OBSTETRICS

Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism–based noninvasive prenatal test

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OBJECTIVE: We sought to determine the ability of single-nucleotide polymorphism–based noninvasive prenatal testing (NIPT) to identify triploid, unrecognized twin, and vanishing twin pregnancies.

STUDY DESIGN: The study included 30,795 consecutive reported clinical cases received for NIPT for fetal whole-chromosome aneuploidies; known multiple gestations were excluded. Cell-free DNA was isolated from maternal blood samples, amplified via 19,488-plex polymerase chain reaction, and sequenced. Sequencing results were analyzed to determine fetal chromosome copy number and to identify the presence of additional fetal haplotypes.

RESULTS: Additional fetal haplotypes, indicative of fetal triploidy, vanishing twin, or undetected twin pregnancy, were identified in 130 (0.42%) cases. Clinical confirmation (karyotype for singleton pregnancies, ultrasound for multifetal pregnancies) was available for 58.5% (76/130) of cases. Of the 76 cases with confirmation, 42.1% were vanishing twin, 48.7% were viable twin, 5.3% were diandric triploids, and 3.9% were nontriploid pregnancies that lacked evidence of co-twin demise. One pregnancy had other indications suggesting triploidy but lacked karyotype confirmation. Of the 5 vanishing twin cases with a known date of demise, 100% of losses occurred in the first trimester; up to 8 weeks elapsed between loss and detection by NIPT.

CONCLUSION: This single-nucleotide polymorphism–based NIPT successfully identified vanished twin, previously unrecognized twin, and triploid pregnancies. As vanishing twins are more likely to be aneuploid, and undetected residual cell-free DNA could bias NIPT results, the ability of this method to identify additional fetal haplotypes is expected to result in fewer false-positive calls and prevent incorrect fetal sex calls.

Key words: noninvasive prenatal testing, single-nucleotide polymorphism, triploidy, vanishing twin

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The recent introduction of cell-free DNA (cfDNA)-based noninvasive prenatal testing (NIPT) has offered pregnant women a more accurate method for detecting fetal aneuploidies than traditional serum screening methods.[1-12] NIPT noninvasively determines fetal chromosome copy number by interrogating cfDNA isolated from maternal plasma, with the fetus contributing anywhere from <2% to >30% of the total cfDNA.[3,7,13] Other NIPT approaches use quantitative "counting" methods where fetal chromosome copy number is determined by comparing the absolute number of sequence reads from the chromosome(s) of interest (eg, chromosome 21) to reference chromosome(s), and inferring fetal trisomy when this ratio is above a predetermined threshold. This approach cannot determine the source of DNA (fetal or maternal) and is therefore unable to detect additional fetal haplotypes associated with triploidy or vanishing twins. Vanishing twins were reported to account for 15% of false positives in a

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recent counting-based NIPT study.[14] This likely results in unnecessary invasive prenatal testing. A more recent approach using a single-nucleotide polymorphism (SNP)-based method along with sophisticated informatics can resolve this potential source of false-positive results. This approach identifies the presence of additional fetal haplotypes, indicative of a triploid or dizygotic multifetal pregnancy, and determines parental origin.[10,12]

Using the SNP-based approach, the prevalence of cases found to have additional fetal haplotypes within 30,795 consecutive cases undergoing routine clinical NIPT was determined, and is reported here. Clinical follow-up of these cases is also described.

Materials and Methods

Patients

The current study included all samples from participating centers received for commercial testing from March 1, through Nov. 30, 2013, that received an NIPT result. This study received a notification of exempt determination from an institutional review board (Ethical and Independent Review Services, no. 14064-01). All samples were analyzed at Natera's Clinical Laboratory Improvement Act–certified and College of American Pathologists–accredited laboratory in San Carlos, CA. Analysis was performed for all samples on chromosomes 13, 18, 21, X, and Y, and included detection of trisomy 21, trisomy 18, trisomy 13, monosomy X, sex chromosome abnormalities (47,XXX/XXY/XYY), fetal sex, and additional fetal haplotypes.

Sample collection and NIPT

Maternal blood samples (>13 mL) were collected in Streck (Omaha, NE) blood collection tubes and processed at Natera (San Carlos, CA) within 6 days of collection. All samples were accompanied by a requisition form from the ordering clinician, and included the following patient information: gestational age, maternal date of birth, maternal weight, whether it was a multigestation pregnancy, and whether a paternal buccal swab was included. Ordering clinicians determined indication(s) for testing. Cases accepted for analysis were indicated as singleton pregnancies by ordering clinicians. Results were reported directly to the ordering clinician or distribution partners.

Samples were considered outside of the specifications for testing and were not analyzed if there was insufficient blood volume or the wrong tube was used, the sample was damaged, the sample was received at the laboratory >6 days after collection, the gestational age was <9 weeks, the patient used an egg donor, or the patient had a confirmed multiple gestation.[15] Testing was performed on all samples with sufficient blood volume (>13 mL) as described previously using validated laboratory methodologies (cfDNA isolation, polymerase chain reaction amplification targeting 19,488 SNPs, high-throughput sequencing, and analysis using the Next-generation Aneuploidy Test Using SNPs [NATUS] algorithm).[9-12,15] Samples were subject to a stringent set of quality-control metrics[9-13,15] before reports were sent to ordering clinicians.

The NATUS algorithm incorporates parental genotypic information, uses numerous quality control metrics, and determines a sample-specific accuracy for each interrogated chromosome.[9-12,15] Briefly, the algorithm considers parental genotypic information, crossover frequency data, and possible fetal chromosome copy numbers (monosomy/disomy/trisomy) at 19,488 evaluated polymorphic loci. By comparing the observed fetal allele distributions from the sequencing data to the predicted distributions, the algorithm determines the fetal ploidy state with the maximum likelihood for each interrogated chromosome; this maximum likelihood probability is incorporated into a risk score for reporting purposes.[15] The NATUS algorithm is currently only validated to call aneuploidy in singleton gestations. However, the algorithm is able to determine when cfDNA sequencing results do not match the modeled fetal copy numbers with a high likelihood, and can identify the presence of additional fetal haplotypes that indicate either fetal triploidy or the presence of an undetected dizygotic multiple gestation. The presence of an additional fetal haplotype was identified when all tested chromosomes failed to match the disomy hypothesis, and when the additional haplotype was apparent from allele distributions. At this time, the algorithm cannot distinguish dizygotic twin gestations from triploidy pregnancies due to similar allele distributions (Figure 1); therefore these are reported as a single call. Specifically, in a euploid singleton pregnancy, where the maternal alleles are AA (with dimorphic alleles arbitrarily labeled as A and B), the 2 expected fetal genotypes include AA and AB. By contrast, in dizygotic twin and triploid pregnancies where the maternal alleles are AA, there are 3 expected fetal genotypes for both triploid (AAA, AAB, ABB) (Figure 1, A) and dizygotic twin (AA/AA, AA/AB, AB/AB) (Figure 1, B) pregnancies. This results in equivalent B allele distributions (0, 1, or 2 B alleles), and very similar A allele distributions in triploid (1, 2, or 3) and dizygotic twin (2, 3, or 4) pregnancies.

For cases with an identified additional fetal haplotype, a report was sent to the ordering clinician or laboratory indicating that the results were consistent with a possible triploid or vanishing twin pregnancy, and recommending follow-up counseling and testing; after report delivery, a Natera genetic counselor contacted the ordering clinician/provider to answer questions related to the NIPT findings.

Clinical outcomes

Follow-up information on cases identified with an additional fetal haplotype was requested by telephone at regular intervals from ordering clinicians and partner laboratories. All information detailing ultrasound findings and pregnancy outcomes were recorded in the laboratory follow-up database. Follow-up information directly reported to Natera by providers was also recorded. Multifetal pregnancies were confirmed by ultrasound, which is consistent with how they are clinically diagnosed in practice. Cases were categorized as follows: (1) "confirmed vanishing twin pregnancy" if ultrasound detected a second empty sac or second sac containing a deceased fetus;