Clinical experience with noninvasive prenatal testing (NIPT) for rare autosomal trisomies

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Introduction

- A whole-genome sequencing approach for NIPT has the advantage of allowing analysis of all 24 chromosomes.
- Genome-wide NIPT found that trisomies 21/18/13 combined comprise around two thirds of all chromosome aberrations.
- Although other autosomal trisomies are individually rare, collectively they make up a substantial number of chromosome abnormalities. Thus, limiting screening to only trisomies 21/18/13 misses many of these chromosome aberrations.
- Our laboratory began offering rare autosomal trisomy (RAT) screening, in addition to the common aneuploidies, in 2017.
- This study presents our initial clinical experience with RAT screening.

Methods

- Maternal blood samples from over 10,000 singleton pregnancies were submitted to the CLIA-certified Illumina Laboratory (Redwood City, CA) for the Verifi™ Plus Prenatal Test.
- Cell-free DNA was extracted from maternal plasma and sequenced post library preparation.
- Whole-genome sequence data was computationally processed with quantitative scores for each chromosome determined using chromosomal sequence coverage and fetal fraction.
- Classification thresholds for each chromosome were derived to maximize specificity while accounting for differences in prevalence for each RAT.
- Outcome information was requested for all positive cases. Additionally, we encourage all clients and health care providers (HCP) to provide outcome information on false negative results.

Results

- A total of 43 cases (0.4%) were reported as RAT screen positive.
- The chromosome distribution of RAT calls is shown in Figure 1.
- The average maternal age (35.0 years) and gestational age (12.4 weeks) of the screen positive cohort were similar to the whole study cohort. (Table 1)
- The most common reported indication for RAT testing was AMA (30.4%), followed by positive serum screening (5%), and abnormal ultrasound (3.86%).
- More cases in the screen positive cohort were noted to have indications of abnormal ultrasound (1.8x) or history suggestive of increased risk for chromosome aneuploidy (5.5x) compared with the whole study population. (Table 2)
- Clinical outcome information was available in 8 cases (18.6%): 2 confirmed positives (1 full trisomy 9; 1 segmental 9p duplication), 2 false positives, 3 miscarriages, and 1 elective termination without confirmatory testing.

Conclusion

- The 0.4% screen positive frequency observed for RATs is consistent with previous studies.
- The most common trisomy identified was trisomy 22, followed by trisomy 7 and trisomy 9 (Figure 1).
- There is an increased risk for confined placental mosaicism (CPM), leading to intrauterine growth restriction (IUGR) or uniparental disomy (UPD) related birth defects. Thus, results obtained through NIPT early in pregnancy can be valuable for clinical management.
- As the rate of diagnostic testing decreases with more patients opting for screening, identifying RATs can be valuable for pregnancy management.
- Ongoing outcome collection will provide more insight into the biological aspects of RATs.

Table 1. Sample Demographics

<table>
<thead>
<tr>
<th>RAT Screen Positives</th>
<th>Total Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>43</td>
</tr>
<tr>
<td>Maternal Age, years</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>35.0</td>
</tr>
<tr>
<td>Min-max</td>
<td>21-46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestational Age, weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Min-max</td>
</tr>
</tbody>
</table>

Table 2. Sample Indication for Testing

<table>
<thead>
<tr>
<th>Indication for Testing</th>
<th>RAT Screen Positives</th>
<th>Whole Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced maternal age</td>
<td>13/43 (31.0%)</td>
<td>3429/10702 (30.4%)</td>
</tr>
<tr>
<td>Abnormal ultrasound</td>
<td>3/43 (6.97%)</td>
<td>413/10702 (3.86%)</td>
</tr>
<tr>
<td>Personal History*</td>
<td>4/43 (9.3%)</td>
<td>177/10702 (1.65%)</td>
</tr>
</tbody>
</table>

*Hx suggestive of increased risk for chromosomal aneuploidy

Figure 1. Positive RATs by Chromosome Number

0 1 2 3 4 5 6
0 1 2 3 4 5 6
Chrm 1  Chrm 2  Chrm 3  Chrm 4  Chrm 5  Chrm 6  Chrm 7  Chrm 8  Chrm 9  Chrm 10  Chrm 11  Chrm 12  Chrm 13  Chrm 14  Chrm 15  Chrm 16  Chrm 17  Chrm 18  Chrm 19  Chrm 20  Chrm 22  Multiple
Number of Positive Calls

Figure 1. Positive RATs by Chromosome Number

0 1 2 3 4 5 6
0 1 2 3 4 5 6
Chrm 1  Chrm 2  Chrm 3  Chrm 4  Chrm 5  Chrm 6  Chrm 7  Chrm 8  Chrm 9  Chrm 10  Chrm 11  Chrm 12  Chrm 13  Chrm 14  Chrm 15  Chrm 16  Chrm 17  Chrm 18  Chrm 19  Chrm 20  Chrm 22  Multiple
Number of Positive Calls