Clinical Cytogenetic Testing: Applications in Constitutional and Oncology Settings

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Learning Objectives

• List the areas of medicine that overlap with clinical cytogenetics and common indications for testing across these disciplines

• Explain the basic methodologies, technical capabilities and limitations of chromosome analysis, FISH and genomic microarray

• List common cytogenetic abnormalities encountered across different clinical contexts, including childhood developmental phenotypes, prenatal and perinatal diagnosis, pregnancy loss and in cancer
What is Cytogenetics?

- The study of chromosomes and genomic structure, function, and variation and their role in human disease and heredity
- Clinical cytogenetics overlaps with several areas of medicine: pathology, pediatrics, neurology, endocrinology, psychiatry, obstetrics and gynecology, hematologic oncology, other areas of medical oncology

reprinted from Jorde et al. *Medical Genetics* 3rd Ed 2006
Constitutional versus cancer cytogenetics

- Constitutional cytogenetics: diagnosis of heritable genetic abnormalities in children, adults, pregnancy, and fetal loss
  - Abnormalities may be inherited or de novo

- Cancer cytogenetics: detection of acquired or somatic (versus germline/constitutional) genetic abnormalities for the diagnosis, prognosis, therapy, and/or monitoring of many types of cancer (especially leukemia and lymphoma)
Indications for Constitutional Cytogenetic Testing

• Postnatal, childhood growth and development
  – Perinatal: Birth defects, malformations, dysmorphisms, ambiguous genitalia
  – Growth: failure to thrive, growth delay, short stature
  – Developmental delay (fine and gross motor, speech)
  – Cognitive: intellectual disability, learning disability
  – Neurological: hypotonia, seizures, ataxia
  – Behavioral: autism, OCD, psychiatric illness

Tissues studied: Peripheral blood, buccal swab, skin biopsy
Indications for Constitutional Cytogenetic Testing

• Adolescent, adult sexual development and fertility
  – Amenorrhea, primary or secondary ovarian failure, premature menopause
  – Azoospermia, oligospermia, hypogonadism
  – History of infertility or spontaneous abortions
  – Birth of a child with a chromosomal abnormality

Tissues studied: Peripheral blood
Indications for Constitutional Cytogenetic Testing

- Prenatal
  - Abnormal maternal serum screening (first or second trimester)
  - Abnormal cell-free DNA testing (cfDNA), non-invasive prenatal testing (NIPT)/screening (NIPS)
  - Abnormal ultrasound findings: cystic hygromas/hydrops, cardiac defects, other malformations, IUGR, etc.
  - Advanced maternal age (AMA), generally ≥ 35 yrs
  - Parental or familial chromosome abnormality

- Fetal or neonatal demise (products of conception, POC)

Tissues studied: Amniotic fluid, chorionic villus sampling, fetal tissues
Indications for Cancer Cytogenetic Testing

• Hematologic oncology
  – Myeloid: Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Myelodysplastic syndromes (MDS), Myeloproliferative neoplasms (MPN)
  – Lymphoid: Acute lymphoblastic leukemia/lymphoma (ALL), Chronic lymphocytic leukemia (CLL), Non-Hodgkin lymphoma (NHL), Plasma cell neoplasms (Multiple Myeloma, MM)

• Bone marrow transplant

• Other areas of oncology (solid tumors)

Tissues studied: bone marrow, peripheral blood, lymph nodes, solid tumor, pleural fluid, spinal fluid
Techniques for Cytogenetic Studies

Chromosome analysis/karyotyping

Fluorescence in situ hybridization (FISH)

Genomic microarray analysis (GMA)
Preparation of metaphase chromosomes

- 5 mL venous blood
- Add phytohemagglutinin and culture medium
- Culture at 37°C for 3 days
- Add colcemid and hypotonic saline
- Cells fixed
- Digest with trypsin and stain with Giemsa
- Spread cells onto slide by dropping
- Analyze “metaphase spread”

Modified from Preparation of a karyotype. From Mueller and Young, 2001

Amethopterin, Thymidine, Ethidium bromide
Karyotyping

Karyogram

Metaphase spread

Karyotype: 46,XY
Overview of chromosome analysis

- Generally, 20 cells are analyzed from multiple cultures

- Definition of a clone:
  - At least two metaphase cells with the same extra chromosome or structural abnormality
  - At least three metaphase cells with the same chromosome loss

Dewald et al., Cytogenetic Studies in Neoplastic Hematologic Disorders 2nd Ed.
Differences in level of resolution by sample type

350
BM

400-425
AF
POC

550-700
PB

3

3

3

3

7

7

7

7

7

7
Pros and Cons of Chromosome Analysis

**Advantages**
- Genome-wide approach
- Detects both numerical and structural abnormalities
- Gold standard: well-established technology

**Disadvantages**
- Resolution is limited
- Requires culturing
  - Some tissues/cell types do not grow well in culture
  - Potential for *in vitro* artifacts
- Analysis is subjective
Common Constitutional Numerical Abnormalities

**Aneuploidy**

- 47,XXY (Klinefelter syndrome)
- 45,X (Turner syndrome)
- 47,XX,+21 (Down syndrome)
- 47,XY,+18 (Edwards syndrome)
- 47,XY,+13 (Patau syndrome)
- 47,XX,+16

**Polyploidy**

- Triploidy (e.g. 69,XXY)
- Tetraploidy (e.g. 92,XXYY)
# Observed frequencies of chromosomal abnormalities in gametes and pregnancy

## Incidence of aneuploidy during development

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>0</th>
<th>6-8</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage</strong></td>
<td>Sperm</td>
<td>Oocytes</td>
<td>Pre-implantation embryos</td>
<td>Spontaneous abortions</td>
</tr>
<tr>
<td><strong>Incidence of aneuploidy</strong></td>
<td>1-2%</td>
<td>~20%</td>
<td>~20%</td>
<td>35-50%</td>
</tr>
<tr>
<td><strong>Most common aneuploidies</strong></td>
<td>Various</td>
<td>Various</td>
<td>Various</td>
<td>45,X, +16, +21, +22, Triploidy</td>
</tr>
</tbody>
</table>

Table modified from Hassold and Hunt, 2001, Nat Rev Genet
Chromosome size and gene content correlates with incidence of *postnatal* trisomy
## Incidence of aneuploidy detected in newborns

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Rate/1000</th>
<th>Rate (1/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal Trisomies (All)</td>
<td>1.62</td>
<td>617</td>
</tr>
<tr>
<td>13</td>
<td>0.04</td>
<td>24,058</td>
</tr>
<tr>
<td>18</td>
<td>0.21</td>
<td>4,812</td>
</tr>
<tr>
<td>21</td>
<td>1.37</td>
<td>730</td>
</tr>
<tr>
<td>Sex Chromosome Aneuploidies (All)</td>
<td>2.70</td>
<td>375</td>
</tr>
<tr>
<td>45,X and variants</td>
<td>0.29</td>
<td>3,509</td>
</tr>
<tr>
<td>47,XXX and 47,XXX/46,XX</td>
<td>0.50</td>
<td>2,000</td>
</tr>
<tr>
<td>47,XXY and variants</td>
<td>0.72</td>
<td>1,400</td>
</tr>
<tr>
<td>47,XYY and 46,XY/47,XYY</td>
<td>0.53</td>
<td>1,887</td>
</tr>
</tbody>
</table>

Data from: Milunsky and Milunsky, Genetic Disorders of the Fetus, 6th Ed. (2010). Benn, Chp. 6

- Incidence of sex chromosome aneuploidy is higher
- True rates are underestimated, especially for sex chromosome aneuploidies, which may be unrecognized at birth
## Parental Origins of Aneuploidy

### Table 1. Summary of studies of the origin of human trisomies

<table>
<thead>
<tr>
<th>Trisomy</th>
<th>n</th>
<th>Maternal MI (%)</th>
<th>Maternal MII (%)</th>
<th>Paternal MI (%)</th>
<th>Paternal MII (%)</th>
<th>PZM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acrocentrics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>74</td>
<td>56.6</td>
<td>33.9</td>
<td>2.7</td>
<td>5.4</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>36.5</td>
<td>36.5</td>
<td>0.0</td>
<td>19.2</td>
<td>7.7</td>
</tr>
<tr>
<td>15</td>
<td>34</td>
<td>76.3</td>
<td>9.0</td>
<td>0.0</td>
<td>14.7</td>
<td>0.0</td>
</tr>
<tr>
<td>21</td>
<td>782</td>
<td>69.6</td>
<td>23.6</td>
<td>1.7</td>
<td>2.3</td>
<td>2.7</td>
</tr>
<tr>
<td>22</td>
<td>130</td>
<td>86.4</td>
<td>10.0</td>
<td>1.8</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Non-acrocentrics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>53.4</td>
<td>13.3</td>
<td>27.8</td>
<td>0.0</td>
<td>5.6</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>17.2</td>
<td>25.7</td>
<td>0.0</td>
<td>0.0</td>
<td>57.1</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>50.0</td>
<td>50.0</td>
<td>0.0</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>16</td>
<td>104</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>18</td>
<td>150</td>
<td>33.3</td>
<td>58.7</td>
<td>0.0</td>
<td>0.0</td>
<td>8.0</td>
</tr>
<tr>
<td>XXX</td>
<td>46</td>
<td>63.0</td>
<td>17.4</td>
<td>0.0</td>
<td>0.0</td>
<td>19.6</td>
</tr>
<tr>
<td>XXY</td>
<td>224</td>
<td>25.4</td>
<td>15.2</td>
<td>50.9</td>
<td>0.0</td>
<td>8.5</td>
</tr>
<tr>
<td>X</td>
<td>~30</td>
<td>~30</td>
<td>~70</td>
<td>~70</td>
<td>~70</td>
<td>~70</td>
</tr>
</tbody>
</table>

*a Adapted from Hall et al. (6). MI, meiosis I; MII, meiosis II; PZM, post-zygotic mitotic.*

Table: Hassold, Hall and Hunt, 2007, Hum Mol Genet
Images modified, source: [http://learn.genetics.utah.edu/content/chromosomes/readchromosomes/](http://learn.genetics.utah.edu/content/chromosomes/readchromosomes/)
Oogenesis vs Spermatogenesis

Hassold and Hunt (2001) Nat Rev Genet
Down Syndrome and Maternal Age

Risk of Down syndrome in live births (%)

Maternal age (years)

0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

Ovum from a woman in her 20’s

Ovum from a woman in her 40’s


Battaglia et al., 1996
Incidence of aneuploidy detected prenatally with various ultrasound findings

<table>
<thead>
<tr>
<th>Defect</th>
<th>Overall frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic hygroma</td>
<td>133/211 (63%)</td>
</tr>
<tr>
<td>Tracheo -esophageal atresia</td>
<td>25/40 (63%)</td>
</tr>
<tr>
<td>Congenital heart defect</td>
<td>166/339 (49%)</td>
</tr>
<tr>
<td>Agenesis of corpus collosum</td>
<td>8/21 (38%)</td>
</tr>
<tr>
<td>Limb anomalies</td>
<td>205/549 (37%)</td>
</tr>
<tr>
<td>Neural tube defect</td>
<td>7/96 (7%)</td>
</tr>
<tr>
<td>Choroid plexus cyst</td>
<td>55/656 (8%)</td>
</tr>
</tbody>
</table>

Table 6.11 Ultrasound abnormalities and frequency of fetal aneuploidy

<table>
<thead>
<tr>
<th>Defect</th>
<th>Overall frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal wall defect</td>
<td>1/30</td>
</tr>
<tr>
<td>Agenesis of corpus collosom</td>
<td>0/4</td>
</tr>
<tr>
<td>Cystic hygroma</td>
<td>0/4</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>0/0</td>
</tr>
<tr>
<td>Duodenal atresia</td>
<td>0/0</td>
</tr>
<tr>
<td>Echogenic bowel</td>
<td>0/0</td>
</tr>
<tr>
<td>Facial cleft</td>
<td>0/0</td>
</tr>
<tr>
<td>Holoprosencephaly</td>
<td>0/0</td>
</tr>
<tr>
<td>Hydrocephaly</td>
<td>0/0</td>
</tr>
<tr>
<td>Hydrogenesrosis</td>
<td>0/0</td>
</tr>
<tr>
<td>Hydrops (nonimmune)</td>
<td>0/0</td>
</tr>
<tr>
<td>IUGR</td>
<td>0/0</td>
</tr>
<tr>
<td>Limb anomalies</td>
<td>0/0</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>0/0</td>
</tr>
<tr>
<td>NTD</td>
<td>0/0</td>
</tr>
<tr>
<td>Nuchal fold/thickness/edema</td>
<td>0/0</td>
</tr>
<tr>
<td>Oligohydramnos</td>
<td>0/0</td>
</tr>
<tr>
<td>Polycydrnomos</td>
<td>0/0</td>
</tr>
<tr>
<td>Renal anomalies</td>
<td>0/0</td>
</tr>
<tr>
<td>SF/E</td>
<td>0/0</td>
</tr>
<tr>
<td>Two-vessel cord</td>
<td>0/0</td>
</tr>
</tbody>
</table>

Structural Abnormalities

• Definition: Breakage and rejoining of chromosomes or chromosome segments

• May be either balanced or unbalanced

• Breakpoints can disrupt gene expression (within a gene or regulatory element)

• Can create gene fusions or affect gene expression (↑↓) by position effect
  – Common in cancer
Structural Chromosome Abnormalities
(Abnormal chromosome is on the right)

Deletions
- Terminal
- Interstitial

Duplications
- 8

Insertions
- 12
- 13

Reciprocal Translocations
- Balanced
- Unbalanced

Robertsonian Translocations
- Balanced
- Unbalanced
Structural Chromosome Abnormalities

(Abnormal chromosome is on the right)

Inversions

- Pericentric
- Paracentric

Ring chromosomes

- Recombinant chromosomes
- Isochromosomes
### Incidence of chromosome abnormalities detected in newborns

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<td>Sex Chromosome Aneuploidies</td>
<td>2.70</td>
<td>375</td>
</tr>
<tr>
<td>Balanced Structural Rearrangements</td>
<td>2.04</td>
<td>490</td>
</tr>
<tr>
<td>Translocations, insertions</td>
<td>0.97</td>
<td>1,028</td>
</tr>
<tr>
<td>Inversions</td>
<td>0.16</td>
<td>6,331</td>
</tr>
<tr>
<td>Robertsonians</td>
<td>0.91</td>
<td>1,099</td>
</tr>
<tr>
<td>Unbalanced Structural Rearrangements</td>
<td>0.63</td>
<td>1,587</td>
</tr>
<tr>
<td>Translocations, insertions, inversions</td>
<td>0.09</td>
<td>10,935</td>
</tr>
<tr>
<td>Robertsonians</td>
<td>0.07</td>
<td>13,366</td>
</tr>
<tr>
<td>Deletions, rings</td>
<td>0.06</td>
<td>17,184</td>
</tr>
<tr>
<td>+Markers (e.g. isochromosomes)</td>
<td>0.41</td>
<td>2,455</td>
</tr>
</tbody>
</table>

Data from: Milunsky and Milunsky, Genetic Disorders of the Fetus, 6th Ed. (2010). Benn, Chp 6

~1/500 is a carrier of a balanced rearrangement
Some syndromic microdeletion and duplication regions

- 1p36 del
- 2q37 del BDMR
- 7q11.23 del (WBS)/dup
- 13q14 del RB1
- 15q11-13 del pat/mat & dup mat
- 20p12 del Alagille
- 18p- 18q-
- 22q11 del (VCFS)/dup
- Inv dup 15
- Wolf-Hirschhorn 4p16.3 del
- 3p15 del
- BWS/RSS 11p15 dup pat/mat
- Pallister-Killian
- Langer-Giedion 8q24 del
- Smith-Magenis/Potocki-Lupski 16p13.3 del
-红酒症
- Langer-Giedion
- Rubenstein-Taybi
- 11p11.2 del
- Jacobsen 11q24 del
- 11p11.2 del/pat mat
- Miller-Dieker 17p13.3 del
- 17p11.2 del/dup
- 17p13.3 del/hnpp/CMT1A
- Cat-eye
- 22q11 del
- Phelan-McDermid
- 5p15 del
- Cri du chat
- 11p13 del
- Pallister-Killian
- 11p11.2 del
- 11q24 del
- Pallister-Killian
- HNPP/CMT1A
- Xp22.31 STS/KAL del

Image modified from Gardner, Sutherland and Shaffer Chromosome Abnormalities and Genetic Counseling 4th ed (2011)
# Incidence of Microdeletion and Duplication Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Incidence</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36 deletion</td>
<td>1:7500</td>
<td>Terminal deletion</td>
</tr>
<tr>
<td>1q21.1 deletion (distal)</td>
<td>1:500</td>
<td>Interstitial deletion (SD)</td>
</tr>
<tr>
<td>4p-/Wolf-Hirschhorn</td>
<td>1:50,000</td>
<td>Terminal deletion</td>
</tr>
<tr>
<td>5p-/Cri du chat</td>
<td>1:50,000</td>
<td>Terminal deletion</td>
</tr>
<tr>
<td>7q11.23/Williams</td>
<td>1:7500</td>
<td>Interstitial deletion (SD)</td>
</tr>
<tr>
<td>15q11q13/Prader-Willi</td>
<td>1:20,000</td>
<td>Interstitial deletion (pat)/ mUPD/methylation defect/mutation</td>
</tr>
<tr>
<td>22q11.2/DiGeorge/VCFS</td>
<td>1:5000</td>
<td>Interstitial deletion (SD)</td>
</tr>
</tbody>
</table>
Chromosome Abnormalities in Cancer

• Numerical
  – Aneuploid: 2n - or + chromosomes
    • Monosomy or trisomy
  – Polyploid: 1n, 2n, 3n, 4n, etc. where n=23 chr.

• Structural
  – Deletions
  – Duplications/amplifications
  – Translocations: balanced or unbalanced
  – Inversions

• Copy-neutral loss of heterozygosity (LOH)
  – Mitotic recombination
  – Mitotic malsegregation: uniparental disomy
Karyotyping in Cancer
e.g. Clinical Utility of Karyotype in ALL

Cytogenetic subtype distribution by age

Harrison. *ASH Education Program* (2013) 118-125
Effects of Translocations

• Constitutional carriers are at risk for infertility, recurrent miscarriage and/or birth of a child with a congenital anomaly syndrome
  – Most risk figures fall into the range of 0-30% for a liveborn child with an abnormality (higher end if previous child)

• May disrupt gene expression (breakpoint within a gene or regulatory element by position effect)
  – In the prenatal setting and if de novo, risk=~6% (Warburton ‘91)

• Create gene fusions and affect gene expression by position effect, especially in cancer
  – e.g. Translocation 9;22 BCR-ABL1 chimeric transcript in CML and ALL
  – e.g. Translocation 11;14 CCND1 upregulation by translocation near the IGH locus regulatory region in MCL and MM
Meiosis in the Balanced Translocation Carrier

A, B: Normal chromosomes
A’, B’: Derivative chromosomes

Gardner, Sutherland and Shaffer. 2012
Meiosis in the Balanced Translocation Carrier

Only alternate segregation will result in normal/balanced gametes.

All other modes of segregation result in unbalanced gametes.

Chromosome Abnormalities and Genetic Counseling. 4th ed. Gardner, Sutherland and Shaffer. 2012
Fluorescence in situ hybridization (FISH)

• A fluorescently labeled DNA fragment is used to detect a chromosome, region or gene in situ

• Advantages:
  – Much higher resolution compared to karyotyping for identifying deletions, duplications, insertions, and translocation breakpoints (down to the 100’s of kb range)
  – Can use cells in any state of the cell cycle (interphase or metaphase), as well as archived tissue
  – Does not require culturing = shorter TAT
  – Greater sensitivity for low-level mosaicism compared to chromosomes (1-5% by interphase FISH)

• Limitation:
  – Targeted approach: only analyzing the region of the genome that is complementary to the FISH probe

FISH for X and Y centromeres on an interphase and metaphase cell
FISH Applications in Constitutional Studies

- Detecting aneuploidy with rapid TAT
- Characterizing structural abnormalities (e.g. translocations)
- Detecting microdeletions/microduplications
  - For undiagnosed patients, genomic microarray is recommended
FISH Applications in Oncology Studies

- Diagnosis: often using panels targeting recurrent and/or prognostic/therapeutic alterations, some cytogenetically cryptic
- Monitoring: using FISH probe(s) specific to the abnormal clone or panels to simultaneously monitor for residual disease and disease progression

1q21/17p13.1  9q34  11q13/14q32  15q22/17q21.2
FISH Applications in Oncology Studies

- Diagnosis: often using panels targeting recurrent and/or prognostic/therapeutic alterations, some cytogenetically cryptic
- Monitoring: using FISH probe(s) specific to the abnormal clone or panels to simultaneously monitor for residual disease and disease progression

15q22/17q21  
9q34/9q34/22q11  
14q32

\[ \text{der(22)} \]

\[ \text{15q22/17q21} \]

\[ \text{9q34/9q34/22q11} \]

\[ \text{14q32} \]

\[ 5' \text{IGH} \]

\[ 3' \text{IGH} \]
Genomic SNP Microarray (SNP-A)

Tiu et al., Leukemia, 2007
Genomic Alterations Detected by SNP-A

Deletion

Duplication

Region of Homozygosity (ROH)
Pros and Cons of Genomic Microarray (GMA)

**Advantages**
- High resolution technology
  - Down to 10’s of kb range (compared to 3-5 Mb by 550-band chromosomes, 100’s kb by FISH)
- No cell culturing or cell preparation required
  - Can use on archived tissues: frozen or formalin-fixed paraffin-embedded (FFPE)
- Objective analysis
- Detection of absence or loss of heterozygosity (AOH/LOH) if SNP genotyping is incorporated

**Limitations**
- Cannot detect balanced structural abnormalities (i.e. translocations, inversions)
- Cannot interrogate repetitive DNA sequence

**Considerations**
- May uncover findings unrelated to the indication for testing (incidental findings)
Increased Genome-Wide Absence of Heterozygosity (AOH)
• There is clinical utility in the detection of genomic AOH, even when the % is quite low (<3%)
  • Risk for autosomal recessive disease
• Cases with >10% genomic AOH have the potential of uncovering a situation of familial abuse
• Laboratories are encouraged to develop a reporting policy in conjunction with their ethics review committee and legal counsel
Single large region of homozygosity (ROH) …

…may indicate inheritance of both chromosomes from the same parent (i.e. uniparental disomy, UPD)

- Usual observation is ROH on a single chromosome
- Results from an error during meiosis or mitosis

ROH on chr. 15 = 19.6 Mb
Uniparental disomy (UPD)

• Biparental inheritance: the normal situation; one chromosome is inherited from each parent

• Uniparental disomy: both chromosome copies come from a single parent
  • Risk for recessive disease for genes in the homozygous chromosome segment
  • Risk for imprinting disorder if involving chromosomes that contain imprinted genes, differentially expressed dependent on parent of origin

Images modified from Yamazawa et al., 2010, Am J Med Gen C
Imprinted chromosomes and human disease due to uniparental disomy (UPD)

Image from: http://carolguze.com/text/442-10-nontraditional_inheritance.shtml

Velissariou, Balkan J Med Gen
Clinical Utility of GMA in Postnatal Studies

Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies


- International standards for cytogenomic arrays (ISCA) consortium: reviewed evidence from 33 studies, including >21,000 patients tested by GMA

  - For genetic testing of individuals with unexplained developmental delay, intellectual disability, autism or multiple congenital anomalies, this technology offers a much higher dx yield (between 15-20%) compared to ~3% by karyotype and excluding other recognizable chromosome syndromes
Detection of submicroscopic, small pathogenic CNVs
Clinical Utility of GMA in Prenatal Studies

Clinically relevant findings in cases with normal karyotype:

<table>
<thead>
<tr>
<th>Indication</th>
<th>Total Clinically Relevant</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA (n=1966)</td>
<td>34 (1.7%)</td>
<td>1.2 – 2.4</td>
</tr>
<tr>
<td>Positive Serum Screen (n=729)</td>
<td>12 (1.6%)</td>
<td>0.9 – 2.9</td>
</tr>
<tr>
<td>Ultrasound Anomaly (n=755)</td>
<td>45 (6.0%)</td>
<td>4.5 – 7.9</td>
</tr>
</tbody>
</table>

Wapner et al., NEJM 2012
Clinical Utility of GMA in Prenatal Studies and in Pregnancy Loss

- Use in prenatal diagnosis: in patients with a fetus with one or more structural abnormalities identified on ultrasound, patients undergoing invasive prenatal diagnostic testing, not restricted to women aged 35+

- Use in intrauterine fetal demise or stillbirth: when further cytogenetic analysis is desired, not recommended for first or second trimester losses due to limited data on utility
Case: IUFD 24 weeks, fetal tissue, CHR: no grow

Chromosome 13
Maternal chromosome analysis: 45,XX,der(13;14)(q10;q10)

- GMA cannot characterize the structure of copy number changes
- Consideration for recurrence risk should be incorporated into interpretation
Which types of cancers should be studied by GMA?

- Those characterized by recurrent copy number changes
- Those that typically have a normal karyotype (do not grow well in culture or have poor mitotic activity compared to nonmalignant cells)

Examples: ALL, CLL, MDS, MM
Recurrent cytogenetic findings in MDS

Schanz et al., 2012 J Clin Oncol (Table 2)

Image source: Nybakken and Bagg, JMD 2014
SNP-A increases the diagnostic yield in MDS from 50% to 70-80%.

Normal karyotype (n=296, composite of multiple studies)

SNP-A Abnormal (42%)
SNP-A Normal (58%)

Image source: modified from Kulasekararaj, Br J Haematol 2013

See references: Gondek et al., 2008; Heinrichs et al., 2009; Tiu et al., 2011; others
Example: ALL with no karyotype results due to poor growth in culture, SNP-A shows hypodiploidy
Multiple techniques are employed for the detection of different cytogenetic abnormalities

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</thead>
<tbody>
<tr>
<td>Chromosome analysis</td>
<td>3-5 Mb (550 bands)</td>
<td>10-15%</td>
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<tr>
<td>Metaphase FISH</td>
<td>100’s kb</td>
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<td>No</td>
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<tr>
<td>Interphase FISH</td>
<td>100’s kb</td>
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<td>No</td>
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<tr>
<td>Genomic microarray analysis</td>
<td>10-100’s kb</td>
<td>10-20%</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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