Detection and validation of subchromosomal aberrations detected as additional findings in routine noninvasive prenatal testing for common trisomies

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Low coverage whole genome sequencing routinely used in noninvasive prenatal testing is able to detect not only most commonly screened chromosomal aneuploidies but also nontargeted subchromosomal aberrations. Aim of the work was validation of detection of nontargeted subchromosomal aberrations reported as additional findings in samples analyzed in routine noninvasive prenatal testing.
From April 2016 till December 2018, more than 9500 samples were analyzed as part of routine noninvasive prenatal testing. In tested cohort of patients 42 different subchromosomal aberrations were detected in 38 cases, in 18 cases with 18 detections confirmatory testing follow up was not available, in 6 cases with 9 detected aberrations all were confirmed by diagnostic testing and in 14 cases 15 detections were not confirmed and so evaluated as false positives. Positive predictive value of reported additional finding was therefore 37.5%.
Keywords: NIPT, Trisomy test, additional findings, low coverage whole genome sequencing, subchromosomal aberrations detection

Detekcia a validácia subchromozómových aberácií detegovaných ako doplnkové zistenia v rámci rutinného neinvazívneho prenatálneho skríningu na najčastejšie trizómie
Celogenómovým sekvenovaním s nízkym pokrytím, ktoré sa rutinne používa v rámci neinvazívneho prenatálneho skríningu na najčastejšie chromozómové aneuploidie, je možné necielene detegovať subchromozómové aberácie na celogenómovej úrovni.
Cieľom práce bola validácia necielenej detekcie subchromozómových aberácií reportovaných ako doplnkové zistenia vo vzorkách analyzovaných ako súčasť neinvazívneho prenatálneho skríningu.
Od apríla 2016 do decembra 2018 bolo v rámci rutinného prenatálneho skríningu analyzovaných vyše 9 500 vzoriek. V tomto súbore vzoriek bolo detegovaných a reportovaných 42 rôznych subchromozómových aberácií u 38 testovaných jedincov, z toho u 18 testovaných jedincov s 18 detekciami neboli dostupné výsledky konfirmačného diagnostického testovania, u 6 vzoriek s 9 detekciami boli reportované aberácie potvrdené diagnostickým testom a u 14 vzoriek s 15 detekciami ich prítomnosť u plodu nebola potvrdená následným testovaním. Pozitívna prediktívna hodnota reportovaných doplnkových nálezov bola teda 37,5%.
Kľúčové slová: NIPT, Trisomy test, doplnkové nálezy, celogenómové sekvenovanie s nízkym pokrytím, detekcia subchromozómových aberácií

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Introduction

Identification of fetal DNA in maternal circulation led to redefinition of possibilities in the field of noninvasive prenatal testing (NIPT)[(1)]. From the detection of qualitative fetal genetic markers (e. g. fetal gender) with advances of sequencing technologies also quantitative analysis of fetal DNA became possible (e. g. detection of chromosomal aneuploidies)[(2,3)]. With increase of availability of genomic sequencing, NIPT based on analysis of circulating cell-free fetal DNA focused on detection of most common chromosomal aneuploidies (trisomy 13 – T13, trisomy 18 – T18 and trisomy 21 – T21) has become integral part of prenatal genetics and in last few years different laboratories made such NIPT available worldwide. Low coverage whole genome sequencing, the mostly used method in NIPT routine, is able to detect not only most commonly screened chromosomal aneuploidies but also different subchromosomal aberrations.

Aim of the study

Aim of the work was retrospective validation of detection of subchromosomal aberrations of fetal origin identified and reported as additional findings in routine noninvasive prenatal screening focused on most common trisomies.

Materials and methods

From July 2016 till April 2019, more than 9500 samples of pregnant women were analyzed using Trisomy test. For detection of high-risk samples low coverage whole genome

scan was used in association with in-house designed bioinformatic pipeline and proprietary biostatistical approach. In more details, peripheral blood was collected to EDTA or Streck tubes. DNA was extracted using QIAamp DNA Blood Mini Kit from Qiagen. Extracted DNA was quantified using Qubit dsDNA HS Assay Kit from Thermo Fisher Scientific. DNA libraries were prepared using TruSeq Nano DNA Library Prep Kit from Illumina as previously published[4]. For sequencing of libraries NextSeq 500/550 High Output Kit v2 (75 cycles) and NextSeq 500 platform from Illumina were used. Gained genomic data were analyzed by in-house bioinformatic algorithm, which calculated fetal fraction and z-scores (algorithm is patent pending - PCT/EP2016/064604).

Results and discussion

In tested cohort of patients different subchromosomal aberrations were detected. In 38 samples 42 additional findings were reported. In samples with detections fetal fraction varied between 5.1 and 19.1% (median = 10.35%). The smallest reported aberration has 1 megabase (Mb) and the largest one approx. 80 Mb (median = 14 Mb) ***(Graph 1)***. Aberrations were detected on chromosomes 1, 2, 4, 5, 6, 7, 8, 10, 13, 15, 16, 18, 20, 21 and 22. In 18 cases confirmatory testing was refused by the patient, we were not able to get sample for confirmatory analysis or feedback from the further management of the patient was not available. In 20 cases samples from chorionic villus sampling or amniocentesis were available for confirmatory testing, that was performed by different diagnostic laboratories subsequently by karyotyping or comparative genomic hybridization (aCGH). In 4 cases 2 different aberrations were detected in parallel, in one case ring chromosome represented by two deletions of both ends of chromosome 18 was recorded ***(Figure 1)***. In the 20 cases (24 detected aberrations) with available material from invasive procedures 9 findings were confirmed and 15 were evaluated as false positives ***(Graph 1)***. According to the results of the confirmatory testing the positive predictive value (PPV) of subchromosomal aberrations detection by our test reached 37.5% (9 of 24). If currently used criteria for additional findings reporting were applied (fetal fraction above 10% and size of detected aberration above 3 Mb) PPV of our test reached 64.3% (9 of 14). Usage of this limits for future detection and reporting of additional findings will decrease the proportion of false positives. But it is necessary to add, that these cannot be completely avoided as proportion of false positives could be result of feto-placental mosaicisms, but for estimation of this proportion placental samples from these cases have to be analyzed.

Conclusions

In this prospective study the possibility of utilization of low coverage genomic sequencing for detection of subchromosomal aberrations on the whole genome level was confirmed. For proper estimations of sensitivity, specificity, NPV and PPV larger studies are needed as samples significantly differ in crucial factors that are represented by fetal fraction, position and size of detected aberrations. Hence in the real life screening utilizing this approach different chromosomal aberrations could be identified, their clinical significance should be considered in details and the positive results or

Graph 1. *Correlation between subchromosomal aberration size and fetal fraction. In samples with fetal fraction above 10% (vertical dotted line) and size of detected aberration above 3 Mb (horizontal dotted line) is PPV significantly higher than in samples with fetal fraction below this limits. N/A – confirmatory analysis results not available*

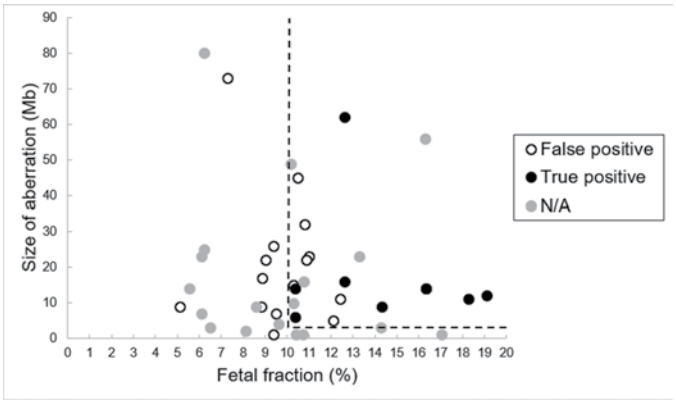
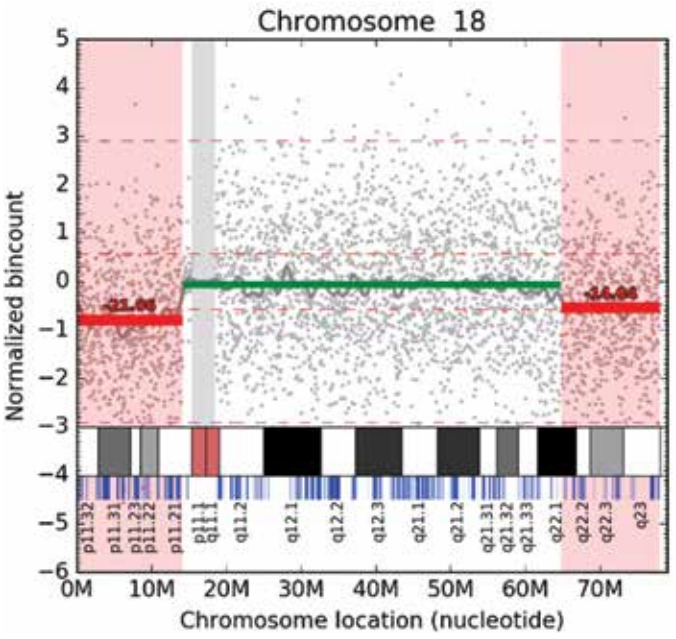


Figure 1. *Two deletions on both ends of chromosome 18 confirmed by aCGH and detected as ring chromosome 18 by karyotyping after amniocentesis*



detections have to be consulted with clinical geneticist or genetic counselor with appropriate experience and usage of up to date information from specialized databases, e.g. ClinVar or Decipher or relevant studies and publications. Moreover, to give clinicians supporting information we are currently preparing clinical decision supporting bioinformatical tool, that should summarize such clinically relevant information in a web-based and user-friendly interface.

Conflict of interest

Regarding this study Sekelská M., Izsáková A., Kubošová K., Tilandyová P., Csekes E., Kúchová Z., Hýblová M. and Minárik G., are employees of Trisomy test Ltd. and Medirex Inc.; Lukačková R. Landlová D. and Križan P. are employees of Medirex Inc.; Haršányová M., Budiš J., Kucharík M. and Szemes T. are employees of Geneton Ltd.

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