

GESTATIONAL AGE ASSESSMENT BY METHYLATION AND SIZE PROFILING OF MATERNAL PLASMA DNA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/410,108, filed on October 19, 2016, the content of which is incorporated herein by reference in its entirety for all purposes.

BACKGROUND

[0002] The discovery of cell-free fetal deoxyribonucleic acid (DNA) in maternal plasma has opened up new possibilities for noninvasive prenatal diagnosis (YMD Lo et al. *Lancet* 1997; 350:485-487). This technology has been rapidly translated into clinical applications, with the detection of fetal-derived, paternally-inherited genes or sequences, e.g., for fetal sex determination and for fetal Rhesus D (RHD) status determination, and, in particular, for screening fetal chromosomal aneuploidies (RWK Chiu et al. 2008 Proc Natl Acad Sci USA; 105:20458-2046). The biological properties of the fetal-derived cell-free DNA in the plasma of a pregnant woman have been demonstrated to exhibit a number of differences from the maternal-derived DNA. For example, the cell-free fetal DNA is generally shorter than the maternal DNA molecules (YMD Lo et al. 2010 Sci Transl Med; 2: 61ra91) and the overall methylation level of the cell-free fetal DNA in the plasma of a pregnant woman is generally lower than the overall methylation level of maternal-derived DNA (FMF Lun et al. Clin. Chem. 2013; 59:1583-94).

BRIEF SUMMARY

[0003] Various examples are directed to applications (e.g., diagnostic and treatment applications) of the analysis of a cell-free DNA sample including fetal and maternal DNA (e.g., plasma DNA) from a female pregnant with at least one fetus. Examples of one application can use the temporal changes in one or more characteristics measured from a cell-free DNA sample to estimate a gestational age of the at least one fetus. Example characteristics include the methylation level measured from the cell-free DNA sample, the size of DNA fragments measured from the cell-free DNA sample (e.g., proportion of fetal-derived DNA fragments longer than a specified size), and patterns for where ends of DNA fragments align to a reference genome.

[0003a] In one aspect, the present invention refers to a method of analyzing a biological sample from a female subject pregnant with a fetus, the biological sample including cell-free DNA molecules from the female subject and the fetus, the method comprising: analyzing the cell-free DNA molecules from the biological sample, wherein analyzing a cell-free DNA molecule includes: determining a location of the cell-free DNA molecule in a genome of the fetus or the female subject; and determining whether the cell-free DNA molecule is methylated at one or more sites; for each of the one or more sites: determining a respective number of cell-free DNA molecules that are methylated at the site; calculating a measured methylation level of cell-free DNA molecules in the biological sample based on the respective numbers of cell-free DNA molecules methylated at the one or more sites; obtaining one or more calibration data points, wherein each calibration data point specifies a gestational age corresponding to a calibration methylation level, and wherein the one or more calibration data points are determined from a plurality of calibration samples with known gestational ages and including cell-free DNA molecules; comparing the measured methylation level to a calibration methylation level of at least one calibration data point; and estimating a gestational age of the fetus based on the comparing.

[0003b] In another aspect, the present invention refers to a method of analyzing a biological sample from a female subject pregnant with a fetus, the biological sample including cell-free DNA molecules from the female subject and the fetus, the method comprising: analyzing the cell-free DNA molecules from the biological sample, wherein analyzing each cell-free DNA molecule of the cell-free DNA molecules includes: determining a genomic position in a reference genome corresponding to at least one end of the cell-free DNA molecule; measuring a sample vector for the biological sample, wherein each value of the sample vector corresponds to a frequency that the cell-free DNA molecules end on a respective site of a set of sites, wherein the set of sites is determined based on numbers of cell-free DNA molecules that end on various sites in one or more first calibration samples; obtaining a plurality of representative vectors, wherein each representative vector corresponds to a gestational age and includes representative frequencies of cell-free DNA molecules that end on the set of sites, and wherein the plurality of representative vectors are determined from one or more second calibration samples with known gestational ages; comparing the sample vector to the plurality of representative vectors; and estimating the gestational age of the fetus based on the comparing.

[0003c] In yet another aspect, the present invention refers to a computer product comprising a computer readable medium storing a plurality of instructions for controlling a computer system to: analyze a cell-free DNA molecules from a biological sample from a female subject pregnant with a fetus, the biological sample including cell-free DNA molecules from the female subject and the fetus, wherein analyzing a cell-free DNA molecule includes: determining a location of the cell-free DNA molecule in a genome of the fetus or the female subject; and determining whether the cell-free DNA molecule is methylated at one or more sites; determine, for each of the one or more sites, a respective number of cell-free DNA molecules that are methylated at the site; calculate a measured methylation level of cell-free DNA molecules in the biological sample based on the respective numbers of cell-free DNA molecules methylated at the one or more sites; obtain one or more calibration data points, wherein each calibration data point specifies a gestational age corresponding to a calibration methylation level, and wherein the one or more calibration data points are determined from a plurality of calibration samples with known gestational ages and including cell-free DNA molecules; compare the measured methylation level to a calibration methylation level of at least one calibration data point; and estimate a gestational age of the fetus based on the comparing.

[0003d] In yet another aspect, the present invention refers to a computer product comprising a computer readable medium storing a plurality of instructions for controlling a computer system to perform an operation of any of the methods above.

[0003e] In yet another aspect, the present invention refers to a system comprising: the computer product of the present disclosure; and one or more processors for executing instructions stored on the computer readable medium.

[0003f] In yet another aspect, the present invention refers to a system comprising means for performing any of the methods as disclosed herein.

[0003g] In yet another aspect, the present invention refers to a system configured to perform any of the above methods as disclosed herein.

[0003h] In yet another aspect, the present invention refers to a system comprising modules that respectively perform the steps of any of the above methods as disclosed herein.

[0004] In further examples, the estimated gestational age may be compared with a gestational age determined using another technique for the biological sample, such as the gestational age determined based on medical history or based on ultrasonic diagnosis. An alarm message can be generated when the estimated gestational age does not match the gestational age determined using other techniques. A corrective action may be taken if the mismatch is caused by administrative errors. Further diagnosis and treatment may be conducted if the mismatch may be caused by pathological reasons, such as pregnancy-associated disorders, such as preeclampsia, preterm labor, intrauterine growth restriction (IUGR), or fetal chromosomal aneuploidies.

[0005] Other examples are directed to systems and computer readable media associated with methods described herein.

[0006] A better understanding of the nature and advantages of embodiments of the present invention may be gained with reference to the following detailed description and the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 a plot of a size distribution of circulating cell-free DNA in maternal plasma, according to certain embodiments of the present invention.

[0008] FIG. 2A shows a plot of size distributions of DNA fragments in two maternal plasma samples (1st trimester pregnancies) with different fractional fetal DNA concentrations, according to certain embodiments of the present invention. .

[0009] FIG. 2B shows a plot of size distributions of DNA fragments in two maternal plasma samples (2nd trimester pregnancies) with different fractional fetal DNA concentrations, according to certain embodiments of the present invention.

[0010] FIG. 3 is a plot showing a proportion of DNA fragments that have 150 or less base pairs for samples having various fetal DNA percentage in maternal plasma, according to certain embodiments of the present invention.

[0011] FIG. 4A shows a bar chart of percentage of methylated CpG sites in Autosomes among samples collected during pregnancy, according to certain embodiments of the present invention.