

NON-INVASIVE PRENATAL TESTING METHOD USING MATERNAL CELL-FREE DNA FRAGMENT

Technical Field

The present invention relates to a non-invasive prenatal testing method using a cell-free DNA fragment derived from maternal blood, and more specifically, to a non-invasive prenatal testing method capable of separating maternal cell-free DNA fragments and fetal cell-free DNA fragments on the basis of a difference in length between the maternal and fetal DNA fragments and determining fetal aneuploidy or maternal mosaicism on the basis of z-scores therefor.

Background Art

One of the important efforts in human medical research is the discovery of genetic anomalies underlying adverse health outcomes. The prenatal testing for the detection of these genetic anomalies determines and diagnoses the presence of fetal diseases before birth through fetal aneuploidy.

In general, pregnant women over the age of 35, pregnant women with a history of inherited diseases or congenital malformations in themselves or their immediate family members, and pregnant women with multiple births are classified as high-risk pregnant women. In recent years, the number of high-risk pregnant women has been steadily increasing, which is the largest cause due to an increase in the average age of childbirth for women. If classified as high-risk pregnant women, a lot of attention is required in prenatal care for the safety of the mother and the fetus, and there is a need for prenatal testing.

The prenatal testing is divided into invasive prenatal testing and non-invasive prenatal testing (NIPT). The invasive prenatal testing includes amniocentesis, chorionic villi sampling, cordocentesis, etc. However, the invasive prenatal testing may cause miscarriage, diseases, or malformations by impacting the fetus during the testing process, and a non-invasive prenatal testing method has recently been developed to overcome these problems.

On the other hand, a general non-invasive prenatal testing method distinguishes normal and aneuploidy chromosomes by calculating z-scores through a normalization process that divides the number of nucleotide sequence fragments arranged on each chromosome by the number of total nucleotide sequence fragments. However, if a fetal fraction (FF) is low or DNA derived from the mother is amplified or deleted, it is difficult to make an accurate determination.

Recently, as a massively parallel signature sequencing (MPSS) method, which is a next generation sequencing (NGS) technology, has been introduced and cell-free fetal DNA (cffDNA) was discovered from cell-free DNA (cfDNA) present in maternal blood, a non-invasive prenatal testing method using this has been developed. However, since the amount of fetal cffDNA present in the maternal blood is relatively very small, a method of generating and discriminating a large number of NGS reads is generally used, resulting in an economic problem. In addition, there is a need to develop a more accurate and sensitive testing method without errors in diagnosing fetal malformations such as false positive (FP) or false negative (FN).

In this regard, in Korean Patent Publication No. 10-2017-0036648, there is provided a non-invasive fetal chromosome analysis method capable of reducing the probability of false positive by improving the reliability and specificity of the results, in which in order to determine fetal aneuploidy from DNA sequence information obtained from a maternal blood sample, the average number of reads of a specific chromosome for which aneuploidy is to be determined and the average number of reads of other chromosomes excluding the chromosome are compared with each other to remove an inter-experimental deviation and a ratio of the number of inter-chromosomal reads weighted and averaged as a coefficient of variation (CV) value is used.

Therefore, the present inventors developed an analysis method capable of more accurately determining fetal aneuploidy or maternal mosaicism by separating the total cell-free DNA fragments isolated from the maternal blood into maternal or fetal cell-free DNA fragments depending on the length of the DNA fragment and using z-scores calculated therefrom while studying to develop an accurate non-invasive prenatal testing method, and completed the present invention.

Detailed Description of the Invention

Technical Problem

An object of the present invention is to provide non-invasive prenatal testing method and apparatus using a cell-free DNA fragment derived from maternal blood.

Technical Solution

In order to achieve the object, the present invention provides a non-invasive prenatal testing method including the steps of: producing total nucleotide sequence fragments from cell-free DNA extracted from maternal blood; arranging the produced total nucleotide sequence

fragments at homologous positions of a human reference genome sequence; dividing the arranged total nucleotide sequence fragments into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length and calculating z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments, respectively; and determining maternal mosaicism or fetal aneuploidy by comparing the calculated z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments.

Further, the present invention provides a non-invasive prenatal testing apparatus including a production unit for producing total nucleotide sequence fragments from cell-free DNA extracted from maternal blood; an arrangement unit for arranging the produced total nucleotide sequence fragments at homologous positions of a human reference genome sequence; a calculation unit for dividing the arranged total nucleotide sequence fragments into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length and calculating z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments, respectively; and a determination unit for determining maternal mosaicism or fetal aneuploidy by comparing the calculated z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments.

Advantageous Effects

According to the present invention, since the method may more accurately determine fetal aneuploidy or maternal mosaicism by separating the total cell-free DNA fragments isolated from the maternal blood into maternal or fetal cell-free DNA fragments depending on the length of the DNA fragment, and using z-scores calculated therefrom, the method can be usefully used in non-invasive prenatal testing.

Brief Description of the Drawings

FIG. 1 is a graph confirming the lengths of fragments obtained from total cell-free DNA isolated from pregnant or non-pregnant women (non-pregnancy.avar: average value of non-pregnant women, pregnancy.avar: average value of pregnant women).

FIG. 2 is a diagram showing a heatmap for a result of confirming trisomy present on a maternal chromosome 21 using z-scores for the number of fragments obtained from total cell-free DNA, maternal cell-free DNA, or fetal cell-free DNA isolated from maternal blood of a pregnant woman (Original: original data, Fetus: fetal data, Mom: maternal data).

FIG. 3 is a diagram showing a heatmap for a result of confirming trisomy present on a maternal chromosome 21 using z-scores for the number of fragments obtained from total cell-free DNA, maternal cell-free DNA, or fetal cell-free DNA isolated from maternal blood of a pregnant woman (Original: original data, Fetus: fetal data, Mom: maternal data).

FIG. 4 is a graph showing average values of z-scores for the number of fragments obtained from total cell-free DNA, maternal cell-free DNA, or fetal cell-free DNA isolated from maternal blood, which are divided into cases of being determined as trisomy or maternal mosaicism (Original: original data, Fetus: fetal data, Mom: maternal data).

FIG. 5 is a diagram showing a heatmap for a result of confirming monosomy present on a fetal X chromosome using z-scores for the number of fragments obtained from total cell-free DNA, maternal cell-free DNA, or fetal cell-free DNA isolated from maternal blood of a pregnant woman (Original: original data, Fetus: fetal data, Mom: maternal data).

FIG. 6 is a diagram showing a heatmap for a result of confirming monosomy present on a maternal X chromosome using z-scores for the number of fragments obtained from total cell-free DNA, maternal cell-free DNA, or fetal cell-free DNA isolated from maternal blood of a pregnant woman (Original: original data, Fetus: fetal data, Mom: maternal data).

FIG. 7 is a graph showing average values of z-scores for the number of fragments obtained from total cell-free DNA, maternal cell-free DNA, or fetal cell-free DNA isolated from maternal blood, which are divided into cases of being determined as monosomy or maternal mosaicism (Original: original data, Fetus: fetal data, Mom: maternal data).

Best Mode For Carrying Out Invention

Hereinafter, the present invention will be described in detail.

The present invention provides a non-invasive prenatal testing method including the steps of: producing total nucleotide sequence fragments from cell-free DNA extracted from maternal blood; arranging the produced total nucleotide sequence fragments at homologous positions of a human reference genome sequence; dividing the arranged total nucleotide sequence fragments into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length and calculating z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments, respectively; and determining maternal mosaicism or fetal aneuploidy by comparing the calculated z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments.

In one aspect, the non-invasive prenatal testing method according to the present invention provides the step of producing the total nucleotide sequence fragments from cell-free DNA extracted from the maternal blood.

As used herein, the term “non-invasive prenatal testing” refers to a testing method capable of confirming fetal chromosomal abnormalities without an invasive surgical procedure, unlike a prenatal testing method such as amniocentesis or chorionic villi sampling. The non-invasive prenatal testing method may be performed through sequencing analysis of cell-free fetal DNA present in the maternal blood.

In the method according to the present invention, the blood may be serum or plasma, specifically plasma.

As used herein, the term “cell-free DNA (cfDNA)” refers to a DNA fragment that is not present in the nucleus of a cell, but is floating in the blood, and has been currently used for prediction of the onset, metastasis, recurrence, response to an anticancer agent, etc. The cfDNA may be isolated from blood by a method known in the art. The isolated cfDNA may be produced as a nucleotide sequence fragment by using a massively parallel signature sequencing method. At this time, the number of produced nucleotide sequence fragments may be 1 million to 10 million.

The term “massively parallel signature sequencing (MPSS)” as a next generation sequencing (NGS) method, refers to a sequencing method of rapidly decoding vast genomic information by producing nucleotide sequences of DNA, RNA, etc. as fragments and then amplifying the fragments to read several fragments at the same time and combining the sequence data obtained above using bioinformatics.

The MPSS may be performed according to a method known in the art, and the method may be appropriately modified by those skilled in the art in some cases. Specifically, the MPSS may be performed by a 454 platform (Margulies, etc., Nature, 2005, 437:376-380), an illumina genome analyzer, illumina HiSeq2000, HiSeq2500, MiSeq, NextSeq500, Life Tech Ion PGM, Ion Proton, Ion S5, Ion S5, Ion S5XL, SOLiD (Applied Biosystems), Helicos True Single Molecule DNA sequencing technology (Harris, etc., Science, 2008, 320:106-109), single molecule or real-time (SMARTTM) technology of Pacific Biosciences, etc.

In this case, the nucleotide sequencing may be performed by a paired-end sequencing or single-read sequencing method, specifically, the paired-end sequencing method.

In another aspect, the non-invasive prenatal testing method according to the present

invention provides the step of arranging the produced total nucleotide sequence fragments at homologous positions of a human reference genome sequence.

The human reference genome sequence is a sequence known as a human genome sequence and stored in a nucleotide sequence database, and may be used to compare sequences of nucleotide sequence fragments produced by the method according to the present invention. The human reference genome sequence may be known sequences such as build 37 (GRCh37), hg18, hg19, hg38, etc., and may be hg18 according to an embodiment of the present invention.

In the step, it is possible to remove the duplicated nucleotide sequence fragments while arranging the total nucleotide sequence fragments at the homologous positions of the human reference genome sequence. In this case, in the nucleotide sequence amplification process of one fragment, only data in which short sequences arranged in forward and reverse directions are derived from the same nucleotide sequence fragment may be taken and used in a subsequent step.

In yet another aspect, the non-invasive prenatal testing method according to the present invention provides the step of dividing the arranged total nucleotide sequence fragments into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length and calculating z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments, respectively.

The fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments may be divided according to the length of the arranged nucleotide sequence fragment. In this case, the fetal nucleotide sequence fragment may be 150 bp or less, and the maternal nucleotide sequence fragment may be 185 bp or more. The nucleotide sequence fragments are divided into the fetal or maternal nucleotide sequence fragments according to the length to be used to determine whether a result derived by the non-invasive prenatal testing method according to the present invention is maternal mosaicism or fetal aneuploidy.

The z-scores may be calculated by a method well-known in the art, and may be calculated by an appropriately modified method in some cases. In the testing method according to the present invention, the z-scores may be calculated by using the number of DNA fragments of the chromosome for which aneuploidy is to be confirmed in the arranged nucleotide sequence. The chromosome may be an autosome or a sex chromosome. The autosome may be at least one selected from the group consisting of chromosomes 1 to 22 in the case of a human, and the sex chromosome may be an X or Y chromosome in the case of the

human.

Specifically, the z-scores may be calculated by Equation 1 below:

[Equation 1]

$$Z\text{-score}_{(chromosome\ i)} = \frac{Normalized\ Value_{(target\ i)} - Mean_{(reference\ i)}}{Standard\ Deviation_{(reference\ i)}}$$

(Wherein, i refers to chromosomes 1 to 22, and an X chromosome or Y chromosome, reference refers to a normal sample group, and target means a maternal sample to be tested).

In still another aspect, the non-invasive prenatal testing method according to the present invention provides the step of determining maternal mosaicism or fetal aneuploidy by comparing the calculated z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments.

As used herein, the term “maternal mosaicism” refers to a case in which since fetal chromosomes are normal, but a mother has an abnormal karyotype, it is determined to have aneuploidy as a result of non-invasive prenatal testing. That is, the non-invasive prenatal testing method according to the present invention may improve the accuracy of the result by reducing false-positive errors caused by maternal mosaicism.

As used herein, the term “aneuploidy” means that the number of chromosomes is different from the number (two) of normal chromosomes, that is, 0, 1, 3 or more. This aneuploidy may be used to determine rare genetic diseases in the fetus.

In the testing method according to the present invention, maternal mosaicism or fetal aneuploidy may be determined as follows:

- i) if the z-score for the fetal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is trisomy;
- ii) if the z-score for the maternal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined as maternal mosaicism;
- iii) if the z-score for the fetal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is monosomy; or
- iv) if the z-score for the maternal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined as maternal mosaicism.

That is, maternal mosaicism or fetal aneuploidy may be determined as follows:

- i) if a value obtained by subtracting the z-score for the total nucleotide sequence

fragment from the z-score for the fetal nucleotide sequence fragment is greater than 0, it is determined that the fetus is trisomy;

ii) a value obtained by subtracting the z-score for the total nucleotide sequence fragment from the z-score for the maternal nucleotide sequence fragment is greater than 0, it is determined as maternal mosaicism;

iii) if a value obtained by subtracting the z-score for the total nucleotide sequence fragment from the z-score for the fetal nucleotide sequence fragment is less than 0, it is determined that the fetus is monosomy; or

iv) a value obtained by subtracting the z-score for the total nucleotide sequence fragment from the z-score for the maternal nucleotide sequence fragment is less than 0, it is determined as maternal mosaicism.

Specifically, the fetal aneuploidy may be determined as follows:

i) if the z-score for the fetal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, and

ii) if the z-score for the maternal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is trisomy.

In addition, the fetal aneuploidy may be determined as follows:

i) if the z-score for the fetal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, and

ii) if the z-score for the maternal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is monosomy.

The testing method according to the present invention may determine autosomal abnormality, sex chromosomal abnormality, or autosomal deletion. For example, the autosomal abnormality may be a case where the number of chromosomes is three or more, specifically three. Diseases that may be caused by the autosomal abnormality may be Down syndrome, Edward syndrome, Patau syndrome, trisomy 9, trisomy 16, or trisomy 22. Meanwhile, the sex chromosome abnormality may be the addition or deletion of an X or Y chromosome. Diseases that may be caused by the sex chromosomal abnormality may be specifically, Turner syndrome, Triple X syndrome, Klinefelter syndrome or Jacobs syndrome. In addition, the autosomal deletion may be a disease in which a part of the autosomal chromosome is deleted. Diseases that may be caused by the autosomal deletion may be specifically 1p36 deletion syndrome, 2q33.1 deletion syndrome, Wolf-Hirschhorn syndrome,

cat crying syndrome, Williams syndrome, Jacobson syndrome, Prader-Willi/Angelman syndrome or DiGeorge syndrome.

Further, the present invention provides a non-invasive prenatal testing apparatus including a production unit for producing total nucleotide sequence fragments from cell-free DNA extracted from maternal blood; an arrangement unit for arranging the produced total nucleotide sequence fragments at homologous positions of a human reference genome sequence; a calculation unit for dividing the arranged total nucleotide sequence fragments into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length and calculating z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments, respectively; and a determination unit for determining maternal mosaicism or fetal aneuploidy by comparing the calculated z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments.

The non-invasive prenatal testing apparatus may have the same characteristics as the non-invasive prenatal testing method described above. For example, the nucleotide sequence fragments may be produced by a massively parallel signature sequencing method, and the produced nucleotide sequence fragments may be classified into fetal or maternal nucleotide sequence fragments according to their lengths. In addition, maternal mosaicism or fetal aneuploidy may be determined as follows by using z-scores calculated from each of the total nucleotide sequence fragment, the fetal nucleotide sequence fragment, and the maternal nucleotide sequence fragment:

- i) if the z-score for the fetal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is trisomy;
- ii) if the z-score for the maternal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined as maternal mosaicism;
- iii) if the z-score for the fetal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is monosomy; or
- iv) if the z-score for the maternal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined as maternal mosaicism.

The non-invasive prenatal testing apparatus according to the present invention may determine autosomal abnormality, sex chromosomal abnormality, or autosomal deletion. Specifically, the autosomal abnormality may cause Down syndrome, Edward syndrome, Patau

syndrome, trisomy 9, trisomy 16, or trisomy 22. The sex chromosomal abnormality may cause Turner syndrome, Triple X syndrome, Klinefelter syndrome or Jacobs syndrome. The autosomal deletion may cause 1p36 deletion syndrome, 2q33.1 deletion syndrome, Wolf-Hirschhorn syndrome, cat crying syndrome, Williams syndrome, Jacobson syndrome, Prader-Willi/Angelman syndrome or DiGeorge syndrome.

Mode For Carrying Out Invention

Hereinafter, the present invention will be described in detail by the following Examples. However, the following Examples are just illustrative of the present invention, and the contents of the present invention are not limited to the following Examples. Anything that has substantially the same configuration as the technical idea described in the appended claims of the present invention and achieves the same operations and effects is included in the technical scope of the present invention.

Example 1. Confirmation of cell free DNA (cfDNA) obtained from pregnant and non-pregnant women

1-1. Isolation of cfDNA from blood

In order to compare the fragment lengths of cfDNA obtained from pregnant and non-pregnant women, first, blood was collected from pregnant or non-pregnant women.

Specifically, 10 ml of blood was taken from subjects and placed in a cell-free DNA blood collection tube manufactured by VanGenes Co., Ltd., and then centrifuged for 15 minutes at room temperature under a condition of 1,900 g. After centrifugation, a supernatant was transferred to a 1.5 ml tube, and centrifuged again for 15 minutes at room temperature under a condition of 16,000 g to obtain the supernatant again. CfDNA was isolated from 2 ml of the obtained supernatant (plasma) according to a manufacturer's protocol using a QIAasympyphony DSP Virus/Pathogen Midi Kit (Qiagen).

1-2. Confirmation of length of cfDNA fragment

100 ng of cfDNA isolated from pregnant or non-pregnant women in Example 1-1 was prepared as each fragment library set through 75-cycle paired-end sequencing using Illumina NextSeq500. Thereafter, the fragments prepared using a BWA (version 0.7.10) program were arranged at homologous positions of a human reference genome sequence (hg19). At this time, duplicated nucleotide sequence fragments were removed using a Picard (version 1.81) program. Among the arranged fragments, in the case where short sequences arranged in forward and reverse directions were derived from the same nucleotide sequence fragment, only data were

collected (referred to as properly paired read).

The number of nucleotide sequence fragments arranged on each chromosome was calculated using a bam file and a SAMtools (version 0.1.18) program obtained as described above, and the distribution of the lengths of each fragment was shown in a graph in FIG. 1.

As shown in FIG. 1, nucleotide sequence fragments having lengths of 160 bp or less were present in a high ratio in cfDNA isolated from pregnant women compared to non-pregnant women. On the other hand, in pregnant women having a large number of short fragments of 160 bp or less, fragments having lengths of 160 bp or more were relatively low compared to non-pregnant women (FIG. 1). That is, from the above, it was found that the length of the cfDNA fragment of the pregnant woman was short by cfDNA included in cfDNA of the pregnant woman.

Example 2. Isolation of cfDNA from maternal blood

In order to diagnose fetal aneuploidy through non-invasive prenatal testing, cfDNA was first isolated from maternal blood by the following method. At this time, blood was collected from 630 pregnant women, and among them, 195 pregnant women had trisomy aneuploidy on chromosome 21. cfDNA was isolated by the same condition and method described in Example 1-1, except that the blood was obtained from the pregnant women.

Example 3. Arrangement of cfDNA fragments and calculation of number of nucleotide sequence fragments

By using 100 ng of cfDNA isolated in Example 2, cfDNA fragments were arranged under the same condition and method as described in Example 1-2, and the number of arranged nucleotide sequence fragments was calculated. At this time, the calculated data was stored as original data. On the other hand, by using a bam file and a SAMtools (version 0.1.18) program, only sequences having the nucleotide sequence length shorter than 150 bp were taken to calculate the number of fragments, and stored as fetal data. In addition, only sequences having the nucleotide sequence length longer than 185 bp were taken to calculate the number of fragments, and stored as maternal data. At this time, the nucleotide sequences having the length of 150 bp to 185 bp is a combined region that is not suitable for distinguishing a fetus and a mother, and was not used for analysis of fetal or maternal data.

Experimental Example 1. Trisomy 21 analysis of Chromosome 21

The trisomy of Chromosome 21 was analyzed using the z-scores calculated using the original data, the fetal data, and the maternal data calculated in Example 3. General z-scores

were calculated using Equation 1 below for a specific chromosome by using a maternal blood sample in which fetal aneuploidy was not found as a normal sample group, and at this time, an analysis algorithm was performed with reference to a known document (Hyuk Jung Kwon et al., Open Journal of Genetics, 2017, 7, p.1-8).

[Equation 1]

$$Z-score_{(chromosome\ i)} = \frac{Normalized\ Value_{(target\ i)} - Mean_{(reference\ i)}}{Standard\ Deviation_{(reference\ i)}}$$

wherein, i refers to chromosomes 1 to 22, and an X chromosome or Y chromosome, reference refers to a normal sample group, and target means a maternal sample to be tested.

In the results obtained above, if the value obtained by subtracting the z-score calculated from the original data from the z-score calculated from the fetal data was greater than 0, it was determined that the fetus had trisomy, and if the value obtained by subtracting the z-score calculated from the original data from the z-score calculated from the maternal data was greater than 0, it was determined as maternal mosaicism. The results were shown in FIG. 1 by preparing a heatmap graph. At this time, one column of sections of chromosome 21 represents each z-score value, which is on the basis for determining aneuploidy by comparing the z-score value with a threshold value.

As shown in FIG. 2, a fragment obtained from cfDNA of a pregnant woman having trisomy on chromosome 21 had a high z-score in chromosome 21 section (horizontal basis) (FIG. 2).

On the other hand, as shown in FIG. 3, the z-scores were not clearly enough to determine fetal aneuploidy in the chromosome 21 section (horizontal basis) of the original data. However, when the z-scores were confirmed to be divided into maternal or fetal data, it was confirmed that the z-scores were significantly high in the chromosome 21 section (horizontal basis) of the fetal data, but not high in the maternal data (FIG. 3). Therefore, from this, it could be confirmed that the fetus had aneuploidy on chromosome 21.

Thus, as described above, the z-scores of the original data obtained from each pregnant woman's blood, and the maternal or fetal data were confirmed, and the graph for z-scores was prepared and shown in FIG. 4 to be divided into cases determined as fetal trisomy or maternal mosaicism.

As shown in FIG. 4, the z-scores in all cases determined as trisomy or maternal

mosaicism showed valid values. When determined as the maternal mosaicism, in the original data and the fetal data, a z-score difference was not shown, but the z-score of the maternal data was remarkably high (blue line). On the other hand, when determined as the fetal trisomy, the z-score of the fetal data was significantly high (red line).

Experimental Example 2. Monosomy X analysis of sex chromosome

First, monosomy of a X chromosome as a sex chromosome was analyzed using the z-scores calculated using the original data, the fetal data, and the maternal data calculated in Example 3. In the results obtained above, if the value obtained by subtracting the z-score calculated from the original data from the z-score calculated from the fetal data was less than 0, it was determined that the fetal X chromosome was monosomy, and if the value obtained by subtracting the z-score calculated from the original data from the z-score calculated from the maternal data was less than 0, it was determined as maternal mosaicism. The results were shown in FIGS. 5 and 6 by preparing a heatmap graph.

As shown in FIG. 5, fragments obtained from cfDNA of a pregnant woman having monosomy on the fetal X chromosome had a low z-score value in an X chromosome section (horizontal basis). That is, the results indicated that the fetal X chromosome was monosomy. In addition, as a result of comparative analysis by dividing the original data into fetal or maternal data, it was confirmed that the result was aneuploidy shown in the fetus by having a low z-score in the fetal data compared to the maternal data (FIG. 5).

On the other hand, as shown in FIG. 6, it was confirmed that in the fragments obtained from the cfDNA of one pregnant woman having monosomy on the maternal X chromosome, a z-score value in the X chromosome section (horizontal basis) was lower in the maternal data than the fetal data, so that the fetus had a normal chromosome (FIG. 6).

In addition, as described above, the z-scores of the original data obtained from each pregnant woman's blood, and the maternal or fetal data were confirmed, and the graph for the z-scores was prepared and shown in FIG. 7 to be divided into cases determined as the fetal monosomy or maternal mosaicism.

As shown in FIG. 7, the z-scores in all cases determined as the monosomy or maternal mosaicism showed valid values. In the case of being determined as the maternal mosaicism, in the original data and the fetal data, a z-score difference was not shown, but the z-score of the maternal data was remarkably low (blue line). On the other hand, when determined as the fetal monosomy, the z-score of the fetal data was significantly low (red line).

Therefore, in conclusion, the method according to the present invention can be applied to determine abnormalities or deletions of autosomal chromosomes such as not only chromosome 21, but also chromosomes 9, 13, and 18 and sex chromosomes.

WHAT IS CLAIMED IS:

1. A non-invasive prenatal testing method comprising the steps of:

1) producing total nucleotide sequence fragments from cell-free DNAs extracted from maternal blood;

2) arranging the total nucleotide sequence fragments produced in step 1) at homologous positions of a human reference genome sequence;

3) dividing the total nucleotide sequence fragments arranged in step 2) into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length, and calculating z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments, respectively; and

4) determining maternal mosaicism or fetal aneuploidy by comparing the z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments which are calculated in step 3).

2. The non-invasive prenatal testing method of claim 1, wherein the nucleotide sequence fragments are produced by a massively parallel signature sequencing method.

3. The non-invasive prenatal testing method of claim 1, wherein in the process of dividing the total nucleotide sequence fragments into the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments according to a length, the fetal nucleotide sequence fragments are isolated by 150 bp or less.

4. The non-invasive prenatal testing method of claim 1, wherein in the process of dividing the total nucleotide sequence fragments into the fetal nucleotide sequence fragments and the maternal nucleotide sequence fragments according to a length, the maternal nucleotide sequence fragments are isolated by 185 bp or more.

5. The non-invasive prenatal testing method of claim 1, wherein the maternal mosaicism or fetal aneuploidy is determined as follows:

i) if the z-score for the fetal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is trisomy;

ii) if the z-score for the maternal nucleotide sequence fragment is greater than the z-

score for the total nucleotide sequence fragment, it is determined as maternal mosaicism;

iii) if the z-score for the fetal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is monosomy; or

iv) if the z-score for the maternal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined as maternal mosaicism.

6. The non-invasive prenatal testing method of claim 1, wherein the fetal aneuploidy is determined as follows:

i) if the z-score for the fetal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, and

ii) if the z-score for the maternal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is trisomy.

7. The non-invasive prenatal testing method of claim 1, wherein the fetal aneuploidy is determined as follows:

i) if the z-score for the fetal nucleotide sequence fragment is smaller than the z-score for the total nucleotide sequence fragment, and

ii) if the z-score for the maternal nucleotide sequence fragment is greater than the z-score for the total nucleotide sequence fragment, it is determined that the fetus is monosomy.

8. The non-invasive prenatal testing method of claim 1, wherein the z-scores are calculated by the following Equation 1:

[Equation 1]

$$Z-score_{(chromosome\ i)} = \frac{Normalized\ Value_{(target\ i)} - Mean_{(reference\ i)}}{Standard\ Deviation_{(reference\ i)}}$$

wherein, i refers to chromosomes 1 to 22, and an X chromosome or Y chromosome, reference refers to a normal sample group, and target means a maternal sample to be tested.

9. The non-invasive prenatal testing method of claim 1, wherein autosomal abnormality, sex chromosomal abnormality, or autosomal deletion is determined.

10. The non-invasive prenatal testing method of claim 9, wherein the autosomal abnormality causes Down syndrome, Edward syndrome, Patau syndrome, trisomy 9, trisomy 16, or trisomy 22.

11. The non-invasive prenatal testing method of claim 9, wherein the sex chromosomal abnormality causes Turner syndrome, Triple X syndrome, Klinefelter syndrome or Jacobs syndrome.

12. The non-invasive prenatal testing method of claim 9, wherein the autosomal deletion causes 1p36 deletion syndrome, 2q33.1 deletion syndrome, Wolf-Hirschhorn syndrome, cat crying syndrome, Williams syndrome, Jacobson syndrome, Prader-Willi/Angelman syndrome or DiGeorge syndrome.

13. A non-invasive prenatal testing apparatus comprising:

1) a production unit configured to produce total nucleotide sequence fragments from cell-free DNA extracted from maternal blood;

2) an arrangement unit configured to arrange the total nucleotide sequence fragments produced in step 1) at homologous positions of a human reference genome sequence;

3) a calculation unit configured to divide the total nucleotide sequence fragments arranged in step 2) into fetal nucleotide sequence fragments and maternal nucleotide sequence fragments according to a length and calculate z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments, respectively; and

4) a determination unit configured to determine maternal mosaicism or fetal aneuploidy by comparing the z-scores for the total nucleotide sequence fragments, the fetal nucleotide sequence fragments, and the maternal nucleotide sequence fragments which are calculated in step 3).