Cytogenetic and Molecular Diagnosis in Gestational Disorders

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Overview

• Discuss the role of molecular genetic testing in evaluating pathologic conditions related to pregnancy
  – Viable pregnancy: prenatal screening and invasive diagnostic testing for genetic abnormalities
  – Pregnancy loss
  – Molar pregnancy

• Review laboratory testing strategies
1. PRENATAL SCREENING AND INVASIVE DIAGNOSTIC TESTING FOR GENETIC ABNORMALITIES
Meiotic errors are correlated with the number and location of recombination events.
Screening tests for genetic abnormalities of the embryo/fetus

• Noninvasive tests for common trisomies (21, 18, 13)
  – Ultrasound
    • First trimester – nuchal translucency
    • Second trimester – anatomy scan (18-20 weeks)
  – Maternal serum screening
    • Biochemical markers of aneuploidy (PAPP-A, beta-hCG, AFP, uE3, inhibin A)
    • Cell free fetal DNA

• Ethnicity-based (parental carrier) screening for single gene disorders
  – Cystic fibrosis (Caucasian, Ashkenazi Jewish)
  – Tay Sachs disease and others (Ashkenazi Jewish)
  – Hemoglobinopathies (Mediterranean descent)
Non-invasive prenatal testing (NIPT)

• Cell free fetal DNA
  – Short fragments of DNA circulating in maternal serum, small proportion (<10%) of total DNA
  – Detectable in maternal serum by 5\textsuperscript{th} week; short half life (disappears within minutes postpartum)

• Companies currently offering NIPT (massively parallel sequencing)
  – Sequenom MaternaT21\textsuperscript{TM} Plus
  – Verinata Health - verifi\textsuperscript{®} Prenatal Test
  – Ariosa Diagnostics - Harmony\textsuperscript{TM} Prenatal Test

• Other technologies in development

• The National Society of Genetic Counselors (NSGC) recommends genetic counseling with NIPT, and follow up of abnormal results with a conventional diagnostic procedure (amniocentesis)
Non-invasive prenatal testing (NIPT): current controversies

- Variably regarded as “diagnostic” vs. “screening” test
- Concern over direct-to-consumer marketing
- Large preclinical trials have been performed, but more clinical validation studies are needed
- Lower sensitivity and specificity for trisomy 13
- Changing landscape of prenatal genetic testing
  - No longer restricted to women > 35
  - Shift toward increased patient autonomy
- Destigmatization of Down syndrome
- State-led initiatives to restrict abortions, concern over potential increase in pregnancy termination rate
Definitive (diagnostic) tests for genetic abnormalities of the embryo/fetus

• Direct fetal sample is obtained via invasive procedure:
  – Chorionic villus sampling (11 - 13 weeks)
  – Amniocentesis (15 – 20 weeks)
• Offered to all pregnant women, along with genetic counseling, regardless of age (ACOG Practice Bulletin No. 77, 2007)
• Preferred method of follow up for abnormal ultrasound and/or abnormal prenatal screening (risk > 1:250 – 1:300)
• Cytogenomic testing methodologies:
  – Karyotype (chromosome analysis, cytogenetics)
    • All indications, including maternal preference for diagnostic testing
  – Genomic microarray for copy number variations, including microdeletion syndromes
Definitive (diagnostic) tests for genetic abnormalities of the embryo/fetus

• Targeted molecular genetic testing
  – FISH or microsatellite genotyping for aneuploidy
  – Single gene / gene panel testing for Mendelian disorders in the fetus
Chromosomal microarray

- DNA-based testing for copy number variations (CNVs) throughout the genome
- Size of detectable CNVs depends on array design and probe coverage

Affymetrix
CytoScan SNP array

- log2 ratio (copy#)
- “smooth signal” (copy#)
- allele difference track (SNP probes)
Microarray showing Trisomy 21
Microarray - 1q21 microdeletion (346.8 kb within 2 Mb common microdeletion region)
2. PREGNANCY LOSS
Pregnancy loss is common in humans

- 15-20% of clinically recognized pregnancies
  - Recurrent miscarriage (≥2): 1%
  - Most losses (90%) occur during the first trimester
- 30-40% of chemically detected pregnancies
  - In a 1988 *NEJM* study*, women who were trying to achieve pregnancy were monitored by daily urine beta hCG levels. A total of 31% of detected pregnancies were lost.
    - 2/3 of the lost pregnancies were undetectable clinically
- Unknown % of pre-implantation pregnancies
  - Cytogenetic studies of IVF pre-embryos at day 3 has shown that about 50% are genetically abnormal
- Probably <50% of conceptions result in live birth

Etiology

• An etiology for the loss can be determined in only about half of unintended pregnancy losses

• Genetic abnormality
• Immunologic factors
• Infections
• Endocrine
• Environmental agents
• Uterine anatomic abnormalities

• Cytogenetic abnormalities are identified in up to 50% of first trimester losses
Value of genetic testing on pregnancy loss: for patient care

Provides closure for families after a loss

Identifies abnormalities associated with a risk for recurrence

Identification of a genetic abnormality prevents additional costly workup for infertility
Fig. from Paxton CN et al, *Prenatal Diagnosis* 32, 1–7 [Epub 8 NOV 2012]
Distribution of abnormalities by mechanism

- Nondisjunction in meiosis: 75%
- Abnormal fertilization: 16%
- Error in zygote cleavage: 3%
- Breakage, aberrant recombination, and/or malsegregation: 6%
Cytogenetic Findings in POCs

- Aneusomy for virtually every chromosome
- 2-3% of trisomies associated with a potentially heritable translocation
- Secondary aneusomy in polyploidy
  - Triploidy: 9% have secondary trisomy or monosomy
  - Tetraploidy: 14% have secondary trisomy or monosomy
- Mosaicism (17% among all abnormal karyotypes)
  - Eliminating cases of likely maternal cell contamination, the true rate of mosaicism is probably 6%
<table>
<thead>
<tr>
<th>Anomaly</th>
<th>POCs at ARUP*</th>
<th>Spontaneous abortions**</th>
<th>Live births**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 13</td>
<td>1.56%</td>
<td>1.10%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>1.19%</td>
<td>0.84%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>2.86%</td>
<td>2.00%</td>
<td>0.11%</td>
</tr>
<tr>
<td>Trisomy 16</td>
<td>3.04%</td>
<td>5.58%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Other trisomies</td>
<td>9.52%</td>
<td>11.81%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>5.47%</td>
<td>8.35%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Sex chromosome trisomies</td>
<td>0.07%</td>
<td>0.33%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Triploids</td>
<td>5.50%</td>
<td>5.79%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Tetraploids</td>
<td>0.98%</td>
<td>2.39%</td>
<td>0.00%</td>
</tr>
<tr>
<td># Karyotyped</td>
<td>2,763*</td>
<td>3,353**</td>
<td>31,521**</td>
</tr>
</tbody>
</table>

*ARUP data estimated over 32 months of data

When Is a Karyotype Insufficient?

• Suspected molar pregnancy
• Nonviable tissue
  – Culture failure rate for fresh tissue: ~14%
  – Only paraffin embedded tissue available
• Uncharacterized structural abnormality
  – Marker chromosome
  – Additional material of unknown origin
  – Submicroscopic copy number variants (CNVs)
Chromosome 1-22: 4 allele tracks, copy number 2, consistent with triploidy
X chromosome shows 3 tracks, as for 46,XX, but copy number approx. 1.3
Chromosome 9 shows copy number approx. 1.7...

...consistent with mosaicism for -9
68<3n>,XX (55%)/67<3n>,XX,-9 (45%)
Characterization of a marker chromosome
45,XY,-21[8]/46,XX[11]
Chromosome 9 gain (copy number = 3)
Chromosome 21 mosaic loss (copy number approx. 1.85)

(Prevalence of cell line with monosomy 21 is about 15% in this sample)
Chromosome X shows copy number = 1, Y copy number = 1 (not shown), consistent with pure male population (direct sample).
Fetal demise – alobar holoprosencephaly

2 Mb loss on 2p21: arr 2p21(43,461,488-45,412,846x1) (hg19)
Metaphase FISH on POC material (used as positive control) RP11-489K22 BAC (Empire Genomics)

Mother carries same deletion by FISH
Absence of heterozygosity affecting 22.5% of genome
Stretches of homozygosity

• May indicate:
  – Uniparental disomy if present on a single chromosome
  – Identity by descent if present in numerous independent regions of the genome
  – Complete hydatidiform mole if 100% homozygous (monospermy) or ~50% homozygous (dispermy)

• Associated with increased risk for autosomal recessive condition
  – In pregnancy loss / fetal demise, consider potentially lethal AR condition
Microarray for pregnancy loss: summary

• Chromosomal microarray can be successfully applied to pregnancy loss samples, may yield different information than that provided by the karyotype, and is more sensitive and specific for characterizing clinically relevant genomic imbalances.

• Karyotype remains the most versatile method for detecting mosaicism and some polyploidy (tetraploidy); microarray is most valuable as a reflex test for POCs with a normal karyotype or POCs which fail to grow in culture.
3. MOLAR PREGNANCY
Hydatidiform Mole

• Associated risk for persistent trophoblastic disease

• Complete mole is often detected clinically, whereas partial mole is more often detected by the pathologist
  – BUT: histologic diagnosis is unreliable

• 3 major ancillary diagnostic tests (FFPE):
  – p57 immunohistochemistry (complete mole)
  – Flow cytometry (partial mole)
  – Microsatellite genotyping (complete and partial mole)
### Molar Pregnancy, 16 DNA Markers: 0051755

**Mnemonic:** MOL PREG

<table>
<thead>
<tr>
<th>Ordering Recommendation</th>
<th>Methodology</th>
<th>Performed</th>
<th>Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnose complete or partial molar pregnancy.</td>
<td>Polymerase Chain Reaction/Fragment Analysis</td>
<td>Mon-Fri</td>
<td>Within 14 days</td>
</tr>
</tbody>
</table>

**Collect:** Products of conception.

**Specimen Preparation:** Formalin fix and paraffin embed tissue containing areas of villi and decidua. In some cases, decidua may not be present in sufficient amounts, and a maternal blood sample may be requested. Transport tissue block.

**Storage/Transport Temperature:** Room temperature. Ship in a cooled container during the summer months.
Complete hydatidiform mole

- Diploid
- Complete uniparental disomy across entire genome
- No maternal DNA
- 15% risk for persistent gestational trophoblastic disease
46,XX
5 STR loci: comparison of genotypes of villi ("fetus") and decidua

Fig. 1 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. *Archives of Pathology and Laboratory Medicine* [In Press]
Partial hydatidiform mole

- Triploid
- 2:1 paternal: maternal DNA contribution
- 0.5% risk for persistent gestational trophoblastic disease
- not a risk factor for choriocarcinoma?
4 STR loci: comparison of genotypes of villi (“fetus”) and decidua

Fig. 2 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. Archives of Pathology and Laboratory Medicine [In Press]
Maternally derived triploidy (digyny): diploid egg

- Triploid
- 1:2 paternal: maternal DNA contribution
- Fetal anomalies
- NO risk for persistent gestational trophoblastic disease
Nonmolar hydropic abortion (HA)

Autosomal trisomy, sex chromosome aneuploidy, Mendelian disorders, non-genetic causes

May simulate molar pregnancy by histopathology
- Trophoblastic proliferation particularly +7, +15, +21, or +22
- Trophoblastic hypoplasia may also be seen (e.g. trisomy 18)
- Villous edema / hydrops
Incidental Trisomy Detection
(10/54 non-molar cases; not reported)
Unusual Case of Chimerism (Androgenetic / Biparental)

Fig. 4 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. 
*Archives of Pathology and Laboratory Medicine* [In Press]
Fig. 5 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. *Archives of Pathology and Laboratory Medicine* [In Press]
Conclusions

• New technology has introduced new options and new testing algorithms for prenatal screening and diagnosis

• Cytogenetic analysis is the most versatile method for whole genome analysis of pregnancy loss samples
  – Microarray is a useful adjunct method, largely because it can yield results in samples that fail to grow in culture

• Accurate diagnosis of hydatidiform mole relies upon ancillary testing