Genetics and What You Need to Know
(or at least what we have time to talk about!)

SC Midlands Perinatal Association
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Discussion topics
• The basics of genetics
• Genetic tests and how to read them and what they mean
• Application to perinatal care
• Fetal autopsy

Exciting Developments in Genetics
• Success of the Human Genome Project
  – New initiatives to understand how genes, chromosomes, and biological pathways contribute to development and disease
  – Importance of genetic variation (SNPs and CNVs)
• Rapid pace of new discoveries in genetics and biology
• Emergence of new genetic approaches and technologies
  – Array comparative genomic hybridization (array CGH)
  – Gene panels
  – Next generation sequencing → ES and WGS
• Treatment initiatives
  – Gene editing and replacement
The genetic material, together with environmental influences before, during, and after birth, determine the attributes of an individual.

Diagnosis

- Provides framework for all subsequent medical decisions
- Must be accurate and specific
- Most genetic units have only 40-50% success rate in making a specific diagnosis

Birth Defects Are Common

1 in 33 (2-3%) of all babies are born with a serious, structural birth defect

This does not include babies with low birth weight, prematurity, developmental delays or eventual ID
**Frequency of Congenital Malformations in Liveborns**

Spanish Collaborative Study of Congenital Malformations

- Total Population: 564,616 liveborns
- CM 11,421 (2%)
  - Isolated 78.3%
  - MCA 21.7%


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**Known Causes of Birth Defects**

- Genetic
  - chromosomal and single gene events
- Environmental
  - chemical, infectious, metabolic, mechanical, and radiation
- Multifactorial
  - most common (NTD, CL/CP, CHD, etc)
- Unknown

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**Suggested Causes of Birth Defects**

- Messages from the supernatural
  - prediction of the future
  - sign of displeasure
- Maternal impressions
- Cohabitation with devils, monsters, animals
Mechanisms of Birth Defects

- Deformation
- Disruption
- Dysplasia
- Malformation

Deformation

- Aberrant mechanical force that distorts otherwise normal structures
- Usually occurs late in gestation, intrauterine constraint
- Most involve cartilage, bone, joints
- Many correct spontaneously after abnormal mechanical force is removed

Disruption

- Destruction of previously normal tissue
- Can be caused by mechanical forces, ischemia, hemorrhage
- Usually affects different tissue types in a distinct anatomic region
Amniotic Band Disruption Complex

Incidence
1/5000

Genetics
Mechanical disruption

Dysplasia

• Abnormal cellular organization or function within a specific tissue type throughout the body
• Often abnormal cellular biochemistry
• Often major mutant gene as cause
• Continuing course - clinical effects continue as long as tissue grows or functions

Malformation

• Failure of completion of one or more embryonic processes
• Usually occur early in gestation
• May involve a single anatomic region, entire organ system or multiple body systems
• Chromosomal abnormalities, mutant genes, teratogens
Syndrome

- Particular set of anomalies that repeatedly occur in a consistent pattern - a recognizable pattern
- Implies a common etiology
- Example: Down syndrome (Trisomy 21)

Known Causes of Birth Defects

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Indications For Cytogenetic Analysis

- Individuals with
  - intellectual disability
  - unusual facial appearance
  - multiple congenital defects
  - abnormalities of growth
  - certain types of malignancies
- Couples with
  - repeated spontaneous abortions
  - infertility
### Prenatal Ultrasound

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Risk of AA (%)</th>
<th>Risk of Aneuploidy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anencephaly</td>
<td>5</td>
<td>Negligible</td>
</tr>
<tr>
<td>Ventriculomegaly</td>
<td>85</td>
<td>10</td>
</tr>
<tr>
<td>Cystic Hygroma</td>
<td>85-90</td>
<td>60-70</td>
</tr>
<tr>
<td>Diaphragmatic Hernia</td>
<td>30-40</td>
<td>5-10</td>
</tr>
<tr>
<td>Duodenal Atresia</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>80</td>
<td>20-30</td>
</tr>
</tbody>
</table>

### Samples for Cytogenetic/Molecular Studies

- **Peripheral Blood** - stimulated with phytohemagglutinin
- **Skin Biopsy** - tissue culture
- **Autopsy** - tissue culture
- **Amniotic Fluid** – tissue culture
- **Chorionic Villi** – tissue culture

### Metaphase Chromosomes – 100X
Chromosome Classification

- Size
- Shape
- Position of the centromere
- Presence of satellites
- Banding patterns

Chromosome Structure

Chromosome Number
Chromosome Arm
Chromosome Region
Chromosome Band

Idiogram

- Pictorial display
- Autosomes numbered
  - length
  - centromere pos
  - 1 to 22
- Sex chromosomes
  - X and Y
Pairs 1 – 22 are termed the autosomes and are homologous (same).

23rd pair are the sex chromosomes
XX - female
XY - male

Karyotype

Array of chromosomes of a single cell

Karyotype Nomenclature

- Total number of chromosomes
- Sex chromosomes
- Numerical aberrations
- Structural aberrations

Examples of Karyotypes

- 46,XX
- 46,XY
- 45,X,-Y
- 47,XXY
- 47,XX,+21
- 46,XX,t(9;22)(q34;q11)
- 47,XY,+8,t(15;17)(q22;q11)
### Frequency of Chromosomal Abnormalities

<table>
<thead>
<tr>
<th>Group</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Birth</td>
<td>0.5 – 0.6</td>
</tr>
<tr>
<td>Intellectual Disability</td>
<td>3 – 10</td>
</tr>
<tr>
<td>ID w/ Malformations</td>
<td>10 – 40</td>
</tr>
<tr>
<td>Stillbirths and Early Neonatal Death</td>
<td>4 – 6</td>
</tr>
<tr>
<td>Intrauterine Growth Retardation</td>
<td>4 – 6</td>
</tr>
<tr>
<td>Infertile Males</td>
<td>2 – 3</td>
</tr>
<tr>
<td>Spontaneous Abortions</td>
<td>40 – 60</td>
</tr>
</tbody>
</table>

Source: de Grouchy and Turleau (1984); Evans (JMG, 1977)

### Chromosome Aberrations in Liveborn Infants

- **T - 21**: 1:700 X 1:2500 females
- **T - 18**: 1:8000 XXY 1:800 males
- **T - 13**: 1:5000 XXX 1:1200 females
- **5p-**: 1:50,000 XYY 1:900 males

Source: de Grouchy and Turleau (1984); Evans (JMG, 1977)

### Maternal Age Related Increase

[Graph showing maternal age related increase]
Chromosomes

- Down syndrome
- Fetal Ultrasound


CPC

- 0.4-3.6% of all 2nd trimester US demonstrate a CPC
- Approximately 1-2% of CPC are associated with a chromosome abnormality, most commonly Trisomy 18
- 30% of fetuses with T-18 have a CPC
- ? Of increased risk of Trisomy 21
- No correlation of size, bilaterality, or disappearance
- Does an isolated CPC warrant amniocentesis?
### Sonographic Features in Aneuploidy

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Trilogy 13</th>
<th>Trilogy 10</th>
<th>Trisomy 13</th>
<th>45, Xy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonographic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Facial Features</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Digital Homelessness</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nasal Root Deviation</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Micrognathia</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urologic Anomalies</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Prenatal CHD</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac Abnormalities</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diaphragmatic Hernia</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dextrocardia</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Abnormal Aorta</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prenatal Growth</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reflexes</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skull Fusions</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Breaks in Bone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Terminal Deletions
- del
- Most common structural abn
- Only one break

### Interstitial Delelions
- del plus breakpoints
- Two breaks
- Microdeletion syndromes
  - Williams – del 7q11.23
    - Langer-Giedion – del 8q24.1
    - Wilms-Aniridia (WAGR) – del 11p13
    - Angelman/Prader Willi – del 15q11.2q13.2
    - Smith-Magenis – del 17p11.2
    - Miller-Dieker – del 17p13
    - Rubinstein-Taybi – del 16p13.1
    - Alagille – del 20p11.23p12.1
    - DiGeorge – del 22q11
Interstitial Deletion
Angelman / Prader Willi Syndrome

Chromosomal Microarray
- The array is made from small pieces of DNA attached to a glass slide or tiny beads in a specific order.
- The patient and control DNA attaches to specific, matching areas on the array.
- Because there are equal amounts of patient and control DNA, they should attach about the same and the two dye colors should balance each other out (1-to-1 ratio).
- When there is extra or missing genetic material, it shows up as a dye imbalance.
Factors that affect interpretation of NIPT results

1. Population Frequency and Positive Predictive Value
2. Fetal fraction
3. Incidence of specific microdeletion conditions
4. Multiples
5. Maternal Weight
6. Failure rate and association with aneuploidy
7. Maternal conditions such as cancer and chromosome abnormalities
Table 1. Cell-free DNA Test Performance Characteristics in Patients Who Receive an Interpretable Result*

<table>
<thead>
<tr>
<th></th>
<th>Age 25 years</th>
<th>Age 40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>99.3</td>
<td>99.8</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>97.4</td>
<td>99.8</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>91.6</td>
<td>99.9</td>
</tr>
<tr>
<td>Sex chromosome aneuploidy</td>
<td>91.0</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Abbreviation: PPV, positive predictive value.

What needs to be covered in a Nutshell

- NIPT is a screening test with false + and false -
- NIPT is good for T21, T18, T13, X,Y in high risk patient
- NIPT in low risk woman has LOW PPV
- NIPT is not appropriate for sex determination
- NIPT has unknown validity in multiples
- NIPT may fail and the risk is higher when woman is obese

What needs to be covered in a Nutshell

- Failed NIPT may indicate a higher risk of aneuploidy
- NIPT for any other condition other than the common aneuploidies must be separately consented
- NIPT results should be confirmed before making management decisions
- Seek Genetic Counseling for the confusing, the unusual and the patient with questions
Fetal/Perinatal Autopsy

- Autopsy remains the standard for confirmation and further delineation of prenatal diagnoses by prenatal ultrasonography and thus determining the cause of fetal loss.
- The studies comparing the ultrasound findings with fetal autopsy reveal the change in diagnosis or additional findings in 22% to 76% of cases.
- In addition, the incidental findings may alter the genetic counseling.
- With the increasing awareness and attitude towards prenatal diagnosis, prospective parents now wish to take informed decisions and request as comprehensive information as possible for the present as well as future pregnancies.
Single Gene Disorders

The Central Dogma of Genetics

Gene Structure
Single Gene Disorders
AKA “Mendelian”

- Autosomal
  - Dominant
  - Recessive
- X-linked
  - Dominant
  - Recessive
- Mitochondrial
Next Generation Sequencing

- **DNA sequencing** is the process of determining the precise order of *nucleotides* within a *DNA* molecule. It includes any method or technology that is used to determine the order of the four bases—*adenine*, *guanine*, *cytosine*, and *thymine*—in a strand of DNA.

Whole Exome Sequencing

- *Exons* are short, functionally important sequences of *DNA* which represent the regions in genes that are translated into protein and the untranslated region (UTR) flanking them.
- In the human genome there are about 180,000 exons: these constitute about 1% of the human genome, which translates to about 30 megabases (Mb) in length.
- It is estimated that the protein coding regions of the human genome constitute about 85% of the disease-causing mutations.
Classification of sequence alterations

• Previously reported, known pathogenic
  • Found in literature and/or Human Gene Mutation Database
• Previously unreported, expected pathogenic
  • Nonsense mutation (premature stop codon)
  • Consensus splice site (-2/-1/+1/+2 positions)
  • Insertion or deletion that causes frameshift in protein
• Previously unreported, possibly pathogenic
  • In-frame insertion or deletion (multiple of 3 basepairs)
  • Missense mutation
    • Use PolyPhen & SIFT to predict pathogenicity
  • Intronic change near splice site

• Previously unreported, likely benign
  • "Silent" change (does not alter the amino acid)
    • could still alter splicing
  • Intronic changes far from splice site (exon/intron boundary)
• Previously reported, known benign variant
  • NCBI dbSNP database or published literature
  • Allele frequency >1% in general population
Gene Panels

- Genetically heterogeneous disorders are cumbersome to molecularly diagnose due to overlapping, non-specific features that make targeting a specific gene difficult or impossible.
- Syndromes are not always easily identified!
- Comprehensive gene panels provide clinically relevant and cost effective genetic testing to help establish a patient diagnosis and provide clinical management.

Gene Panels

- Complete coverage of all clinically relevant genes for a particular phenotype and a reduction in variants of unknown clinical significance and incidental findings eases delivery of results to the patient and requires less counseling time.
- Molecular confirmation of a clinical diagnosis aids in determining the associated health conditions for which an individual should be monitored, guides proper therapeutic intervention and reduces the cost of continuing through the diagnostic odyssey. In addition, identification of a mutation allows for appropriate recurrence risk counseling of prenatal and/or pre-implantation diagnostic options.

Clinical Diagnosis
Microarray and Gene Panel Confirmation

The Molecular Diagnostic Laboratory at the Greenwood Genetic Center has developed a targeted Next Generation Sequencing panel for genes associated with autosomal recessive disorders. The panel was submitted to an external site for testing at an investigational research basis during the validation phase of the test. The abnormal allele is reported as a novel change that is predicted to be pathogenic and is consistent with a clinical diagnosis of the disorder. The laboratory may continue to validate additional genes associated with the same phenotype. Further testing of the parents and any siblings will be performed at no charge.

<table>
<thead>
<tr>
<th>Variant allele</th>
<th>DNA alteration</th>
<th>Protein effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TCTAGA</td>
<td>c.664A&gt;G (Dianagnosis)</td>
<td>nonsense</td>
</tr>
</tbody>
</table>
Clinical Diagnosis
Microarray and Gene Panel Confirmation

The Genetic Team
- Pediatrician; Ob/Gyn
- Medical geneticist
- Genetic counselor
- Ultrasonographer
- Nurse
- Case worker/ social worker
- Medical assistants/Lab technicians
- Other medical specialists

When to Refer for a Genetic Evaluation
- Abnormal growth pattern
- Delayed development/intellectual disability
- Sensory impairment
- Birth defects(s)
- History of exposure to known teratogen
- Dysmorphic features
- Family history of genetic condition