(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 524 321 B2

(12) **NEW EUROPEAN PATENT SPECIFICATION**
After opposition procedure

(45) Date of publication and mention of the opposition decision:
23.07.2014 Bulletin 2014/30

(45) Mention of the grant of the patent:
01.07.2009 Bulletin 2009/27

(21) Application number: **03405742.2**

(22) Date of filing: **16.10.2003**

(51) Int Cl.:
C12Q 1/68 *(2006.01)*

(54) **Non-invasive detection of fetal genetic traits**

Nicht invasiver Nachweis fötaler genetischer Merkmale

Détection non invasive de traits génétiques létaux

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PT RO SE SI SK TR

(43) Date of publication of application:
20.04.2005 Bulletin 2005/16

(60) Divisional application:
09011315.0

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Description

[0001] The presence of circulatory extracellular DNA in the peripheral blood is a well established phenomenon. In this context, it has been shown that in the case of a pregnant woman extracellular fetal DNA is present in the maternal circulation and can be detected in maternal plasma or serum. Studies have shown that this circulatory fetal genetic material can be used for the very reliable determination, e.g. by PCR (polymerase chain reaction) technology, of fetal genetic loci which are completely absent from the maternal genome. Examples of such fetal genetic loci are the fetal RhD gene in pregnancies at risk for HDN (haemolytic disease of the fetus and newborn) or fetal Y chromosome-specific sequences in pregnancies at risk for an X chromosome-linked disorder e.g. haemophilia or fragile X syndrome.

[0002] The determination of other, more complex fetal genetic loci (e.g. chromosomal aberrations such as aneuploidies or chromosomal aberrations associated with Down's syndrome, or hereditary Mendelian genetic disorders and, respectively, genetic markers associated therewith, such as single gene disorders, e.g. cystic fibrosis or the haemoglobinopathies) is, however, more problematic. The reason for this difficulty is that the major proportion (generally >90%) of the extracellular DNA in the maternal circulation is derived from the mother. This vast bulk of maternal circulatory extracellular DNA renders it difficult, if not impossible, to determine fetal genetic alternations such as those involved in chromosomal aberrations (e.g. aneuploidies) or hereditary Mendelian genetic disorders (e.g. cystic fibrosis or the hemoglobinopathies) from the small amount of circulatory extracellular fetal DNA.

[0003] An examination of circulatory extracellular fetal DNA and circulatory extracellular maternal DNA in maternal plasma has now shown that, surprisingly, the majority of the circulatory extracellular *fetal* DNA has a relatively small size of approximately 500 base pairs or less, whereas the majority of circulatory extracellular *maternal* DNA in maternal plasma has a size greater than approximately 500 base pairs. Indeed, in certain instances the circulatory DNA material which is smaller than approximately 300 base pairs appears to be almost entirely *fetal*. Circulatory extracellular fetal DNA in the maternal circulation has thus been found to be smaller in size (approximately 500 base pairs or less) than circulatory extracellular maternal DNA (greater than approximately 500 base pairs).

[0004] This surprising finding forms the basis of the present invention according to which separation of circulatory extracellular DNA fragments which are smaller than approximately 500 base pairs or less provides a possibility to enrich for fetal DNA sequences from the vast bulk of circulatory extracellular maternal DNA.

[0005] This selective enrichment, which is based on size discrimination of circulatory DNA fragments of approximately 500 base pairs or less, leads to a fraction which is largely constituted by fetal extracellular DNA. This permits the analysis of fetal genetic traits including those involved in chromosomal aberrations (e.g. aneuploidies or chromosomal aberrations associated with Down's syndrome) or hereditary Mendelian genetic disorders and, respectively, genetic markers associated therewith (e.g. single gene disorders such as cystic fibrosis or the hemoglobinopathies), the determination of which had, as mentioned above, so far proved difficult, if not impossible. Size separation of extracellular fetal DNA in the maternal circulation thus facilitates the non-invasive detection of fetal genetic traits, including paternally inherited polymorphisms which permit paternity testing.

[0006] Clinical Chemistry, 1999, Vol. 45(9), pages 1570-1572 and The Australian & New Zealand Journal of Obstetrics & Gynaecology, Feb. 2003 (02-2003), Vol. 43(1), pages 10-15 describe a sample of blood plasma of a pregnant woman in which extracellular fetal DNA of less than 500 base pairs is enriched by PCR, is separated by gel electrophoresis and fetal male DNA (fetal Y-chromosome-specific sequence) is detected.

[0007] The present invention provides

- a fraction of a sample of the blood plasma or serum (which preferably is substantially cell-free) of a pregnant woman in which, as the result of said sample having been submitted to a size separation, the extracellular DNA present therein substantially consists of DNA comprising 500 base pairs or less;
- the use of such sample-fraction for the non-invasive detection of fetal genetic traits; and
- a process for performing non-invasive detection of fetal genetic traits which comprises subjecting a sample of the blood plasma or serum of a pregnant woman to a size separation so as to obtain a fraction of said sample in which the extracellular DNA present therein substantially consists of DNA consisting of 500 base pairs or less, and determining in said sample-fraction the fetal genetic trait(s) to be detected.

Said serum or plasma sample is preferably substantially cell-free, and this can be achieved by known methods such as, for example, centrifugation or sterile filtration.

The size separation of the extracellular DNA in said serum or plasma sample can be brought about by a variety of methods, including but not limited to

- chromatography or electrophoresis such as chromatography on agarose or polyacrylamide gels, ion-pair reversed-phase high performance liquid chromatography (IP RP HPLC, see Hecker KH, Green SM, Kobayashi K, J. Biochem. Biophys. Methods 2000 Nov. 20; 46(1-2): 83-93), capillary electrophoresis in a self-coating, low-viscosity polymer matrix (see Du M, Flanagan JH Jr, Lin B, Ma Y, Electrophoresis 2003 Sep; 24(18): 3147-53), selective extraction in microfabricated electrophoresis devices (see Lin R, Burke DT, Bum MA, J. Chromatog. A. 2003 Aug 29; 1010

(2): 255-68), microchip electrophoresis on reduced viscosity polymer matrices (see Xu F, Jabasini M, Liu S, Baba Y, Analyst. 2003 Jun; 128(6): 589-92), adsorptive membrane chromatography (see Teeters MA, Conrardy SE, Thomas BL, Root TW, Lightfoot EN, J. Chromatogr. A. 2003 Mar 7; 989(1):165-73) and the like;

- density gradient centrifugation (see Raptis L, Menard HA, J. Clin. Invest. 1980 Dec; 66(6): 1391-9); and
- methods utilising nanotechnological means such as microfabricated entropic trap arrays (see Han J, Craighead HG, Analytical Chemistry, Vol. 74, No. 2, January 15, 2002) and the like.

[0008] The sample-fraction thus obtained not only permits the subsequent determination of fetal genetic traits which had already been easily detectable in a conventional manner such as the fetal RhD gene in pregnancies at risk for HDN (hemolytic disease of the fetus and the newborn), or fetal Y chromosome-specific sequences in pregnancies at risk for an X chromosome-linked disorder such as hemophilia, fragile X syndrome or the like, but also the determination of other, more complex fetal genetic loci, including but not limited to

- chromosomal aberrations (e.g aneuploidies or Down's syndrome) or hereditary Mendelian genetic disorders and, respectively, genetic markers associated therewith (e.g. single gene disorders such as cystic fibrosis or the hemoglobinopathies); and
- fetal genetic traits which may be decisive when paternity is to be determined.

[0009] Such determination of fetal genetic traits can be effected by methods such as, for example, PCR (polymerase chain reaction) technology, ligase chain reaction, probe hybridisation techniques, nucleic acid arrays (so-called "DNA chips") and the like.

[0010] The following Examples are further illustrating the invention but are not to be construed as limitating its scope in any way.

Example 1: Detection of male fetal DNA in maternal plasma by real-time quantitative Polymerise Chain Reaction (PCR) after size fractionation of DNA by agarose gel electrophoresis

Materials and Methods

Subjects and Sample processing

[0011] Seven women pregnant in the third trimester with a male fetus were recruited for this study. 16 - 18 ml blood samples were collected into EDTA tubes. 6 - 9 ml of plasma were obtained after centrifugation at 1600 g for 10 minutes and a second centrifugation of the supernatant at 16000 g for 10 minutes.

DNA isolation

[0012] DNA from 5 - 7 ml plasma was extracted using the QIAgen Maxi kit, according to the manufacturers' protocol. DNA was eluted in a volume of 1.5 ml.

DNA precipitation

[0013]

1. To the plasma DNA were added: 1/10 volume NaAc (3M, pH 5.2), 2 volumes absolute ethanol, $MgCl_2$ to a final concentration of 0.01 M and Glycogen to a final concentration of 50 μg / ml. The solution was thoroughly mixed by vortexing.
2. The solution was stored overnight at -70° C.
3. The DNA was recovered by centrifugation at 20000 g for 30 minutes at 4° C.
4. The supernatant was carefully removed and the pellet washed with 500 μl 70% ethanol.
5. The pellet was air dried and dissolved in 35 μl distilled water.

DNA separation

[0014]

1. A 1 % agarose Gel (Invitrogen, Cat No: 15510-027)was prepared for DNA electrophoresis.
2. 28 μl DNA solution were loaded on the gel.

3. The gel was electrophoresed at 80 Volt for 1 hour.
4. The Gel was cut into pieces corresponding to specific DNA sizes according to the DNA size markers (New England Biolabs, 100 bp ladder and Lamda Hind III digest). The DNA sizes contained by the specific gel fragments were: 90 - 300 bases, 300 - 500 bases, 500 - 1000 bases, 1.0 - 1.5 kilobases ("kb"), 1.5 - 23 kb and > 23 kb.
5. The DNA was purified from the agarose gel pieces using the QIAEX II Gel Extraction kit (Qiagen, Cat No. 20021) and eluted in 35 μl Tris-HCl (pH 8.0, 10mM).

Real-time PCR

[0015] Sequences from the Y chromosome (SRY) and from chromosome 12 (GAPDH gene) were amplified with the Applied Biosystems (ABI) 7000 Sequence Detection System by real-time quantitative PCR to quantify amounts of fetal and total DNA in the size-separated fractions. The TaqMan system for SRY consisted of the amplification primers SRY_Fwd: TCC TCA AAA GAA ACC GTG CAT and SRY_Rev: AGA TTA ATG GTT GCT AAG GAC TGG AT and a FAM labeled TaqMan MGB (Minor Groove Binder) probe SRY_MGB: TCC CCA CAA CCT CTT. The TaqMan System for the GAPDH gene consisted of the following primers and VIC-labeled probe: GAPDH_Fwd: CCC CAC ACA CAT GCA CTT ACC, GAPDH_Rev: CCT AGT CCC AGG GCT TTG ATT and GAPDH_MGB: TAG GAA GGA CAG GCA AC.

[0016] TaqMan amplification reactions were set up in a total reaction volume of 25 μl, containing 6 μl of the sample DNA solution, 300 nM of each primer (HPLC purified, Mycrosynth, Switzerland) and 200 nM of each probe (ABI) at 1 x concentration of the Universal PCR reaction mix (ABI). Each sample was analyzed in duplicate for each of the two amplification systems. A standard curve containing known amounts of genomic DNA was run in parallel with each analysis.

[0017] Thermal cycling was performed according to the following protocol: An initial incubation at 50 °C for 2 minutes to permit Amp Erase activity, 10 minutes at 95 °C for activation of AmpliTaq Gold, and 40 cycles of 1 minute at 60 °C and 15 seconds at 95 °C.

[0018] Amplification data collected by the 7000 Sequence Detection System was quantified using the slope of the standard curve as calculated by the sequence detection software and the results of a standard DNA solution used in the dilution curve with similar DNA copy numbers as the sample reactions as a reference sample for copy number calculations.

Results

[0019] Table 1 shows that in the five pregnancies examined, DNA fragments originating from the fetus were almost completely of sizes smaller than 500 base pairs with around 70 % being of fetal origin for sizes smaller than 300 base pairs.

[0020] These results demonstrate that free DNA of fetal origin circulating in the maternal circulation can be specifically enriched by size separation of the total free DNA in the maternal blood. Depending on the downstream application the DNA size chosen for the enrichment of fetal DNA will be smaller than 300 or smaller than 500 bases.

Table 1

Size of DNA	% of fetal DNA in each fragment	% of maternal DNA in each fragment
<0.3 kb	73.2 (22.22-87.06)	26.8 (12.94-77.78)
0.3-0.5 kb	18.95 (6.43-31.42)	81.05 (68.58-93.57)
0.5-1 kb	2.81 (0.00-7.75)	97.19 (92.25-100)
1.0-1.5 kB	0.00 (0.00-12.50)	100 (87.5-100)
1.5-23 kb	0.00 (0.00-8.40)	100 (100-100)

[0021] The abbreviation "kb" appearing in the first column of this table stands for 1000 base pairs, and the figures given in its second and the third column are the median values of the percentages and, in brackets, the ranges.

Example 2: Detection of fetal DNA after agarose gel electrophoresis by Polymerase Chain Reaction (PCR) of microsatellite markers, also called "Short Tandem Repeats" (STRs)

Materials and Methods

Subjects and samples

[0022] 18 ml blood samples from pregnant women were collected into EDTA tubes and plasma separated by centrifugation as described in example 1.The maternal buffy coat (i.e. the white colored top layer of the cell pellet obtained after the first centrifugation of 1600g for 10min.) was washed twice with PBS.

DNA isolation

[0023] DNA from the plasma was extracted using a modification of the High Pure DNA template kit from Roche, the whole sample was passed through the filter usually used for 200 μl using a vacuum. The DNA was eluted in a volume of 50 μl elution buffer.

[0024] Maternal DNA was isolated from the buffy coat, using the High Pure DNA template kit, and eluted into 100 μl.

DNA separation

[0025] The DNA was size-separated by electrophoresis on an agarose gel and purified as described in Example 1.

PCR specific for Short Tandem Repeats

[0026] From the fraction of sizes smaller than 500 bases, sequences from tetranucleotide repeat markers on Chromosome 21 were amplified in a PCR reaction as described in Li et al. Clinical Chemistry 49, No. 4, 2003. Because of the low concentration of plasma DNA, the fetal DNA in maternal plasma was examined by using a semi-nested PCR protocol.

[0027] The maternal and paternal pairs were genotyped using total genomic DNA to monitor microsatellite markers on chromosome 21.

[0028] The STR markers used were:

D211 S11;
D21S1270;

[0029] The resulting DNA fragments were then size separated by capillary electrophoresis on a sequencer, and the peak areas representing each allele for a specific marker were measured by the software.

Results

[0030]

Table 2

Detection of fetal alleles specific for the microsatellite marker (Short Tandem Repeat) D21S11 on chromosome 21.		
	Maternal alleles detected (D21S11)	Fetal alleles detected (D21S11)
Maternal genomic DNA	232 bp 234 bp	N/A
Total extracellulear DNA (unseparated)	232 bp 234 bp	No fetal alleles detectable
Size-separated extracellular DNA (<300 bp)	232 bp 234 bp	228 bp 232 bp
Size-separated extracellular DNA (300-500 bp)	232 bp 234 bp	228 bp 232 bp

[0031] Only in the size-separated fractions (<300 bp and 300-500 bp) could the fetal alleles for D21 S 11 be detected, namely the paternally inherited 228 bp allele and the maternally inherited 232 bp allele, i.e. one allele from each parent.

Discussion

[0032] Analysis of the STR fragments can allow for the detection of paternal alleles that are distinct in length from the maternal repeat sequences, and by calculating the ratios between the peak areas it can be possible to identify patterns that are not consistent with a normal fetal karyotype. The identification of fetal STR allele in the maternal circulation can allow the detection of certain chromosomal aberrations non-invasively e.g. trisomy 21. Also paternity testing can be accomplished prenatally in a non-invasive manner.

Claims

1. A fraction of a sample of the blood plasma or serum of a pregnant woman in which, as the result of said sample having been submitted to a DNA extraction, followed by a size separation, of the extracellular DNA, the extracellular DNA present therein substantially consists of DNA consisting of 500 base pairs or less.

2. A sample-fraction according to claim 1 which, before extraction and size separation of the extracellular DNA, is substantially cell-free.

3. A sample-fraction, according to claim 1 or 2 wherein the size separation was carried out by chromatography or electrophoresis, by density gradient centrifugation or by methods utilising nanotechnological means.

4. A sample-fraction according to claim 3 wherein the chromatography and, respectively, electrophoresis was chromatography on agarose or polyacrylamide gels, ion-pair reversed-phase high performance liquid chromatography (IP RP HPLC), capillary electrophoresis in a self-coating, low-viscosity polymer matrix, selective extraction in microfabricated electrophoresis devices, microchip electrophoresis on reduced viscosity polymer matrices or adsorptive membrane chromatography.

5. A sample-fraction according to claim 3 wherein the method utilizing nanotechnological means was making use of microfabricated entropic trap arrays.

6. The use of a sample-fraction according to any one of claims 1 to 5 for the non-invasive detection of fetal genetic traits.

7. The use according to claim 6 wherein the fetal genetic trait to be detected is the fetal RhD gene in a pregnancy at risk for HDN (hemolytic disease of the fetus and the newborn) or a fetal Y chromosome-specific sequence in a pregnancy at risk for an X chromosome-linked disorder.

8. The use according to claim 6 wherein the fetal genetic trait to be detected is a chromosomal aberration, a hereditary Mendelian genetic disorder and, respectively, a genetic marker associated therewith, or a fetal genetic trait which may be decisive when paternity is to be determined.

9. The use according to claim 7 wherein the X chromosome-linked disorder is hemophilia or fragile X syndrome.

10. The use according to claim 8 wherein the chromosomal aberration is aneuploidy.

11. The use according to claim 8 wherein the chromosomal aberration is associated with Down's syndrome.

12. The use according to claim 8 wherein the hereditary Mendelian genetic disorder is a single gene disorder.

13. The use according to claim 12 wherein the single gene disorder is cystic fibrosis or a hemoglobinopathy.

14. The use according to any one of claims 6 to 13 wherein the detection of the fetal genetic traits is carried out by PCR (polymerase chain reaction) technology, ligand chain reaction or probe hybridisation techniques or by means of nucleic acid arrays.

15. A process for performing non-invasive detection of fetal genetic traits which comprises subjecting a sample of the

blood plasma or serum of a pregnant woman to a DNA extraction, followed by a size separation, of the extracellular DNA so as to obtain a fraction of said sample in which the extracellular DNA present therein substantially consists of DNA consisting of 500 base pairs or less, and determining the fetal genetic trait(s) to be detected by submitting such fraction to PCR (polymerase chain reaction) technology, ligase chain reaction or probe hybridisation techniques, or to nucleic acid arrays.

16. A process according to claim 15 wherein the fetal genetic trait to be detected is the fetal RhD gene in a pregnancy at risk for HDN (haemolytic disease of the fetus and the newborn) or a fetal Y chromosome-specific sequence in a pregnancy at risk for an X chromosome-linked disorder.

17. A process according to claim 15 wherein the fetal genetic trait to be detected is a chromosomal aberration, a hereditary Mendelian genetic disorder and, respectively, a genetic marker associated therewith, or a fetal genetic trait which may be decisive when paternity is to be determined.

18. A process according to claim 16 wherein the X chromosome-linked disorder is hemophilia or fragile X syndrome.

19. A process according to claim 17 wherein the chromosomal aberration is an aneuploidy.

20. A process according to claim 17 wherein the chromosomal aberration is associated with Down's syndrome.

21. A process according to claim 17 wherein the hereditary Mendelian genetic disorder is a single gene disorder.

22. A process according to claim 21 wherein the single gene disorder is cystic fibrosis or a hemoglobinopathy.

Patentansprüche

1. Fraktion einer Probe von Blutplasma oder von Blutserum einer schwangeren Frau in welcher, als Resultat einer mit besagter Probe durchgeführten DNA Extraktion, gefolgt von einer Grössentrennung, der extrazellulären DNA, die darin anwesende extrazelluläre DNA im Wesentlichen aus DNA besteht, welche aus 500 Basispaaren oder weniger besteht.

2. Fraktion einer Probe nach Anspruch 1, welche vor der Extraktion und der Grössentrennung der extrazellulären DNA im Wesentlichen zellfrei ist.

3. Fraktion einer Probe nach Anspruch 1 oder 2, wobei die Grössentrennung durch Chromatographie oder Elektrophorese, durch Dichtegradientenzentrifugation oder durch Methoden, welche nanotechnologische Mittel verwenden, ausgeführt wurde.

4. Fraktion einer Probe nach Anspruch 3, wobei die Chromatographie beziehungsweise die Elektrophorese Chromatographie auf Agarose oder Polyacrylamidgelen, Ionenpaar-Umkehrphasen-Hochleistungsflüssigchromatographie (IP RP HPLC), Kapillarelektrophorese in einer selbst-beschichtenden, niederviskosen Polymermatrix, selektive Extraktion in mikrohergestellten Elektrophorese-Vorrichtungen, Mikrochip-Elektrophorese auf Polymermatrizen mit verminderter Viskosität oder Membran-Adsorptions-Chromatographie war.

5. Fraktion einer Probe nach Anspruch 3, wobei in der Methode, welche nanotechnologische Mittel einsetzt, sich mikrohergestellter entropischer Fallenarrays bediente.

6. Verwendung einer Fraktion einer Probe nach einem der Ansprüche 1 bis 5 für den nicht invasiven Nachweis fötaler genetischer Merkmale.

7. Verwendung nach Anspruch 6, wobei das nachzuweisende fötale genetische Merkmal das fötale RhD Gen in einer Schwangerschaft mit Risiko des Morbus haemolyticus neonatorum (hämolytische Krankheit des Fötus und des Neugeborenen) oder eine fötale Y-Chromosomspezifische Sequenz in einer Schwangerschaft mit Risiko einer mit dem X-Chromosom in Verbindung stehende Störung ist.

8. Verwendung nach Anspruch 6, wobei das nachzuweisende fötale genetische Merkmal eine chromosomale Aberration, eine hereditäre genetische Mendelsche Störung beziehungsweise ein damit assoziierter genetischer Marker,

oder ein fötales genetisches Merkmal ist, welches entscheidend sein kann, wenn die Vaterschaft bestimmt werden soll.

9. Verwendung nach Anspruch 7, wobei die mit dem X-Chromosom in Verbindung stehende Störung Hämophilie oder das Fragile-X-Syndrom ist.

10. Verwendung nach Anspruch 8, wobei die chromosomale Aberration eine Aneuploidie ist.

11. Verwendung nach Anspruch 8, wobei die chromosomale Aberration mit dem Down-Syndrom assoziiert ist.

12. Verwendung nach Anspruch 8, wobei die hereditäre genetische Mendelsche Störung eine monogenetische Störung ist.

13. Verwendung nach Anspruch 12, wobei die monogenetische Störung zystische Fibrose oder eine Hämoglobinopathie ist.

14. Verwendung nach einem der Ansprüche 6 bis 13, wobei der Nachweis der fötalen genetischen Merkmale mittels der PCR Methode (Polymerase-Kettenreaktion), Ligase-Kettenreaktionsverfahren oder Sondenhybridisierungsverfahren oder mittels Nukleinsäurearrays ausgeführt wird.

15. Verfahren zum Ausführen des nicht invasiven Nachweises fötaler genetischer Merkmale, welches beinhaltet, eine Probe von Blutplasma oder Blutserum einer schwangeren Frau einer DNA Extraktion, gefolgt von einer Grössentrennung, der extrazellulären DNA zu unterziehen, um eine Fraktion der besagten Probe zu erhalten, in welcher die darin anwesende extrazelluläre DNA im Wesentlichen aus DNA besteht, welche aus 500 Basispaaren oder weniger besteht, und das (die) nachzuweisende(n) fötale(n) genetische(n) Merkmal(e) zu bestimmen, indem eine solche Fraktion der PCR Methode (Polymerase-Kettenreaktion), dem Ligase-Kettenreaktionsverfahren oder dem Sondenhybridisierungsverfahren oder Nukleinsäurearrays unterzogen wird.

16. Verfahren nach Anspruch 15, wobei das nachzuweisende fötale genetische Merkmal das fötale RhD Gen in einer Schwangerschaft mit Risiko des Morbus haemolyticus neonatorum (hämolytische Krankheit des Fötus und des Neugeborenen) oder eine fötale Y-Chromosomspezifische Sequenz in einer Schwangerschaft mit Risiko einer mit dem X-Chromosom in Verbindung stehende Störung ist.

17. Verfahren nach Anspruch 15, wobei das nachzuweisende fötale genetische Merkmal eine chromosomale Aberration, eine hereditäre genetische Mendelsche Störung beziehungsweise ein damit assoziierter genetischer Marker, oder ein fötales genetisches Merkmal ist, welches entscheidend sein kann, wenn die Vaterschaft bestimmt werden soll.

18. Verfahren nach Anspruch 16, wobei die mit dem X-Chromosom in Verbindung stehende Störung Hämophilie oder das Fragile-X-Syndrom ist.

19. Verfahren nach Anspruch 17, wobei die chromosomale Aberration eine Aneuploidie ist.

20. Verfahren nach Anspruch 17, wobei die chromosomale Aberration mit dem Down-Syndrom assoziiert ist.

21. Verfahren nach Anspruch 17, wobei die hereditäre genetische Mendelsche Störung eine monogenetische Störung ist.

22. Verfahren nach Anspruch 21, wobei die monogenetische Störung zystische Fibrose oder eine Hämoglobinopathie ist.

Revendications

1. Fraction d'un échantillon de plasma ou de sérum de sang d'une femme enceinte dans laquelle, à la suite de ledit échantillon ayant été soumis à une extraction d'ADN, suivi par une séparation de taille, de l'ADN extracellulaire, l'ADN extracellulaire ci-présent se compose substantiellement d'ADN constitué de 500 paires de bases ou moins.

2. Fraction d'échantillon selon la revendication 1, qui, avant l'extraction et la séparation de taille de l'ADN extracellulaire, est essentiellement libre de cellules.

3. Fraction d'échantillon selon les revendications 1 ou 2, la séparation de taille étant réalisée par chromatographie ou électrophorèse, par centrifugation en gradient de densité ou par des méthodes utilisant des moyens nanotechnologiques.

4. Fraction d'échantillon selon la revendication 3, la chromatographie ou bien l'électrophorèse étant une chromatographie sur des gels d'agarose ou de polyacrylamide, une chromatographie liquide à haute performance de paires d'ions en phase inverse (IP RP HPLC), une électrophorèse capillaire dans une matrice polymère auto-enrobante à faible viscosité, une extraction sélective dans des dispositifs d'électrophorèse microfabriqués, une électrophorèse sur puce sur des matrices polymères de viscosité réduite ou une chromatographie d'adsorption sur membrane.

5. Fraction d'échantillon selon la revendication 3, la méthode utilisant des moyens nanotechnologiques ayant fait l'usage d' arrangements de piéges entropiques microfabriqués.

6. Utilisation d'une fraction d'échantillon selon l'une quelconque des revendications 1 à 5 pour la détection non invasive de traits génétiques foetaux.

7. Utilisation selon la revendication 6, le trait génétique foetal à détecter étant le gène RhD foetal lors d'une grossesse à risque du Morbus haemolyticus neonatorum (maladie hémolytique du foetus et du nouveau-né) ou une séquence foetale spécifique au chromosome Y lors d'une grossesse à risque d'un trouble lié au chromosome X.

8. Utilisation selon la revendication 6, le trait génétique foetal à détecter étant une aberration chromosomique, une trouble génétique héréditaire mendélien ou bien un marqueur génétique ci-associé, ou un trait génétique foetal qui peut être décisif au cas où la paternité doit être déterminée.

9. Utilisation selon la revendication 7, le trouble lié au chromosome X étant l'hémophilie ou le syndrome de l'X fragile.

10. Utilisation selon la revendication 8, l'aberration chromosomique étant une aneuploïdie.

11. Utilisation selon la revendication 8, l'aberration chromosomique étant associée avec le syndrome de Down.

12. Utilisation selon la revendication 8, le trouble génétique héréditaire mendélien étant un trouble monogénique.

13. Utilisation selon la revendication 12, le trouble monogénique étant la fibrose cystique ou une hémoglobinopathie.

14. Utilisation selon l'une quelconque des revendications 6 à 13, la détection des traits génétiques foetaux étant effectuée par la technologie PCR (réaction en chaîne par polymérase), des techniques de réaction de ligature en chaîne ou d'hybridation de sonde ou au moyens d'arrangements d'acides nucléiques.

15. Procédé pour la réalisation de la détection non invasive de traits génétiques foetaux qui comprend un échantillon de plasma ou de sérum de sang d'une femme enceinte étant soumis à une extraction d'ADN, suivi par une séparation de taille, de l'ADN extracellulaire, afin d'obtenir une fraction de ledit échantillon dans laquelle l'ADN extracellulaire ci-présent se compose substantiellement d'ADN constitué de 500 paires de bases ou moins, et un (les) trait(s) génétique(s) foetal (foetaux) à détecter étant déterminé(s) en soumettant une telle fraction à la technologie PCR (réaction en chaîne par polymérase), à des techniques de réaction de ligature en chaîne ou d'hybridation de sonde, ou à des arrangements d'acides nucléiques.

16. Procédé selon la revendication 15, le trait génétique foetal à détecter étant le gène RhD foetal lors d'une grossesse à risque de Morbus haemolyticus neonatorum (maladie hémolytique du foetus et du nouveau-né) ou une séquence foetale spécifique au chromosome Y lors d'une grossesse à risque d'un trouble lié au chromosome X.

17. Procédé selon la revendication 15, le trait génétique foetal à détecter étant une aberration chromosomique, un trouble génétique héréditaire mendélien ou bien un marqueur génétique ciassocié ou un trait génétique foetal qui peut être décisif quand la paternité doit être déterminée.

18. Procédé selon la revendication 16, le trouble lié au chromosome X étant l'hémophilie ou le syndrome de l'X fragile.

19. Procédé selon la revendication 17, l'aberration chromosomique étant une aneuploïdie.

20. Procédé selon la revendication 17, l'aberration chromosomique étant associée avec le syndrome de Down.

21. Procédé selon la revendication 17, le trouble génétique héréditaire mendélien étant un trouble monogénique.

22. Procédé selon la revendication 21, le trouble monogénique étant la fibrose cystique ou une hémoglobinopathie.

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	胎儿遗传性状的非侵入性检测		
公开(公告)号	EP1524321B2	公开(公告)日	2014-07-23
申请号	EP2003405742	申请日	2003-10-16
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IPC分类号	C12Q1/68 G01N33/53 B01J20/281 C12N15/09 G01N30/88 G01N37/00		
CPC分类号	C12Q1/6883 C12Q1/6806 C12Q2600/156		
其他公开文献	EP1524321B1 EP1524321A1		
外部链接	Espacenet		

摘要(译)

孕妇的血浆含有胎儿和母体循环细胞外DNA，后者形成其主要部分（通常> 90％）。现在已发现大多数循环细胞外胎儿DNA具有约500碱基对或更小的小尺寸，而大多数循环细胞外母体DNA具有大于约500个碱基对的大小。小于约500个碱基对的循环细胞外DNA片段的分离提供了从大量的循环细胞外母体DNA中富集胎儿DNA序列的可能性。因此，提供了孕妇的血浆或血清（优选基本上无细胞）的样品，其中富含包含500个碱基对或更少的细胞外DNA;通过对孕妇的血浆或血清样品进行大小分离以便富集包含500个碱基对或更少的细胞外DNA来制造这种样品;以及这种样品用于胎儿遗传性状的非侵入性检测的用途。

Size of DNA	% of fetal DNA in each fragment	% of maternal DNA in each fragment
<0.3 kb	73.2 (22.22-87.06)	26.8 (12.94-77.78)
0.3-0.5 kb	18.95 (6.43-31.42)	81.05 (68.58-93.57)
0.5-1 kb	2.81 (0.00-7.75)	97.19 (92.25-100)
1.0-1.5 kB	0.00 (0.00-12.50)	100 (87.5-100)
1.5-23 kb	0.00 (0.00-8.40)	100 (100-100)