Outline: Apr 30

- Overview of Non-invasive Prenatal Testing (NIPT)
- Small group discussion of papers
- Regroup as a class

Notes:
- Office hours TODAY 3:15-5pm
- Project meetings in lab on Thursday
Prenatal Testing Background
Causes of aneuploidy

abnormal number of chromosomes (usually 1 or 3 instead of 2)

"Mitotic nondisjunction" by Wpeissner - https://commons.wikimedia.org
Motivation: aneuploidy testing

Autosomal chromosomes:
- Trisomy 13: Patau syndrome
- Trisomy 18: Edwards syndrome
- Trisomy 21: Down syndrome
Motivation: aneuploidy testing

Autosomal chromosomes:
- Trisomy 13: Patau syndrome
- Trisomy 18: Edwards syndrome
- Trisomy 21: Down syndrome

Sex chromosomes:
- X0 (one X chromosome): Turner’s syndrome
- XYY or XXX: normal male or female phenotype
- XXY: Klinefelter Syndrome
Older women are more at risk

![Graph showing the prevalence of Down Syndrome by mother's age](Image)
Current testing procedures

- **cFTS** (combined First-Trimester Screening), 12-14 weeks
  - *Looks at biomarkers and other data*

- **Amniocentesis** (invasive)
  - 16-22 weeks, uses karyotyping to determine aneuploidy

- 1997: proof of fetal DNA in maternal blood (Y-chromosome)

- ≈2011: companies offer Non-Invasive Prenatal Testing (NIPT)
  - *Verinata, Harmony, NIFTY*
  - *All using next-generation sequencing*
Basic Procedure

1) Sample maternal blood
   - contains cell-free fetal DNA (cffDNA)

2) Low-coverage sequencing (0.1x - 4x)

3) Read alignment/mapping to human genome (using BWA or similar)
   - save reads that map uniquely

4) Compute coverage for each chromosome

5) t-test for aneuploidy (coverage differences)
fetal DNA?

maternal DNA?

of re positives!
Direct to Consumer (DTC) testing
Continuous innovation

Increased safety and peace of mind for your patients
Swift acceptance of the verifi® prenatal test has made a world of difference to high-risk patients across the country:

- SAFE—Routine blood draw, just one tube (7-10 ml)
- ACCURATE—Directly analyzes cell-free fetal DNA with our proprietary SAFeR™ algorithm
- EASY—Test as early as 10 weeks, no limitations in reference to patient ethnicity, BMI, ART, or egg donor cases
- FAST—Results reported in 3-5 business days after sample receipt

The basic verifi® test detects:

- T21 (Down syndrome)
- T18 (Edwards syndrome)
- T13 (Patau syndrome)

Now a wider option is available for sex chromosomes at no extra charge:

- Monosomy X (MX; Turner syndrome)
- XXX (Triple X)
- XXY (Klinefelter syndrome)
- XYY (Jacobs syndrome)
- Fetal sex (XX or XY)—aids in stratifying the risk for X-linked disorders such as hemophilia, Duchenne muscular dystrophy, or cases of ambiguous genitalia, such as congenital adrenal hyperplasia
99% confidence (3.09)

known trisomy 21

FIG. 12
## DNA Sequencing versus Standard Prenatal Aneuploidy Screening


<table>
<thead>
<tr>
<th>Type</th>
<th>false positive (sequencing)</th>
<th>false positive (standard)</th>
<th>false negative (sequencing)</th>
<th>num positives</th>
<th>PPV (sequencing)</th>
<th>PPV (standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T21</td>
<td>0.30%</td>
<td>3.60%</td>
<td>0</td>
<td>5</td>
<td>45.50%</td>
<td>4.20%</td>
</tr>
<tr>
<td>T18</td>
<td>0.20%</td>
<td>0.60%</td>
<td>0</td>
<td>2</td>
<td>40.00%</td>
<td>8.30%</td>
</tr>
<tr>
<td>T13</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Probability Considerations
Clinical Trials Example

- Disease affects 1/100 people: $P(\text{disease}) = 0.01$
- Test for the disease with 90% accuracy
  - $P(\text{positive|disease}) = 0.9$
  - $P(\text{negative|healthy}) = 0.9$
Clinical Trials Example

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$$P(\text{disease} | \text{positive}) = \frac{P(\text{positive} | \text{disease}) P(\text{disease})}{P(\text{positive})}$$
Clinical Trials Example

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\[
P(\text{disease} | \text{positive}) = \frac{P(\text{positive} | \text{disease}) \cdot P(\text{disease})}{P(\text{positive})}
\]

\( \approx 8.3\% \)
Clinical Trials Example

P(disease) = P(D) = \frac{1}{100}

test with 90% accuracy

P(\text{pos|D}) = \frac{9}{10}

P(\text{neg|H}) = \frac{9}{10}

P(D|P) = \frac{P(D)P(\text{P|D})}{P(D)P(\text{P|D}) + P(P,H)}

= \frac{\frac{1}{100} \cdot \frac{9}{10} + \frac{9}{100} \cdot \frac{99}{100} \cdot \frac{1}{10}}{\frac{9}{100} \cdot \frac{9}{10} + \frac{9}{100} \cdot \frac{99}{100} \cdot \frac{1}{10}}

= \frac{9}{108} = \frac{1}{12}

PPV positive predictive value

lots of false positives!
Bayesian Model

Input data are read counts for each chromosome (1,2,...,n):

\[ q_1, q_2, \cdots, q_n = \bar{q} \]

\[ \sum_{i=1}^{n} q_i = N \]
Bayesian Model

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Goal:

\[
\mathbb{P}(T_{21} | \vec{q}) = \frac{\mathbb{P}(\vec{q} | T_{21}) \cdot \mathbb{P}(T_{21})}{\mathbb{P}(\vec{q})}
\]

\[
= \frac{\mathbb{P}(\vec{q} | T_{21}) \cdot \mathbb{P}(T_{21})}{\mathbb{P}(\vec{q} | T_{21}) \cdot \mathbb{P}(T_{21}) + \mathbb{P}(\vec{q} | T_{21}^C) \cdot \mathbb{P}(T_{21}^C)}
\]
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\mathbb{P}(T_{21} | \vec{q}) = \frac{\mathbb{P}(\vec{q} | T_{21}) \cdot \mathbb{P}(T_{21})}{\mathbb{P}(\vec{q})} \\
= \frac{\mathbb{P}(\vec{q} | T_{21}) \cdot \mathbb{P}(T_{21})}{\mathbb{P}(\vec{q} | T_{21}) \cdot \mathbb{P}(T_{21}) + \mathbb{P}(\vec{q} | T_{21}^C) \cdot \mathbb{P}(T_{21}^C)}
\]
<table>
<thead>
<tr>
<th>Maternal Age</th>
<th>Trisomy 21</th>
<th>All Trisomies</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1 in 1,667</td>
<td>1 in 526</td>
</tr>
<tr>
<td>21</td>
<td>1 in 1,429</td>
<td>1 in 526</td>
</tr>
<tr>
<td>22</td>
<td>1 in 1,429</td>
<td>1 in 500</td>
</tr>
<tr>
<td>23</td>
<td>1 in 1,429</td>
<td>1 in 500</td>
</tr>
<tr>
<td>24</td>
<td>1 in 1,250</td>
<td>1 in 476</td>
</tr>
<tr>
<td>25</td>
<td>1 in 1,250</td>
<td>1 in 476</td>
</tr>
<tr>
<td>26</td>
<td>1 in 1,176</td>
<td>1 in 476</td>
</tr>
<tr>
<td>27</td>
<td>1 in 1,111</td>
<td>1 in 455</td>
</tr>
<tr>
<td>28</td>
<td>1 in 1,053</td>
<td>1 in 435</td>
</tr>
<tr>
<td>29</td>
<td>1 in 1,000</td>
<td>1 in 417</td>
</tr>
<tr>
<td>30</td>
<td>1 in 952</td>
<td>1 in 384</td>
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<tr>
<td>31</td>
<td>1 in 909</td>
<td>1 in 384</td>
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<tr>
<td>32</td>
<td>1 in 769</td>
<td>1 in 323</td>
</tr>
<tr>
<td>33</td>
<td>1 in 625</td>
<td>1 in 286</td>
</tr>
<tr>
<td>34</td>
<td>1 in 500</td>
<td>1 in 238</td>
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<td>35</td>
<td>1 in 385</td>
<td>1 in 192</td>
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<td>1 in 175</td>
<td>1 in 102</td>
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<td>1 in 137</td>
<td>1 in 83</td>
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<tr>
<td>40</td>
<td>1 in 106</td>
<td>1 in 66</td>
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<tr>
<td>41</td>
<td>1 in 82</td>
<td>1 in 53</td>
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<tr>
<td>42</td>
<td>1 in 64</td>
<td>1 in 42</td>
</tr>
<tr>
<td>43</td>
<td>1 in 50</td>
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<td>48</td>
<td>1 in 14</td>
<td>1 in 10</td>
</tr>
<tr>
<td>49</td>
<td>1 in 11</td>
<td>1 in 8</td>
</tr>
</tbody>
</table>

Prior:

\[ P(T_{21}) \]
“A couple in which the man carries the mutation for Huntington's disease request prenatal testing during their first pregnancy. Though they would not terminate an affected pregnancy, they would like the information. There is no treatment available that can change the course of the disease so the diagnosis will not result in medical benefit for the child.”

Viewpoint 1: not to test
Viewpoint 2: to test
Discussion Questions

■ Form small groups (3-4 people)
■ Discuss questions below (or anything else you find interesting/relevant about this topic)
■ Choose a representative to mention an interesting part of your discussion to the class

1) Which side of the second article (about Huntington’s Disease) do you find most compelling?

2) Considering the potential of NIPT to resolve the entire fetal genome, what would be your recommendation about how to use this technology?