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Case Report

Screening for 22q11.2 deletion syndrome by two non-invasive prenatal testing methodologies: A case with discordant results

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ABSTRACT

Objective: Non-invasive prenatal testing (NIPT) through the analysis of cell-free DNA in maternal plasma has bee expanded to include clinically-relevant microdeletions such as the 22q11.2 deletion syndrome (22q11.2DS).

Case report: We present a pregnancy where the fetus was affected with 22q11.2DS based on chromosome microarray analysis. Discordant results were obtained through two different NIPT methodologies. The pregnancy was identified as high risk by a SNP-based approach but low risk using a genome-wide counting methodology. A review of the technical methods used for these tests provides insight into why they may provide conflicting results and emphasizes the importance of chromosome microarray studies for diagnostic confirmation and defining the deletion.

Conclusion: Currently available NIPT for 22q11.2DS use different technologies that are not equivalent. The genome-wide counting methodology has the potential to detect deletions outside the critical 22q11.2 A–D region but current data suggests it may have a lower sensitivity for deletions within the critical region.

Introduction

The 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge or velocardiofacial syndrome, results from submicroscopic deletion of a defined part of chromosomal band 22q11.2 known as the A–D region [1]. With a reported population-wide frequency of 1 in 3000–6000 live births and an estimated prenatal prevalence of 1 in 1000 pregnancies [2–4], 22q11.2DS is the most common microdeletion syndrome. 22q11.2 DS has a heterogeneous clinical presentation that can affect multiple organ systems with varying degrees of severity. Common features include conotruncal heart anomalies, palatal abnormalities, characteristic facial dysmorphic features, immune deficiency, congenital hypocalcemia, urogenital abnormalities, cognitive impairment and psychiatric disorders [1].

Traditional prenatal screening modalities such as serum markers do not reliably identify pregnancies that are at an increased risk for 22q11.2DS. Although routine ultrasound examination may detect some cases with cardiovascular or other abnormalities associated with 22q11.2DS, many will be undetected [5,6]. Furthermore, given that the majority of patients (~90%) have deletions that are de novo in origin [7] and that the risk for microdeletions is independent of maternal age [1], family history and maternal age are poor predictors of risk. When 22q11.2DS is suspected prenatally, definitive diagnosis requires chromosomal microarray (CMA) performed on samples following invasive testing by amniocentesis or chorionic villus sampling (CVS). Use of fluorescence in situ hybridization (FISH) with a probe specific to 22q11.2 can also be used although this is considered sub-optimal for the detection of all relevant deletions [8]. Because of the invasive nature of amniocentesis and CVS, this follow-up testing may not be acceptable to all patients.

The availability of non-invasive prenatal testing (NIPT) through the analysis of cell-free DNA (cfDNA) in maternal plasma offers alternative approaches to identifying pregnancies at high risk for

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sub-chromosomal abnormalities. Advances in NIPT technology have allowed coverage of a set of microdeletions with high penetrance and severe phenotype, including 22q11.2DS [3,9,10]. Multiple NIPT methodologies are commercially available to screen for fetal 22q11.2DS. These include methods that use genome-wide sequencing to count the relative number of maternal plasma DNA fragments for various regions throughout the genome including at 22q11.2 ("genome-wide counting") [11,12], a microarray based approach that similarly quantifies plasma DNA fragments but limits analysis to targeted regions of interest ("targeted counting") [9] and a sequencing-based approach that uses single nucleotide polymorphisms (SNP) to assess the fetal and maternal copy number at specific regions of interest ("SNP-based") [3].

This Case report demonstrates differences in the performance of some of these tests.

Case presentation

A 39-year-old healthy woman with no significant history of genetic disorders and unremarkable pregnancy course received amniocentesis at 18 weeks gestational age and a whole-genome CMA was performed on the uncultured amniocytes. This showed the presence of a chromosome 22 deletion involving base-pairs 18,661,749–21,808,980 (hg19) i.e. a 3.15 Mb deletion at the 22q11.21 A–D region, consistent with a clinical diagnosis of 22q11.2DS. A level II ultrasound examination was then performed at 21 weeks revealing a singleton fetus with multiple abnormalities including Tetralogy of Fallot, left renal pelviectasis, relative polyhydramnios, and borderline brachycephaly. The woman subsequently opted to terminate the pregnancy that was completed without complications.

At 23 weeks, prior to the termination, the physician obtained permission from the patient to test a blood sample for fetal 22q11.2 deletion by NIPT. The purpose of this additional analysis was to gather more information about non-invasive 22q11.2 deletion screening and was not intended for decision-making. This testing was carried out using two laboratories; one using the genome-wide counting approach and the second utilizing the SNP-based methodology. Neither laboratory was aware of the fact that there was a confirmed 22q11.2 deletion that had been established through CMA. The genome-wide counting method indicated that the patient was "low risk" for the 22q11.2 deletion and did not report a fetal fraction estimate. The laboratory using the SNP-based methodology reported the patient as "high risk" for the 22q11.2 deletion, with a risk score of 1/19, with a fetal fraction 7.4%.

Discussion

Several validation studies have demonstrated the potential of NIPT as a prenatal screening modality for 22q11.2DS [3,4,9–11,13] and, in addition, several studies have documented clinical experience in the identification of affected pregnancies [4,10,13].

This report describes a case with discordant results for the detection of the 22q11.2 deletion by two different NIPT methodologies. The patient was identified as high risk for the 22q11.2 deletion by the targeted SNP-based methodology [4,13]. In contrast, she received a low-risk call for the presence of the deletion by the genome-wide counting methodology. While this represents a single case evaluated for discrepancy between two laboratories and does not therefore reflect a rigorous comparison of tests, it is instructive to consider why different NIPT technologies might yield contradictory results.

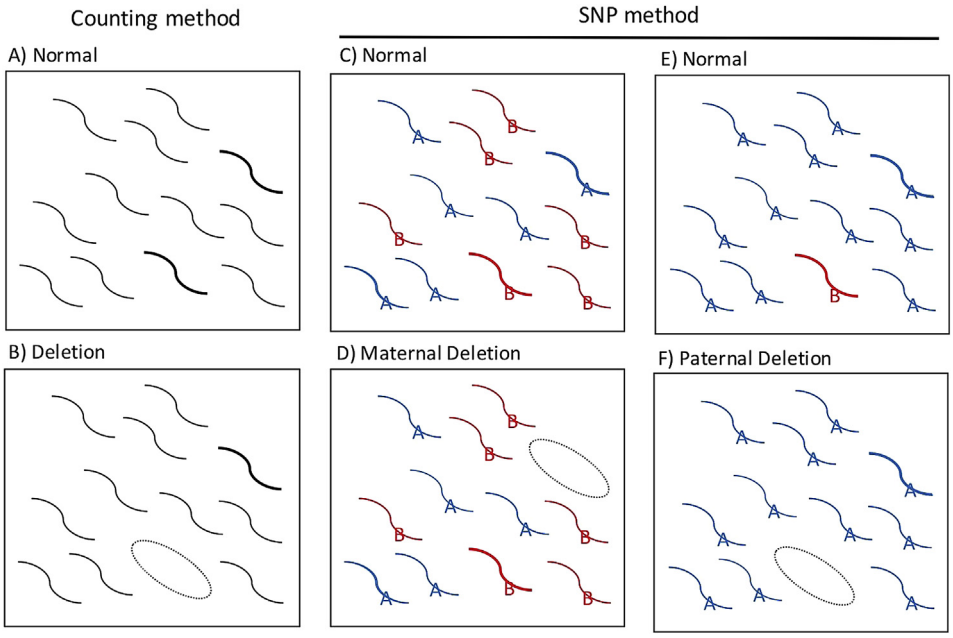

Fig. 1. Visual explanation of how the two methods identify deletions. The cartoons show how the two methods are able to detect deletions. In each box, the wavy line represents cfDNA from the 22q11.2 region; the thin wavy line is cfDNA from the mother, and the bold wavy line is cfDNA from the fetus. The examples depict cases with a fetal fraction of ~16%. The left two boxes (A – normal, B – deletion) show how the counting method identifies a deletion by detecting the ~8% drop in cfDNA over the deletion region. Boxes C–F illustrate how the SNP method detects a deletion by analyzing the maternal and fetal SNP profiles. Multiple SNP evaluations are carried out within regions of interest with some SNP combinations informative for detecting a deletion. The middle two boxes (C – normal, D – maternal deletion) show cfDNA from an informative SNP combination where the mother and fetus are heterozygous (AB), and identification of the deletion is based on detection of the ~16% drop in one of the maternally inherited alleles. The right two boxes (E – normal, F – paternal deletion) show cfDNA from a locus where the mother is homozygous (AA) and the fetus is heterozygous (AB), and identification of the deletion is based on recognizing the absence of the paternally inherited allele.

Fig. 1 summarizes the underlying principles of the two NIPT approaches used in this case. Genome-wide counting interrogates the entire genome for regions where there is an apparent excess or deficiency in the relative number of DNA fragments and does not necessarily distinguish between those which are present in apoptotic maternal cells, those that present in trophoblasts ("fetal"), or both. This method will potentially identify rare 22q11.2 deletions outside the A–D critical region that are associated with variable phenotypes that are not considered part of 22q11.2DS [14]. Currently available published clinical experience of screening for 22q11.2DS by genome-wide counting methods suggests that even small deletions present in the maternal genome can be identified at a frequency similar to that expected [10]. However, the frequency of detected de novo fetal 22q11.2 deletions identified (12 in 175, 393 or approximately 1/15,000) would seem to be substantially less than that expected (approximately 1–2 per thousand) [2–4]. This is probably because microdeletions make up a relatively small fraction of the genome, about 0.1% in the case of the 22q11.2 A–D deletion, thus this method requires a high depth of sequencing, especially when fetal fraction is low [12].

Targeted approaches can overcome this limitation by enriching for specific DNA sequences, allowing a deeper analysis of copy number in commonly deleted or duplicated regions. The SNP-based approach targets a 2.9 Mb region in the 22q11.2 region. This method has been validated to detect the A–D deletion, which makes up 85% of 22q11.2 deletions, with high sensitivity [3]; it has not been validated to detect the nested and distal deletions that make up 15% of cases. Rare deletions that substantially include the target region but extend beyond the A-D region should be detected, but the boundaries of the deleted segment will not be defined. For the various deletions within 22q11.2 A–D region, there appears to be differences in phenotype or penetrance depending on the breakpoints [15]. These considerations underscore the importance of CMA, instead of FISH, as the optimal follow-up test for NIPT 22q11.2DS screen-positive pregnancies [1].

Prenatal screening for 22q11.2DS through NIPT, early referral for fetal ultrasound, and subsequent confirmatory diagnostic testing offers the opportunity for intervention in cases with a severe phenotype. For ongoing pregnancies, early diagnosis can result in improved postnatal care. For example, congenital heart diseases lead to approximately 87% of deaths associated with 22q11.2 DS, most of which occur in the first year of life [1]. Prompt cardiac care at birth positively impacts the clinical outcome of cases with the 22q11.2 deletion by reducing mortality by up to 12% [15].

Clinicians providing screening for 22q11.DS need to be aware of the differences in the tests available, the highly variable phenotypes that can be present even for cases with identical deletions, and the appropriate follow-up testing for test-positive patients.

Disclosures

Peter Benn is a paid consult and holds stock options in Natera, Inc. There are no other conflicts of interest.

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