Disclosures

None
[@Summer Pierson] Looks great! I made a few comments. Also, would you please remove all the Myriad stuff from the masters. Thanks!
Jim Goldberg, 2021/03/03

Will do.
Summer Pierson, 2021/03/03

[Jim Goldberg] double check that the solution I used to hide the branding watermarks works when you download this to your computer. I tried something "new"
Summer Pierson, 2021/03/12

[Summer Pierson] Looks good. Can you remove the “more info” from the lower right corner of #7 and do a build for #9. Thanks!
Jim Goldberg, 2021/03/12

[Jim Goldberg] I animated 9 to have the plots appear as you advance... Is that what you meant by "build"?
Summer Pierson, 2021/03/12

Yep!
Jim Goldberg, 2021/03/12
Prenatal testing continues to evolve

- History of prenatal aneuploidy screening
- Fetal fraction enrichment
- The future of prenatal aneuploidy screening

1973
Alpha-fetoprotein (AFP) for neural tube defect

1984
AFP for Trisomy 21

1990
Triple screen for T21

1993
Triple screen for Trisomy 18

1996
Inhibin for Trisomy 21

1997
Discovery of fetal cfDNA in maternal blood

1999
Imaging matures: NT Screening

2010
Noninvasive prenatal screening (NIPS) available for high risk

2016
Guidelines recommend NIPS counseling for all patients

2020
ACOG PB #226 supports NIPS in average risk patients


2020 ACOG PB #226 supports NIPS in average risk patients

Prenatal testing continues to evolve
No. 348 Joint SOGC-CCMG Guideline: Update on testing for Fetal Aneuploidy, Fetal Anomalies & Adverse Pregnancy Outcomes September, 2017

Ontario & British Columbia, along with Yukon Territory currently offer NIPS in high-risk patients

High-risk Criteria:
- Advanced maternal age (40+ at delivery)
- Abnormal serum screen i.e. FTS/IPS/MSS
- Nuchal translucency measurement of 3.5mm +
- Previous pregnancy/child with T21,18 or 13
- Ultrasound abnormalities suggestive of aneuploidy
- Multiple soft markers on ultrasound
- At risk of carrying a male fetus with an X-linked condition.

NIPS: Highest Detection AND Lowest False Positive Rates

<table>
<thead>
<tr>
<th>Maternal Age</th>
<th>T21 Detection Rates</th>
<th>T21 False Positive Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional screen</td>
<td>~2.4%</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Sequential NIPS</td>
<td>~3.4%</td>
<td>5%</td>
</tr>
<tr>
<td>Quad</td>
<td>~81%</td>
<td>5%</td>
</tr>
</tbody>
</table>

1. ACOG Practice Bulletin #226: Screening for Fetal Chromosomal Abnormalities’ ’Obstet Gynecol 2020;136
SP10  [@Jim Goldberg] [@Susan Hancock]  Did some digging. Is this “fair” Susan?
Summer Pierson, 2021/03/12

SH45  I would use 3.4% for sequential/1st tri from the NEXT study. That mat age one looks right from the paper we looked at together, Summer. I don’t know for Quad but trust you if you found one!
Susan Hancock, 2021/03/12

SH46  I would also use 81% PPV for NIPS from the NEXT study. It would be really cool to make a whole new slide and make the bars represent PPV rather than overlaying on the sensitivity bar chart. Really hammers home the difference! or could replace FP bar chart with the PPV chart.
Susan Hancock, 2021/03/12

SP13  Thanks, [@Susan Hancock]! [@Jim Goldberg] Built a PPV based version of the graph on the next slide. I loose some symmetry with the FPR graphic though. It’s up to you.
Summer Pierson, 2021/03/15

JG19  I think I like #6 best.
Jim Goldberg, 2021/03/15

SP14  You got it! I’ll bury the others.
Summer Pierson, 2021/03/15
• Fetal Fraction: Percentage of circulating DNA attributed to the fetus.

• The DNA identified as “fetal” originates from trophoblastic cells from the placenta.

• cffDNA co-mingles with maternal cfDNA in maternal circulation.

What is whole-genome sequencing?

An example of one bin count read:

If a similar increase in reads is observed along the length of the chromosome (i.e. across thousands of bins) = high risk for aneuploidy
Fetal fraction can impact test failures

**FETAL FRACTION** is the amount of fetal DNA from placenta circulating in the mother's blood.

Patients with **low fetal fraction** are more likely to receive test failures thus requiring further counseling and invasive diagnostic testing.

Patients who may have low fetal fraction:

- Those with high body mass index (BMI)
- Those screened at an early gestational age
- Those with certain aneuploidies (T13/T18)
Due to low fetal fraction, BMI can result in disparities in care for pregnancy management

- ~50% of pregnant patients present as overweight or obese to their OBGYN (BMI>25)\(^1\). Further complicating the problem is that BMI is not evenly distributed across ethnicities\(^2\).

- Current strategies to manage patients with high BMI include maternal serum screening or offering NIPS later in a patient's pregnancy. This creates a disparity in care.

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STRATEGIES TO DEAL WITH LOW FETAL FRACTION LEAD TO WORKFLOW CHALLENGES AND DISPARITY IN CARE

CALL FOR INNOVATION TO ADDRESS LOW FETAL FRACTION SAMPLES

"Addressing the problem of low fetal fraction is a high priority. Many approaches have been proposed to enrich fetal fraction by optimizing sequencing conditions, capitalizing on the biological differences between fetal and maternal DNA, and/or developing new statistical algorithms..."
High-throughput fetal fraction amplification increases analytical performance of noninvasive prenatal screening

Noah C. Welker, PhD¹, Albert K. Lee, PhD², Rachel A. S. Kjolby, PhD², Helen Y. Wan, BS, MBA², Mark R. Theilmann, AB², Diana Jeon, BA², James D. Goldberg, MD², Kevin R. Haas, PhD², Dale Muzzey, PhD ☞² and Clement S. Chu, PhD²


Fetal fraction amplification (FFA) technology increases fetal fraction (FF) across all BMI levels
Fetal fraction amplification (FFA) technology increases fetal fraction (FF) across all BMI levels.

FFA directly increases the concentration of fetal-derived cfDNA in samples undergoing NIPS.
HOW FFA TECHNOLOGY WORKS:
Increasing fetal fraction by leveraging differences in cfDNA length
FFA increases FF for 99.8% of samples tested and most appreciably for low-FF samples.

FFA increases z-scores for positive samples but not for negative samples.
CASE EXAMPLE: FFA ENABLES MORE SENSITIVE DETECTION OF MICRODELETIONS

INCREASED CONFIDENCE IN ANEUPLOIDY CALLS

<table>
<thead>
<tr>
<th></th>
<th>Analytical sensitivity</th>
<th>Analytical specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common aneuploidies (aggregate)</td>
<td>99.988% ± 0.004%</td>
<td>99.968% ± 0.005%</td>
</tr>
<tr>
<td>- T21</td>
<td>99.990% ± 0.005%</td>
<td>99.996% ± 0.001%</td>
</tr>
<tr>
<td>- T18</td>
<td>99.990% ± 0.002%</td>
<td>99.996% ± 0.001%</td>
</tr>
<tr>
<td>- T13</td>
<td>99.978% ± 0.005%</td>
<td>99.976% ± 0.005%</td>
</tr>
<tr>
<td>RAAs (aggregate)</td>
<td>99.695% ± 0.305%</td>
<td>99.981% ± 0.010%</td>
</tr>
<tr>
<td>Microdeletions (aggregate)</td>
<td>97.172% ± 0.054%</td>
<td>99.767% ± 0.012%</td>
</tr>
<tr>
<td>- DiGeorge Syndrome (22q11.2)</td>
<td>95.633% ± 0.071%</td>
<td>99.949% ± 0.005%</td>
</tr>
</tbody>
</table>
SUBSTANTIAL DECREASE IN FALSE NEGATIVES

<table>
<thead>
<tr>
<th></th>
<th>NIPS Without AMPLIFY</th>
<th>Prequel With AMPLIFY Technology</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with low Fetal Fraction &lt;4%</td>
<td>1 in 20 samples</td>
<td>&lt;1 in 1000 samples</td>
<td>50X</td>
</tr>
</tbody>
</table>

False negatives per sample screened

<table>
<thead>
<tr>
<th></th>
<th>NIPS Without AMPLIFY</th>
<th>Prequel With AMPLIFY Technology</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common aneuploidies</td>
<td>1 in 18,300</td>
<td>1 in 833,300</td>
<td>45X</td>
</tr>
<tr>
<td>Expanded aneuploidies</td>
<td>1 in 1,400</td>
<td>1 in 66,800</td>
<td>48X</td>
</tr>
<tr>
<td>5 common microdeletions</td>
<td>1 in 4,000</td>
<td>1 in 36,000</td>
<td>9X</td>
</tr>
<tr>
<td>~ 22q11.2 (DiGeorge)</td>
<td>1 in 6,000</td>
<td>1 in 46,000</td>
<td>8X</td>
</tr>
</tbody>
</table>

Data based on analytical test performance.

IMPACT OF FFA IN A REAL-WORLD CLINICAL LABORATORY SETTING

- >20K clinical lab samples
- Test failure rate 0.16%
- Average FF with AMPLIFY: 20.5% (without AMPLIFY 8.1%)
HOW DOES FETAL FRACTION AMPLIFICATION SET THE STAGE FOR FUTURE INNOVATIONS?

Higher fetal fraction amplification allows for increased sensitivity for small changes to the genome, including:

- Higher sensitivity for the microdeletion syndromes, like 22q deletion.
- Increased resolution allows for the increased accuracy in the calling of novel deletions and duplications throughout the genome, which can help in the diagnostics of other syndromes.

What questions do you have?