the pregnancy-related state of the subject with an Area Under Curve (AUC) of at least about 0.99.

[0061] In some examples, the subject is asymptomatic for one or more of: pre-term birth, onset of labor, pregnancy-related hypertensive disorders (e.g., preeclampsia), eclampsia, gestational diabetes, a congenital disorder of a fetus of the subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications (e.g., post-partum depression, hemorrhage or excessive bleeding, pulmonary embolism, cardiomyopathy, diabetes, anemia, and hypertensive disorders), hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions (e.g., anemia, apnea, bradycardia and other heart defects, bronchopulmonary dysplasia or chronic lung disease, diabetes, gastroschisis, hydrocephaly, hyperbilirubinemia, hypocalcemia, hypoglycemia, intraventricular hemorrhage, jaundice, necrotizing enterocolitis, patent ductus arteriosis, periventricular leukomalacia, persistent pulmonary hypertension, polycythemia, respiratory distress syndrome, retinopathy of prematurity, and transient tachypnea), and abnormal fetal development stages or states (e.g., abnormal fetal organ function or development). In some examples, the trained algorithm is trained using at least about 10 independent training samples associated with the pregnancy-related state. In some examples, the trained algorithm is trained using no more than about 100 independent training samples associated with the pregnancy-related state. In some examples, the trained algorithm is trained using a first set of independent training samples associated with a presence of the pregnancy-related state and a second set of independent training samples associated with an absence of the pregnancy-related state. In some examples, the method further comprises using the trained algorithm to process the first dataset to determine the presence or susceptibility of the pregnancy-related state . In some examples, the method further comprises using the trained algorithm to process a set of clinical health data of the subject to determine the presence or susceptibility of the pregnancy-related state .

[0062] In some examples, (a) comprises (i) subjecting the first cell-free biological sample to conditions that are sufficient to isolate, enrich, or extract a first set of ribonucleic acid (RNA) molecules, deoxyribonucleic acid (DNA) molecules, proteins, or metabolites, and (ii) analyzing the first set of RNA molecules, DNA molecules, proteins, or metabolites using the first assay to generate the first dataset. In some examples, the method further comprises extracting a first set of nucleic acid molecules from the first cell-free biological sample, and subjecting the first set of nucleic acid molecules to sequencing to generate a first set of sequencing reads, wherein the first dataset comprises the first set of sequencing reads. In some examples, the method further comprises extracting a first set of metabolites from the first cell-free biological sample, and assaying the first set of metabolites to generate the first dataset In some examples, (b) comprises

(i) subjecting the second cell-free biological sample to conditions that are sufficient to isolate, enrich, or extract a second set of ribonucleic acid (RNA) molecules, deoxyribonucleic acid (DNA) molecules, proteins, or metabolites, and (ii) analyzing the second set of RNA molecules, DNA molecules, proteins, or metabolites using the second assay to generate the second dataset. In some examples, the method further comprises extracting a second set of nucleic acid molecules from the second cell-free biological sample, and subjecting the second set of nucleic acid molecules to sequencing to generate a second set of sequencing reads, wherein the second dataset comprises the second set of sequencing reads. In some examples, the method further comprises extracting a second set of metabolites from the second cell-free biological sample, and assaying the second set of metabolites to generate the second dataset. In some examples, the sequencing is massively parallel sequencing. In some examples, the sequencing comprises nucleic acid amplification. In some examples, the nucleic acid amplification comprises polymerase chain reaction (PCR). In some examples, the sequencing comprises use of simultaneous reverse transcription (RT) and polymerase chain reaction (PCR).

[0063] In some examples, the method further comprises using probes configured to selectively enrich the first set of nucleic acid molecules or the second set of nucleic acid molecules corresponding to a panel of one or more genomic loci. In some examples, the probes are nucleic acid primers. In some examples, the probes have sequence complementarity with nucleic acid sequences of the panel of the one or more genomic loci. In some examples, the panel of the one or more genomic loci comprises at least one genomic locus selected from the group consisting of ACTB, ADAM12, ALPP, ANXA3, APLF, ARG1, AVPR1A, CAMP, CAPN6, CD180, CGA, CGB, CLCN3,CPVL, CSH1, CSH2, CSHL1, CYP3A7, DAPP1, DCX, DEFA4, DGCR14, ELANE, ENAH, EPB42, FABP1, FAM212B-AS1, FGA, FGB, FRMD4B, FRZB, FSTL3, GH2, GNAZ, HAL, HSD17B1, HSD3B1, HSPB8, Immune, ITIH2, KLF9, KNG1, KRT8, LGALS14, LTF, LYPLAL1, MAP3K7CL, MEF2C, MMD, MMP8, MOB1B, NFATC2, OTC, P2RY12, PAPPA, PGLYRP1, PKHD1L1, PKHD1L1, PLAC1, PLAC4, POLE2, PPBP, PSG1, PSG4, PSG7, PTGER3, RAB11A, RAB27B, RAP1GAP, RGS18, RPL23AP7, S100A8, S100A9, S100P, SERPINA7, SLC2A2, SLC38A4, SLC4A1, TBC1D15, VCAN, VGLL1, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, the panel of the one or more genomic loci comprises at least 5 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 10 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises a genomic locus associated with pre-term birth, wherein said genomic locus is selected from the group consisting of ADAM12, ANXA3, APLF, AVPR1A, CAMP, CAPN6, CD180, CGA, CGB, CLCN3,CPVL, CSH2, CSHL1, CYP3A7, DAPP1, DGCR14, ELANE, ENAH, FAM212B-AS1, FRMD4B, GH2, HSPB8, Immune, KLF9,

KRT8, LGALS14, LTF, LYPLAL1, MAP3K7CL, MMD, MOB1B, NFATC2, P2RY12, PAPPA, PGLYRP1, PKHD1L1, PKHD1L1, PLAC1, PLAC4, POLE2, PPBP, PSG1, PSG4, PSG7, RAB11A, RAB27B, RAP1GAP, RGS18, RPL23AP7, TBC1D15, VCAN, VGLL1, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, the panel of the one or more genomic loci comprises a genomic locus associated with gestational age, wherein said genomic locus is selected from the group consisting of ACTB, ADAM12, ALPP, ANXA3, ARG1, CAMP, CAPN6, CGA, CGB, CSH1, CSH2, CSHL1, CYP3A7, DCX, DEFA4, EPB42, FABP1, FGA, FGB, FRZB, FSTL3, GH2, GNAZ, HAL, HSD17B1, HSD3B1, HSPB8, ITIH2, KNG1, LGALS14, LTF, MEF2C, MMP8, OTC, PAPPA, PGLYRP1, PLAC1, PLAC4, PSG1, PSG4, PSG7, PTGER3, S100A8, S100A9, S100P, SERPINA7, SLC2A2, SLC38A4, SLC4A1, VGLL1, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, the panel of said one or more genomic loci comprises a genomic locus associated with due date, wherein the genomic locus is selected from the group of genes listed in **Table** 1 and **Table 7**. In some examples, the panel of said one or more genomic loci comprises a genomic locus associated with gestational age, wherein the genomic locus is selected from the group of genes listed in **Table 2**. In some examples, the panel of said one or more genomic loci comprises a genomic locus associated with pre-term birth, wherein the genomic locus is selected from the group of genes listed in **Table 5**, genes listed in **Table 6**, genes listed in **Table 8**, RAB27B, RGS18, CLCN3, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, the panel of the one or more genomic loci comprises at least 5 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 10 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 25 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 50 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 100 distinct genomic loci. In some examples, the panel of the one or more genomic loci comprises at least 150 distinct genomic loci. In some examples, the first cell-free biological sample or the second cell-free biological sample is processed without nucleic acid isolation, enrichment, or extraction. In some examples, the report is presented on a graphical user interface of an electronic device of a user. In some examples, the user is the subject.

[0064] In some examples, the method further comprises determining a likelihood of the determination of the presence or susceptibility of the pregnancy-related state of the subject. In some examples, the trained algorithm comprises a supervised machine learning algorithm. In some examples, the supervised machine learning algorithm comprises a deep learning algorithm, a support vector machine (SVM), a neural network, or a Random Forest. In some examples, the method further comprises providing the subject with a therapeutic intervention for the presence

or susceptibility of the pregnancy-related state . In some examples, therapeutic intervention comprises a progesterone treatment such as hydroxyprogesterone caproate (e.g., 17-alpha hydroxyprogesterone caproate (17-P), LPCN 1107 from Lipocine, Makena from AMAG Pharma), a vaginal progesterone, or a natural progesterone IVR product (e.g., DARE-FRT1 (JNP-0301) from Juniper Pharma); a prostaglandin F2 alpha receptor antagonist (e.g., OBE022 from ObsEva); or a beta2-adrenergic receptor agonist (e.g., bedoradrine sulfate (MN-221) from MediciNova). Therapeutic interventions may be described by, for example, "WHO Recommendations on Interventions to Improve Preterm Birth Outcomes," World Health Organization, 2015, which is hereby incorporated by reference in its entirety. In some examples, the method further comprises monitoring the presence or susceptibility of the pregnancy-related state , wherein the monitoring comprises assessing the presence or susceptibility of the pregnancy-related state of the subject at a plurality of time points, wherein the assessing is based at least on the presence or susceptibility of the pregnancy-related state determined in (d) at each of the plurality of time points. In some examples, a difference in the assessment of the presence or susceptibility of the pregnancy-related state of the subject among the plurality of time points is indicative of one or more clinical indications selected from the group consisting of: (i) a diagnosis of the presence or susceptibility of the pregnancy-related state of the subject, (ii) a prognosis of the presence or susceptibility of the pregnancy-related state of the subject, and (iii) an efficacy or non-efficacy of a course of treatment for treating the presence or susceptibility of the pregnancy-related state of the subject. In some examples, the method further comprises stratifying the pre-term birth by using the trained algorithm to determine a molecular sub-type of the pre-term birth from among a plurality of distinct molecular subtypes of pre-term birth. In some examples, the plurality of distinct molecular subtypes of pre-term birth comprises a molecular subtype of pre-term birth selected from the group consisting of history of prior pre-term birth, spontaneous pre-term birth, ethnicity specific pre-term birth risk (e.g., among an African-American population), and pre-term premature rupture of membrane (PPROM).

[0065] In another example, the present disclosure provides a computer system for identifying or monitoring a presence or susceptibility of the pregnancy-related state of a subject, comprising: a database that is configured to store a first dataset and a second dataset, wherein the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a specificity greater than the first dataset; and one or more computer processors operatively coupled to the database, wherein the one or more computer processors are individually collectively programmed to: (i) use a trained algorithm to process at least the second dataset to determine the presence or susceptibility of the pregnancy-related state , which trained algorithm has an accuracy of at least about 80% over 50 independent samples; and (ii) electronically

output a report indicative of the presence or susceptibility of the pregnancy-related state of the subject.

[0066] In some examples, the computer system further comprises an electronic display operatively coupled to the one or more computer processors, wherein the electronic display comprises a graphical user interface that is configured to display the report.

[0067] In another example, the present disclosure provides a non-transitory computer readable medium comprising machine-executable code that, upon execution by one or more computer processors, implements a method for identifying or monitoring a presence or susceptibility of the pregnancy-related state of a subject, the method comprising: (a) obtaining a first dataset, and a second dataset, wherein the second dataset is indicative of the presence or susceptibility of the pregnancy-related state at a specificity greater than the first dataset; (b) using a trained algorithm to process at least the second dataset to determine the pregnancy-related state, which trained algorithm has an accuracy of at least about 80% over 50 independent samples; and (c) electronically outputting a report indicative of the presence or susceptibility of the pregnancy-related state of the subject.

[0068] In another example, the present disclosure provides a method for identifying a presence or susceptibility of pregnancy-related state of a subject, comprising (i) assaying a first cell-free biological sample derived from the subject with a first assay to generate a first dataset, (ii) assaying a second cell-free biological sample derived from the subject with a second assay to generate a second dataset that is indicative of the presence or susceptibility of the pregnancy-related state at a specificity greater than the first dataset, and (iii) using a trained algorithm to process at least the second dataset to determine the presence or susceptibility of the pregnancy-related state at an accuracy of at least about 80%. In some examples, the accuracy is at least about 90%. In some examples, the pregnancy-related state is selected from the group consisting of pre-term birth, full-term birth, gestational age, due date, onset of labor, pregnancy-related hypertensive disorders (e.g., preeclampsia), eclampsia, gestational diabetes, a congenital disorder of a fetus of the subject, ectopic pregnancy, spontaneous abortion, stillbirth, post-partum complications (e.g., post-partum depression, hemorrhage or excessive bleeding, pulmonary embolism, cardiomyopathy, diabetes, anemia, and hypertensive disorders), hyperemesis gravidarum (morning sickness), hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa (placenta covering the cervix), intrauterine/fetal growth restriction, macrosomia (large fetus for gestational age), neonatal conditions (e.g., anemia, apnea, bradycardia and other heart defects, bronchopulmonary dysplasia or chronic lung disease, diabetes, gastroschisis, hydrocephaly, hyperbilirubinemia, hypocalcemia, hypoglycemia, intraventricular hemorrhage,

jaundice, necrotizing enterocolitis, patent ductus arteriosis, periventricular leukomalacia, persistent pulmonary hypertension, polycythemia, respiratory distress syndrome, retinopathy of prematurity, and transient tachypnea), and fetal development stages or states (e.g., normal fetal organ function or development, and abnormal fetal organ function or development).

[0069] In another example, the present disclosure provides a method for determining that a subject is at risk of pre-term birth, comprising assaying a cell-free biological sample derived from the subject to generate a dataset that is indicative of the pre-term birth risk at a specificity of at least 80%, and using a trained algorithm that is trained on samples independent of the cell-free biological sample to determine that the subject is at risk of pre-term birth at an accuracy of at least about 80%. In some examples, the accuracy is at least about 90%.

[0070] In another example, the present disclosure provides a method for detecting a presence or risk of a prenatal metabolic genetic disease of a fetus of a pregnant subject, comprising: assaying ribonucleic acid (RNA) in a cell-free biological sample derived from said pregnant subject to detect a set of biomarkers; and analyzing said set of biomarkers with an algorithm (e.g., a trained algorithm) to detect said presence or risk of said prenatal metabolic genetic disease.

[0071] In another example, the present disclosure provides a method for detecting at least two health or physiological conditions of a fetus of a pregnant subject or of said pregnant subject, comprising: assaying a first cell-free biological sample obtained or derived from said pregnant subject at a first time point and a second cell-free biological sample obtained or derived from said pregnant subject at a second time point, to detect a first set of biomarkers at said first time point and a second set of biomarkers at said second time point, and analyzing said first set of biomarkers or said second set of biomarkers with a trained algorithm to detect said at least two health or physiological conditions.

[0072] In some examples, said at least two health or physiological conditions are selected from the group consisting of pre-term birth, full-term birth, gestational age, due date, onset of labor, a pregnancy-related hypertensive disorder, eclampsia, gestational diabetes, a congenital disorder of a fetus of said subject, ectopic pregnancy, spontaneous abortion, stillbirth, a post-partum complication, hyperemesis gravidarum, hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa, intrauterine/fetal growth restriction, macrosomia, a neonatal condition, and a fetal development stage or state. In some examples, said set of biomarkers comprises a genomic locus associated with due date, wherein said genomic locus is selected from the group consisting of genes listed in **Table 1** and **Table 7.** In some examples, said set of biomarkers comprises a genomic locus associated with gestational age, wherein said genomic locus is selected from the

group consisting of genes listed in **Table 2.** In some examples, said set of biomarkers comprises a genomic locus associated with pre-term birth, wherein said genomic locus is selected from the group consisting of genes listed in **Table 5**, genes listed in **Table 6**, genes listed in **Table 8**, RAB27B, RGS18, CLCN3, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, said set of biomarkers comprises at least 5 distinct genomic loci.

[0073] In another example, the present disclosure provides a method comprising: assaying one or more cell-free biological samples obtained or derived from a pregnant subject to detect a set of biomarkers; and analyzing said set of biomarkers to identify (1) a due date or a range thereof of a fetus of said pregnant subject and (2) a health or physiological condition of said fetus of said pregnant subject or of said pregnant subject.

[0074] In some examples, the method further comprises analyzing said set of biomarkers with a trained algorithm. In some examples, said health or physiological condition is selected from the group consisting of pre-term birth, full-term birth, gestational age, due date, onset of labor, a pregnancy-related hypertensive disorder, eclampsia, gestational diabetes, a congenital disorder of a fetus of said subject, ectopic pregnancy, spontaneous abortion, stillbirth, a post-partum complication, hyperemesis gravidarum, hemorrhage or excessive bleeding during delivery, premature rupture of membrane, premature rupture of membrane in pre-term birth, placenta previa, intrauterine/fetal growth restriction, macrosomia, a neonatal condition, and a fetal development stage or state. In some examples, said set of biomarkers comprises a genomic locus associated with due date, wherein said genomic locus is selected from the group consisting of genes listed in **Table 1** and **Table 7.** In some examples, said set of biomarkers comprises a genomic locus associated with gestational age, wherein said genomic locus is selected from the group consisting of genes listed in **Table 2.** In some examples, said set of biomarkers comprises a genomic locus associated with pre-term birth, wherein said genomic locus is selected from the group consisting of genes listed in **Table 5**, genes listed in **Table 6**, genes listed in **Table 8**, RAB27B, RGS18, CLCN3, B3GNT2, COL24A1, CXCL8, and PTGS2. In some examples, said set of biomarkers comprises at least 5 distinct genomic loci.

[0075] In another example, the present disclosure provides a method comprising: assaying one or more cell-free biological samples obtained or derived from a pregnant subject to detect a set of nucleic acids of non-human origin; and analyzing said set of nucleic acids of non-human origin to detect a health or physiological condition of a fetus of said pregnant subject or of said pregnant subject. In some examples, the nucleic acids of non-human origin comprise DNA or RNA of a non-human organism. In some examples, the non-human organism is a bacteria, a virus, or a parasite. In some examples, the method further comprises analyzing said set of nucleic acids of non-human origin using a trained algorithm.

[0076] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0077] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0078] Additional examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0079] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

WHAT IS CLAIMED IS:

1. Use of a drug in the manufacture of a medicament for treating a pregnant subject for a pregnancy-related hypertensive disorder, wherein:

(a) a cell-free blood sample is to be obtained from said pregnant subject;

(b) nucleic acid molecules obtained or derived from said cell-free blood sample are to be assayed to determine at least one ribonucleic acid (RNA) level of at least one pregnancy-associated genomic locus, wherein said at least one pregnancy-associated genomic locus is differentially expressed in a first population of subjects with said pregnancy-related hypertensive disorder as compared to a second population of subjects without said pregnancy-related hypertensive disorder, wherein said assaying comprises selectively enriching said nucleic acid molecules for said at least one pregnancy-associated genomic locus, wherein said selectively enriching comprises use of nucleic acids having sequence complementarity with a nucleic acid sequence of said at least one pregnancy-associated genomic locus;

(c) said at least one RNA level of said at least one pregnancy-associated genomic locus determined in (b) is to be computer processed (i) against at least one reference RNA level of said at least one pregnancy-associated genomic locus or (ii) using a trained machine learning algorithm; wherein

(d) said pregnant subject is determined to have or be at an elevated risk of having said pregnancy-related hypertensive disorder based at least in part on said computer processing in (c); and wherein

(e) the drug is to be administered to said pregnant subject to treat said pregnancy-related hypertensive disorder, based on said determination in (d).

2. The use of claim 1, wherein ribonucleic acid (RNA) molecules derived from said cell-free blood sample are to be further reverse transcribed to produce complementary deoxyribonucleic acid (cDNA) molecules; and said cDNA molecules or derivatives thereof are to be assayed to determine said at least one RNA level of said at least one pregnancy-associated genomic locus.

3. The use of claim 1, wherein said assaying comprises use of nucleic acid sequencing.

4. The use of claim 1, wherein said pregnancy-related hypertensive disorder is selected from the group consisting of preeclampsia and eclampsia.

5. The use of claim 1, wherein said cell-free blood sample comprises a plasma sample.

6. The use of claim 1, wherein said pregnant subject is asymptomatic for said pregnancy-related hypertensive disorder.

7. The use of claim 1, wherein said computer processing in (c) comprises using said trained machine learning algorithm.

8. The use of claim 7, wherein said trained machine learning algorithm is selected from the group consisting of a linear regression, a logistic regression, an analysis of variance (ANOVA) model, a deep learning algorithm, a support vector machine (SVM), a neural network, a Random Forest, and a combination thereof.

9. The use of claim 1, wherein said pregnant subject is to be further monitored for said pregnancy-related hypertensive disorder at least in part by assessing said pregnancy-related hypertensive disorder of said pregnant subject at a plurality of time points, wherein said assessing is based at least in part on determining whether said pregnant subject has said pregnancy-related hypertensive disorder at each of said plurality of time points.

10. The use of claim 9, wherein a difference in said assessing of said pregnancy-related hypertensive disorder of said pregnant subject among said plurality of time points is indicative of one or more clinical indications selected from the group consisting of: (i) a diagnosis of said pregnancy-related hypertensive disorder of said pregnant subject, (ii) a prognosis of said pregnancy-related hypertensive disorder of said pregnant subject, and (iii) an efficacy or non-efficacy of a therapeutic intervention for treating said pregnancy-related hypertensive disorder of said pregnant subject.

11. The use of claim 4, wherein said pregnancy-related hypertensive disorder comprises said preeclampsia.

12. The use of claim 1, wherein said drug is selected from the group consisting of a corticosteroid, an antibiotic, a tocolytic drug, a calcium channel blocker, a cyclo-oxygenase inhibitor, an oxytocin antagonist, a betamimetic drug, magnesium sulfate, magnesium chloride, and magnesium oxide.

13. The use of claim 12, wherein said drug is said corticosteroid.

14. The use of claim 12, wherein said drug is said tocolytic drug.

15. The use of claim 12, wherein said drug is said magnesium sulfate.

16. The use of claim 1, wherein delivery of a fetus of said pregnant subject is to be performed.

17. The use of claim 11, wherein said at least one pregnancy-associated genomic locus is differentially expressed by subjects with preeclampsia as compared to subjects without preeclampsia, wherein said at least one pregnancy-associated genomic locus comprises a member selected from the group consisting of PAPPA2, FABP1, GPSM2, CRH, and HP.

18. The use of claim 17, wherein said at least one pregnancy-associated genomic locus comprises three or more members selected from the group consisting of PAPPA2, FABP1, GPSM2, CRH, and HP.

19. The use of claim 4, wherein said pregnancy-related hypertensive disorder comprises said eclampsia.

20. The use of claim 7, wherein said trained machine learning algorithm is trained using a training dataset comprising: a first set of pregnant subjects with said pregnancy-related hypertensive disorder, and a second set of pregnant subjects without said pregnancy-related hypertensive disorder.

21. The use of claim 8, wherein said trained machine learning algorithm comprises said linear regression.

22. The use of claim 8, wherein said trained machine learning algorithm comprises said ANOVA model.

23. The use of claim 1, wherein said assaying comprises use of array hybridization.

24. The use of claim 1, wherein said assaying comprises use of polymerase chain reaction (PCR).

25. The use of claim 1, wherein said selectively enriching further comprises amplifying at least a portion of said nucleic acid molecules.

26. The use of claim 1, wherein said pregnant subject is to be further determined to have said pregnancy-related hypertensive disorder with a clinical sensitivity or clinical specificity of at least about 70%.

27. The use of claim 1, wherein said nucleic acids are primers or probes having sequence complementarity with said nucleic acid sequence of said at least one pregnancy-associated genomic locus.

28. The use of claim 27, wherein said nucleic acids are said probes having sequence complementarity with said nucleic acid sequence of said at least one pregnancy-associated genomic locus.