The Genetics Consultation in OB-GYN:
Reproductive pathologies and prenatal diagnosis

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Etiologies of malformations / genetic disorders

- **Chromosomal** (monosomies, trisomies)
- **Monogenic** (dominant, recessive, X-linked)
- **Exogenous** (pre- or postnatal environment)
- **Multifactorial** (predisposition + environment)
Genetic resolution levels

- **HUMAN GENOME**
  - about 30,000 genes
    - (3,000,000 kilobases of DNA)

- **AVERAGE CHROMOSOME**
  - about 1500-2000 genes (150,000 kilobases)

- **AVERAGE METAPHASE BAND**
  - about 100 genes (7500 kilobases)
Prenatal diagnosis in 2001: detection of disorders of genetic etiology

- **Chromosomal anomalies**
  - all chromosomes studied (all but subtle structural aberrations detected); high-risk populations tested

- **monogenic disorders**
  - direct analysis (mutations defined)
  - indirect (gene localisation known)

- **multifactorial etiology**
  - diagnosis by indirect means (US; mat. serum)
Prenatal diagnosis: Limitations

- We can diagnose, but rarely treat
- We can diagnose presence of the disorder, but rarely its severity
- Test which give the most information carry risks for the pregnancy
- Results available “late” in pregnancy
- No sure means of prenatal diagnosis for the majority of malformations / syndromes
Non-invasive Prenatal diagnosis:

- Ultrasound
Types of prenatal diagnosis

- **Non-invasive tests**
  - Ultrasound
  - Maternal serum screening

- **Invasive tests**
  - Amniocentesis
  - Chorionic villus sampling (CVS)
  - Cord blood sampling (cordocentesis)
Ultrasound markers (for Down syndrome)

- Nuccal translucency (1st trimester)
- Intestinal symptoms
- Skeletal alterations (including growth)
- Malformations
“Noninvasive” prenatal diagnosis

- Maternal serum screening
  - in the second trimester
  - in the first trimester
  - combined screening
Second trimester serum markers

- Alpha-fetoprotein (AFP)
- Human chorionic gonadotropin (HCG)
- Unconjugated estriol (uE3)
First trimester serum markers

- Pregnancy-associated plasma protein A (PAPP-A)
- free beta-HCG
- Others??
  - Schwanger-shafts protein 1 (SDP-1)
  - prostate-specific antigen (PSA)?
Maternal genes

Maternal Weight

Fetal genes

Fetus & Placenta: production of “serum markers”

Ethnic group

Maternal disease

Medications?

?????
Performance of Down syndrome screening at 8-14 weeks gestation
(from Kennard & Wald, 1996)

<table>
<thead>
<tr>
<th>Risk cut-off level</th>
<th>Maternal age detection rate (%)</th>
<th>False-positive rate (%)</th>
<th>OAPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:100</td>
<td>45</td>
<td>1.5</td>
<td>1:25</td>
</tr>
<tr>
<td>1:200</td>
<td>57</td>
<td>3.5</td>
<td>1:47</td>
</tr>
<tr>
<td>1:300</td>
<td>63</td>
<td>5.5</td>
<td>1:67</td>
</tr>
<tr>
<td>1:400</td>
<td>68</td>
<td>7.4</td>
<td>1:84</td>
</tr>
<tr>
<td>1:500</td>
<td>72</td>
<td>9.5</td>
<td>1:102</td>
</tr>
</tbody>
</table>
Down syndrome rates and false-positive rates
(using triple test at different ages; risk cut-off 1/250)
(from Kennard & Wald, 1995 & 1996)

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>Detection rate (%)</th>
<th>Positive Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>35</td>
<td>66</td>
<td>13</td>
</tr>
<tr>
<td>40</td>
<td>89</td>
<td>41</td>
</tr>
<tr>
<td>45</td>
<td>99</td>
<td>79</td>
</tr>
</tbody>
</table>
Maternal serum analysis

(hormones and fetal products in maternal circulation)

**Advantages**
- No risk for the pregnancy
- May reduce the number of invasive tests requested
- *Screening* test which indicates at risk pregnancies
- Can be applied on a large scale (general population)

**Disadvantages**
- Gives only a risk of being affected; requires additional analyses
- Second trimester screening gives late results (later amniocentesis)
- Knowledge of physiological factors affecting markers still incomplete
Counseling issues in serum screening

- Who to screen?
  - all pregnancies?
  - all but those who will have amniocentesis?
  - only women who would have amniocentesis and potential abortion?

- How to inform?
  - explained by gynecologist before test?
  - explained by gynecologist only after results?
  - by brochure or video, questions afterwards?
  - during prenatal consultation with geneticist?
Counseling difficulties linked to serum screening

- Time and timing (for counseling)
- Generation of anxiety
- False positives and false negatives
- Scientific unknowns
- Insurances and financial factors
- Disagreement within couples
- How far to go?
Analysis of fetal cells in maternal circulation

- A promising technique en theory, but:
  - technically difficult to separate fetal cells
  - genetic testing is limited by cell number
  - “misuse” of methods to be anticipated!

- Current research concentrating on isolation of free circulating fetal DNA
Invasive methods

- Amniocentesis
Cytogenetics: Indications for testing

- Prenatal tests
  - advanced maternal age
  - precedent gene or chromosomal disorder
  - previous abnormal infant or pregnancy
  - positive serum screening, anxiety

*Trisomies, monosomies, and major structural anomalies detected; no routine gene analysis*
Mosaicism on amniocentesis
(from Hsu et al. PRENAT DIAGN 12:555-573)

<table>
<thead>
<tr>
<th>Origin of data</th>
<th>Cases studied</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>US survey</td>
<td>62,279</td>
<td>0.25</td>
</tr>
<tr>
<td>European sur.</td>
<td>44,170</td>
<td>0.10</td>
</tr>
<tr>
<td>Canadian sur.</td>
<td>12,386</td>
<td>0.30</td>
</tr>
<tr>
<td>PDL data</td>
<td>12,000</td>
<td>0.20</td>
</tr>
</tbody>
</table>
FISH: current uses in prenatal diagnosis

- family history of microdeletion/duplication
- determining chromosomal content of abnormal chromosomes
  - chromosomal paints
  - centromeric probes
- searching for mosaicism when abnormal cell(s) have been seen
- screening for common aneuploidies using uncultured cells (interphase)
Interphase FISH showing three copies of chromosome 2 (yellow). Control probe is chromosome 4 (red).
Invasive methods

- Choriocentesis
  (chorionic villus sampling- CVS)
CVS is the preferred method of prenatal testing for DNA (gene) analysis!
Chromosomal mosaicism detected by Chorionic Villus Sampling

occurs in 1-2% of CVS

-- necessitates further analyses to determine whether it is fetal or only in placenta and adnexes (further culture, amniocentesis, ultrasound)

-- the majority of mosaic cases are LIMITED TO THE PLACENTA, but...

-- depending on the chromosome, may predispose to reproductive pathologies (growth retardation, etc) even if confined to placenta
True Mosaicism or CPM?

- diploid
- aneuploid

? ?
## Amniocentesis vs. Choriocentesis

<table>
<thead>
<tr>
<th><strong>Amniocentesis</strong></th>
<th><strong>Choriocentesis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>higher success rate</td>
<td>results available earlier</td>
</tr>
<tr>
<td>superior cytogenetic image</td>
<td>preferred method for DNA analysis</td>
</tr>
<tr>
<td>results reflect fetal karyotype</td>
<td>option to verify by amniocentesis</td>
</tr>
<tr>
<td>very few false negatives or positives</td>
<td>problem of placental mosaicism</td>
</tr>
</tbody>
</table>
Invasive methods:

- Fetal blood and tissue sampling
The choice of a method of prenatal diagnosis...

- depends on what type of analyses are planned
- for cytogenetic studies:
  - amniocentesis or choriocentesis
  - each method has advantages and disadvantages
- for molecular (DNA) analysis:
  - choriocentesis is the preferred method
    - for technical reasons and early results
  - amniocentesis can also be used
    - results come late in pregnancy
Genetic prenatal diagnosis in the near future

- Increase in the number of disorders we can diagnose
  - monogenic etiologies
  - genetic predispositions

- Earlier and less invasive screening tests
  - separation of fetal cells from maternal blood
  - better biochemical markers from maternal circulation

- Potential for fetal therapy?
Preimplantation diagnosis

- Requires assisted reproductive technologies
- Genetic analysis done on 1-3 cells (blastomeres)
- “Healthy” embryos are chosen for implantation
The Genetics Consultation in OB-GYN :
Reproductive pathologies
Genetics and sterility (primary)

- **sex chromosome anomalies**
  - numerical (ex: 47,XXX; mosaic XXY or XO)
  - structural (ex: deletions of X or Y)

- **autosomal chromosome anomalies**
  - structural (ex: translocations in males)

- **single gene disorders**
  - autosomal dominant (ex: Steinert M.D. in male)
  - autosomal recessive (ex: cystic fibrosis mutations)
  - X-linked disorders (ex: androgen resistance)
Genetics and sterility (secondary)

- **sex chromosome anomalies**
  - numerical (ex: mosaic XXY or XO)

- **autosomal chromosome anomalies**
  - structural (ex: translocations, inversions)

- **single gene disorders**
  - autosomal dominant (ex: Steinert M.D. in female)
  - autosomal recessive (ex: sickle cell anemia)
  - X-linked disorders (ex: focal dermal hypoplasia)
Genetic testing in infertility: Goals

- Determine origin of the infertility
- Identify syndromic causes (prophylaxis for the affected individual)
- Better estimate the probability of success with ART
- Offer genetic counseling
  - concerning ART methods
  - concerning prenatal diagnosis
<table>
<thead>
<tr>
<th><strong>Question</strong> (proband or family)</th>
<th><strong>Disorder</strong> (genetic transmission)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pulmonary or digestive symptoms?</td>
<td>cystic fibrosis (AR)</td>
</tr>
<tr>
<td>Masculine infertility /</td>
<td>immotile cilia syndrome (AR)</td>
</tr>
<tr>
<td>sexual ambiguity</td>
<td>partial androgen resistance (XLR)</td>
</tr>
<tr>
<td>Neuromuscular symptoms</td>
<td>Kennedy disease (XLR)</td>
</tr>
<tr>
<td></td>
<td>Steinert myotonic dystrophy (AD)</td>
</tr>
</tbody>
</table>
Azoospermia

- 15% of males have a chromosomal abnormality
- 13% of males have a *de novo* deletion of the azoospermia factor (AZF) region on Yq11
- 1-2% of males have congenital bilateral absence of the vas deferens (CBAVD)
Genetic Counseling for Azoospermia - Chromosomal Etiology (numerical)

- Discuss clinical features
- Discuss karyotypic findings
- Reproductive options
  - adoption
  - donor sperm
  - MESA/ICSI/IVF/PGD
- Theoretical recurrence risks
Genetic Counseling for A/Oligospermia - Chromosomal Etiology (structural)

- Discuss karyotypic findings
- Provide empirical risks
- Discuss implications for other family members
Genetic Counseling for Azoospermia - Y Deletion

- Discuss AZF deletion versus polymorphism with no adverse phenotypic effect
- Reproductive options if de novo deletion of AZF
  - adoption
  - donor sperm
  - MESA/ICSI/IVF with/without prenatal dx
Genetic Counseling for Azoospermia - CBAVD

- Offer cystic fibrosis (CF) testing
  - 10-20% with 2 CF mutations
  - 40-60% with CF mutation
- Discuss further medical evaluations
Genetic Counseling for CBAVD Due to CF Mutation(s)

- Ascertain patient’s understanding of diagnosis
- Discuss genetics and relationship of CF and CBAVD
- Offer CF carrier screening to partner
- Risk assessment
### CFTR mutations in males with abnormal spermograms

(13 mutations CF)

(van de Ven et al. (1996) HUM REPROD 11:513-517)

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>Number mutations /no.chrom.</th>
</tr>
</thead>
<tbody>
<tr>
<td>normospermia</td>
<td>0 / 52 (0%)</td>
</tr>
<tr>
<td>anomalies</td>
<td>18/202 (8.9%)</td>
</tr>
<tr>
<td>azospermia</td>
<td>4 / 42 (9.5%)</td>
</tr>
<tr>
<td>asthenospermia</td>
<td>5 / 54 (9.2%)</td>
</tr>
<tr>
<td>teratospermia</td>
<td>1 / 8 (12.5%)</td>
</tr>
<tr>
<td>oligospermia</td>
<td>0 / 8 (0%)</td>
</tr>
<tr>
<td>OA</td>
<td>1 / 10 (10%)</td>
</tr>
<tr>
<td>OT</td>
<td>1 / 14 (7.1%)</td>
</tr>
<tr>
<td>OAT</td>
<td>5 / 32 (15.6%)</td>
</tr>
<tr>
<td>AT</td>
<td>1 / 34 (2.9%)</td>
</tr>
</tbody>
</table>
Pre-ICSI counseling
(while awaiting more definitive data…..)

Bonduelle et al., HUM REPROD 14:243-264

*Before any treatment is started, patients should be informed of available data:*

- risk of transmitting (parental) chromosomal aberrations
- risk of de novo, mainly sex chromosome, anomalies
- risk of transmitting fertility problems to offspring
- no increased incidence of congenital malformations
1082 fetuses tested prenatally

**ANOMALIES**

18 *de novo* (1.66%)
- 9 sex chromosome ano.
- 5 autosomal trisomies
- 4 structural anomalies
10 inherited

- 690 amnios
  - 15 abnormal
- 382 CVS
  - 13 abnormal
Abnormal fetal karyotypes after ICSI
(Bondeulle et al., 1999, HUM REPROD 14:243-264)

- 18 cases had de novo aberrations
- not expected because of maternal age (mean of 32.5)
- value of 0.83% of sex chromosomal anomalies is 4x higher than in newborns
- linked to male infertility in most of these couples
- increased incidence of de novo structural aberrations (0.36%) as well (3-4x more than expected)
THE END

plus some image