False positivity and false negativity as a standard part of noninvasive prenatal testing

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Background

Noninvasive prenatal testing (NIPT) is becoming highly accepted as a routine screening tool in most developed countries. With advanced versions of NIPT tests covering aneuploidies of all chromosomes and subchromosomal aberrations, false positives and negatives appear more frequently and should be analysed further.
To get comprehensive information about the reason for NIPT result falseness methods such as qPCR, aCGH, FISH, karyotyping, and genomic sequencing in diagnostic settings have been used for analyses of DNA extracted from chorionic villi, amniotic fluid, amniotic cells, placental tissue, fetal tissue, maternal blood taken after delivery, and other relevant biological specimens obtained before as well as after delivery were used additionally to original low coverage whole genome sequencing performed on circulating DNA extracted from plasma of pregnant women.

Study aim

This research was focused on the determination of primary causes of false negative and false positive results of noninvasive prenatal testing performed by low coverage whole genome sequencing-based Trisomy test® routinely used in an unselected population cohort of pregnant women.

Results

Out of 19159 NIPT tests evaluated after routine clinical laboratory testing for basic trisomies, 9 analyses were reported as false negative (2) and false positive (7), which in statistics focused solely on chromosomes 21, 18, and 13 trisomies represent less than 0.05% of cases. When focusing on all chromosomes and also subchromosomal aberrations, the frequency of such false results is higher.
From the portfolio of potential biological reasons, we detected and confirmed by subsequent diagnostic analyses false results related to confined placental mosaicism (Fig. 1), true fetal mosaicism (Fig. 2), a maternal aberration in the full and mosaic state (Fig. 3), precancerous maternal aberration (Fig. 4), incorrect anamnestic data of the mother (Fig. 5). For technical reasons, gray zone results (Fig. 6), insufficient coverage issues (Fig. 7), and syndrome-specific information related (Fig. 8) issues were detected as primary reasons for the potential false negativity of NIPT.

Figure 1. Confined placental mosaicism (T22). Chromosome 22 trisomy detected in circulating DNA of pregnant woman (A), not confirmed in amniotic fluid based diagnostic analyses but confirmed in mosaic form in placenta (B – 1-2).

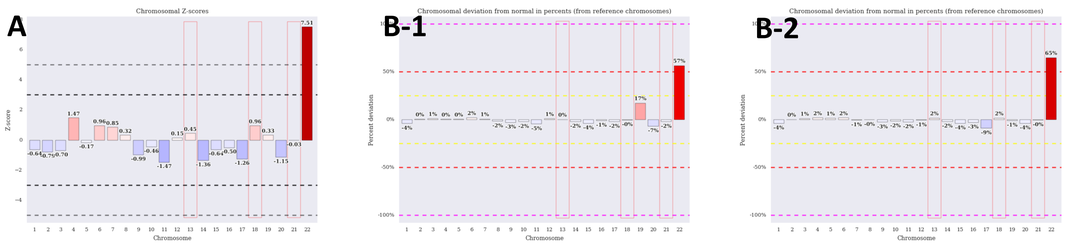


Figure 2. True fetal mosaicism (T18). Chromosome 18 trisomy not detected in maternal circulating DNA (A), detected with ultrasound and confirmed in amniotic fluid based diagnostic analyses and fetal tissue (B), subsequently detected in mosaic form showing decrease of T18 corresponding signal in placenta (C – 1-4). Fetal fraction with T18 at only 1.53%.

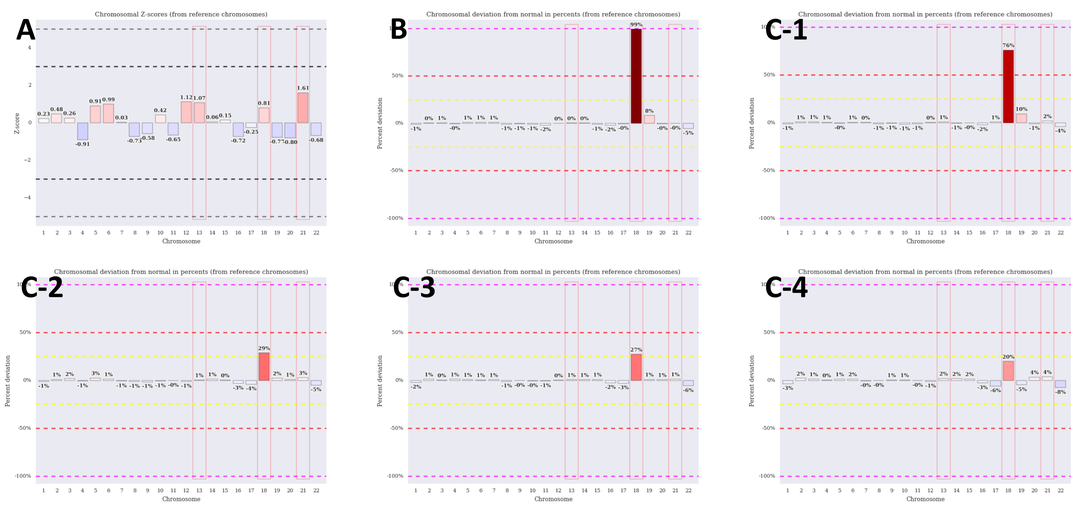


Figure 3. Maternal aberration in full and mosaic state. Detected high risk of Monosomy X as a consequence of maternal subchromosomal deletion on chrX (A – 1-2) and as a consequence of maternal mosaicism for monosomy X as detected during pregnancy (B – 1) as well as after delivery (B – 2), but not in placenta (B – 3).

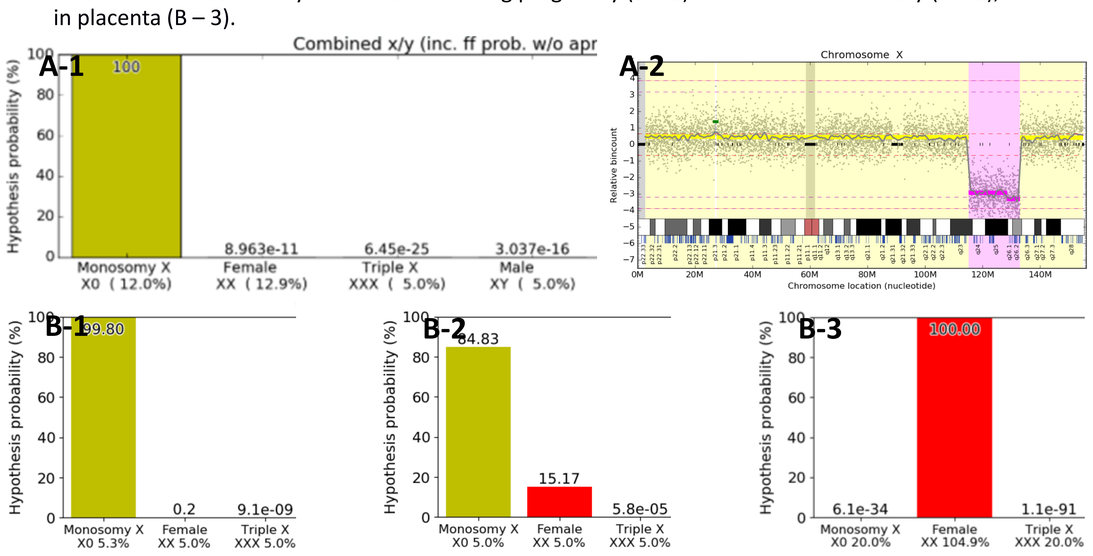


Figure 4. Precancerous maternal aberration (T8). Chromosome 8 trisomy detected in maternal circulating DNA with signal extremely above the limit for aberration or aneuploidy originated in fetus (placenta) visualized using Chromosomal Z scores (A) and CNV caller (B). T8 was confirmed with subsequent karyotyping, where fetal (placental) signal should not be present.

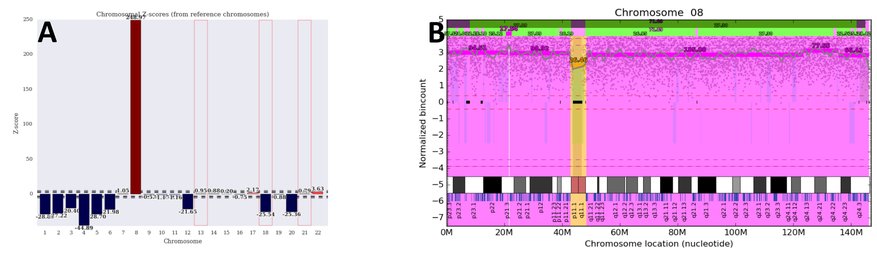


Figure 5. Incorrect anamenstic data and previous diagnostic karyotyping. Chromosome X aberration 46,XX,der(X)t(X;12)(q23;p11.1) detected as maternal duplication (chr12 – A) and deletion (chrX - B) during NIPT testing not recorded in patient anamnestic data with special information regarding „normal female karyotype“. Repeated karyotyping confirmed presence of translocation.

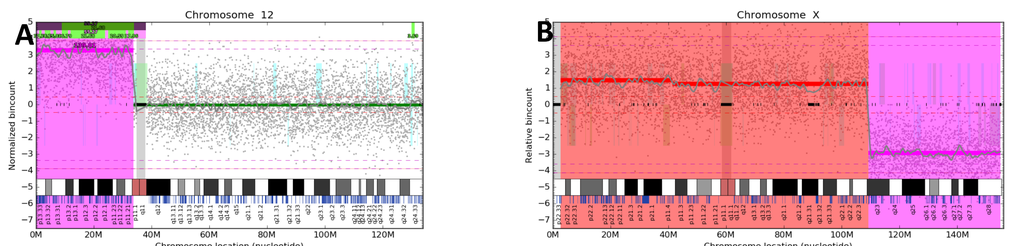


Figure 6. Gray zone result of detection of 22q11.2 deletion (DiGeorge syndrome). Deletion 22q11.2 detected repeatedly in circulating DNA with gray zone signal and so bellow limit for standard reporting (A – 1-2) and detected in analysis od DNA extracted from amniotic fluid (B).

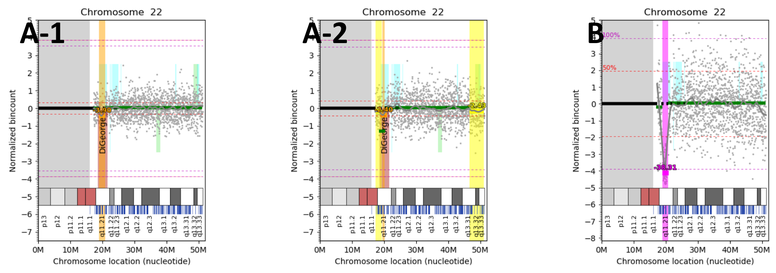
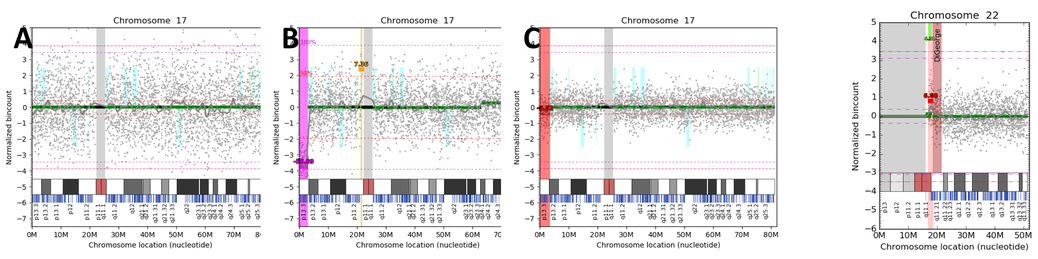


Figure 7. Insufficient coverage (basic test) of the region of interest (17p13.3) without detection in primary analysis (A). After delivery Miller-Dieker syndrome phenotype of newborn and diagnostic detection of the deletion (B). Repeated retrospective analysis of original plasma with higher coverage (advanced test) confirmed presence of the deletion (C).

Figure 8. Detection related to cat eye syndrome, caused by 22q11.1q11.21 tetraplication, with size 1.12 Mb detected at 10.8% fetal fraction)



Conclusions

False negative and false positive NIPT results are reported every day all over the world and need to be addressed with comprehensive supplementary diagnostic testing. Very likely, the „falseness“ of such results is caused by biological and technical limitations of samples and methods used in non-invasive prenatal testing, and principally, „falseness is false“.
Consensus and laboratory standards addressing procedures related to such „false“ results should be created and routinely used and international guidelines not only for additional laboratory procedures but also for clinical geneticist consultation should reflect this needs, too.

Samples and Methods

Samples

Circulating plasma based DNA samples from pregnant women with singleton pregnancies analyzed in routine Trisomy test screening were used in the study. In the case of false positive or false negative result, additional biological specimens (e.g. amniotic fluid, placenta, fetal tissue, maternal blood) were obtained from women before or after delivery. All the participating women were recruited at prenatal diagnostic centers and ob-gyn ambulances and clinics in Brno, Novy Jicin (both Czech Republic) and Bratislava (Slovakia) between July 2016 and June 2021. All the pregnant women gave their informed consent for inclusion before participation. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved on 30 June 2015 by the Ethics Committee of the Bratislava Self-Governing Region (03899/2015/HF).

Methods

Circulating plasma DNA was extracted using DNA Blood Mini Kit (Qiagen). Genomic libraries were prepared using TruSeq Nano LP Kit (Illumina). Sequencing was performed using Illumina NextSeq 500/550 platforms (Illumina) with 2x35 bp paired-end sequencing protocol. Sequencing data were demultiplexed, mapped to the human reference genome (hg19 and hg38) using the Bowtie2 algorithm. Fetal fractions were calculated from the Y chromosome in pregnancies carrying a male fetus and using the in-house Combo method for female fetuses. To determine samples that are of high risk for whole chromosomes aneuploidies method based on Z score calculations, further tuned and validated in house, was used (Chromosomal Z scores). Similarly, for detection of sex chromosomal aneuploidies in-house developed tool was used (XYzer). Additionally, for identification of subchromosomal aberrations, we grouped the reads per bin (20 kb bin size) and performed two-step normalization based on LOESS-based correction and PCA normalization. Finally, the signal was split into regions with equal level signals using the circular binary segmentation algorithm from the R package DNAcopy. For each detected CNV, the corresponding CNV fraction value was calculated and the result was visualized using an in-house tool (CNV caller). These figures are automatically generated for each chromosome, including X and Y.

Acknowledgements

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