Non Invasive Prenatal Testing (NIPT)

New Era for Genetic Testing

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If you currently do not know a lot about this topic, you are not alone

Survey of OB providers
(87% MDs, DOs; 11% RNs, NPs, CNMs)

Knowledge level
“not high”

85%

Prevalence of Aneuploidy

Live birth + IUFD + termination = 1 in 228 (0.4%)
98.6% of all invasive procedure have normal karyotypes

53%

16%

5%

8%

5%

13%

Down syndrome
Trisomy 18
Trisomy 13
45,X
XXX, XXY, XYY
Other

Sayers, et al. 2011

Screening and invasive testing for aneuploidy should be available to all women who present for prenatal care before 20 weeks REGARDLESS of maternal age.

Screening tests calculate risks based on phenotypic factors

hCG
AFP
uE3
Inhibin
PAPP-A
NT

Types of Screening Tests

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Detection Rate</th>
<th>Screen Positive Rate</th>
<th>Risk in Positives</th>
<th>Procedure Related Losses per 150,000 screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Screening Tests</td>
<td>69%</td>
<td>5%</td>
<td>1 in 50</td>
<td>67</td>
</tr>
<tr>
<td>2nd Trimester Screen</td>
<td>81%</td>
<td>5%</td>
<td>1 in 45</td>
<td>45</td>
</tr>
<tr>
<td>First Trimester Screen</td>
<td>91%</td>
<td>5%</td>
<td>1 in 17</td>
<td>33</td>
</tr>
<tr>
<td>Age</td>
<td>91%</td>
<td>1 in 120</td>
<td>1 in 300</td>
<td>300</td>
</tr>
</tbody>
</table>

**Goals of Screening / NIPT**

- Reduced risk to fetus
- Reduced anxiety
- Increased detection
- Reduced false positive
- (Available to all women)

**DNA based test (NIPT)**

- NIPT estimates risks based on DNA
- However, the DNA is derived from placental cells, so similar problems to CVS

**Sources of Fetal DNA in Maternal Blood**

- Cells (~1 fetal cell per 1 billion cells)
  - Persist between pregnancies
- Cell Free DNA
  - 100-250 bp in length
  - By 10 weeks’ gestation
  - ~90% of total cfDNA is maternal
  - Primarily from sponges of blood cells
  - ~10% is from the pregnancy
  - Primarily from sponges of placental cells
  - Half-life ~20 minutes
  - Undetectable ~2 hours postpartum

**Performance Comparison**

<table>
<thead>
<tr>
<th></th>
<th>CVS</th>
<th>Amnio</th>
<th>FT5</th>
<th>NIPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timing</td>
<td>12-14 weeks</td>
<td>16+ weeks</td>
<td>11-14 weeks</td>
<td>&gt;10 weeks</td>
</tr>
<tr>
<td>Risk</td>
<td>1% miscarriage</td>
<td>0.3% miscarriage</td>
<td>None to pregnancy</td>
<td>None to pregnancy</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>&gt;99.9% for aneuploidy</td>
<td>&gt;99.9% for aneuploidy</td>
<td>99.1% for Down syndrome</td>
<td>99.1% for Down syndrome</td>
</tr>
<tr>
<td>Specificity</td>
<td>&gt;98% for aneuploidy (PPV &lt;2%)</td>
<td>&gt;99% for aneuploidy (PPV &lt;1%)</td>
<td>95% for Down syndrome (PPV 5%)</td>
<td>99% for Down syndrome (PPV 0.2%)</td>
</tr>
<tr>
<td>Turn Around</td>
<td>2 weeks</td>
<td>&lt;2 weeks</td>
<td>&lt; 1 week</td>
<td>&lt; 2 weeks</td>
</tr>
</tbody>
</table>

**Sequencing Technology**

- <10 cents per 1 million base pairs
- 1/2012

**Companies Offering NIPT**

- SEQUENOM
- Verinata
- VeriFi
- Integrated Genetics
- Ariosa
- Harmony
- natera
- Launching early 2013
**HOW IS THE TEST PERFORMED?**

**Mapping Reads**
- 1 read = 1 fragment of contiguous nucleotide sequence
- Chromosomal origin of each fragment is determined by comparison to genome map

**Shotgun vs. Targeted Sequencing**

**Shotgun**
- Amplification and data from all chromosomes
- Most not needed for analysis
- Requires >20 million reads for sufficient data
- Large sequencing machines required

**Targeted**
- Amplification of specific (targeted) chromosome regions of interest
- Data only from chromosomes of interest
- Almost all data obtained is used in analysis
- Requires <<20 million reads
- Benchtop sequencers

**Library Preparation**
- PCR primers and indexes added to DNA sample
- Several libraries will be pooled (run in parallel), so each given unique index
- Pooled libraries loaded into flow cell

**Test Methodologies**
- Massively Parallel (Shotgun) Sequencing (Sequenom, Verinata)
  - All fragments are run (>20 million total)
- Targeted Sequencing (Ariosa, Natera)
  - Fragments with specific sequences identified before testing via PCR using specific primers
  - Selection of nonpolymorphic loci (Ariosa)
    - ~400 loci per chromosome of interest
    - Each 56 bp in length
  - Selection of polymorphic SNPs (Natera)
    - SNP = single nucleotide polymorphism
      - Well characterized, common
      - ~10,000 over all chromosomes of interest

**Massively Parallel Sequencing**
- Clusters are sequenced simultaneously
- One by one, fluorescently-labeled reversible terminator nucleotides are incorporated and fluoresced by laser
- Each base (A, T, C, G) has its own color
- Each cycle is captured digitally
Quantifying Fragments

Factors Influencing Sequencing

- Depth of sequencing coverage
  - Average number of times each base pair is sequenced
  - Number of reads varies \(10^3\) to \(10^5\)
  - Number of samples per lane

- GC content
  - GC nucleotide pairs held together by 3 bonds, AT pairs by 2
  - When GC content is high or low, DNA forms hairpin turns and polymerase does not bind well

Fetal Fraction and expected separation

- Mother is assumed to be euploid
- As fetal fraction increases, the accuracy increases
- Accurate analysis requires \(\geq 4\%\) fetal fraction

<table>
<thead>
<tr>
<th>Fetal Fraction</th>
<th>Fetal Karyotype</th>
<th>Chromosome 21 DNA as % of total DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any (0-100%)</td>
<td>Euploid</td>
<td>1.5%</td>
</tr>
<tr>
<td>0%</td>
<td>Trisomy 21</td>
<td>1.5%</td>
</tr>
<tr>
<td>4%</td>
<td>Trisomy 21</td>
<td>1.53%</td>
</tr>
<tr>
<td>10% (typical)</td>
<td>Trisomy 21</td>
<td>1.58%</td>
</tr>
<tr>
<td>20%</td>
<td>Trisomy 21</td>
<td>1.65%</td>
</tr>
</tbody>
</table>

More reads = More reliable results

Detecting Down syndrome

More difficult because need to test for quantity (3 copies of chromosome 21 instead of 2)
Other factors influencing sequencing

- **Fetal Fraction**
  - Affected by obesity
  - Likely due to apoptosis of adipose tissue and larger blood volume
  - Unaffected by
  - Maternal age
  - Ethnicity
  - Gestational age
  - NT measurement

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 Fayette et al., 2011
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Fetal fraction and test performance

- **All tests, regardless of methodology**
  - Will not provide an answer if the fetal fraction is <4%
  - Will be less reliable if the fetal fraction is low (5-9%)
  - Will be most reliable if the fetal fraction is high (>15%)

**Data Interpretation**

- Normalizing chromosome fragments
  - Variability intra- and inter- chromosome which can obscure aneuploidy
  - Compare number of reads mapping to a certain chromosome to that of reference chromosomes and calculate standard deviations
  - **Z-score** if using all chromosomes as reference
  - **Normalized Chromosome Value (NCV) score** if reference chromosomes similar biochemically to chromosome of interest

**Interpreting the Data**

- **Normalized Chromosome Value (Verinata)**
- **Z-Score (Sequence, Arisox)**

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Bonhe et al., 2012
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Palomaki et al., 2011
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**Challenge of Threshold Results**

- **Dual Threshold Method (Verinata)**
- **Single Threshold Method (Sequnom)**

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Altman et al., 2011
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**SNP data (Natera)**

- **Analyzing Allelic Distribution**
- Algorithms subtract out maternal allelic distribution (and paternal if available), taking possible crossover events into account, and selects result with highest significant likelihood

- **Quantification of reads not needed**
- **Mapping not needed because location of SNPs known**
Practical Issues

Comparison of NIPT technologies

<table>
<thead>
<tr>
<th>Chromosome Anomaly</th>
<th>Detection Rate (False Positive Rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>99.5% (0.3%)</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>99.2% (0.7%)</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>99.1% (0.4%)</td>
</tr>
<tr>
<td>13,14,21</td>
<td>99.1% (0.5%)</td>
</tr>
<tr>
<td>Not evaluated</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Not enough data</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Y chromosome</td>
<td>99.4% (0.4%)</td>
</tr>
<tr>
<td>Recall Rate</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

Likelihood ratios

<table>
<thead>
<tr>
<th>Test</th>
<th>True Positive</th>
<th>False Negative</th>
<th>False Positive</th>
<th>Unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choo et al. 2011</td>
<td>234/337</td>
<td>7/207</td>
<td>3/146</td>
<td>1/1166</td>
</tr>
<tr>
<td>Smith et al. 2010</td>
<td>296/312</td>
<td>3/471</td>
<td>3/140</td>
<td>1/1066</td>
</tr>
<tr>
<td>Total</td>
<td>334/337</td>
<td>7/207</td>
<td>3/146</td>
<td>1/1166</td>
</tr>
</tbody>
</table>

How do results impact the risks

<table>
<thead>
<tr>
<th>Prior risk based on conventional screening</th>
<th>Final Risk following MaterniT21 test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1:10</td>
<td>29:1 (96%)</td>
</tr>
<tr>
<td>1:100</td>
<td>2:1 (73%)</td>
</tr>
<tr>
<td>1:270</td>
<td>1:1 (50%)</td>
</tr>
<tr>
<td>1:1000</td>
<td>1:3 (29%)</td>
</tr>
</tbody>
</table>

Clinical Management

<table>
<thead>
<tr>
<th>CCFDNA results</th>
<th>Ultrasound Findings</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>None</td>
<td>No further assessment for aneuploidy</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Invasive testing</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Invasive testing</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Invasive testing with prenatal microarray</td>
</tr>
</tbody>
</table>

Benefits

- Eliminates risk to pregnancy from invasive testing
- Diagnosis earlier in pregnancy
- Safer terminations
- Reduction of parental anxiety
- Decreased medical costs
Practical Issues
- Currently, most women receive limited information prior to screening
- Pretest counseling should be provided for every woman, but limited manpower
- Targets few chromosome abn., so would miss other chromosome abnormalities
- Lay person assumes NIPT is as good as invasive testing for aneuploidy

Practical Issues cont.
- Other screening, e.g. ONTDs
- Should first trimester nuchal translucency be done?
- Limited data in twins, donor egg, trisomy 13

Ethical Issues
- Abortion
- Economic benefits from reduced numbers of genetic disorders
- Discrimination against a minority group
  - In US, number of DS births unchanged
- Could increase stigma of having a genetic disorder
- First step to screening for other traits
- Noninvasive prenatal paternity testing
- Direct to consumer marketing already a problem with sex testing

Patient resources by NCHPEG and NSGC

The International Society of Prenatal Diagnosis Position Statement (ISPD) on Prenatal Detection of Down Syndrome
- Population Screening: Need additional trials and evidence that MPS is broadly applicable, cost effective, and also can be combined with other elements of prenatal screening
- MPS may be helpful for women determined to be high risk by one of the previously recommended strategies
- Genetic counseling must be provided
  - Does not detect all DS, etc.
  - Test failure rate
  - MPS test + women will be very high risk but still need confirmatory testing
  - MPS limitations with respect to detection of Mendelian disorders, microdeletion syndromes, other chromosomal imbalances, etc.
NONINVASIVE PERNATAL TESTING/NONINVASIVE PERNATAL DIAGNOSIS (NPT/NPD): The National Society of Genetic Counselors currently supports Noninvasive Prenatal Testing/Noninvasive Prenatal Diagnosis (NPT/NPD) as an option for patients whose pregnancies are considered to be of increased risk for certain chromosome abnormalities. NACGC urges that NPT/NPD only be offered in the context of informed consent, education, and counseling by a qualified provider, such as a certified genetic counselor. Patients whose NPT/NPD results are abnormal, or who have other factors suggestive of a chromosome abnormality, should receive genetic counseling and be given the option of standard confirmatory diagnostic testing. (Adapted February 18, 2012)

COMMITTEE OPINION

• Patients at increased risk of aneuploidy can be offered testing
  (AMA, advanced age, anxiety, prior affected pregnancy, positive risk screen, parent carries Robertsonian translocation involving chromosome 13 or 21).
• Should not be part of routine prenatal laboratory assessment, but an
  informed patient choice after appropriate pretest counseling.
• Should not be offered to low-risk women or women with multiple
  gestations.
• If a fetal structural abnormality is identified on ultrasound, invasive prenatal
  diagnosis should be offered.
• A patient with a positive test result should be referred for genetic
  counseling and offered invasive prenatal diagnosis for confirmation of test
  results.
• Does not replace the accuracy and diagnostic precision of CVS or
  amniocentesis, which remain an option for women.