Unlocking the Diagnostic Potential of Genomic Medicine – The Beaumont DNA Array Experience

Mark Micale, Ph.D., F.A.C.M.G.
Medical Director, Clinical Cytogenomics Laboratory
Department of Pathology and Laboratory Medicine
Beaumont Health System
Associate Professor of Pathology and Laboratory Medicine
Oakland University William Beaumont School of Medicine

Our Current Knowledge of Genetics and Genomics

Karyotyping Has Been the Gold Standard for Identifying Chromosome Abnormalities

- The era of modern cytogenetics began in 1956 with the discovery by Tijo and Levan that the human diploid chromosome number was 46.
- In 1958, Lejeune reported on the chromosomal basis of Down syndrome.
- In 1960, Nowell and Hungerford demonstrated an association between chromosomes and cancer with the discovery of the “Philadelphia chromosome” in chronic myeloid leukemia.
- 1959-1966: Additional associations between chromosome abnormalities and specific phenotypes were made (Turner, Klinefelter, Cri-du-chat, Patau, Edward, Bloom, Fanconi anemia).
- Conventional cytogenetics (karyotyping) represents a whole-genome test but has somewhat limited resolution (5-10Mb).
- Karyotyping is great at identifying larger chromosome abnormalities, but is not good at identifying smaller rearrangements associated with intellectual disability, multiple congenital anomalies, and autism/ASD.

Molecular Cytogenetics (FISH) Provides High-Resolution Targeted Genomic Analysis

- FISH was introduced into the clinical lab in the late 80s, and prenatal FISH aneuploidy detection became routine in the mid 1990s.
- FISH is far more sensitive than karyotyping, but only interrogates a specific genomic region of interest.
- FISH is a great test in those clinical settings whereby the phenotype provides some differential diagnosis:
  - Common microdeletion/microduplications syndromes
  - Fetal chromosome aneuploidy
  - FISH panels for hematolymphoid disorders; detection of specific rearrangements in AML, CML, etc.

Resolution of Conventional and FISH Chromosome Analysis

<table>
<thead>
<tr>
<th>Method</th>
<th>Resolution</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotyping</td>
<td>&gt;5-10Mb</td>
<td>Complete</td>
</tr>
<tr>
<td>FISH (interphase)</td>
<td>&gt;20kb</td>
<td>Probe specific</td>
</tr>
<tr>
<td>FISH (metaphase)</td>
<td>&gt;100kb</td>
<td>Probe specific</td>
</tr>
<tr>
<td>Chromosome microarray/Single nucleotide polymorphism (SNP) array analysis</td>
<td>50kb/SNP 1 bp</td>
<td>Complete</td>
</tr>
</tbody>
</table>

Increasing the Sensitivity of Genomic Assays Permits a Higher Resolution Study of the Human Genome

DNA sequencing
FISH
Chromosome banding

Chromosome Microarray (CMA) or Single Nucleotide Polymorphism (SNP) Analysis

Other highly-sensitive molecular assays: Massively parallel sequencing, exome or whole-genome sequencing (NextGen sequencing), mutation detection
Chromosome Microarray Analysis (CMA) – Molecular Karyotyping

- Analyzes multiple genetic loci at higher resolution than available by conventional cytogenetics (100kb vs. 5-10Mb)
- Compares DNA content between two differentially labeled chromosomes – patient and reference DNA

A Normal CMA Study

CMA Results - Characterization of Chromosome 18q21.2 Deletion

18q21.2 2.5Mb deletion
75 probes 10 genes

CMA in the Diagnosis of Constitutional Genetic Syndromes

- DiGeorge Syndrome
- Prader-Willi/Angelman Syndrome
- Potocki-Lupski Syndrome

My Very Own CMA Abnormality - Chromosome 18q23 Duplication

18q23 copy gain
~210.5kb duplication
24 probes 2 genes
- CTDP1 gene
- KCNQ2 gene

CMA Sensitivity is Based on Array Element Distribution
Copy Number Variants (CNVs)

- Genomic deletions or duplications that may represent rare or common benign variants or pathologic variants (1kb - several Mb in size)
- May encompass as much as 12% of the genome
- CNV may be clinically significant in child but have no phenotype in carrier parent
- Add environmental and genomic factors needed
- CNV may be variably penetrant
- By constructing arrays that eliminate highly variable regions, frequency of benign variants can be minimized
  - Currently more of a problem with oligo arrays
  - Most resolved by parental studies
- Databases are needed for proper interpretation of CNVs – Proprietary, Open access Database of Genomic Variants
- This is a problem for prenatal CMA

Is a CNV Benign or Pathogenic?

- Size
- Location
- Genomic content
- Comparison with other cases
- Inherited or de novo

Example of a Benign CNV

Benign 518.66kb chromosome 9p23 deletion. Notice that there are no genes in the deleted region.

Is a CNV Benign or Pathogenic?

Clinical Genetic Testing: Patients with unexplained DD, MR, RSD, AGL+

- Clinical Presentation
- Structural/Chromosomal
  - Variants in copy number variations
  - Traditional methods
  - Array-based methods (SNV, CNV)
- Mosaic
- Copy number variations
- Whole genome
- Whole exome
- Targeted panel

Miller B et al (2010), AJHG 86, 749-764
Child presents with developmental delay and microcephaly.

CMA Has Been Transformational to the Practice of Clinical Genetics

CMA Identifies Three Genomic Abnormalities Resulting in Gain of 50 Genes

- Derivative chromosome 4 with a 2.15 Mb single copy gain involving chromosome 4q21.22-4q21.31 as well as a 196 kd X-linked copy gain within the segment band 4q11.21.
- Derivative chromosome 4 with a 5.07 Mb single copy gain involving the pericentromeric region of the X chromosome.
- Derivative X chromosome with a single copy gain of a 1.28 Mb segment involving Xp11.22 (HSD17B10, HUWE1, and PHF8 genes all associated with intellectual disability) and (b) a single copy gain of a 5.07 Mb segment involving the paracentromeric region of the X chromosome.

42.95kb Chromosome 22q Deletion Involving the SHANK3 Gene

- Seven year old referred for developmental delay, ADHD, and speech apraxia.
- 42.95kb deletion identified that knocks out one gene - SHANK3.
- This defines the Phelan-McDermid (22q13.3 microdeletion) syndrome; the deletion explains the child's phenotype.
- Identification provides accurate genetic counseling and accurate risk assessment in future pregnancies.

CMA identifies a chromosome 3p triplication in a child with developmental delay.

CMA has Identified Novel Microdeletion/Microduplication Syndromes

Reported diagnostic yield of CMA is 8-17% depending on methodology and selected patient population; most of these patients had a normal karyotype.

- CMA detects de novo CNVs in 10% of patient's with autism (16p11.2 autism locus).
- CMA identifies more microdeletions than microduplications (may be technical or patient selection bias due to milder phenotype in duplications).
- Determination of the clinical significance of CNVs remains an issue.

CMA studies on children with developmental delay, mental retardation, and multiple congenital anomalies

- Number of studies performed: 330 (October 2009-present)
- Number of cases with one or more clinically significant abnormalities: 74 (22.4% yield)
  - Smallest imbalance: 42.95 kb (SHANK3 gene - Phelan McDermid Syndrome)
  - Largest Imbalance: 32.8 Mb (Cri-du-Chat syndrome)
- 16p deletion/duplication: 6/74 cases
- 8p inverted duplication/deletion syndrome: 4/74 cases
- Rare genetic syndromes identified: Pitt-Hopkins syndrome (535kb)
  - Opitz/GBBB syndrome (140.58kb)
- Number of cases with abnormality(s) of uncertain clinical significance: 54 (16.4%)
The parental anxiety created by identifying CNVs of unknown significance
The need to do parental CMA studies to further characterize uncertain results
Familial variants are not always benign due to reduced penetrance and variable expressivity
The need for pretest and posttest genetic counseling
The identification of a genomic imbalance that is not related to the reason for referral nor to the observed phenotype, but rather to late-onset disorders, infertility, neurological, or cancer predisposition loci
Will (and should) CMA replace standard karyotyping and FISH, and when?

Considerations in Prenatal CMA Analysis

Applications of SNP Array Analysis in Oncology

- Chronic lymphocytic leukemia
- Myelodysplastic syndrome
- Plasma cell myeloma
- Acute lymphoblastic leukemia
- Acute myeloid leukemia
- Renal cell carcinoma
- Meduloblastoma
- Glial tumors
- Neuroblastoma

SNP Oligonucleotide Arrays

- SNP arrays identify a set of genetic variants (single nucleotide polymorphisms) in an individual; Estimated one SNP/100-300 bp
- Probes on a SNP array consist of oligonucleotides that contain SNPs; SNP arrays can distinguish parental alleles at heterozygous loci
- 9.2 million SNPs have been reported (HapMap); 300,000-600,000 SNPs contain most of the information about the patterns of genetic variation
- SNP genotyping permits genome-wide high-resolution detection of copy-copy number changes (Affymetrix CytosHD SNP array with 2.697 million DNA markers)

SNP Arrays Detect Copy Number Neutral Loss of Heterozygosity (aUPD)

SNP Arrays Detect Copy Number Neutral Loss of Heterozygosity (aUPD)

- Mitotic Recombination Error Resulting in Segmental UPD

UPD has been described in CLL, PCV leading to JAK2 homozygosity, MDS/MPD, AML, and follicular lymphoma

Principles of Copy Number Detection and Genotyping Using SNP Arrays

- The problem with current SNP arrays is that they can’t detect balanced rearrangements!

The Power of SNP Array Analysis – A Case of Mantle Cell Lymphoma

- Normal karyotype
- Positive for IGH/CCND1 fusion and for monosomy 13 by FISH
The Power of SNP Array Analysis – A Case of Mantle Cell Lymphoma

FISH misidentified this as monosomy 13. In fact, it’s far more complicated!

SNP Array Analysis of Chronic Lymphocytic Leukemia

- CLL is a highly variable disease with life expectancies from a few months to many decades
- CLL FISH panel detects abnormalities that may not be found by karyotype
- CLL SNP array useful as the disease is characterized by genomic gain or loss
- CLL SNP array has high concordance with other studies; however, SNP array can identify 30-76% more abnormalities
- Elevated CLL genomic complexity is an independent and powerful marker to identify patients with aggressive disease and a shorter survival!

SNP Array Analysis in AML

- Copy number alterations are identified in 15-40% of AML patients with a normal karyotype and predict an adverse clinical outcome
- The implementation of SNP array analysis in AML can increase the detection of clinically significant anomalies (including aUPD) from ~50% by karyotyping to ~87%
- Copy number alterations were identified in 40-90% of MDS/AML patients with abnormal clones and nearly 100% of cases with complex karyotypes

SNP Array Analysis in Renal Cell Carcinoma

- Clear cell RCC
- Papillary RCC
- Mucinous, tubular, and spindle cell carcinoma
- Chromophobe RCC
- Oncocytoma

The Significance of Acquired UPD as Detected by SNP Array Analysis

- Copy neutral LOH is known to constitute between 50-80% of LOH in all human cancers
- aUPDs are found in:
  - 20% of AML
  - 30% of MDS
  - 80% of lymphomas
- UPDs are also associated with homozygous mutations of a number of tumor suppressor genes including TET2, CDKN2A/B, TP53, NF1, RB, CEBPa, and RUNX1
- UPDs are also associated with gain-of-function alleles of oncogenes including JAK2, NRAS, c-CBL, and FLT3
Neuroblastoma (NB), the most common extracranial tumor of childhood, can vary in clinical behavior from highly aggressive to spontaneous regression. Children <1 year present with localized tumor and have an excellent outcome while older children have more aggressive disease and a poorer prognosis.

The prognosis is highly dependent on cytogenetic characteristics:

<table>
<thead>
<tr>
<th>Good Prognosis</th>
<th>Poor Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near-triploidy</td>
<td>Numerical only</td>
</tr>
<tr>
<td>Numerical only</td>
<td>Near-diploidy with segmental rearrangements</td>
</tr>
<tr>
<td>MYCN gene amplification</td>
<td></td>
</tr>
</tbody>
</table>

Genome-wide microarray analysis is an excellent tool to assess cytogenetic status and is important to fully evaluate risk, aid diagnosis, and guide treatment.

del(11q) NBs constitute a unique group of unfavorable tumors with distinct features that differ from the other high-risk NB group (MYCN-amplified).

Other Molecular Technologies May Supplant CMA

Ion Torrent Proton Sequencer

The $1000 Genome Will Transform Medicine (and Make it a Lot More Complicated!)

Thank You