



# Genetic Testing and Screening: Implications for Birth Defects Programs

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# Chromosomes

- Consist of chromatin (DNA with histones and nonhistone proteins)
- In somatic cells, there are 22 pairs of autosomes (1-22) and one pair of sex chromosomes (X and Y)
- Chromosome structures occur with condensation or tight packaging of chromatin during cell division (mitosis and meiosis)

## Metaphase chromosomes



- Metaphase chromosomes can be arranged into a karyotype from a film or digital microscope study

# Karyotype

- Ordering the chromosomes from largest to smallest, with the sex chromosomes placed separately



# Normal Chromosome Pattern in Females

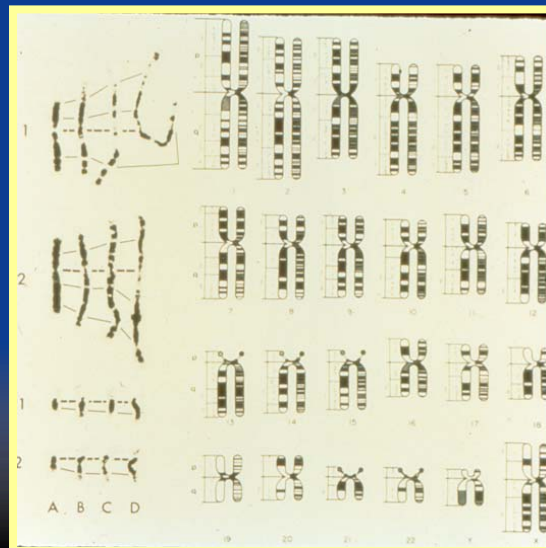


## Normal Chromosome Pattern in Males



## Chromosome Features

- All human chromosomes have two arms
  - ◆ The shorter arm is called “p” for petit
  - ◆ The longer arm is called “q” since the letter follows “p” in the alphabet
- The **centromere** separates the p from q arms

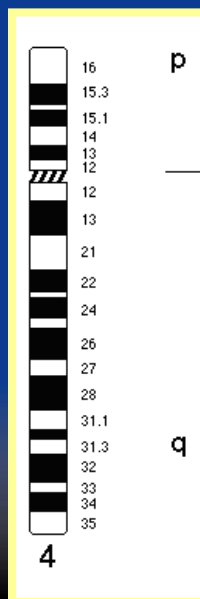


# Chromosome Banding

- Laboratory method used to stain condensed chromatin
- G-banding is the most common type of staining; giemsa produces the pattern of alternating light and dark bands

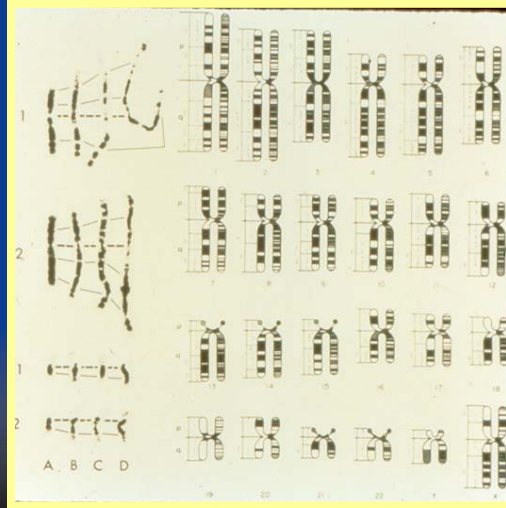


# Chromosome Banding



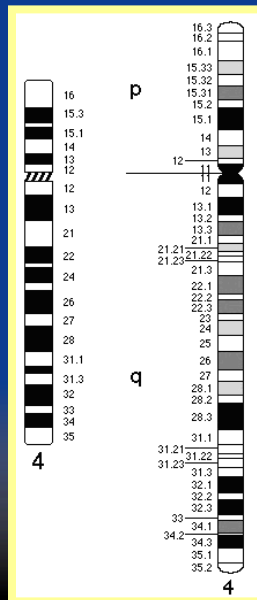
# Chromosome Banding

- A standard karyotype contains approximately 550 bands
- The number of sub-bands increases in extended chromosome studies; approximately 800 bands can be visualized when mitosis is arrested in prometaphase instead of metaphase



# Chromosome Banding

Normal Banding



Extended Banding

## Nomenclature for Chromosome Patterns

- A shorthand nomenclature is used to describe normal and abnormal chromosome patterns
  - ◆ 46,XX Normal female
  - ◆ 46,XY Normal male
  - ◆ 45,X Turner syndrome
  - ◆ 47,XXY Klinefelter syndrome
  - ◆ 47,XX,+21 Down syndrome (trisomy 21) female
  - ◆ 46,XY,del(5p15.2)  
Male with a deletion of part of the short arm of one chromosome #5; this abnormality is seen in patients with Cri-Du-Chat syndrome

## Nomenclature for Chromosome Patterns

- del Deletion of bands (can be terminal or interstitial)
- der Derivative (composite, rearranged chromosome)
- dup Duplication of bands (can be terminal or interstitial)
- i Isochromosome (has only 2 p arms or 2 q arms)
- ins Insertion (extra bands)
- inv Inversion (flipped bands)
- mar Marker (unidentified free chromosomal material)
- ring Both pter and qter lost with fusion of the free ends
- t Translocation of part of one chromosome to another
- ter Terminus or end of the chromosome (pter or qter)
- + Plus or extra (Example: 47,XY,+21)
- - Minus or loss
- . Precedes sub-bands (Example: q11.2)
- / Separates mosaic cell lines (Example: 46,XX/45,X)

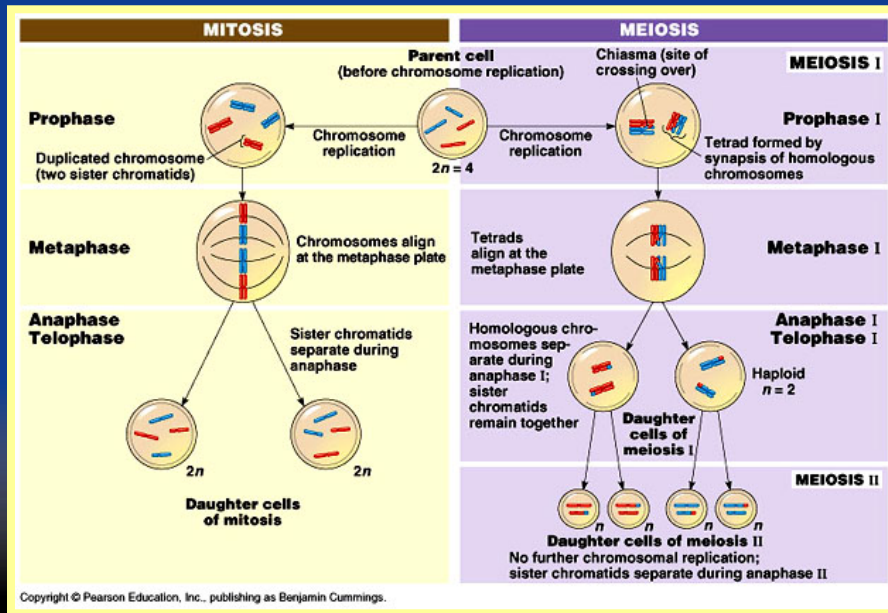
## Chromosomes

- There are 20,000 to 25,000 genes in humans
- The average-sized chromosome will contain about 1400 genes
- The average-sized chromosomal band (550 band level) will contain 40-45 genes

## Chromosome Abnormalities

- Chromosome abnormalities result from mistakes in cellular processes during meiosis or mitosis

# Mitosis and Meiosis

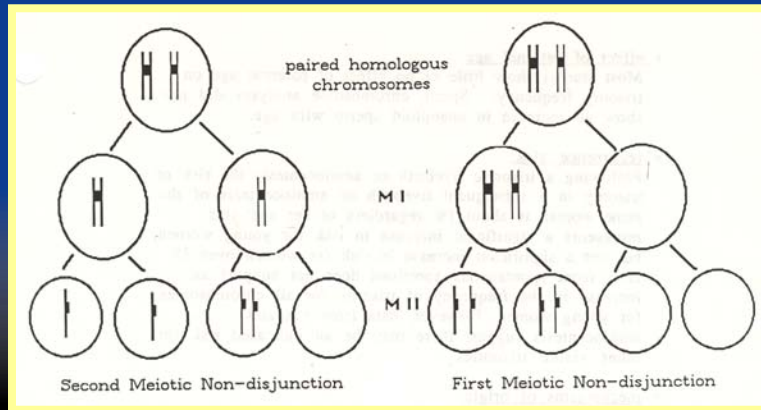


## Chromosome Abnormalities

- Chromosome abnormalities result from mistakes in cellular processes during meiosis or mitosis
- Since an average chromosome contains approximately 1400 genes, **aneuploidy** (additional or missing chromosomes) would result in large imbalances
- Chromosome abnormalities are present in
  - ◆ 50% of all first trimester miscarriages
  - ◆ 7-10% of all clinically recognized pregnancies
  - ◆ 0.7% of all live born infants

# Nondisjunction

- Failure of homologous chromosomes to separate during meiosis I or meiosis II, resulting in gametes with either 2 or zero copies of a particular chromosome



# Nondisjunction

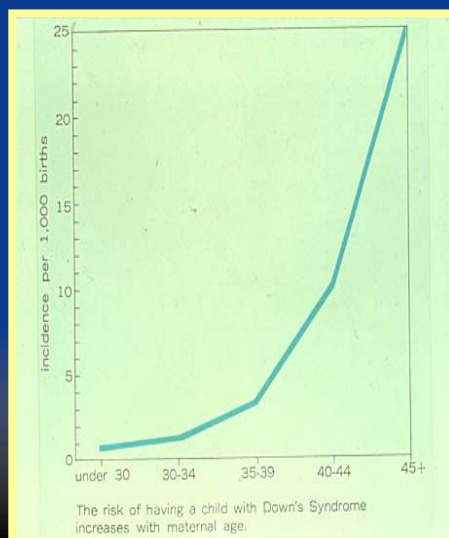
- If a gamete with 2 copies of a chromosome is used for fertilization, the conceptus will be trisomic for the particular chromosome (e.g. trisomy 21)
- If a gamete with zero copies of a chromosome is used for fertilization, the conceptus will be monosomic for the particular chromosome (e.g. Turner syndrome or 45,X)

# Nondisjunction

- Nondisjunction accounts for the great majority of aneuploidy
- Aneuploidy occurs in approximately 7% of all recognized pregnancies
- The cause of nondisjunction is not known
- The frequency of trisomies from nondisjunction increases with advancing maternal age

## Advanced Maternal Age in Down Syndrome

- At age 35, the risk for trisomy 21 is approximately equal to the risk of a miscarriage from an amniocentesis (0.4%)
- At age 40 and 45, the risks for trisomy 21 increase to 1% and 2.5%, respectively



## **Advanced Maternal Age in Down Syndrome**

- More infants with Down syndrome are born to women younger than 35 years than to women over 35 years

## **Advanced Maternal Age in Down Syndrome**

- More infants with Down syndrome are born to women younger than 35 years than to women over 35 years because fewer women over 35 years of age are having children

## Chromosome Abnormalities

- Constitutional--arise before, at, or very shortly after conception
- Acquired--arise in somatic cells some time after conception or in the child or adult (mosaicism)

## Mosaicism

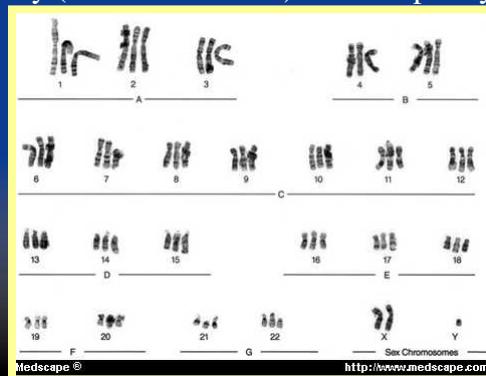
- Mitotic nondisjunction is an error of the normal disjoining of chromatids during mitosis
- Mitotic nondisjunction can lead to a mixture of two or more cell lines in an individual, called mosaicism
- Somatic mosaicism can lead to an abnormal phenotype depending upon
  - ◆ Which chromosome is involved
  - ◆ The tissues that are affected
  - ◆ The percentage of cells with the abnormal chromosome pattern (level of mosaicism)

# Mosaicism

- 46,XX/45,X (mosaic Turner syndrome)
- Mosaic Turner syndrome has also been observed in individuals with 46,XY/45,X
- 46,XY/47,XY+21 (mosaic Down syndrome)

# Other Numerical Chromosome Abnormalities

- Approximately 2-3% of conceptuses are **polyploid** (multiples of 23 chromosomes)
- Most polyploid conceptuses abort spontaneously
- Examples include triploidy (69 chromosomes) and tetraploidy (92 chromosomes)



## Structural Chromosome Abnormalities

- DNA or chromosomes are frequently damaged or broken
- DNA damage is quickly repaired
- Chromosome damage improperly repaired in germline cells produces a variety of *de novo* structural rearrangements
- Alternatively, nonhomologous recombination can lead to structural rearrangements

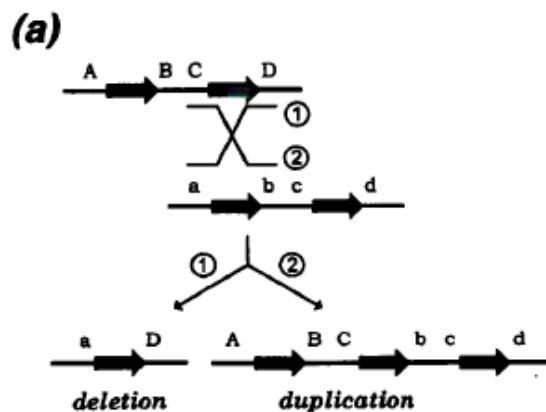
## Structural Chromosome Abnormalities

- Terminal deletions
- Interstitial deletions
- Inversions
  - ◆ Paracentric inversions (inverted region does not include the centromere)
  - ◆ Pericentric inversions (inverted region includes the centromere)
- Reciprocal translocations
- Robertsonian translocations

# Chromosome Deletions and Duplications

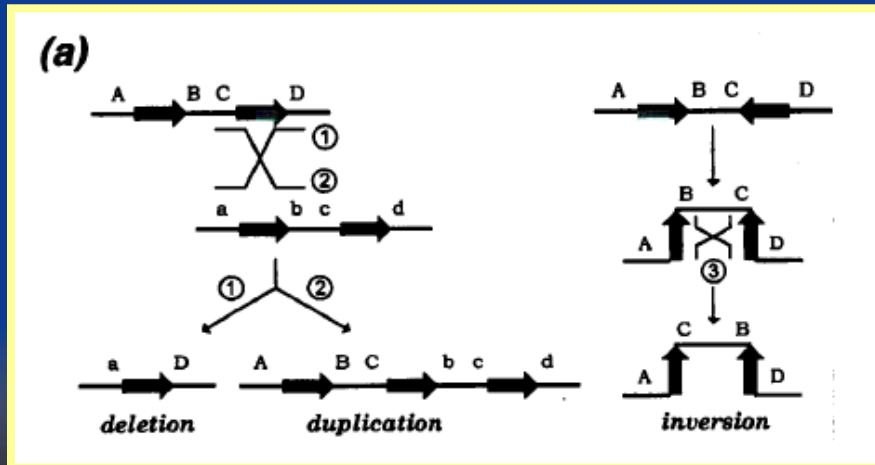
- Terminal deletions result from a break in one chromosome arm, and loss of the terminal acentric (without a centromere) segment
- Interstitial deletions result from two breaks in one chromosome arm, the sticky ends of the break rejoin, and the interstitial acentric fragment is lost
- Interstitial deletions can also result when non-homologous recombination occurs
- Duplications usually result when nonhomologous recombination occurs

# Chromosome Deletions and Duplications



Shaffer LG and Lupski JR. Molecular mechanisms for constitutional chromosomal rearrangements in humans. *Annu Rev Genet.* 2000;34:279-329

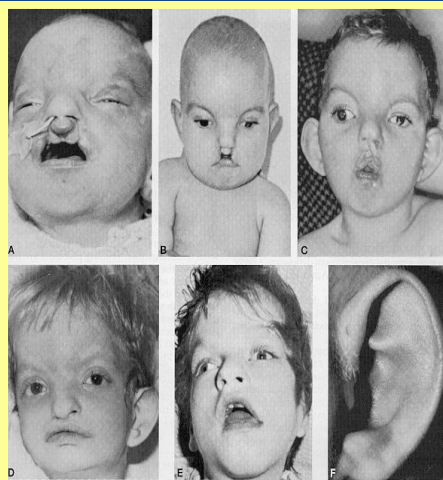
## Chromosome Deletions and Duplications



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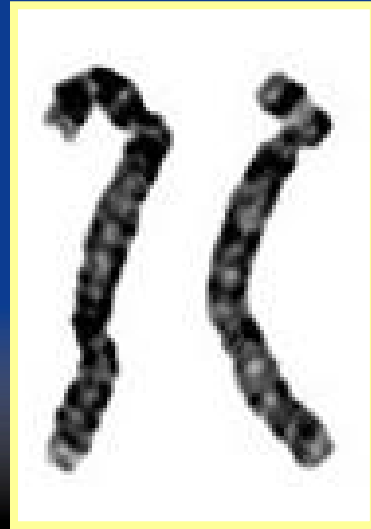
## Wolf-Hirschhorn Syndrome (4p Deletion)

- A well-recognized chromosomal deletion syndrome
- Clinical features include cutis aplasia, cataracts, ear anomalies, “Greek helmet” appearance, cleft lip/palate, growth retardation, seizures, and mental retardation



## Wolf-Hirschhorn Syndrome (4p Deletion)

- Most deletions are visible by standard cytogenetic approaches, although some deletions in Wolf-Hirschhorn patients are quite small and only identified by high-resolution chromosome analysis



## Velocardiofacial/DiGeorge Syndrome (22q11.2 Deletion)

- Presence and severity of the clinical features vary considerably from patient to patient, even within the same family
- Classic triad of features in DiGeorge syndrome are
  - ◆ Congenital heart disease
  - ◆ Hypocalcemia from parathyroid hypoplasia
  - ◆ Thymic hypoplasia (T-cell deficiency)
- Clinical features in Velocardiofacial syndrome (VCFS) are similar to DiGeorge syndrome, but are often milder

## Velocardiofacial/DiGeorge Syndrome (22q11.2 Deletion)

- Other features in VCFS patients include dysmorphic facial features (bulbous nose with hypoplasia of the alae nasi and prominent or protuberant ears), milder congenital heart defects, cleft palate or pharyngeal insufficiency (velopharyngeal incompetence), learning disabilities, and often developmental delay



## Velocardiofacial/DiGeorge Syndrome (22q11.2 Deletion)

- Majority of the clinical features are felt to be due to an embryonic defect involving the 3rd and 4th pharyngeal pouches
- The chromosome 22q11.2 deletion is often *de novo*, but rarely it is inherited from a parent who has a milder phenotype
- Deletions of the 22q11.2 critical region are rarely identified by standard or high-resolution chromosome analysis, but must be visualized by FISH

## Williams Syndrome (Submicroscopic Deletion in 7q11.23)

- Clinical features include supra-aortic stenosis, hypercalcemia, dysmorphic facial features, mental retardation, hoarse voice, and “Cocktail party” personality



## Williams Syndrome (Submicroscopic Deletion in 7q11.23)

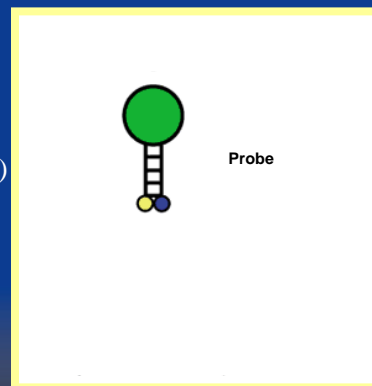
- Deletions of the 7q11.23 critical region are never identified by standard or high-resolution chromosome analysis, but only by FISH

## Fluorescence In Situ Hybridization (FISH)

- A cytogenetic technique that determines the number and location of specific DNA sequences within intact chromosomes
- FISH can identify specific chromosomal regions that have been duplicated or deleted
- FISH can be applied to metaphase chromosomes or interphase nuclei

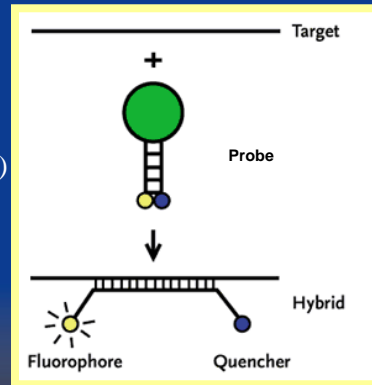
## Fluorescence In Situ Hybridization (FISH)

- FISH technique
  - ◆ Label the probe DNA (fluorescent tag)
  - ◆ Prepare and denature the sample (target) DNA (metaphase chromosomes or interphase nuclei)
  - ◆ Hybridize the probe DNA to the target DNA

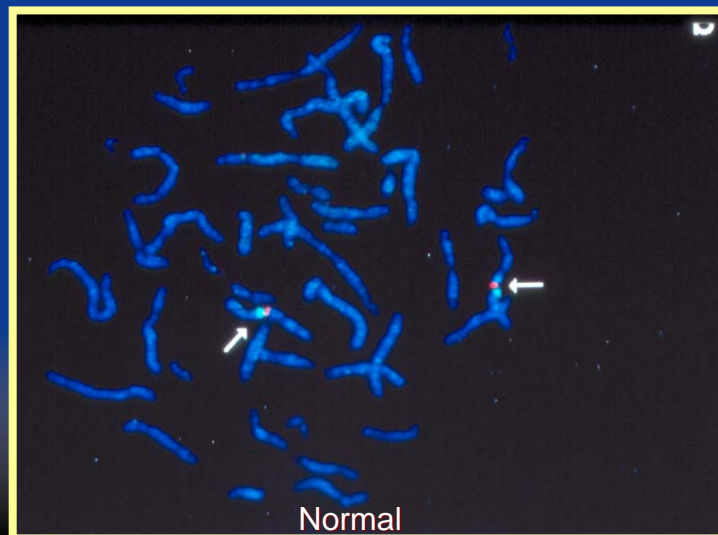


# Fluorescence In Situ Hybridization (FISH)

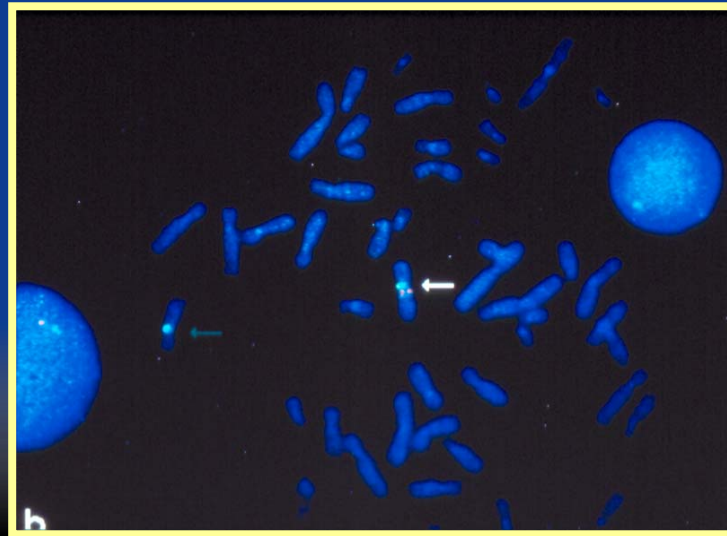
- FISH technique
  - ◆ Label the probe DNA (fluorescent tag)
  - ◆ Prepare and denature the sample (target) DNA (metaphase chromosomes or interphase nuclei)
  - ◆ Hybridize the probe DNA to the target DNA
  - ◆ Wash away unbound or weakly bound probe DNA
  - ◆ Detect the resulting probe DNA::target DNA hybrid molecules under fluorescent microscopy



# Fluorescence In Situ Hybridization (FISH)



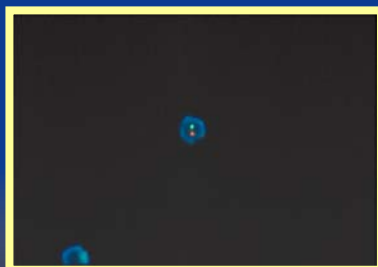
## Fluorescence In Situ Hybridization (FISH)



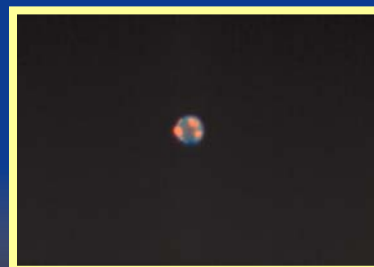
## Fluorescence In Situ Hybridization (FISH)

- Rapid interphase FISH (approximately 4 hours)

X and Y Chromosomes



Trisomy 18



## Nomenclature for FISH Results

- nuc ish FISH performed on interphase nuclei
- ish FISH performed on metaphase chromosome spread
- x1 One fluorescent signal was present
- x2 Two fluorescent signals were present
- x3 Three fluorescent signals were present
- - One expected signal from the probe was missing

**Example:** Normal interphase FISH for chromosomes 13, 18, and 21  
nuc ish 13q14 (RB1x2), 18cen(D18Z1x2), 21q22.13-q22.2  
(D21S259x2,D21S341x2,D21S342x2)

RB1 is a probe in band 13q14

D18Z1 is a probe at the centromere of chromosome 18

D21S259, D21S314, and D21S342 are 3 probes in band 21q22.13-q22.1

## Nomenclature for FISH Results

- nuc ish FISH performed on interphase nuclei
- ish FISH performed on metaphase chromosome spread
- x1 One fluorescent signal was present
- x2 Two fluorescent signals were present
- x3 Three fluorescent signals were present
- - One expected signal from the probe was missing

**Example:** Trisomy 18 on interphase FISH for chromosomes 13, 18, and 21

nuc ish 13q14 (RB1x2), 18cen(D18Z1x3), 21q22.13-q22.2  
(D21S259x2,D21S341x2,D21S342x2)

## Nomenclature for FISH Results

- nuc ish FISH performed on interphase nuclei
- ish FISH performed on metaphase chromosome spread
- x1 One fluorescent signal was present
- x2 Two fluorescent signals were present
- x3 Three fluorescent signals were present
- - One expected signal from the probe was missing

**Example:** Normal FISH result on metaphase chromosomes for Williams syndrome

ish 7q11.23(ELNx2)

ELN is a probe in band 7q11.23 (the elastin gene)

**Example:** Williams syndrome deletion detected by FISH on metaphase chromosomes

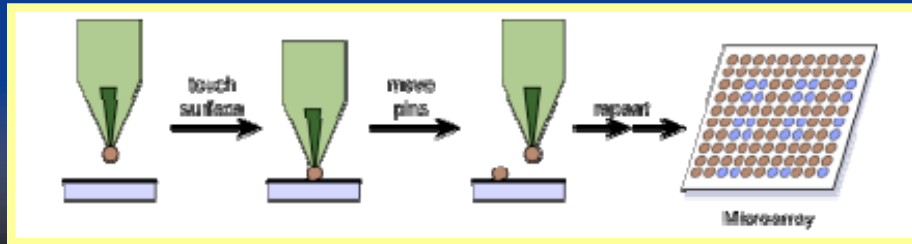
ish del(7)(q11.23q11.23)(ELN-)

## CGH Microarray

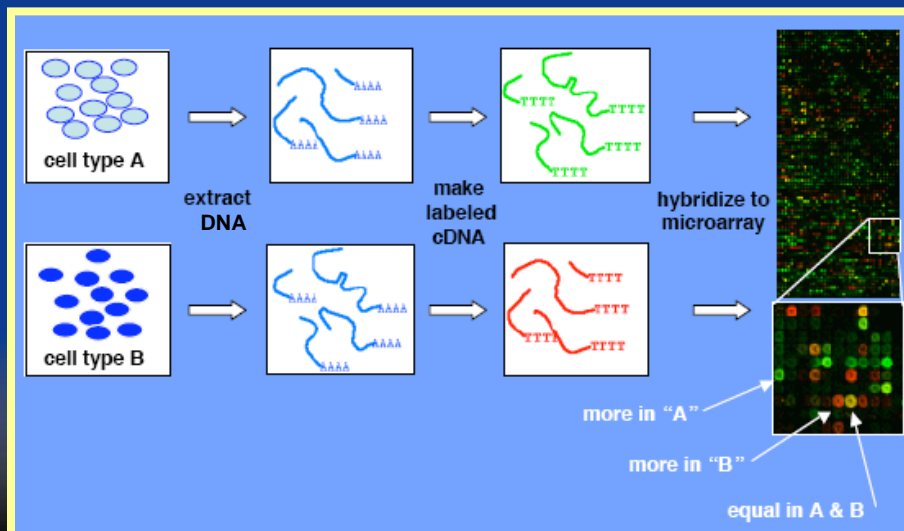
- A newer technique called comparative genomic hybridization microarray (CGH microarray) has been developed
  - ◆ Combines standard chromosome analysis and FISH analysis
  - ◆ Can test for many deletion or duplication disorders in a single test
  - ◆ Tests for the duplication or deletion of tens of thousands of probes in a single analysis

# CGH Microarray

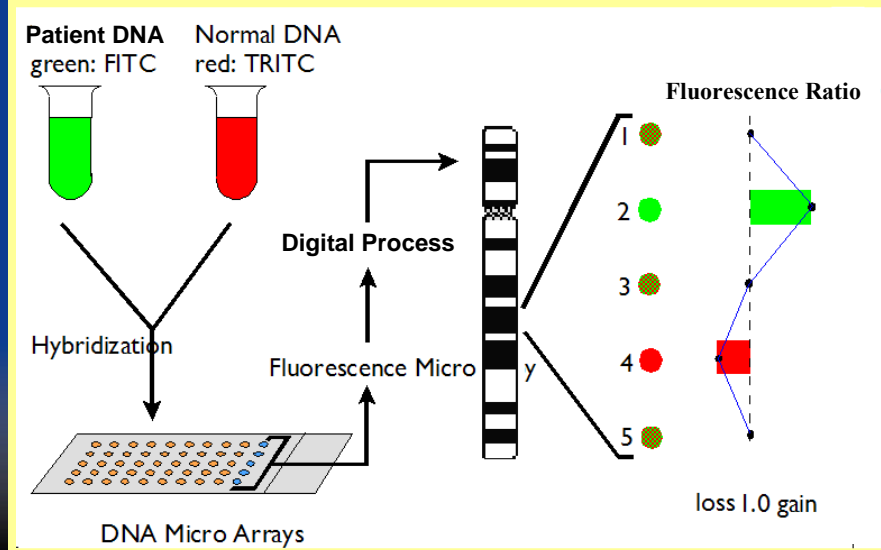
- DNA (acting as probes) from many different genes along the chromosomes is spotted on and attached to a solid support (glass, plastic, or nylon)
- These DNA oligonucleotide or BAC (bacterial artificial chromosome) “probes” are not fluorescent



# CGH Microarray

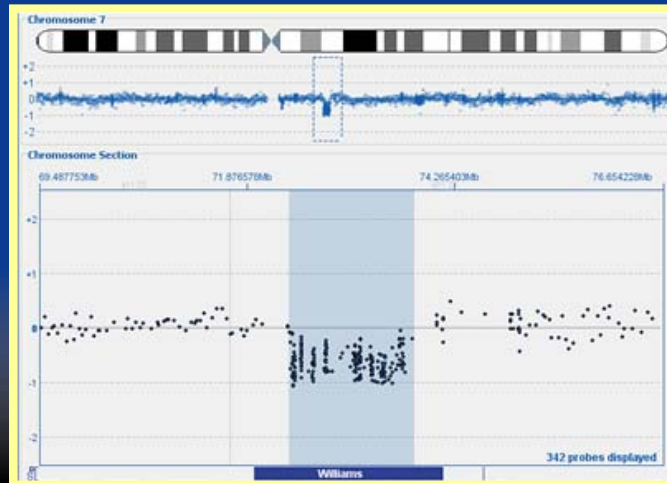


# CGH Microarray



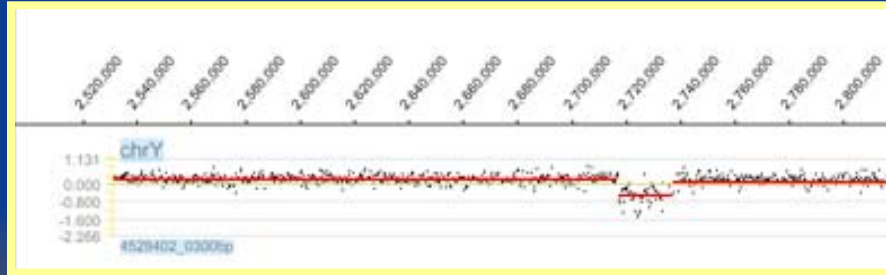
# CGH Microarray

- Williams syndrome deletion by microarray



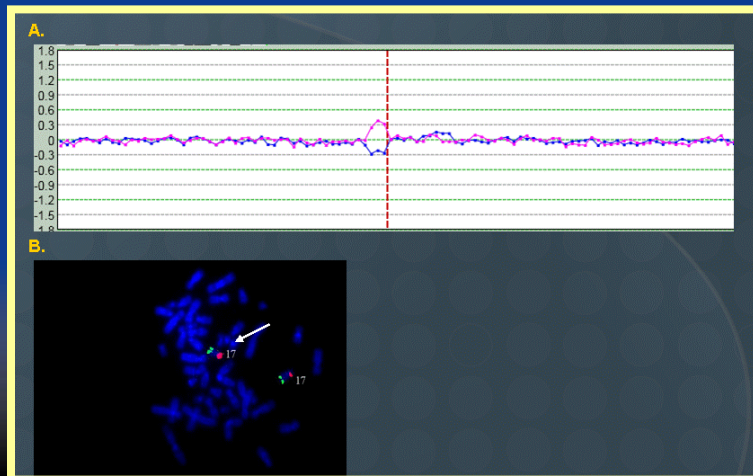
# CGH Microarray

- Y chromosome deletion of 20 kilobases



# CGH Microarray

- Duplication of 17p11.2 on microarray, confirmed by FISH



## Nomenclature for CGH Microarray Results

- arr cgh      CGH microarray performed
- x1            Probes present in one copy (deletion)
- x3            Probes present in three copies (duplication)

**Example:** Deletion detected by CGH microarray in chromosome 8  
arr cgh 8q12.1q12.2 (61,424,674-62,001,209)x1

An interstitial deletion of approximately 576 kb on the long arm of chromosome 8, extending from cytogenetic band 8q12.1 to 8q12.2. The deleted interval contains two genes, CHD7 and RAB2A. Mutations of CDH7 are associated with CHARGE syndrome.

## CGH Microarray Benign versus Pathogenic

- Copy Number Variant (CNV)
  - ◆ DNA region larger than 1 kilobase with a variable copy number (duplication or deletion) compared to a reference genome
  - ◆ Many CNVs contribute to normal human variation and are benign
  - ◆ CNVs may be pathogenic (disease-causing) when they contain gene(s) whose dosage is important for normal function

## CGH Microarray Benign versus Pathogenic

- Pathogenic Copy Number Variants (CNVs)
  - ◆ Parental studies did not show the same microdeletion or microduplication, indicating that the event was most likely *de novo*
  - ◆ The microdeletion or microduplication was previously characterized as being associated with a clinical phenotype
  - ◆ The microdeletion or microduplication contains at least one gene that is known or strongly suspected to be dosage sensitive
  - ◆ The microdeletion or microduplication is of sufficient size to be likely pathogenic (generally >400 kilobases)

## CGH Microarray Benign versus Pathogenic

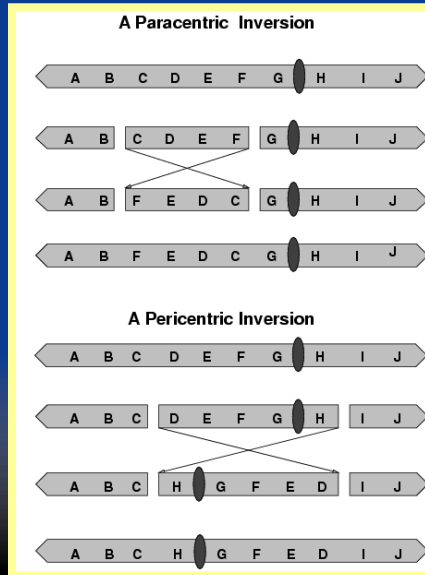
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An interstitial deletion of approximately 576 kb on the long arm of chromosome 8, extending from cytogenetic band 8q12.1 to 8q12.2. The deleted interval contains two genes, CHD7 and RAB2A. Mutations of CDH7 are associated with CHARGE syndrome.

- Parental studies were not available X
- This microdeletion has not been seen before X
- The microdeletion contains at least one gene that is known to be dosage sensitive ✓
- The microdeletion is of sufficient size to be likely pathogenic (generally >400 kilobases) ✓

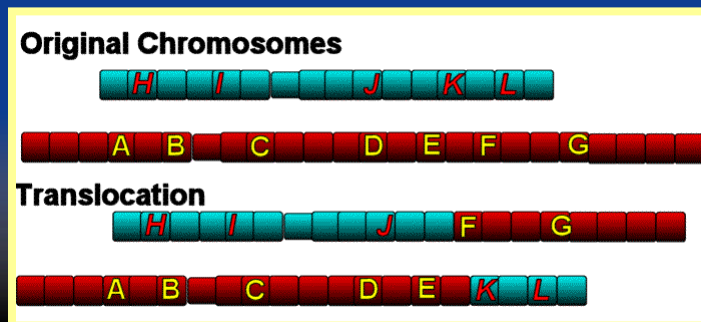
# Chromosome Inversions

- Inversions result from two breaks in one chromosome, inversion of the chromosome piece between the breaks, and rejoining of the ends
- Inversion results in a balanced rearrangement, with no gain or loss of chromosomal material
- Some chromosome inversions are normal variants in the general population (E.g. 9 and 16)



# Chromosome Translocations

- Reciprocal translocations
  - ◆ Two breaks occur in two different chromosomes, with exchange and rejoining of the broken ends
  - ◆ Reciprocal translocations result in a balanced rearrangement, with no gain or loss of chromosomal material



## Clinical Indications for Chromosome Analysis

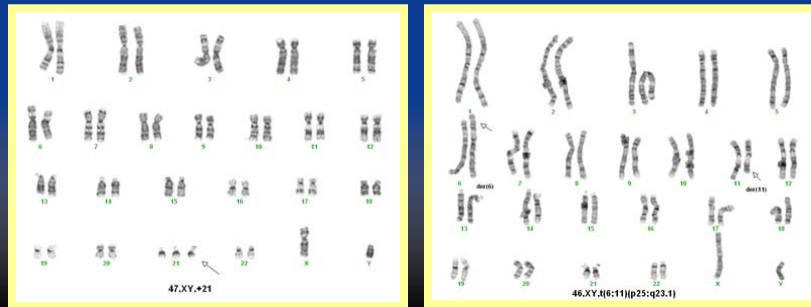
- Features of a chromosomal syndrome
- Multiple malformations, with or without mental retardation
- Psychomotor or growth retardation (or both)
- X-linked disorder occurring in a female
- Ambiguous genitalia
- Hypogonadism, cryptorchidism, small testes
- Primary amenorrhea
- Multiple miscarriages (perform on both parents)
- Infertility

## Clinical Indications for Chromosome Analysis

- Family history of a balanced chromosomal rearrangement
- Prenatal diagnosis for advanced maternal age, positive family history of a balanced chromosomal rearrangement, or abnormal triple/quad screen or ultrasonography result
- Evaluation for a chromosomal breakage syndrome
- Tumor tissue or leukemia cytogenetic evaluation

## Prenatal versus Postnatal Chromosome Analysis

- Amniocytes are cultured for 7-18 days while blood lymphocytes are cultured for 48-72 hours
- Banding of amniocyte chromosomes is generally not more than 450 bands while blood lymphocyte chromosomes are generally 550-600 bands, and extended banding to 800 is possible



## Clinical Indications for FISH

- Diagnosis of suspected deletion or duplication syndromes
- Determining the nature of chromosomal rearrangements
- Rapid assessment of the critically ill newborn for suspected aneuploidy conditions (such as Trisomy 13, Trisomy 18, Trisomy 21)
- Rapid assessment of ambiguous genitalia (probes for X and Y)
- Rapid prenatal diagnosis of abnormal triple/quad screen or ultrasonography result (probes for 13, 18, 21, X, and Y)
- Identify tumor-specific cytogenetic abnormalities

## Clinical Indications CGH Microarray

- Same indications as for a chromosome analysis except
  - ◆ CGH microarray will not detect balanced chromosome rearrangements (inversions, balanced translocations)
- Some programs are now recommending CGH microarray testing in lieu of chromosome analysis because if the chromosome analysis is normal, then CGH microarray is often the next step (paying for 2 tests instead of 1)
- CGH microarray is currently not performed routinely on prenatal samples because interpretation of benign versus pathogenic CNVs is problematic in the prenatal setting

## Abstracting Cytogenetic Testing from the Medical Record

- Was testing performed?
  - ◆ Chromosome analysis?
  - ◆ Fluorescent In Situ Hybridization (FISH)?
  - ◆ Comparative Genomic Hybridization (CGH) Microarray?
- Was testing performed on the parents? Results?
- Correct transcription of nomenclature for the cytogenetic, FISH, or CGH microarray results
- Is the cytogenetic result a normal variant?
- Is the CGH microarray result a benign or pathogenic copy number variant?