Overview

- Central Dogma of Biology
  - Organization of Genetic Information
- ACOG Recommendations Regarding Genetic Screening in Pregnancy
  - Fetal Aneuploidy
  - Ancestry-based Carrier Screening
- Purpose of Screening
- Screening in the First and Second Trimester
- Case Examples
  - Genetic Testing and Special Considerations
- Genetic Resources
Fun Facts

- 50 Trillion cells unpacked
- Fruit Flies to Humans
- Human genome coding and variation
Central Dogma of Biology

1. DNA Polymerase
   - DNA → DNA
   - Replication

2. RNA Polymerase
   - DNA → RNA
   - Transcription

3. Ribosome
   - RNA → Protein
   - Translation
Central Dogma of Biology

Chromatids (10 coils long)

One coil (30 rosettes)

One rosette (6 loops) around nuclear scaffold

One loop (75 kbp)

30 nm chromatin fibre

Beads-on-a-string chromatin fibre wrapped around histones

DNA
ACOG Recommendations

- Screening for select fetal aneuploidy should be offered to all women, regardless of maternal age.
- Counselling should be provided regarding details of screening.
- Screening results enable women and their partners to balance the benefits and risks of invasive diagnostic testing (CVS and amniocentesis).
- Do not perform successive screens unless purposefully designed.
  - Example) Quadruple screening should not be performed following a first trimester screen.

Purpose of Screening

- Screening is to identify pregnancies in a population who are at an *increased risk* for a specific condition such as Down syndrome or open neural tube defect.

- Specific cut-offs are used to determine which pregnancies are considered at “increased risk”.
  - Example) 1 in 300 regarding DS

- Historically, maternal age was used as first screen for Down syndrome.

- False positive
  - Screening result is “positive”, however pregnancy is not at increased risk.
Down syndrome
Trisomy 18
Trisomy 13

- Small head
- Absent eyebrows
- Cleft lip and/or palate
- Dysplastic, or malformed ears
- Clenched hands and polydactyly, or extra fingers
- Undescended or abnormal testes
Screening in the First Trimester

- First Trimester Screen or “NT screen”
- Combines ultrasound markers (nuchal translucency measurement, nasal bone assessment) AND biochemistry (hCG, PAPP-A)

- Detection rates:
  - DS – 90-95%, Trisomy18/13 – 95%

- False positive rate:
  - 2%-5%

- Performed: 9w 0d – 13w 6d
Comparison of screening methods for +21

<table>
<thead>
<tr>
<th>Method of screening</th>
<th>DR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (MA)</td>
<td>30</td>
</tr>
<tr>
<td>MA and maternal serum biochemistry at 15–18 weeks</td>
<td>50–70</td>
</tr>
<tr>
<td>MA and fetal nuchal translucency (NT) at 11–13wks</td>
<td>70–80</td>
</tr>
<tr>
<td>MA and fetal NT and maternal serum free β-hCG and PAPP-A at 11–13wks</td>
<td>85–90</td>
</tr>
<tr>
<td>MA and fetal NT and fetal nasal bone (NB) at 11–13wks</td>
<td>90</td>
</tr>
<tr>
<td>MA and fetal NT and NB and maternal serum free β-hCG and PAPP-A at 11–13wks</td>
<td>95</td>
</tr>
</tbody>
</table>

hCG: human chorionic gonadotropin, PAPP-A: pregnancy-associated plasma protein A

Data from Fetal Medicine Foundation
Non-invasive prenatal testing (NIPT) 
The presence of high concentrations of cell-free fetal DNA in maternal plasma was demonstrated in 1997 using polymerase chain reaction (PCR).

Typically performed after 10 weeks EGA.

Sensitivity (detection rate) for Down syndrome and Trisomy 18 is greater than 99% and 98% respectively.

- Regarding +13: ~90%

Specificity (correctly identify unaffected): >99%

- Less than 0.5% false positive rate

Non-invasive Prenatal Testing (NIPT)

- Counting methods
  - Fetal cell-free DNA in maternal serum

Red = Fetal
Non-invasive Prenatal Testing (NIPT)

- Sequence (via massively parallel sequencing (whole sample or targeted)) and assign

- Approximately 162 bp

- Chromosome 7
- Chromosome 21
- Chromosome Y
- Chromosome 1
- Chromosome 3

Bioinformatics
Non-invasive Prenatal Testing (NIPT)

- Call is made based on subtle difference in ratio of chromosome material in affected vs unaffected populations.
- Another method of NIPT takes into consideration Single Nucleotide Polymorphisms (SNPs) and analyzes for maternal versus fetal predicted genotype.
Non-invasive Prenatal Testing (NIPT)

- Conditions included in screening has expanded to include sex chromosomes X and Y.
  - Can detect abnormalities such as Turner syndrome (monosomy X), Klinefelter syndrome (XXY), etc.
    - ~91-95% detection rate.

- Micro deletion/duplication syndromes
  - 22q11.2 deletion, Angelman/Prader-Willi, etc.

- Future may include single gene disorders such as hemoglobinopathies.
ACOG Recommendations NIPT

- Patients at increased risk of aneuploidy can be offered testing with cell free fetal DNA.
- Pretest counseling should include a review of benefits and limitations.
- A family history should be obtained before the use of this test to determine if the patient should be offered other forms of screening or prenatal diagnosis for familial genetic disease.
A patient with a positive test result should be referred for genetic counseling and offered invasive prenatal diagnosis for confirmation of test results.

Cell free fetal DNA does not replace the accuracy and diagnostic precision of prenatal diagnosis with CVS or amniocentesis, which remain an option for women.

ACOG Committee Opinion, Noninvasive prenatal testing for fetal aneuploidy, No. 545, Dec 2012
Screening in the Second Trimester

- Maternal serum screening (Quad, single marker msAFP, etc)
- Yields risk assessment for +21, +18, and ONTD
- Markers analyzed typically includes: AFP, hCG, uE3, DIA
  - Sensitivity:
    - DS 75-80%, Trisomy 18 ~70%, ONTD 80%
  - False positive rate: 5%
    - Possibly higher in AMA patients
- Maternal serum AFP (msAFP) alone screens for ONTD.
Screening in the Second Trimester

- Ultrasound examination
  - Congenital malformations
  - “Soft markers” for fetal aneuploidy
    - U/S sensitivity for DS: ~66%.
  - Ultrasound has been shown to have a high sensitivity (>95%) for ONTD after 16 weeks EGA.
  - Level II recommended as standard of care.
Screening in the Second Trimester
ACOG Recommendations Ancestry

- Ancestry-based carrier screens should be offered to all women of reproductive age (pregnant or considering pregnancy).
- Specific autosomal recessive conditions are more prevalent in individuals of certain ancestries.
- Cystic Fibrosis (CF) should be offered to all women.

ACOG Committee Opinion, Update on Carrier Screening for Cystic Fibrosis, #486, Vol. 117, No. 4, April 2011
Hemoglobinopathy screening should be offered to patients of African, Mediterranean, and southeast Asian descent.

CBC and hemoglobin electrophoresis is the preferred screen.
- Solubility tests alone are inadequate.

Prenatal diagnosis is best accomplished by DNA analysis of amniocytes or chorionic villi.

Expanded Carrier Screening

- On average, an individual is a carrier for 2-3 significant autosomal recessive conditions.
- Prenatal diagnosis of single gene disorders typically requires the two disease-causing mutations to be known.
- Ancestry-based carrier screening is complicated by a diverse population due to a high degree of mixed ethnicity.
  - Genetics is poor at distinguishing factor regarding ethnic background.
Expanded Carrier Screening

- Single screen to determine patient’s carrier status for a substantial number of single, gene recessive conditions.
  - 75+ conditions
  - CF, SMA, Fragile-X, hemoglobinopathies, and Ashkenazi Jewish disorders
  - Highly cost effective

- Molecular analysis for specific mutations associated with a given condition using whole blood sample.
  - Example: 23+ mutations known to cause CF in CFTR gene
Genetic Testing

- Conventional cytogenetics (the study of chromosomes)
  - Cell culture ➔ stain ➔ examine under microscope
  - Pros: detect changes > 5-10 Mb, diagnostic
  - Cons: Labor intensive, Subjective

- Karyotype: a picture of the number and appearance of chromosomes.

- Vernacular
  - “Please draw a specimen for: chromosomes, karyotyping, cyto, a karyotype, chromes.”
Testing (continued)

Anatomy of a Chromosome

Chromosome Spread

Chromosome

- Telomere
- p arm
- Centromere
- q arm
- Telomere
Normal Female - 46,XX

Chromosomes artificially straightened for illustrative purposes causing some apparent discrepancies in banding patterns of chromosome pairs.
Conventional Cyto. Case

- MM, female.
  - Presents with:
    - Pediatrician hears heart murmur and is unable to detect pulses in groin or legs.
    - Lymphodema of hands and feet.
    - Prominent skin on neck.
  - Echocardiogram reveals coarctation of the aorta.
  - Chromosome analysis is ordered:
Conventional Cyto. Case

Monosomy X - Turner Syndrome 45,X

Chromosomes artificially straightened for illustrative purposes causing some apparent discrepancies in bonding patterns of chromosome pairs.
FISH (fluorescence in situ hybridization)

- FISH uses fluorescent probes that bind to specific parts of a chromosome.
- Targeted analysis for deletions or duplications of specific chromosome material
  - Pros: FAST, targeted
  - Cons: del or dup must be large enough to change hybridization of probe, probe must be available, targeted
Testing (continued)

FISH Analysis of Chromosomal Trisomy

Interphase

Metaphase
FISH Case

- EP, male, maternal age: 29, G1 P1 A0, No prenatal care, ?38 week delivery, Vaginal, weight 30%tile, length 35%tile.

- Presents to L&D with:
  - Dysmorphic face, Hypocalcemia, significant feeding problems.
    - Cleft Palate is identified
  - Echocardiogram reveals ventricular septal defect.

- Chromosome analysis is 46, XY.
FISH Case

- FISH for 22q11.2 shows deletion
- 22q11.2 deletion syndrome
  - AKA:
    - DiGeorge syndrome
    - Velocardiofacial syndrome
Microarray Technologies

- Micro- extremely small; array- orderly arrangement
- Microarrays are small, solid supports onto which the sequences from thousands of different genes are immobilized, or attached, at fixed locations.
  - Glass or silicon slides with spots of genetic information
The location of each spot in the array is used to identify a particular gene sequence.
Microarrays are genome-wide studies:

- Comparative genomic hybridization (CGH)
- Single nucleotide polymorphism (SNP, “snip”) array

Pros: a lot of information (genome wide), detects del/dup >100 kb

Cons: a lot of information, variants of unknown clinical significance, will not detect balanced rearrangements
BO, male, maternal age: 32. G5 P2 A3, 35 week delivery, prenatal ultrasound revealed small for gestational age and abnormal feet positions.

Presents to NICU with:
- Dysmorphic face, hypotonia, poor sucking, feeding difficulties, low birth weight.

Chromosome analysis is 46, XY (ruling out Down syndrome)

DNA methylation testing on chromosome 15 rules out Prader-Willi and Angelman syndrome.
Microarray Case

- Microarray CGH reveals 600 kb heterozygous (1 copy) deletion at chromosome 17q21.31
- 17q21.31 Microdeletion Syndrome:
  - characterized by developmental delay/intellectual disability, dysmorphisms, congenital malformations, and behavioral features.
Microarray Case
Gene sequencing

Genes are coded using four bases: A G T C.

Sequencing typically “reads” each base along the entire coding region of the gene.

- Pros: Will detect single base-pair changes
- Cons: expensive, long turn around time, won’t detect large dup/del
Gene Sequencing Case

- FR, male, 2 days old, uncomplicated delivery, uncomplicated prenatal history.
- Presents to Mother Baby with:
  - lethargy, somnolence, refusal to feed, vomiting.
- Laboratory studies reveal: plasma ammonia concentration 1535 µmol/L
- Newborn screening reveals elevated citrulline.
  - Plasma quantitative amino acid analysis shows absence of argininosuccinic acid and concentration of citrulline greater than 1000 µmol/L.
Gene Sequencing Case

- Urea cycle disorder highly suspected.
- Gene sequencing of ASS1 confirms a diagnosis of Citrullinemia type I (CTLN1).
  - Autosomal recessive, impaired or absent function of argininosuccinic acid synthetase (ASS).
Gene Sequencing Case

HCO$_3$ + NH$_4$ + 2 ATP → Mitochondria

CPSI → N-acetylglutamate

Carbamyl Phosphate → OTC

Citrulline → Cytoplasm

Aspartate → Citrin*

Argininosuccinate → ORNT1*

Ornithine → ASS

Asparagine → Argininosuccinate

Arginine → Fumarate

Urea → ARG

ASL
Newborn Screening

- Urea cycle disorder highly suspected.
- Gene sequencing of ASS1 confirms a diagnosis of Citrullinemia type I (CTLN1).
  - Autosomal recessive, impaired or absent function of argininosuccinic acid synthetase (ASS).
Inheritance Patterns

- **Autosomal (not on sex chromosome) recessive:**
  - Both copies of gene are changed (homozygous).
  - Both parents are typically carriers, chance for each child to be affected is 1 in 4.

- **Autosomal dominant:**
  - One copy of gene is changed.
    - *De novo (new)* mutation in affected individual only.
    - One parent affected, chance for each child to be affected is 1 in 2.
Inheritance Patterns

- X-Linked (on the X chromosome)
  - Men are typically affected.
  - Women are carriers or more mildly affected.
  - Sons of carrier women have a 1 in 2 chance to be affected. Daughters of carrier women have a 1 in 2 chance to be carrier (recessive).
Future testing methods

- Microarrays able to detect smaller gains and losses.
- Multi-gene panels
  - Panels of multiple genes which are sequenced simultaneously using Next-Gen sequencing
- Whole exome sequencing
  - Sequencing all regions of the genome which are expressed.
- Full genome sequencing
  - $1,000 genome goal
Special Considerations

- Mosaicism: the presence of two or more populations of cells with different genotypes within the same individual.
  - Alters prognosis
  - May be tissue specific
    - Example: gonadal mosaicism
Special Considerations

Mosaicism

- Normal Cells
- Cells With Genetic Change

Cell Division

Mosaic Tissue
Genetic Resources

- Me
- Gene Reviews
  - http://www.genetests.org/
- Online Mendelian Inheritance in Man
- March of Dimes
  - http://www.marchofdimes.com/
- Foundations and societies for specific disorders
  - Spinal Muscular Atrophy Foundation,
    http://www.smafoundation.org/
Questions

- Thank you for your time.

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