

CASE REPORT

Failure of NIPT to detect constitutional chromoanasythesis involving chromosome 21 in a case of fetal hydrops—A case report

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Abstract

We report a case of a de novo ring 21 complex chromosomal rearrangement in a fetus presenting with hydrops. Noninvasive prenatal testing (NIPT) failed to detect the imbalance. This case highlights the need to understand the various limitations and strengths of NIPT technology when counseling patients.

KEYWORDS

chromoanasythesis, chromosome 21, complex chromosomal rearrangement, fetal hydrops, noninvasive prenatal testing, prenatal diagnosis

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We report a prenatal case of a de novo ring 21 complex chromosomal rearrangement (CCR) detected by microarray, consistent with constitutional chromoanasythesis of maternal chromosomal origin, in which noninvasive prenatal testing (NIPT) failed to detect an imbalance. Informed consent was obtained from the patient for postmortem evaluation and cytogenetic testing, and for the use of all related data collected in this publication. Our patient, a healthy 38-year-old, G4, T2, P1, SA1, L2, had a positive first-trimester screen for trisomy 21 (T21) at gestational age (GA) 12 weeks and 2 days. The calculated risk for T21 was 1/60. The nuchal translucency (NT) was measured at 3.10 mm. She subsequently underwent HarmonyTM NIPT with a low-risk screening result. No further invasive testing was performed.

At GA of 19 weeks and 0 days, she presented for her second trimester detailed fetal anatomical ultrasound. The fetus was found to have hydrops with significant nuchal edema, skin edema, and ascites. The lungs showed bilateral atelectasis and moderate pleural effusions. Ultrasound imaging of the heart showed no congenital anomalies. Doppler measurements of

the fetal middle cerebral arteries (MCA) were unremarkable, and growth parameters were within normal limits. The initial management of the patient included fetal pleurocentesis and amniocentesis. The fetal pleural effusions reaccumulated and the patient elected to undergo pregnancy termination.

Rapid aneuploidy detection (RAD) was performed on DNA extracted from uncultured amniocytes using quantitative fluorescence polymerase chain reaction (QF-PCR) with a panel of PCR primers specific to chromosomes 13, 18, 21, X, and Y. This analysis demonstrated a normal diploid complement for chromosomes 13 and 18 and showed a sex chromosome amplification pattern consistent with a male fetus. However, analysis was uninformative for chromosome 21 (Figure 1A). One marker was disomic, two markers were trisomic, and four markers were uninformative. Array comparative genomic hybridization (aCGH) demonstrated a complex pattern of gains and losses along chromosome 21, with five large regions of pathogenic copy number changes (CNCs), interspersed with normal copy number (Figure 1B). Region one was a 5.08 Mb copy number gain in region 21q11.2q21.1; region two was a 1.31 Mb copy number gain in region 21q21.1q21.2; region three was a 5.62 Mb copy

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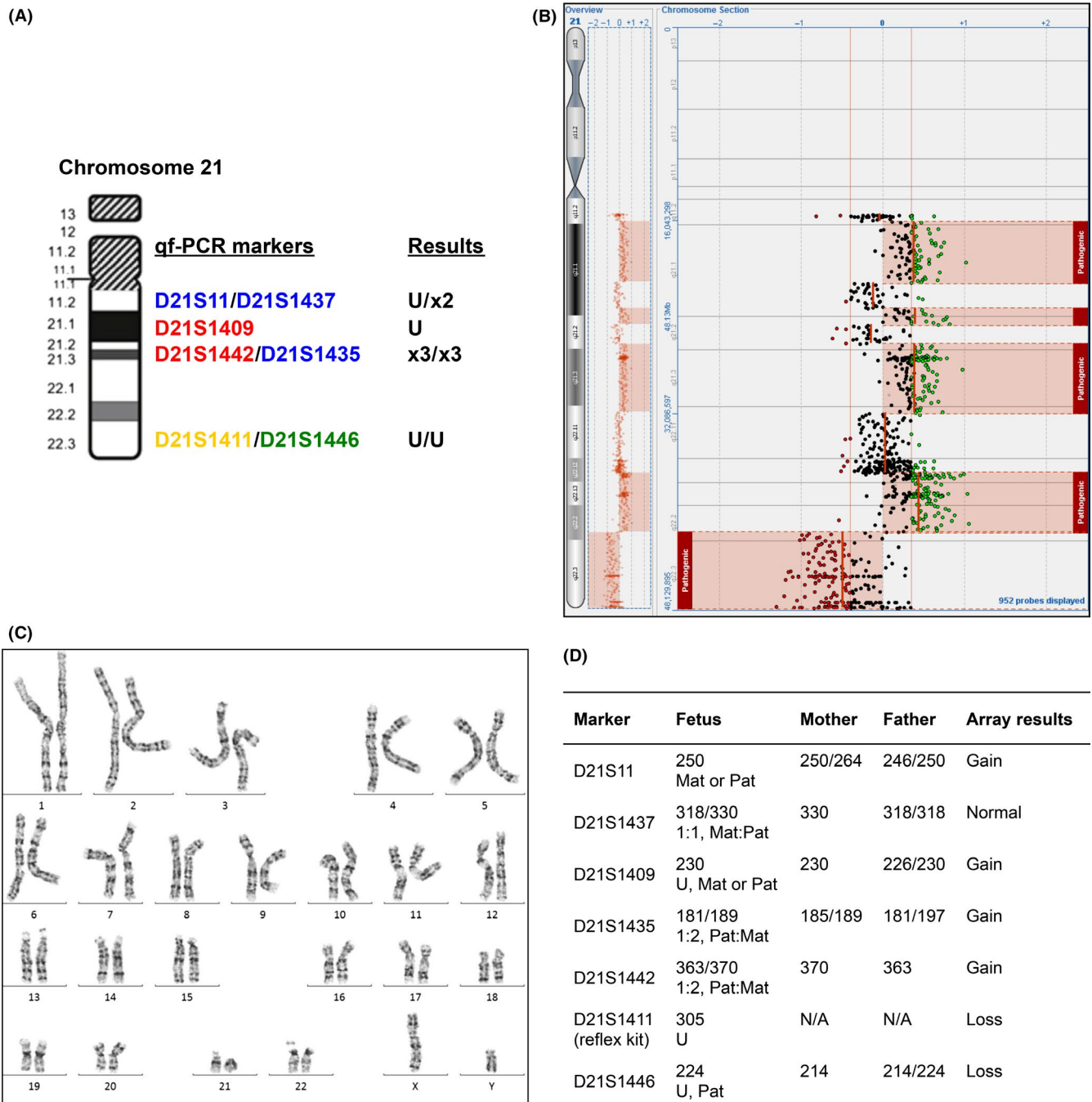


FIGURE 1 A, Rapid aneuploidy detection (RAD) testing of chromosome 21 showed uninformative markers (u) and areas of duplication and normal copy number. B, Array comparative genomic hybridization (aCGH) results showed multiple areas of copy number change along chromosome 21. C, Karyotype showed 46,XY,r(21)(p11.2q22). One copy of chromosome 21 was a complex ring structure. D, Satellite analysis showed the complex ring 21 chromosome was maternally derived

number gain in region 21q21.2q22.11; region four was a 4.89 Mb copy number gain in region 21q22.12q22.2; and region five was a 6.23 Mb terminal copy number loss in chromosome region 21q22.2q22.3. G-banded chromosome analysis was performed on cultured amniotic fluid. All mitoses examined had a structurally abnormal ring chromosome 21, 46,XY,r(21)(p11.2q22) (Figure 1C). Taken together with the aCGH data, this complex ring chromosome had

duplications totaling nearly 17 Mb along the length of its long arm, and a terminal deletion of over 6 Mb. The type of complex pattern of gains and losses within a single chromosome is suggestive of chromoanasythesis. Parental analysis by array CGH and karyotype was normal. Microsatellite genotyping (Elucigene QST[®]R, v2, Hologic Gen-Probe Inc) of parental and fetal DNA determined that the complex de novo ring 21 chromosome present in the fetus was derived from

the maternal chromosome 21 (Figure 1D). On autopsy, there was a limited examination with a few features consistent with trisomy 21 and no obvious features of monosomy 21.

Chromoaniasynthesis is a type of complex chromosomal rearrangement (CCR) confined to one chromosome or locus leading to deletions, duplications, and triplications along a single chromosome.¹⁻⁵ Constitutional chromoaniasynthesis involving up to 33 breakpoints has been described in the literature, likely the result of fork stalling and template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR).^{1,3} With the rapid advancement of chromosomal microarrays and whole genome sequencing technologies, the molecular mechanisms underpinning these complex structural rearrangements are just beginning to be understood. The incidence of these events in constitutional chromosomal rearrangements is unknown. We found no other reports of constitutional chromoaniasynthesis involving chromosome 21.

In addition to a novel chromoaniasynthesis event, our case also demonstrates that NIPT by HarmonyTM was unable to detect the chromosome 21-duplicated regions in this patient. While the various NIPT testing platforms utilize different proprietary technologies and algorithms to determine common chromosomal aneuploidy risk, they are all based on the ability to detect fragments of cell-free fetal DNA (cffDNA) from the cytotrophoblast cells of the placenta in the maternal circulation.⁶ The fundamental principle of such testing is to determine whether there is an excess number of fetal chromosomes or fragments circulating in the maternal plasma. There are several different sequencing technologies used in NIPT including random whole genome or massively parallel sequencing, targeted, or chromosome-selective sequencing and single-nucleotide polymorphism-based sequencing.⁶ There are also different bioinformatics algorithms in use to determine the risk of aneuploidy. The HarmonyTM NIPT test employs a targeted sequencing approach of cell-free DNA (cfDNA) fragments to selected chromosomal regions (DANSRTM technology) and uses a proprietary algorithm (FORTETM) to determine the risk of fetal trisomy 21, 18, and 13.⁷ The assay also makes use of single-nucleotide polymorphisms and microarray technology in the determination of the fetal cfDNA fraction and to distinguish the maternal and fetal fractions. Therefore, it is surprising that this test failed to detect nearly 17 Mb of duplicated region along chromosome 21. The long arm of chromosome 21 is a total of 29 Mb in length, and the duplicated region accounts for approximately 58% of the total chromosomal length.

While NIPT is reported to have a high sensitivity and specificity for trisomy 21, the test remains a screening test and not a diagnostic test. False positive (FP) and false negative (FN) results, in addition to test failure, are possible. The most common cause of a false negative test result is a low cffDNA fraction in the circulating maternal blood.⁸ Previous

studies have reported that a fetal DNA fraction as low as 3%-4% can increase the chance of a FN result.⁸ NIPT laboratories typically have a minimum fetal fraction requirement and a fraction below this cutoff is not used to generate a result. The fetal fraction is affected by the gestational age of the fetus, maternal body mass index (BMI), and placental size and function.⁸

Discordant NIPT results have been published in the literature. FP or FN results for fetal aneuploidies may be due to biological factors surrounding fetal and placental development. Fetal cfDNA originates from the cytotrophoblast cells of the placenta, which forms the outer layer of the placenta, whereas the fetus arises from mesenchymal core, which is derived from the extra-embryonic mesoderm of the blastocyst.⁹ Van Opstal et al⁹ have extrapolated the historical rate of FN results from short-term chorionic villi cultures derived from cytotrophoblast cells to NIPT. In their study, the FN rate for NIPT in a patient population at high risk of fetal aneuploidies was estimated to be 0.2%. Amsterdam et al¹⁰ have published a paper that trisomy 21 due to isochromosome 21q is overrepresented among FN cfDNA prenatal screening results. These authors estimated that 28% of cases of T21 with FN NIPT testing are due to 21q;21q rearrangement compared with 2% of live-born children with T21.¹⁰ The biological mechanism for these FN results may be due to postzygotic origins of the 21q;21q rearrangement, leading to true fetal aneuploidy with a normal placenta or confined placental mosaicism not detected by NIPT testing.¹⁰ Postzygotic chromosomal rearrangements may result in true fetal aneuploidies with normal placental tissue leading to FN NIPT results.

In our case, the chromosomal 21 segmental imbalances were not detected using the HarmonyTM NIPT platform with a result of a low-risk NIPT screen for T21 despite the presence of multiple areas of duplication on the ring chromosome. Harmony NIPT testing uses targeted sequencing, and it is possible that the sequenced areas did not involve the areas of imbalance seen in our case thus resulting in a FN result. It is possible that another method of NIPT testing such as random whole genome or massively parallel sequencing or SNP-based sequencing would have been able to detect the areas of imbalance on the ring 21 chromosome. Another possibility for the failure of NIPT in this case is the CCR may have arisen postzygotically in the mesenchymal core. In this case, NIPT would not detect the areas of imbalance on the ring 21 chromosome since the ring 21 chromosome would not be found in the placental tissue and therefore, would not be found in the cffDNA. Unfortunately, we were unable to obtain placental tissue for array or karyotype analysis.

This case highlights the importance of understanding the limitations of the various NIPT technologies in detecting chromosomal imbalances when counseling patients. Our patient had a positive first-trimester screen for trisomy 21 and felt reassured after her low-risk NIPT result. Although NIPT

is a good test for the detection of common fetal aneuploidies, this case shows that it has its limitations and that NIPT is a screening test and not a replacement for invasive diagnostic testing for the diagnosis of chromosomal abnormalities.

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CONFLICT OF INTEREST

None Declared.

AUTHOR CONTRIBUTIONS

Kathleen Bone and Judy Chernos were involved in cytogenetics data analysis and interpretation. Melissa Jean MacPherson and Julie Lauzon were involved in care and clinical assessment of patient and pregnancy. Kathleen Bone and Melissa Jean MacPherson collected clinical and laboratory data, drafted the manuscript and figures, and revised the manuscript. Judy Chernos and Julie Lauzon reviewed and revised the manuscript.

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