Basic Concepts in Medical Genetics:

Mendelian and Non-Mendelian Genetics and the Rise of the New Technologies

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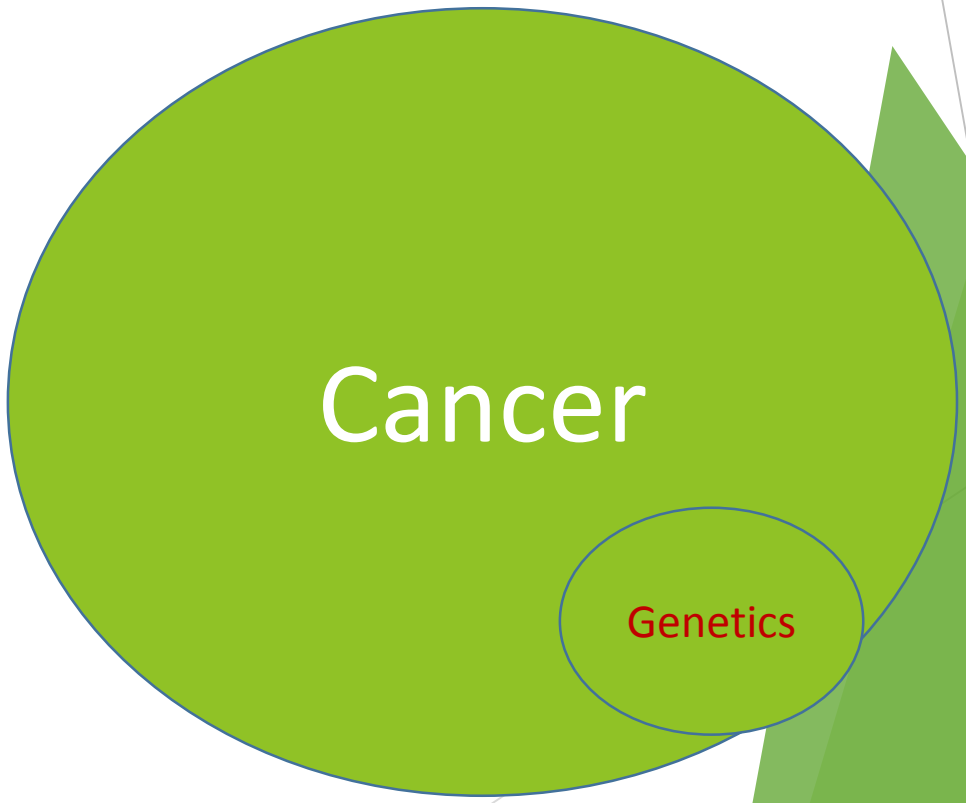
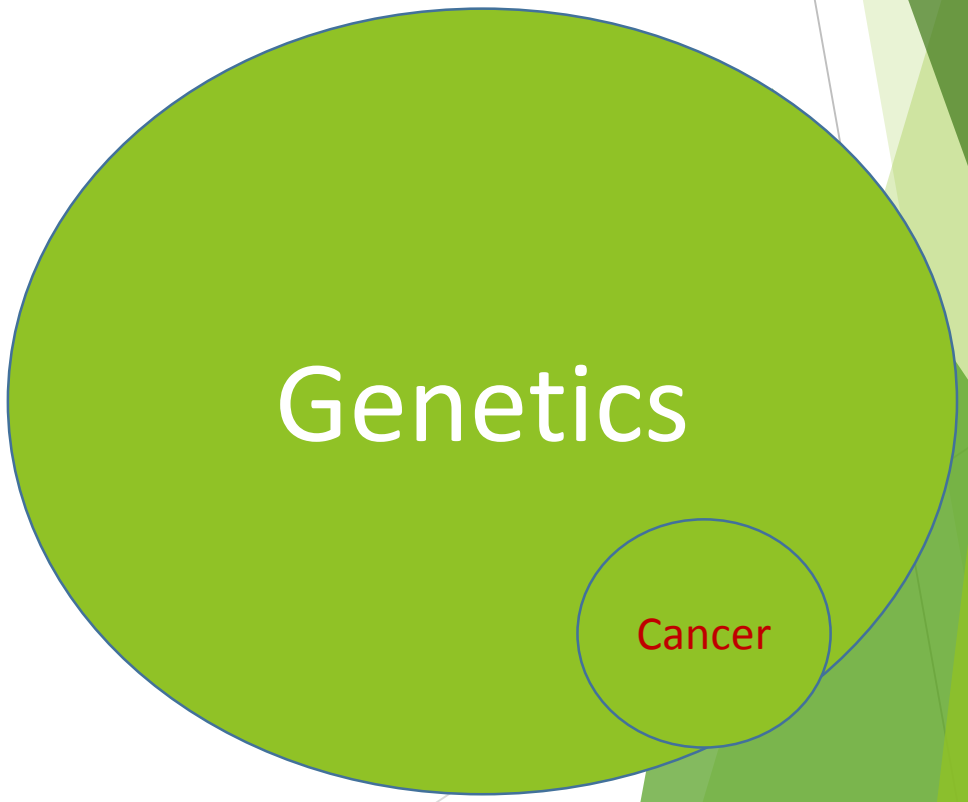
The Oncologist's View of Genetics



The Medical Geneticist's View of Cancer



The Medical Geneticist's Perspective is Unique

- LR is a 52 year old with a recent diagnosis of advanced stage breast cancer (invasive ductal), triple negative (ER, PR, Her2neu); after surgery, her therapeutic options appeared limited
- In an effort to determine the most reasonable management plan, a sample of archived cancer tissue was sent for analysis to a laboratory in Boston
- At the laboratory, 315 cancer related genes and 28 cancer related DNA rearrangements were queried; **tumor genotyping suggested a BRCA2 genomic alteration**
- The oncology team were unsure of the germline (familial) implications of the result and queried Foundation; I was asked to contact the clinicians
- Of the 6663 BRCA2 variants reported in the literature, the variant detected in this patient's tumor had been reported as **likely pathologic**
- Pedigree analysis/germline testing identified **3 immediate family members** at risk for cancer development; surveillance was begun; **an early cancer was found**

The Medical Geneticist's Role

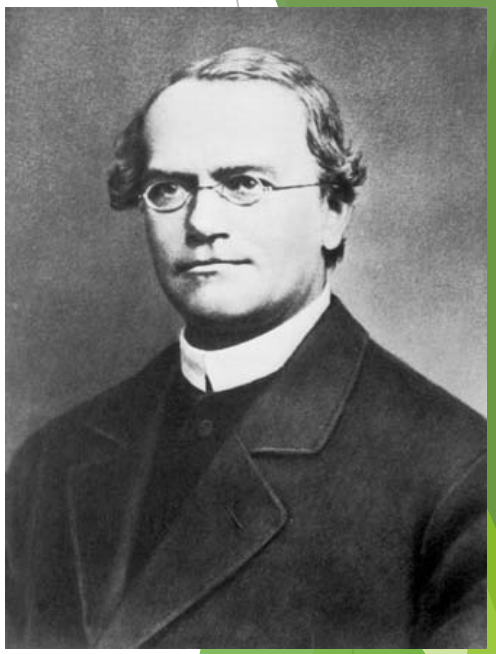
- **Introduction**
  - Personal disclaimer/disclosure
    - Personal history: academics and teaching, research, private practice
    - What I **am not**; What I **am**
    - No disclosures (too bad!!!)
  - Watson's fly

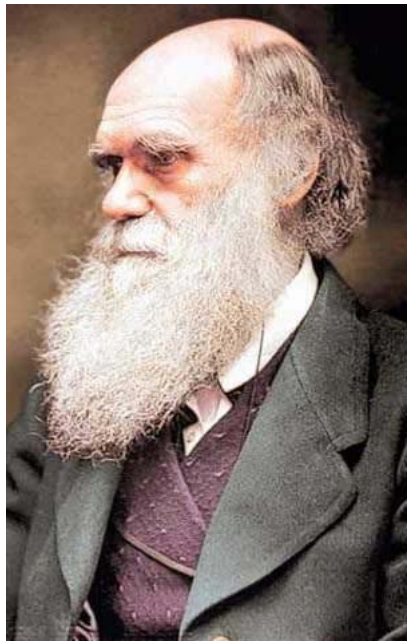
WHY ARE WE DOING THIS?

- **Poor understanding** of medical genetics in clinical community
  - **"…although 98% of physicians know that patient genetic information will influence therapy- less than 10% believe that they are adequately informed about the use of genetic/genomic testing information to apply it in practice"…** AMA 2018
- The technologies are **advancing rapidly** and are **poorly understood**
- The clinical applications are **profound**; we are **all** affected **personally and clinically**
- **All** areas of **medicine** (and of **society**) are touched
- There is a huge risk of **misrepresentation: Question Authority!!**

A LITTLE HISTORY

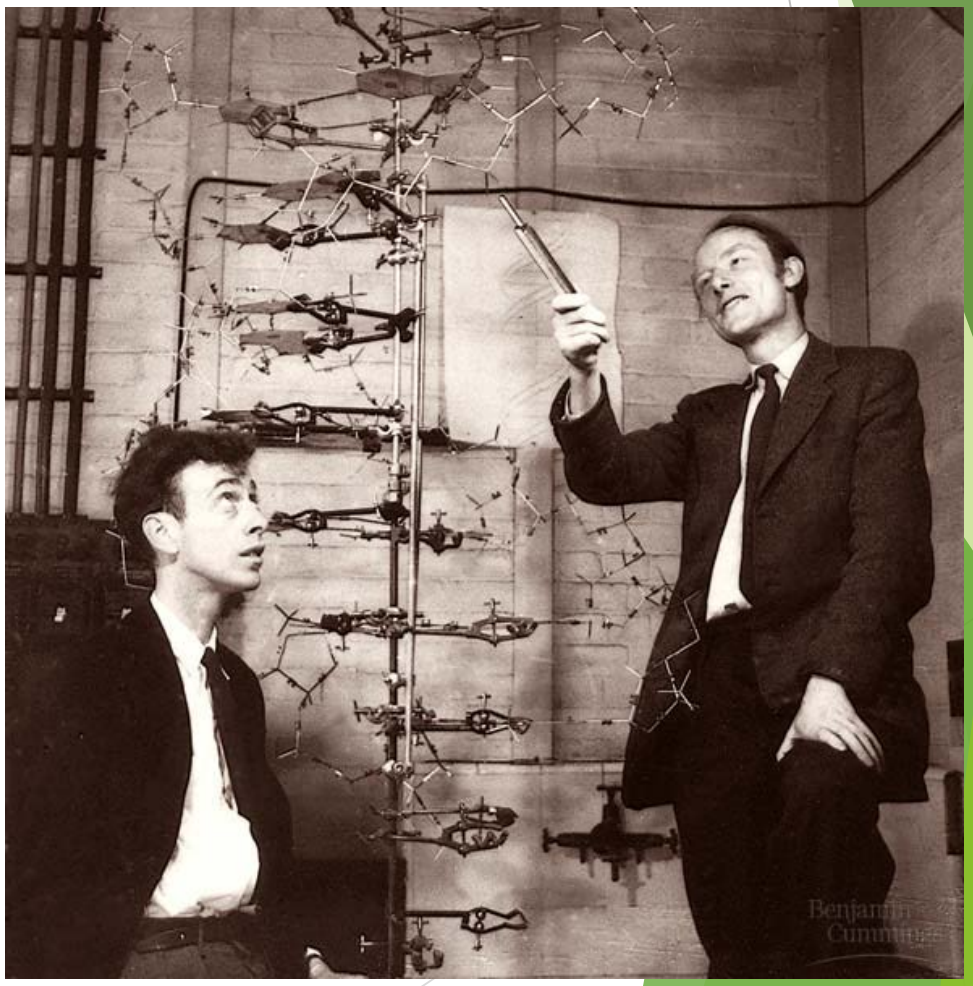
- **Darwin and Mendel**
  - Natural selection
  - Independent assortment of factors
  - Patterns of inheritance



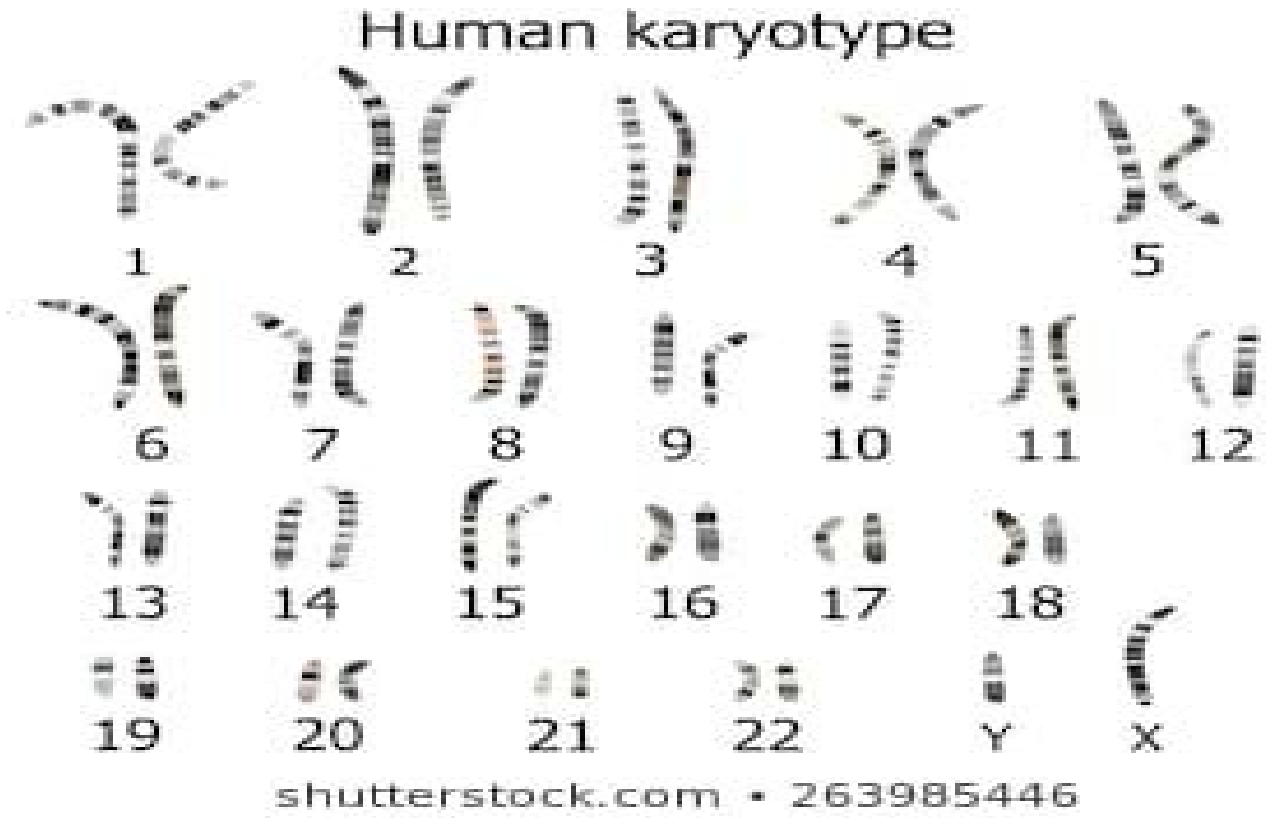


A LITTLE HISTORY

- **Watson and Crick**
  - Structure of DNA (Double Helix)

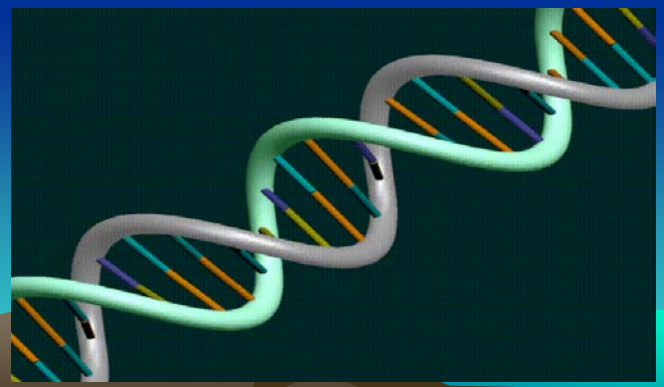


The Human Karyotype



Basic Medical Genetics

- Basic Genetic Concepts
  - 3 billion base pairs of DNA in the nuclear chromosomes
  - Expression of genetic code is in the proteins DNA codes for (exceptions: structural “control” RNAs)
  - Error in DNA (mutation) can result in abnormally made and/or poorly functioning protein, or NO phenotype difference (if the error is in non-functional part of protein)
  - DNA mutation can reside in a “control” site giving phenotype without change in DNA of gene itself
    - Promotor mutation
    - Transcription and splicing mutation
    - Organelle targeting errors
    - Activation and inactivation errors

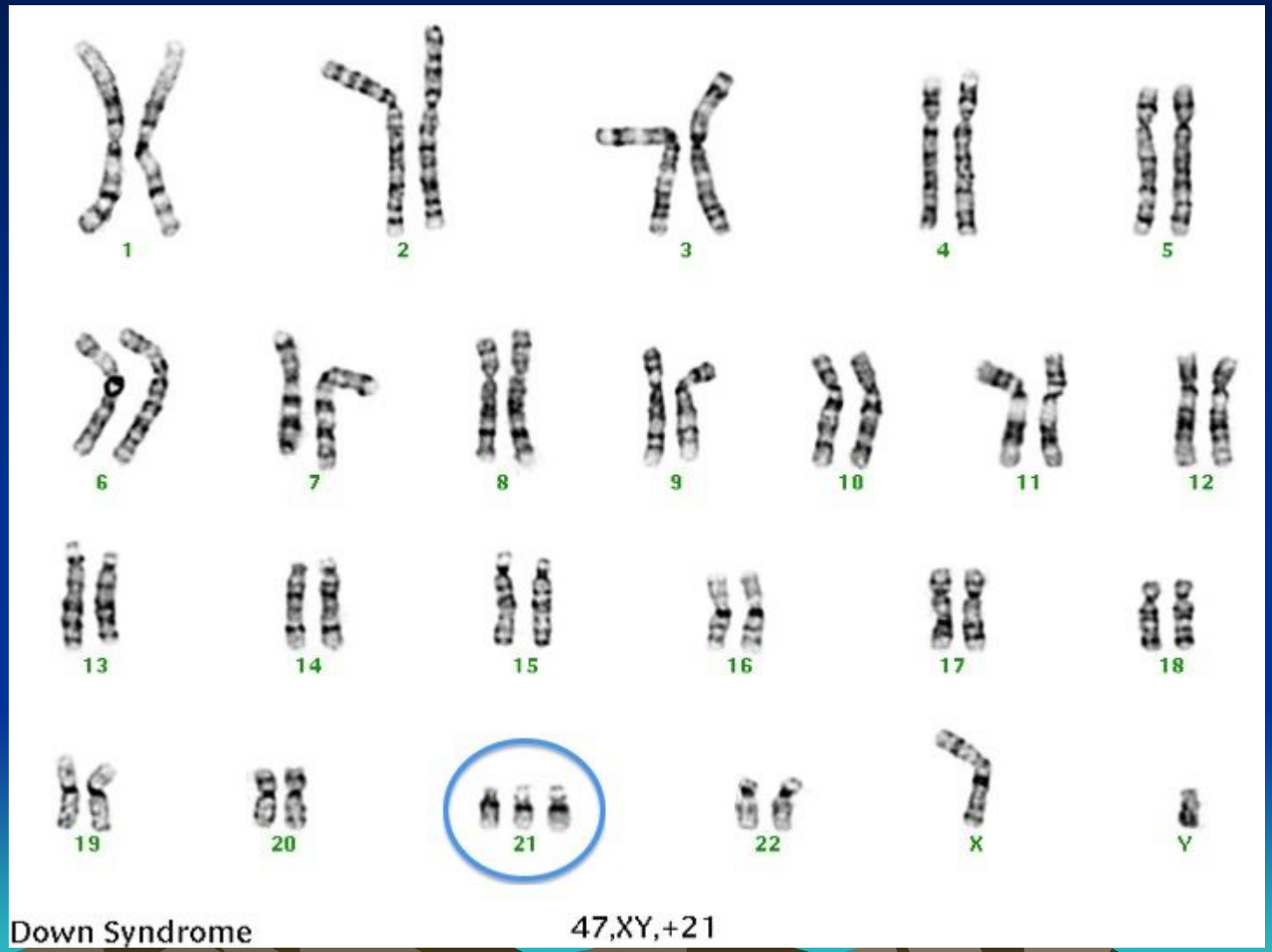


Basic Medical Genet

- **Basic Genetics Concepts**
  - Mendelian Genetics
    - Defined through decades of work in animals and plants
    - One gene: one protein
      - DNA sequence determines RNA sequence which determines protein (amnio acid) sequence and protein function/expression
    - Watson and Crick (1953) : DNA is the molecule of heredity and evolution
    - Much of DNA does NOT code for protein sequences
    - Genotype: phenotype correlation
      - Phenotype must be visible to be recognized as a genetic entity
    - Mutations are passed from parent to offspring unaltered
    - Visibility of mutation dependent on information from other parent and cell type being examined
      - Eg hemoglobin mutation phenotype not visible in skin cell although genotype should be if you look

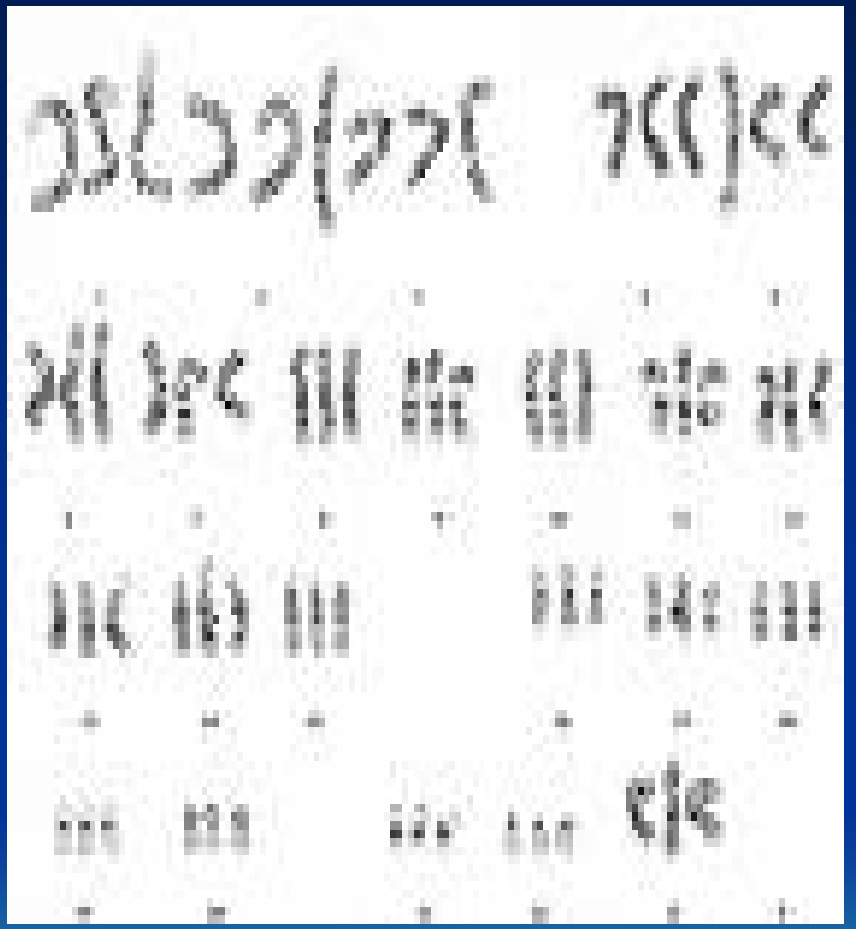
The New Genetics

- Mendelian Genetics
  - Chromosomal basis of inheritance
    - Mitosis, meiosis, nondisjunction
  - Dominant inheritance
    - e.g. achondroplasia, Marfan's syndrome
  - Recessive inheritance
    - e.g. sickle cell disease, Tay-Sachs, cystic fibrosis
  - X linked inheritance
    - e.g. hemophilia (VIII, IX), Duchenne's muscular dystrophy
  - Aneuploidy
    - e.g. Trisomies 21, 18, 13, triploidy



Down Syndrome

47,XY,+21



Basic Mendelian Genetics

- Basic Genetic Concepts
  - Mendelian Genetics
    - Five Crucial Mendelian Concepts
      - It shouldn't matter where (which parent) DNA comes from.
      - DNA is copied faithfully at each round of mitosis/meiosis (no errors);
      - Germline DNA (gametic) is reflective of non-germline DNA (somatic) DNA;
      - All cells in the body have identical DNA.
      - Nuclear (chromosomal) DNA is all the genetic material in a cell.

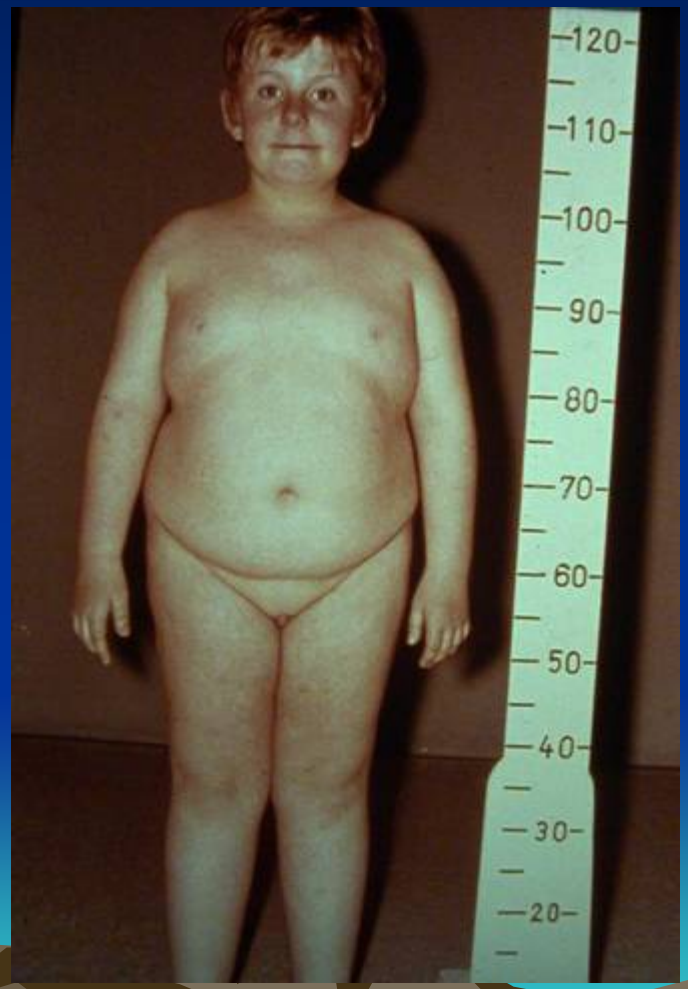
Genomic Imprinting

- Genomic Imprinting
  - An epigenetic process (no permanent DNA change)
  - Involves DNA methylation/histone modification
  - Achieves monoallelic expression without change in DNA and is reversible in opposite sex meiosis
  - Accomplished in germline (all somatic cells affected)
  - Affects ~1% of all therian mammalian genes
  - Many diseases result from imprinting

Genomic Imprinting

- Prader-Willi/Angelman syndromes
  - Both map to same site on 15q11-13
  - Both relates to small deletion of DNA in this region
  - Completely different conditions
    - Prader-Willi Syndrome (deletion from father)
      - Hypotonia, obesity, hypogonadism, MR
    - Angelman syndrome (deletion from mother)
      - Epilepsy, tremors, smiling (“Happy Puppet syndrome”)
- Imprinting results from differential methylation of certain genes in meiosis; different genes are methylated (controlled) differently in male meiosis vs female meiosis

Genomic Imprinting
Prader-Willi Syndrome

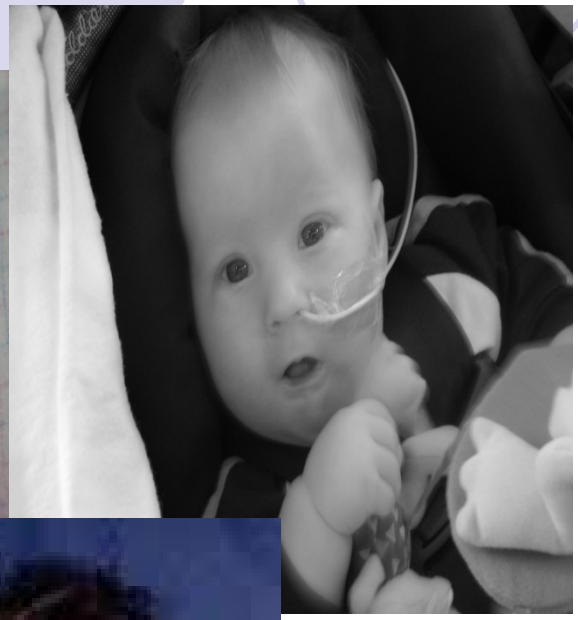


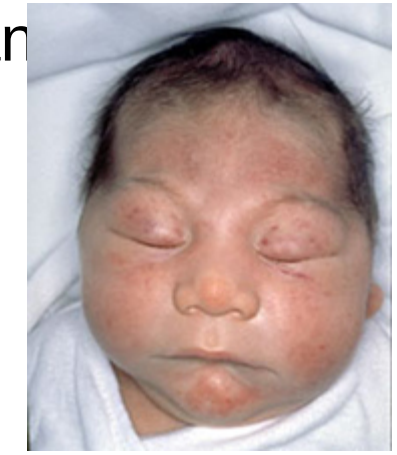
Genomic Imprinting
Angelman Syndrome

- Angelman Syndrome

Disease-Specific Examples

- DiGeorge (22q11 del)
- Cri-Du-Chat syndrome
- Miller-Dieker syndrome
  - Lissencephaly
- Wolf-Hirshhorn
- Williams syndrome
- Prader-Willi/Angelman
- Smith-Magenis syndrome
- X-linked Ichthyosis/Kallman



MICRODELETION ANALYSES

It is standard of care to diagnosis microdeletion syndromes using FISH (fluorescence in situ hybridization).

- **Angelman syndrome** (AS) is a genetic disorder associated with mental retardation. AS is not usually suspected in the first year of life but becomes a consideration between 1-4 years of age. The syndrome is characterized by severe developmental delay, absence of speech, ataxic gait, inappropriate laughter, hand-flapping, and less frequently, microcephaly and seizures.

- **Cri-du-chat** is a genetic disorder characterized by dysmorphic facial features, microcephaly, growth deficiency, mental retardation, speech delay and a characteristic "cat-like" cry.

- **DiGeorge/Velocardiofacial syndrome** analysis tests for DiGeorge and Velocardiofacial syndromes, as both are due to 22q11 deletions. DiGeorge syndrome is a genetic disorder characterized by hypocalcemia, congenital heart defects, cleft lip and/or palate, microcephaly and mild mental retardation. Velocardiofacial syndrome is characterized by facial abnormalities, congenital heart defects, diminished muscle tone, mild small stature, psychomotor retardation and/or learning disabilities.

- **Kallman syndrome** is a genetic disorder that involves the hypothalamus, causing a hormone deficiency. Symptoms of Kallman syndrome are: failure to enter puberty, lack of sexual drive, infertility (non-ovulation in women and azoospermia/oligospermia in men) and no sense of smell.

- **Miller-Dieker syndrome** is a genetic disorder characterized by lissencephaly ("smooth brain"), mental retardation and a distinct facial appearance.

- **Prader-Willi syndrome** (PWS) is a genetic disorder associated with mental retardation. Diagnostic criteria for PWS are different for children under 3 years of age compared with older individuals. Major diagnostic criteria include: hypotonia, failure to thrive, rapid weight gain/obesity between 12 months and 6 years, characteristic facial features, hypogonadism and mild to moderate mental retardation.

- **Smith-Magenis syndrome** is a genetic disorder characterized by flattened mid-face, down-turned mouth, hypotonia, short, broad hands, mental retardation, chronic sleep disturbance and self-injurious behavior.

- **Steroid sulfatase deficiency (X-linked Ichthyosis)** is a genetic disorder that causes an X-linked form of Ichthyosis. Ichthyosis has a variable presentation characterized by dry, scaly skin, sparse hair and conical teeth in affected males.

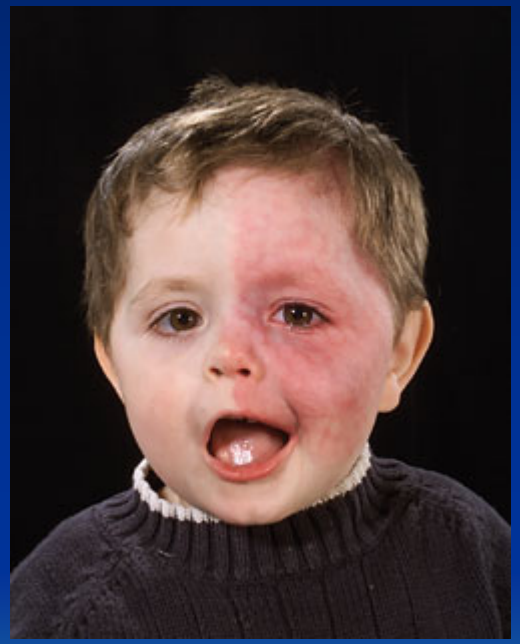
- **Williams syndrome** is a genetic disorder characterized by "elfin" facial features, mental retardation, growth deficiency, hypercalcemia and cardiovascular disease.

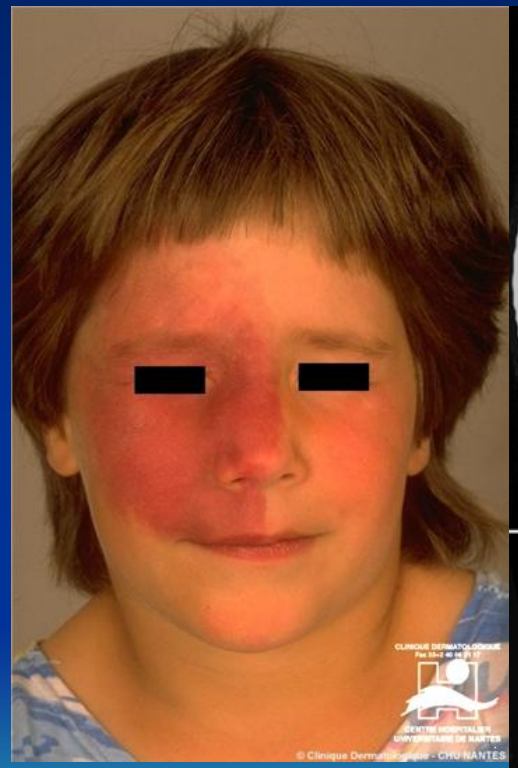
- **Wolf-Hirschhorn syndrome** is a genetic disorder characterized by microcephaly, growth deficiency, mental retardation and characteristic facial features.

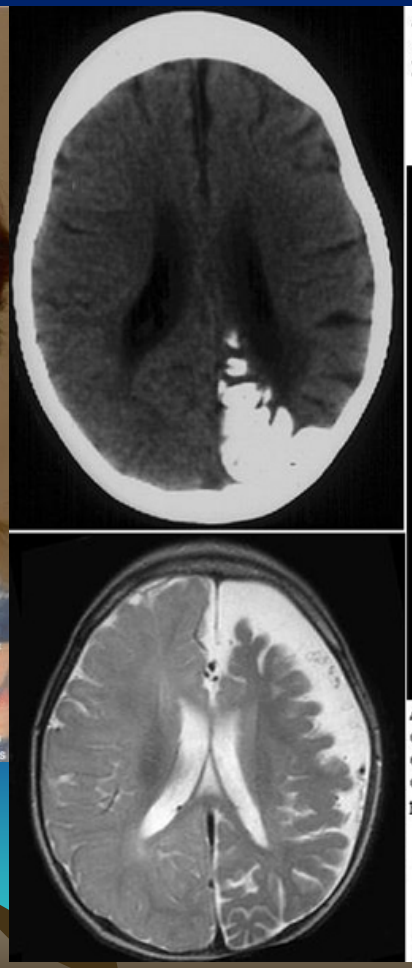
Somatic Mosaicism

- Somatic mosaicism is common event
  - The number of cells in the human body greatly exceeds mutation rate for all disorders (30,000,000,000,000 + cells in the adult!)
  - Mosaicism can be chromosomal, involve single genes, or involve extrachromosomal DNA
  - Phenotype related to
    - Type of mutation and gene involved
      - Missense mutation in regulatory gene vs deletion in structural gene
    - Tissue, cell lineage and cell type involved
      - Proliferation/metastasis in glandular cell vs predestined apoptotic cell
      - **ALL CANCERS (+/-) RESULTS FROM SOMATIC MUTATION EVENTS**
    - When in development somatic mutation occurs
    - Streaky, patchy or otherwise assymetric phenotypes

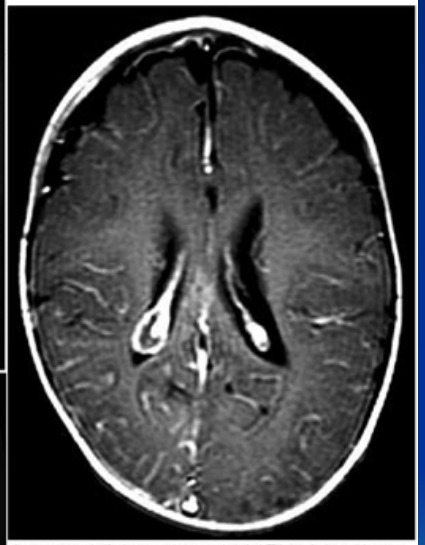
Somatic Mosaicism
Sturge-Weber Syndrome







An axial computed tomographic image revealing abnormal calcifications of the left occipital lobe in a 9 year old male patient.

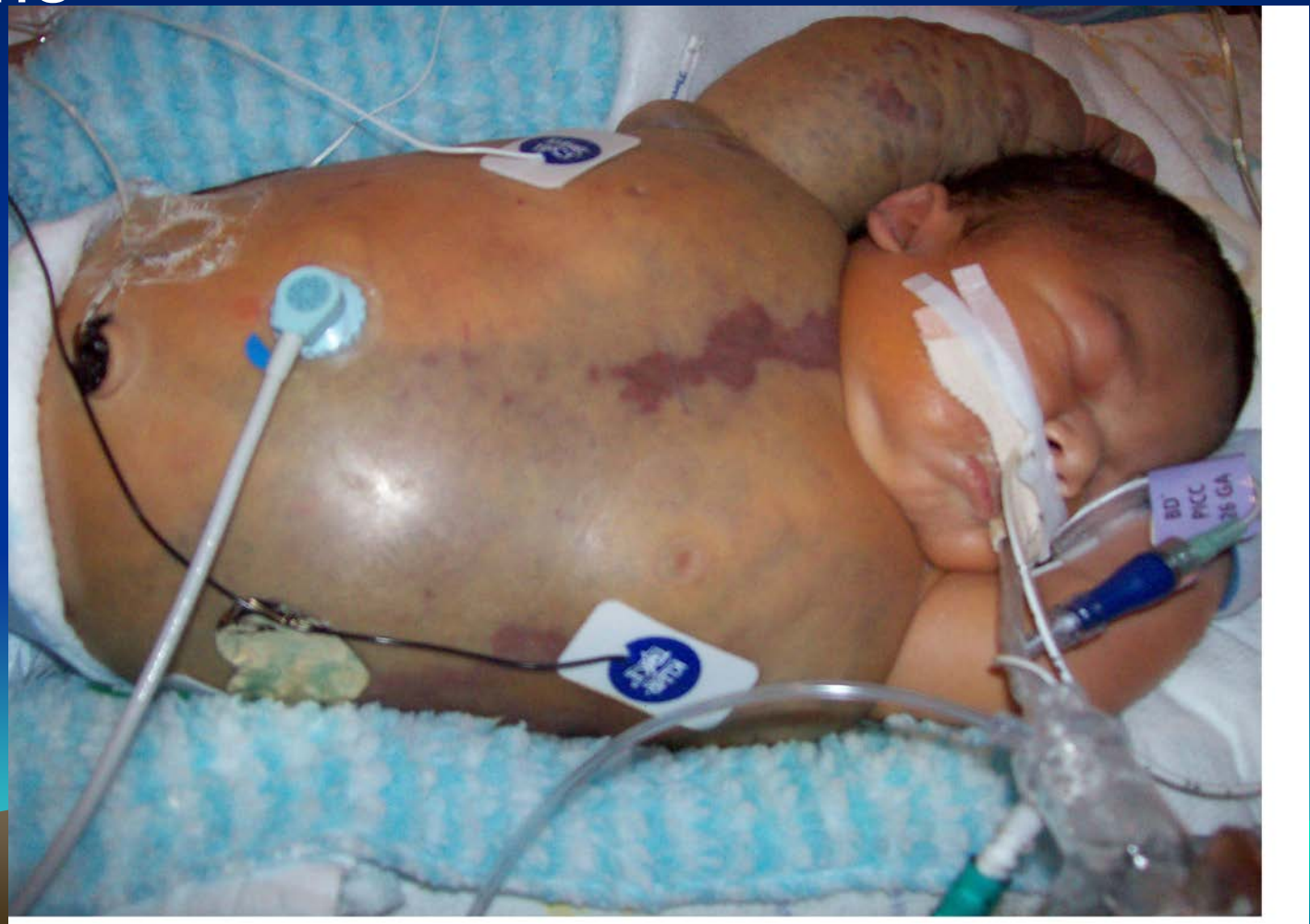


A post gadolinium-enhanced axial T1 weighted image demonstrating prominent leptomeningeal enhancement over the right occipital lobe of a 7 month old male patient. The choriod plexus is enlarged in the posterior horn of the right lateral ventricle.

An axial T2 weighted MRI demonstrating left lateral frontal lobe atrophy in an 11 month old male patient.

Somatic Mosaicism
KTW Syndrome

- KTW syndrome

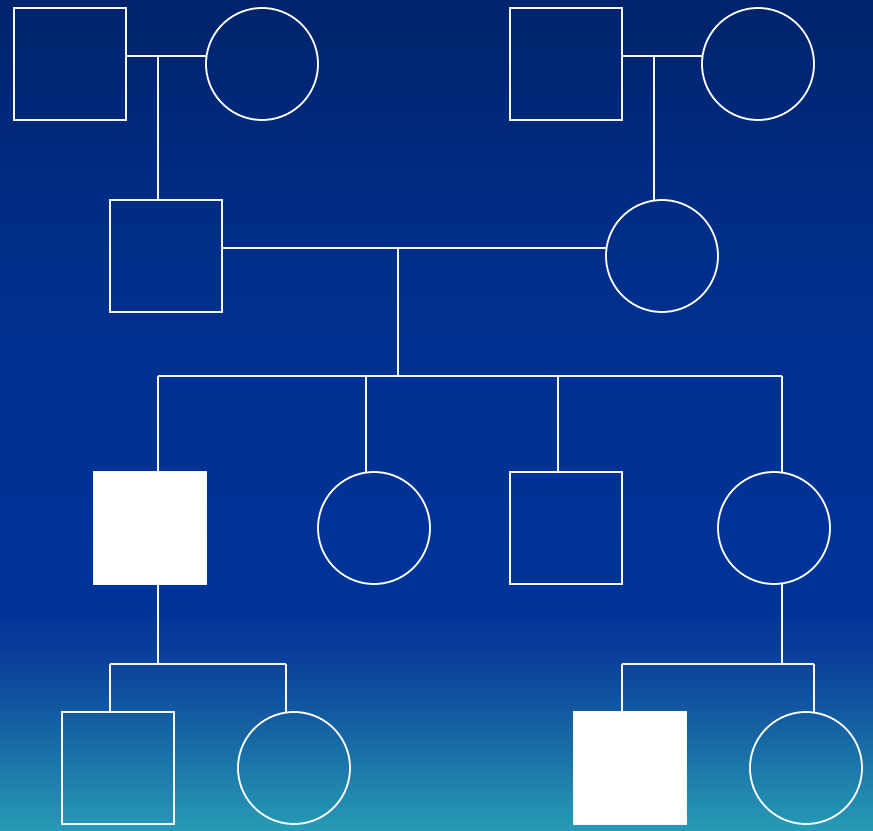


Mitochondrial Inheritance

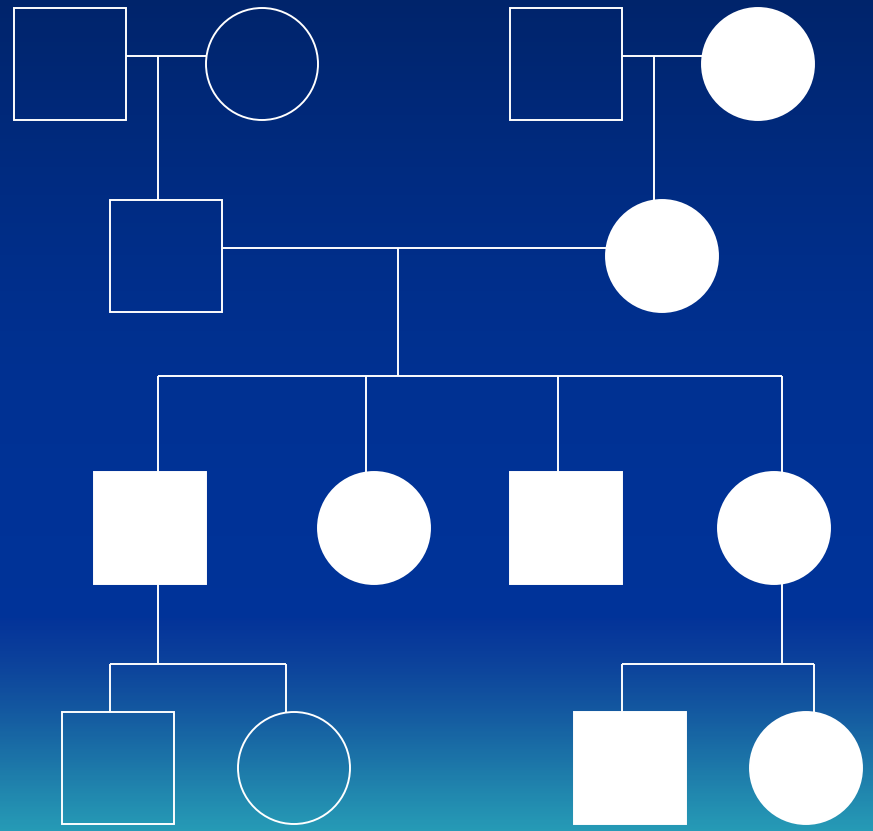
- Mitochondria
  - Cellular organelle important in oxidative phosphorylation and energy production in eukaryotic cells
  - Separate evolutionary origin (bacterial symbiosis?)
  - Each mitochondrion contains 2-10 DNA “chromosomes”
  - Each mt genome has 10-15,000 bp (vs 3 billion bp)
  - 37 genes (13 proteins, 22 tRNA genes)
  - Majority of mt proteins are imported into the mitochondria from the cell

Modes of inheritance

X-linked recessive



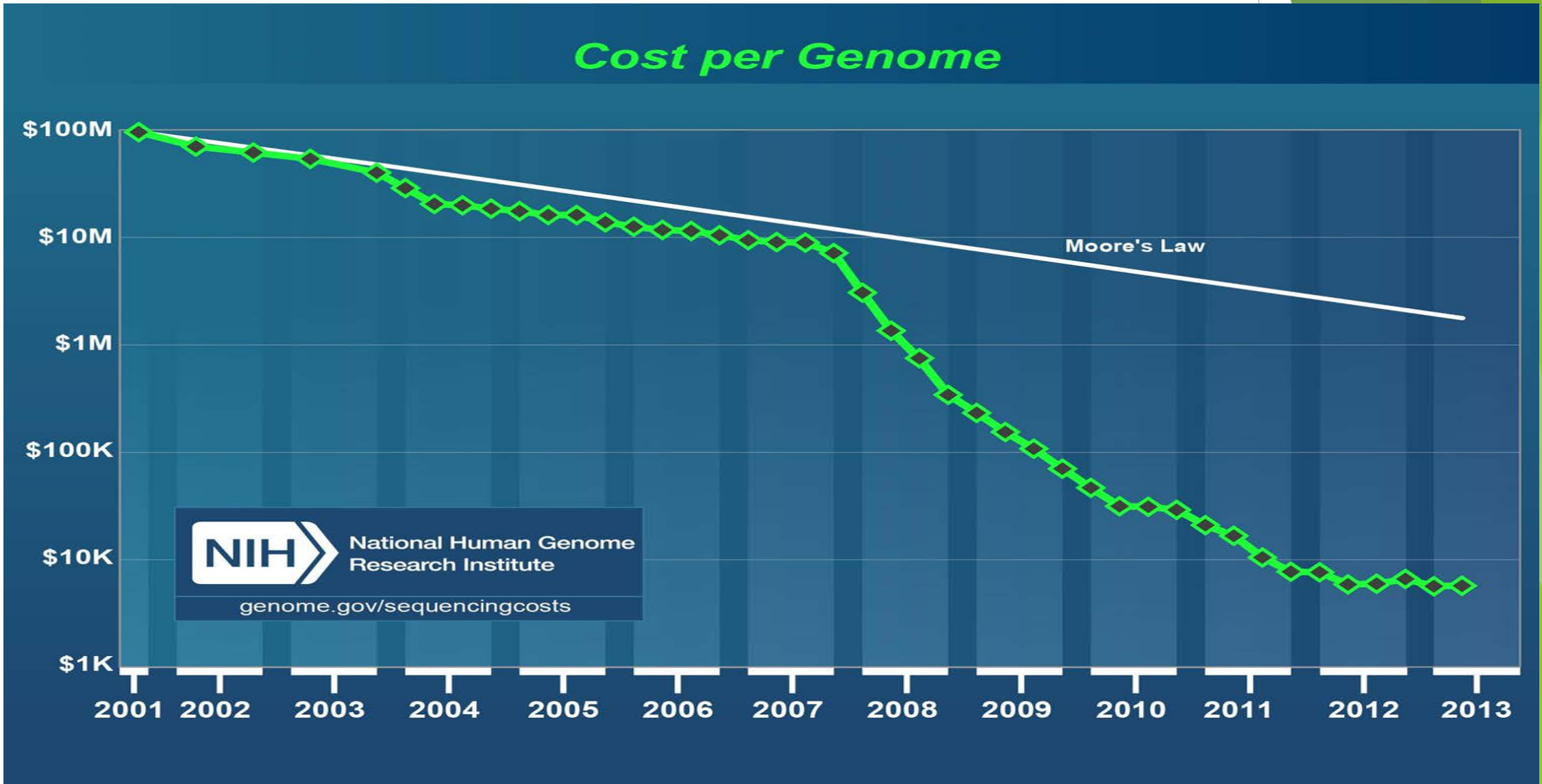
Mitochondrial



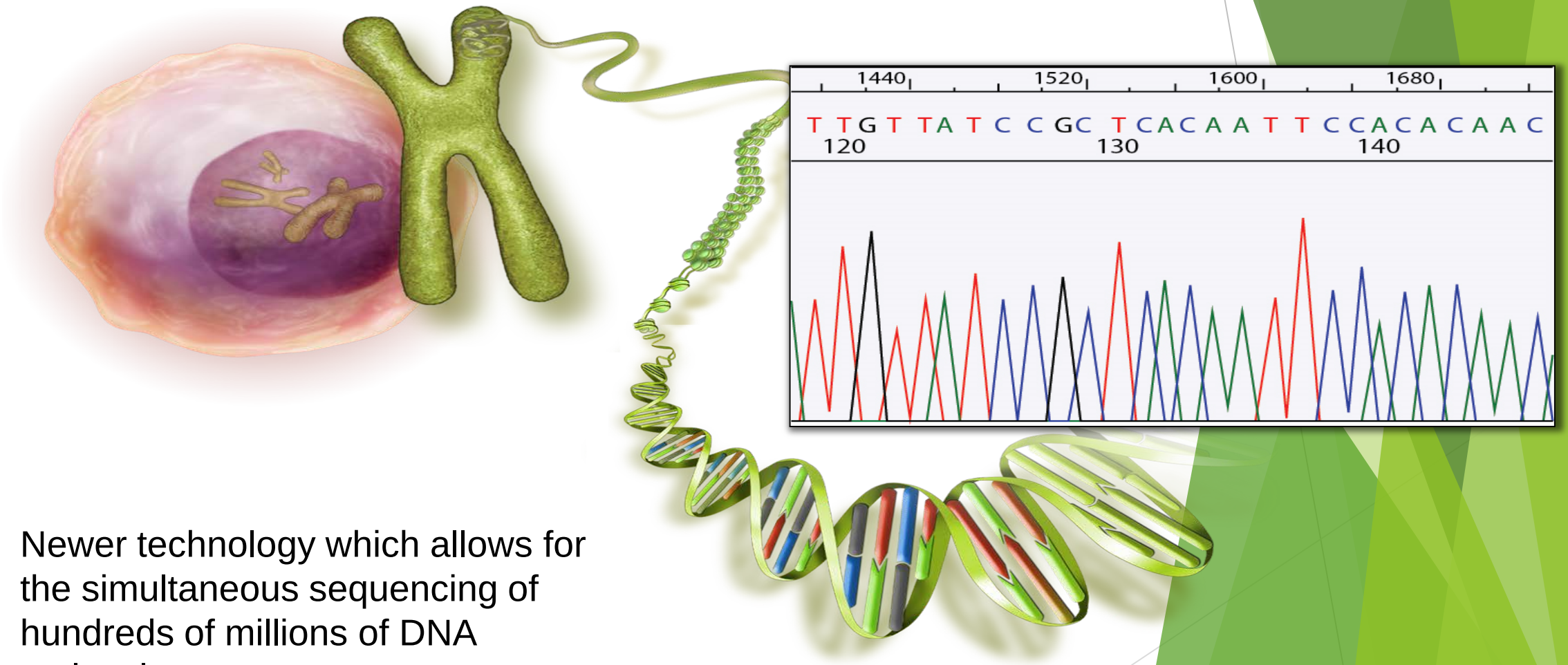
Medical Genetics
New Technology

- The practice of Medical Genetics is dependent today on sequencing technologies
  - Cost
  - Accuracy
  - Clinical suspicion
  - Management and surveillance
- Claims and reality can be very different!

Next Generation DNA sequencing



Next-Generation Sequencing (NGS)



- Newer technology which allows for the simultaneous sequencing of hundreds of millions of DNA molecules at once.

Next Generation DNA sequencing

1. Library preparation

Sequencing library is prepared by random fragmentation of DNA or cDNA sample, followed by 5' and 3' adapter ligation. This greatly increases the efficiency of the preparation and later amplification process. Adapter ligated fragments are then PCR amplified and gel purified

2. Cluster generation

Library is loaded into a flow cell where fragments are captured on a lawn of surface-bound or microsphere-bound oligos complementary to library adapters. Fragments are then amplified into distinct, clonal clusters through (bridge) amplification.

3. Sequencing

Sequencing by synthesis uses a terminator based method that detects single bases as they are incorporated into DNA template strands. As all 4 dNTP's are present in the reaction mix during the sequencing cycle, error rates are minimized resulting in highly accurate base-by-base data with minimal sequence context errors, even in areas of repetition and homopolymers.

4. Data analysis

Sequences can't be "read" and analyzed until fragment data is aligned to a reference genome . Depth of coverage determines quality of data and "meaningfulness" of the generated sequence.

AT EACH STEP ERRORS CAN OCCUR AFFECTING DNA SEQUENCE'S CLINICAL UTILITY!!!

Next Generation DNA sequencing

- Illumina sequencing by synthesis cartoon
- https://www.youtube.com/watch?v=womKfikWlxM

The Problem Today:

- **“MISREPRESENTATION OF A MISUNDERSTOOD TECHNOLOGY TO THE MISINFORMED”**

  Where is the value in DNA testing (and archiving)?

  Why do DNA sequencing companies exist?

Monday December 07, 2015 *Dna Kit Predicts Health Issues*