FALSE POSITIVE TRISOMY 21 NIPT-RESULT DUE TO MATERNAL INHERITED CNV

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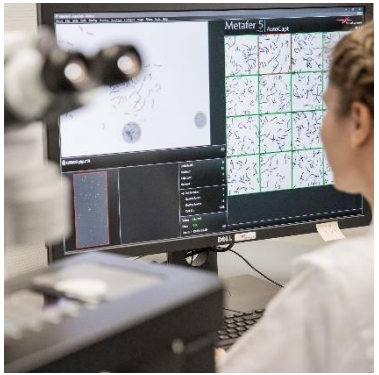

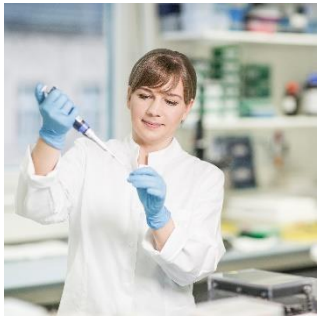

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Introduction

- Noninvasive prenatal testing (NIPT) is a well established option to screen with high specificity and sensitivity for fetal aneuploidies, such as trisomy 13, 18 and 21.
- NIPT is based on the analysis of cell free placental DNA (10% mainly from the cytotrophoblast) in a maternal DNA background (90%). No fetal DNA is analysed.
- Therefore, fetal/placental discrepancies are a common source for false positive and also for false negative results.
- In rare cases, maternal factors such as maternal mosaicism, maternal malignancy and maternal copy number variations (CNV) are also reasons for discrepancies between NIPT-results and true fetal karyotypes.
- Here we present a unique case of a maternal inherited CNV, resulting in reporting of a false positive trisomy 21.
- The fetus himself, showed an inconspiciuous fetal ultrasound and a normal male karyotype following conventional chromosome analysis (CA) of amniotic fluid cells.
- We report about the pregnancy management and follow analyses to get optimal information for genetic counselling and prognosis.

Material and Methods

- **Next Generation sequencing (NGS) of cfDNA**

The molecular genetic analysis of the cell-free fetal DNA (cfDNA) from the mother's blood was carried out by "Next Generation Sequencing" (NGS) using the Praenatest (LifeCodexx AG). For this purpose, cfDNA was extracted from the blood of the pregnant woman. A genomic library of these DNA fragments was generated followed by partial sequencing of the fragments. The quantification of the amount of DNA sequenced and the bioinformatic determination of the so-called z-score value using the PraenaTest® DAP.plus software (LifeCodexx AG). The z-score is used as a measure to differentiate between the existence of a trisomy and the normal number of two chromosomes.

- **Chromosome analysis (CA) from peripheral blood, amniotic fluid and placental cells**

Chromosome preparations were obtained from stimulated lymphocyte cultures (Lymphogrow medium, Cytocell) as well as long-term culture of placental chorion cells (AmnioGrow Plus, Cytogen; Bio-AMF™-2, BI Biological Industries) and GTG-banded after semi-automatic harvesting (Hanabi, ADS Biotec) following standard procedures. The preparations were evaluated using NEON software (Metasystems).

- **Fluorescence-in-situ-Hybridization (FISH) on metaphases and interphases**

For further clarification FISH analyses were performed with a whole chromosome painting probe (XL 21q22/XCP 21, Metasystems) on metaphases from stimulated lymphocytes cultures from heparinized peripheral blood of the parents and the child according to the standard protocol. In addition FISH analyses were performed on interphases nuclei from heparinized peripheral blood and cultured placenta chorion cells of the child with a locus-specific probe for the region 21q22 (DSCR4; XA 13/21, Metasystems).

- **array-CGH Analysis (aCGH)**

After extraction of genomic DNA (umbilical cord), an array CGH was performed using the SurePrint G3 Unrestricted HD-CGH microarray ISCA v2, 4x180k (Agilent Technologies, USA). This chip contains 180,000 60mer oligonucleotides and controls with a genomic resolution of ~ 25 kb in the backbone and <25 kb in the clinically relevant ISCA regions. After labeling, the patient's DNA was hybridized to the microarray together with a male reference DNA (Promega). Data was generated on an Innoscan 710 scanner (Innopsys). The evaluation was carried out using the software Agilent CytoGenomics software (Vers. 4.0.3; Agilent). The genome build GRCh37 [hg19] was used.

- **Quantitative real-time PCR (qPCR)**

To verify and further clarify the array-CGH finding, a region-specific segregation analysis was performed using the five amplicons LTN1 (21q21.2), BACH1 (21q21.2), GRIK1 (21q21.2), CLDN8 (21q22.11) and TIAM1 (21q22.11). The amplicons detect fragments in the chromosomal bands 21q21.2 to 21q22.11. Amplicons for the factor VIII gene and albumin were used as a control and calibrator. The analysis was carried out on the ViiA 7 real-time PCR system from 4 Applied Biosystems. The genome build GRCh37 [hg19] was used.

Results

- NIPT indicated a fetal trisomy 21 (Data not shown)
- in contrast, ultrasound examination and amniocentesis showed normal results (Data not shown)
- reanalysis of NIPT by WISECONDOR algorithm showed a 10 Mb gain on chromosome 21 (Data not shown).
  - The parents declined further diagnostics until birth of an phenotypically unremarkable boy.
- postpartum CA and FISH analyses from peripheral blood (umbilical cord) and placental cells of the child (**Figure 1-2**) as well as CA and FISH analyses from peripheral blood of the parents resulted in normal karyotypes (Data not shown)
- in contrast, aCGH confirmed a 1,44 Mb gain in the chromosomal region 21q21.3q22.11 (**Figure 3**).
- because no comparable cases were described in literature or databases → aberration was classified as variant of unclear significance (VUS)
- qPCR analyses confirmed the gain and showed it was maternal inherited (Data not shown)

➢ therefore, **aberration was reclassified as a familial benign Copy number variant (CNV)**

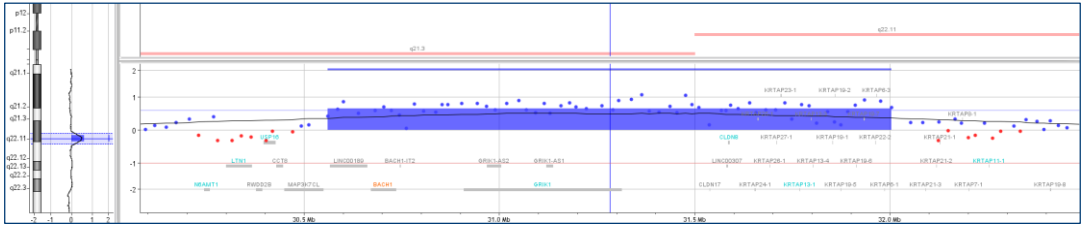

Figure 3: Schematic representation of aCGH result showed a gain in 21q21.3q22.11 in peripheral blood (umbilical cord)

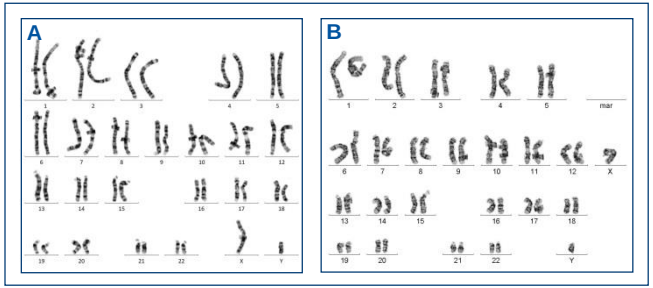

Figure 1: GTG karyotyping revealed a normal karyotype 46,XY [ISCN 2016] in **A** – peripheral blood (umbilical cord) and **B** – placental chorion cells

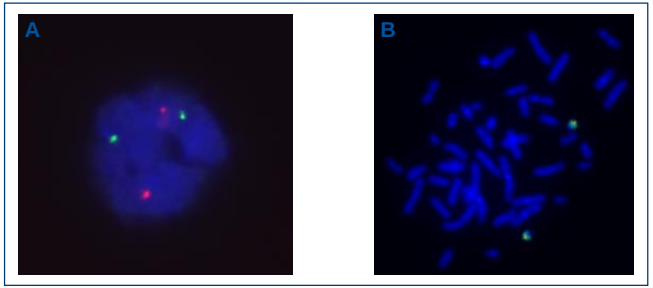

Figure 2: FISH analyses revealed normal signals **A** – on interphase nuclei from heparinized peripheral blood with a locus-specific probe for the region 21q22 (DSCR4) and **B** – on metaphases from stimulated lymphocyte cultures from heparinized peripheral blood with the whole chromosome painting probe XL 21q22/XCP 21.

Conclusion

- To the best of our knowledge, this is the first report of a unique benign maternal chromosome 21 CNV leading to a false positive trisomy 21 NIPT result.
- This case confirms the importance of critical analyses of abnormal NIPT results, especially in genotype/phenotype discrepancies (abnormal genotype/normal phenotype or vice versa).
- Fetal/placental mosaicism is a common source for false positive and false negative results. Such discrepancies can be ruled out by amniocentesis.
- Nevertheless, maternal factors are also a rare source for discrepancies between NIPT results and true fetal karyotypes. In these cases, maternal genetic analysis is necessary to get additional information for adequate genetic counselling.
- Modern algorithms discriminating placental and maternal DNA fragments may give helpful information to detect maternal mosaicism and maternal CNVs in order to optimize NIPT.
- In summary, our case clearly demonstrates the importance of prenatal ultrasound analyses and the need for critical reflection of NIPT results, especially in fetuses with genotype/phenotype discrepancies.

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