DETECTION OF CHROMOSOMAL REARRANGEMENTS IN THE SHORT ARMS OF CHROMOSOMES 4 AND 12 AS AN EXAMPLE OF A WHOLE-GENOME APPROACH TO NONINVASIVE PRENATAL TESTING

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Timely detection of fetal aneuploidy is an important aspect of clinical practice. At present, analytical techniques involving high-throughput sequencing are on the rise. Noninvasive prenatal testing (NIPT) ensures reliable results as early as week 9–11 into pregnancy. This article describes a clinical case of NIPT application and further verification of its results. Using next-generation sequencing, the microarray analysis of cell-free DNA in the amniotic fluid and the cytogenetic analysis of fetal chromosomes, a high risk of chromosomal rearrangements was detected in the short arms of chromosomes 4 and 12. This prediction was verified by molecular karyotyping conducted in both parents. The mother was found to be a balanced carrier of translocations between chromosomes 4 and 12. This case demonstrates the advantages of a whole-genome approach to NIPT over targeted-based.

Keywords: aneuploidy, noninvasive prenatal testing, syndrome, invasive diagnostic test, combined screening

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Compliance with ethical standards: the study was approved by the Ethics Committee of Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology (Protocol № 2015/13).

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ДЕТЕКЦИЯ ХРОМОСОМНЫХ ПЕРЕСТРОЕК В КОРОТКОМ ПЛЕЧЕ 4-Й И 12-Й ХРОМОСОМ КАК ПРИМЕР ПОЛНОГЕНОМНОГО ПОДХОДА ПРИ ПРОВЕДЕНИИ НЕИНВАЗИВНОГО ДНК-СКРИНИНГА

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Своевременное обнаружение анеуплоидий плода очень важно в клинической практике. В настоящее время идет активное развитие аналитических методов с применением высокопроизводительного секвенирования. Благодаря неинвазивному пренатальному ДНК-скринингу (НИПС) достоверные результаты можно получать на сроке 9–11 недель. Описан клинический случай применения НИПС и дальнейшей верификации полученных результатов. С помощью методов высокопроизводительного секвенирования, микроматричного анализа амниотической жидкости и цитогенетического кариотипирования у плода обнаружен высокий риск хромосомных перестроек в коротком плече 4-й и 12-й хромосом. Результаты были подтверждены с помощью молекулярного кариотипирования. Проверка родителей позволила выявить у матери сбалансированные хромосомные перестройки в 4-й и 12-й хромосомах. Данный случай демонстрирует преимущества полногеномного подхода перед таргетным при проведении НИПС.

Ключевые слова: анеуплоидии, неинвазивный пренатальный ДНК-скрининг, синдром, инвазивная диагностика, комбинированный скрининг

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Chromosomal aneuploidy (CA) is a common cause of perinatal death and abnormal fetal development. CA is diagnosed in one in 3 pregnancies ended in missed or spontaneous abortions. The incidence rate of CA in newborn infants is 1 : 300. The most common aneuploidies are trisomies 21, 18 and 13 [1] and sex chromosome aneuploidy. Maternal age is one of the risk factors for CA.

For timely detection of fetal CA, Russia has adopted 1st trimester screening that combines an ultrasound scan and blood biochemistry tests [2]. It has limited specificity and sensitivity because biochemical parameters of the blood are determined by a number of factors that also include the hormonal status of the mother apart from the chromosomal status of the fetus. Women at high risk for aneuploidy are recommended to undergo additional, more accurate tests. CA is confirmed in about 13 to 15% of these women. The tests are invasive (amniocentesis, cordocentesis, chorionic villus sampling) and can cause pregnancy loss in 0.5-2% of patients; therefore, they are contraindicated for women at high risk for pregnancy loss. Noninvasive prenatal testing (NIPT) is an alternative to invasive procedures in cases when gestational age does not exceed 18–19 weeks [3]. NIPT does not have contraindications for high-risk women and can be performed as early as 9–10 weeks into pregnancy. Noninvasive prenatal tests vary in the number of chromosomes they target, which is usually limited to chromosomes 13, 18 and 21 [4,5]. Even in whole-genome-based NIPT, other chromosomes are rarely analyzed (Prenetix, Panorama). There is a test that only looks for trisomy 21 (Down syndrome); it is also referred to as NIPT.

Case study

Below, we describe a case of a 30-year-old pregnant female patient P. The patient already had a 3-year-old daughter diagnosed with disseminated intravascular coagulation and high-pressure hydrocephalus. The patient's BMI was 17.6 kg/m^2.

The patient was pregnant for the third time; the pregnancy was spontaneous. An ultrasound scan performed as part of combined first trimester screening was not suggestive of chromosomal abnormalities or congenital defects. NFT was 1.3 mm; the nasal bone could be visualized. β-HCG = 0.508 MoM; PAPP-A = 0.314 MoM.

The following risks were identified based on the results of first trimester screening:

trisomy 21 — 1 : 10 084 (baseline risk 1 : 585);
trisomy 18 — 1 : 1073 (baseline risk 1 : 1396);
trisomy 13 — 1 : 1372 (baseline risk 1 : 4389).

A blood sample for NIPT was collected at 13 weeks 4 days gestational age. Whole-genome sequencing of the cell-free DNA library was carried out using Ion S5XL (ThermoFisher Scientific; USA); the yielded data were analyzed following a protocol proposed in [6]. The obtained reads were mapped onto the reference genome; CG content was bias-corrected, and uniquely mapped reads were counted. The risk for CA was estimated using the original software developed by the authors of this work [7].

Based on the results of our analysis, the following conclusions were drawn. The prepared whole-genome library was covered by 7.5 million reads; the sex of the fetus was determined as male. Y chromosome sequences made 16% of the total cell-free DNA. No aneuploidy was detected for chromosomes 13, 18 and 21. However, the analysis revealed a high risk of a p16-p14 deletion on the short arm of chromosome with an estimated size of 35 Mb (Fig. 1A) and a high risk of a p13.3–p12.1 duplication (25 Mb in size) on the short arm of chromosome 12 (Fig. 1B). The obtained data had to be further verified using invasive techniques [8, 9].

To verify NIPT results [10], a microarray analysis of the amniotic fluid was performed using CytoScan Optima Array microchips (Affimetrics; USA). Sampling was done at 16 weeks into pregnancy.

Results are presented in Fig. 2 and 3.

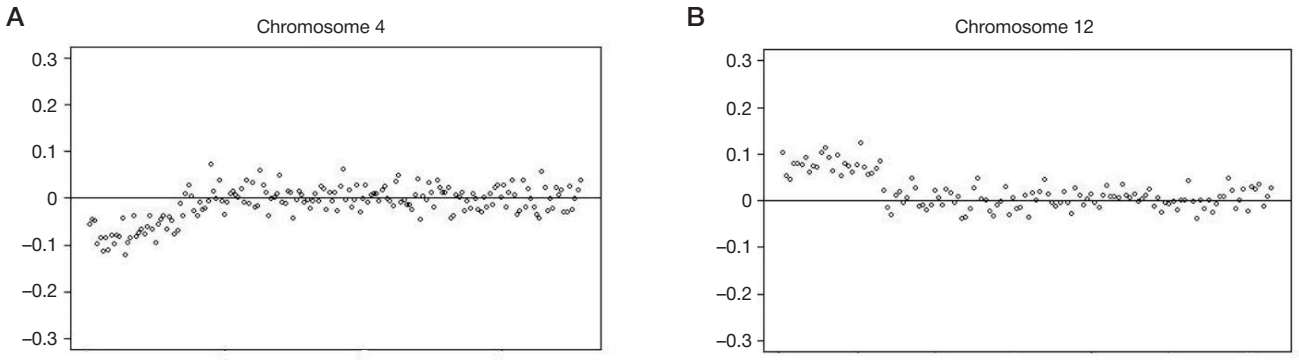

Fig. 1. A visual representation of distribution of reads along the chromosome. The Y axis shows deviations in the read count from the reference values for the normal genotype. **A**. Distribution of reads for chromosome 4. **B**. Distribution of reads for chromosome 12

Fig. 2. Results of the analysis of the amniotic fluid collected from patient P. showing a deletion on the short arm of chromosome 4

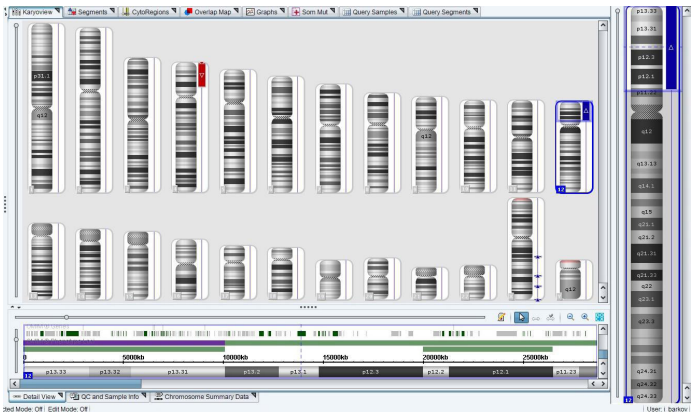

Fig. 3. Results of the analysis of the amniotic fluid collected from patient P. showing a duplication on the short arm of chromosome 12

Specimen type: peripheral blood
Staining type: G-banding
Karyotype: 46,XX,t(4;12)(p15.1;p11.2)
Conclusion: Balanced female carrier of a reciprocal translocation between chromosomes 4 and 12 with breakpoints at 4p15.1 and 12p11.2

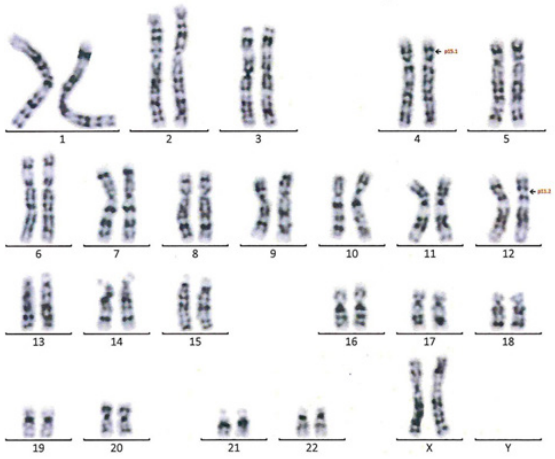

Fig. 4. Patient P.'s karyotype

The embryonic karyotype is described below:

arr[hg19] 4p16.3(68,345-35,195,686)x1 — a 35 million b.p.-long partial deletion of the short arm of chromosome 4 causing the Wolf–Hirschhorn syndrome (OMIM# 194190);

arr[hg19] 12p13.33p11.22(173,786-28,183,286)x3 — a 28 million b.p.-long duplication on the short arm of chromosome 12 causing the Pallister–Killian syndrome (OMIM# 601803) also known as tetrasomy 12p.

The patient was recommended to terminate pregnancy. Pregnancy was terminated at 18 weeks.

DISCUSSION

The analysis revealed detrimental chromosomal rearrangements in the fetus. Therefore, it was recommended that both parents undergo karyotyping. The mother was found to carry a balanced translocation involving chromosomes 4 and 12, the underlying cause of the abnormalities in the fetus (Fig. 4).

We were able to detect such rare chromosomal aberrations only due to the use of whole-genome sequencing during NIPT. There are a few varieties of NIPT. Some of them are based either on the targeted sequencing of chromosomes 13, 18 and 21 or on the selective analysis of only certain chromosomes [11, 12]. Obviously, such approaches do not allow detection of those chromosomal rearrangements that are present on other, untargeted chromosomes. There have been clinical reports of missed partial deletions (22q11, known as DiGeorge syndrome), as well as deletions of the entire short arm of chromosome 5 (cat's cry syndrome), that went unnoticed by NIPT and eventually led to the birth of an unhealthy child.

CONCLUSION

This clinical case had a few minor and major implications. First, due to the high risk of chromosomal abnormalities in future pregnancies, the patient and her husband were recommended to undergo preimplantation genetic profiling. Second, whole-genome NIPT has a few advantages over targeted-based since it covers all chromosomes and does not result in the loss of data from clinically important genome regions.

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