QUANTIFICATION OF FETAL DNA IN THE PLASMA OF PREGNANT WOMEN USING NEXT GENERATION SEQUENCING OF FREQUENT SINGLE NUCLEOTIDE POLYMORPHISMS

Shubina J[1,2] ✉, Jankevic T[2], Goltsov AYu[1,2], Mukosey IS[1], Kochetkova TO[1], Bystritsky AA[1], Barkov IYu[1], Tetruashvili NK[1], Kim LV[1], Trofimov DYu[1,2]

[1] Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Moscow

[2] DNA-Technology LLC, Moscow

Introduced into clinical practice in 2011, non-invasive prenatal testing (NIPT) allows detection of chromosomal aneuploidies in the fetus using maternal blood samples. Multiple studies have shown that one of the key factors affecting the result of this test is the fetal DNA fraction. The aim of this work was to develop a method capable of measuring the fetal DNA fraction based on targeted SNP sequencing. We selected polymorphisms with high frequency of heterozygous genotype from the international HapMap database. To estimate the frequency of these polymorphisms in the Russian population, we used 827 DNA donor samples. Fetal DNA fraction was measured in 87 plasma samples of pregnant women. Sequencing was performed on Ion Proton and Ion S5. We determined the frequencies of the studied polymorphisms in the pooled samples and compared the data on 53 SNPs in the pooled and 87 individual samples. The median difference was 3.4%. The correlation between the results obtained by targeted SNP sequencing and Y chromosome read count was 0.7. Thus, the proposed method can be used to estimate the fetal DNA fraction using SNP genotyping regardless of the fetus's sex.

Keywords: non-invasive prenatal testing, fetal DNA fraction, single nucleotide polymorphisms, chromosome aneuploidy

Funding: this work was supported by the Ministry of Education and Science of the Russian Federation (Agreement 14.607.21.0136, Project ID RFMEFI60715X0136).

✉ **Correspondence should be addressed:** Jekaterina Shubina
Akademika Oparina 4, Moscow, 117997; jekaterina.shubina@gmail.com

Recieved: 15.04.2018 **Accepted:** 18.04.2018

DOI: 10.24075/brsmu.2018.031

ОПРЕДЕЛЕНИЕ ДОЛИ ПЛОДОВОЙ ДНК В ПЛАЗМЕ КРОВИ БЕРЕМЕННОЙ ЖЕНЩИНЫ С ПОМОЩЬЮ ВЫСОКОПРОИЗВОДИТЕЛЬНОГО СЕКВЕНИРОВАНИЯ НАБОРА ЧАСТОТНЫХ ОДНОНУКЛЕОТИДНЫХ ПОЛИМОРФИЗМОВ

Е. Шубина[1,2] ✉, Т. Янкевич[2], А. Ю. Гольцов[1,2], И. С. Мукосей[1], Т. О. Кочеткова[1], А. А. Быстрицкий[1], И. Ю. Барков[1], Н. К. Тетруашвили[1], Л. В. Ким[1], Д. Ю. Трофимов[1,2]

[1] Национальный медицинский исследовательский центр акушерства, гинекологии и перинатологии имени В. И. Кулакова, Москва

[2] ООО «НПФ ДНК-Технология», Москва

Неинвазивный пренатальный ДНК-скрининг (НИПС) анеуплоидий по крови матери применяется для выявления хромосомных анеуплоидий (ХА) с 2011 г. Многочисленные клинические исследования показали, что важным параметром при проведении НИПС является доля плодовой ДНК. Целью работы была разработка тест-системы для оценки доли плодовой ДНК с помощью таргетного секвенирования однонуклеотидных полиморфизмов (SNP). По данным исследований международного проекта HAPMAP были отобраны полиморфизмы с высокой частотой встречаемости гетерозиготного генотипа. Для оценки частоты встречаемости отобранных полиморфизмов в российской популяции использовали 827 образцов ДНК доноров. С целью определения доли плодовой ДНК исследовали 87 образцов плазмы крови беременных женщин. Секвенирование проводили на приборах Ion Proton и Ion S5. В ходе работы были определены частоты встречаемости по данным секвенирования пулированных образцов. Проведено сравнение данных о 53 SNP в 87 отдельных образцах. Медиана разницы, полученой различными способами, составила 3,4%. Результаты определения доли плодовой ДНК с помощью SNP сравнивали с данными по Y-хромосоме, корреляция составила 0,7. Таким образом, разработанную тест-систему можно применять для определения доли плодовой ДНК с помощью SNP вне зависимости от пола плода.

Ключевые слова: неинвазивный пренатальный ДНК-скрининг, доля плодовой ДНК, однонуклеотидные полиморфизмы, хромосомные анеуплоидии

Финансирование: работа поддержана Министерством образования и науки Российской Федерации (соглашение № 14.607.21.0136, идентификатор проекта RFMEFI60715X0136).

✉ **Для корреспонденции:** Шубина Екатерина
ул. Академика Опарина, д. 4, г. Москва, 117997; jekaterina.shubina@gmail.com

Статья получена: 15.04.2018 **Статья принята к печати:** 18.04.2018

DOI: 10.24075/vrgmu.2018.031

Introduced into clinical practice in 2011, non-invasive prenatal testing (NIPT) allows detection of chromosomal aneuploidies in the fetus using maternal blood samples [1]. Multiple studies have shown that one of the key factors affecting the result of this test is the fetal DNA fraction [2, 3]. The test loses its sensitivity and can come out false-negative if the amount of fetal DNA in the sample is insufficient [3].

The fetal DNA fraction is easy to measure in women carrying a male fetus. This is done by comparing the number of Y reads with the read counts for autosomal chromosomes. In contrast, pregnancy with a female fetus complicates quantification of fetal DNA.

Currently existing methods for estimating the proportion of fetal DNA in the total cell-free circulating DNA (cfDNA) are based on the detection and quantification of DNA fragments whose origin can be established. Some of these methods make use of Y-chromosome-specific fragments only found in male fetuses. Others are not sex-based and rely on the analysis of differentially methylated cfDNA fragments [4], SNPs [5–7], unequal sizes of fetal and maternal DNA fragments [8], and distribution of fetal DNA fragments across the genome [9–11].

Targeted sequencing of single nucleotide polymorphisms (SNPs) can be employed to determine the fetal DNA fraction and enables genetic-based identification of the sample. Besides, it can be used in non-invasive paternity and prenatal testing [12, 13].

The aim of this work was to develop a method capable of measuring the fetal DNA fraction regardless of the fetus's sex using targeted SNP sequencing.

METHODS

Selection of single nucleotide polymorphisms

Seventy-three polymorphisms were selected from the HapMap database, a product of the large-scale population research studies [14] (http://hapmap.ncbi.nlm.nih.gov/). The frequency of their heterozygous variants is 49–51% for the CEU population (Northern and Western Europe) and 45–55% for the African (ASW), Chinese (CHD, CHB) and Japanese (JPT) populations. These polymorphisms are located on chromosomes 1–12 no less than 20 million b.p. apart. For each of them, specific amplification primers were selected. The intended size of PCR products was 110 b.p.

DNA and plasma samples

The frequency of the selected polymorphisms in the Russian population was estimated based on the analysis of 827 DNA samples isolated from donors' blood. The fetal DNA fraction was measured in 87 plasma samples obtained from 45 women pregnant with a male fetus and 42 women carrying a female fetus.

Frequency of polymorphisms in the Russian population

Because no large-scale population data are available describing the frequency of various polymorphisms in the Russian population and because the polymorphisms we selected represented non-Russian populations, our estimates can differ from the published data. In this work we estimated the frequency of the studied polymorphisms in the Russian population using targeted sequencing of DNA samples pooled at equal concentrations.

Ten pools of 827 samples (51 to 114 samples per pool) were sequenced. Prior to DNA pooling, we determined DNA concentrations in the samples by real-time polymerase chain reaction (PCR). To estimate the frequency of the studied polymorphisms in the Russian population, we added up the frequencies obtained for each sequenced pool, with due account of the number of samples in the pool. The resulting figures were compared with the data generated by the sequencing of 87 individual samples.

Estimation of fetal DNA fraction

The fetal DNA fraction was estimated after sequencing 53 frequent polymorphisms that had been selected based on the preliminary sequence data analysis for pooled samples.

To estimate the fetal DNA fraction, we relied on the polymorphisms for which the frequency of one allele was over 80% but below 99.5%, assuming that the mother had a homozygous genotype and the fetus was heterozygous. The fetal DNA fraction was calculated according to the formula $ff = 2 \cdot B / (A + B)$, where A is a more abundant and B is a less abundant allele. The fetal DNA fraction was presented as a median of values obtained for all informative polymorphisms. The fetal DNA fraction estimated by SNP genotyping was compared to the value obtained by counting the proportion of DNA molecules originating from the Y chromosome.

Sequencing

Libraries of PCR products were prepared according to the manufacturer's protocol (Thermo Fisher Scientific Inc., USA). Sequencing was performed on Ion Proton and Ion S5 (Thermo Fisher Scientific Inc., USA) according to the manufacturer's protocol.

Data analysis

The results were processed using Torrent Server 4.4.3. The sequences were aligned against the reference genome ver. GRCh37/hg19 using TMAP (Thermo Fisher Scientific Inc., USA).Then the reads were counted for each allele located in the genomic regions corresponding to the selected polymorphisms using an original script and the pysam module [15]. Only those fragments were eligible for the analysis for which the alignment quality was >30 and the size was >80% of the expected length.

RESULTS

Sequencing of pooled samples

Data generated by the sequencing of pooled samples are presented in Table 1.

Upon assessing the performance of the method in general and the frequency of the studied polymorphisms, we selected 53 SNPs for further analysis. Infrequent polymorphisms were excluded. Table 2 shows the frequencies of 53 SNPs in the pooled samples and 87 individual samples. The median difference between the "pooled" and "individual" frequencies was 3.4%.

Results of fetal DNA fraction estimation

The average number of polymorphisms with a homozygous genotype in the mother was 28 (25–32), of them 14 (10–18) were informative. Fig. 1 compares the estimates of the fetal DNA fraction obtained through SNP genotyping and Y chromosome read count; the correlation index is 0.7.

Table 1. Data generated by the sequencing of pooled samples

Pool ID	Number of samples	Number of reads	Number of reads/polymorphism med (q1–q3)
1	96	2 681 517	12476 (5195–40260)
2	114	2 002 697	13408 (4724–34127)
3	96	2 711 707	17753 (7810–48959)
4	84	3 037 177	20001 (6742–44910)
5	78	3 884 900	28124 (10032–66108)
6	96	1 677 467	9808 (2860–24624)
7	92	1 592 401	8826 (2503–26345)
8	63	1 759 487	11359 (3629–28146)
9	57	2 340 385	14355 (3983–38147)
10	51	2 403 795	16195 (4686–37680)

Table 2. Comparison of SNP frequencies in the pooled samples and 87 individual samples

SNP	Pooled samples (827)		Individual samples (87)		SNP	Pooled samples (827)		Individual samples (87)	
	Allele 1	Allele 2	Allele 1	Allele 2		Allele 1	Allele 2	Allele 1	Allele 2
rs4846002	0.619	0.381	0.592	0.408	rs1265758	0.599	0.4	0.576	0.424
rs4926658	0.577	0.423	0.529	0.471	rs2143829	0.574	0.426	0.616	0.384
rs9434166	0.576	0.424	0.598	0.402	rs591356	0.438	0.562	0.453	0.547
rs10753750	0.564	0.436	0.586	0.414	rs9373116	0.579	0.421	0.494	0.506
rs1973943	0.532	0.467	0.494	0.506	rs7770051	0.479	0.521	0.407	0.593
rs7597744	0.49	0.51	0.494	0.506	rs16	0.516	0.484	0.506	0.494
rs2121304	0.56	0.44	0.558	0.442	rs12333726	0.564	0.435	0.552	0.448
rs1726025	0.517	0.483	0.517	0.483	rs6958027	0.593	0.407	0.523	0.477
rs11164111	0.513	0.487	0.494	0.506	rs314320	0.724	0.276	0.75	0.25
rs981841	0.49	0.509	0.558	0.442	rs625218	0.597	0.403	0.618	0.382
rs1978346	0.653	0.347	0.698	0.302	rs7005848	0.457	0.542	0.5	0.5
rs9843942	0.565	0.435	0.523	0.477	rs952559	0.547	0.453	0.612	0.388
rs6777416	0.587	0.413	0.616	0.384	rs827584	0.72	0.279	0.7	0.3
rs957303	0.61	0.39	0.593	0.407	rs9987271	0.577	0.422	0.541	0.459
rs1553212	0.514	0.486	0.448	0.552	rs6559467	0.583	0.417	0.612	0.388
rs751834	0.561	0.438	0.663	0.337	rs4132699	0.667	0.332	0.647	0.353
rs6771838	0.645	0.354	0.622	0.378	rs10980011	0.599	0.4	0.571	0.429
rs7696439	0.629	0.37	0.663	0.337	rs2583839	0.603	0.397	0.565	0.435
rs4864809	0.452	0.548	0.517	0.483	rs7904536	0.793	0.207	0.941	0.059
rs17002804	0.484	0.516	0.541	0.459	rs4917915	0.531	0.468	0.453	0.547
rs978373	0.497	0.502	0.459	0.541	rs845085	0.681	0.319	0.724	0.276
rs4621390	0.607	0.393	0.57	0.43	rs4333997	0.522	0.477	0.488	0.512
rs7703985	0.491	0.509	0.442	0.558	rs602991	0.696	0.304	0.747	0.253
rs2962799	0.542	0.456	0.541	0.459	rs2289300	0.716	0.283	0.647	0.353
rs902987	0.52	0.478	0.512	0.488	rs7973612	0.704	0.296	0.765	0.235
rs6859147	0.551	0.449	0.547	0.453	rs7971962	0.606	0.39	0.606	0.394
rs4921132	0.495	0.505	0.494	0.506					

DISCUSSION

We have estimated the frequency of the selected polymorphisms in the studied population using sequencing of pooled samples. We have shown that sequencing of pooled samples and genotyping of individual samples produce comparable results. Targeted sequencing of a small number of frequent polymorphisms is a feasible method for estimating the fetal DNA fraction independent of the fetus's sex. In our work, the correlation between the results obtained by targeted SNP sequencing and Y chromosome read count was lower than in the published studied that used the comparable number of polymorphisms to measure the fetal DNA fraction [7], probably because the authors of that study used molecular identifiers and counted individual molecules.

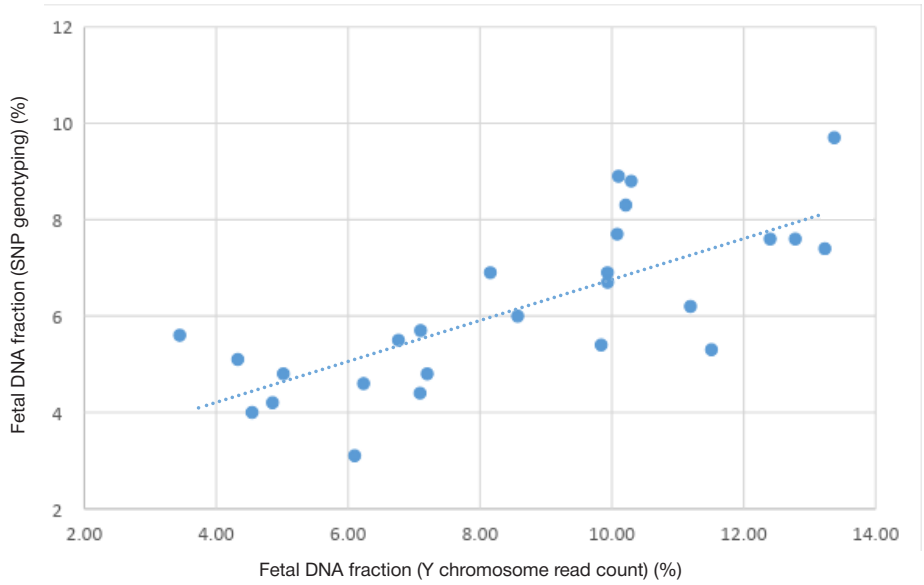

Fig. 1. Comparison of the estimates of the fetal DNA fragment fraction done by targeted SNP sequencing and Y chromosome read count

CONCLUSIONS

The proposed method can be used to estimate the frequency of alleles in frequent polymorphisms. The method allows both estimation of the fetal DNA fraction and genetic identification of the samples and can be used in non-invasive paternity or prenatal screening if the mutation is passed on by the father and is absent in the mother

References

1. Agarwal A, Sayres LC, Cho MK, Cook-Deegan R, Chandrasekharan S. Commercial landscape of noninvasive prenatal testing in the United States. Prenat Diagn. 2013; 33 (6): 521–31.
2. Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. Prenat Diagn. 2013; 33 (7): 667–74.
3. Sukhikh GT, et al. Noninvasive prenatal diagnosis of aneuploidies by next-generation sequencing (NGS) in a group of high-risk women."Obstetrics and Gynecology. 2015; 4: 5–10.
4. Nygren AO, Dean J, Jensen TJ, Kruse S, Kwong W, van den Boom D, et al. Quantification of fetal DNA by use of methylation-based DNA discrimination. Clin Chem. 2010 Oct; 56 (10): 1627–35.
5. Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012 Apr; 206 (4): 319. e1–9.
6. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn. 2013 Jun; 33 (6): 575–9.
7. Song Y, et al. Quantitation of fetal DNA fraction in maternal plasma using circulating single molecule amplification and re-sequencing technology (cSMART). Clin Chim Acta. 2016; 456: 151–6.
8. Yu SCY, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. Proc Natl Acad Sci USA. 2014 Jun; 111 (23): 8583–8.
9. Kim SK, et al. Determination of Fetal DNA Fraction from the Plasma of Pregnant Women using Sequence Read Counts. Prenat Diagn. 2015: n/a–n/a.
10. Straver R, Oudejans CBM, Sistermans EA, Reinders MJT. Calculating the fetal fraction for Non Invasive Prenatal Testing based on Genome-wide nucleosome profiles. Prenat Diagn. 2016: n/a–n/a.
11. van Beek DM, et al. Comparing methods for fetal fraction determination and quality control of NIPT samples. Prenat Diagn. 2017; 37 (8): 769–73.
12. Lv W, et al. Noninvasive prenatal testing for Wilson disease by use of circulating single-molecule amplification and resequencing technology (cSMART). Clin Chem. 2015; 61 (1): 172–81.
13. Meng M, et al. Noninvasive prenatal testing for autosomal recessive conditions by maternal plasma sequencing in a case of congenital deafness. Genet Med. 2014; 16 (12): 972–6.
14. International HapMap Consortium. The International HapMap Project. Nature. 2003; 426 (6968): 789–96.
15. Li H, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009 Aug; 25 (16): 2078–9.

Литература

1. Agarwal A, Sayres LC, Cho MK, Cook-Deegan R, Chandrasekharan S. Commercial landscape of noninvasive prenatal testing in the United States. Prenat Diagn. 2013; 33 (6): 521–31.
2. Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. Prenat Diagn. 2013; 33 (7): 667–74.
3. Сухих Г. Т., Каретникова Н. А., Баранова Е. Е., Шубина Е. С., Коростин Д. О., Екимов А. Н. и др. Неинвазивная пренатальная диагностика анеуплоидий методом высокопроизводительносго секвенирования (NGS) в группе женщин высокого риска. Акушерство и гинекология. 2015; 4: 5–10.
4. Nygren AO, Dean J, Jensen TJ, Kruse S, Kwong W, van den Boom D, et al. Quantification of fetal DNA by use of methylation-based DNA discrimination. Clin Chem. 2010 Oct; 56 (10): 1627–35.
5. Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18.

Am J Obstet Gynecol. 2012 Apr; 206 (4): 319. e1–9.
6. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn. 2013 Jun; 33 (6): 575–9.
7. Song Y, et al. Quantitation of fetal DNA fraction in maternal plasma using circulating single molecule amplification and re-sequencing technology (cSMART). Clin Chim Acta. 2016; 456: 151–6.
8. Yu SCY, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. Proc Natl Acad Sci USA. 2014 Jun; 111 (23): 8583–8.
9. Kim SK, et al. Determination of Fetal DNA Fraction from the Plasma of Pregnant Women using Sequence Read Counts. Prenat Diagn. 2015: n/a–n/a.
10. Straver R, Oudejans CBM, Sistermans EA, Reinders MJT. Calculating the fetal fraction for Non Invasive Prenatal Testing based on Genome-wide nucleosome profiles. Prenat Diagn. 2016: n/a–n/a.
11. van Beek DM, et al. Comparing methods for fetal fraction determination and quality control of NIPT samples. Prenat Diagn. 2017; 37 (8): 769–73.
12. Lv W, et al. Noninvasive prenatal testing for Wilson disease by use of circulating single-molecule amplification and resequencing technology (cSMART). Clin Chem. 2015; 61 (1): 172–81.
13. Meng M, et al. Noninvasive prenatal testing for autosomal recessive conditions by maternal plasma sequencing in a case of congenital deafness. Genet Med. 2014; 16 (12): 972–6.
14. International HapMap Consortium. The International HapMap Project. Nature. 2003; 426 (6968): 789–96.
15. Li H, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009 Aug; 25 (16): 2078–9.