





## Chromosomal Microarray Analysis: Taking a Second Look

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## Case Presentation #1

- 8-month-old female presented for genetics evaluation of hypotonia, infantile spasms and severe developmental delay




## Genetic Family History

- First born child to a 25-year-old healthy woman and her 25-year-old healthy nonconsanguineous husband
- Family history was remarkable for 2 distant paternal relatives with deafness
- There was no known history of birth defects or mental retardation



## Prenatal and Birth History

- Pregnancy complicated by an elevated maternal serum AFP; amniocentesis was declined
- No maternal diseases, infections or exposures to teratogens
- Vaginal vertex delivery at 42 weeks of gestation; birth weight was 6#11
- Infant initially had difficulty nursing but fed well by bottle and was discharged home with mother



## Postnatal History: Medical

- Neurology: developed seizures at age 6 weeks, diagnosed with infantile spasms; started on ACTH and later placed on keppra and trileptol with fair seizure control
- Ophthalmology: delayed visual maturation, otherwise normal
- Audiology: bilateral hearing loss
- Gastroenterology: severe GERD; placed on prilosec



## Developmental History (at age 8 months)

- Severe motor and speech delays
  - Hypotonia with poor head control
  - No attempt to roll
  - Inconsistent visual focus; no visual tracking
  - Coos, smiles and laughs; no razz or babble

## Diagnostic studies prior to age 8 months

- EEG: hypsarrhythmia
- Echocardiogram: normal
- CT brain: normal
- MRI brain: normal
- CT middle and inner ear: narrowed external canals and partial fusion of incus to posterior wall on the left
- Routine karyotype: normal

## Physical Examination

- Growth
  - Height: 71 cm (75th-90th centile)
  - Weight: 8.2 kg (50th-75th centile)
  - OFC: 41.5 cm (5th centile)
- Neurologic examination: severe hypotonia with head lag; unable to lift head in prone; inconsistent visual focus

## Physical Examination

- Craniofacial examination
  - Bitemporal narrowing
  - Flattened occiput with decreased anteroposterior dimension of the head
  - Large anterior fontanelle
  - Deeply set eyes

## Physical examination

- Extremities: short dorsiflexed halluces
- Remainder of physical examination was normal



## Assessment

- 8-month-old female with hypotonia, mild microcephaly, minor malformations, seizure disorder and global developmental delays
- Diagnostic considerations
  - Angelman syndrome
  - Submicroscopic chromosomal abnormality

## Recommended diagnostic studies

- Methylation testing for AS/PWS: normal
- Microarray analysis: abnormal; deletion 1p36

## Case #2

- 3-day-old female presented for genetics evaluation of hypotonia and a large anterior fontanelle

## Genetic Family History

- Third born child to a 23-year-old healthy woman and her 23-year-old healthy nonconsanguineous husband
- Family history was remarkable for a maternal uncle with a VSD; several maternal relatives also had learning disabilities

## Prenatal and Birth History

- Pregnancy was complicated by exposure to varicella at 6 months of pregnancy; mother did not develop chicken pox
- Vaginal vertex delivery at 40 weeks of gestation; birth weight was 5#5
- Infant required transfer to the NICU for transient respiratory distress and hypoglycemia; discharged at 4 days of age

## Postnatal History: Medical

- Cardiology: VSD diagnosed at age 4 months; required surgical closure
- Neurology: parents suspected seizures beginning at age 4 months; negative work-up
- Ophthalmology: pseudostrabismus; otherwise normal
- Audiology: normal hearing

## Developmental History (at age 3 1/2 years)

- Severe motor and speech delays
  - Hypotonia during infancy
  - Walked independently at age 34 months
  - No true speech; 2-3 consistent signs

## Diagnostic Studies prior to age 3 1/2 years

- EEG: normal
- Echocardiogram: VSD and PDA
- MRI brain: normal
- Routine karyotype: normal

## Physical Examination

- Growth
  - Height: 88 cm (<5th centile; 50th centile for 27 months)
  - Weight: 12 kg (3rd centile; 25th-50th centile for 27 months)
  - OFC: 45 cm (<3rd centile; 3rd centile for 27 months)

## Physical Examination

- Craniofacial
  - Straight eyebrows
  - Depressed nasal bridge
  - Short columella
- Extremities
  - Short fifth fingers



## Assessment

- 3 1/2-year-old with growth deficiency, mild microcephaly, hypotonia, VSD, minor malformations and global developmental delays, including absent speech
- Diagnostic considerations: submicroscopic chromosomal abnormality

## Recommended Diagnostic Studies

- Microarray analysis: abnormal; deletion 1p36

## Deletion 1p36: A Recognizable Pattern of Malformation

- Most common terminal chromosomal deletion, occurring in 1/5000 live births
- Accounts for ~1% of cases of unexplained mental retardation
- 2-3% of the general population has mental retardation, 50% of which have no identifiable etiology (genetic or environmental)

## Deletion 1p36: Growth and Development

- Postnatal growth deficiency (85%)
- Feeding difficulties: oropharyngeal dysphagia with failure to thrive
- Neurologic examination
  - Hypotonia (95%)
  - Seizures (45%)
  - Developmental delay (100%)
  - Severe to profound mental retardation with absent or very little speech


## Deletion 1p36 Phenotype

- Craniofacial findings
  - Microcephaly (95%)
  - Large anterior fontanelle
  - Decreased AP dimension of the head
  - Straight eyebrows
  - Deeply set eyes
  - Flat nasal bridge
  - Minor ear anomalies (structure or position)
  - Pointed chin

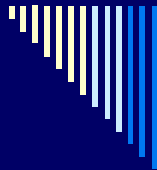
## Deletion 1p36: Other findings

- Cardiac defects (70%)
- Hearing loss (28%)




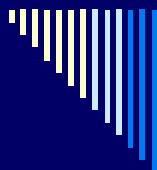


## So What???

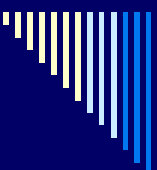


## Prognosis Recurrence Risk


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- ### Importance of Making a Specific Diagnosis
- **Prognosis:** poor; majority of patients with deletion 1p36 have severe to profound mental retardation
  - **Recurrence Risk:** majority are de novo (unrelated to parents' chromosomes); no increased risk for future pregnancies
  - Parental studies indicated in all cases; 6% of parents will have a balanced rearrangement with associated increased risks of recurrence



## Methods of Detecting Chromosomal Abnormalities



## Routine Karyotype

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- ### History of the Routine Karyotype
- 19<sup>th</sup> century: chromosomes first seen and named
  - 1952: Hsu discovered by accident that treatment of cells with a hypotonic solution allowed for improved chromosome spreading & visualization
  - 1956: Tijo and Levan establish human chromosome number as 46

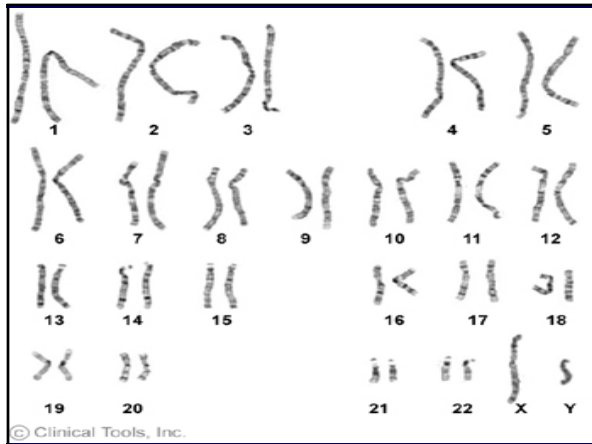
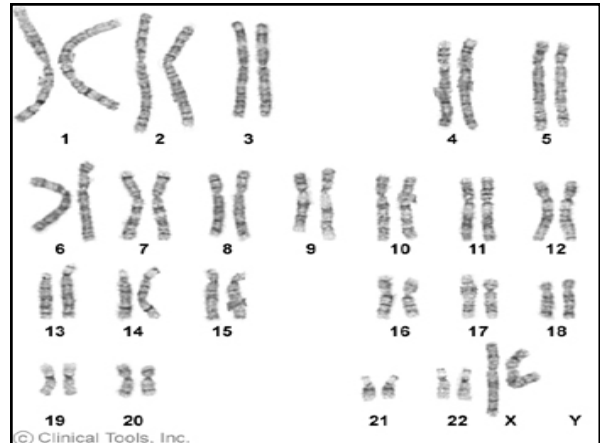
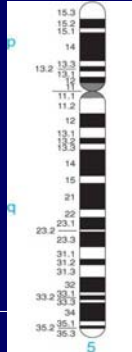
## History of the Routine Karyotype

- 1959: Lejeune discovers that Down syndrome is due to trisomy 21
- 1966: Steele & Breg report cultured cells from amniotic fluid can be used to look at fetal chromosomes
- 1970: Caspersson discovers banding for chromosome identification

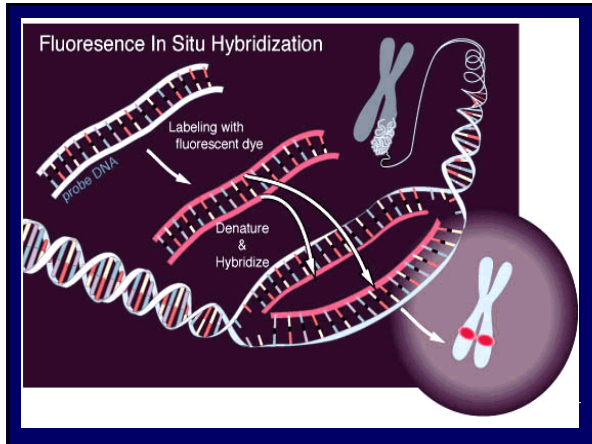
## Routine Karyotype

- Standardized arrangement of all the chromosomes of a cell
- Banding identifies each chromosome uniquely
- Can detect numerical and gross structural abnormalities (trisomy, monosomy, translocation, inversion, marker, ring)
- Resolution to ~5Mb
- Detection rate of ~3% for unexplained developmental delay/MR +/- dysmorphic features

## Structure of a Chromosome

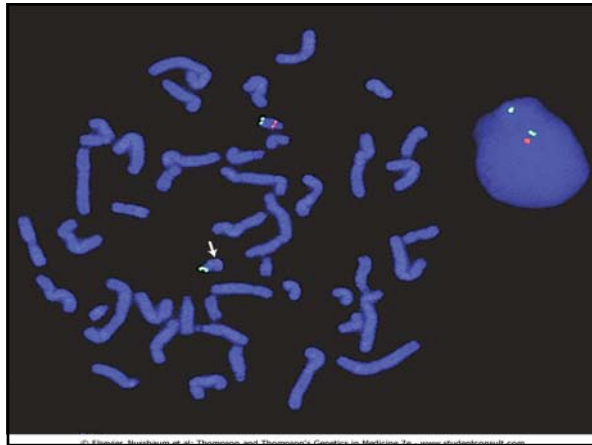


## Fluorescence in situ hybridization (FISH)

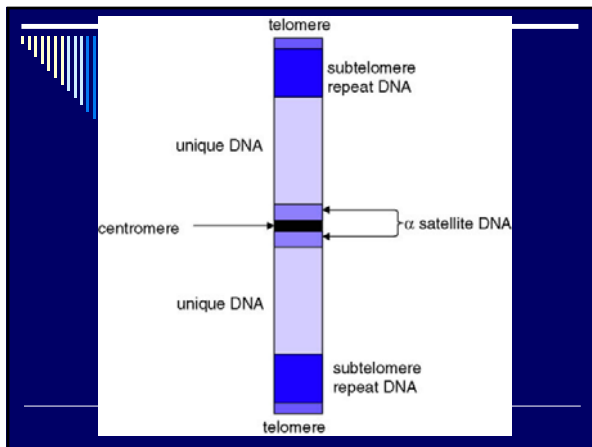


### FISH

- Developed in the 1990s
- Useful for clinically identifiable microdeletion syndromes that cannot be detected using standard cytogenetic methods
  - Deletion 22q11.2 (velocardiofacial syndrome)
  - Williams syndrome
- Also for rapid detection of common aneuploidy in amniotic fluid samples



### Multiprobe Subtelomere FISH

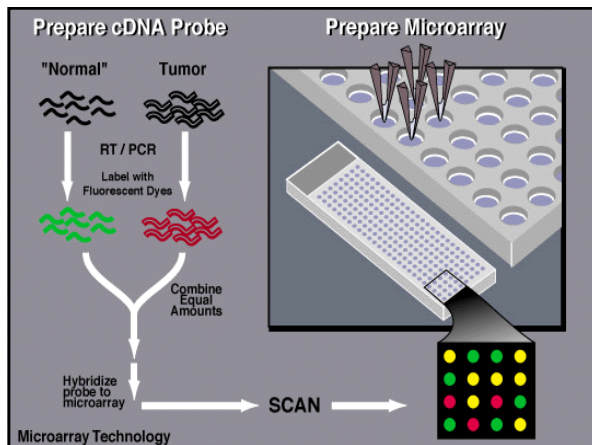


### Multiprobe Subtelomere FISH

- FISH at rearrangement hot-spots
- Developed in late 1990s, commercially available since about 2000-2001
- Typically uses 1 FISH probe per region
- Detection rate of 7-10% for unexplained developmental delay/mental retardation +/- dysmorphic features

# Microarray Analysis

- ## Microarray Analysis
- Relatively new technique examining “hot-spots” for chromosomal deletions and duplications throughout the genome
  - Detects copy number variations at resolution of 1Mb or less
  - Detection rate 10-20% for individuals with normal karyotype, unexplained developmental delay/MR +/- dysmorphic features



- ## Microarray Analysis - Benefits
- Higher resolution than any previously available cytogenetic technique
  - If a specific genomic imbalance is detected, may be able to provide the family with prognostic information and recurrence risk information and MDs with possible medical complications
  - May get a diagnosis for previously undiagnosed individual (parent support groups, information)

- ## Microarray Analysis - Limitations
- New technology; chance of detecting benign copy number changes is high. The clinical significance of copy number changes may be refined through parental analysis
  - For *de novo* rearrangements, prognosis is sometimes unknown (may be novel)
  - Will not detect balanced rearrangements (translocations or inversions) that may interrupt gene(s) causing abnormal phenotype
  - Expensive; \$1500-\$2900
  - No laboratory has NY state approval yet