The Genetics of Pregnancy Loss

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Disclosures

• None

GENETICS
This is How it Works
Objectives

• Review the current technology available for genetic testing in pregnancy loss
• Review the literature regarding incidence of genetic abnormalities in pregnancy loss
• Review recommendations for testing patients with pregnancy loss

Patient Example

• Mrs. Lopez is a 22 year old G1P0 who comes for her first prenatal visit. She has no medical issues. Today, she is 9 weeks gestation by last menstrual period (LMP). You perform an ultrasound – and find an embryo with no cardiac activity, crown-rump length measurement consistent with 8 weeks gestation.
• What do you do for this patient?

What kinds of genetic testing can be done for pregnancy loss?
Step 1 – obtain the sample

Tissue biopsy
Amniocentesis

Karyotype has an 80-100% success rate from amniotic fluid obtained before delivery, 10-30% from skin or umbilical cord, and ~40% for fascia.

Step 2 - analyze the sample

Karyotype
Step 2 - analyze the sample

Karyotype

Pitfalls of karyotype

• Takes 14 days
• Requires live cells for culture
• Normal maternal cells can overgrow abnormal fetal cells
• Doesn’t catch small deletions or duplications that can be very significant

Benefits of karyotype

• Many years of experience with the technology
• Very accurate for aneuploidy
• Relatively inexpensive
Step 2 – Analyze the sample

FISH

Benefits and Pitfalls of FISH

• Of limited utility – helpful when karyotype fails, because it can be done from preserved tissue blocks
• Can only detect what you actively look for; you decide what probes you will use (common trisomies, etc)

Single gene disorders

• Several are associated with stillbirth – hemoglobinopathies, metabolic disorders
• Concern increased if consanguinity
• If predominantly male fetuses lost – consider X-linked disorders (Rett)
Step 2 – Analyze the sample

Microarray

DNA fragmentation and ligation of adapters, PCR amplification, labeling, hybridization, wash to remove unbound DNA →
Scanning and file conversion →
Interpretation using specialized software

Microarray

Benefits of microarray

- Detects very small deletions/duplications and LOH
- Does not require live tissue
- Results available in 7 days
- Very accurate
  - SNP/oligo arrays have improved sensitivity over CGH
Pitfalls of microarray

- Does not detect balanced translocations
- Does not detect tetraploidy
- Some of the findings fall under “VOUS” – variant of unknown significance
- $$

Pitfalls of microarray - VOUS

- Dependent on parameters for “calls”
  - NEJM Stillbirth study - >500kb
  - Ongoing study in loss <20 weeks: software flags at deletions >50kb, duplications >200kb
- Calls compared against databases
  - OMIM
  - Database of Genomic Variants

Pitfalls of microarray – ethical concerns

- Accidental findings (Huntington’s, BRCA)
- Uncertainty of results can be distressing to patients – “Toxic Knowledge”
Other testing

- Whole genome sequencing – not yet widely available, experimental

Testing - Review

Testing summary

- Tissue: amniotic fluid preferred over biopsy
- Testing options:
  - Karyotype/ FISH
  - Microarray
  - Others?
Why do any of these tests?

- Let’s review the available information about incidence of genetic abnormalities in pregnancy loss

Risk factors for chromosomal abnormalities

- Increasing maternal age
- Anatomic abnormalities in the abortus
- Growth restricted on ultrasound

Pre-embryonic and Embryonic losses: <10 weeks gestation

<table>
<thead>
<tr>
<th>Type</th>
<th>Approximate proportion of abnormal karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneuploidy</td>
<td></td>
</tr>
<tr>
<td>Autosomal trisomy</td>
<td></td>
</tr>
<tr>
<td>Autosomal monosomy</td>
<td></td>
</tr>
<tr>
<td>45, X</td>
<td></td>
</tr>
<tr>
<td>Triploidy</td>
<td>16%</td>
</tr>
<tr>
<td>Tetraploidy</td>
<td>6%</td>
</tr>
<tr>
<td>Other</td>
<td>7%</td>
</tr>
</tbody>
</table>
Fetal loss (10-20 weeks)

- Chromosomal abnormalities in 6% to 12%
- Higher in fetuses with structural abnormalities
- Most common: aneuploidy (with the most common being Trisomy 21, 18, and 13; others still present)
- If normal karyotype in fetus but abnormality in the placenta (Confined Placental Mosaicism) – severe IUGR, fetal death
  - Higher risk if AMA
  - Can be from mitotic nondisjunction

Losses at or beyond 20 weeks: Stillbirth

Karyotype versus Microarray Testing for Genetic Abnormalities after Stillbirth

[Image of journal article]
Results

• 532 stillbirths had both karyotype and microarray done
• 70.5% of karyotypes worked; 8.3% of those were abnormal
  – In cases of karyotype failure (N=157), microarray was successful in 86% (N=135)
• 87.4% of microarrays worked; 9.5% were abnormal, 5.4% were VOUS (so possibly abnormal)
  – In cases of microarray failure (N=67), karyotype was successful in 67.2% (N=45)

Ongoing research

• Enrolling patients with pregnancy loss <20 weeks gestation
• Aim is to compare rates of genetic abnormalities across 3 different periods of gestation – pre-embryonic, embryonic, and fetal
• Using microarray (Affymetrix CytoScan Array) for analysis of products of conception
• Collecting parental DNA and analyzing the triad for further information in cases of VOUS in the POC’s
Ongoing research

- Preliminary results (out of 85 enrolled):
  - 36 normal
  - 13 trisomies (22 [2], 21, 18[2], 16 [1], 15 [1], 13[2], 9 [1], 8 [2], X[1-mosaic])
  - 1 monosomy X
  - 4 triploid

Ongoing research

- VOUS
  - 31 total
    - 3 with questionable significance (also aneuploid)
  - 3 in HOX genes, others in regions of possible interest –
    will review with research team after recruitment completed
  - 15 with no result
    - 3 arrays failed
    - 12 no POCs in tissue brought from home

Incidence of genetic abnormalities in pregnancy loss - Review

- Highest in early losses
- More common in later losses if fetal anomalies present
- More common with advancing maternal age
- Single gene disorders can also be associated with loss
So what should I do for Mrs. Lopez?

- Let’s review the recommendations regarding genetic testing in pregnancy loss

Mrs. Lopez

- G1P0 with no medical issues, first pregnancy ends in an embryonic loss
  - No testing necessary
    - Some authors advocate for testing even in sporadic loss, to help families obtain closure, individualized prognosis for future pregnancies
    - With sporadic loss in early 1st trimester – recurrence risk is 12-14%

What about Mrs. Lopez’s cousins?

- What if this was her 3rd loss?
  - Recurrent Pregnancy Loss (RPL) is defined in obstetrics as three embryonic or fetal deaths which are not iatrogenic and having the same paternity, with no more than one live birth
  - Recommend genetic evaluation, both of the parents (looking for translocations) and products of conception
  - Thorough history is important
What about Mrs. Lopez’s cousins?

- What if she were >10 weeks (fetal loss)?
  - Genetic evaluation as part of workup recommended
  - Consider microarray, particularly if anomalies noted on ultrasound

References

Questions?

Thank you!