Non-invasive Prenatal Screening

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Disclosure

• Tiffney Carter is a faculty member of the Department of Molecular and Human Genetics at Baylor College of Medicine
• Lauren Westerfield is a genetic counselor at the Department of Molecular and Human Genetics at Baylor College of Medicine
• The Medical Genetics Laboratories in this department offer extensive genetic laboratory testing, including chromosomal microarray analysis (CMA) and NIPS, and derive revenue from this activity
• We do not receive personal revenue from this activity.

Objectives

Attendees will be able to:
• describe how non-invasive prenatal screening (NIPS) is performed
• list the major indications for NIPS
• describe appropriate follow-up counseling for an abnormal NIPS result
So what is a genetic counselor?

- Health care professionals that work as a member of a multidisciplinary team
  - Specialized masters degree
  - Experience in medical genetics and counseling
- Risk Assessment
- Education
- Support
- Facilitate Decision Making

Where we practice

- Hospitals and affiliated clinics
- University medical centers
- Clinical laboratories
- Research
- Telemedicine
- Nonprofit organizations
How to find services for your patient

– American Board of Genetic Counseling, http://www.abgc.net
– Local specialty clinics such as Maternal Fetal Medicine, Cancer/Oncology, Cardiology/Cardiovascular disease, children’s hospitals

GENETICS REFRESHER
Common chromosome anomalies

- Aneuploidy
  - Monosomy, trisomy, tetrasomy
  - Most common are trisomy 13, trisomy 18, trisomy 21, monosomy X, and Klinefelter syndrome (XXY)
- Polyploidies
  - Triploidy, tetraploidy
- Copy number variants
  - Duplications
  - Deletions
- Abnormalities can be balanced (reciprocal translocation, inversion) or unbalanced

These are generally sporadic conditions – your patient may have no obvious risk factors

Goals of Prenatal Diagnosis and Counseling

- Assess the health of a fetus
- Determine specific risks to fetus
- Manage the pregnancy
- Plan for complications at birth
- Evaluate prenatal diagnostic options
- Diagnosis fetus when desired and possible
- Educate family about diagnosis, likely outcomes, potential and management options
- Discuss risks, benefits, and uncertainties
- Explore family concerns
- Provide risk assessment for other family members
- Provide psychosocial support and follow-up
Traditional maternal serum screening (MSS)

- Available at specific gestational age
- Targets the most common trisomies – 21 and 18
- Influence by maternal factors – age, ethnicity, accurate dating, diabetes, number of fetuses

Detection Rates for Maternal serum screening (MSS) for Down syndrome

<table>
<thead>
<tr>
<th>Method Screening</th>
<th>Weeks performed</th>
<th>Detection Rate for Down syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age (MA)</td>
<td>_____</td>
<td>30%</td>
</tr>
<tr>
<td>Nuchal translucency (NT) alone</td>
<td></td>
<td>64-70%</td>
</tr>
<tr>
<td>First trimester screening (NT + analytes)</td>
<td>11-13+6 wks</td>
<td>91%</td>
</tr>
<tr>
<td>First trimester screening (NT + analytes + nasal bone)</td>
<td>11-13+6 wks</td>
<td>95%</td>
</tr>
<tr>
<td>Second trimester screening</td>
<td>15-21+6 wks</td>
<td>Triple screen 70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quadruple screen 75-81%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penta screen 83%</td>
</tr>
</tbody>
</table>

False positive rates range from 2-5%.

NON-INVASIVE PREGNATAL SCREENING (NIPS)
Technological Evolution

- 1997:cffDNA first described

Research phase

- 2011: Clinical Launch, October 2011
  - Trisomy 21

- 2012: Trisomy 18 and 13, February 2012
  - Twin gestations, May 2012
  - Presence of Y chromosome, August 2012

- 2013: Sex chromosome aneuploidy, triploidy/vanishing twin, February 2013

- 2014: Expanded NIPS launches*, October 2013

- 2015: 3 additional microdeletions**, July 2014

Methods of analysis

- Analysis Methods/Proprietary Algorithm:
  - Z-score - Sequenom
  - Normalized Chromosome Value (NCV) - Verinata
  - Digital Analysis of Selected Regions (DANSR) – Ariosa
  - Next-generation Aneuploidy Testing Using SNPs (NATUS) – Natera

- Reporting
  - positive/negative
  - “low risk”/“high risk”
**HOW DOES IT WORK?**

- Count the fragments (N) from each chromosome:
  - N is proportional to the size of the chromosome
  - N is consistent from sample to sample
  - N is consistent from patient to patient
- If there is fetal trisomy, there is a relative small increase in the number of fragments from that chromosome

Requires various normalization steps

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**Fetal Fraction can affect test performance**

Fetal fraction = 10-20% between 10-21 weeks

<table>
<thead>
<tr>
<th>Increased fetal fraction</th>
<th>Decreased fetal fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crown-rump length</td>
<td>Maternal weight (BMI)</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>African/Caribbean descent</td>
</tr>
<tr>
<td>Beta-hCG</td>
<td>Smoking</td>
</tr>
<tr>
<td>Intrauterine growth restriction</td>
<td></td>
</tr>
</tbody>
</table>

**Aneuploidy:**

- Decrease in T13, T18, mono X
- Increase in T21

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**“Traditional NIPS**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>DR (%)</th>
<th>FPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21 (18 studies)</td>
<td>99.0</td>
<td>0.03 – 0.14</td>
</tr>
<tr>
<td>Trisomy 18 (15 studies)</td>
<td>96.8</td>
<td>0.05 – 0.25</td>
</tr>
<tr>
<td>Trisomy 13 (11 studies)</td>
<td>92.1</td>
<td>0.04 – 0.46</td>
</tr>
<tr>
<td>Monosomy X (12 studies)</td>
<td>88.6</td>
<td>0.05 – 0.24</td>
</tr>
<tr>
<td>Other sex chr. aneuploidy</td>
<td>93.8</td>
<td>0.02 – 0.28</td>
</tr>
<tr>
<td>Twin pregnancy trisomy 21</td>
<td>94.4</td>
<td>0.00 – 1.84</td>
</tr>
</tbody>
</table>

Gil et al. Fetal Diagn Ther 2014;35:156-173
### NIPS for Microdeletion syndromes and other trisomies

<table>
<thead>
<tr>
<th>Deletion</th>
<th>Natera</th>
<th>Sequenom/Quest</th>
<th>Illumina</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q11.2 deletion (DiGeorge)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>15q11-13 deletion (Angelman/Prader-Willi)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>5p (Cri-du-chat)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>4p (Wolf-Hirschhorn)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>1p36 deletion</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>8q (Langer-Giedion)</td>
<td>●</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11p (Jacobsen)</td>
<td>●</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trisomy 9</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Trisomy 22</td>
<td>●</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Currently offered in the US by five companies
- Different reporting and ordering algorithms

### Expanded NIPS

Microdeletions are rare – their incidence is not linked to maternal age
- Limited validation studies, mostly case reports and proof of concept studies

The larger the deletion, the easier it is to detect.
- Deletions <7 Mb, sensitivity estimated at 60-85%
- Deletions >7 Mb (which might be cytogenetically visible), sensitivity estimated at >85%

### First purely clinical outcome study

<table>
<thead>
<tr>
<th>Deletion</th>
<th>True Positive DR(%)</th>
<th>Suspected DR(%)</th>
<th>False Positive DR(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q11.2</td>
<td>23/32 (71.9%)</td>
<td>8/32 (25.0%)</td>
<td>unknown</td>
</tr>
<tr>
<td>15q</td>
<td>4/9 (44.0%)</td>
<td>1/9 (11.1%)</td>
<td>unknown</td>
</tr>
<tr>
<td>1p36</td>
<td>3/5 (60.0%)</td>
<td>1/5 (20.0%)</td>
<td>1/5 (20.0%)</td>
</tr>
<tr>
<td>5p</td>
<td>4/6 (66.7%)</td>
<td>0/1</td>
<td>2/6 (33.3%)</td>
</tr>
<tr>
<td>4p</td>
<td>1/1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11q</td>
<td>1/1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8q</td>
<td>1/1 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hume et al. 2015. Clinical outcome of subchromosomal events detected by whole-genome non-invasive prenatal testing.
NIPS: Concerns with microdeletions

Limited to recurrent, well-characterized deletions
• not designed for smaller or atypically located ones

Variability in detection by company
• Fetal fraction, sequence reads, etc all play a role, but algorithms vary

Potential to incidentally diagnose a parent
• 8-28% of 22q11.2 deletions are inherited and parent may not know they’re affected
• 25 of 55 identified microdeletions in 2015 Sequenom study had maternal contribution

Value of NIPS

• A step towards the ultimate goal of non-invasive amniocentesis/chromosome microarray, but not a replacement for diagnostic testing
  – Positive result can help direct management, but does not confirm diagnosis.
  – Negative results does not exclude condition or possible other chromosome rearrangement

• When multiple ultrasound anomalies are seen, karyotype with addition of chromosome microarray has the highest diagnostic yield

However....

A test can have both a high sensitivity and specificity without being a good predictor of whether the condition is actually present

It’s all about the PPV!
### Calculating PPV for NIPS

- Hypothetical population of 35 y.o. women = 100,000 births annually

<table>
<thead>
<tr>
<th></th>
<th>Down syndrome</th>
<th>Trisomy 18</th>
<th>Trisomy 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>1/250</td>
<td>1/2000</td>
<td>1/5000</td>
</tr>
<tr>
<td>Affected fetuses</td>
<td>400</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99.5%</td>
<td>98.0%</td>
<td>90.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.9%</td>
<td>99.6%</td>
<td>99.8%</td>
</tr>
<tr>
<td>Total test positives</td>
<td>498</td>
<td>449</td>
<td>218</td>
</tr>
<tr>
<td>True test positives</td>
<td>398</td>
<td>49</td>
<td>18</td>
</tr>
<tr>
<td>False positives</td>
<td>100</td>
<td>400</td>
<td>200</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>80%</td>
<td>11%</td>
<td>8%</td>
</tr>
</tbody>
</table>

### NIPS: Discrepant Results  Etiologies

- No test is perfect:
  - Test statistics and fetal fraction
    - 2.6 – 5.4% test failure (many due to low FF)
    - Lab error
  - Pregnancy factors
    - Confined placental mosaicism ~1%?
    - NIPS measures the genome of the entire cytotrophoblast
    - True fetal mosaicism
    - Co-twin demise (“vanishing twin”)
  - Maternal factors
    - sex chromosome abnormality (may increase with age)
    - marker chromosome
    - deletion or duplication
    - tumor with high levels of cffDNA
• The use of NIPS continues to expand:
  – General (low risk) population screening
  – Rarer conditions such as microdeletion syndromes

• The positive predictive value is dependent on the background incidence of the condition being tested.
  – High risk populations/common conditions = higher PPV
  – Low risk populations/rare conditions = lower PPV
Even if sensitivity and specificity are the same!

OFFERING TESTING AND DISCUSSING RESULTS

For those women who are at increased risk of a child with a prenatally diagnosable disorder with Mendelian pattern of inheritance,
microdeletion syndrome, and some other conditions, amniocentesis or CVS would still be indicated.
Guidelines for offering

NIPS can be offered to patients at increased risk of fetal aneuploidy

- Maternal age 35 years or older at delivery
- Fetal ultrasound findings indicating an increased risk of aneuploidy
- History of a prior pregnancy with aneuploidy
- Abnormal maternal serum screen for aneuploidy (first trimester, sequential, integrated, quad)
- Parental balanced robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21

ACOG and SMFM Committee Opinion 640, 2015
ACMG statement on NIPS for fetal aneuploidy, 2013

Guidelines for offering

- Conventional screening methods remain the most appropriate first-line choice for the general obstetric population*
- NIPS is not recommended for women with multiple gestations, because it has not been sufficiently evaluated in these groups**
- Routine NIPS screening for microdeletion syndromes should not be performed

ACOG and SMFM Committee Opinion 640, 2015
ACMG statement on NIPS for fetal aneuploidy, 2013

Other recommendations

- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed.
- Unreportable, indeterminate, or uninterpretable ("no call") results:
  - Increased risk of aneuploidy
  - receive further genetic counseling and be offered ultrasound evaluation and diagnostic testing

ACOG and SMFM Committee Opinion 640, September 2015
Other

- NIPS does not replace the utility of a first-trimester ultrasound examination, which has been proven to be useful for:
  - accurate gestational dating
  - assessment of the nuchal translucency region to identify a fetus at increased risk for a chromosome abnormality
  - identification of twins and higher-order pregnancies, placental abnormalities, and congenital anomalies.

- NIPS does not screen for open neural tube defects. Maternal serum α-fetoprotein testing should still be offered at 15–20 weeks gestation to screen for open neural tube defects even when NIPS is performed.

Talking to patients

Pre-test discussion

- Benefits and limitations – this is a screening test and does not look for all genetic conditions
- Explain test in context of clinical situation – what will a positive result mean? A negative result?
- Cost
- NIPS is NOT just a gender test

Talking to patients

Post-test discussion - Negative result

- Reassuring, but does not eliminate risk
  - Does not reduce risk of other abnormalities
  - Residual risk of a chromosome abnormality in the case of an abnormal serum screen and normal NIPS is reported to be about 2%
- Still recommend msAFP and anatomy ultrasound
Talking to patients

Post-test discussion - Positive result

- Increased risk, but is not diagnostic
  - Provide individualized risk if possible (PPV)
- Refer for genetic counseling
- Referrals to other specialists as needed
  - MFM, echo, MRI, fetal centers, neo, etc
- Confirmatory testing (CVS, amniocentesis, neonatal studies) is recommended
- Management decisions, including termination, should not be based on NIPS results alone

CASE EXAMPLES

Case 1

- 35 y.o. G2P0010 who conceived via in-vitro fertilization. No preimplantation genetic screening performed
  - Family history noncontributory
  - Declined genetic screening in the first trimester
- Second trimester anatomy ultrasound at 19 weeks detected bilateral ventriculomegaly and a heart defect
- Fetal echo confirmed complete balanced AV canal defect. Additional findings included a two-vessel cord.
AV canal defects

Etiology

• 40-50% - Down syndrome
• 32% - nonsyndromic
• 17.5% - other genetic syndrome
  – Deletion 8p, Noonan syndrome, VACTERL, Ellis-van Creveld, etc.

Outcome

• NIPS was drawn at 19 weeks and results were positive for trisomy 21
• Patient elected to continue pregnancy and due to NIPS results received consultation with the pediatric Down syndrome clinic during her pregnancy
• Confirmatory testing planned at delivery

Case 2

• 39 y.o. G2P1001 who was referred at 28w2d GA for fetal cardiac anomaly
  – Ultrasound findings consistent with Tetralogy of Fallot. No other abnormalities seen
• Prior NIPS drawn at 12 weeks was
  – Negative for aneuploidy of 13, 18, 21, X, or Y
  – No evidence of 22q, 15q, 5p, or 1p microdeletion
• Family history noncontributory
• Fetal echo at 28 weeks confirmed Tetralogy of Fallot with pulmonary atresia and a right aortic arch
Tetralogy of Fallot

• 4 to 5 per 10,000 live births
• 7 to 10% of cases of congenital heart disease
• Approximately 15 percent of patients with TOF present with associated syndromes:
  – Down syndrome
  – Alagille syndrome
  – DiGeorge and velocardiofacial syndromes (deletion on chromosome 22q11)
• Pulmonary atresia and/or arch anomaly more significantly associated with DiGeorge/22q11.2 deletion syndrome

Outcome

• Delivered at 33w GA
• Sample collected at birth and sent for CMA
  – 2.247 Mb deletion at 22q11.2
• False negative NIPS
  – Best option for prenatal testing based on ultrasound would have been CMA
• Maternal FISH was negative for the deletion
• Paternal genetic status unknown at this time

Case 3

• 15 y.o. G1P0 who was referred at 35w0d gestation due to multiple fetal brain anomalies, possibly consistent with Dandy-Walker malformation
• Family history noncontributory
• Patient offered and declined amniocentesis. Expanded NIPS drawn
• Results: increased risk for trisomy 9
  – No data available to provide a positive predictive value for trisomy 9
Trisomy 9

- Non-mosaic or complete trisomy 9 is a lethal diagnosis, with most fetuses dying prenatally or during the early postnatal period.
  - Few cases reported to have been detected by prenatal ultrasound.
  - Most cases result in first trimester spontaneous miscarriage.
  - Longest reported surviving case of full trisomy 9 less than 1 month.
- Most of individuals that survive to term are mosaic.
  - Multiple malformations, dysmorphic features, severe developmental and intellectual disability.

Again offered amniocentesis for diagnostic testing and to inform neonatal management. Patient declined. Postnatal genetics evaluation was arranged.

Outcome

- Delivered at 40 weeks 5 days gestation.
- Sample collected for chromosome analysis.
  - Karyotype: 46,XX normal female.
- CMA: 4.544 Mb deletion of 6p25.3p25.1 including the FOXC1 gene.
  - Deletions of FOXC1 have been reported in patients with Axenfeld-Rieger syndrome, type 3.
  - Terminal deletion is within the chromosome 6pter-p24 deletion syndrome region.
  - Similar sized deletions have been reported in patients with intellectual disability, developmental delay, ocular abnormalities, hearing loss and a characteristic facial appearance.

Predictions for the future

- Additional conditions.
- Additional populations.
- Technology evolution.
  - Intact fetal cells.
  - Non-invasive microarray/karyotype.

Things will continue to evolve at a rapid pace.
References


References cont’d

• Hume JH, Wardrop J, Boomer T, et al. Clinical outcome of subchromosomal events detected by whole-genome non-invasive prenatal testing. Accepted and pending publication.

THANK YOU!