Brought to you by

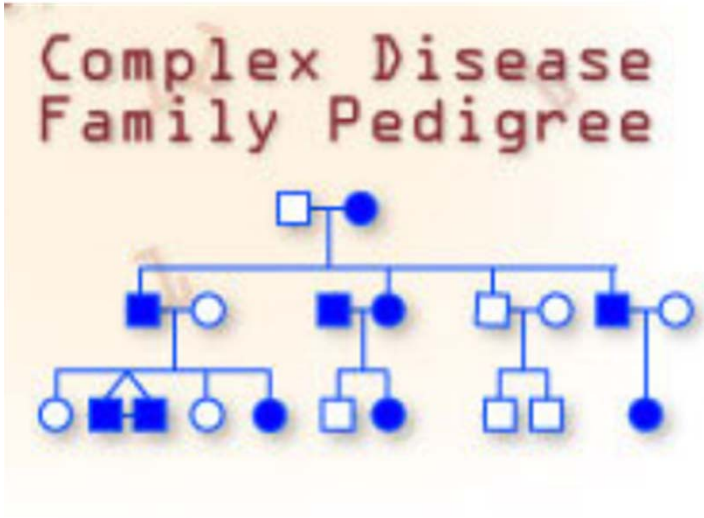
AMERICAN SOCIETY OF Cytopathology FOUNDATION

American Society of Cytopathology Core Curriculum in Molecular Biology

Copyright 2010 American Society of Cytopathology

Brought to you by

AMERICAN SOCIETY OF Cytopathology FOUNDATION



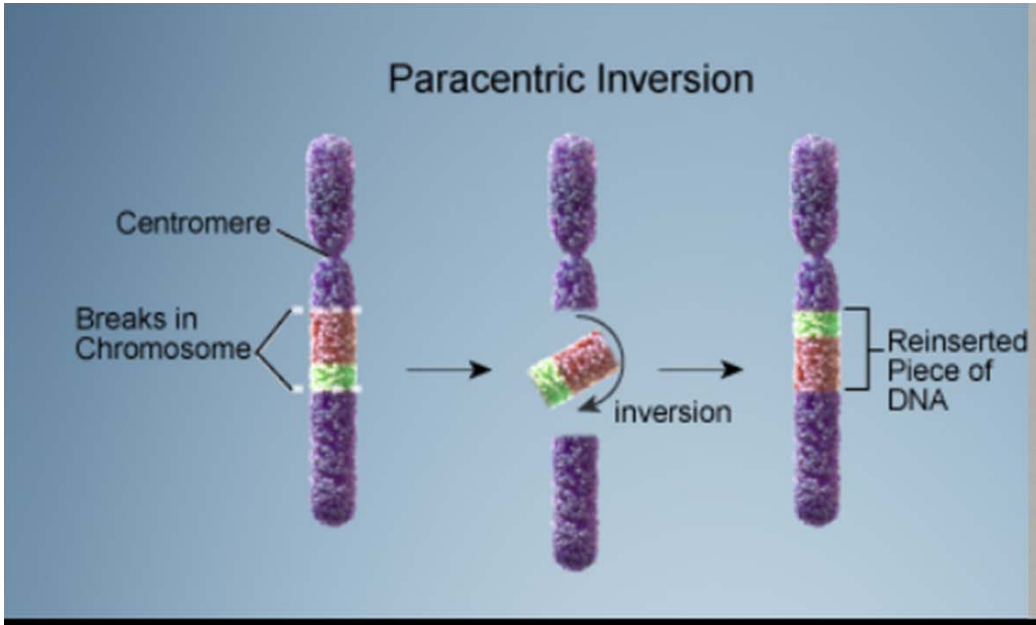
American Society of Cytopathology Core Curriculum in Molecular Biology

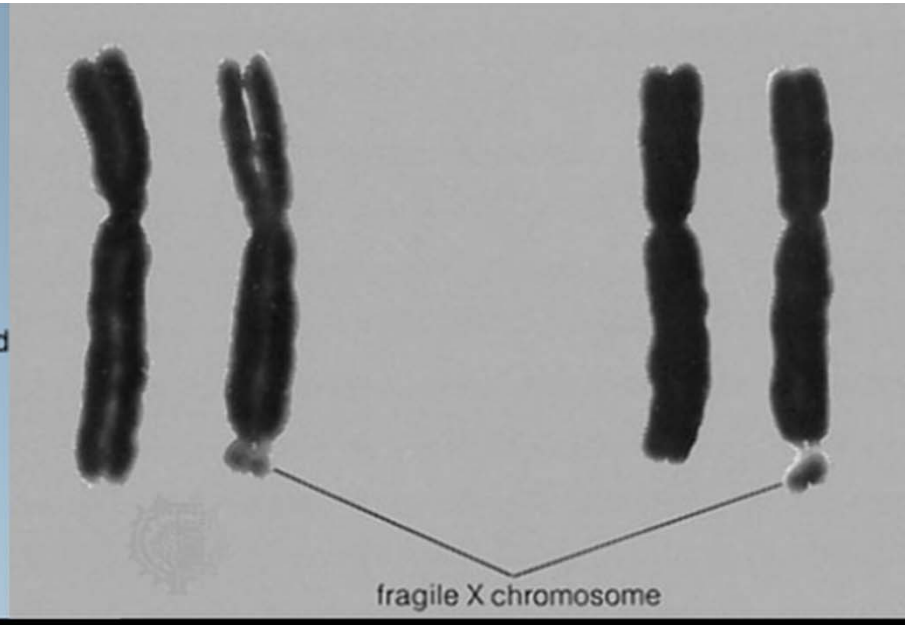
Chapter 5

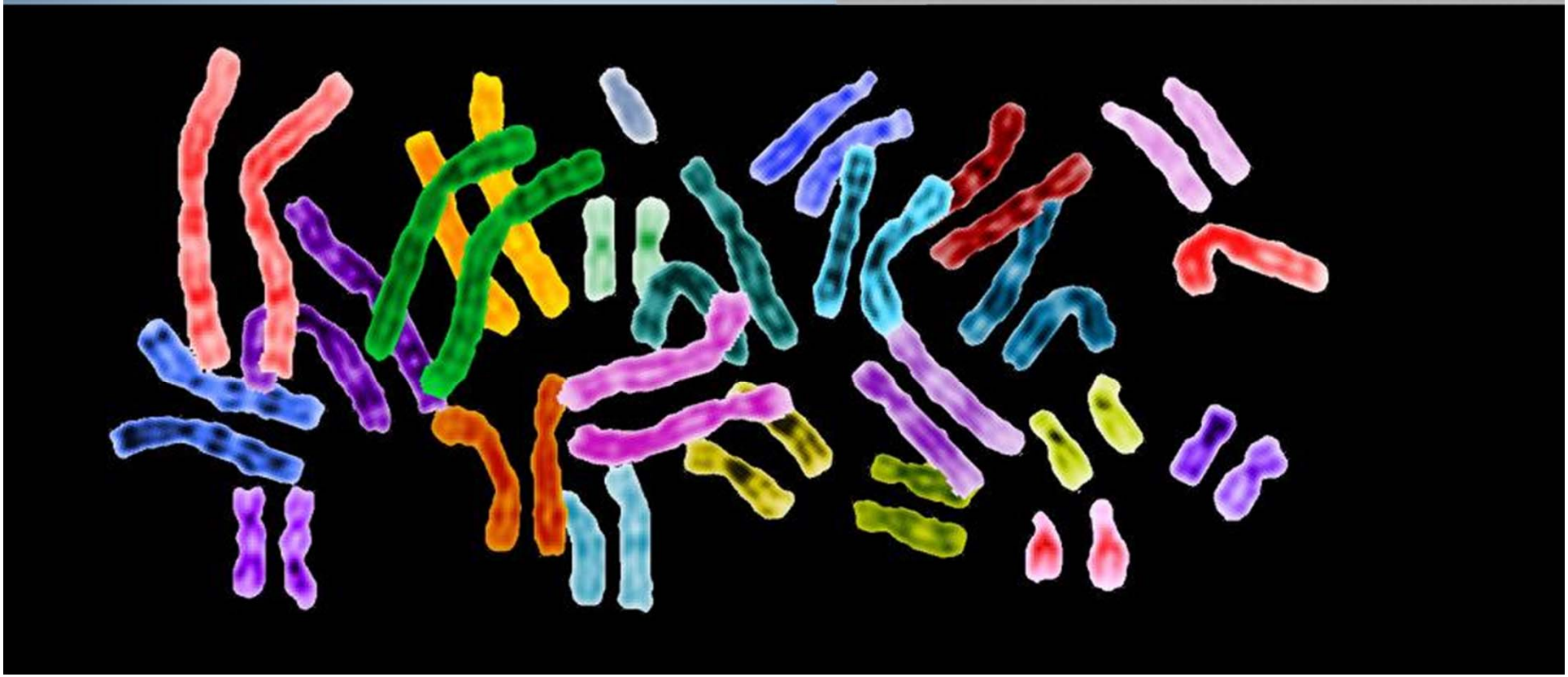
Applications of Molecular Testing
Molecular Testing for Genetic Diseases

Marilee Means, PhD, SCT(ASCP)
University of Kansas Medical Center
Kansas City, Kansas

Copyright 2010 American Society of Cytopathology


Paracentric Inversion
Centromere
Breaks in Chromosome
inversion
Reinserted Piece of DNA


fragile X chromosome



The dance of the chromosomes…

Although most times, the chromosomes accurately transmit their genetic material to the next generation of cells, sometimes a genetic defect, an inversion of a critical gene, or other genetic abnormality is passed on to the daughter cells. While these may have no effect at all, other times this leads to serious genetic diseases. Molecular methods are well suited to detect these abnormalities.

Copyright 2010 American Society of Cytopathology

Objectives

- Describe the three main patterns of mendelian inheritance
- Discuss laboratory methods to detect common single-gene disorders
- Discuss non-mendelian inheritance
- Discuss how genomic imprinting can affect disease phenotype

Copyright 2010 American Society of Cytopathology

Inheritable Diseases

- Mutations range from single nucleotide changes to abnormal number of copies of chromosomes.
- Some of these may lead to disease and others may have no effect on the patient
- Germ cell mutation results in inherited disease
- Somatic cell mutation results in cancer or congenital malformations

Copyright 2010 American Society of Cytopathology

Epigenetic Changes

- Do not affect the primary DNA sequence
- DNA methylation– stops RNA transcription
- Genomic imprinting-inactivates regions of chromosome (such as X chromosome)
- Chromatin remodeling- protein binding and histone modification
- Turns “on” or “off” certain genes but does not alter their order

Copyright 2010 American Society of Cytopathology

Mouse Epigenetics

Agouti yellow, mottled and brown mice all have the same gene with different levels of DNA methylation. Mice with unmethylated DNA express yellow coloring, ones with highly methylated DNA are brown, and ones with variable levels of methylation are mottled. High levels of methylation "turn off" the agouti gene so the yellow coloring disappears in the mouse.

http://epigenome.eu/media/images/large/1067.jpg

Copyright 2010 American Society of Cytopathology

Chromosomal Abnormalities

- Detected by:
  - Karyotyping
  - Flow cytometry (ploidy studies)
  - FISH (fluorescent in situ hybridization)
- **Aneuploidy** (gain or loss of any autosome) is caused by chromosomal non-disjunction
- **Triploidy/monoploidy** (3 or one copy of a chromosome rather than 2)

Copyright 2010 American Society of Cytopathology

Autosomal Triploidy/Monoploidy

- Caused from fertilization of gamete with an extra or missing chromosome
- Usually incompatible with life
- Sex chromosome aneuploidy more frequently tolerated (compatible with life) but creates phenotypical abnormalities

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Types of Abnormalities

- **Mosaicism** – 2 or more genetically distinct populations of cells from one zygote in an individual
- **Chimerism** – 2 or more genetically distinct populations from different zygotes in an individual
- Derived from mutation events in germ or somatic cells

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Chromosomal Terminology

- 22 pairs of somatic chromosomes, plus the X and Y sex chromosomes for 23 pairs
- Sorted by size and labeled from 1-22, X and Y
- Banding patterns after staining by Giemsa results in the following subclassifications:
- p arm is the shorter segment from the centromere outward, q is the longer
- By convention, p arm is always orientated "upward" in karyotypes

Copyright 2010 American Society of Cytopathology

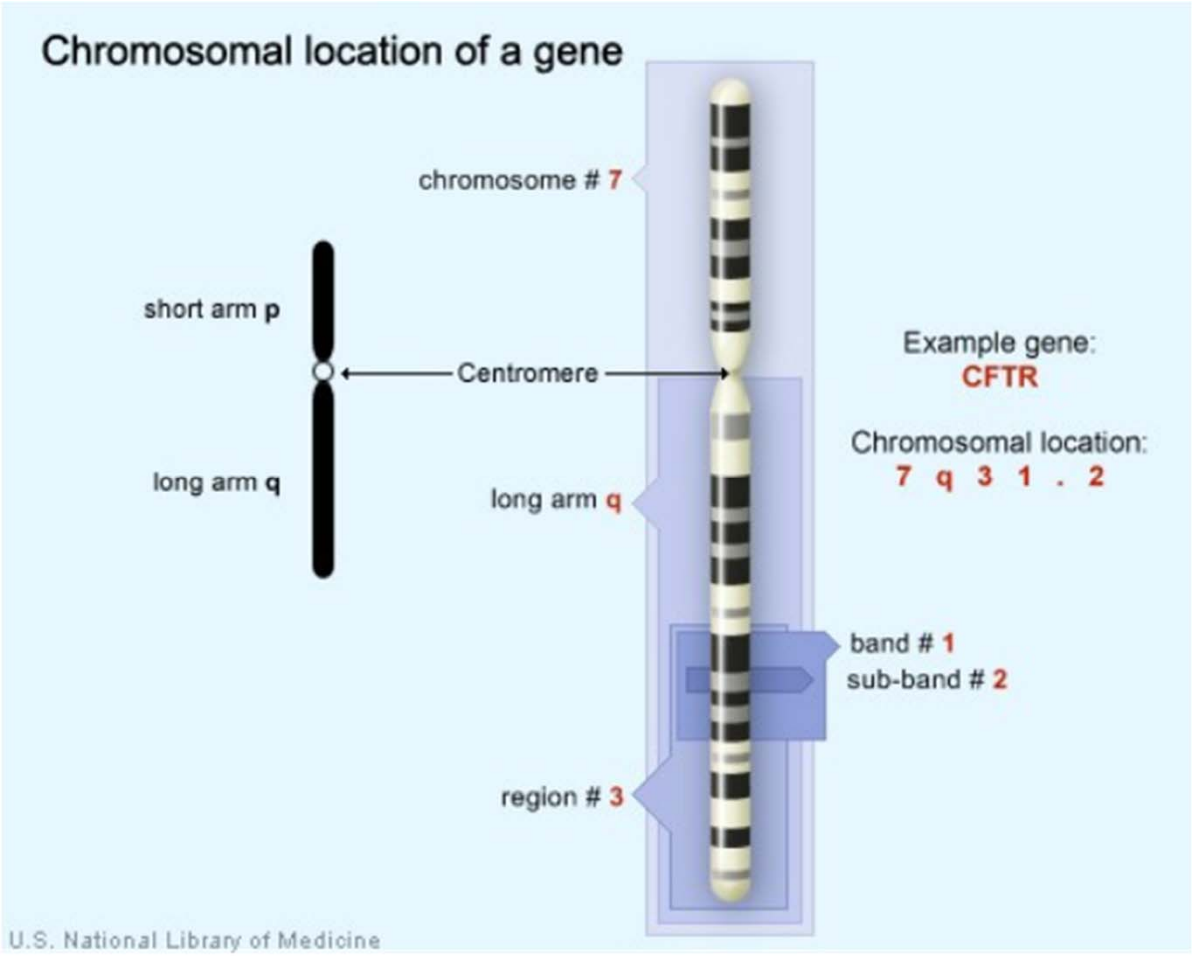
Chromosomal Terminology

- The next subdivision is the region; the one closest to the centromere is region 1
- The next subdivision is the band, and then the subband, again labeled outward from the centromere
- Thus, a site on the long (q) arm of chromosome 7 would be termed 7q31.2 indicating region 3, band 1, and subband 2 of the long arm of chromosome 7

Copyright 2010 American Society of Cytopathology

Location of CFTR gene on chromosome 7: 7q31.2

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION



Copyright 2010 American Society of Cytopathology

More Terminology

- When indicating a normal human karyotype, the terminology would be 46, XY (male) or 46,XX (female)
- A male with a trisomy 21 would be indicated as 47,XY +21
- A male with a deletion of short arm of chromosome 5 would be written 46,XY,(del5p)
- Absence of second X in a Turner's patient would be indicated as 45,X
- Translocations are written as t(8;12)(q21,q31)

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Other terminology

- inv = inversion, the breakage and reattachment of a length of chromosome "upside down" to its normal arrangement
- ins = insertion of a section of another chromosome into a section of the original chromosome
- der = derivative chromosome in which there is more than one rearrangement in one chromosome or rearrangements between two or more chromosomes

Copyright 2010 American Society of Cytopathology

Examples of Genome Mutations Sex Chromosomes

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- Mosaicism somewhat common in sex chromosomes (for example: 45,X/47,XXX)
- Klinefelter's – 47,XXY
- XYY syndrome – 47,XYY
- Turner's syndrome – 45,X and variants
- Multi X females – 47,XXX; 48,XXXX

Copyright 2010 American Society of Cytopathology

Examples of Genome Mutations Trisomy and Deletions

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Down's syndrome –Trisomy 21, 47,XY,+21
Edward's syndrome-Trisomy 18, 47,XY,+18
Patau's syndrome- Trisomy 13,47,XY,+13
DiGeorge's syndrome - del(22q)
Cri du chat syndrome – del(5p)
Contiguous gene syndrome – del(11p)

Copyright 2010 American Society of Cytopathology

Patterns of Inheritance– Single Gene Disorders

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- Transmission pattern – determined by family history
- **Pedigree** – diagram of inheritance pattern
- **3 main types of patterns:**
  - Autosomal-dominant
  - Autosomal-recessive
  - X-linked or sex-linked recessive

Copyright 2010 American Society of Cytopathology

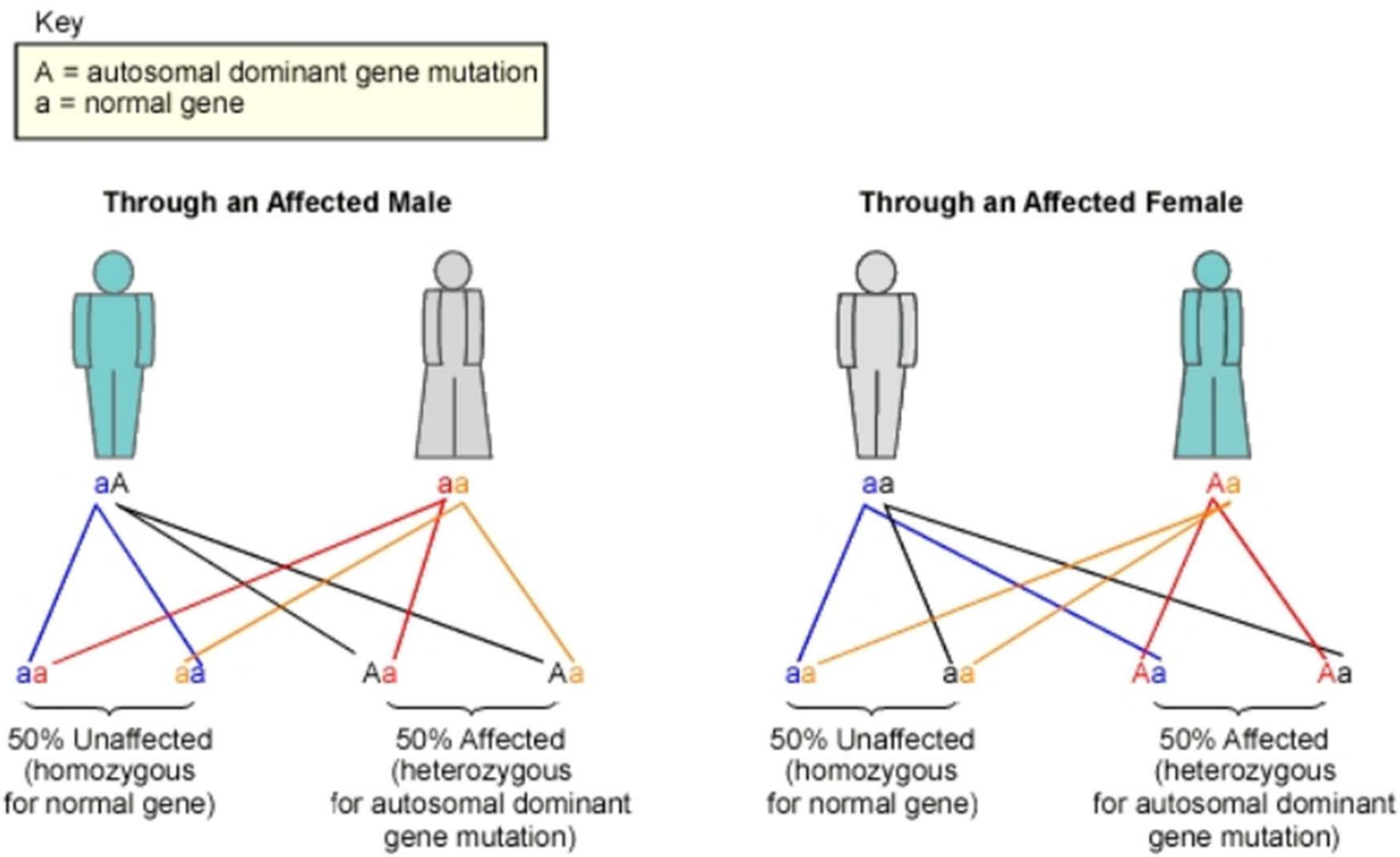
Autosomal-dominant pattern

- **Autosomal-dominant transmission**: heterozygous individuals express trait
- **Loss of function** mutation affects protein which may interfere with normal protein function produced by normal chromosome resulting in a autosomal-dominant pattern
- **Gain of function** mutation result from new allele having new properties which result in inappropriate site or time for gene products

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Autosomal Dominant Pattern



www.ncbi.nlm.nih.gov/bookshelf/picrender.fcgi...

Copyright 2010 American Society of Cytopathology

Autosomal-recessive pattern

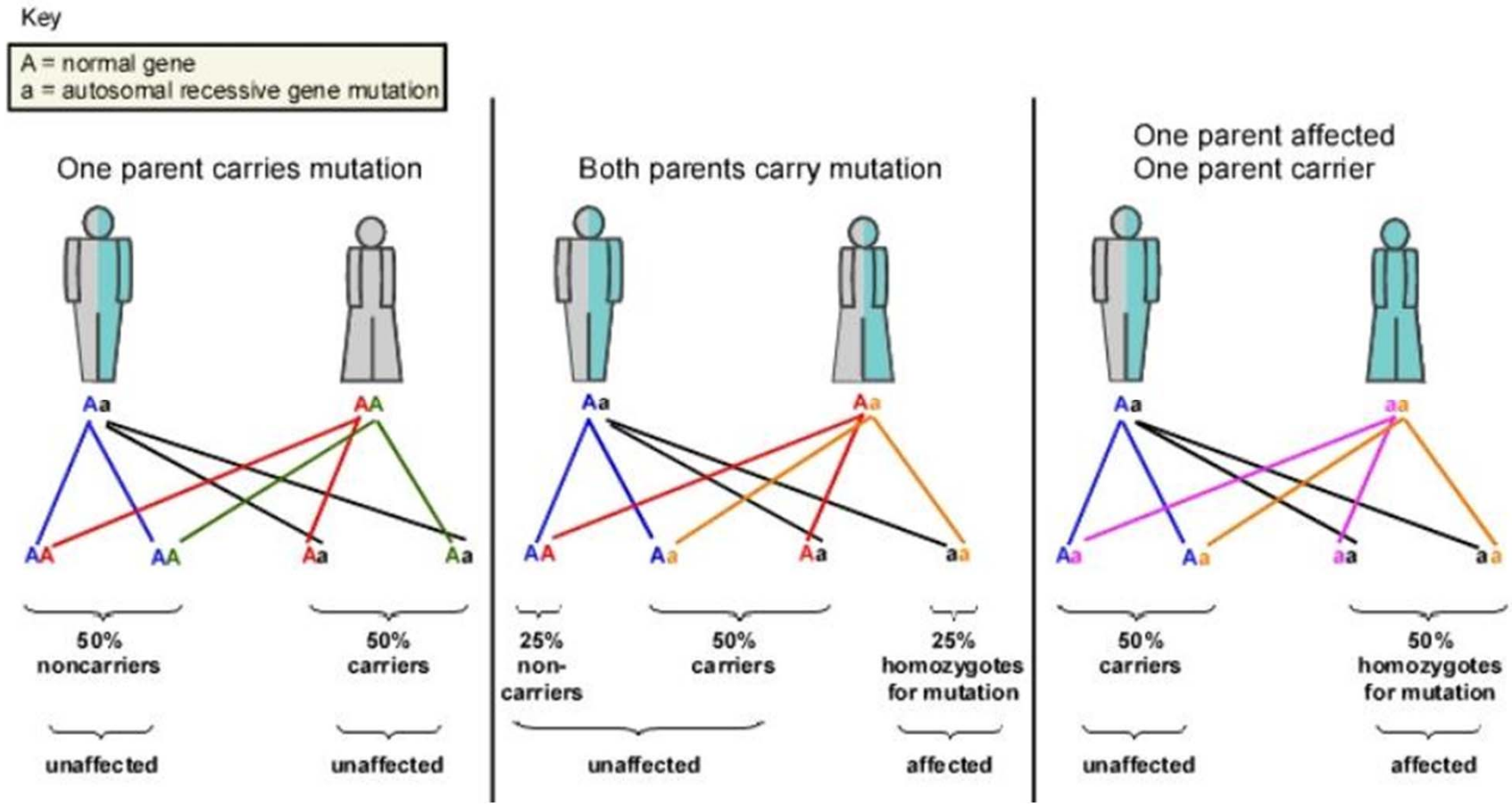
- **Autosomal-recessive transmission**: to express trait, must be homozygous or lose one normal allele expression
- Largest category of mendelian disorders
- Recessive mutation is present in both alleles (homozygous) OR
- Normal allele is lost (loss of heterozygosity)
- Tumor suppressor gene mutations (risk factors for neoplasia) are autosomal-recessive pattern

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Autosomal Recessive Pattern

Odds of transmitting to offspring



www.ncbi.nlm.nih.gov/bookshelf/picrender.fcgi...

Copyright 2010 American Society of Cytopathology

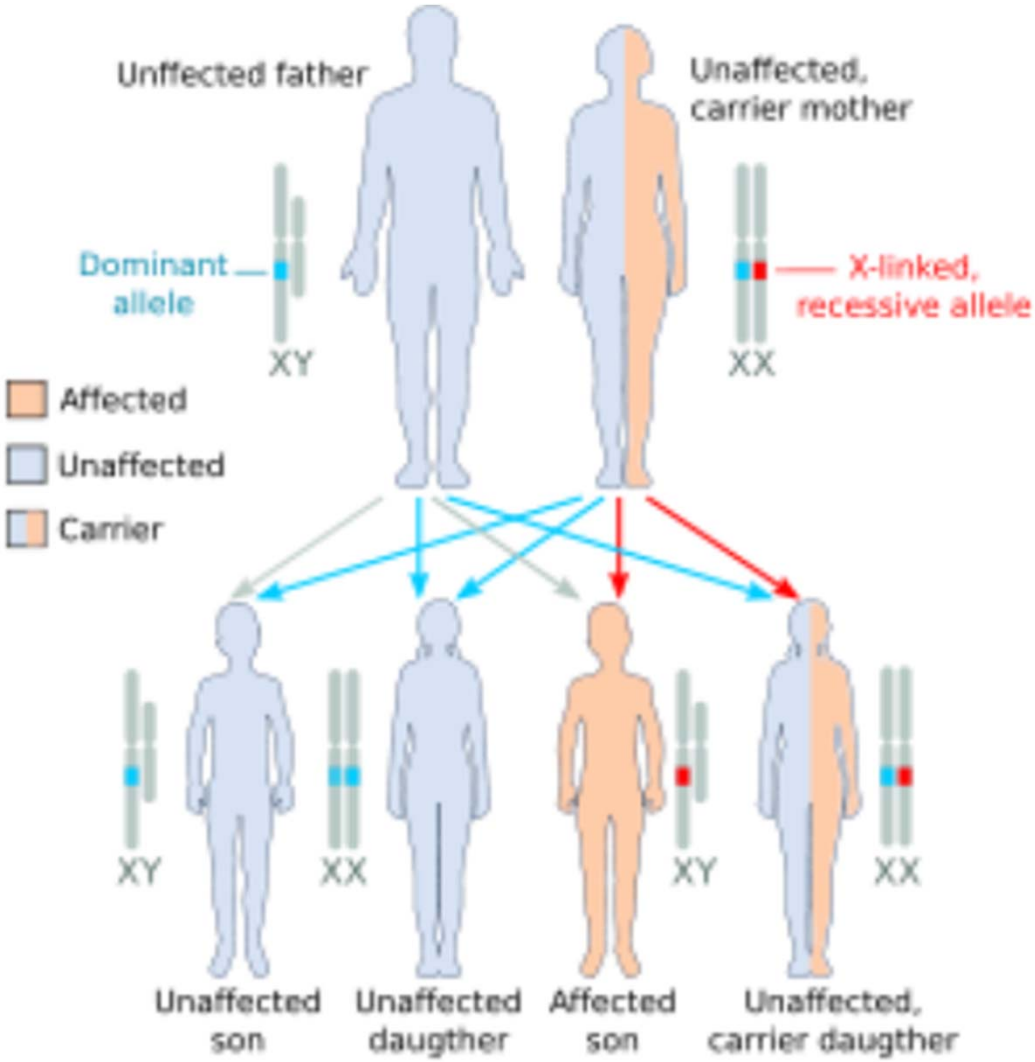
Sex-linked (X-linked) disorders

- Usually recessive
- X chromosome inactivated in females, but is reversible so normal allele can be available so that trait is not expressed
- Males have only one copy of X, thus are **hemizygous**
- Thus, males are more likely to manifest trait
- Trait carried by females but expressed usually in males

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

X-linked recessive pattern



http://en.wikipedia.org/wiki/Sex_linkage

Copyright 2010 American Society of Cytopathology

Penetrance

- Frequency of expression of disease phenotype in individuals with a mutation
- **Complete penetrance:** expression of trait in every individual with mutated gene
- **Variable expressivity:** a range of phenotypes in individuals with the same gene mutation

Copyright 2010 American Society of Cytopathology

Examples of single-gene disorders

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

Protein Example Gene location Molecular Method

Structural Sickle cell anemia (11p15.5) Sequencing, PCR-RFLP

Cell growth Neurofibromatosis (17q11.2) Sequencing, linkage regulators analysis

Cell growth Li-Fraumeni (17p13) Sequencing, SSCP regulators syndrome p53 TSG DGGE

Copyright 2010 American Society of Cytopathology

Lysosomal Storage Diseases

- Storage disorder diseases result from incompletely digested macromolecules due to loss of enzyme function
- Tay-Sachs
- Niemann-Pick disease
- Gaucher's disease
- Screen by measuring ability of serum enzymes to digest test substrates
- Mutations detected by direct sequencing

Copyright 2010 American Society of Cytopathology

Molecular Diagnosis of Single-Gene Disorders

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- **Factor V Leiden** – hypercoagulable phenotype – F5 (1q23)
- Heterozygous form in 4% - 7% of population
- Homozygous in 0.06% - 0.25%
- PCR-RFLP, SSP-PCR, Invader
- F2 prothrombin gene may also occur independently and leads to increased risk
- Multiplex PCR-RFLP can test for both mutations
- Gel electrophoresis banding patterns show the normal or mutant genotypes

Copyright 2010 American Society of Cytopathology

Hemochromatosis

- Autosomal-recessive pattern – over absorption of iron, causing damage to pancreas, liver, heart, etc.
- Dysfunction of HFE gene or HLA-H (6p21.3) causes loss of function of protein that binds with cells in intestine leading to over absorption of iron from food
- Usual mutation is C282Y, detectable with PCR-RFLP

Copyright 2010 American Society of Cytopathology

Cystic Fibrosis

- Autosomal-recessive – severe lung damage and nutritional deficiencies
- Affects secretions to make them sticky and thick (mucus, sweat, saliva, digestive juices)
- Loss of function of CF transmembrane conductance regulator CFTR gene (7q31.2). F508del is one of many mutations associated with the disease.

Copyright 2010 American Society of Cytopathology

Methods for CF detection

- RFLP, PCR-RFLP, heteroduplex analysis, temporal temperature gradient gel electrophoresis, SSCP, SSP-PCR, Invader, bead array technology, and direct sequencing.
- Early diagnosis and prenatal counseling aided by molecular testing.

Copyright 2010 American Society of Cytopathology

Single-Gene Disorders with Nonclassical Inheritance Patterns

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- Types of non-classical patterns
  - **Mitochondrial mutations** – maternal inheritance
  - **Genomic imprinting** – specific expression of genes in different cells and tissues
  - **Gonadal mosaicism** – new mutations in germ line cells; gives rise to eggs or sperm carrying the mutation; suspected if phenotypically normal parents have an autosomal-dominant phenotype in a child

Copyright 2010 American Society of Cytopathology

Mutations in Mitochondrial Genes

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- Mitochondria contain their own circular DNA, inherited maternally
- Mutations affect energy production and affect organs that require high levels of energy, such as muscle and nervous system.
- Detection by Southern blot for large deletions, point mutations by PCR-RFLP

Copyright 2010 American Society of Cytopathology

Trinucleotide Repeat Expansion Disorders

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- Triplet repeats are short tandem repeats (STRs) with 3 bp repeating units
- These can contract or expand in length during DNA replication
- Fragile X and Huntington's disease are two examples

Copyright 2010 American Society of Cytopathology

Fragile X

- CGG expansion in noncoding region close to the fragile X mental retardation gene, FMR-1
- Results in methylation of the region and shuts down FMR-1
- Normal repeats are 5-55, premutations (carrier) are 56-200, and full mutations (affected) are 200 - >2000 repeats of CGG

Copyright 2010 American Society of Cytopathology

Fragile X

- Symptoms increase in severity with each generation in a family
- Karyotyping as well as PCR and southern blot can detect.
- Full fragile X requires Southern blot as mutations can be so large
- Mosaicism can be present with both premutations and full fragile X in same patient

Copyright 2010 American Society of Cytopathology

Huntington's Disease

- Expansion of (4p16.3) CAG expands from 9-37 (normal)
- Onset in 30s-40s
- Child has 50% chance of inheriting from parent
- Repeats take place within the coding region and add protein plaques in nervous tissues, causing neurologic symptoms
- Detectable by PCR methods

Copyright 2010 American Society of Cytopathology

Idiopathic Congenital Central Hypoventilation Syndrome

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

- CCHS –hypoventilation while asleep
- Triplet expansion within PHOX2b gene (4p12)13
- Severity of disease increases with number of repeats
- Detected by PCR

Copyright 2010 American Society of Cytopathology

Genomic Imprinting

- Transcriptional silencing through histone or DNA modification
- Occurs during germ cell production
- One or the other allele of a gene is lost
- Uniparental disomy/deletion illustrates imprinting on chromosome 15

Copyright 2010 American Society of Cytopathology

Prader-Willi vs. Angelman's

- Prader-Willi syndrome is caused by **paternal** 15 deletion: del(15)(q11q13)
- Loss of same region from **maternal** chromosome 15 causes Angelman's syndrome which has very different symptoms

Copyright 2010 American Society of Cytopathology

Brought to you by AMERICAN SOCIETY OF Cytopathology FOUNDATION

4 methods of inheritance

- Deletion on the paternal or maternal chromosome 15
- Mutation on the paternal or maternal chromosome 15
- Translocation with loss of critical region from one chromosome
- Maternal or paternal uniparental disomy, i.e. both chromosomes are inherited from one parent and not the other

Copyright 2010 American Society of Cytopathology

Methods for detection

- Cytogenetic methods are useful to detect
- High resolution can detect smaller deletions
- FISH with label probes to deleted region
- PCR of RFLP or STR can demonstrate uniparental disomy

Copyright 2010 American Society of Cytopathology

Summary

- Patterns of inheritance
- Molecular basis of single-gene disorders
- Factor V Leiden, hemochromatosis, cystic fibrosis
- Non-classical pattern of inheritance
  - Mitochondrial genes
  - Trinucleotide repeat expansion disorders
  - Genomic imprinting

Copyright 2010 American Society of Cytopathology