Objectives:

Brief discussion of two remaining placental topics:
Review timing of common placental pathologies.
Review the associations between poor perinatal outcomes and placental pathologies.

Perinatal Pathology Update:
Karyotype versus microarray analysis in perinatal medicine.
APPROXIMATE TIMING OF PLACENTAL PATHOLOGIES

Acute placental lesions (less than 6-12 h)
APPROXIMATE TIMING OF PLACENTAL PATHOLOGIES

Subacute placental lesions (at least 6-12 h and less than 1 week)
Acute chorioamnionitis with substantial fetal inflammatory response.
Prolonged meconium exposure (numerous meconium-laden macrophages
deep in chorion or associated myonecrosis.
Organizing fetal vascular thrombi.
Retroplacental hemorrhage with associated histologic findings (infarct).
FTV with villous stromal vascular karyorrhexis.
Recurrent intermittent UC compression.
Fetomaternal hemorrhage (some).
APPORXIMATE TIMING OF PLACENTAL PATHOLOGIES

Chronic placental lesions (>1 week)
MVU (small placenta, infarcts, distal villous hypoplasia, etc).
Fetal vascular obstruction (avascular villi).
Chronic villitis (VUE) +/- obliterative fetal vasculopathy.
Increased perivillous fibrinoid (affecting >10% villi).
Chronic abruption (chorionic hemosiderosis).
Placental Pathology: A Systematic Approach with Clinical Correlations

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PLACENTAL LESIONS ASSOCIATED ADVERSE OUTCOMES

**Preterm birth:** acute chorioamnionitis, chronic deciduitis with plasma cells, MVU, acute and chronic abruption.

<table>
<thead>
<tr>
<th>Gestational age (wks)</th>
<th>N</th>
<th>ACA</th>
<th>MV</th>
<th>Marginal abruption</th>
<th>Chronic abruption</th>
<th>LPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25</td>
<td>62</td>
<td>41 (66)a</td>
<td>3 (5)</td>
<td>18 (29)</td>
<td>6 (10)</td>
<td>9 (14)</td>
</tr>
<tr>
<td>26–31</td>
<td>124</td>
<td>63 (51)</td>
<td>24 (19)</td>
<td>24 (19)</td>
<td>9 (7)</td>
<td>14 (11)</td>
</tr>
<tr>
<td>32–36</td>
<td>226</td>
<td>51 (23)</td>
<td>43 (19)</td>
<td>39 (17)</td>
<td>20 (9)</td>
<td>14 (6)</td>
</tr>
</tbody>
</table>

ACA = acute chorioamnionitis, MV = maternal vascular underperfusion, partial or complete, and LPD = lymphoplasmacytic deciduitis.


* Number positive (percent positive).
PLACENTAL LESIONS ASSOCIATED ADVERSE OUTCOMES

IUGR: MVU, fetal vascular obstruction (FTV), HGCV, MVU, chronic abruption, increased perivillous fibrinoid.

<table>
<thead>
<tr>
<th>Placental lesions</th>
<th>IUGR, N = 66</th>
<th>No IUGR, N = 543</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;37 weeks</td>
<td>54%</td>
<td>55%</td>
</tr>
<tr>
<td>Maternal vascular obstruction</td>
<td>31 (47)b***</td>
<td>107 (20)</td>
</tr>
<tr>
<td>Fetal vascular obstruction</td>
<td>7 (11)*</td>
<td>19 (4)</td>
</tr>
<tr>
<td>Villitis of unknown etiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>4 (6)</td>
<td>30 (6)</td>
</tr>
<tr>
<td>Patchy/diffuse</td>
<td>13 (20)**</td>
<td>26 (5)</td>
</tr>
<tr>
<td>Perivillous fibrin(oid) deposition</td>
<td>11 (17)**</td>
<td>16 (3)</td>
</tr>
<tr>
<td>Chronic abruption</td>
<td>5 (8)</td>
<td>24 (4)</td>
</tr>
</tbody>
</table>

***p < 0.001, *p < 0.05 (Chi Square or Fisher’s exact test).  
Brigham and Women’s Hospital 1989—1990, Redline, unpublished data.

a Birth weight less than 10th percentile for gestational age.

b Number positive (percent positive).
Neurodisability: FTV, HGCV with obliterative vasculopathy, ACA with severe fetal inflammatory response, meconium +/- vascular wall myonecrosis, pathologic UC lesions.

**PLACENTAL LESIONS ASSOCIATED ADVERSE OUTCOMES**

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Neurodisability in term infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental lesions</td>
<td>Neurologic impairment, N = 125</td>
</tr>
<tr>
<td>Fetal thrombo-occlusive disease</td>
<td></td>
</tr>
<tr>
<td>&lt;15 Avascular villi/slide</td>
<td>18 (14)**</td>
</tr>
<tr>
<td>≥15 Avascular villi/slide</td>
<td>23 (18)**</td>
</tr>
<tr>
<td>Villitis of unknown etiology</td>
<td></td>
</tr>
<tr>
<td>No obliterative vasculopathy</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Obliterative vasculopathy</td>
<td>22 (18)**</td>
</tr>
<tr>
<td>Acute chorioamnionitis</td>
<td></td>
</tr>
<tr>
<td>No fetal vasculitis</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Fetal vasculitis</td>
<td>11 (9)</td>
</tr>
<tr>
<td>Severe fetal vasculitis</td>
<td>11 (9)*</td>
</tr>
<tr>
<td>Chorionic plate meconium</td>
<td></td>
</tr>
<tr>
<td>No fetal vascular necrosis</td>
<td>14 (11)*</td>
</tr>
<tr>
<td>Fetal vascular necrosis</td>
<td>17 (14)**</td>
</tr>
<tr>
<td>Pathologic umbilical cord lesion</td>
<td>19 (15)**</td>
</tr>
</tbody>
</table>

**p < 0.001, *p < 0.05 (Chi Square or Fisher’s exact test).**


a Number positive (percent positive).
PLACENTAL LESIONS ASSOCIATED ADVERSE OUTCOMES

**Maternal recurrent pregnancy loss:** MVU, chronic deciduitis with plasma cells, CV (VUE), MPVFD, chronic histiocytic intervilloitis.
PLACENTAL LESIONS ASSOCIATED ADVERSE OUTCOMES


Birth asphyxia defined by low cord pH and elevated base excess with resulting neonatal encephalopathy (NE).
PLACENTAL LESIONS ASSOCIATED WITH NEUROLOGIC DYSFUNCTION AND OTHER ADVERSE OUTCOMES

I. Sentinel event: severe placental perfusion defects resulting in asphyxia.
   * Abruptio placenta or uterine rupture
   * Acute umbilical cord occlusion
   * Acute fetal hemorrhage
   * Maternal hypotension

II. Placental pathology associated with CNS injury.
   * Fetal Vasculopathy:
     A - FTV;
     B - Chronic villitis with obliterative fetal vasculopathy (HGCV).
   * Prolonged partial asphyxia/chronic intermittent hypoxia:
     A – Chronic partial/intermittent UC compression:  abnormal insertion; decreased Wharton’s jelly or hypercoiling (>5 coils/10 cm); Entanglements.
     B – Subacute and/or chronic abruption.
   * Uteroplacental Insufficiency/Decreased Placental Reserve:
     A – Maternal malperfusion (MVU)
     B – Distal villous immaturity (delayed maturation).
     C – Pathologic perivillous fibrinoid deposition.

III. Placental Biomarkers for potential poor outcomes.
   * Fetal inflammatory response to AFI.
   * Meconium fetal vascular myonecrosis
   * Increased circulating nucleated fetal red blood cells.
PERINATAL PATHOLOGY ANNOUNCEMENTS

Useful Pediatric Pathology App for your cell phone.

Go to Apps store and type in PedsPath. This will bring up Peds Path Measurements (Free) by Adrian Arva. Very helpful to have handy organ weights and growth parameters.
POSSIBLE PERINATAL TOPICS FOR DISCUSSION

I. The Perinatal Autopsy

Perinatal pathology: practice suggestions for limited-resource settings.
PMID: 23721272 [PubMed - indexed for MEDLINE]


II. Stillbirth
Classification schemes for the causes of stillbirth (INCODE).

III. **Karyotyping versus Microarray**
THE UTILITY OF KARYOTYPE VS. MICROARRAY IN ASSESSING FOR GENETIC ABNORMALITIES.
A CASE

Clinical History:
• 32-34 week live born male neonate delivered via C-section at an outside institution.
• 28 year old G5P4 mother with no prenatal care and a history of drug use.
• Mother presented 12 hours prior to delivery with bleeding and foul smelling discharge.
• Ultrasound revealed a large abdominal wall defect and agenesis of the lower spine in fetus.
• At delivery Apgars were 7 and 8.
• Intubated/pressors and transferred to Lurie Children’s Hospital.
• Mother decided to pursue palliative measures and infant expired at 24 hours of age.
• A full autopsy was requested.
Post-mortem X-ray

- 9 pairs of ribs with partial fusion of right 9<sup>th</sup> and 10<sup>th</sup> ribs
- Probable vertebral segmentation anomaly in the lower thoracic spine
- Agenesis of the lumbosacral spine
- Pelvic bones unremarkable
- Normal mineralization
• Foot length and weight appropriate for gestational age of 30-32 wks.
• 5.3 cm central abdominal wall defect
  – Extraabdominal small intestines and portion of rt lobe of liver, no covering.
  – Gastrochisis vs. omphalocele
• 3 V UC at base of defect
• Bilateral popliteal pterygia
• Bilateral club feet
• Abnormally posteriorly positioned patent anus
Malrotated small bowel.
Polysplenia.
Absent pancreatic tail.
Bilateral cryptorchidism.
Bilateral absence of psoas muscles.
Chronic serositis with extensive granulation tissue entire GI tract.
Ectopic pancreas in submucosa of duodenum.
Kidney

• Single horseshoe kidney with cystic change.
• Bilateral hydroureter with focal areas of stricture.
Kidney with focal cystic renal dysplasia secondary to obstruction.
Infra-diaphragmatic vascular abnormalities: Arterial

AORTA BELOW THE DIAPHRAGM

DIAPHRAGM

UPPER GI TRACT

LEFT UMBILICAL ARTERY

RIGHT UMBILICAL ARTERY

PROBABLE RENAL ARTERIES

STRicture

RIGHT COLON

PANCREATIC BRANCH

LOOPS OF SMALL BOWEL IN GASTROCHISIS

LOOPS OF SMALL BOWEL
Infra-diaphragmatic vascular abnormalities: Venous

• No definitive inferior vena cava.
• A confluence of veins from kidneys, GI, adrenals formed a vein that passed separately and centrally through the diaphragm.
Other Findings

- Left hand with single transverse palmar crease.
- Grossly normal heart and lungs.
- Aberrantly low set right lobe of thyroid.
CNS

- Cortical dysplasia.
- Partial fusion of thalami.
- Spinal cord with lumbosacral fusion of grey matter with dysplasia – consistent with sacral agenesis.
- Mild hypoxic ischemic injury (ponto-subiculum neuronal necrosis with periventricular leukomalacia).
Acute Findings

• Small pulmonary artery branches with thrombi.
Placenta with chorioamnionitis

- Maternal inflammatory response, stage 2
- Fetal inflammatory response, stage 2
Summary

• Multiple congenital anomalies

• Evidence of acute chorioamnionitis in placenta

• Evidence of neonatal DIC
Differential Diagnosis?
DIFFERENTIAL DIAGNOSIS:

Chromosomal anomaly (normal male karyotype 46,XY)
More subtle cytogenetic anomaly (CGH results).
Caudal regression syndrome:
  - abdominal wall defects
  - kidney anomalies
  - others
  - not reported polysplenia, pancreas, thyroid anomalies

Other syndrome (OMIM search negative).
Amniotic band disruption sequence.
Infection (TORCH).
Lower mesodermal defects sequence.
Teratogen exposure (diabetes, retinoic acid, minoxidil).
Defect in normal vasculogenesis/angiogenesis.
Mastroiacovo et al. (2007) analyzed 3,322 cases of gastroschisis from 24 birth defect registries worldwide and found that
   - 469 (14.1%) cases were registered as 'nonisolated,' including 41 chromosomal syndromes, 24 other syndromes, and 404 multiple congenital anomalies (MCA).
     • Among MCA cases, 4 groups of anomalies were most frequent:
       - CNS (4.5%),
       - cardiovascular (2.5%),
       - limb (2.2%),
       - kidney anomalies (1.9%).
   - Two patterns emerged:
     - 26 MCA cases resembling limb-body wall complex
     - 26 others resembling the omphalocele-exstrophy-imperforate anus-spinal defects complex
     - in both situations, omphalocele rather than gastroschisis is more commonly reported,
     - and the authors noted that these cases may represent misdiagnoses of the abdominal wall defect.
Additional Molecular Workup by:

Lawrence Jennings MD, PhD
Katrin Carlson Leuer PhD
This was a very unusual case.

Initially we did chromosome analysis on peripheral blood that showed a normal male karyotype 46,XY in 20 cells.

Then the CGH microarray results came back with an abnormality dup(3)(q23q29).

Phenotype is not the same as seen in chromosome 3 duplication q/ deletion p syndrome (craniofacial and cardiac defects).

CGH array results showing dup(3)(q23q29) 58Mb contains 346 genes

Dr. Larry Jennings (Head of Lurie Children’s Molecular Diagnostics Laboratory)
This abnormality is relatively large by microarray and it should be present in a large % of cells (ratio ~ .3).

Why did the karyotype come back as normal?

Drs. Jennings and Carlson-Leuer decided to use a chromosome 3 painting probe and see where this 3q material was inserted in the genome.
FISH for chromosome 3:
An abnormal extra chromosome 3 was found in about 1 out of 100 metaphase spreads.
47,XY,+mar  "mar"=marker chromosome on chromosome of unknown origin
The "mar" was found in only one cell of more than 100 analyzed.
A typical analysis looks at 20 cells.
There were two confusing issues:

1) The marker chromosome was much larger than the duplicated segment identified by microarray would have predicted it to be.

2) Only 1 in 100 metaphase spreads showed the marker chromosome and this does not seem to account for the quantity seen by CGH.
Marker chromosome is much larger than duplicated segment predicted by array.

To resolve this we performed FISH with a single locus probe (BCL6)
We performed FISH with a probe to the BCL6 gene which maps to 3q27.
Given that BCL6 hybridized twice to the marker we conclude that the marker chromosome contains at least 2 copies (possibly more) of the 3q23-q29 region. The marker chromosome does not represent a simple translocation event and is larger because it involves multiple copies of 3q23-q29.

Why is the % of cells containing the marker chromosome so low?
1) The marker may contain more than 2 extra copies?

2) The BCL6 marker was also assessed in interphase cells and about 47% of the cells showed duplicated signals. This suggests true mosaicism for this marker chromosome in about half of all cells (c/w CGH). Cell culture artifact?
FINAL DIAGNOSES:

Multiple congenital anomalies due to mosaicism for an extra chromosome containing duplications of part of chromosome 3q. (male karyotype 46,XY + mar)

Preterm premature delivery due to acute chorioamnionitis.

Diffuse microthrombi in pulmonary artery branches.
KARYOTYPE VS MICROARRAY

When do you perform karyotype vs. microarray?

What are the advantages and disadvantages of microarray?

What are the current recommendations for microarray?

How do the recommendations affect pediatric pathologists and the perinatal autopsy?
Background:

*Karyotype* has limited success of obtaining viable cells:
Prenatal amnio/CVS 84%
Stillbirth <50%

Karyotype turn-around time >3 weeks.

Karyotype detects gene defects (amplifications/deletions) 3-10Mb.

*Chromosome microarray* success >85% (can use FFPET)

CMA turn-around time 1 week.

CMA detects defects of 50-200kb (higher diagnostic yield).
Chromosomal Microarray (CMA) Background:

CMA: uncultured amniocytes, CVS cells, fetal cells, cell free fetal DNA or formalin fixed paraffin embedded tissue. Can use even more highly degraded DNA.

Copy number variants (CNV): deleted or duplicated DNA fragments detected by CMA. Human disease is associated with CNVs in 15% of cases. Some CNVs clearly associated with known disorders but other CNVs are of unknown significance (variants of unknown significance – VOUS).

Methods of CMA:
Comparative genomic hybridization (CGH). Single nucleotide polymorphisms (SNP).
ADVANTAGES of CMA vs. KARYOTYPING:

1 – *Higher diagnostic yield* due to smaller changes detected by CMA.

Multiple studies have shown CNVs in 2-3% of normal prenatal patients and in 6-8% of fetuses with structural abnormalities detected by US – all with normal karyotypes.

2 – *Faster turnaround time* for CMA (no cells need to be grown).
DISADVANTAGES CMA vs. KARYOTYPING:

1 – CMA does not detect balanced translocations (rare and often no pathology).

2 – CGH CMA does not detect triploidy.

3 – CMA has variable ability to detect low level mosaicism (SNPs better).

4 – CMA detect variants of unknown significance:
   - parental anxiety
   - mandates both pre- and post-CMA genetic counseling ***
   - VOUS occur in 1-2% of patients (number decreasing with time)

5 - CMA is more expensive than karyotype (2-3 fold higher) – cost of counseling not included.

6 – CMA demonstrates variable clinical sensitivity (point mutations missed).
Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis


CONCLUSIONS

In the context of prenatal diagnostic testing, chromosomal microarray analysis identified additional, clinically significant cytogenetic information as compared with karyotyping and was equally efficacious in identifying aneuploidies and unbalanced rearrangements but did not identify balanced translocations and triploidies.
Authors used a SNP microarray and frozen unfixed tissue samples.

Failed testing
Normal result
VOUS – variant of unknown significance
Pathologic CNV (copy number variant)
Aneuploidy
CMA results on those with normal karyotype

Failed testing
Normal result
VOUS – variant of unknown significance
Pathologic CNV (copy number variant)
Aneuploidy

<table>
<thead>
<tr>
<th>Result</th>
<th>Percentage</th>
<th>Count (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failed</td>
<td>12.5%</td>
<td>43</td>
</tr>
<tr>
<td>Normal</td>
<td>80.8%</td>
<td>278</td>
</tr>
<tr>
<td>VOUS</td>
<td>4.1%</td>
<td>14</td>
</tr>
<tr>
<td>Pathogenic CNV</td>
<td>2.6%</td>
<td>9</td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>0.0%</td>
<td>0</td>
</tr>
</tbody>
</table>
CMA results on patients with abnormal karyotype

Failed testing
Normal result
VOUS – variant of unknown significance
Pathologic CNV (copy number variant)
Aneuploidy

Abnormal
5.8% (N=31)
8.3% (N=31/375)

Failed 6.5% (N=2)
Normal 6.5% (N=2)
VOUS 3.2% (N=1)
Pathogenic CNV 3.2% (N=1)
Aneuploidy 80.6% (N=25)
Summary of microarray results for stillbirths with aneuploidy, VOUS, or pathogenic variants in which karyotyping failed or was normal:

**Failed karyotyping:**
2 cases  Trisomy 18  
2 cases  Trisomy 21 (one with XXY as well)  
3 cases  Monosomy X  
2 partial deletions  1q; 22q  
10 partial duplications  6 cases 19p13; 2 cases 21q; 1 case Xq27; 1 case 5p15

**Normal karyotyping:**
6 partial deletions  22q11; Xp22; 7q11; Yq11; 1p35; 16p11  
1 mixed (dup & del)  4q32(del) & 17p13(dup)  
16 partial duplications  22q11; 18p11; 2 cases 16p13; 17q21; 3 cases 19p13; 15q12; 19p12; 6p25; 10q23; 19q13; 8q24; 3p21; 11p13

No 3q duplication described.

**Overall:** 1 - stillbirths with congenital anomalies - genetic abnormalities IDed by microarray 30% compared to karyotype 19%, P=0.008.  
2 – useful results from 87.4% microarrays and 70.5% karyotyping P<0.001.

**Limitations:** Balanced rearrangements can be missed by microarray.  
Low level mosaicism can be missed by microarray analysis.  
Cost of microarray > karyotyping.
INDICATIONS FOR CMA:

**Stillbirth:** (>20 weeks). CMA detects increased CNVs and especially useful if abnormalities present. ** Care must be taken in ordering CMA from autopsy material (need informed consent (beyond standard autopsy permission). Currently we demand that the OB order the CMA and we simply provide the autopsy tissue for analysis.

**Post-natal:** CMA useful in identifying CNVs in patients with autism, altered cognitive abilities and congenital anomalies (includes CHD).

**Pre-natal:** ACOG Bulletin.
The Use of Chromosomal Microarray Analysis in Prenatal Diagnosis
ACOOG BULLETIN SUMMARY:

1 – CMA should replace karyotype for patients with a fetus with >1 anomaly detected by US and undergoing invasive prenatal diagnosis.

2 – CMA or karyotype can be used after invasive prenatal diagnosis if normal fetus by US.

3 – Maternal age is not related to CMA.

4 – IUFD or stillbirth CMA is recommended due to higher success rate.

5 – Usefulness of CMA in first and second trimester loss is limited and does NOT recommend CMA at this time (more studies needed).

6 – Comprehensive pretest and posttest genetic counseling must be Offered. CMA should not be ordered without informed consent. Counseling should involve discussion of VOUS, nonpaternity, consanguinity, and ID of adult onset diseases.
Box 1. Information to Share With Patients Before Prenatal Chromosomal Microarray Analysis

- Chromosomal microarray analysis will identify almost all of the abnormalities that are identified by fetal karyotyping and may identify additional specific genetic diseases. It will not identify all genetic disorders.
- Diseases may be identified for which the clinical presentation may vary greatly and range from mild to severe. It may not be possible to predict what the outcome will be in a given patient.
- The test may identify consanguinity (a close blood relationship or incest) or nonpaternity.
- Genetic changes may be identified that may or may not cause disease. Samples from both parents may be required to help understand the significance of these results.
- Test results may identify adult-onset diseases that will not affect health during the newborn period or childhood but may have unknown severity later in life. Identification of such findings may also indicate that one of the parents has the same adult-onset disease but has not yet developed symptoms.
CONCLUSION AND TAKEHOME MESSAGE

Microarray analysis is more likely than karyotype analysis to provide a genetic diagnosis, especially in the setting of multiple congenital anomalies.
REFERENCES:


