Chorionic Villus Sampling and Amniocentesis:

*When did invasive testing get so dangerous??*

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Professor, Obstetrics Gynecology and Reproductive Sciences
Director, Maternal Fetal Medicine
Icahn School of Medicine at Mount Sinai
Increasing risk of chromosomal abnormalities with advancing maternal age.
Proportion of 1ˢᵗ births by maternal age
Technique and experience with transabdominal amniocentesis in 50 normal patients.
PARRISH HM, ROUNTREE ME, LOCK FR.

PMID: 13508758 [PubMed - indexed for MEDLINE]
TROPHOBLAST SAMPLING IN EARLY PREGNANCY. CULTURE OF RAPIDLY DIVIDING CELLS FROM IMMATURE PLACENTAL VILLI

BY

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DULCIE V. COLEMAN, Senior Lecturer, Clinical Cytology and Cytogenetics
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AND

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ACOG Practice Bulletin 88  2007 (reaffirmed 2013) :
Invasive diagnostic testing for aneuploidy should be available to all women, regardless of maternal age
Loss Rates Associated with *invasive (diagnostic)* procedures

- Amniocentesis: initially 1%
- Higher in CVS?
  - Concern for limb defects
- Later 1/200
- Even later 1/300-1/500 and equal with transabdominal CVS and amniocentesis
- More recently 1/300 – 1/500 but probably lower in experienced centers
- And now?... We will get to it
WHO’S FOR AMNIOCENTESIS?

Virtually all chromosomal aberrations and many biochemical disorders can be detected by amniocentesis and prenatal diagnosis. Although errors do occur in cytogenetic and biochemical investigations,¹,² there is a strong case for prenatal diagnosis, but the value of the test must be considered in relation to the potential complications involved.
Karyotype

Banding Resolution

Chromosome 12

Chromosome 11

Resolution:

>7-10 Million Base Pairs

(7-10 Mb)

Chromosomal Microarray (CMA)

Resolution:

< 0.5 Million Base Pairs

(< 500 kb)

Compliments of Dr. Ron Wapner
Chromosomal Microarray vs. Karyotype

ACOG Committee Opinion 581 2013

Microarray analysis:
- Higher resolution
- Yields more genetic information
- Can be obtained from uncultured cells (faster)
- Useful in fetal demise/stillbirth because does not require dividing cells
## Microdeletion Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Chromosome Region</th>
<th>Size (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiGeorge</td>
<td>22q11 Deletion</td>
<td>3.5</td>
</tr>
<tr>
<td>Miller Dieker</td>
<td>17p13.3 deletion</td>
<td></td>
</tr>
<tr>
<td>Prader Willi</td>
<td>15q11-13 deletion</td>
<td>4</td>
</tr>
<tr>
<td>Smith Magenis</td>
<td>17p11.2 deletion</td>
<td>5</td>
</tr>
<tr>
<td>Wolf Hirshhorn</td>
<td>4p16.3 deletion</td>
<td>1.9</td>
</tr>
<tr>
<td>Williams-Beuren</td>
<td>7q11.23 Deletion</td>
<td>1.5</td>
</tr>
</tbody>
</table>

## Non-Syndromic Micro Del /Dups

<table>
<thead>
<tr>
<th>Region</th>
<th>Condition</th>
<th>Size (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16p11.2</td>
<td>Autism</td>
<td>0.55</td>
</tr>
<tr>
<td>1q21.1</td>
<td>ID, microcephaly, cardiac, cataracts</td>
<td>0.8</td>
</tr>
<tr>
<td>16p13.11</td>
<td>Autism, ID, and schizophrenia</td>
<td>0.8</td>
</tr>
</tbody>
</table>

## Postnatal Studies

15-20% yield by CMA in children with unexplained developmental delay/ID, and congenital anomalies compared to ~3% with karyotype.
Incidence of pathologic microarray

1/90 fetuses
NOT age related
Trends in utilization of CVS and Amniocentesis

Turner et al Obstet and Gynecol May 2014
# Yearly Change in Volume of Prenatal Diagnostic Procedures 2008-2013

<table>
<thead>
<tr>
<th>Year</th>
<th>% Change</th>
<th>2008</th>
<th>2009</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>amnio</td>
<td></td>
<td>-8.5</td>
<td>-9.9</td>
<td>-13.8</td>
<td>-26.5</td>
</tr>
<tr>
<td>cvs</td>
<td></td>
<td>14.9</td>
<td>13.8</td>
<td>-5.5</td>
<td>-26</td>
</tr>
</tbody>
</table>
Is knowledge what women want?

- Randomized study of computer based counseling vs routine management
- Informed women given options chose to have LESS invasive testing than women who had usual care

Kupperman M et al JAMA 2014: 312(12)
Is NIPS the culprit?
cfDNA vs NT + serum markers

- 16,000 average risk women (76% < age 35)
- Trisomy 21 (primary outcome)
  - Detected in 100% (38/38) women in cfDNA vs 79% (30/38) in standard-screening group
  - FP rate 0.06% cfDNA vs 5.4% standard screen

Norton, M et al NEJM April 2015
• Trisomy 18
  – Detected in 90% (9/10) women in cfDNA vs 8/10 (80%) in standard screen
  – FP rate 0.01% cfDNA vs 0.31%

• Trisomy 13
  – Detected 100% (2/2) cfDNA vs 1 / 2 (50%) with standard screen
# NIPS for Common Trisomies

<table>
<thead>
<tr>
<th></th>
<th>Trisomy 18</th>
<th></th>
<th>Trisomy 13</th>
<th></th>
<th>Trisomy 21</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detection rate</td>
<td>False positive</td>
<td>Detection rate</td>
<td>False positive</td>
<td>Detection rate</td>
<td>False positive</td>
</tr>
<tr>
<td>Sequenom - Lo</td>
<td>84%</td>
<td>2%</td>
<td>44%</td>
<td>6%</td>
<td>100%</td>
<td>2.1%</td>
</tr>
<tr>
<td>Sequenom - Lo (GC correct)</td>
<td>92%</td>
<td>2%</td>
<td>100%</td>
<td>1%</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Sequenom – Palomaki</td>
<td>100%</td>
<td>0.3%</td>
<td>92%</td>
<td>1%</td>
<td>98.6%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Sequenom – Palomaki (GC correct)</td>
<td>100%</td>
<td>0.7%</td>
<td>92%</td>
<td>0.5%</td>
<td>99.1%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Verinata – Bianchi*</td>
<td>97%</td>
<td>0%</td>
<td>79%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Verinata – Bianchi (all samples**)</td>
<td>97%</td>
<td>0.6%</td>
<td>81%</td>
<td>0%</td>
<td>100%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Ariosa -Sparks</td>
<td>98%</td>
<td>0.1%</td>
<td>N/A</td>
<td>N/A</td>
<td>100%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

*with the “no call zone”  **at z=2.5 cut-off c/o Dr. Ronald Wapner

• False negatives
  – Inadequate sample
  – Fetal cfDNA at too low concentration
  – Confined placental mosaicism

• False positives
  – Maternal aneuploidy
  – Maternal cancer
  – Vanishing twin
  – Confined placental mosaicism
  – Failure of complex bioinformatics to generate result

• Not a diagnostic test
• Down syndrome comprise only 50% of aneuploidies
• Screening with NIPS alone or NT alone will miss other genetic disorders
• Currently CMA can only be done with “invasive” procedures
Are They Making an Informed Decision?

- widespread misconception that all intellectual disability = Down syndrome and that’s all we can test for...
Invasive testing is NOT obsolete
Advanced Maternal Age: Residual Risk for a Cytogenetic Abnormality after cffDNA

Amniocentesis Performed for AMA

Ferguson-Smith, M.A. Prenatal Diag 1984

<table>
<thead>
<tr>
<th>Maternal Age</th>
<th>Freq of Chrom Abn</th>
<th>% Trisomy 21,18,13</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 35</td>
<td>0.93%</td>
<td>18%</td>
</tr>
<tr>
<td>35</td>
<td>1.2%</td>
<td>37%</td>
</tr>
<tr>
<td>40</td>
<td>2.1%</td>
<td>68%</td>
</tr>
<tr>
<td>45</td>
<td>6.6%</td>
<td>78%</td>
</tr>
</tbody>
</table>

Data adapted from Wellesley, D, et al., Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. *Eur J of Hum Gen*, 11 January 2012.

c/o Dr. Ronald Wapner
Positive First Trimester Screen Residual Risk for a Karyotype Abnormality after NIPT

State of California Screening Program
N = 1,324,607 Screened

Screen Positive
68,990 (5.2%)

Invasive Testing
26,059 (37.7%)

Abnormal
2992 (11.5%)

Detectable by NIPT
2499 (74.9%)
(Includes Sex Chr 247 (8.2%))

Not Detectable by NIPT
504 (16.8%)

Mosaic 186 (6.2%)
Other Tris 92 (3.1%)
Ins/Del 88 (2.9%)
Structural 101 (3.3%)
Balan 97
(Unbal 3 (0.1%)
Triploid 29 (1.0%)
Marker 9 (0.3%)

For Patients with Positive First Tri Screen Residual Risk of Chromosome Abnormal after Normal NIPT
1:52

Norton: SMFM
What Patients Should Know

Basic Information

- Cytogenetic abnormalities occur in approximately 2% of pregnancies
  - Whole chromosome abnormalities 0.6%
  - Microdeletions and duplications 1.1%

- All cytogenetic abnormalities have clinical consequences varying from mild to severe
  - Both whole chromosome abnormalities and microdeletions can have very severe consequences
Advantages of Identifying an Etiology

- Pregnancy termination
- Referral to appropriate specialists & social services
- Targeted treatment
- Identify special education resources
- Appropriate surveillance & long term follow up
- More accurate recurrence risk estimate
- Prenatal diagnosis in subsequent pregnancies
- Referral to parent support groups and on-line resources
Should We Offer Microarray To All Pregnant Women
Copy Number Variants

Other Chromosome Abnormalities

Improved Screening

Diagnostic Testing

More Information

Diagnostic Procedure

Compliments of Dr. Wapner
So how do we counsel patients about DIAGNOSTIC (aka invasive) testing?
What do Societies Say?

- Royal College of Obstetricians and Gynecologists (RCOG) states that the additional risk of miscarriage from an AC is about 1% and the additional risks from a CVS may be slightly higher, about 1-2%.

- ACOG says that the procedure-related loss rate after AC is < 1 in 300-500 and that loss rates for CVS may be the same as AC.

- Committee opinion from the Society of Obstetricians and Gynaecologists of Canada (SOGC) states that the risks of loss following AC is unique to an individual and is based on multiple variables but may range from 0.19% to 1.53%.
Is that the truth?

“You can’t handle the truth!”
What are the CURRENT loss rates of diagnostic testing?

All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident.

- Arthur Schopenhauer (1788-1860)
Amniocentesis

- 7 studies 1986 – 2007
- Loss rate after amniocentesis
  - 394/39,065 = 1.0%
- Loss rate after no procedure
  - 852/98,439 = 1.0%
- Loss rate after amniocentesis: 1/1,000
What about Chorionic Villus Sampling?

Many women are scared by thought of doing CVS
Reasons to consider earlier diagnosis

- CVS only diagnostic procedure available in 1\textsuperscript{st} trimester
- Of those women who would consider pregnancy termination, 50\% would only undergo TOP in 1\textsuperscript{st} trimester
- Maternal death rate 1/100,000 after 1\textsuperscript{st} trimester TOP vs. 7-10/100,000 in mid-trimester
- Benefit for multiple gestations considering reduction of selective termination
Why is accurate data on CVS loss rates so difficult to come by?

- Little information on background loss rates
- Different definitions of fetal loss
- Majority of data about CVS loss rates are from studies comparing CVS to AC
  - Look at TOTAL loss rate (background + procedure)
    - Higher due to earlier GA when risk of spontaneous loss is higher
## CVS compared to no procedure

<table>
<thead>
<tr>
<th>Year</th>
<th>Study Design</th>
<th>Primary indication</th>
<th>Definition of loss</th>
<th>CVS (% loss)</th>
<th>No procedure (% loss)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 Caughey</td>
<td>Retrospective cohort TA/TC vs no procedure: Only last 5 years</td>
<td>Elevated MSAFP, AMA</td>
<td>Recent losses &lt; 28 weeks 1998-2003</td>
<td>1.2</td>
<td>0.3</td>
<td>ns</td>
</tr>
<tr>
<td>2008 Odibo</td>
<td>Retrospective cohort TA/TC vs. no procedure 1990-2006</td>
<td>AMA, abnormal screen</td>
<td>Fetal loss &lt; 24 weeks</td>
<td>2.7</td>
<td>3.3</td>
<td>ns</td>
</tr>
</tbody>
</table>

After controlling for potential confounders, and when data limited to pregnancies since 1998, loss rate following CVS were NOT significantly increased about background rate.
Amniocentesis and CVS: Systematic reviews and meta-analyses

• **Cochrane review 2003 of AC vs. CVS:**
  - total pregnancy loss rate following TA CVS = 2\textsuperscript{nd} trimester AC

• **Systematic Review Mujezinovic et al 2007:**
  - 29 observational studies of AC and 16 studies of CVS all published > 1995
  - Loss rate for CVS vs. AC:
    • 0.7% vs 0.6% within 2 w PP
    • 1.3% vs. 0.9% up to 24 w
• Risks loss < 24 w: **AC 0.81% and CVS 2.18%**
• Background risks with no procedure: **AC 0.67% and CVS 1.79%**
• Weighted pool procedure-related risks loss:
  – **AC:** 0.1% (95% CI -0.04 to 0.26%)
  – **CVS:** 0.2 (95% CI -0.71 to 1.16%)
• Concluded no significant risk of miscarriage < 24 in women who undergo AC or CVS and in those who do not have any invasive testing

• The added procedure-related risk after AC and CVS are in the nature of 0.1% and 0.2% and may be unrelated to the invasive procedure but may reflect the pregnancy characteristics of the women undergoing invasive testing

• Experience of individual performing procedure needs to be considered
Importance of counseling

• For all patients
  – All should be informed of options/limitations

• For multiples
  – Counseling should include discussion of MPR/ST

• Options for microarray
  – Increased detection of genetic disorders
  – Consideration of variants of unknown significance
  – Can also detect other maternal conditions, non-paternity, etc
My humble opinion...

• With almost NO risk, and a huge amount of information obtained, everyone should be offered (AND GET) CVS or amniocentesis
Thank you!!

GENOME PROJECT

WHEN I ASKED WHAT LITTLE GIRLS ARE MADE OF, I WAS HopING HE WOULD SAY "SUGAR AND SPICE."