Contemporary Prenatal Diagnosis – The Clinician’s Perspective

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Noninvasive Prenatal Testing (Screening)

- Introduced commercially October 2011
- High sensitivity and specificity in the high risk population
- Two types
  - Massive Parallel Shotgun Sequencing
  - Targeted Fetal DNA Sequencing
Criteria

- Currently: High risk population
  - 35 and above
  - Ultrasound findings
  - Increased risk via other screening
  - Family history

- Prevalence = 1/8 vs. 1/600 (low risk population)
- PPV 90% vs. 11%
Which is Best?

- Tough question
- MPSS (Sequenom, Vernata)
- Targeted Fetal DNA Sequencing
  - Ariosa DANSR/FORTE: hybridize, amplify, sequence
  - Natera: Massive multiplex isolation with SNP analysis
Sensitivities & Specificities

• All have high sensitivities
  – >99% DS and T18
  – More variable for T13
  – Less data for sex chromosomal abnormalities

• All have low false positive rates
False Negatives

- Gestational age (<10 wks)
- Fetal fraction
  - Maternal Weight
- Genetic Variants
- Failure to extract adequate material
- Individual variation in cfDNA amount
- GC rich regions
False Positives

- Contamination
- Vanishing twin
- Placental mosaicism (more in T13, 18, 21)
- Low level mosaicism (esp. sex chromo)
  - Maternal mosaicism (loss of X in older women)
- Maternal Cancers
  (only a few cases, no specific pattern)
## Failure Rates

<table>
<thead>
<tr>
<th>Trial</th>
<th>Failure Rate</th>
<th>DS Detection</th>
<th>FP rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiu et al. 2011</td>
<td>11/764 (1.4%)</td>
<td>86/86</td>
<td>3/146</td>
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<tr>
<td>Ehrich et al. (2011)</td>
<td>18/467 (3.8%)</td>
<td>39/39</td>
<td>1/410</td>
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<tr>
<td>Palomaki et al. (2011)</td>
<td>13/1696 (0.8%)</td>
<td>209/212</td>
<td>3/1471</td>
</tr>
<tr>
<td>Bianchi et al. (2012)</td>
<td>148/532 (3.0%)</td>
<td>89/89</td>
<td>0/404</td>
</tr>
<tr>
<td>Norton et al. (2012)</td>
<td>148/3228 (4.6%)</td>
<td>81/81</td>
<td>1/2888</td>
</tr>
<tr>
<td>Zimmerman et al. (2012)</td>
<td>21/166 (12.6%)</td>
<td>11/11</td>
<td>0/145</td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td><strong>424/6687 (3.2%)</strong></td>
<td><strong>424/427 (99.3%)</strong></td>
<td><strong>8/5319 (0.15%)</strong></td>
</tr>
</tbody>
</table>

*Note: Not all study designs the same, different techniques, variety of FP rates, thresholds to call DS risk have different methodologies*
Remember

- There is no free lunch
  - Nothing in biology is 100%
  - Are we going backwards in PNDx?
  - Does not detect many things... yet
ACOG, ACMG, ISPD, NSGC: Common Themes

- Great sensitivities and specificities for T21 & T18
- Not diagnostic
- Needs Genetic Counseling (pre- and post)
- Should only be used in validated groups
- More studies needed for the general population
Shifting Paradigms

Does NIPT replace other screening tests available today?

- Better sensitivity but... look what we are missing....
- First & second trimester ultrasound benefits
  - Increased NT, early defects, cardiac esp.
  - Other anomalies seen in embryological progression (cranial, skeletal, cardiac)

- Serum screening benefits
  - Unexplained increased MSAFP
  - Low uE3 (SLO, X linked ichthyosis, sulfatase deficiency, congenital adrenal hypoplasia, Zellweger, Antley Bixler, POMC deficiency, other cholesterol metabolism, IUGR, SAB)
  - Low PAPP-A
  - Combination of abnormal biochemical markers
Future

- Twin and population data
- Aneuploidy in all chromosomes
- Targeted microdeletion and microduplication syndromes
- “Low density” microarray (>10mB)
- Single gene defects (CF, β-thal, many others)
- Whole genome sequencing (ultimate goal)
Chromosomal Microarray (CMA)

- Introduced in the prenatal arena circa 2005
- Results and counseling still from postnatal databases
- Unknowns (VOUS)
- Comparative array hybridization vs. SNP oligo-array
CMA has changed Prenatal Diagnosis

- Increased detection of chromosomal variation
- Ability to detect absence of heterozygosity (SNP Oligo-array)
  - Consanguinity
  - UPD (heterodisomy is harder to detect)
  - Inherited disorders (AR, AD, X-linked)
  - Triploidy
- Both miss true balanced translocations (0.08-0.09%) and other balanced rearrangements
Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies

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Increases Detection

- Shaffer et al. Prenatal Diagnosis 2012
- 2004-2011
- N = 5003 prenatal cases, various reasons
- All known aneuploidy excluded from karyotype
- No fetal demises
- Detection of an additional 5.3% abnormals (6.5% & 8.2% for abnormal US and demise, respectively)
- 0.39% de novo copy number variations noted

- 71% found below the resolution of karyotype (<10Mb). Thus 29% should have been detected via karyotype!
Specific Ultrasound Anomalies

Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound  

n=2858 cases

- Clinically significant genomic alterations were identified in cases with a single ultrasound anomaly (n=99/1773, 5.6%)

- Anomalies in two or more organ systems (n=77/808, 9.5%), isolated growth abnormalities (n=2/76, 2.6%), and soft markers (n=2/77, 2.6%).

- High detection rates: holoprosencephaly (n=9/85, 10.6%), posterior fossa defects (n=21/144, 14.6%), skeletal anomalies (n=15/140, 10.7%), ventricular septal defect (n=14/132, 10.6%), hypoplastic left heart (n=11/68, 16.2%), and cleft lip/palate (n=14/136, 10.3%)

Shaffer et al, Prenatal Diagnosis 2012, 32: 986–995 (free)
“GENERAL” POPULATION?

• Issues:
  – Wapner et al. (NEJM 2012) showed 1.7% (1:60) of patients with abnormal CMA (aCGH) for AMA alone (no ultrasound findings) or abnormal serum screening
  – Positive Predictive Values decreases significantly
  – “Unknowns” – more so with Whole Genome/Exome Sequencing
The Unknowns – this is truly not unique to us

Copy number loss and gain

– Parentally inherited?
– Incompletely penetrant/variable phenotype?
– What genes are involved? Significance of these genes? Inherited disorders (AR, AD) involved? Does it agree with the phenotype?
– How large? Does this make a difference?
– What about future findings at this site? Are we obligated to follow up in the future? Who will take this responsibility?
How Do We Navigate Now?

- Talk with the patient: “nothing”, “everything” or “don’t know”
- Are patients truly informed?
- Find out the patient’s perception of risk and their comfort level
- The information (and decision) can be overwhelming for patients
- Time constraints for patient education (not everyone is at the same level)
- When to educate? Prenatal is ideal.
Paradox vs. Paradigm

- Noninvasive vs. Diagnostic (none vs. slight risk)
- Less vs. detailed information
- Missing clinically significant disorders vs. VOUS
- Explaining FP and FN with all tests
- Pleotropic phenotypes with all genetic disorders (or findings)
- Education for professionals and lay public
Thank you.

Questions?