

biological factors influencing placental RNA levels in maternal plasma. In addition, transcripts that are expressed and released by non-placental tissues in response to pregnancy could not be identified by this method. A sensitive and high-throughput methodology that allows the direct profiling of the maternal plasma transcriptome would therefore be very desirable.

5 [0004] Similarly, direct plasma RNA profiling may be useful in other scenarios where there is a mixture of RNA molecules from two individuals, such as for organ transplantation. The circulation of transplantation recipients contains nucleic acid molecules from both the donor and recipient. A change in the relative profile of RNA molecules contributed by the donor or the recipient may reveal pathologies in the transplanted organ or recipient, such as graft rejection.

10 SUMMARY

[0004a] In one aspect, the present disclosure provides for the use of a gene listed in FIG. 21 or FIG. 26 for diagnosing a pregnancy-associated disorder in a female subject pregnant with a fetus by comparing an expression level of the gene with a control value determined from one or more other female subjects, each pregnant with a healthy fetus, wherein the gene is not CSH1, KISS1,
15 TFPI2, PLAC4, CSHL1, SERPINE1, PAPP, ADAM12, GH2, INHBA, PSG9, PLAC1, SERPINB2, CRH, GCM1, CSH2, CSHL1, SERPINE1, ADAM12, or INHBA.

[0005] Methods, systems, and apparatuses are provided for diagnosing pregnancy-associated disorders, determining allelic ratios, determining maternal or fetal contributions to circulating transcripts, and/or identifying maternal or fetal markers using a sample from a
20 pregnant female subject. In some examples, the sample is blood plasma containing a mixture of maternal- and fetal-derived RNA molecules.

[0006] The RNA molecules are analyzed (e.g., sequenced) to obtain a plurality of reads, and the locations of these reads in a reference sequence are identified (e.g., by sequence alignment). Informative loci are identified that are homozygous for a first allele in either the
25 mother or fetus, and heterozygous for a first allele and a second allele in the other one of the mother and fetus. The informative loci are then filtered and reads located at (e.g., aligning to) the filtered informative loci are further analyzed. In some examples, a ratio of reads corresponding to first alleles and second alleles is calculated and compared to a cutoff to diagnose a pregnancy-associated disorder. In some examples, a portion of RNA in the
30 sample that is of fetal origin is determined using reads located at filtered informative maternal loci. In some examples, a ratio of reads corresponding to the first allele and the second allele is calculated for an individual filtered informative locus, and the ratio is compared to a cutoff to designate the locus as a maternal or fetal marker.

[0007] The present methods are not limited to prenatal diagnostics and can be applied to
35 any biological sample that contains a mixture of RNA molecules derived from two

WHAT IS CLAIMED IS:

1. Use of a gene listed in FIG. 21 or FIG. 26 for diagnosing a pregnancy-associated disorder in a female subject pregnant with a fetus by comparing an expression level of the gene with a control value determined from one or more other female subjects,
5 each pregnant with a healthy fetus, wherein the gene is not CSH1, KISS1, TFPI2, PLAC4, CSHL1, SERPINE1, PAPP, ADAM12, GH2, INHBA, PSG9, PLAC1, SERPINB2, CRH, GCM1, CSH2, CSHL1, SERPINE1, ADAM12, or INHBA.
2. The use of claim 1, wherein the pregnancy-associated disorder is associated with the female subject.
- 10 3. The use of claim 1, wherein the pregnancy-associated disorder is associated with the fetus.
4. The use of claim 3, wherein the pregnancy-associated disorder is pre-eclampsia or pre-term birth.
5. The use of claim 1, wherein the pregnancy-associated disorder is
15 diagnosed if the expression level exceeds the control value.
6. The use of claim 1, wherein the pregnancy-associated disorder is diagnosed if the expression level is below the control value.
7. The use of claim 1, wherein the female subject exhibits an elevated expression level of the gene while pregnant as compared with after delivery.
8. The use of claim 1, wherein the expression level of the gene in a female subject is measured by
obtaining a blood plasma sample from the female subject,
receiving a plurality of reads, wherein the reads are obtained from an analysis of RNA molecules obtained from the sample;
identifying, by a computer system, locations of the reads in a reference sequence; and
counting the reads located at the gene.

9. The use of claim 1, wherein the fold-change increase from the transcript level of the gene in post-delivery plasma and the transcript level of the gene in pre-delivery plasma is greater than 20 in FIG. 21.

10. The use of claim 1, wherein the fold-change increase from the transcript level of the gene in plasma of women with normal pregnancies to the transcript level of the gene in plasma of women with pre-eclampsia is greater than 20 in FIG. 21.