Genetics in the NICU Setting

Audrey Harris Neonatal Conference of Northwest Arkansas

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I. Congenital Anomalies

Birth Defects

- March of Dimes reports 6% risk of one anomaly in worldwide population
- Account for one of leading causes of death in infants
- Regardless of race, location, socioeconomic status

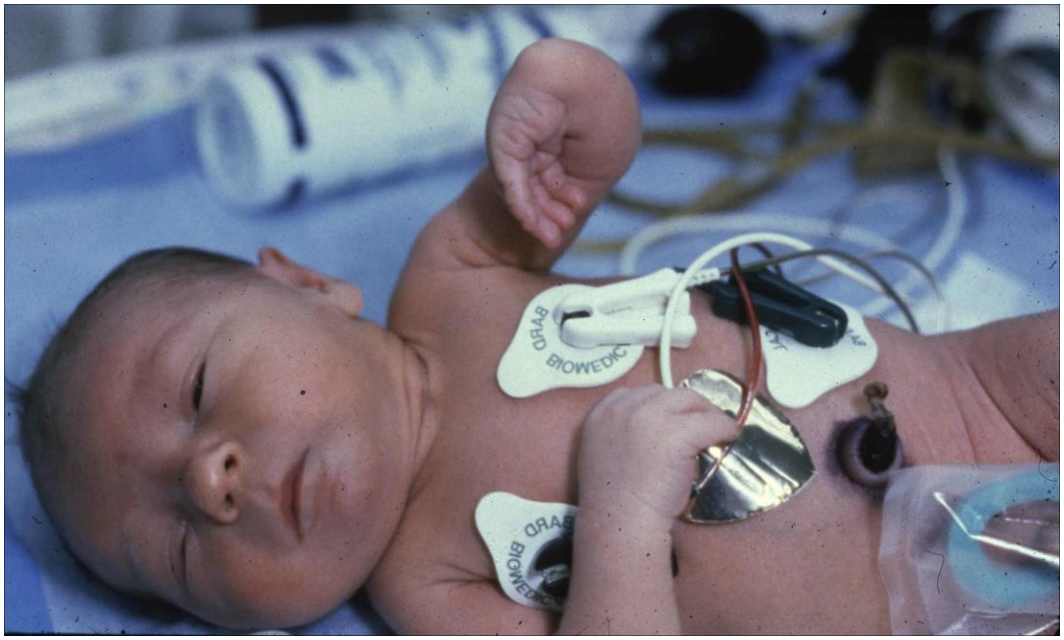
Birth Defects

- Structural or functional
- Genetic, infectious, nutritional, environmental→ Combination of these
- When one anomaly is present, 50% risk of a second anomaly
- Heavy burden on individuals, families, healthcare, societies

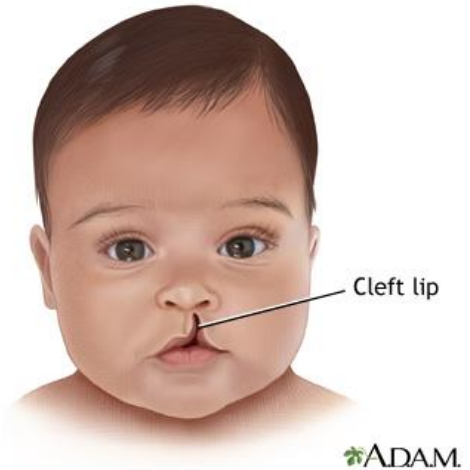
Birth Defects in Prenatal Setting

- Identified in prenatal setting
- Neonatology consult during pregnancy to prepare the family
- Birthing centers may have fetal care teams
  - Obstetrics, neonatology, radiology, subspecialties depending on needs
- Consideration of method of delivery and birth at medical center with NICU access

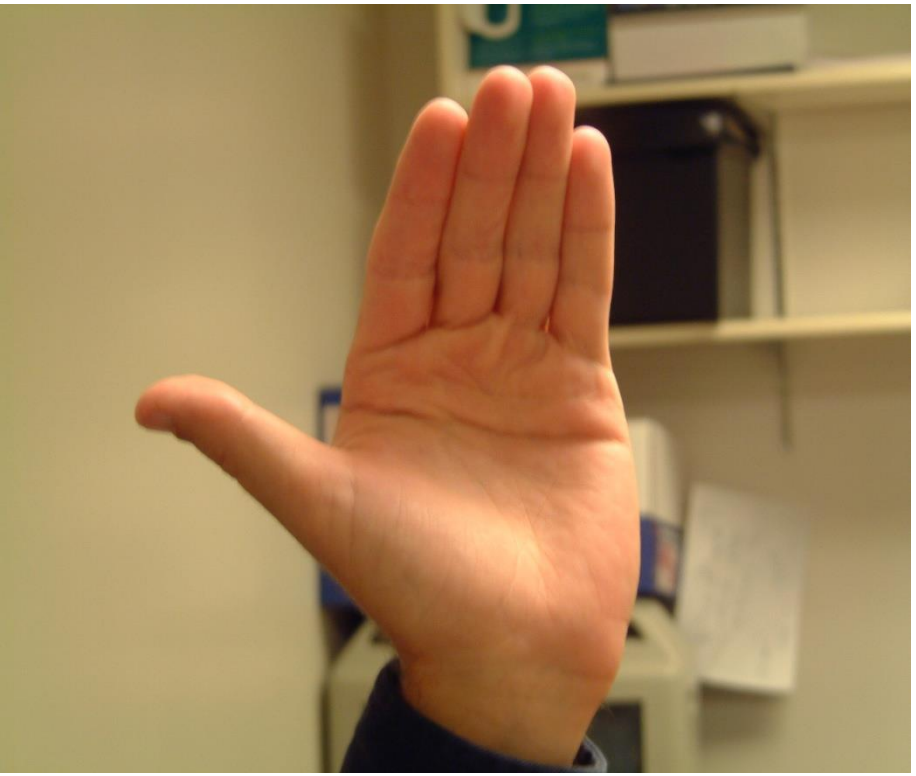
Major Malformation

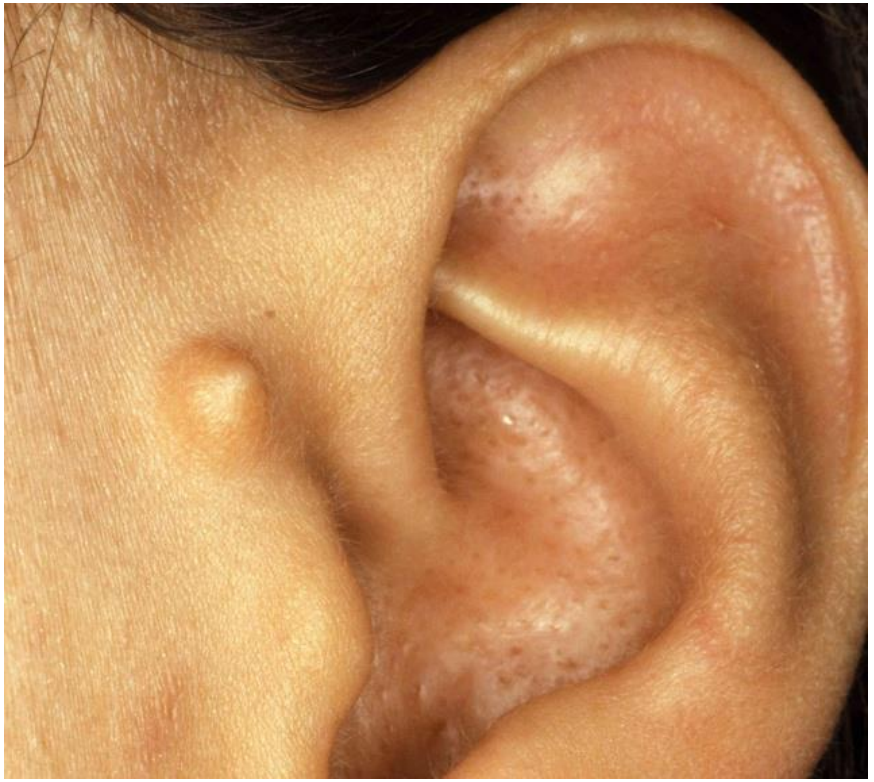


Absent Radius



Minor Malformation





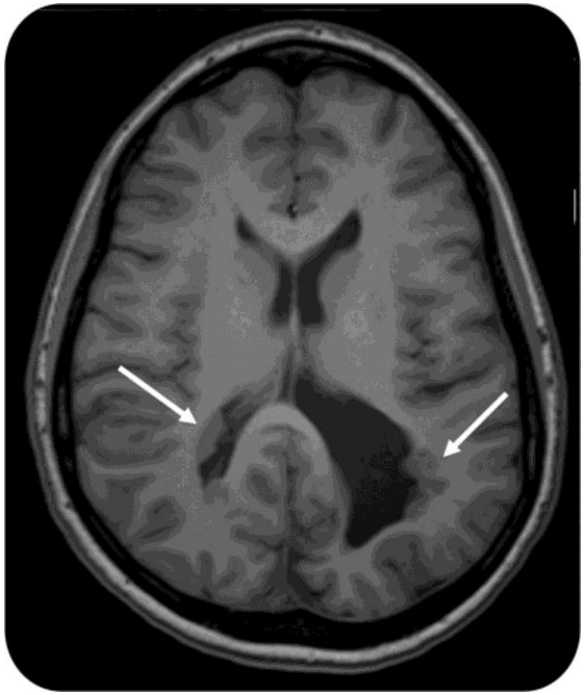
Classification of Birth Defects

Classified Based on Mechanism

- *Malformations*
  - Intrinsically abnormal development
- *Deformations*
  - External forces secondarily deform tissue
- *Disruptions*
  - Secondary breakdown of tissue
- *Dysplasia*
  - Abnormal organization of cells into tissues

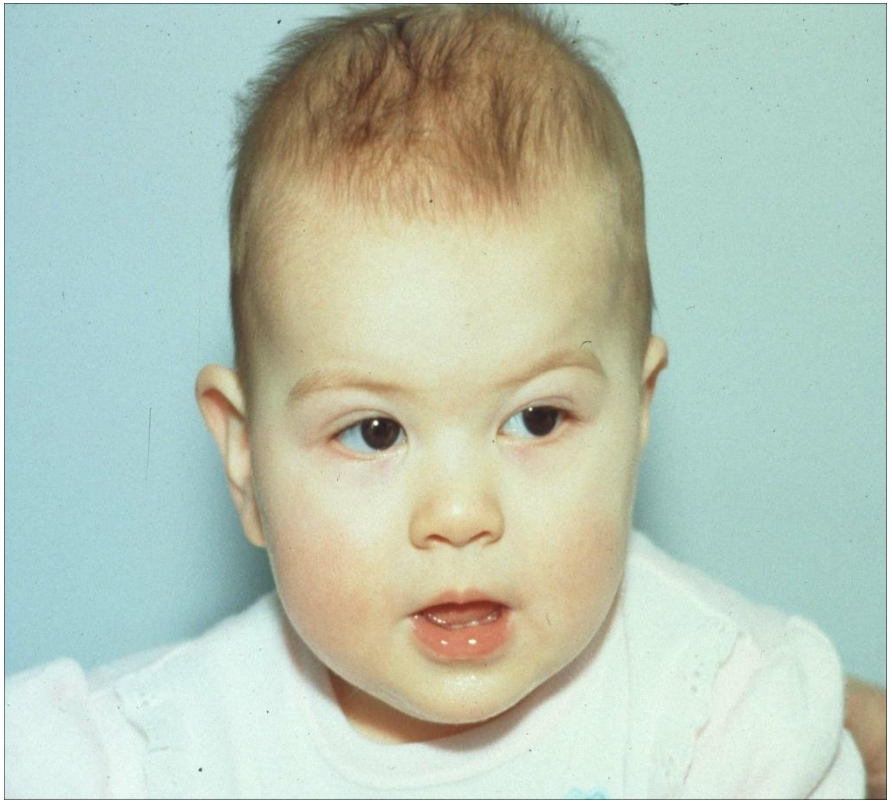
Malformations

- Agenesis, hypogenesis, heterotopias, ectopia.

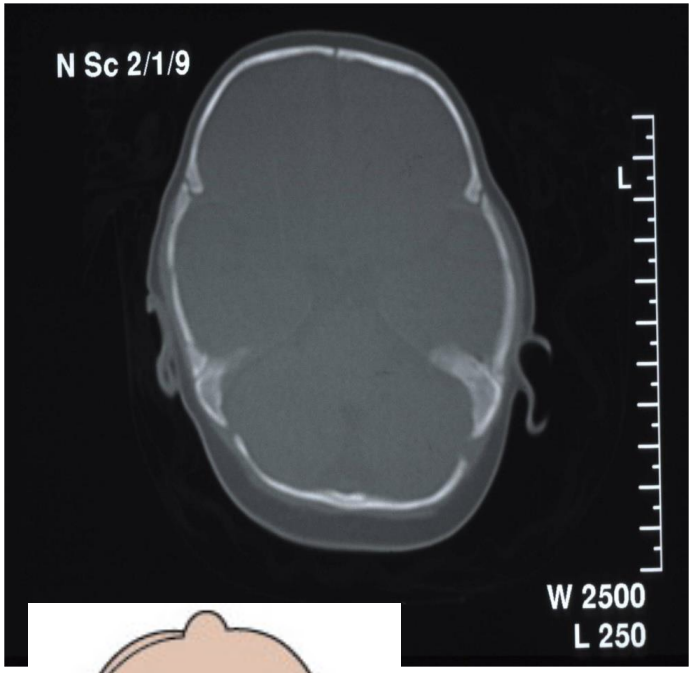


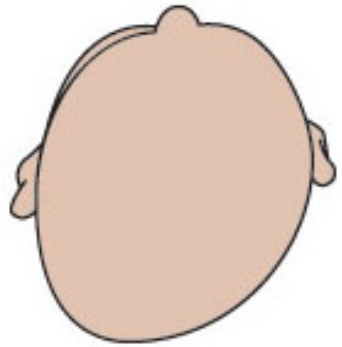
Subependymal Heterotopia

Deformations



Plagiocephaly





Plagiocephaly

Deformations

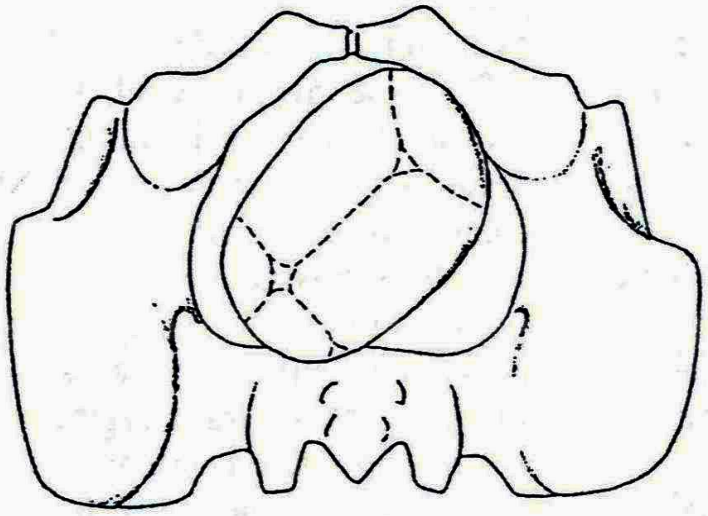


FIG. 7. Drawing, from a caudal view, of the pelvis of an infant engaged in the left occipital anterior (LOA) position. Deformational frontal plagiocephaly, which is more common on the left side, may be secondary to prolonged compression against the sacral prominence. Note also the flattening of the contralateral posterior cranium against the iliopectineal region of the pubis (see Fig. 4, *right*).

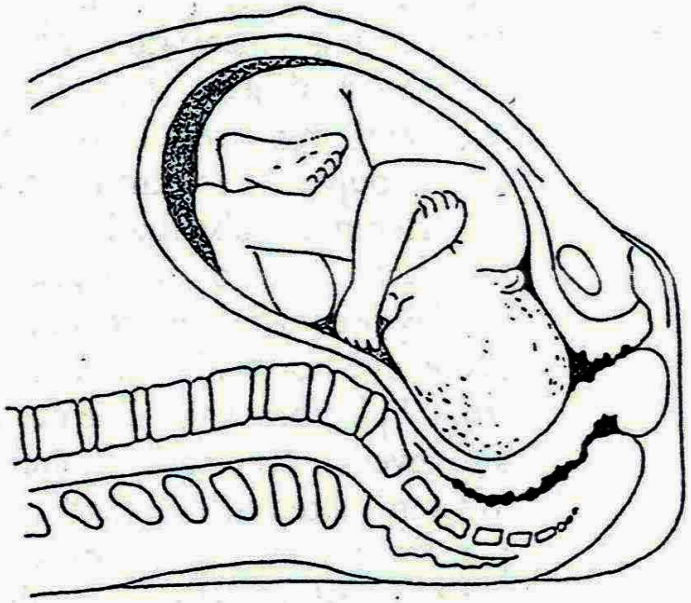
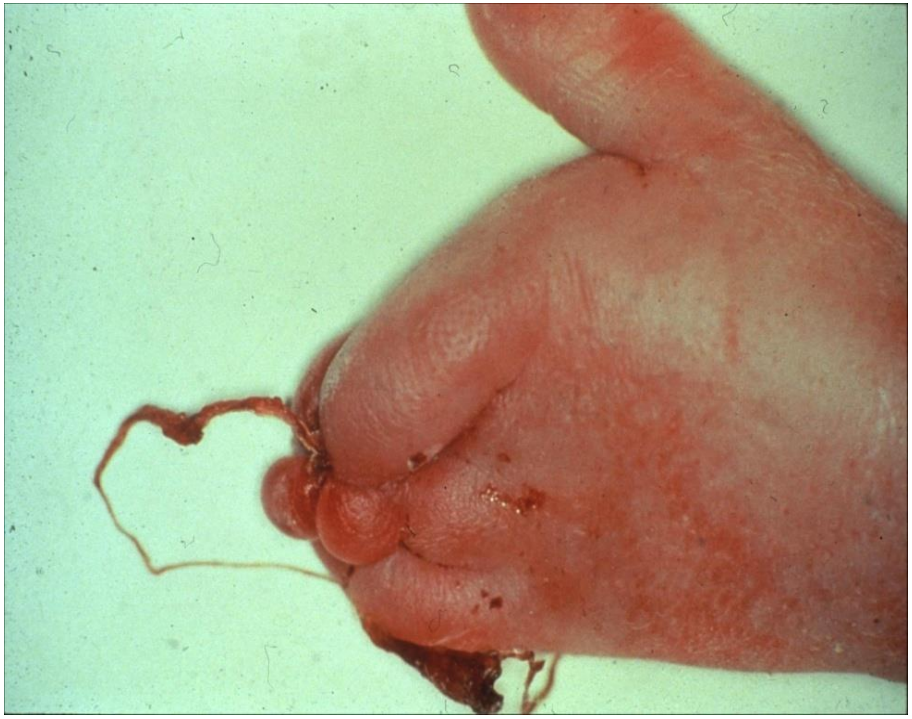


FIG. 8. Sagittal drawing of a fetus descended in the pelvis to illustrate a possible mechanism for deformational frontal plagiocephaly. The left neck is kinked; the left forehead is compressed against the sacrum, resulting in left deformational frontal plagiocephaly and ipsilateral torticollis.

Disruptions

- Secondary breakdown of tissue
- Major factors responsible for disruptions:
  - Vascular (occlusion, hemorrhage)
  - Ischemia
  - Ionizing radiation
  - Infection
  - Early amnion rupture

Disruptions



Amniotic Band Syndrome:

-Single or multiple anomalies

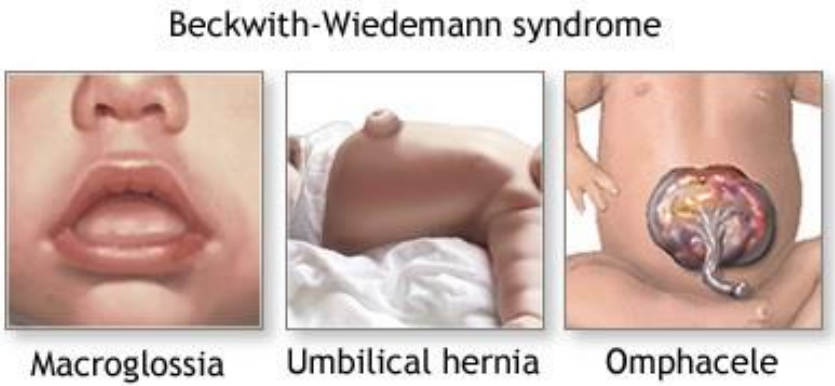
-Inner layer (amnion) of sac ruptures exposing fetus to fibrous strands that float free or remain tethered to amniotic sac

Dysplasia

- Abnormal organization of cells into tissues
- Typically later during gestation
- Independent of morphogenesis
- Dysplastic tissues often predispose tissue to cancer later in life

Dysplasia

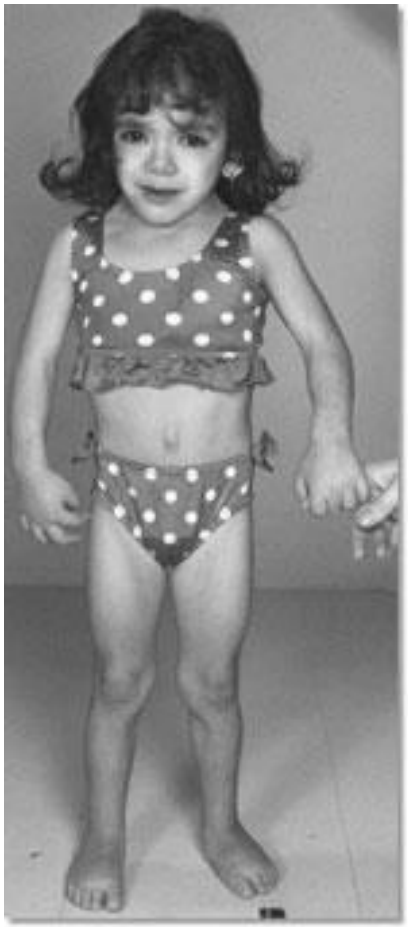
- Beckwith Wiedemann Syndrome
  - Overgrowth and hypoglycemia
  - Limb size discrepancy
  - Omphalocele
  - Macroglossia
  - Wilms Tumor and Hepat

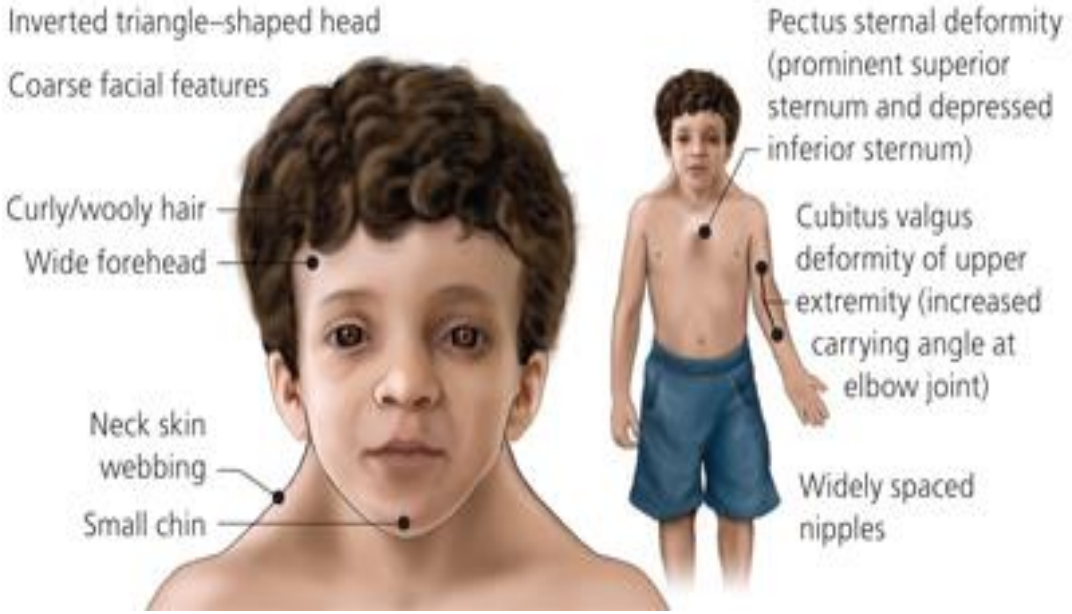


Patterns of Congenital Anomalies

- Syndromes
  - Multiple anomalies of 2 or more organ systems with a *common cause*
- Associations
  - Nonrandom occurrence of anomalies that occur together more often than by chance.
- Sequences
  - Series of anomalous findings attributable to an early abnormality of embryogenesis with a *cascading* effect

Syndrome




Inverted triangle–shaped head
Coarse facial features
Curly/wooly hair
Wide forehead
Neck skin webbing
Small chin
Pectus sternal deformity (prominent superior sternum and depressed inferior sternum)
Cubitus valgus deformity of upper extremity (increased carrying angle at elbow joint)
Widely spaced nipples

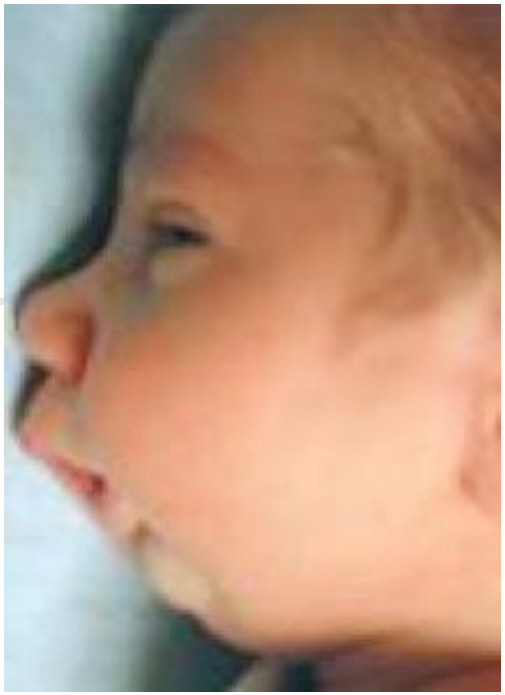
Association

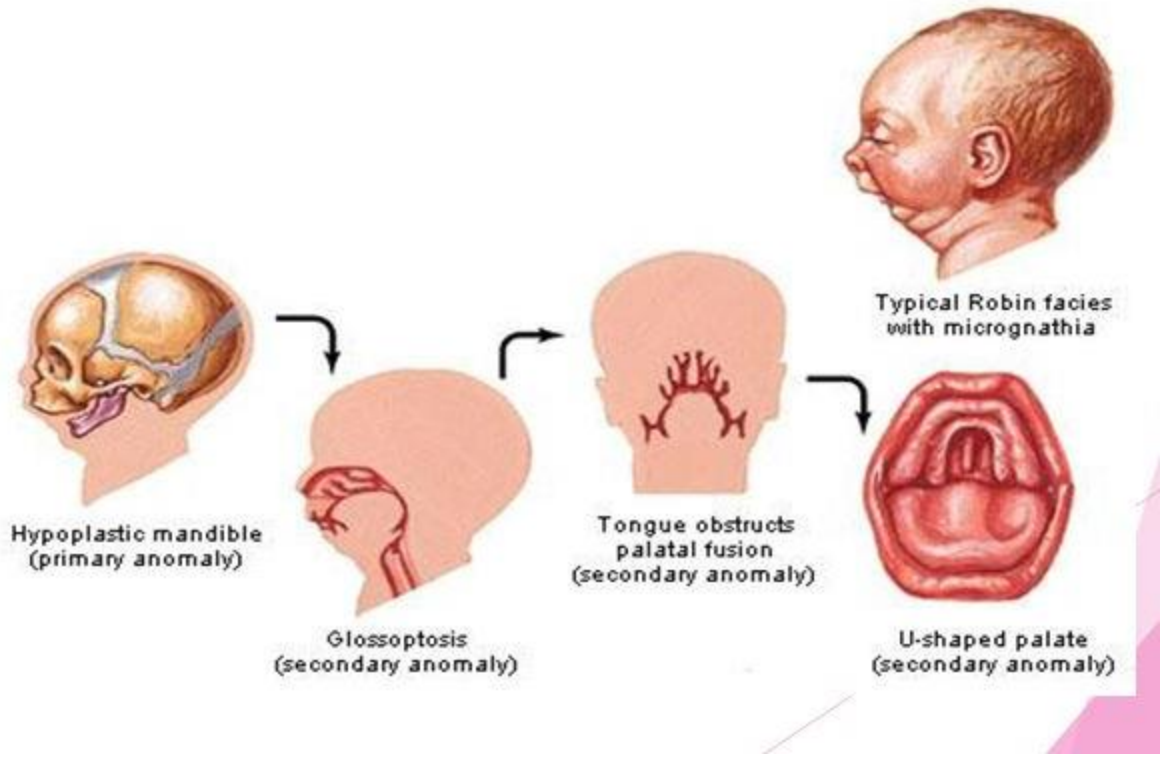
- *VACTERL* association
- Vertebral
- Anal atresia
- Cardiac malformations
- Tracheal-Esophageal fistula
- Renal anomalies
- Limb anomalies

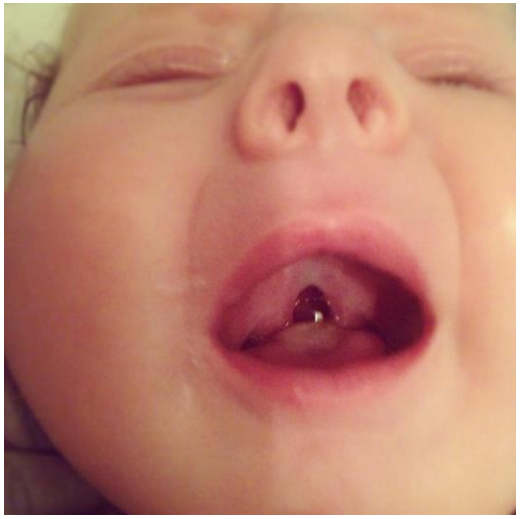
Need 3 Anomalies for Dx.

Sequence

- Pierre Robin Sequence







Newborn Screening

- Early detection of potentially fatal conditions or conditions that may affect health long-term
- Window to treat
- 54 Conditions
  - SCID recently added
- *NOT PKU TEST!!!!!!!*

Arkansas Numbers

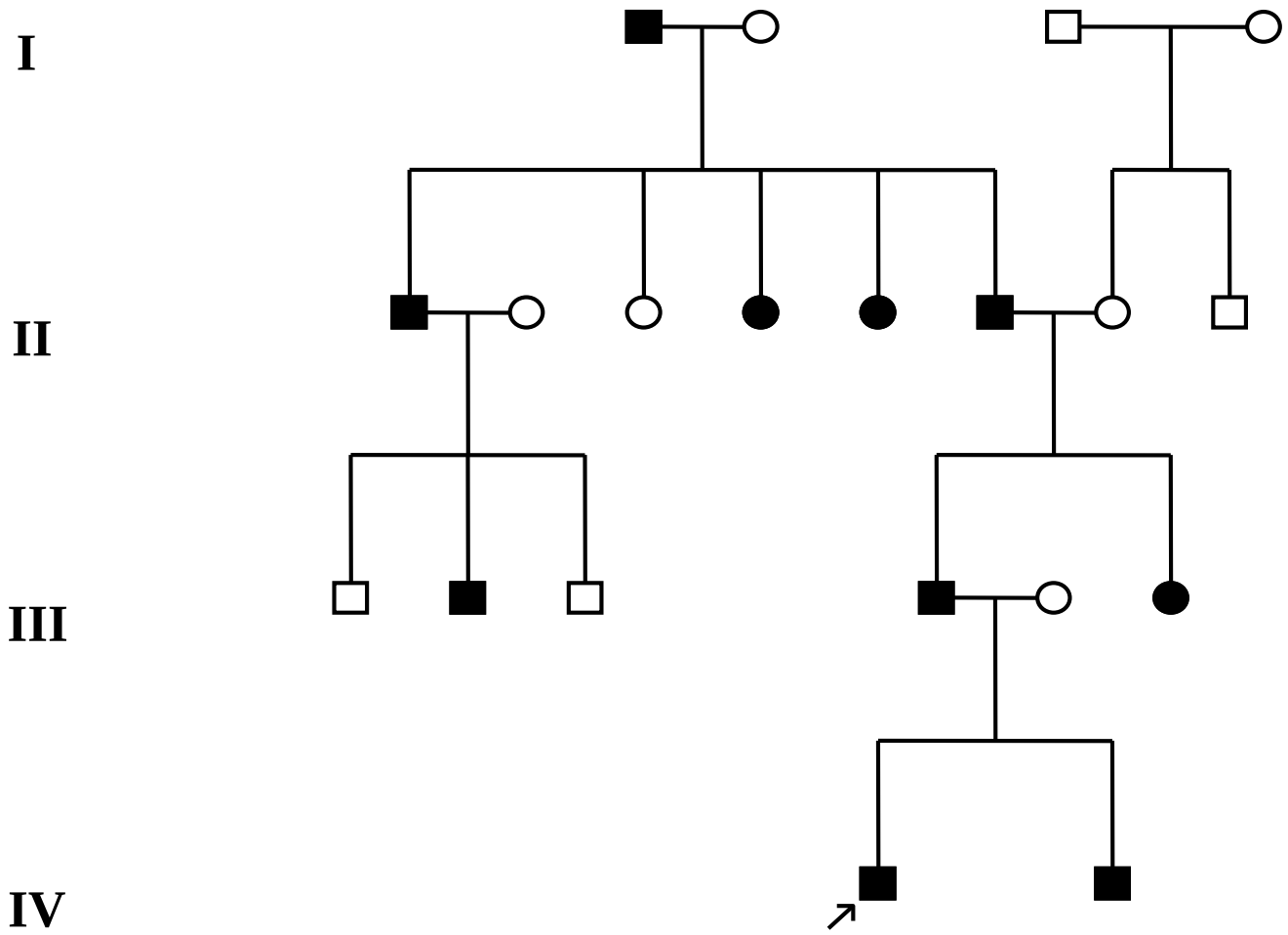
- 40,000 babies screened
- 2,000 Positives
  - 1,200 Hgb traits
- 140 Diagnosed with other conditions
  - 70 Metabolic
  - 70 Hearing loss

Inheritance

- Why we take a family history
- Provides clues about what conditions to consider

II. Single Gene Inheritance

Autosomal Dominant Pedigree



Variable Expression

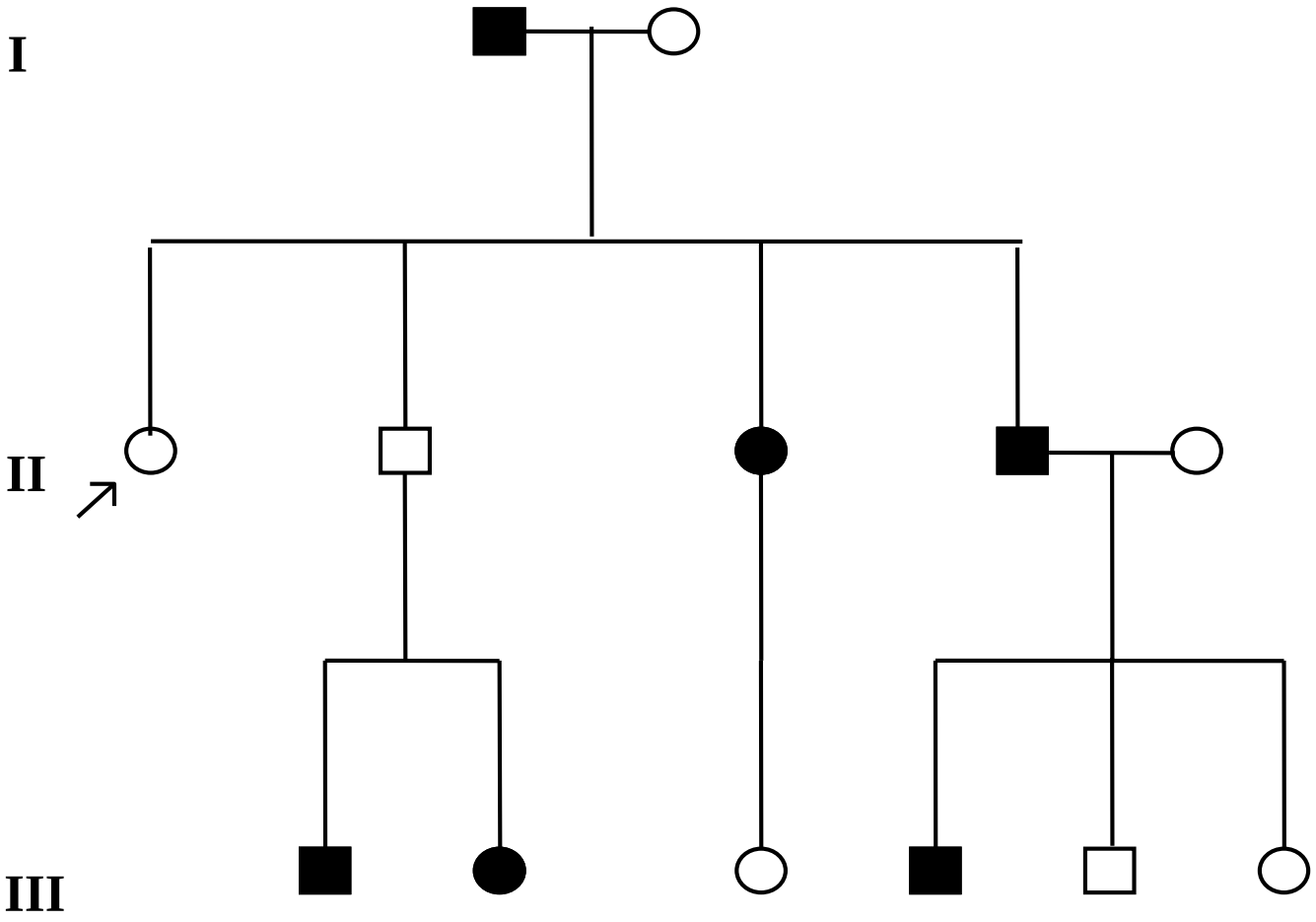
- Even when penetrance of a condition is complete, the *severity* of the disease may vary greatly.

- Possible causes: environmental factors, modifier genes.

Incomplete Penetrance

- An individual who has the genotype for a disease may not exhibit the disease phenotype *at all*, even though he or she can transmit the disease gene to the next generation.
- Penetrance rates are estimated by examining a large number of families to determine what proportion of obligate carriers (AD) or homozygotes (AR) develop the disease phenotype.
- This is how you get "skipping generations".

Incomplete Penetrance

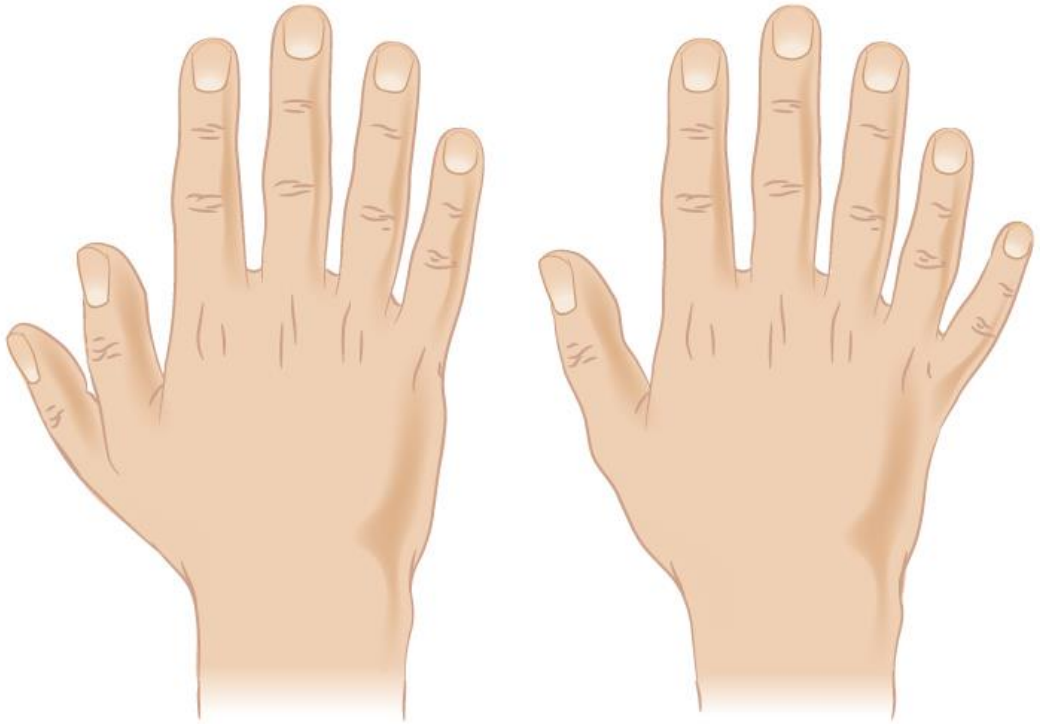


Polydactyly

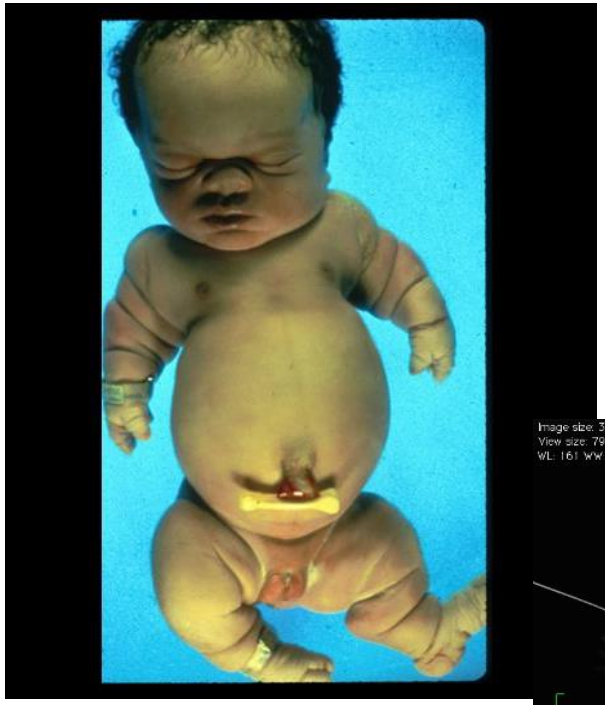
Often Exhibits Autosomal Dominant Inheritance

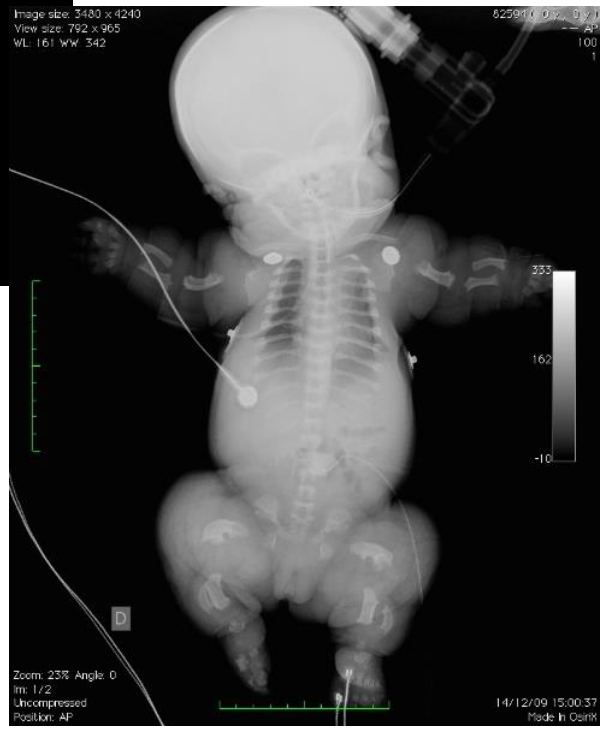
Often Incomplete Inheritance

Pre-axial



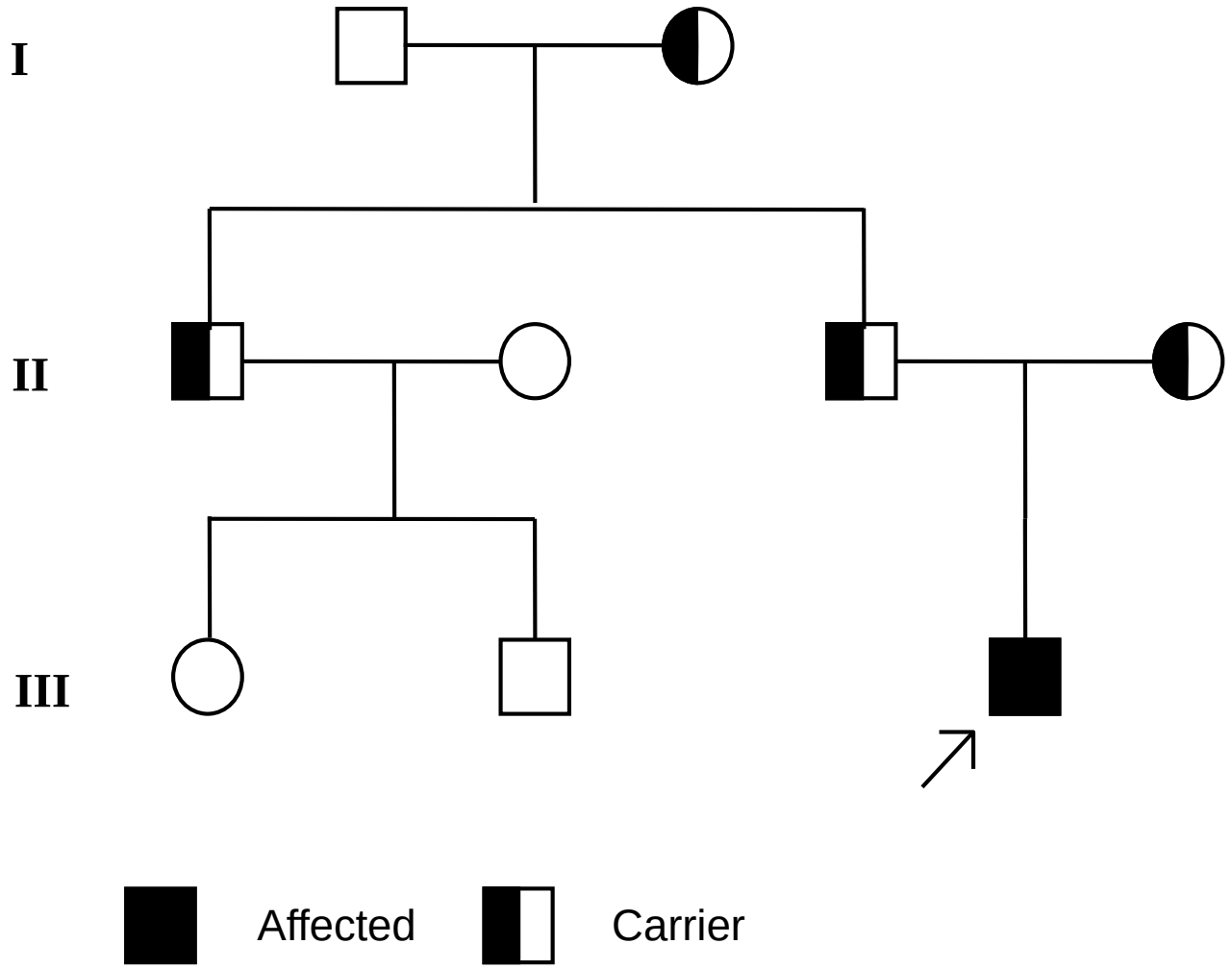
Thanatophoric Dysplasia





- AD Condition
- 100% Penetrance
- Diagnosed on Xray
- 99% with specific exons
- (FGFR3)

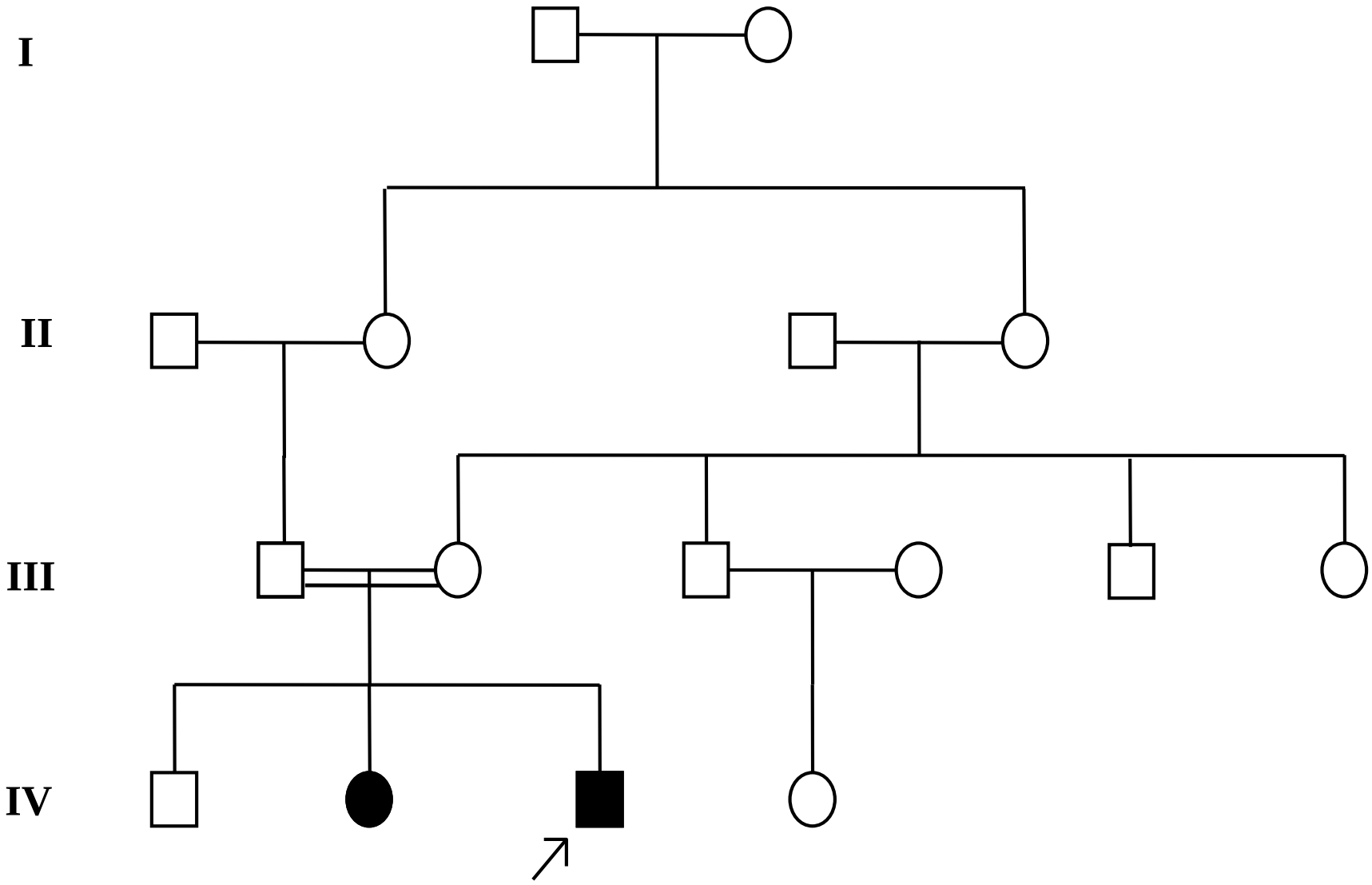
Autosomal Recessive Pedigree



Consanguinity

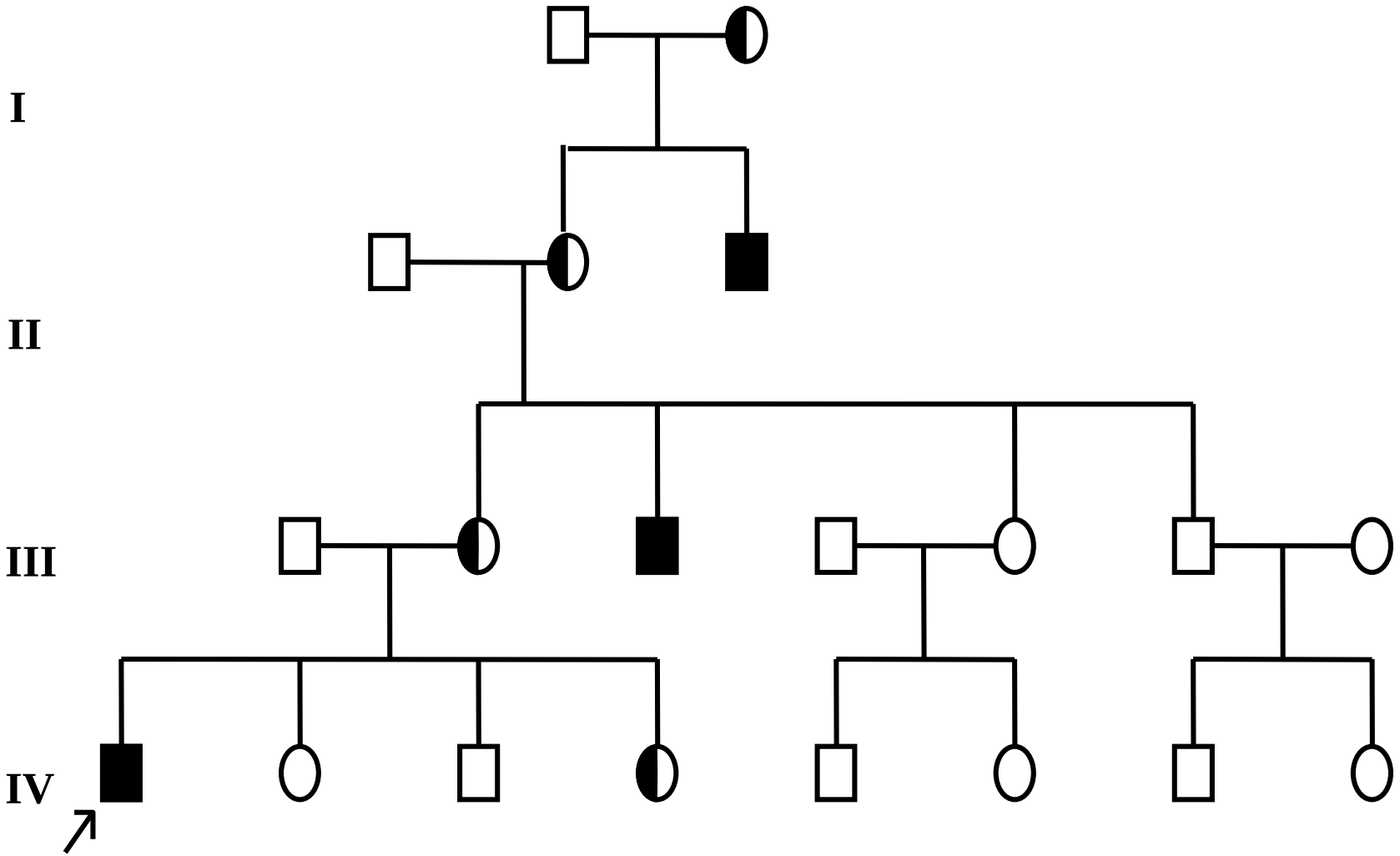
- Relationship by descent from a common ancestor.
  - Inbreeding: Breeding btw individuals closely related compared to random mating
- Increases risk of autosomal recessive disorders

Consanguinity

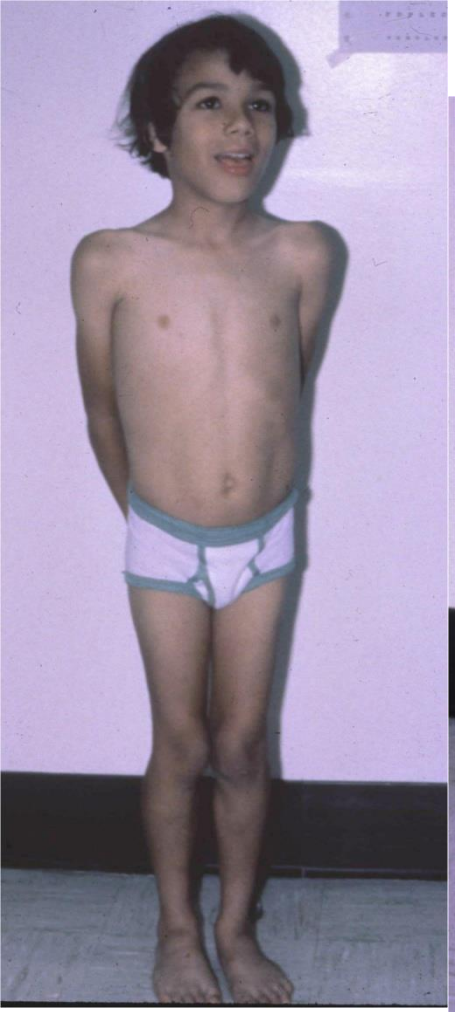
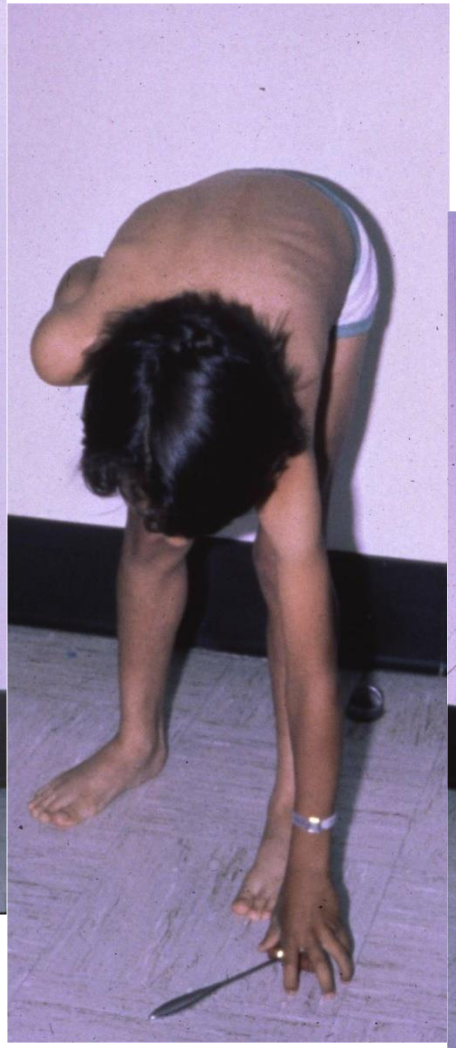
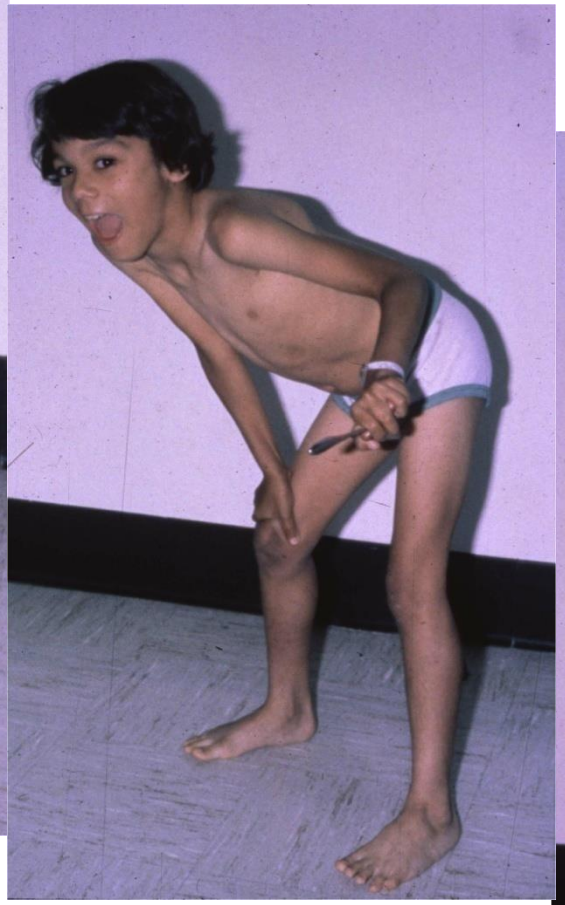
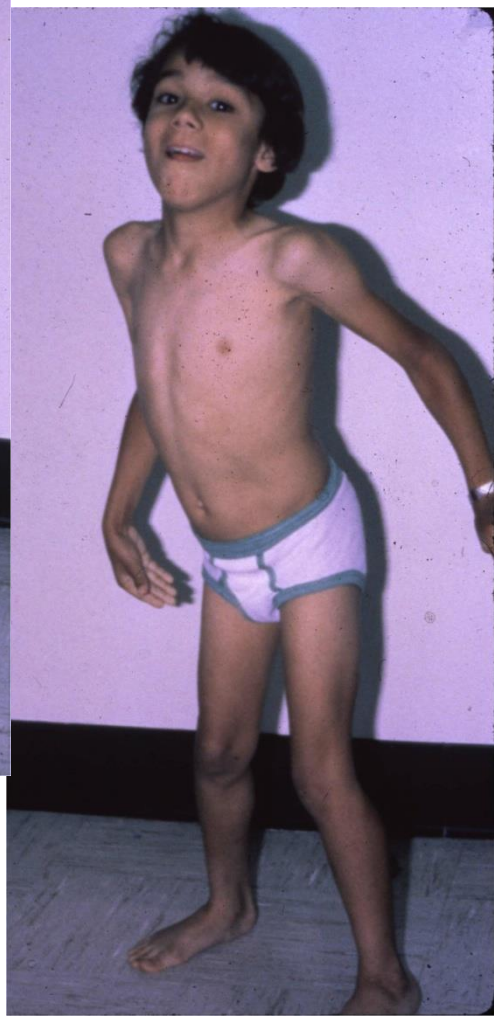


X-linked Inheritance

- X-linked mutant genes are fully expressed in males, who have only a single X chromosome, i.e., are hemizygous for X-linked genes.
- Fathers must transmit their Y chromosome to their sons, thus there is no male-to-male transmission of X-linked genes.

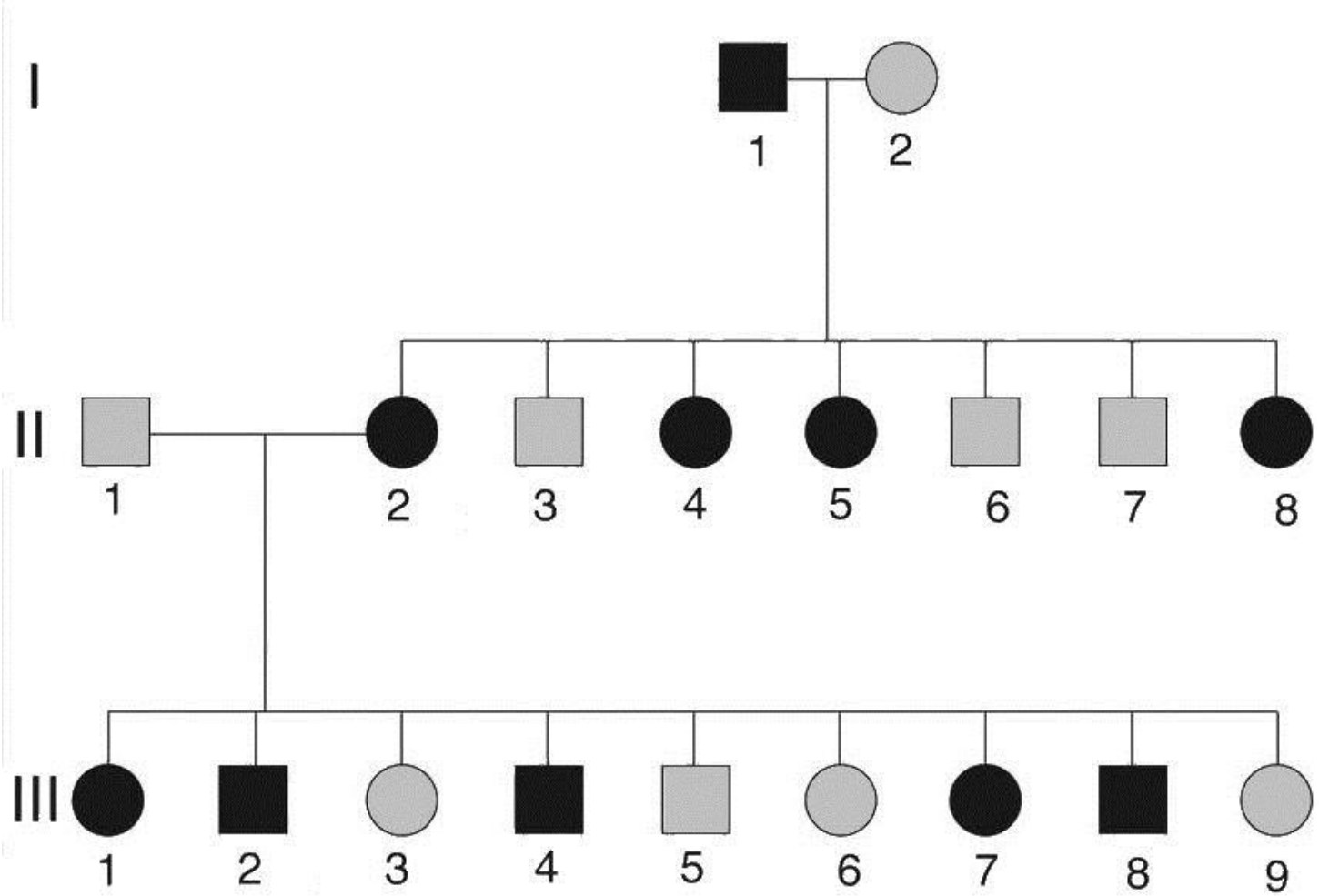


X-Linked Recessive Pedigree

X-linked *Dominant* Inheritance

- Males or females may be affected.
- The expression in heterozygous females may be variable.
- Often the clinical expression is more consistent and severe in hemizygous males than in heterozygous females, with some conditions causing lethality in males
  - Example : Incontentia Pigmenti

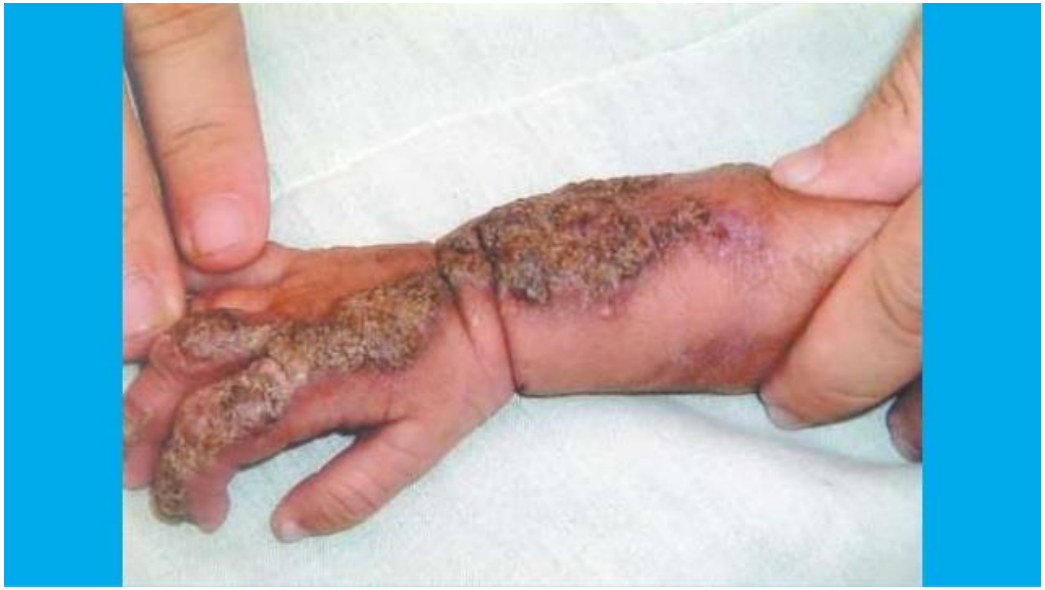


X linked Dominant:

Males and Females

Looks AD but affected males to ALL females and NO males.

Incontentia Pigmenti

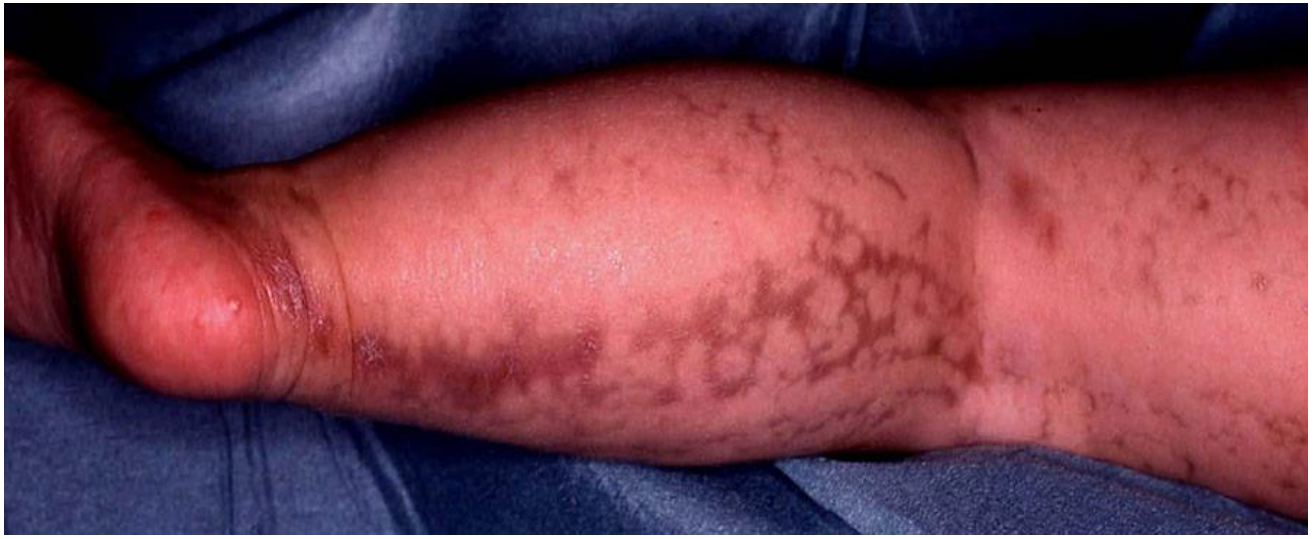


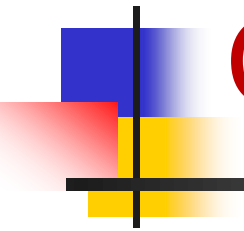
I. Blistering

II. Wart-like

III. Swirling macules hyperpigmentation

IV. Linear Hyperpigmentation





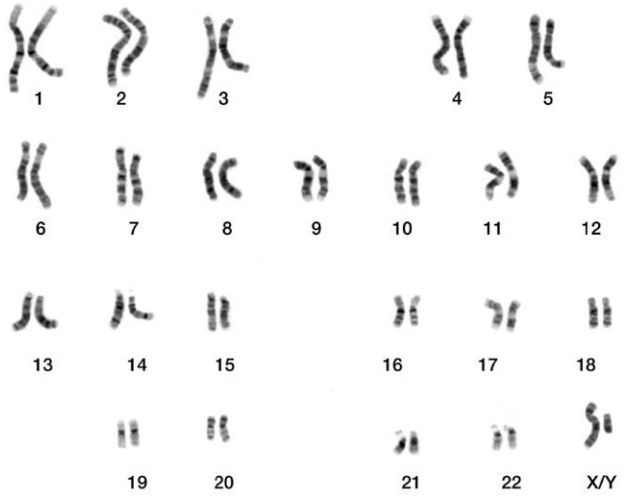
Genetic Testing

Karyotype

1956 discovered 46 chromosomes.

Tissues for Analysis

- Blood
- Bone Marrow
- Skin Biopsy (fibroblasts)
- Muscle Biopsy
- Tumor Biopsy
- Products of Conception
- Gonad Tissue
- Amniotic Fluid



Down Syndrome

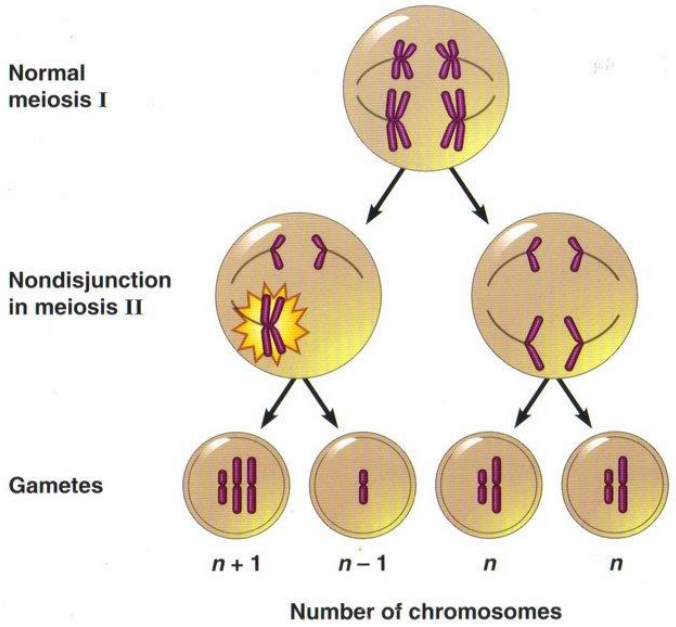
- Signs: Hypotonia, Brachydactyly, Flat Midface, Up-slanted palpebral fissures, Epicanthal folds
- Common Medical Problems:
  - Hearing Problem (75%)
  - Vision Problem (60%)
  - Cataract (15%)/Refractive (50%)
  - OSA (50-75%)
  - Otitis Media (50-70%)
  - Congenital Heart Dz (50%)
  - GI Atresia (12%)
  - Thyroid Dz (18%)
  - Transient Myeloproliferative DO (10%)
  - Leukemia (1%)
  - Celiac (5%)
  - Atlantoaxial Instability (2%)

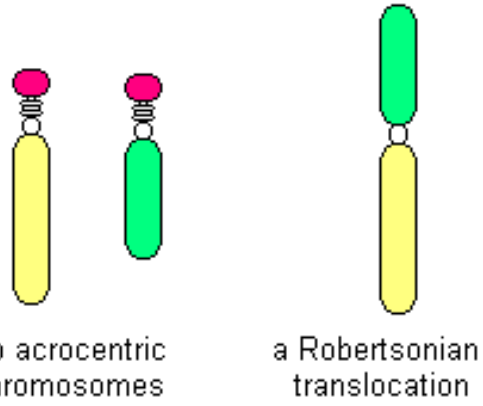
Down Syndrome

- Initial work up:
  - Karyotype (not FISH)
  - ECHO (Some discussion if this needs repeating if fetal ECHO done)
  - CBC and Thyroid (Part of NB Screen)
  - Monitor Feeding
  - Red Reflex

Down Syndrome

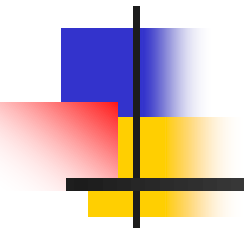
- Duplication of 21q22
- 95% of cases result from nondisjunction of chromosome 21.
- 5% split between Robertsonian translocations and mosaic Down syndrome.





Turner Syndrome

- Work Up
  - Karyotype
  - If any kind of marker chromosome, need to FISH for Y.
    - Risk for gonadoblastoma
  - ECHO
  - Renal US (30%)/Pelvic US
  - Watch BP (40% HTN)
  - Hearing Evaluation (25%)
  - Eye exam
  - Thyroid
  - Growth Hormone



CGH/ Microarray

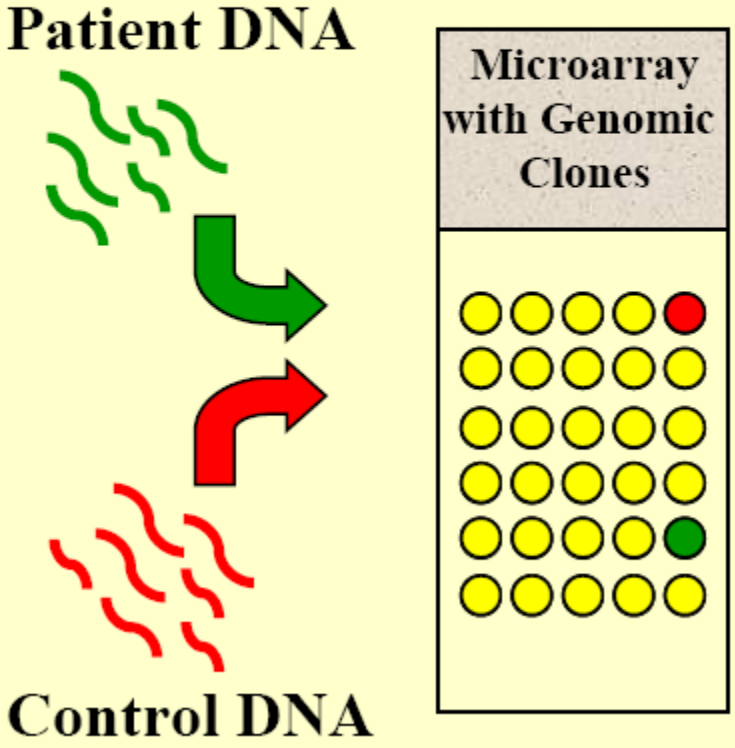
2001

Definitions

- **Microarray**
  - A wafer of glass/plastic/silicon onto which different NA have been spotted in a pattern- CHIP

- **Comparative genomic hybridization**
  - A technique that is used to detect chromosome gain or loss by hybridizing DNA from a patient and from a control cell that are *differentially labeled* with unique fluorescence
  - Look at ratio of light emitted
  - NEED PATIENT AND CONTROL

Array-based
Comparative Genomic Hybridization



→ Loss: ratio < 0.8

→ Normal: ratio 0.8 - 1.2

→ Gain: ratio > 1.2

SNP 'Array'

- **SNP** (Single Nucleotide Polymorphism) Most common type of polymorphism.
  - Very common—up to 10,000,000 variant positions between humans.
  - Make humans 'genetically unique'
  - But just because they are common, does not mean all are neutral. Disease *susceptibility* rather than directly cause illness.

Targeted Array

Focus on Specific Areas:

- Subtelomeres
- Pericentromeres
- Microdeletion/Microduplication regions

Whole Genome Array

Analyze entire Genome:

- BAC arrays (32,000 BACs)
- Oligo arrays (44,000 to 2M probes)

But now we have….

- Backbone coverage with high-density coverage for clinically relevant genes.
- Interspersed with SNPs between the probes to give us deletion, duplication, UPD, LOH information
- Because of this power we now ask for consent

Obtaining Consent

- Imperfect Test
  - Not testing for all disorders
- Different types of results
  - Variants of Uncertain Significance
  - Detect unrelated information
- Can detect consanguinity

Main Advantages of Cytogenomic Arrays

- Far superior for genomic copy number changes (gains or losses) that are below resolution of G-banding.
- Remember resolution dependent on:
  - Size of DNA probes on the arrays
  - Distance between probes
- Equivalent to thousands of FISH at once
- Does not require dividing cells (no culture)
- SNPs can detect loss of heterozygosity

Limitations of Cytogenomic Arrays

- Inability to detect BALANCED rearrangements
  - Inversions, balanced translocations
- Cannot detect single point mutations
- Relatively expensive
- Not all copy number changes are clinically significant (benign, pathogenic, or of unknown significance).

Deletion 22q11.2

- No established diagnostic criteria!
- Congenital Heart Dz (75%)→ Conotruncal
- Immunodeficiency (75%)→ Thymus hypoplasia
- Palate defects (75%)→ Cleft or VPI
- Hypocalcemia (50%)
- Renal Anomalies
- Dysphagia
- Developmental Delay (90%)

Deletion 22q11.2

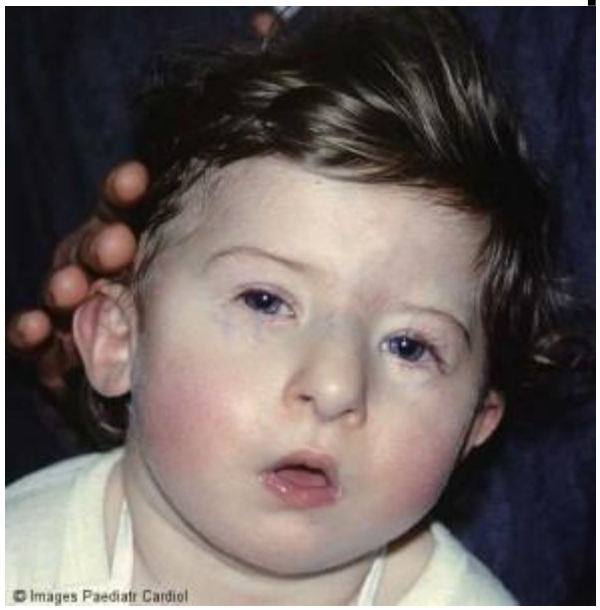
- The facial features often not helpful

Prominent ears

Fullness to nasal tip

Small mouth

Thin upper lip



Deletion 22q11.2

- 90% have same 3Mb deletion
- 30 genes
- Small percentage have smaller deletion
- Wide variability- even in families, even in identical twins
- >90% new deletions
  - Some family members not identified
- Autosomal Dominant

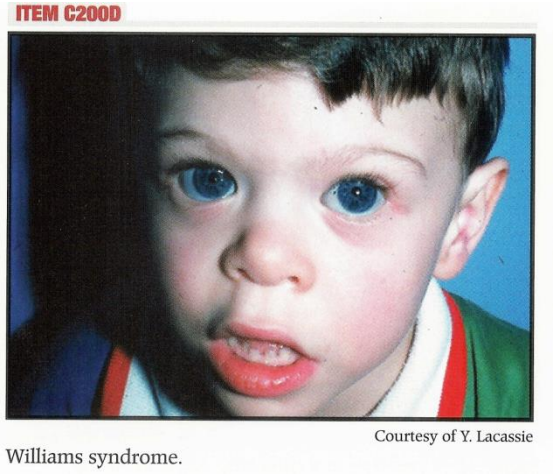
Williams Syndrome

Features:

- Supravalvar aortic stenosis (progressive) (75%)
- Dysmorphic features
- Idiopathic hypercalcemia (15%) and hypercalciuria (30%)
- Developmental Delay
- Short stature and Feeding issues
- Connective tissue abnormalities
- Personality: empathy, overfriendly, anxiety, attention issues

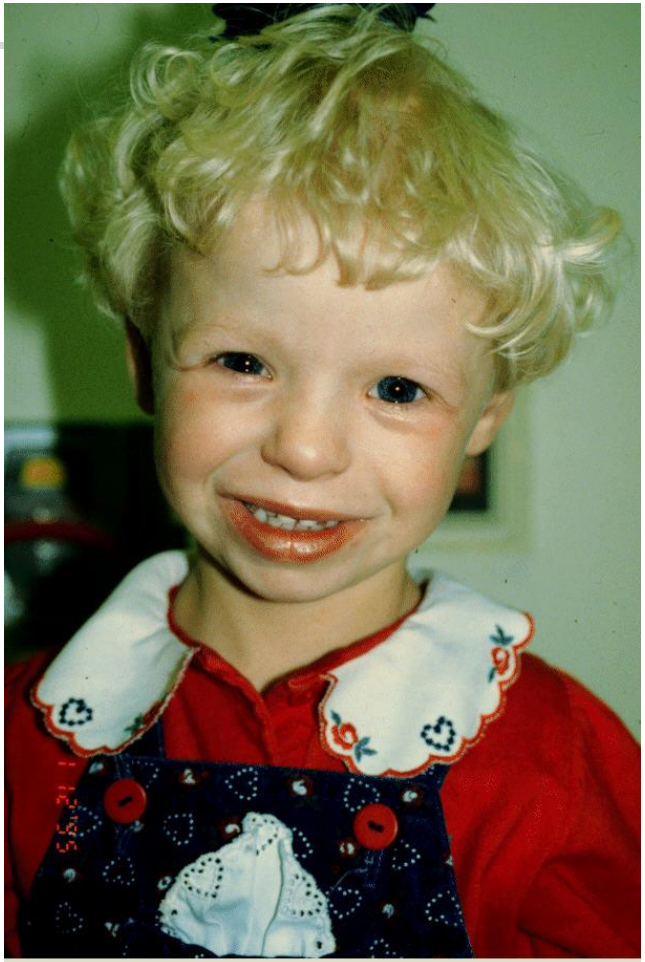
Williams Syndrome

- Dysmorphic facial features (100%)
  - Periorbital fullness
  - Stellate irises
  - Short nose with bulbous tip
  - Long philtrum
  - Full lips
  - Wide mouth



Courtesy of Y. Lacassie

Williams syndrome.



The face of Williams syndrome.

Reproduced from http://medgen.genetics.utah.edu

Williams Syndrome

- Deletion 7q11.23
  - Includes *ELN* gene→ Supravalvar AS
  - *LIMK1* gene → Cognitive profile
- FISH or microarray
- Autosomal Dominant
- Majority are de novo– low recurrence risk

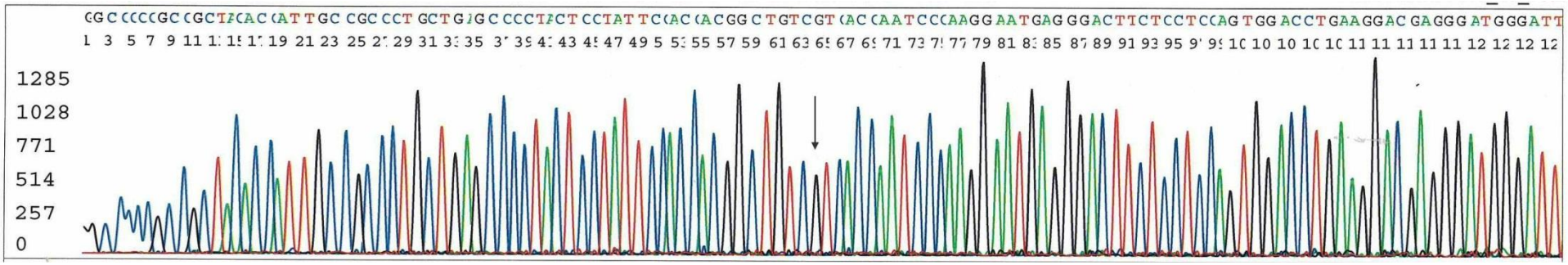
Gene Sequencing

Gene Sequencing

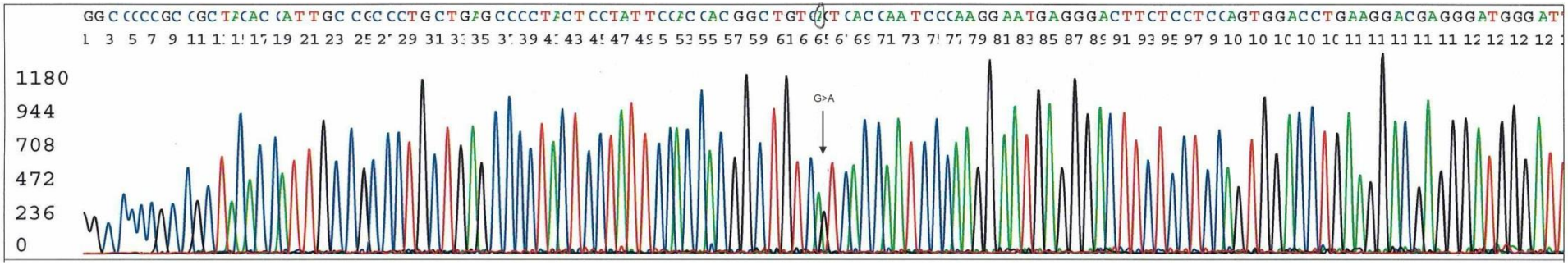
- FISH and microarray tests are very helpful in identifying duplication / deletions of *specific loci*.
- Won't detect small changes, point mutations, etc.
- Often the only method to make a diagnosis is to sequence the gene.
- Still, it is very expensive and time consuming to sequence large genes

Sanger Sequencing

Normal control

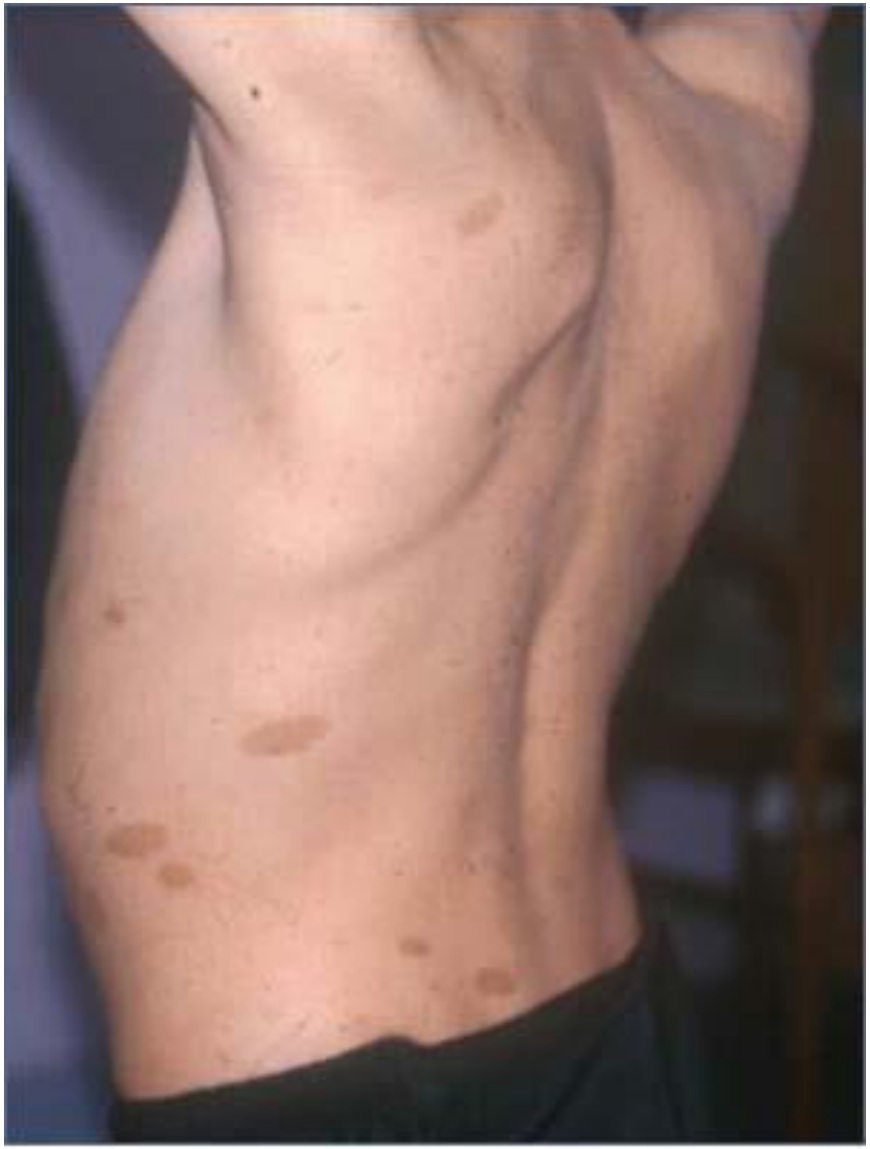


Mutation identified

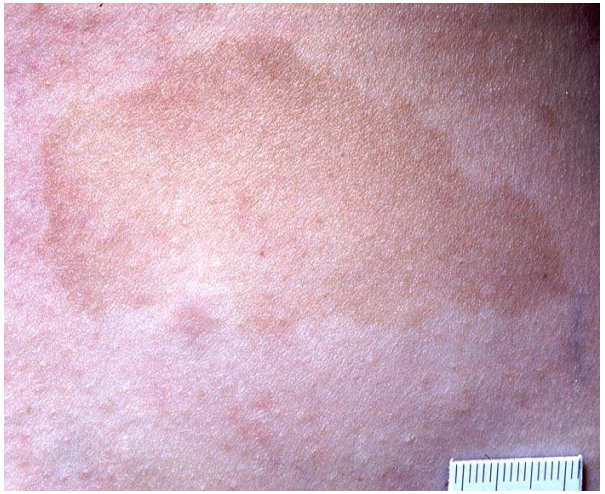


Neurofibromatosis 1

- Two or more of the following
  - 6 or more *café-au-lait macules*
    - >5 mm in greatest diameter in pre-pubertal individuals
    - >15 mm in greatest diameter in post-pubertal individuals
  - *Freckling* in the axillary or inguinal regions
  - Two or more *Lisch nodules* (iris hamartomas)
  - Two or more *neurofibromas* of any type or 1 *plexiform neurofibroma*
  - *Optic glioma*
  - Distinctive *osseous lesion*
  - *First degree relative* (parent, sibling, or offspring) with NF1



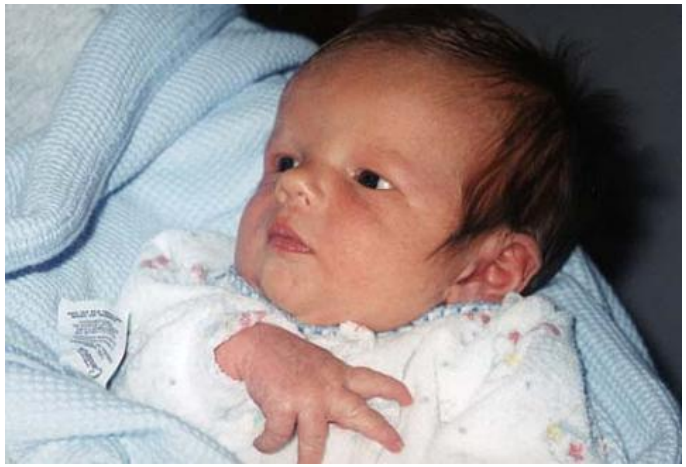
this picture shows cafe au lait marks

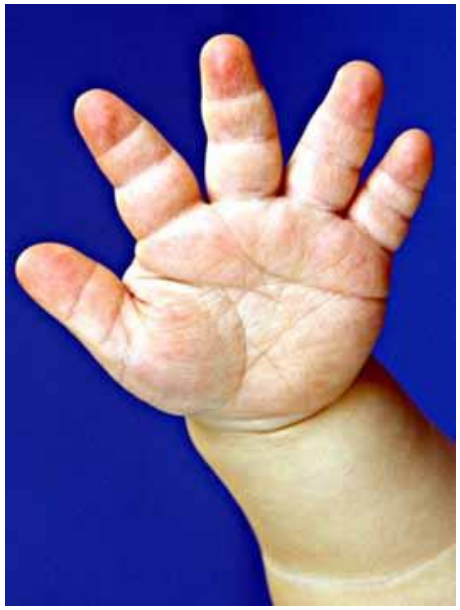


Achondroplasia

- Birth:
  - Rhizomelic shortening of arms and legs
  - Long, narrow trunk
  - Trident hands
  - Macrocephaly with midface hypoplasia and prominent forehead
- Small cranial base, obstructive apnea, dental crowding, otitis media
- Lumbar scoliosis and lordosis
- 3-7% die in first year due to brainstem compression or obstructive apnea

Achondroplasia





- Rhizometric micromelia (shortened limbs, proximal>distal shortening)
- Inability to fully extend elbows
- Genu varum (bow legs)/Knee instability

Achondroplasia

- Fibroblast Growth Factor Receptor 3 (*FGFR3)*
  - 98% have 1138G>A mutation
  - 1% have 1138G>C mutation
- Coordinate growth of chondrocytes
- Autosomal Dominant, fully penetrant
  - Paternal age effect
  - Homozygous lethality
- Can diagnose with skeletal survey

Whole Exome Sequencing

Whole Exome Sequencing (WES)

- Sequences all known coding regions (exomes) of human genome
  - Exome 1-2% of human genome
  - Thought to harbor majority of pathogenic mutations
- Expensive, time consuming

Rapid WGS

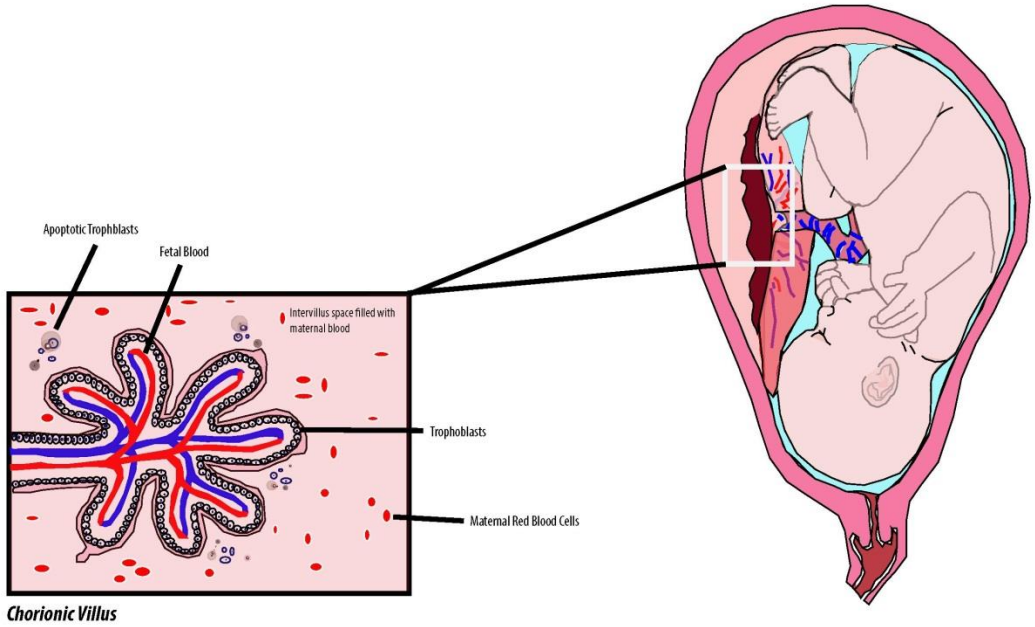
- Places are trialing a rapid test
  - 26-hour test has been reported (50% yield)
  - Clinically, we use a rapid test with 7 day verbal result
- Goal to decrease infant mortality and facilitate parental decision making
- Studies in some populations show faster discharge (more comfortable with palliative care)

Cell-Free Fetal DNA (Prenatal)

2011

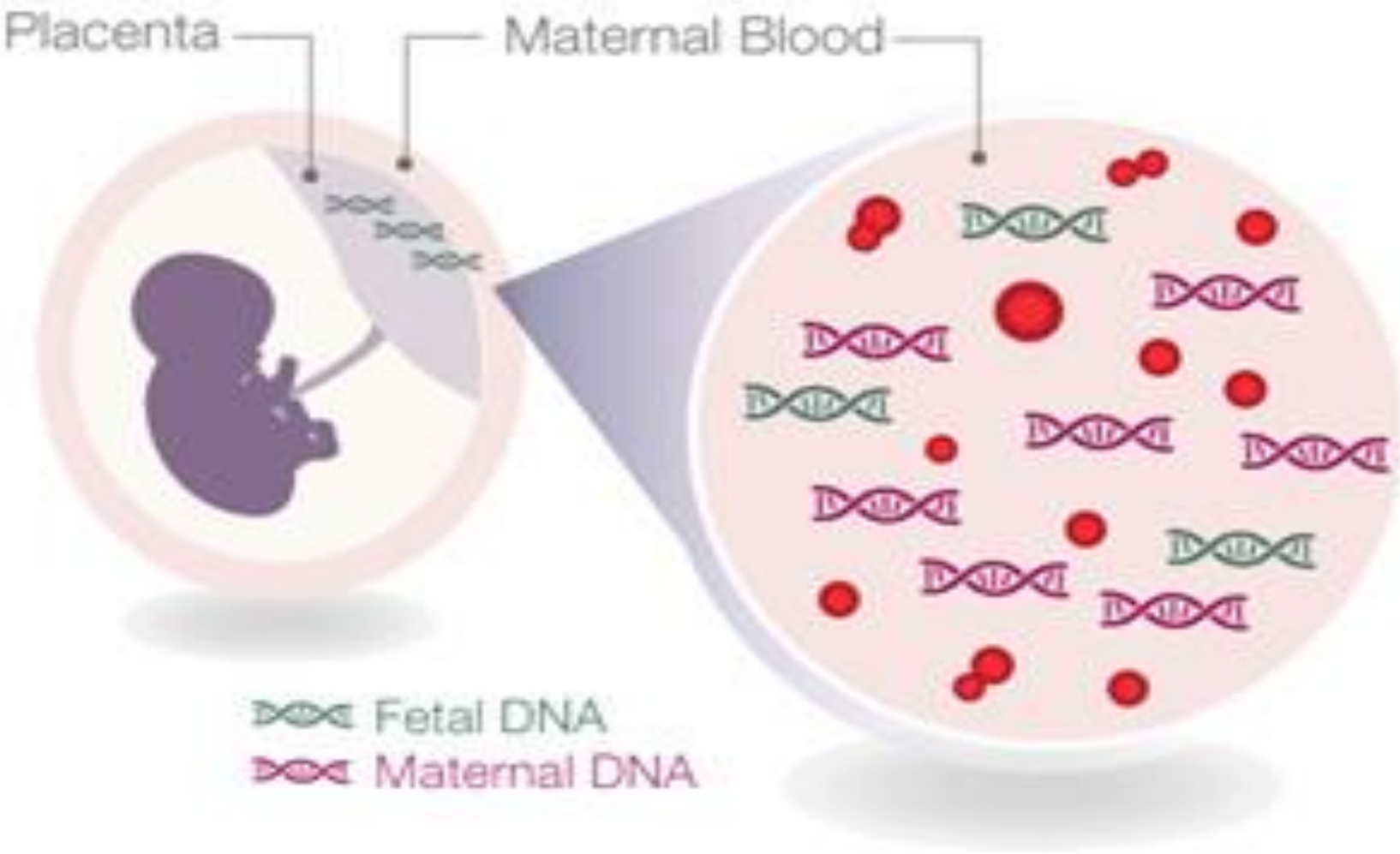
Cell-Free Fetal DNA

- Fetal **cells** pass between mother and fetus
- Scarce! (20 fetal cells per 20 ml blood)
- Difficult to extract and
- Persist after delivery



Cell-Free Fetal DNA

- "Cell-Free" nucleic acids found in 1947
- *Fragments of DNA* without cell membrane (pancreatitis, cancer, lupus)
- Placenta apoptosis
  - Present early in gestation: 5-7 weeks
  - ffDNA 3-5% of total DNA in maternal circulation
  - Cleared within hours of delivery of placenta
  - ffDNA can be isolated with high fidelity

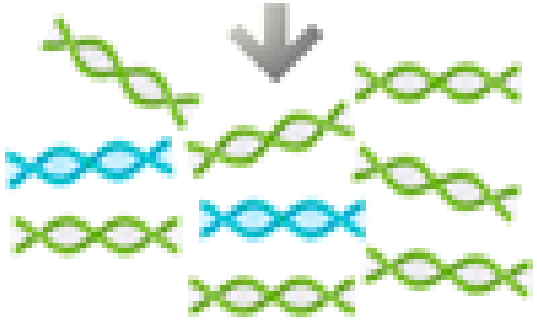

Placenta
Maternal Blood
Fetal DNA
Maternal DNA

Cell-Free Fetal DNA

- Use Massively Parallel Genomic Sequencing….
- Use NextGen to rapidly ‘count’ fragments and see proportion
- Screening test– NOT *diagnostic*

MASSIVELY PARALLEL SEQUENCING (MPS)

MATERNAL BLOOD SAMPLE

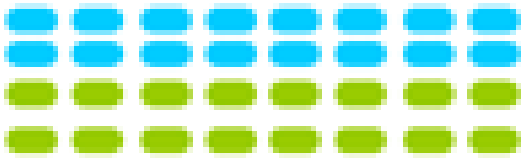


MATERNAL AND FETAL CELL-FREE DNA

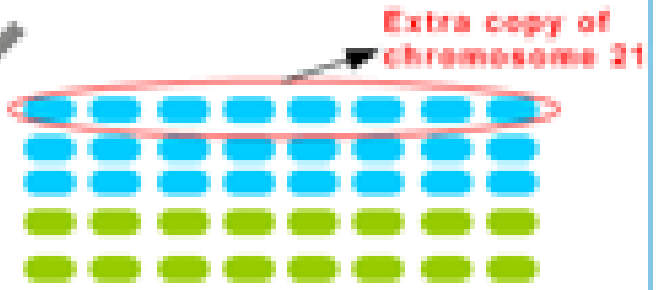
CELL-FREE DNA SEQUENCED VIA MPS

CCCTTAGCTTTAACGTACGTAAAAACCCTT
AACGTACGTAAAAAACGGGGGTCAAAGGTCCC
GACTTAAAATCGGAATCGATGCCCAAACTT
GAATCGATGCCCAAAACGGGGGTCAAAGTTCCC

ALIGNMENT AND COUNTING



Chromosome 21
No Aneuploidy



Chromosome 21
Aneuploidy

Positive Predictive Value

Positive Predictive Value	Wang et al.	Bianchi et al.	Choy et al.	Meck et al.	Norton et al.
Trisomy 21	38/41 (93%)	5/11 (45.5%)	52/55 (95%)	29/30 (97%)	9/47 (80.1%)
Trisomy 18	16/25 (64%)	2/5 (40%)	6/12 (50%)	3/5 (60%)	9/10 (90%)
Trisomy 13	7/16 (44%)		4/7 (57%)	1/4 (25%)	2/4 (50%)
Sex Chromosome Aneuploidy	6/16 (38%)		4/6 (67%)	1/7 (14%)	

The PPV is higher for women over 40 years than 20 years.

Please Remember…

- Largely validated for Trisomy 21
- Most data on high-risk women NOT general population
- Still recommended to confirm with amnio
- Not validated for microdeletions (but is being used in this way)
- Not recommended for sex determination
- Limitations– maternal obesity

- Better than standard screening (quad screen)

Outcomes

- Each child with congenital anomalies and/or genetic syndrome is unique
- Outcomes vary
  - 20% of infant deaths due to congenital or chromosome anomalies
  - Many infants do not leave the NICU
- Advances in genetic testing have allowed for better diagnosis and management
  - Either while in the NICU or in follow up
- A genetic diagnosis expedites *appropriate* treatment and empowers families to make the best decisions for their child.

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