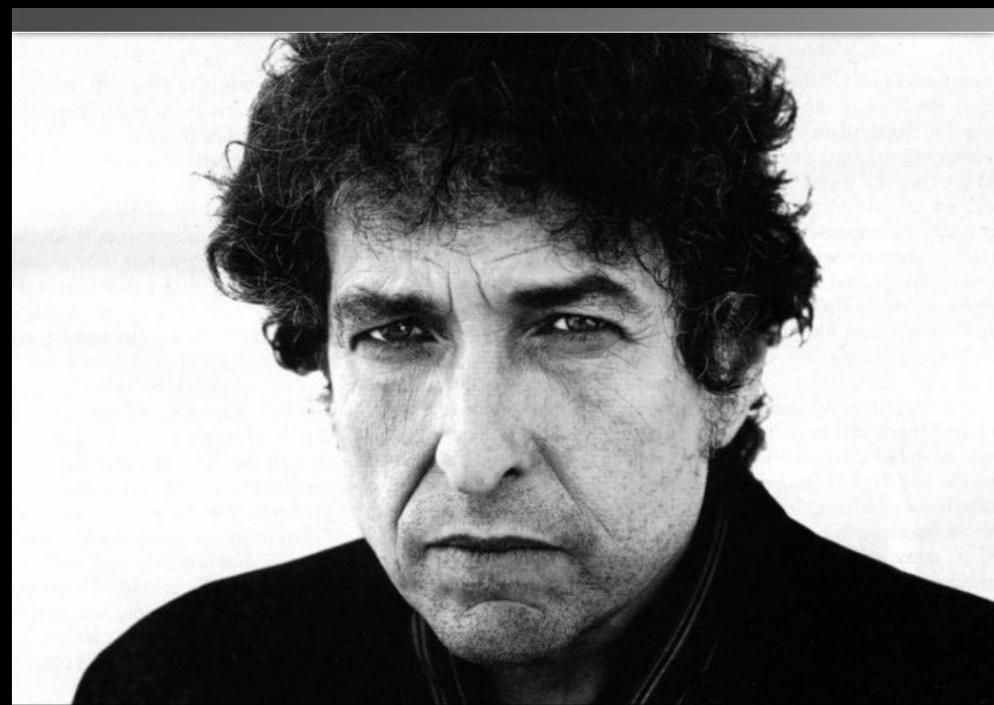


Come gather 'round people  
Wherever you roam  
And admit that the waters  
Around you have grown  
And accept it that soon  
You'll be drenched to the bone.  
If your time to you  
Is worth savin'  
Then you better start swimmin'  
Or you'll sink like a stone  
**For the times they are a-changin'.**



Performing Invasive Testing For Maternal Age Alone  
Can No Longer Be Justified

Down Syndrome Screening Has Moved From The  
Second To The First Trimester

More Accurate

Safer

Patients Prefer

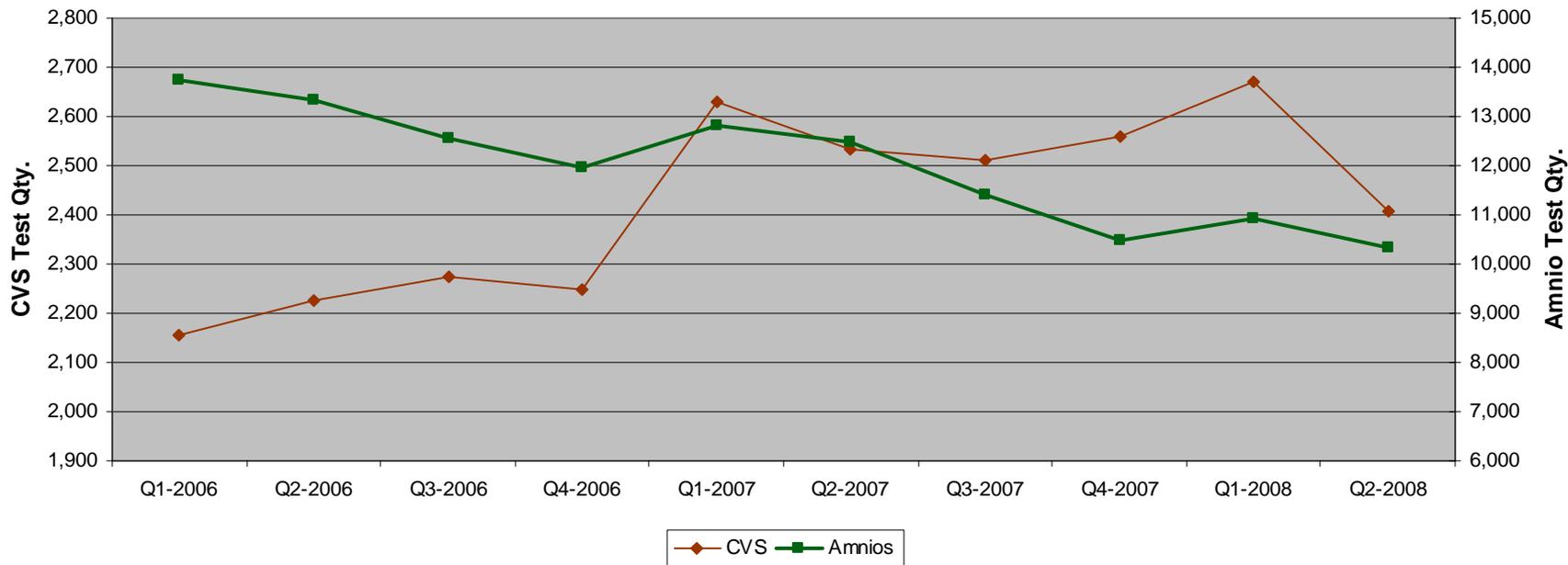
Invasive Prenatal Testing is moving to the first  
trimester

# 1<sup>st</sup> Trimester Screening National Genetic Laboratory

Year	1 <sup>st</sup> Trim Screening
2005	1
2006	2.11 X 2005
2007	2.0 X 2006
2008*	1.6 X 2007

\*: 2008 rates are almost 7 folds higher than those of 2005

Trend: CVS Testing vs. Amnio Testing - Q1'06 - Q2'08



Test Quantity	Q1-2006	Q2-2006	Q3-2006	Q4-2006	Q1-2007	Q2-2007	Q3-2007	Q4-2007	Q1-2008	Q2-2008	% Change Q2'06 to Q2'08
CVS	2,155	2,225	2,275	2,247	2,629	2,532	2,511	2,558	2,671	2,406	8%
Amnio	13,750	13,336	12,546	11,972	12,806	12,498	11,403	10,483	10,932	10,322	-23%

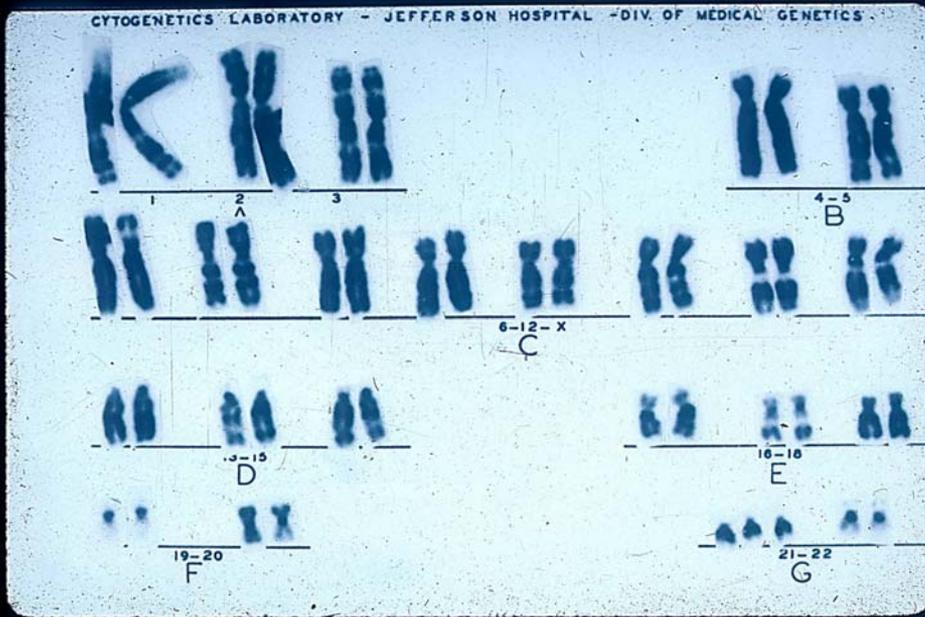
Percent of live births  
with Amnio

2006	2007
7.30%	6.60%

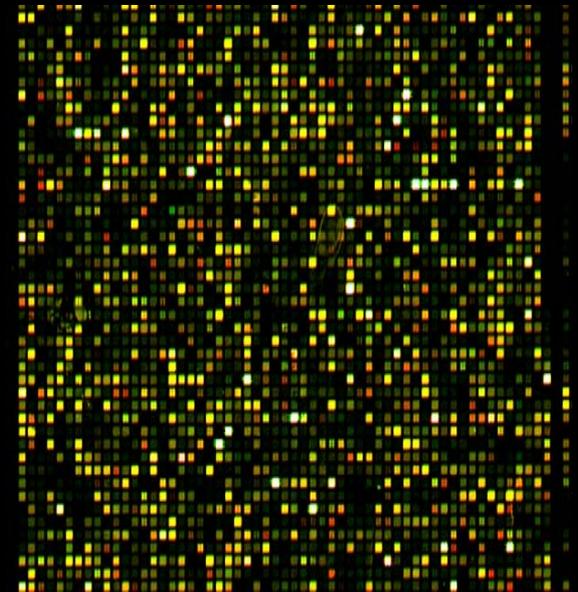
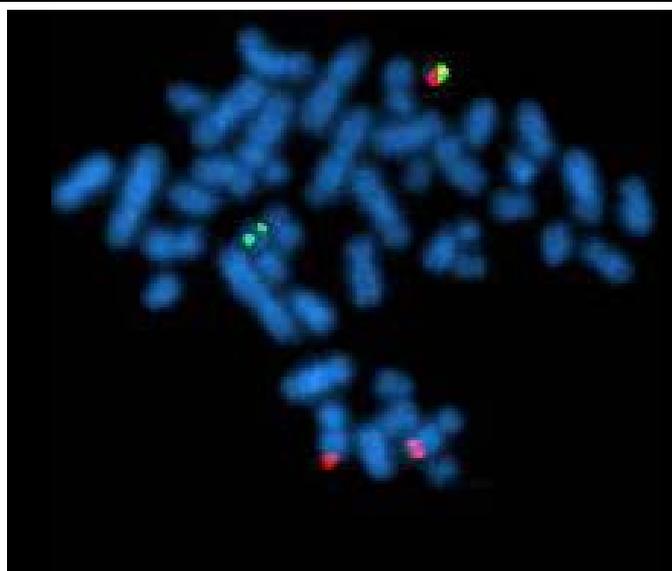
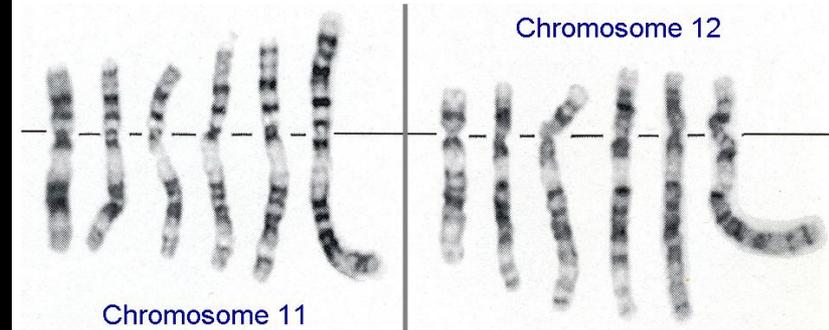
Percent of live births  
with CVS

1.20%	1.36%
-------	-------

# The Evolution of Laboratory Prenatal Diagnosis



## Banding Resolution



# Human Genetic Variation

## Copy Number Variation

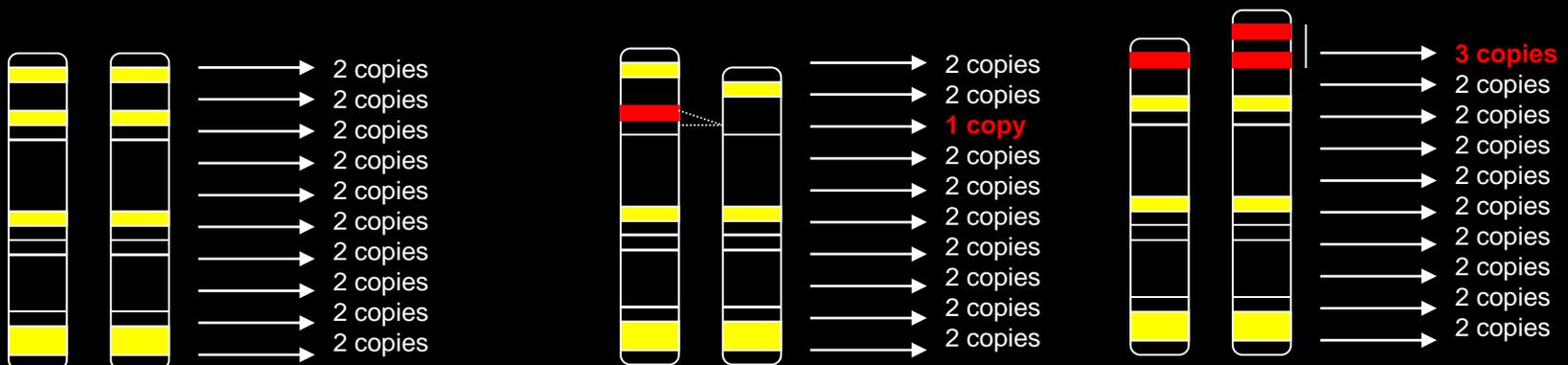
A CNV is a DNA segment (usually larger than 1 kb) present at an altered copy number in comparison with a reference genome

- Whole chromosome aneuploidy
- Segmental Aneuploidy

Deletions

Duplications

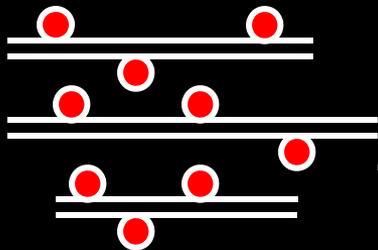
Copy number polymorphisms



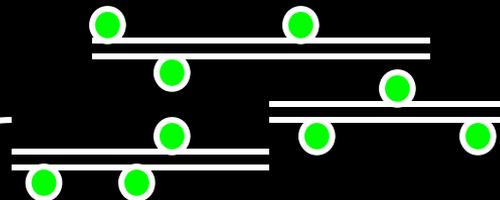
# Comparative Genomic Hybridization

## Differential labeling of DNA

Reference (Normal Genomic) DNA

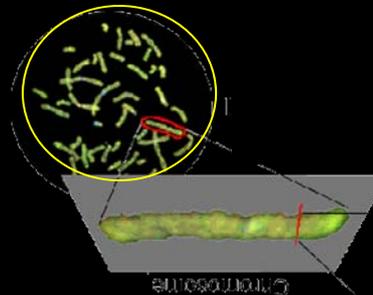
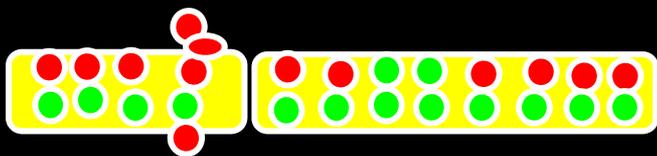


Test DNA from Patient

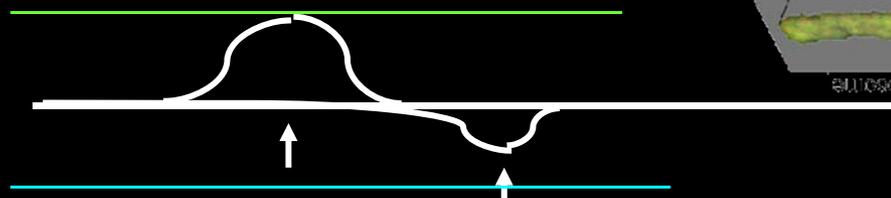


+ Human cot-1 DNA

Denature and preanneal  
Hybridize to normal chromosomes on slides



Ratio profile



excess of test  
DNA

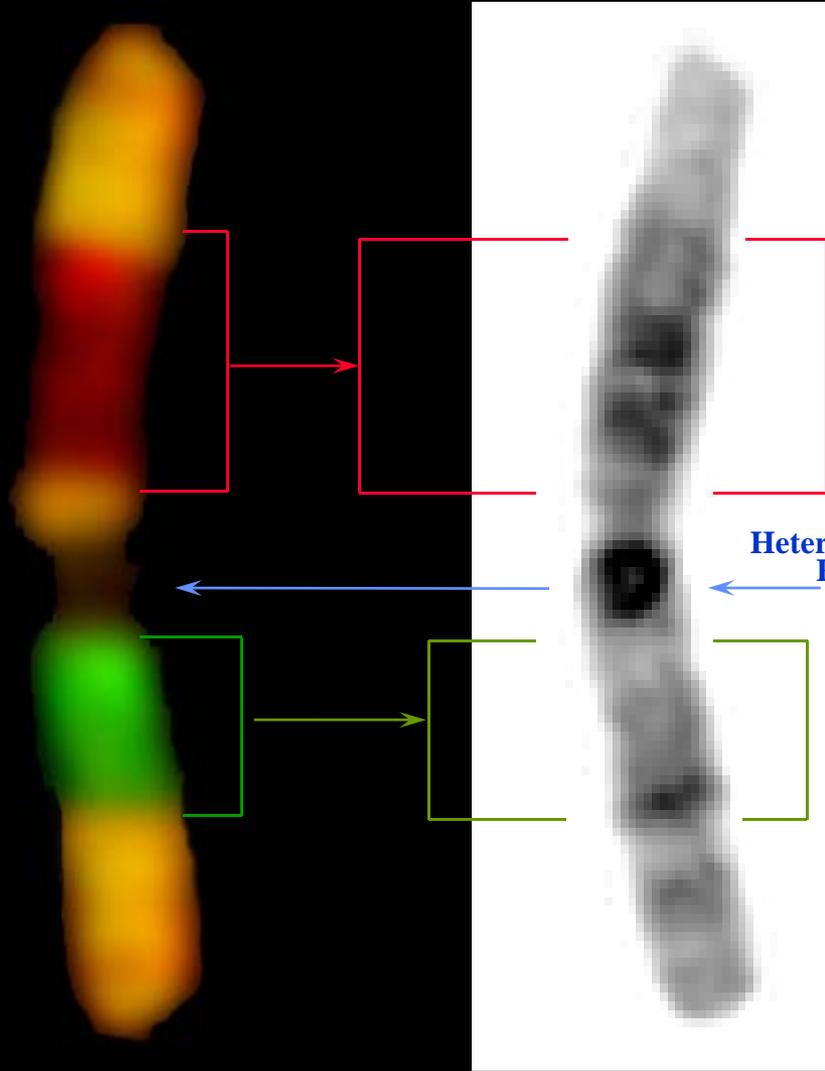
deficiency of  
test DNA

Duplication

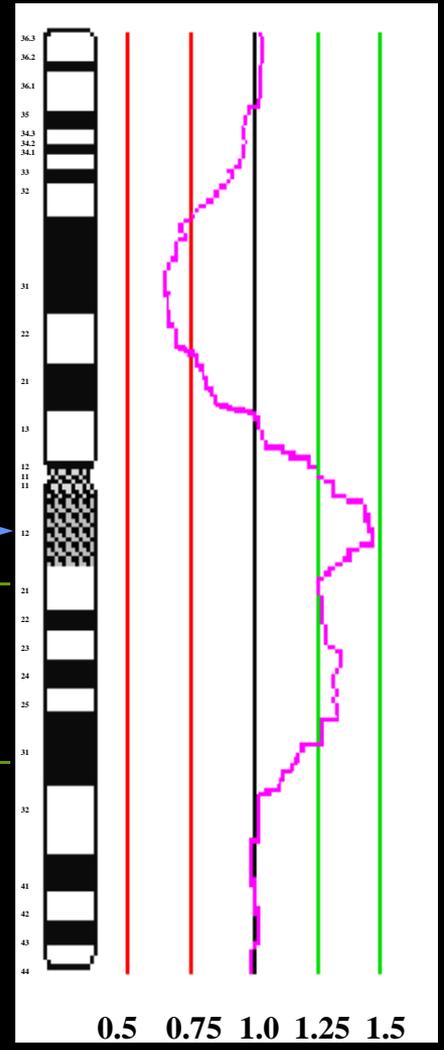
Deletion

LOSS

GAIN



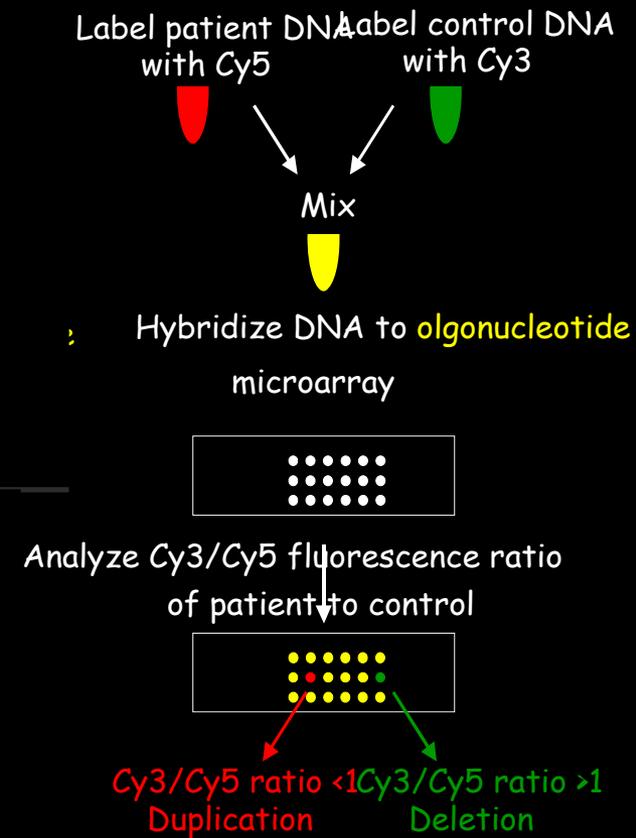
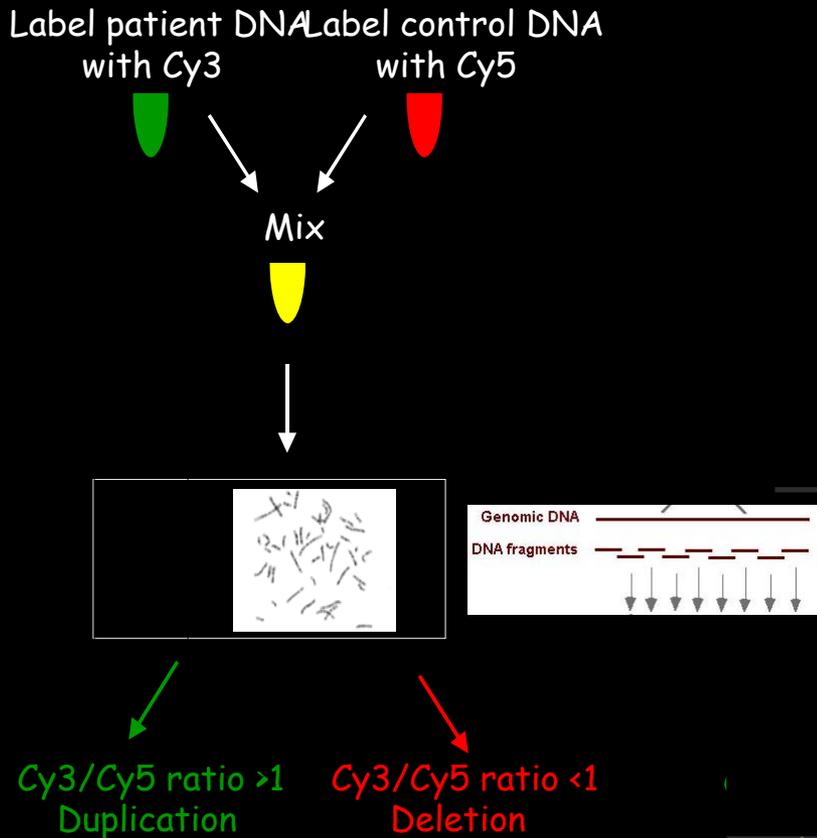
Heterochromatic Region



Chromosome 1

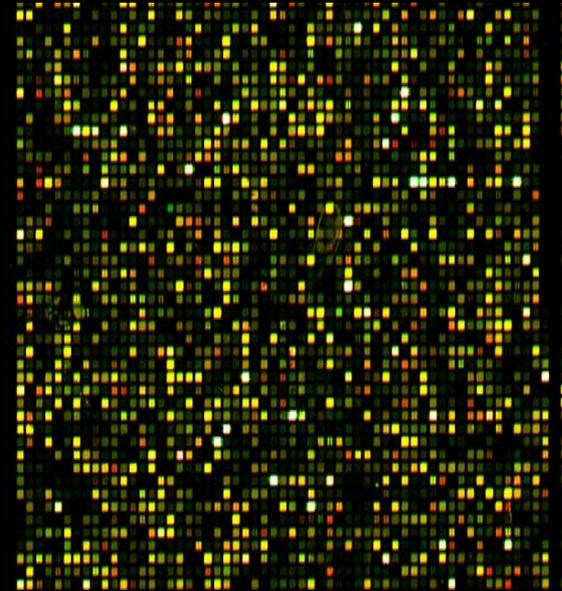
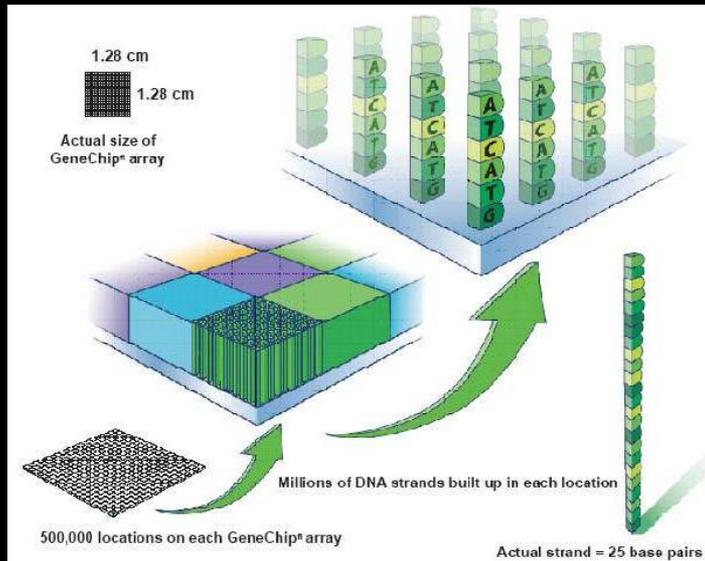
# Conventional CGH

# Array CGH (aCGH)



# Oligonucleotide-Based Microarrays

Oligos are synthesized directly on slides



Short target sequences pretested for hybridization efficiency

Resolution limited only by number of oligos

# Post Natal Arrays For Children With Normal Karyotype, Dysmorphic Features And /Or Developmental Delay

## High Resolution Arrays

		N	% with significant CNV	Familial CNV or CNV of unknown Significance
DeVries 2005	Whole genome tiling-path	100	10	97% with CNV
Friedman 2006	100kb	100	11	100% with CNV 30 CNV per case
Menten 2006	1.0Mb	140	13.6	

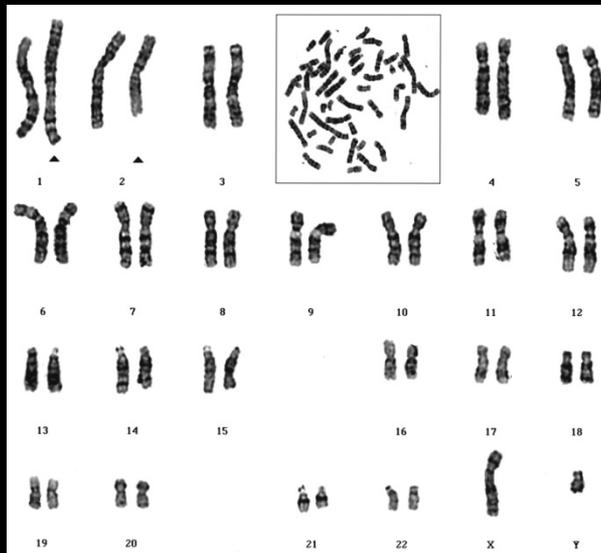
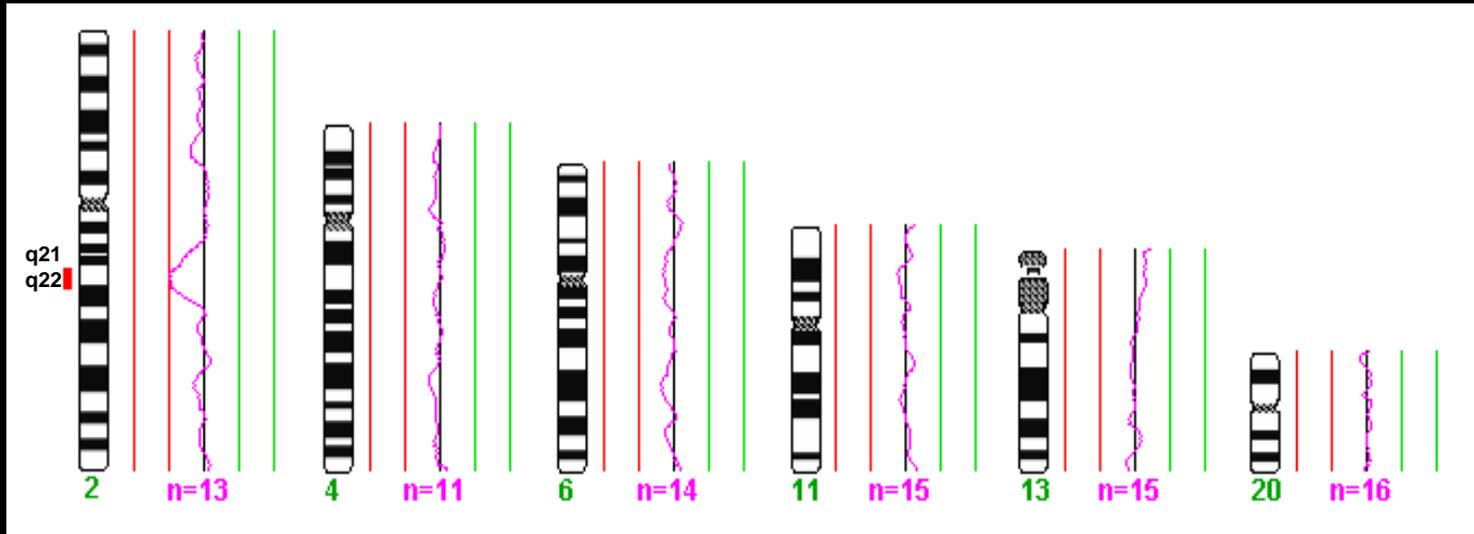
## Targeted Arrays

Poss 2006	Targeted	121	9.6	
Aylor 2006	Targeted	1200	7.0	
Schaeffer	Targeted	1500	5.6	3.3% with CNV

# Differences Between Prenatal and Postnatal Cytogenetic Testing

- **Completeness and Accuracy of Phenotype**
  - Limitations of Ultrasound
  - Prenatal Testing Being Done Before Phenotype Develops
- **Use of Information**
- **Desires of Parents**

# CGH in a patient with an "Apparently Balanced Translocation" and Clinical Abnormalities

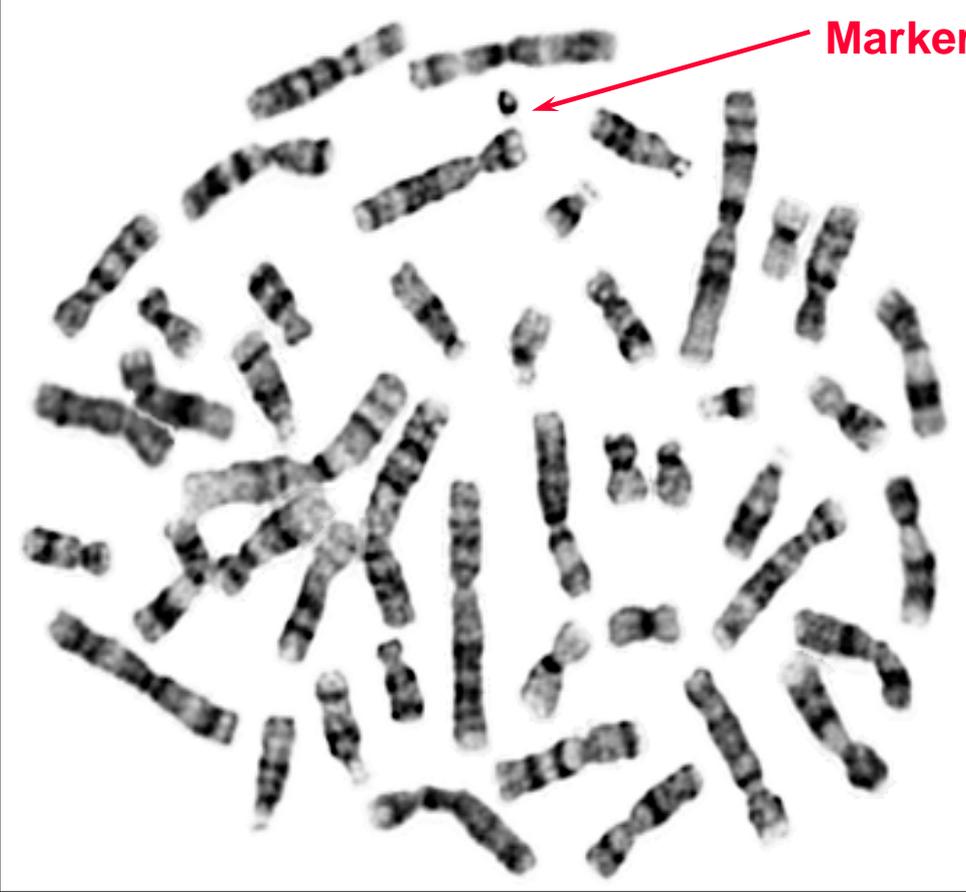


Initial Karyotypic Designation  
46,XY,t(1;2)(p22;q14.1)



# Apparently Balanced Reciprocal Translocation by Karyotype

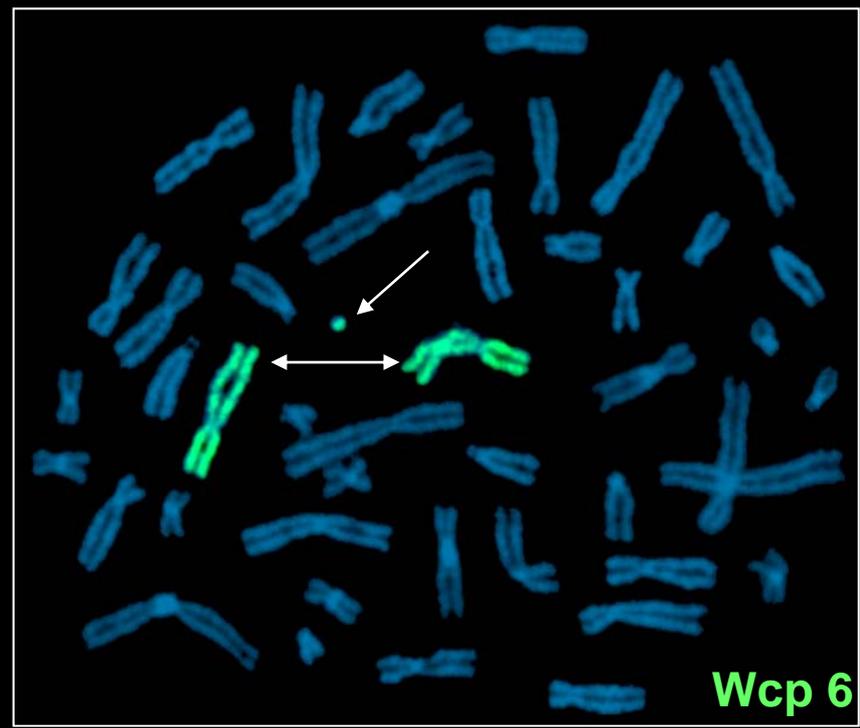
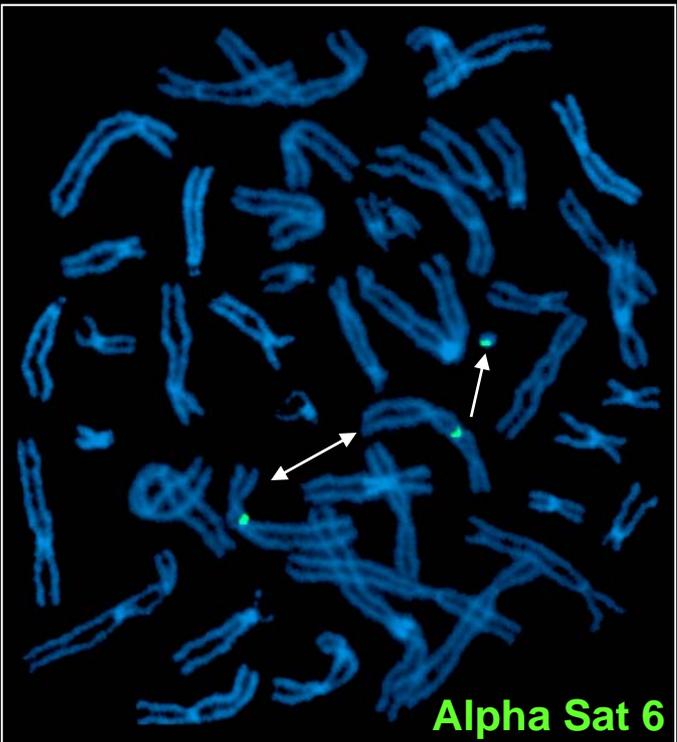
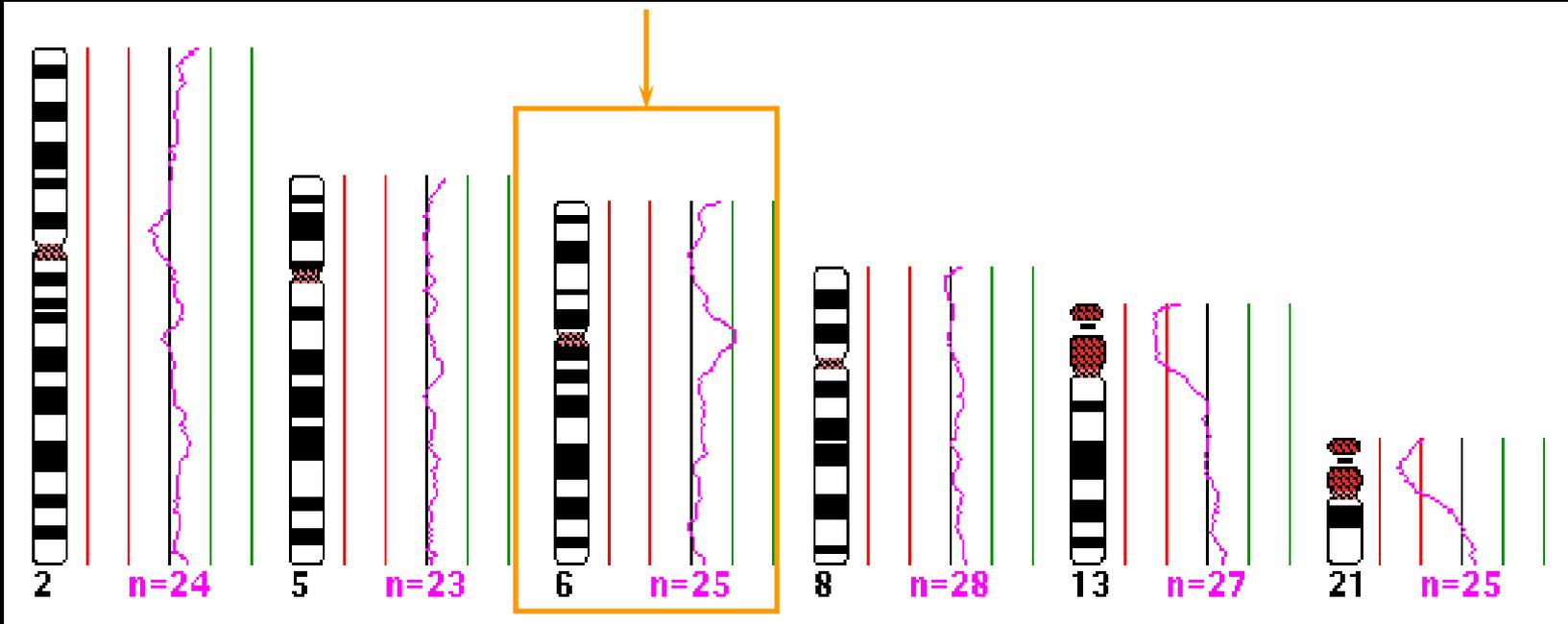
	<u>Unbalanced by aCGH</u>
Abnormal Phenotype	15/40 (37%) 11/27 (40%)
Prenatal Diagnosis	0/14 (2 with phenotype abn)



## Risk of Abnormal Phenotype with Marker Chromosome

Non- Satellited	14.7%
Satellited	10.9%

Acrocentric vs non-acrocentric  
Heterochromatin vs euchromatin



# Evaluation of Stillbirth



Abnormal Karyotype:

Structurally Normal Fetus

4-5%

Abn/Macerated/ Fetus

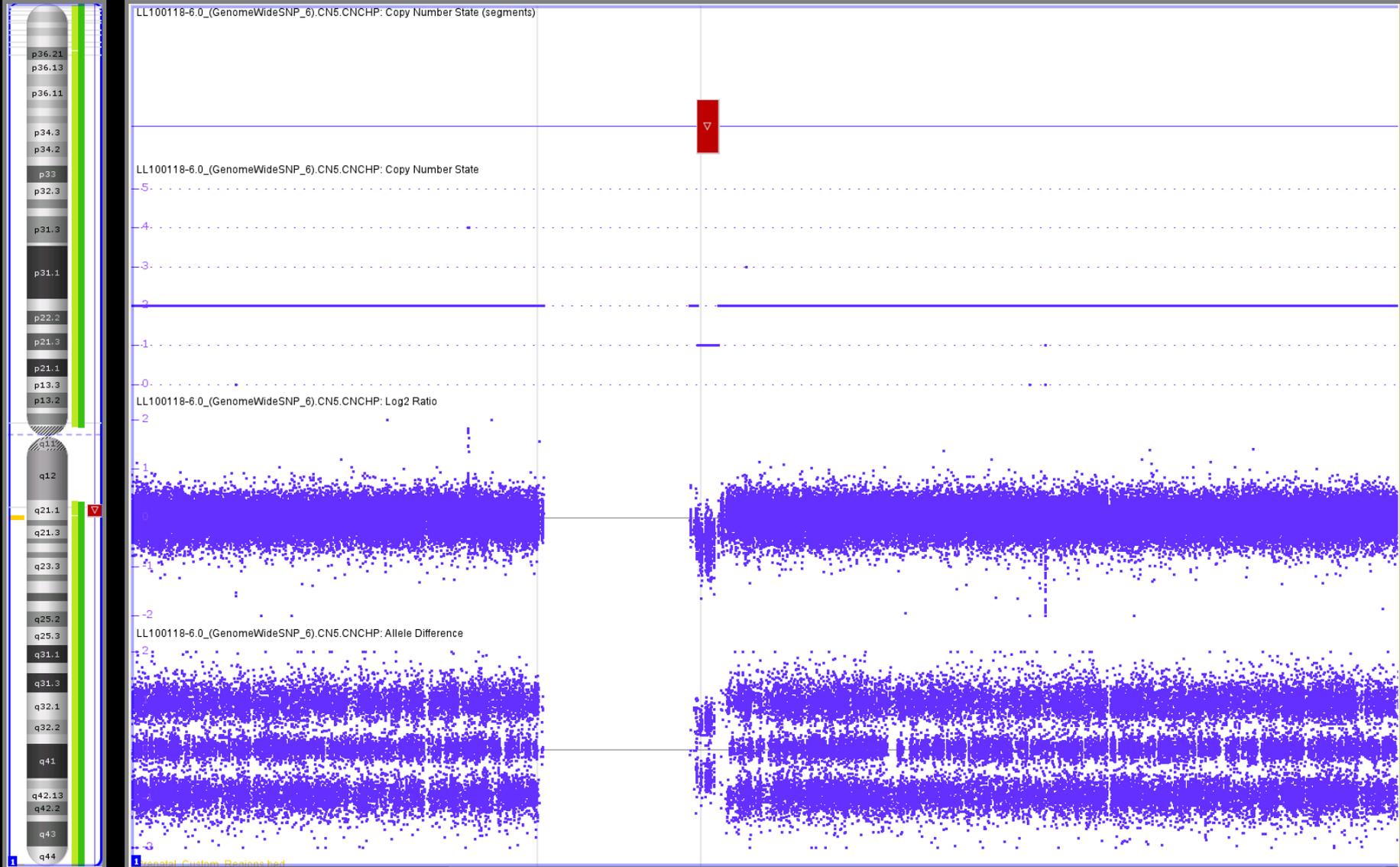
35-40%

Success of Karyotype in stillborn  
pregnancies = 50%

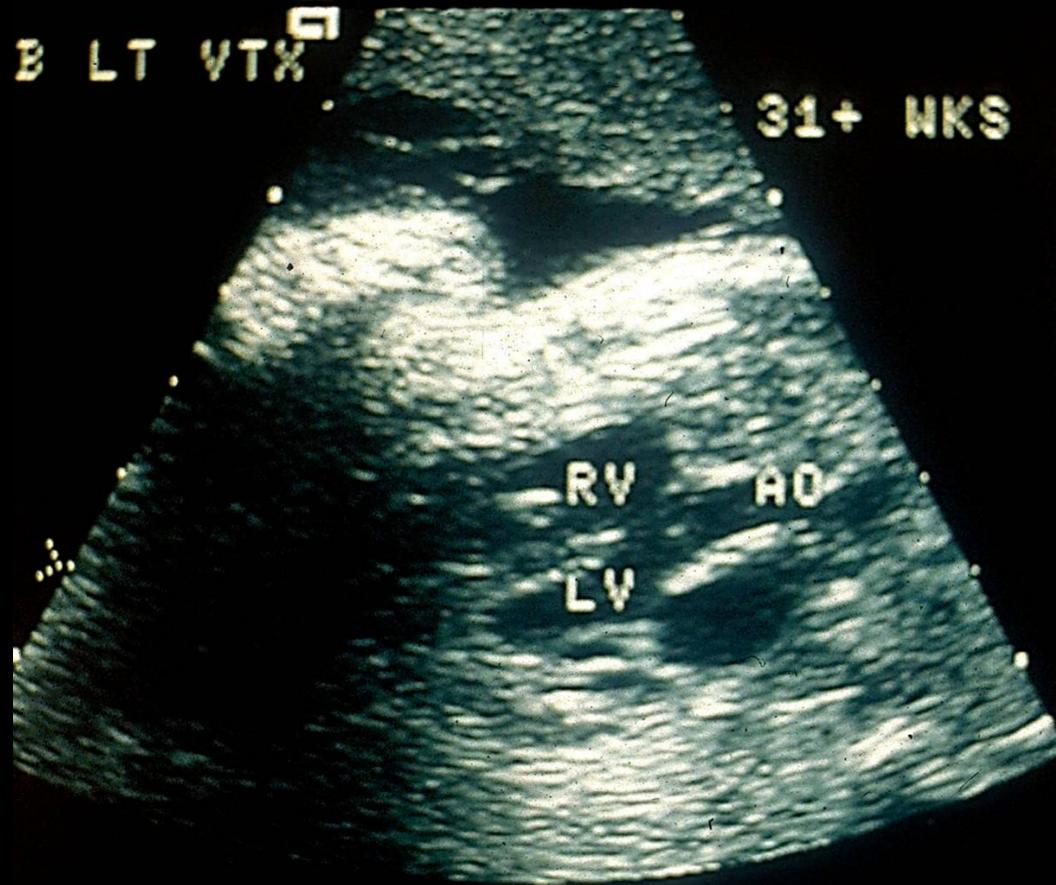
Less in macerated Fetuses

# Stillbirth: Normal Karyotype

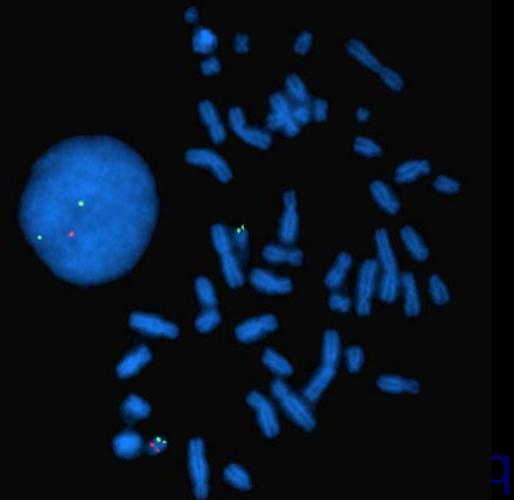
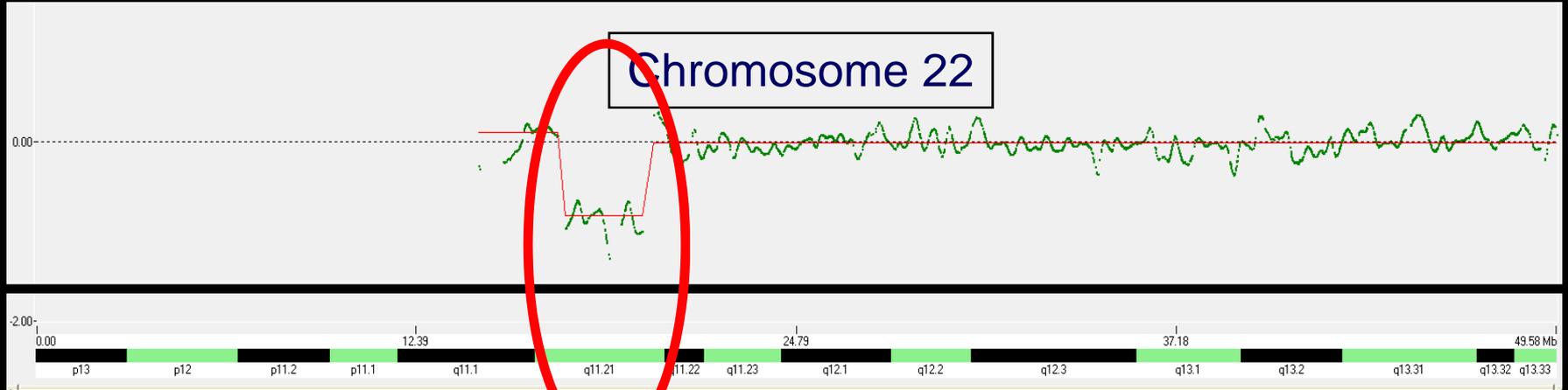
arr 1q21.1(143,845,772 – 146,838,707)x1



# Evaluation of Structural Anomaly Seen on Ultrasound



# 22q Deletion



p

q

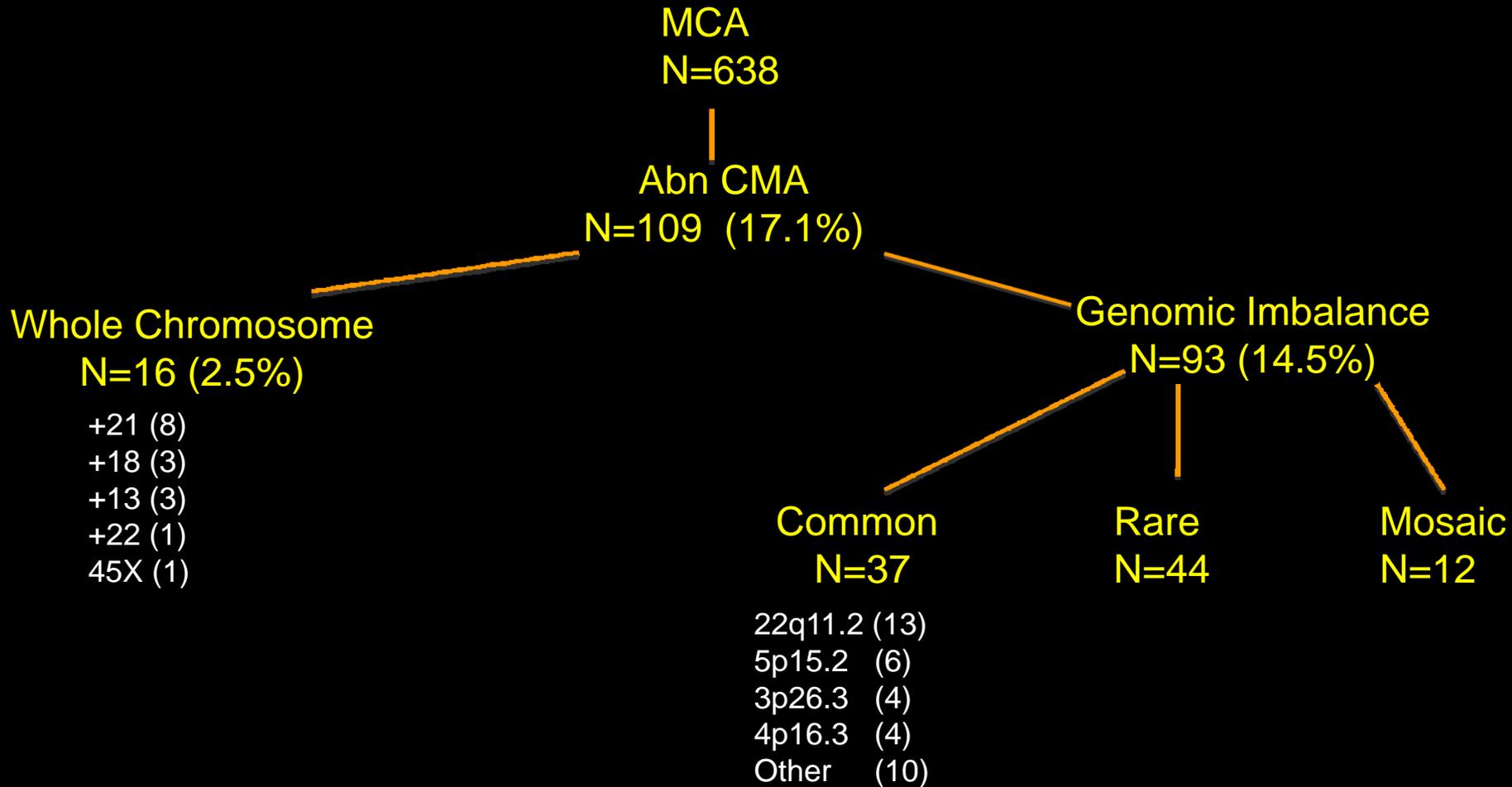
# Microdeletion Syndrome

## 22 q 11 Deletion Spectrum of Clinical Features N = 900

	<u>%</u>
<b>Learning Disabilities</b>	
-none/mild-	62%
-moderate/severe	30%
<b>Cardiac Defects</b>	75%
<b>Genitourinary Defects</b>	36%
<b>Palate Anomalies</b>	76%%
-cleft palate	9%
-velopharyngeal insufficiency	67%
<b>Abnormal facial features</b>	frequent
<b>Growth Delay (&lt;3rd %)</b>	36%
<b>Psychosis /Behavior Problems</b>	25%
<b>Hypoparathyroid</b>	60%

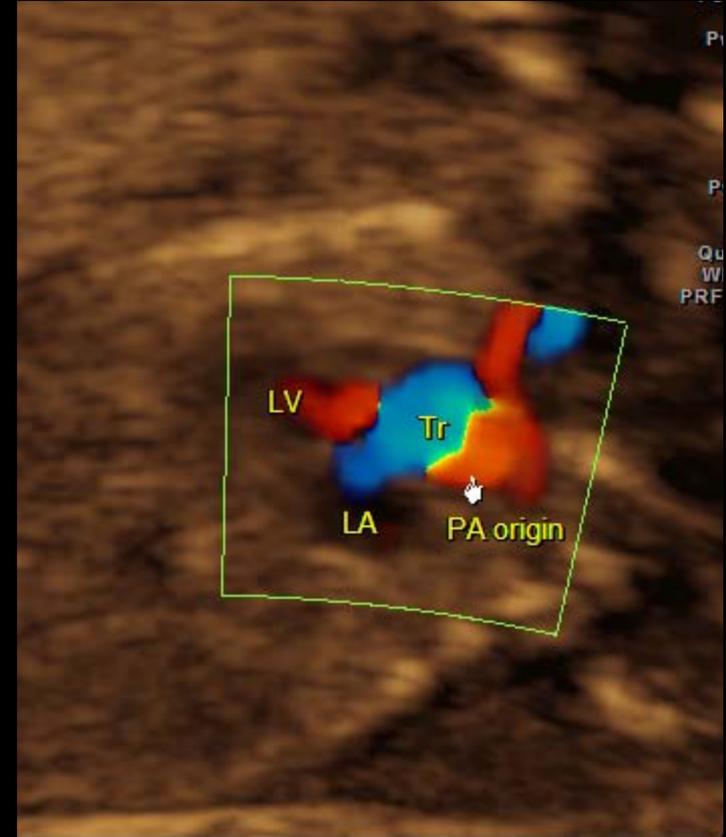
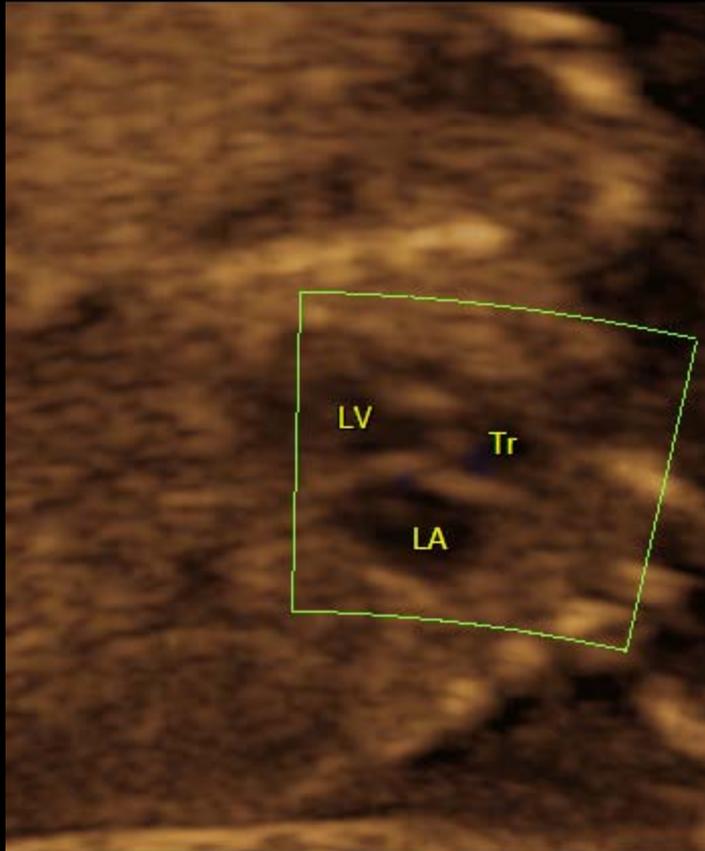


# Frequency and Type of Pathologic CNV with Neonatal Congenital Anomalies



# CVS: Normal Karyotype

Array: arr 22q11.21(17,299,941-19,770,515)x1



15 week fetal echocardiogram



13465-08-11-07-5

AB 2-7/OB

MI 1.3

10.4cm / 37Hz

TIs 0.3

11/07/2008 02:26:14 PM



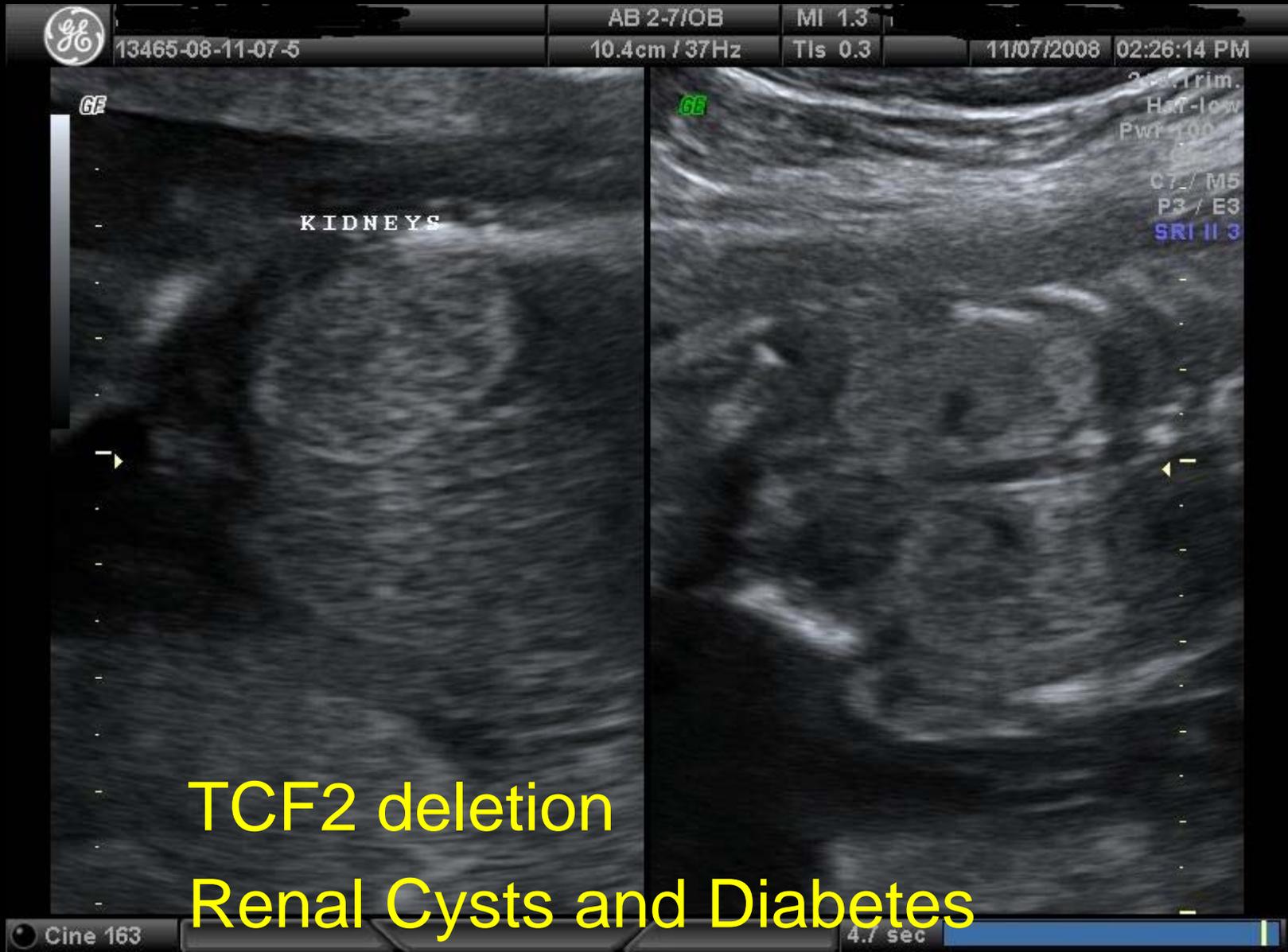
2.5cm. Prim.  
Hat-low  
Pwr 100  
C7 / M5  
P3 / E3  
SRI II 3

Cine 163

4.7 sec

# CVS: Normal Karyotype

Array: arr 17q12(31464079-33406373)x1



TCF2 deletion

Renal Cysts and Diabetes

# Should Molecular Karyotyping by aCGH Replace Karyotyping

**Higher resolution independent of the ability of the cells to grow and/or generate good metaphase spreads**

**Standard Karyotype 5Mb Resolution**

**aCGH 1 Mb to 100 or less Kb Resolution**

**Direct mapping of aberrations to the genome sequence**

**Single step global genome scan prevents FISHing expedition**

**Amenable to automation and quality control procedures**

**Higher throughput and shorter reporting times**

**Better and Cheaper**

# NICHD Prenatal Array CGH Study

## Specific Aims

### Clinical

Demonstrate the performance of CNV-microarray analysis as a clinical method for prenatal cytogenetic diagnosis :

- Accuracy in the detection of the common autosomal and sex chromosomal aneuploidies (trisomies 13, 18, 21, 45,X, 47,XXY, etc)
- Ability to diagnosis less common, but clinically significant, cytogenetic aneusomies currently not detected by the conventional cytogenetic microscopy method.
- Evaluation of the utility of aCGH in specific clinical scenarios such as ultrasound detection of congenital anomalies and growth disorders.

# Specific Aims

## Laboratory

Evaluate the appropriate construction of prenatal diagnostic CNV array devices

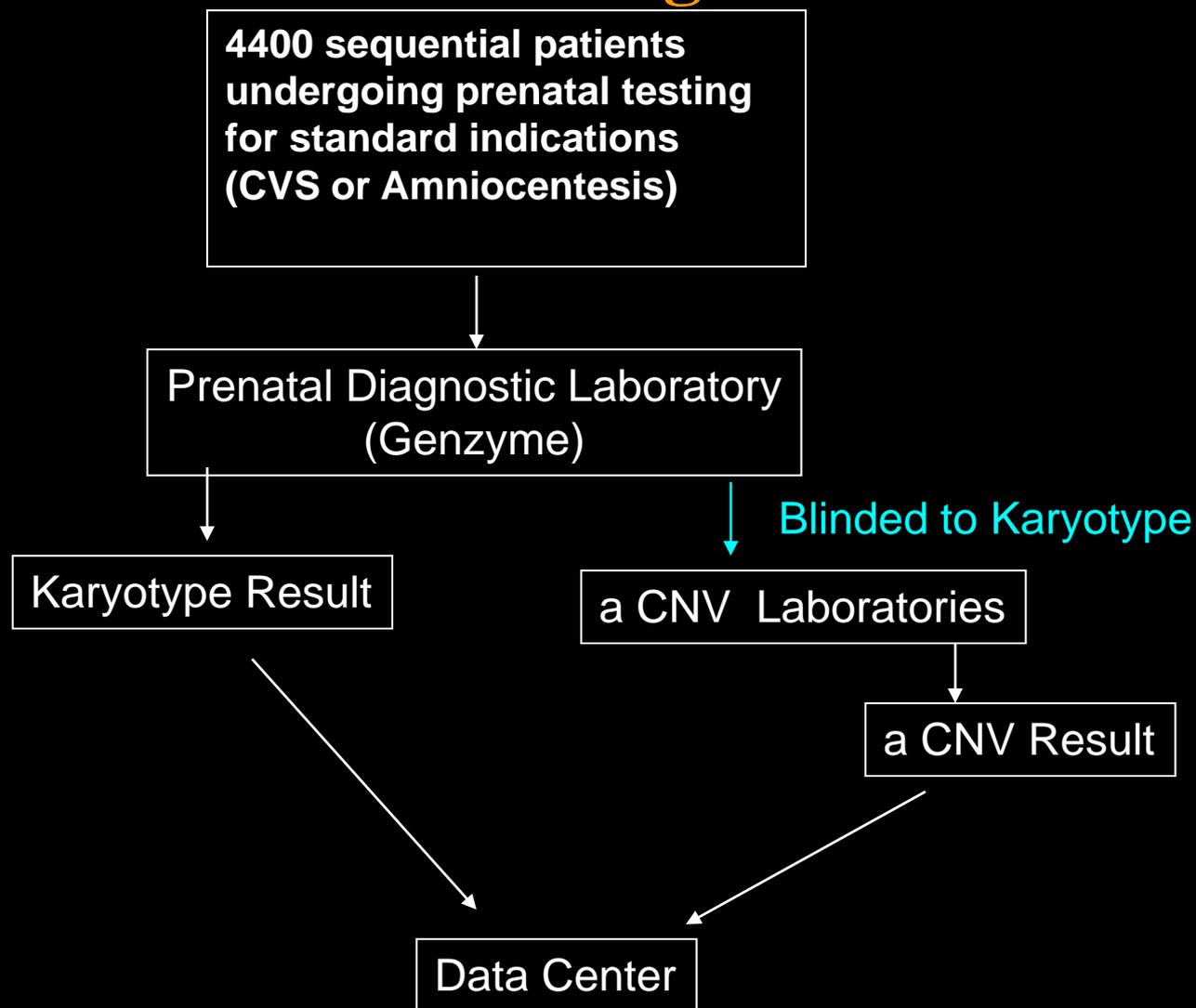
- Maximal detection of clinically relevant information
- Minimal detection of unexpected and difficult to interpret polymorphisms which have no clinical significance but might provoke patient anxiety.

## Specific Aims

- Evaluate the feasibility and cost-effectiveness of using microarrays as a primary prenatal diagnostic tool:
  - Processing Time
  - Logistics Of Access (Sample Collection, Transport Etc)
  - Cost
  - Patient And Provider Acceptability.

Does aCGH improve patient care

# Outline of Study Evaluating aCNV for Prenatal Diagnosis



# Management of Results When Array and Karyotype are Discordant

Normal Karyotype  
+  
Abnormal aCNV

(CNVs found in the backbone that are < 1mb are not reported to the patient)

Results of Known Clinical Significance

Confirmed by FISH

Results to BCC

Results to patient/MD by Coordinator

Results of unknown Clinical Significance

Evaluate Parental Blood

+ CNV Parental Blood

- CNV Parental Blood

Confirmed by FISH

Clinical Advisory Committee

Report to MD

Normal Controls → 2 year Follow-up

# Should Microarray Technology Replace Metaphase Karyotyping for Prenatal Diagnosis

Results of Unknown Clinical Significance  
Copy Number Variants

Ability to detect Mosaicism

What should a prenatal diagnostic array contain  
Targeted Array vs whole Genome Screen

Understanding Full Phenotypic Spectrum of  
Microdeletions and Duplications

# Normal Copy Number Variation

## Widespread in the Human Genome

Cover approximately 12% (360 Mb) of the human genome

CNV varies from 6% to 19% of any chromosome

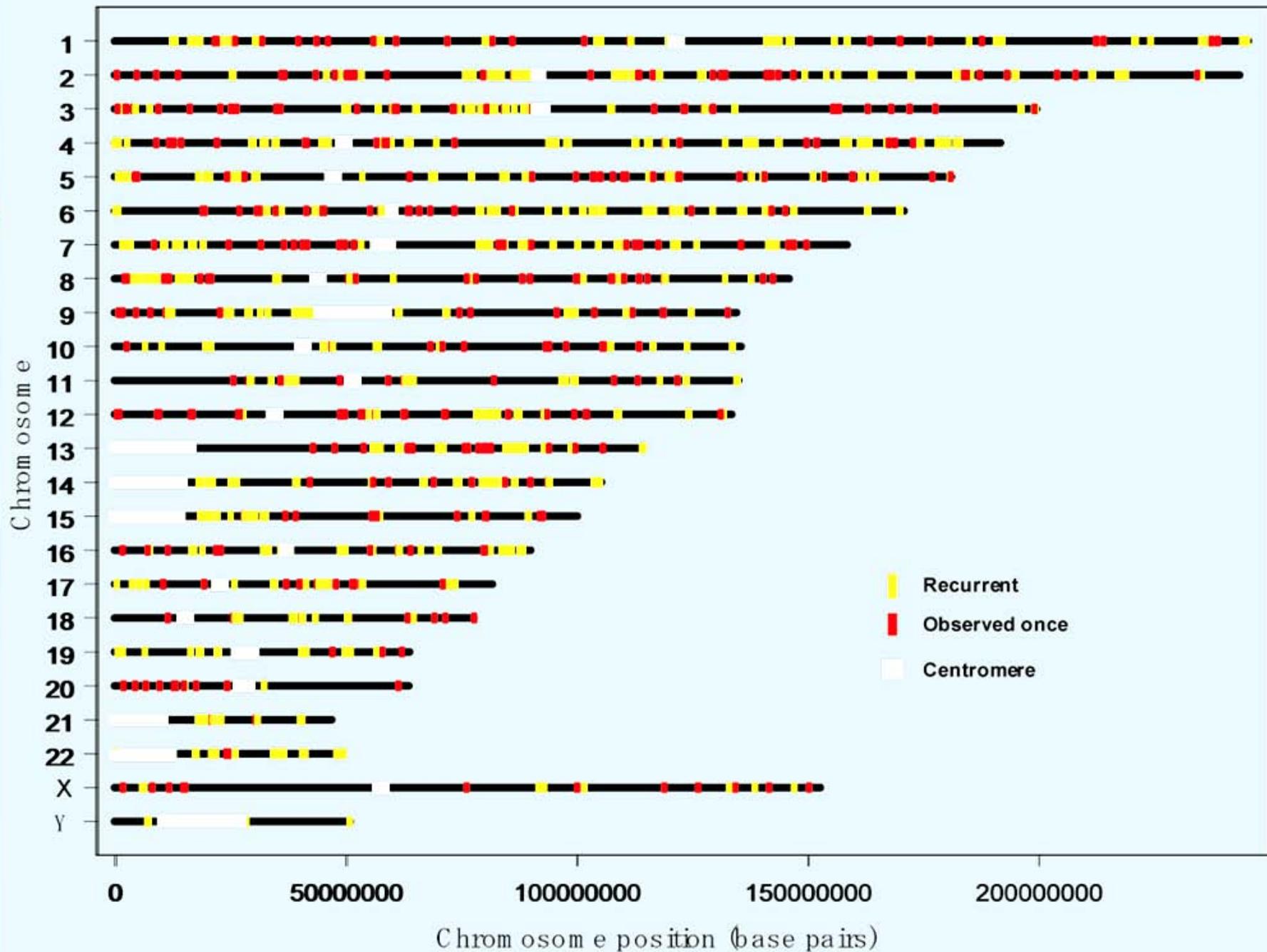
Average of about 12 CNVs per person with 35k resolution

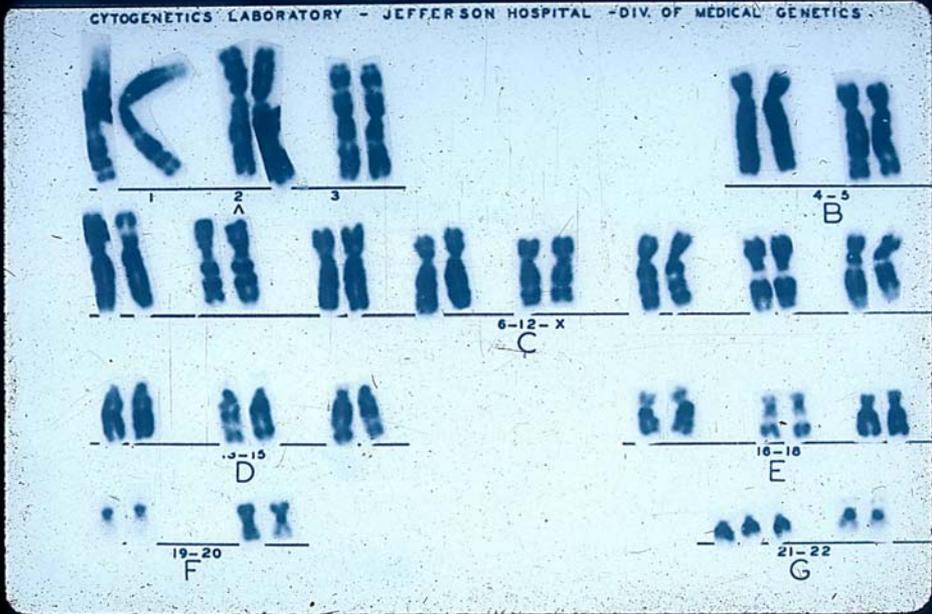
## Not associated with obvious pathology

May contribute to phenotypic variation.

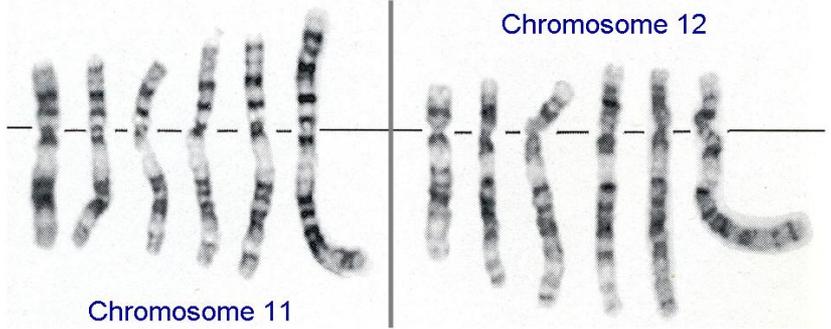
## Almost always Inherited

## May contain known genes





# Banding Resolution



# Should Microarray Technology Replace Metaphase Karyotyping for Prenatal Diagnosis

Results of Unknown Clinical Significance  
Copy Number Variants

Ability To Detect Mosaicism

What should a prenatal diagnostic array contain  
Targeted Array vs whole Genome Screen

Understanding Full Phenotypic Spectrum of  
Microdeletions and Duplications

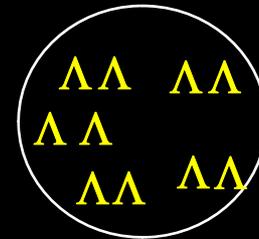
# Impact of Mosaicism in Prenatal Diagnosis

- True Fetal Mosaicism 0.1% of Fetuses

# Mosaicism and CVS

Confined placental mosaicism (CPM) is present in approximately 1.0% - 2.0% of cases

**Embryo**



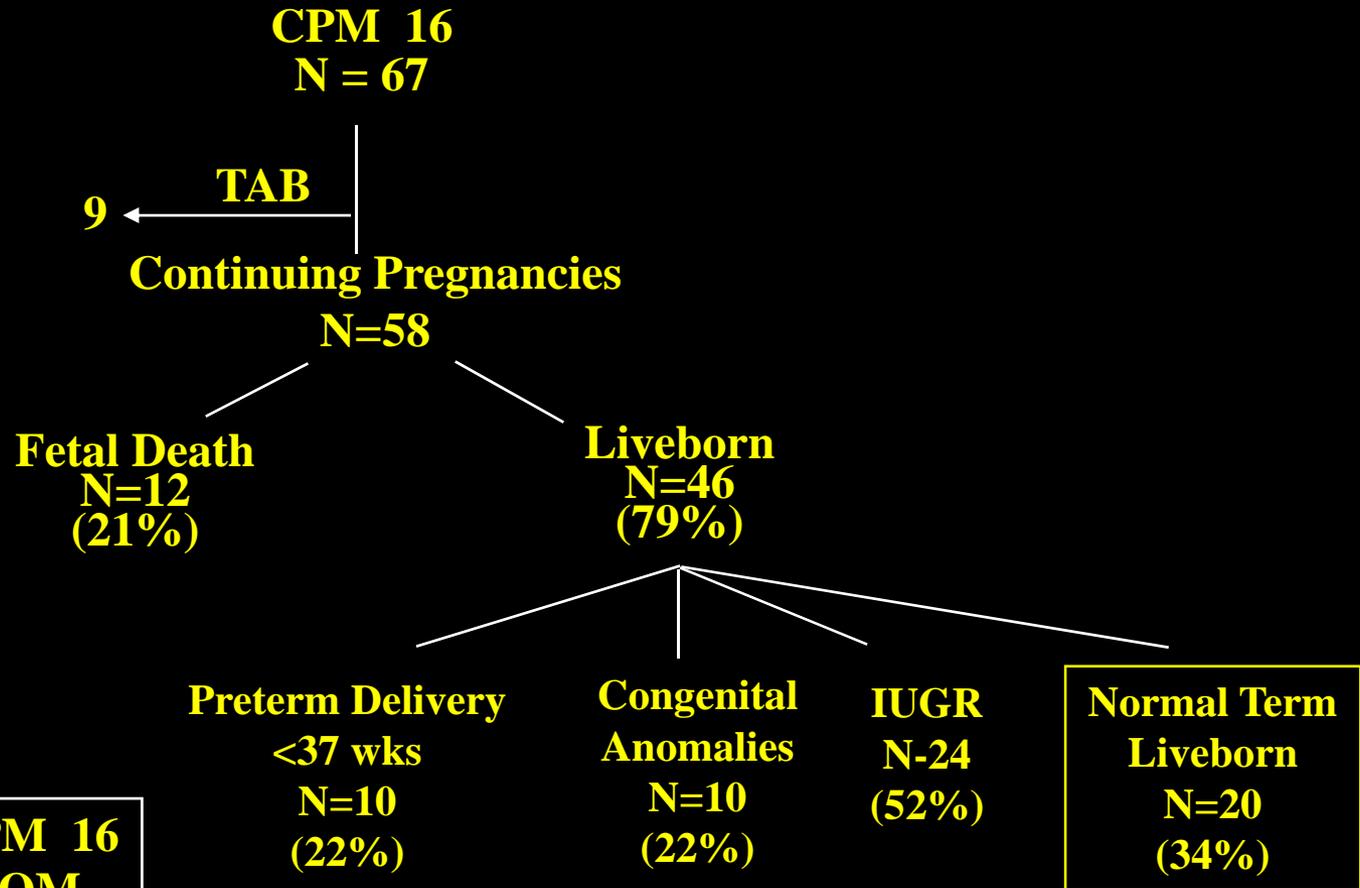
**Placenta**



## Clinical Significance of CPM

- 15-20% of cases have altered perinatal outcome
- Increased risk of spontaneous abortion, fetal death, IUGR
- Perinatal outcome dependent on:
  - chromosome involved
  - percent abnormal cells
  - tissue involved
  - persistence of abnormal cell line
- Presence of uniparental disomy (e.g., 7, 14, 15)

# Impact of Trisomy 16 Mosaicism Confined to the Placenta



**Triple Screen - CPM 16**  
 $\bar{X}$  HCG = 8.6 MOM  
 $\bar{X}$  AFP = 2.9 MOM



# Clinical Significance of CPM

- Presence of uniparental disomy (e.g., 7, 14, 15)



# Level of Detection of Mosaicism in Prenatal Testing

## Microarray

Whole Chromosome:	10-30%
Segmental:	20-50%

# Identification of Mosaicism by Karyotype

95% Certainty	
Cells Counted	Mosaicism Excluded
15	>20%
20	>14%

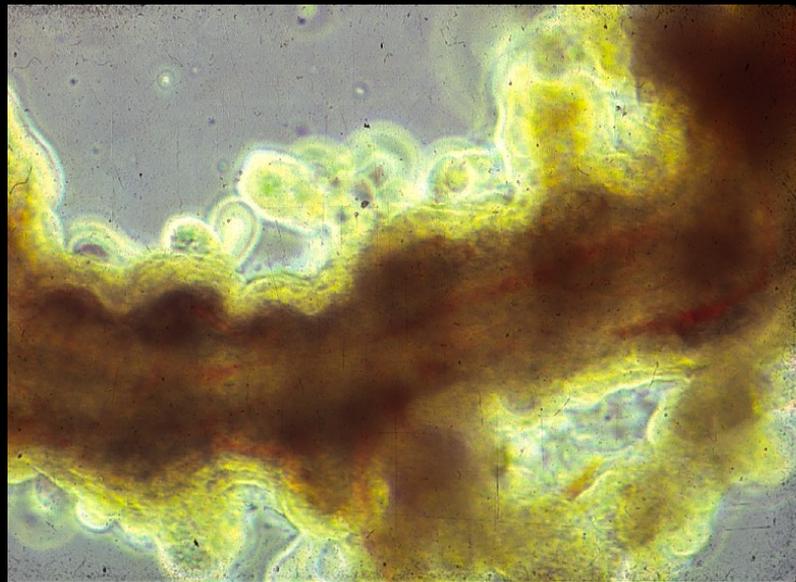
  

Cells Counted	Clones Counted		
	5	10	30
15	> 47%	> 32%	
20	>46%	>30%	
50	> 20%		

Mosaicism Excluded

# Detection of Mosaicism by Prenatal Testing

Discordant Results between Cultured and Uncultured Villi  
occurs in **BOTH** Karyotype and aCGH



# Should Microarray Technology Replace Metaphase Karyotyping for Prenatal Diagnosis

Results of Unknown Clinical Significance

Copy Number Variants

Mosaicism

What should a prenatal diagnostic array contain

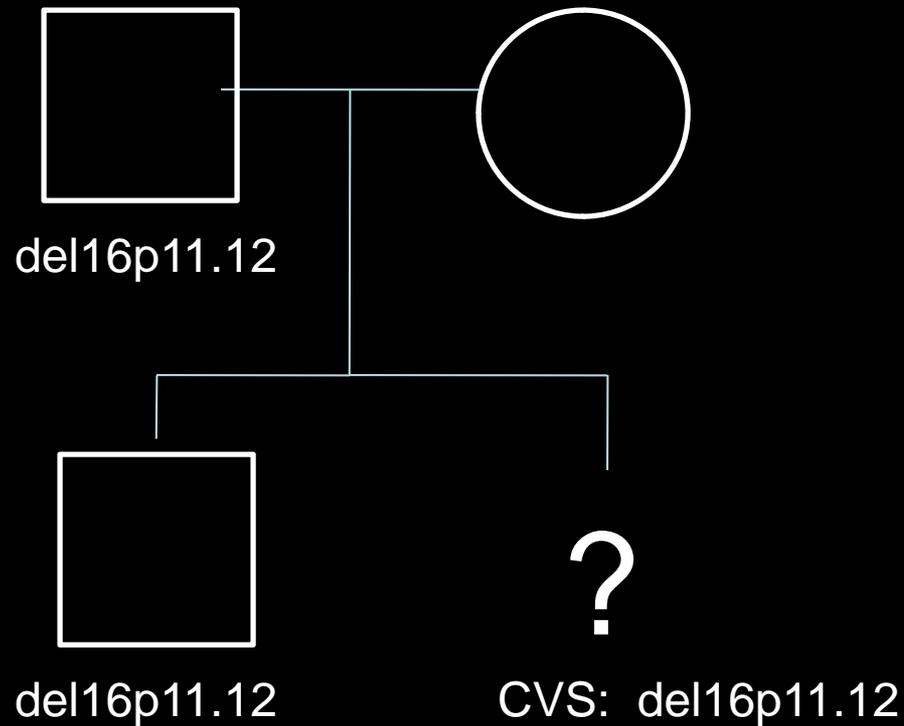
Targeted Array vs whole Genome Screen

Understanding Full Phenotypic Spectrum of Microdeletions and Duplications

# **Prenatal Counseling Difficulties Found in the NICHD Study**

- **Atypical Deletions In Areas Of Known Pathologic Variants**
- **Duplications In Areas Of Known Pathologic Deletions**
- **Incomplete Penetrence And Variable Expressivity**
- **Lack of guidance From the Literature Because of Ascertainment Bias From Abnormal Postnatal Cases**

# Should Microarray Replace Metaphase Karyotyping



# Should Microarray Technology Replace Metaphase Karyotyping for Prenatal Diagnosis

Results of Unknown Clinical Significance

Copy Number Variants

Mosaicism

What should a prenatal diagnostic array contain

Targeted Array vs whole Genome Screen

Understanding Full Phenotypic Spectrum of Microdeletions and Duplications

# Targeted Array

## 43 Unique Pericentric Regions:

Marker Chromosomes that contain a centromere

## 41 Unique Telomere Regions:

Subtelomere deletions or duplications

## Backbone of approximately 1Mb density:

Locations with minimal variation along each chromosome to determine genomic imbalance outside the targeted regions

## Specific Disease Loci (approximately 70)

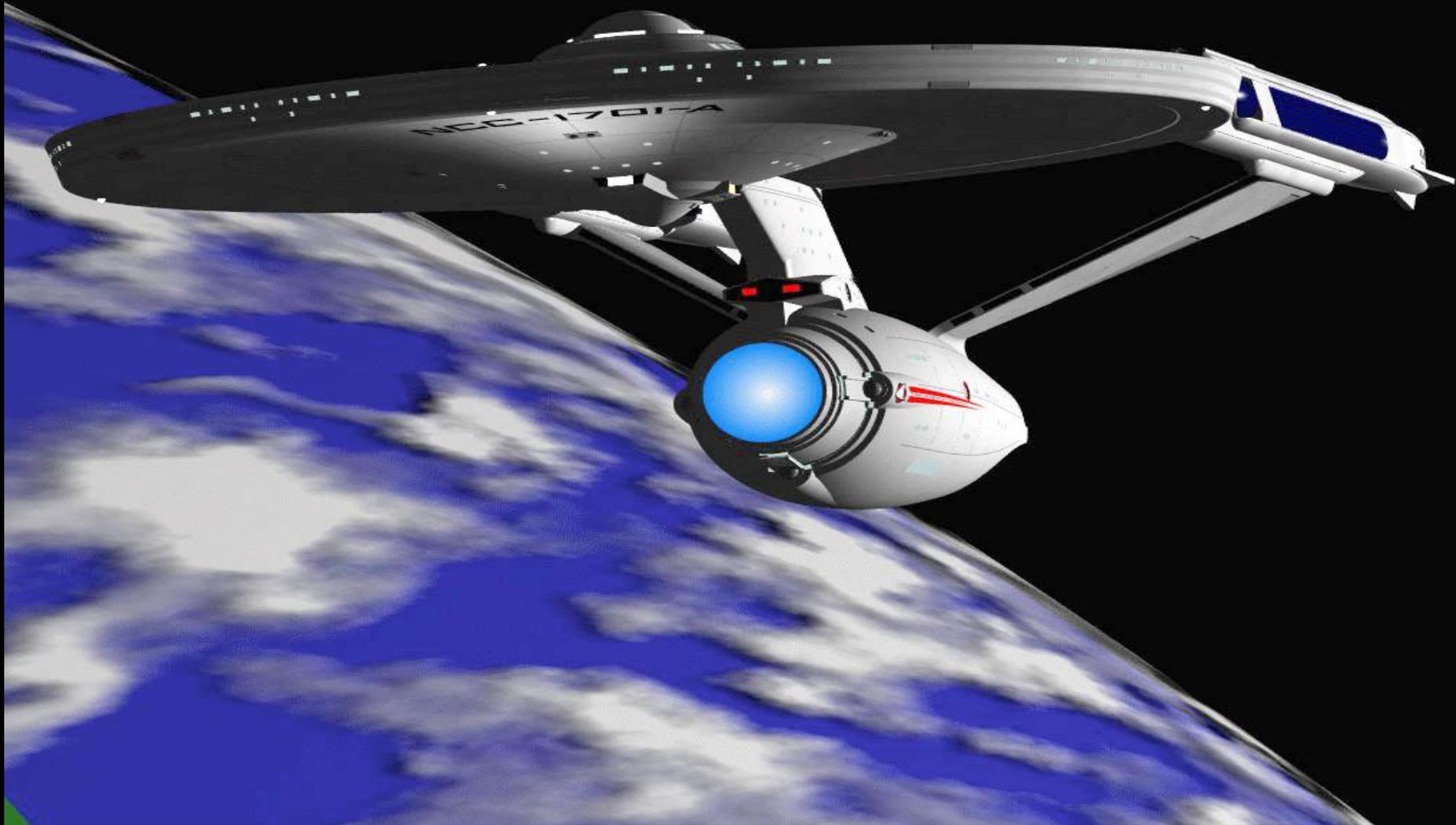
# Criteria for Disease Loci on Microarray

- A significant proportion of cases caused by deletions or duplications
- Disease of significant clinical importance
- Spectrum of disease understood
- Associated with clinical findings

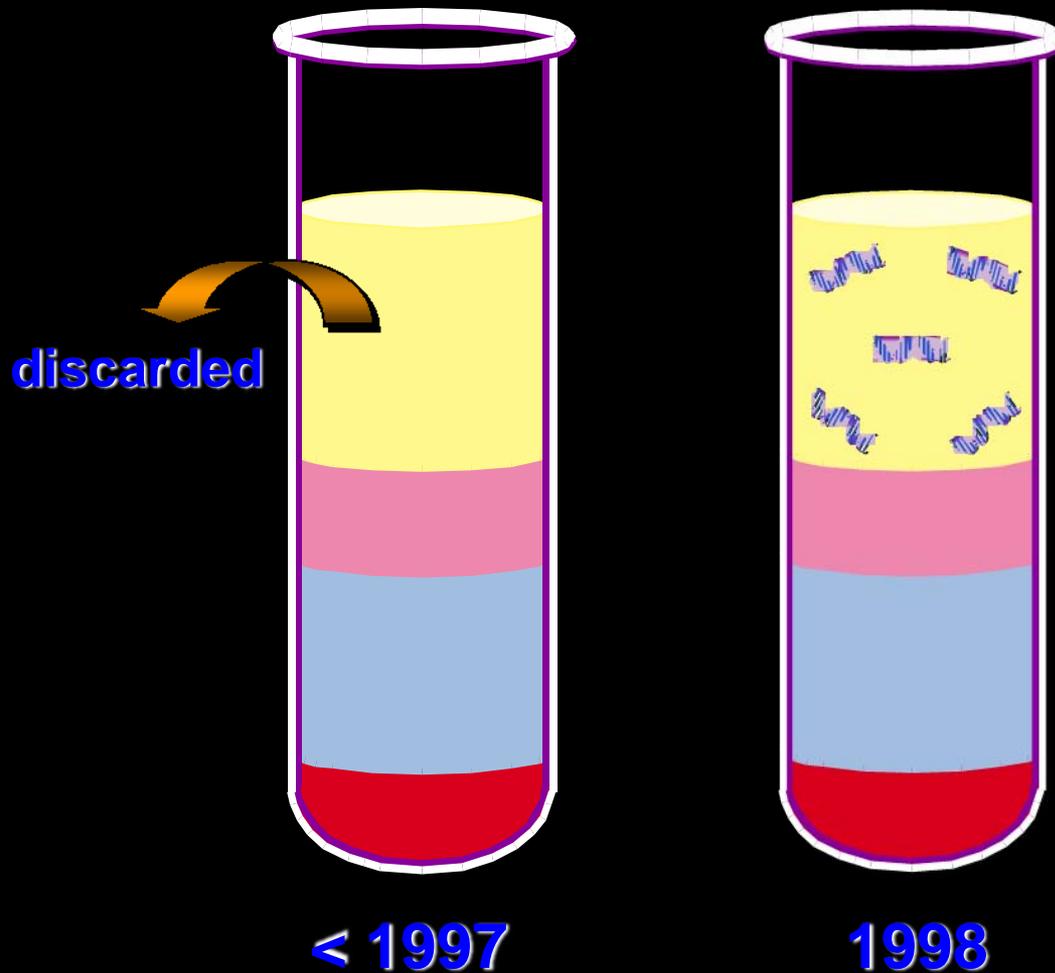
## Some Suggested Disease Specific Loci

- Angelman
- Aniridia / Wilms Tumor
- Cat-eye
- Cri-du-Chat
- DiGeorge/Velocardiofacial (VCF)
- Jacobsen/11q terminal deletion disorder
- Kallmann / Steroid Sulfatase deficiency
- Langer-Giedion / Trichorhinophalangeal
- Miller-Dieker / Isolated Lissencephaly
- Prader-Willi
- Potocki-Shaffer
- Retinoblastoma/MR
- Rett syndrome
- Smith-Magenis
- Sotos
- SRY - Testis determining factor
- Williams-Beuren
- Wolf-Hirschhorn

# THE NEXT GENERATION



# Evolving Appreciation of the Top Layer of the Gradient



# Mass Parallel (Shotgun) Sequencing

