**Arizona Society of Pathologists meeting, 4/13/13: 1:45-2:30PM, Brothman – outline/ summary**

# The New Cytogenomics Era

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## (Very) quick history of cytogenetics:

| | |
|---|---|
| Boveri-Sutton chromosome theory - 1904 | FISH (Pinkel) - 1986) |
| Hsu – discovers hypotonic - 1952 | Sanger sequencing - 1977 |
| Tjio and Levan – "human 2n=46" - 1956 | CGH (Kallioniemi - 1993) CMA (Pinkel - 1998) |
| Q-banding (Caspersson) - 1970 | Mayer and Farinelli - "NGS" - 2000 |
| G-banding (Seabright) - 1971 | Human Genome Project complete – 2001 |

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## What is cytogenomics? Evaluation of the whole structural genome

**Cytogenetics:** original whole genome analysis

*Analysis of chromosomes from a tissue of interest to identify large scale genomic alterations; G-banded karyotype*

**Molecular cytogenetics:** analysis of small regions for imbalances and rearrangements*: FISH (fluoresecence in situ hybrization , CMA (cytogenomic microarray)*

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## American College of Medical Genetics and Genomics

Official name change of the College in March, 2012 to reflect the increasingly central role of medical genomics and its importance alongside genetics in fulfilling the mission of the College.

"*The vastly increased power of the genomic approach has made it more and more vital to the practice of medical genetics and recognizes the current importance of genomics as well as its future roles in both the clinical and laboratory practices*

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## Objectives

- Review the main technologies discussed below and answer the questions:
- Chromosomes – are conventional cytogenetics still necessary?
- FISH – is FISH still necessary, prudent and sufficient?
- CMA – where does this fit in?
- Brief introduction to next generation sequencing – are we ready for this in diagnostics?

**Cytogenomics, two components:**

- Utilization for **constitutional** (germline) studies – CMA becoming accepted as first tier, already has replaced some studies
- Utilization for **acquired** (oncology) studies – CMA gaining acceptance to complement conventional cytogenetics and FISH

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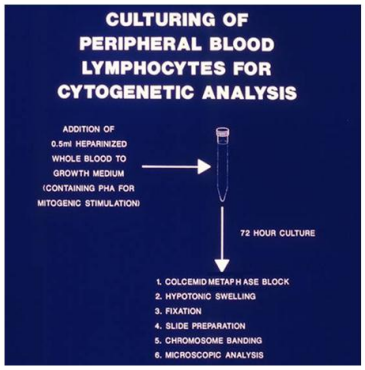
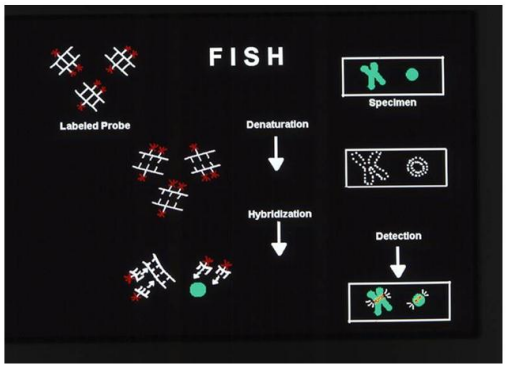
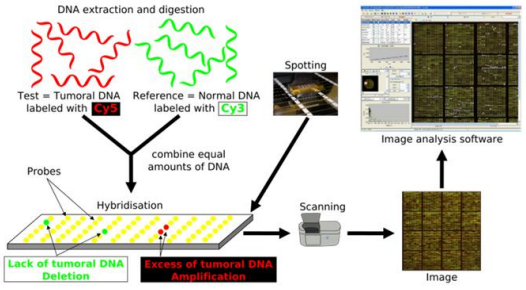
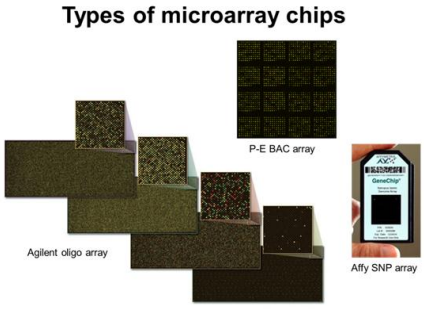
## Techniques: "all you need to know"

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**Whole genome analysis: conventional karyotype: resolution ~5 Mb, CMA can increase ~1000 fold!**

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**Examples of constitutional abnormalities seen by conventional cytogenetics, FISH and CMA**

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**First time a recommended test suggested to replace conventional karyotyping**

Position paper published by ISCA consortium recommending arr as first tier testing for developmental and congenital abnormalities. *Miller et al. AJHG 86:749, 2010*

**CMA gaining acceptance in prenatal medicine, too much information?**

**Clinically significant CNVs in first multicenter prenatal cohort**

Table 3. Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

| Indication for Prenatal Diagnosis | Normal Karyotype | Common Benign | Pathogenic | Uncertain Clinical Significance (N=130) | | Total Known Pathogenic and Potential for Clinical Significance* |
|---|---|---|---|---|---|---|
| | | | | Likely to Be Benign | Potential for Clinical Significance | |
| | *no.* | | *no. (%)* | | | *no. (%) [95% CI]†* |
| Any | 3822 | 1234 (32.3) | 35 (0.9) | 69 (1.8)‡ | 61 (1.6) | 96 (2.5) [2.1–3.1] |
| Advanced maternal age | 1966 | 628 (31.9) | 9 (0.5) | 37 (1.9) | 25 (1.3) | 34 (1.7) [1.2–2.4] |
| Positive on Down's syndrome screening | 729 | 247 (33.9) | 3 (0.4) | 13 (1.8) | 9 (1.2) | 12 (1.6) [0.9–2.9] |
| Anomaly on ultrasonography | 755 | 247 (32.7) | 21 (2.8) | 16 (2.1) | 24 (3.2) | 45 (6.0) [4.5–7.9] |
| Other§ | 372 | 112 (30.1) | 2 (0.5) | 3 (0.8) | 3 (0.8) | 5 (1.3) [0.6–3.1] |

*Wapner et al NEJM 367,215, 2012*

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**Need to distinguish between pathogenic and benign CNVs:**

**Factors influencing the risk assessment of a CNV**

| MAJOR CRITERIA | | Characteristics of a CNV that is: | |
|---|---|---|---|
| | | Pathogenic | Benign |
| 1. | a. Inherited from a healthy parent | | ✓ |
| | b. Inherited from an affected parent | ✓ | |
| 2. | a. Similar to a CNV in a healthy relative | | ✓ |
| | b. Similar to a CNV in an affected relative | ✓ | |
| 3. | CNV overlaps a genomic imbalance in a CNV database for healthy individuals (e.g. Database of genomic variants) | | ✓ |
| 4. | CNV overlaps a genomic imbalance in a CNV database for clinical patients (e.g. DECIPHER) | ✓ | |
| 5. | CNV contains morbid OMIM genes | ✓ | |
| 6. | a. CNV is gene-rich | ✓ | |
| | b. CNV is gene-poor | | ✓ |

*from: Lee, Iafrate, Brothman Nat Genet:39:S48,2007*

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**Acquired abnormalities: Cancer is a clonal disease and all cancers have some genetic component**

The first clear understanding of a mechanism in cancer came from the identification of the "Philadelphia chromosome" – t(9;22). This resulted in the development of the first "tailor made" drug to treat CML: Gleevac (Imatinib) – a specific tyrosine kinase inhibitor asssociated with the translocation breakpoints – or fusion gene. **Cytogenetics, FISH and now CMA play major roles in cancer diagnosis, prognosis and (as noted above) treatment.**

**Examples of acquired abnormalities seen by conventional cytogenetics, FISH and CMA**

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## Cancer Cytogenomics Microarray Consortium

- 500 international institution members
- Share data and interpretive information
- Preliminary studies indicate >90% concordance in cytogenetic, FISH and histology for MDS, CLL and renal cell carcinomas
- www.cancergenomics.org

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**Need for public databases and review of copy number variation (currently ~29,000 reported CNVs)**

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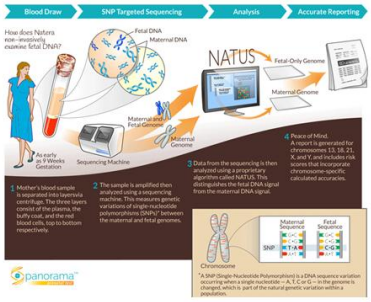
## Beyond cytogenomics – DNA sequencing

- First human genome sequence took ~11 years (1990 to 2001) to complete at a cost of ~$3-billion.
- Now multiple genomes can be sequenced in a few days at a cost of <$2000.
- Initial technology: Sanger (capillary electrophoresis) sequencing (1975)
- Next generation (massive parallel sequencing) – 2008.
- Has led to commercially available testing, including prenatal, cell-free screening for aneusomies.
- 87 abstracts at this year's ACMG meeting!

One example: Non-invasive prenatal testing using cell free fetal DNA from maternal serum – **SCREENING TEST**




**Offered by:Sequenom MaternaT21™ Plus, Verinata Health - verifi® Prenatal Test, Ariosa Diagnostics - Harmony™ Prenatal Test, Natera Panorama™ Prenatal Test**

## Recent position on "incidental findings" (mutation based, by whole genome sequencing) by ACMG

**Highlights:**

**Pretesting counseling to define incidental findings**

**Limit initial clinical interpretations to well defined genes (57) expect ~2% of the population to have a mutation in one of the genes.**

**Patients and their families cannot "opt out" of knowing result ("duty to warn" more important that autonomy); inform parents and children of result. Negative result not "normal"**

**Genetic counseling for patients and their families critical**

*Green et al. (Genetics in Medicine): American College of Medical Genetics and Genomics recommendations for reporting of incidental findings in clinical exome and genome sequencing. Position statement 3/22/13.*

## Summary

- Review the main technologies discussed below and answer the questions:
- Chromosomes – are conventional cytogenetics still necessary? **YES!**
- FISH – is FISH still necessary, prudent and sufficient? **YES – for balanced and targeted copy number abnormalities!**
- CMA – where does this fit in? **Constitutional first tier, gaining speed in oncology.**
- Brief intro. to next generation sequencing – are we ready for this in diagnostics? **?**

## Some Key (and hopefully useful) References:

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