Noninvasive prenatal screening for fetal aneuploidy in twin pregnancies: A clinical laboratory experience

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Noninvasive cfDNA screening for chromosomal aneuploidy has been available for twin pregnancies for eight years on several commercial platforms. We examined all twin pregnancy outcomes performed at Progeny, Inc. using an internally developed, proprietary MPS method. Here we summarize clinical laboratory experience in screening 1,477 twin pregnancies and contribute to the growing body of literature supporting the use of cfDNA technology for twin gestations.

Objectives

Noninvasive cfDNA screening for fetal aneuploidy in twin pregnancies has an estimated 50% detection rate and 5% false positive rate for Down syndrome.

Background

Current American College of Obstetricians and Gynecologists and Society for Maternal-Fetal Medicine guidelines do not recommend cell-free DNA (cfDNA) analysis in multiple gestation pregnancies due to “limited evidence regarding its efficacy.” However, laboratories have been offering cfDNA screening in twin pregnancies for years. Previous reports and data from laboratories have indicated test performance in twin gestations is similar to singleton pregnancies. Patients continue to choose cfDNA analysis in twin pregnancies to avoid invasive procedures such as chorionic villus sampling and amniocentesis and associated risks. The use of maternal serum screening for fetal aneuploidy detection is declining, likely due to inferior performance compared to cfDNA analysis. Second trimester serum screening has an estimated 50% detection rate and 5% false positive rate for Down syndrome.

Study Design

A retrospective analysis was performed for 1,477 twin gestations referred for cfDNA screening after 10 weeks’ gestation. Nucleic acid was extracted using a proprietary method and used to prepare a DNA library for next-generation sequencing. Sequencing was performed in a multiplex using the Illumina NovaSeq 6000™ platform. Sequenced reads were aligned to the reference genome and quantitative analysis was performed using a proprietary algorithm to determine risk for fetal aneuploidy. In twin pregnancies, risk for aneuploidy of chromosomes 13, 18, and 21, and the presence or absence of Y chromosome material was assessed.

For twin gestation results determined to be “Aneuploidy Detected” (positive), outcome data is routinely collected through faxed requests to the ordering provider. The first fax is sent approximately eight weeks after sample submission. If no response, a second fax is sent after expected delivery. Discordant results were tracked, including fetal sex discordant results.

Table 1. Review of twin gestations with positive results indicating possible aneuploidy in one or both fetuses.

<table>
<thead>
<tr>
<th>Case Number</th>
<th>cfDNA Test Result</th>
<th>Diagnostic Testing Results</th>
<th>Ultrasound Findings</th>
<th>Pregnancy Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Down syndrome</td>
<td>Twin A: Trisomy 21</td>
<td>No data available</td>
<td>Termination of pregnancy</td>
</tr>
<tr>
<td>2</td>
<td>Down syndrome</td>
<td>Twin A: Trisomy 21</td>
<td>No data available</td>
<td>Live birth</td>
</tr>
<tr>
<td>3</td>
<td>Down syndrome</td>
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<td>No data available</td>
<td>Live birth</td>
</tr>
<tr>
<td>4</td>
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<td>No data available</td>
<td>Live birth</td>
</tr>
<tr>
<td>5</td>
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<td>No data available</td>
<td>Live birth</td>
</tr>
<tr>
<td>6</td>
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<td>No data available</td>
<td>Live birth</td>
</tr>
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</tr>
<tr>
<td>8</td>
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</tr>
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<td>9</td>
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<td>Live birth</td>
</tr>
<tr>
<td>10</td>
<td>Down syndrome</td>
<td>Twin A: Trisomy 21</td>
<td>No data available</td>
<td>Live birth</td>
</tr>
</tbody>
</table>

Results

1,477 twin gestation cases reviewed for this study had the following results:

Negative results: 96.5% (n=1,425)
Positive results: 0.7% (n=10)
Failed analysis: 2.6% (n=42) non-reportable
Fetal outcomes were requested for all ten positive results, along with a randomly selected 1% of negative results. The positive cases are reviewed in more detail in Table 1.

Of the 1,477 cases, there was one reported false positive result, giving an observed false positive rate of 0.07%. There were no reported false negatives.

Y chromosome was detected, indicating at least one twin was male, in 71.8% of cases (n=1,031).
Fetal sex discordance was reported in two cases, giving a fetal sex discordance rate of 0.14%.
Of the 42 failures representing 39 patients, 23 had a negative repeat NIPT result and/or normal birth outcome reported, 15 had unknown outcomes, and 1 reported a loss at 21 weeks gestation with a normal anatomy ultrasound.

Demographics

Table 1. Range of values:

- Maternal Age: 15 – 50 years, Mean: 31.3 years
- BMI: Range: 15.6 – 70.1, Mean: 29.7
- Fetal Fraction: Range: 1 – 21%, Mean: 8.9%

<references>
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