Objective: To determine the ability of single-nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) to identify unrecognized vanishing twin pregnancies that could otherwise confound NIPT results.

Methods: The study included 30,795 consecutive reported clinical cases received for NIPT for fetal aneuploidies as well as triploidy and multiple gestations were excluded. Cell-free DNA was isolated from maternal blood samples, amplified using a 19,488plex PCR approach, and sequenced. Two possible fetal genotypes AA, AB, and BB were used to determine if the fetal cfDNA reads were in the maternal background or if there was a maternal, fetal, or mixed result. Based on these calculations, the unique ability of this method to identify additional fetal haplotypes has the potential to decrease the false positive rate. As triploidy has substantial clinical implications for patients, including the risk for gestational trophoblastic neoplasia, SNP-based NIPT may impact care beyond aneuploidy detection.

Results: Additional fetal haplotypes, indicative of fetal triploidy, vanishing twin, or undetected twin pregnancy, were identified among 11 of 13 (84.6%) cases. The results were also validated using the 2nd and 3rd trimester NIPT sequencing method. There were 32 (42.1%) cases where the fetal cfDNA reads were confirmed as AA/BB or BB/BB, which were triploid cases. We also validated the results using 3rd trimester NIPT sequencing method. The method has been validated for detection of various whole chromosome aneuploidies as well as identification of cases with additional fetal haplotypes (twins/triploidy). Based on the results reported here, fetal cfDNA from a vanished twin was detectable for up to 8 weeks following co-twin demise. This method has been validated for detection of various whole chromosome aneuploidies as well as identification of cases with additional fetal haplotypes (twins/triploidy). Based on the results reported here, fetal cfDNA from a vanished twin was detectable for up to 8 weeks following co-twin demise. This method has been validated for detection of various whole chromosome aneuploidies as well as identification of cases with additional fetal haplotypes (twins/triploidy). Based on the results reported here, fetal cfDNA from a vanished twin was detectable for up to 8 weeks following co-twin demise. This method has been validated for detection of various whole chromosome aneuploidies as well as identification of cases with additional fetal haplotypes (twins/triploidy).

Conclusions: This SNP-based approach successfully identified vanished twin, previously unrecognized twin, and triploid pregnancies. As triploidy has substantial clinical implications for patients, including the risk for gestational trophoblastic neoplasia, SNP-based NIPT may impact care beyond aneuploidy detection.

Table 1. Follow-up information on twins/triploidy calls.

<table>
<thead>
<tr>
<th>Maternal Age (years)</th>
<th>Gestational Age (weeks)</th>
<th>Fetal Fraction</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.5</td>
<td>12.1</td>
<td>1.1</td>
<td>0.018</td>
</tr>
<tr>
<td>33.0</td>
<td>13.0</td>
<td>1.1</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Table 2. Demographics of confirmed multifetal pregnancies.

<table>
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Figure 1: The SNP-based Non-Invasive Prenatal Testing (NIPT)/NIPT Method. The NIPT algorithm considers parental genotypes, HapMap crossover frequency data, and possible fetal chromosome copy number to calculate expected allele distributions for a large number of hypothetical possible fetal genotypes and haplotypes. The algorithm also determines when cDNA 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 an undetected dizygotic multiple gestation (ongoing or vanishing twin). This method has been validated for detection of various whole chromosome aneuploidies as well as identification of cases with additional fetal haplotypes (twins/triploidy).

Figure 2: SNP data for a euploid fetus (A), a triploid fetus (B), and a vanishing twin case (C). Data is presented as points plotting the ratios of the two most likely alleles (A and B). X-axes: Linear SNP pattern along each chromosome. Y-axes: fetal alleles (maternal alleles)/fetal alleles. Maternal genotype is indicated by color: maternal AA alleles (RED), maternal BB alleles (BLUE), and maternal AB alleles (GREEN). The vertical location of each point represents the sum of maternal and fetal cDNA A allele reads. Where the maternal alleles are AA, the two possible fetal genotypes of a euploid fetus (AA, AB, and BB) are easily distinguishable from the three potential fetal genotypes of a triploid twin (AA/AA, AA/AB, or BB/BB), as shown by vanishing twin 1 fetus (1/2) pregnancy; at this time the algorithm does not distinguish between the very similar alleles A allele distributions in triploid (one, two, or three) and dizygotic twin (two, three, or four) pregnancies. As such, identified cases are labeled as “twins or triploidy.” Note that this is not how the algorithm makes ploidy calls, but is one method for visualizing the data.

References:

This SNP-based NIPT identified vanishing twin, unrecognized ongoing twin, and triploid pregnancies. Identification of part molar (diandric triploidy) pregnancies is important because of the substantial clinical implications for patients, including the risk for gestational trophoblastic neoplasia and chorioncarcinoma. In the results reported here, fetal cfDNA from a vanished twin was detectable for up to 8 weeks following co-twin demise. There is the potential to reduce the false positive rate. As triploidy has substantial clinical implications for patients, including the risk for gestational trophoblastic neoplasia, SNP-based NIPT may impact care beyond aneuploidy detection.

Conclusions: This SNP-based approach successfully identified vanished twin, previously unrecognized twin, and triploid pregnancies. As triploidy has substantial clinical implications for patients, including the risk for gestational trophoblastic neoplasia, SNP-based NIPT may impact care beyond aneuploidy detection.

Figure 3: Breakdown of Twin/Triploidy Calls with Known Outcomes.