Most of the diseases have either major or minor genetic contribution

Thus diseases may be divided into:

1. Traditional category of genetic diseases: genetic contribution is particularly marked (e.g. Down’s syndrome)
2. Other conditions: Significant but variable genetic contribution (e.g. cancers)

Importance of Genetics to Medicine

- Globally, at least 7.6 million children are born annually with severe genetic or congenital malformations
- 90% of these infants are born in mid- and low-income countries.
- In the developed world, genetic and congenital disorders are the second most common cause of infant and childhood death

Prevalence of more common conditions for referral

- Down syndrome (1/600 live births and increases with advanced maternal age)
- Cystic Fibrosis (1/2,500 Caucasian Americans)
- Fragile X syndrome (1/1,000 males and 1/800 female carriers of which 30% will be mentally retarded)
- Sickle cell disease (1/500 of African American births)
- Hemophilia - Factor VIII Deficiency (48/100,000 male births)
- Duchenne muscular dystrophy (200/million male births)
- Hemochromatosis (1/450 individuals)
- Breast cancer (1/8 women of which 5-10% of will have a genetic predisposition)
**Type of genetic disease**

- Chromosomal (Cytogenetics)
- Single gene (mendelian)
- Multifactorial

---

**CHROMOSOME FEATURES**

- **Chromosomes** are long coiled pieces of DNA, with supporting proteins.
- **Genes** are short regions of this DNA that hold the information needed to build proteins.

---

**Central Dogma of Genetics**

1. **Replication**
   - DNA: CCTGAGCCAACTATTGATGAA
2. **Transcription**
   - RNA: CCGAGCCAAGCCUGUGAAGAA
3. **Translation**
   - Protein: PEPTIDE

---

**Chromosome**

- **Interphase**
- **Metaphase**
**Structure of chromosome**

- **CHROMATIDS**: two duplicated mitotic prophase chromosomes, each called a chromatid as long as it remains connected to "sister" chromatid.

**Morphology of chromosomes.**

- **Centromere**: holds 5 chromatids together and delineates the chromosome into a short arm (p) and a long arm (q). Allows each copy to be "pulled" to the new cell.
- **Telomere**: Allow the ends of the chromosome to be replicated
  - DNA sequence (thousands of copies of TTAGGG) at the end of all chromosomes
  - Satellite: Small segments of chromatin distal to the secondary constriction on the 'p' of the acrocentric
  - Ch. 13, 14, 15, 21 & 22.

**Chromosome Regions**

- **CENTROMERE** (primary constriction) is the area which holds the chromatids together and delineates the chromosome into a short arm (p) and a long arm (q). Allows each copy to be "pulled" to the new cell.
- **Telomere**: Protect the DNA from digestion by nucleases
  - DNA sequence (thousands of copies of TTAGGG) at the end of all chromosomes
  - Satellite: Small segments of chromatin distal to the secondary constriction on the p of the acrocentric
  - Ch. 13, 14, 15, 21 & 22.

**Chromosome classification**

- **Categories depending on the position of the centromere.**
  - **metacentric**: centromere in the middle, with arms of equal length. Ch. 1
  - **acrocentric**: centromere near one end, with arms of very different lengths Ch. 6
  - **sub-metacentric**: centromere near the middle, with arms of slightly different lengths. Ch. 6
  - Telocentric: centromere at one end, with only 1 arm. Such telocentric chromosomes are not seen in human cells.

**DNA packing**

- The haploid human genome contains approximately 3 billion base pairs of DNA packaged into 23 chromosomes.
- 6 billion base pairs of DNA per cell.
- Each base pair is around 0.34 nanometers long
- Each diploid cell therefore contains about 2 meters of DNA.
- Moreover, the human body contains about 50 trillion cells—which works out to 100 trillion meters of DNA per human.
- Now, consider the fact that the Sun is 150 billion meters from Earth.
- This means that each of us has enough DNA to go from here to the Sun and back more than 300 times, or around Earth’s equator 2.5 million times!
- How is this possible?
On staining pattern - 2 types of areas on chromosomes seen in nucleus

- **EUCHROMATIN**
  - When DNA is in its least condensed form
  - Transcribed
  - Replicated early

- **HETERO CHROMATIN**
  - When DNA is in its most condensed form
  - Devoid of genes or has inactive genes
  - Not transcribed
  - Replicated late

**HETERO CHROMATIN**

- **Constitutive heterochromatin**
  - Centromere
  - q of Y chromosome
  - Satellite of acrocentric Ch.

- **Facultative heterochromatin**
  - Transcriptionally inactive stage e.g. Barr body

**Chromosome number in different mammals**

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Genus and Species</th>
<th>Diploid Chromosome Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>Felis catus</td>
<td>38</td>
</tr>
<tr>
<td>Human</td>
<td>Homo sapiens</td>
<td>46</td>
</tr>
<tr>
<td>Donkey</td>
<td>Equus asinus</td>
<td>62</td>
</tr>
<tr>
<td>Pig</td>
<td>Sus scrofa</td>
<td>38</td>
</tr>
</tbody>
</table>

The number as well as the size and shape of the chromosomes of a species is usually constant and is called its karyotype.
Chromosome classification

- Chromosome classification is based on International System for Human Cytogenetic Nomenclature (ISCN) from 1985.

Normal Human Karyotype

- Autosomes are divided into:
  - Groups A (1 to 3),
  - B (4 & 5),
  - C (6 to 12),
  - D (13 to 15),
  - E (16 to 18),
  - F (19 & 20) and
  - G (21 & 22).

- Sex chromosome XX or XY

Human Chromosomes

- 46 chromosomes, or 23 pairs.
- 44 of them are called autosomes and are numbered 1 through 22. Chromosome 1 is the longest, 22 is the shortest.
- The other 2 chromosomes are the sex chromosomes: the X chromosome and the Y chromosome.
- Males have an X and a Y; females have 2 X’s: XY vs. XX.

Abnormal Karyotypes

- ABNORMALITY IN NUMBER
  - 45, X
  - 48, XXXY
  - 47, XY,+21
  - 46, XY+18, -21
  - 70, XXY,+22
  - 45, X/46, XX/47, XXX

- STRUCTURALLY ALTERED CHROMOSOMES
  - 46, X,i(Xq)
  - 46, XY,t(2;12)(p24;q15)
  - 46, XY,r(4)(p16q34

Visualizing chromosomes

- Obtain tissue from person
  - Fetal tissue: amniocentesis
  - chorionic villi sampling
  - fetal cell sorting
  - Adult tissue: blood (white blood cells)
  - cheek swab (buccal cells)
  - skin cells
  - tissue biopsy

CHROMOSOME ANALYSIS OR CYTOGENETIC STUDIES

- TECHNIQUE: Collect venous blood → Isolate lymphocytes → Culture → Add PHA → Add Colchicine → Add hypotonic saline → Fix cells → Spread on slides → Stain → Photograph → Karyotype

- STAINS:
  - G banding by Giemsa stain Commonly used
  - OTHER
    - Q banding
    - R banding
    - High resolution banding
Chromosome banding

- CHROMOSOME BANDS: Alternate light & dark stained areas, constant morphology between chromosomes and individuals.
- Each is numbered & starts with 11. e.g., 11.1
- G banding: produced by staining with Giemsa after digesting the chromosomes with trypsin

End of lecture 1

Genetic mutation

- Chromosomal mutations:
- Are large scale mutation
- Arise
  - spontaneously
  - induced by chemicals or radiation.
- Different from Small scale mutation

Types of chromosomal disorders

- Abnormalities in number
- Abnormalities in structure
Ploidy
- **Ploidy** is the number of sets of chromosomes in the nucleus of a biological cell
- The **haploid number** \( (n) \) is the number of chromosomes in a gamete.
- Two gametes form a **diploid** zygote with twice this number \( (2n) \).

**Euploidy**
- **Euploidy** the state or condition of having a variation in chromosome number that is an exact multiple of the haploid number
- **Polyploidy** is the state where all cells have multiple sets of chromosomes beyond the basic set, usually 3 or more.
- Specific terms are **triploid** (3 sets), **tetraploid** (4 sets), pentaploid (5 sets), hexaploid (6 sets), heptaploid or septaploid (7 sets) octoploid (8 sets), nonaploid (9 sets), decaploid (10 sets), undecaploid (11 sets), dodecaploid (12 sets), tridecaploid (13 sets), tetradecaploid (14 sets) etc.

**EUPLOID CHANGES IN HUMAN**
Variation involving entire sets of chromosomes

<table>
<thead>
<tr>
<th>Euploid Type</th>
<th>( n )</th>
<th>Chromosome Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploid or monoploid</td>
<td>One (1)</td>
<td>1, 2, 3...</td>
</tr>
<tr>
<td>Diploid</td>
<td>Two (2n)</td>
<td>1,1, 2,2, 3,3...</td>
</tr>
<tr>
<td>Polyploid</td>
<td>More than two</td>
<td>1,1,1, 2,2,2, 3,3,3...</td>
</tr>
<tr>
<td>--Triploid</td>
<td>Three (3n)</td>
<td>1,1,1, 2,2,2, 3,3,3...</td>
</tr>
<tr>
<td>--Tetraploid</td>
<td>Four (4n)</td>
<td>1,1,1,1, 2,2,2,2, 3,3,3,3...</td>
</tr>
</tbody>
</table>

**Two types of polyploidy**
- **Autopolyploidy**: all of the chromosome sets come from the same species.
  - Failure of cell division \( (2N \rightarrow 4N) \)
  - Produce diploid (not haploid) gametes
- **Allopolyploidy**: the chromosome sets come from two or more different species, usually a plant
  - 2 different species hybridize

**Allopolyploidy**
- All of the chromosome sets come from two or more different species.
Triploidy

**TRIPLOIDY 69+XXX or XXY or XYY**

- 20% of chromosomally abnormal abortions
- 1st trimester - focal trophoblastic hyperplasia, partial mole
- 2nd trimester - growth retardation, foetal defects
- Live births are rare, survive for only brief period.

**Pathogenesis**

- Fertilization error e.g. dispermy
- Failure of meiosis in germ cells i.e. fertilization of a diploid ovum by a haploid sperm & vice versa.

- extra set of chromosomes is of paternal origin, resulting from dispermy
- extra set of chromosomes is of maternal origin, resulting from failure of extrusion of the polar body.

Asymmetry between the head and abdomen in 2nd trimester triploidy.
Partial mole

Unless you have bad

Aneuploidy

TETRAPLOIDY 92+ XXXX or XXYY
- Karyotype 92+XXXX or XXYY
- Chromosomally abnormal abortions
- Most are lost during 1st trimester
- Ongoing pregnancy rare
- Growth retardation, multiple malformation
- Pathogenesis: Failure of the 1st cleavage division resulting in doubling in number immediately after fertilization.

**NO RECURRENCE RISK**

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of chromosomes</th>
<th>Chromosome example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disomic (normal diploid)</td>
<td>2n</td>
<td>1, 2, 2, 3, 3, 4, 4,...</td>
</tr>
<tr>
<td>Monosomic</td>
<td>2n – 1</td>
<td>1, 2, 2, 3, 3, 4, 4,...</td>
</tr>
<tr>
<td>Nullisomic</td>
<td>2n – 2</td>
<td>1, 2, 2, 0, 0, 4, 4,...</td>
</tr>
<tr>
<td>Polysomic</td>
<td>3n+1</td>
<td>1, 2, 2, 3, 3, 4, 4,...</td>
</tr>
<tr>
<td>Trisomic</td>
<td>3n+1</td>
<td>1, 2, 2, 3, 3, 4, 4,...</td>
</tr>
<tr>
<td>Tetrasomic</td>
<td>3n+2</td>
<td>1, 2, 2, 3, 3, 4, 4, 4, 4,...</td>
</tr>
</tbody>
</table>
Frequency of common chromosomal numerical disorders among live born infants

**Autosomal**
- Trisomy 13: 1:15,000
- Trisomy 18: 1:5,000
- Trisomy 21 (Down syndrome): 1:800

**Sex chromosome**
- Klinefelter syndrome (47,XXY): 1:700 M
- Triple X syndrome (47,XXX): 1:1,000 F
- Turner syndrome (45,X; other): 1:1,500 F
- XYY Syndrome (47,XYY): 1 in 800 M

**Aneuploidy is almost always harmful.**
Imbalanced gene dosage causes the negative effects of aneuploidy.

**Aneuploidy can affect any chromosome**

The reason only trisomy 13, 18 or 21 is seen in live births is because other autosomal aneuploides are embryonic lethal conditions.

---

Mitotic Nondisjunction and Chromosome Loss Lead to Somatic Cell Aneuploidy

Aneuploidy is usually due to **nondisjunction**
Non-disjunction during meiosis

Spontaneous fetal loss for autosomal trisomies

Most chromosomal aneuploidies do not survive to birth

**Trisomy 21**
**Down Syndrome**

Every life has value.
The best known human aneuploidy is Trisomy 21 (Down Syndrome, 47, +21).

This was the first chromosomal mutation to be associated with a particular genetic disease in humans.

- **Occurs worldwide**
- **Most common of chromosomal disorders (1 in 800 live birth in the US)**

### Clinical Features

- **Prenatal** → Cystic hygroma, Low maternal α-feto protein level
- **Infancy** → Characteristic physical features
- **Childhood & Adult** → 40% have congenital heart disease
- **Mental retardation (IQ 25-50)**
- **40 to 20 fold increased risk of Acute lymphomas.**
- **Presenile dementia (changes like Alzheimer’s)**
- **Abnormal immune response (serious infections, thyroid autoimmunity)**

### Incidence of Some Associated Medical Complications in Persons with Down Syndrome

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental retardation</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Early Alzheimer’s disease</td>
<td>Affects 75% by age 60</td>
</tr>
<tr>
<td>Congenital heart defects</td>
<td>40</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>40 to 75</td>
</tr>
<tr>
<td>Ophthalmic disorders</td>
<td>60</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>5 to 10</td>
</tr>
<tr>
<td>Gastrointestinal malformations</td>
<td>5</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>5</td>
</tr>
<tr>
<td>Leukemia</td>
<td>1</td>
</tr>
<tr>
<td>Atlantoaxial subluxation with spinal cord compression</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Increased susceptibility to infection</td>
<td>Unknown</td>
</tr>
<tr>
<td>Infertility</td>
<td>&gt;99% in men; anovulation in 30% of women</td>
</tr>
</tbody>
</table>

### Incidence of Down Syndrome Increases with Maternal Age

- **Incidence of Down Syndrome Increases with Maternal Age**

- **The incidence, or risk, of Down syndrome is related to maternal age as the following chart shows:**

<table>
<thead>
<tr>
<th>Mother’s age</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1/1550</td>
</tr>
<tr>
<td>25</td>
<td>1/1050</td>
</tr>
<tr>
<td>30</td>
<td>1/1200</td>
</tr>
<tr>
<td>35</td>
<td>1/350</td>
</tr>
<tr>
<td>40</td>
<td>1/70</td>
</tr>
<tr>
<td>45</td>
<td>1/25</td>
</tr>
<tr>
<td>48</td>
<td>1/9</td>
</tr>
</tbody>
</table>

- **Most often occurs by nondisjunction of chr. 21 during meiosis; in theory could occur in either father or mother, but 95% of these trisomies have defective ovum as source**

- **The reasons for this maternal age effect are not known but it is seen in all aneuploidies, for all chromosomes**
  - All ova are formed by birth and arrested in meiosis;
  - increased age and the syndrome due to more nondisjunction in older ovum
**KARYOTIPES in Dawn’s Synd:**
- 95% are TRISOMY 21
- 4% Robertsonian translocation of 21q to ch. 14 or 22 (t (14q 21a)).
- 1% are Mosaics (usually 46/47 mosaics)

- Obligate Dawn’s Synd. region is 21q 22.2 & 21q 23.3
- Recurrence risk – 1/200 – 1/100,

**Prenatal Screening for Down Syndrome**
- **Screening tests** for “high risk” pregnancies
  - If +ve then further diagnostic testing.
    - quick and easy
    - more chances of “false-positives” or “false-negatives”
  - **Diagnostic tests**: +ve result very likely the patient has Down baby.
    - More expensive and require an elaborate procedure

**Maternal Serum Screening**

Combination of different markers on mother’s blood
- **Triple test**: alpha-fetoprotein (AFP), unconjugated estriol (uE3), and human chorionic gonadotropin (hCG)
- Quadruple screen: inhibin A is added
- These are done in the 15th to 18th week of pregnancy.

**Ultrasound Screening**

- The main usefulness of ultrasound is to confirm the gestational age of the fetus
- a strong association between the size of a collection of fluid at the back of the fetal neck, called nuchal translucency, and the risk of Down syndrome
- Several other items that can be found during an ultrasound exam (echogenic bowel, echogenic intracardiac focus, and dilatation of the kidneys (pyelectasis))
- However, these markers as a sign of Down syndrome are still controversial
- Even the best combination of ultrasound findings and other variables is only predictive and not diagnostic.
  - For confirmatory diagnosis, the chromosomes of the fetus must be examined (Amniocentesis, Chorionic Villus Sampling)

**Chromosome analysis in Foetal Down Syndrome**

<table>
<thead>
<tr>
<th>Diagnostic procedure</th>
<th>Gestational age when test is done (weeks)</th>
<th>Risk of fetal loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chorionic villus sampling</td>
<td>10 to 12</td>
<td>0.5 to 1.5</td>
</tr>
<tr>
<td>Early amniocentesis</td>
<td>12 to 15</td>
<td>1.0 to 2.0</td>
</tr>
<tr>
<td>Second-trimester amniocentesis</td>
<td>15 to 20</td>
<td>0.5 to 1.0</td>
</tr>
</tbody>
</table>

**Other autosomal trisomies**

- **Trisomy-13** Produces Patau syndrome
  - Frequency: 2 in 10,000 live births
  - Features:
    - Cleft lip and palate
    - Small eyes
    - Polydactyly
    - Developmental retardation
    - Most die before 3 months
- **Trisomy-18** Produces Edwards syndrome
  - Incidence: 2.5 in 10,000 live births
  - About 80% are female
  - Features:
    - Elongated skull
    - Low-set malformed ears
    - Mental and developmental retardation
    - 90% of infants with Edwards syndrome die within 6 months
Sex Chromosome Aneuploidy

<table>
<thead>
<tr>
<th>Situation</th>
<th>Oocyte</th>
<th>Sperm</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>X Y</td>
<td>46, XY normal male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X X</td>
<td>46, XX normal female</td>
<td></td>
</tr>
<tr>
<td>Female Nondisjunction</td>
<td>XX Y</td>
<td>47, XXXKlinefelter syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XX X</td>
<td>47, XXX triplo-X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>45, Y nonviable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>45, X Turner syndrome</td>
<td></td>
</tr>
<tr>
<td>Male Nondisjunction</td>
<td>X</td>
<td>45, X Turner syndrome</td>
<td></td>
</tr>
<tr>
<td>(meiosis I)</td>
<td>XX</td>
<td>47, XXX triplo-X</td>
<td></td>
</tr>
<tr>
<td>Male Nondisjunction</td>
<td>X</td>
<td>47, XYY Jacobs syndrome</td>
<td></td>
</tr>
<tr>
<td>(meiosis II)</td>
<td>YY</td>
<td>45, XYY Jacobs syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>45, X Turner syndrome</td>
<td></td>
</tr>
</tbody>
</table>

Summary: Aneuploidy of the sex chromosomes

1. Need at least one X chromosome (44, −X) and (45, −Y) not even seen in spontaneous abortions
2. Single X (45, X) – viable, but not fertile
   - need at least 2X for normal female
3. Extra X or Y interferes with normal development
   - ranges from very mild (near normal) to severe
   - severity increases as the multiples Xs and Ys increase

MONOSOMY

- Autosomal monosomies are lethal
- X chromosome monosomies are seen

Nondisjunction of X chromosome

Nondisjunction also occurs with autosomes

TURNER SYNDROME

- Complete or partial monosomy of X chromosome
- Characterized by hypogonadism in phenotypic females
- Karyotype
  - 57% are 45X0
  - Deletion of small arm 46Xi (Xq)
  - Deletion of portions of small or long arm
  - Mosaics 45X/46XY, 45X/47XXX
  - Only 1% fetuses with 45X0 survive, 99% aborted
  - Karyotypic heterogeneity is responsible for significant variation in phenotype

Turner syndrome (45, X)

- 1 in 2,000 female births
- 99% of fetuses die before birth
- 75% of all cases are thought to originate in the father
- Need two XX chromosomes for normal female sexual development. One X is enough for other traits.
Turner syndrome
- 1st indication is delayed sexual development
- Sexual infantilism, short stature, webbing of neck
- Primary and secondary amenorrhea.
- Rarely fertile, offsprings increased chance of Ch. abnormality
- Phenotypes include short stature, webbing at back of neck, incomplete sexual development, hearing impairment

Klinefelter Syndrome (47, XXY)
- Males with an extra X-chromosome
- 1 in 1000 live births
- Includes XXXY, XXXY, XXXXY
- Most Klinefelter syndrome males appear normal
- Phenotypes include incomplete sexual development (rudimentary testes and prostate), long limbs, large hands and feet, some breast tissue development.
- Most discovered with evaluation of male infertility.
- Effective treatment - testosterone injections

Triplo- X (47, XXX)
- 1:1000 females are XXX
- Phenotype - tallness and menstrual irregularities
  - maybe slightly less intelligent then their siblings
- Protected through X-inactivation

XYY syndrome (Jacobs Syndrome)
1 in 1000 male births
First described in 1961
1965 - Patricia Jacobs
- Studied 197 inmates in Scotland
- Seven had an extra Y chromosome
Frequency of XYY males in penal and mental institution is significantly higher than that in the population at large.

Is violent and aggressive behavior linked to a YY condition?

Today
- Know that 96% of all XYY males are apparently normal
- Modest phenotype includes
  - Tendency to have great height
  - Acne problems
  - Speech and reading problems
- Studies suggesting some increase in aggressive behaviors remain controversial.
Females are XX, males are XY

What is the consequence for females of having two X chromosomes, while males have only one?

Do XX females produce twice the amount of X-linked gene products (proteins) as XY males?

No!

because XX females “compensate” by inactivating one of their X chromosomes to make a single “dosage” of X-linked genes (Dosage Compensation)

- Measure the expression of X-linked genes revealed:
  - The level of mRNA or protein for various X-linked genes (like autosomal genes) are similar between males and females
  - Example – Factor VIII

How is the dosage for X-linked genes adjusted to be equivalent in males and females?

X Inactivation

- one X chromosome in each female cell is inactivated
- inactivation is a random process
  - Some cells - turn off paternal X
  - Some cells - turn off maternal X

Summary: Lyons Hypothesis

- Only one X chromosome is active in somatic cells
- Inactivated X can be either the maternal or paternal chromosome
- Inactivation occurs early in embryonic development
- Inactivation is permanent in all daughter cells of somatic cells
- Random inactivation makes male and female cells equivalent for X-linked genes
- Exception - germ line cells – both X remain active

In 1961 Mary Francis Lyon - British geneticist

- studied color coat in mice
- knew that coat color was X-linked
Advantage of X-inactivation for females
- Usually protects against deleterious recessive X-linked genes
- However, female heterozygotes can express an X-linked recessive trait
  - color blindness
  - anhidrotic ectodermal dysplasia
  - hemophilia

If normal XX female has one X inactivated, why is a X Turner female not normal?
Similarly, if XXY male has one X inactivated, why does he have Klinefelter syndrome?

Perhaps not complete inactivation
Or inactivation does not happen immediately, then some overexpression of X-linked genes

Many of the genes on X escape inactivation
eg. MIC-2

Genes inactivated are DMD, G 6PD, HPRT etc.


Barr Body
Inactivated X chromosome can be seen in females cells as the Barr body - Murray Barr (1949)

Barr Bodies are Inactivated X Chromosomes in Females

Normal male, Turner female
0 1

Normal female, Klinefelter male
2 3

# Barr bodies = N-1 rule

HERMAPHRODITISM
What determines maleness and femaleness?

Two kinds of sex determination.

1. Environmental sex determination
2. Genotypic sex determination

XX normal female
XY normal male

Genetic sex in humans

XX - normal female
X - female phenotype –infertile (Turner’s)
XXX - normal female (triplo-X)

- the X chromosome relates to the female phenotype
- minimum of XX for normal female

XY - normal male
XXX - “normal male” – (Klinefelters)
XXXXY - severe Klinefelters syndrome

- male phenotype

Y - monosomy Y - embryonic lethal

What is so different between the X and Y chromosomes?

X - over 1000 genes identified
Y - 330 genes identified, many are inactive

What is it about the Y chromosome that causes the indifferent gonad to begin developing into a testis?

Genes on the Y chromosome

There are three classes of genes on the Y.

Genes shared with X chromosome define the pseudoautosomal regions (PAR)

Genes similar to X chromosome genes are X-Y homologs

Genes unique to the Y including SRY gene

SRY (Sex-determining Region Y) is a sex-determining gene on the Y chromosome in humans

SRY – starts male development by
- turning on testis-determining genes
- turning off ovary-determining genes

Phenotypic males that were XX - sterile
Phenotypic females who were XY - turner’s syndrome
**Summary of TDF**

1. Initiates the process that directs the indifferent gonads toward testis development
2. Activates Sertoli cells to produce Mullerian inhibiting hormone, causing Mullerian duct degeneration
3. Stimulates Leydig cells to secrete testosterone, which then directs development of the Wolffian ducts towards epididymides, vas deferens and seminal vesicles
   - Testosterone conversion to dihydrotestosterone (DHT) - directs development of the urethra, prostate gland and penis

**What happens in XX?**

- Y chromosome (SRY region; TDF gene) is not present.
- no TDF to tell it to form testis
- gonadal tissue develops towards ovary formation
- In the absence of testosterone – Wolffian duct system degenerates
- In absence of MIH – Mullerian ducts continue to develop towards fallopian tubes, uterus, and upper vagina.

**What is an abnormal sexual phenotypes?**

There is an inconsistency between the observed genetic sex, gonadal sex and sexual differentiation

**Abnormal Development**

**Hermaphroditism**

- **True hermaphroditism:**
  - possessing both male and female sexual anatomy
  - example: one ovary, one testis, vaginal opening and penis
- **Pseudohermaphroditism:**
  - ovaries or testes, but not both
  - if ovaries, then male external sexual anatomy
  - if testes, then female external sexual anatomy

**TRUE HERMAPHRODITISM**

- Very rare
- Have both TESTICULAR and OVARIAN tissue.
- Internal & External sex organs variable
- Sex hormones also variable
- Majority XX, some XY some XX/XY
PSEUDO HERMAPHRODITISM
- Have gonad of one sex i.e. testis OR ovary
- Ambiguous genitalia
- Various cause (cytogenetic, mendelian, Teratogenic)

MALE PSEUDO HERMAPHRODITISM
- Heterogenous group. genetically as well as clinically
- TESTICULAR FEMINZATION
  - X Linked disorder
  - genetic males (XY) with a female phenotype
  - gonadal sex correct - gonads differentiate to testis
  - produce MIH – females duct system has degenerated
  - produce testosterone and DHT

TESTICULAR FEMINZATION
- No uterus, Fallopian tube or ovary
- TESTIS intrabdominal or in inguinal canal
- Breast develop at puberty, sparse pubic / axillary hair
- child appears to be a girl
  - raised as girls
  - at puberty, genetically driven male phenotype emerges from an apparent female phenotype

TESTICULAR FEMINZATION
- DEFECT is absence of androgen receptors.
  - gene that encodes the androgen receptor defective
    - can't bind testosterone
    - X-linked trait
  - development proceeds as if no testosterone is present
    - Wolffian ducts degenerated into an indifferent female plan
  - Unlike 5-α reductase deficiency
    - can’t respond to the androgen surge at puberty
    - puberty have breast development, but no menstruation
**FEMALE PSEDOHERMAPHRODITISM**

- **CONGENITAL ADRENAL HYPERPLASIA** (Adrenogenital synd.)
  - Several genetic & clinical forms, all are AR
  - Block in a specific step in cortisol biosynthesis
  - Increased ACTH secretion
  - Hyperplasia of adrenal gland
  - Masculinization of female fetus

**CONGENITAL ADRENAL HYPERPLASIA**

- Most common form is 21 – hydroxylase deficiency
- Results in 3 different clinical presentations:
  - Salt losing
  - Simple virilizing
  - Late onset virilization
- Diagnostic clues – Absence of testis in scrotum
  - Presence of a uterus
  - Elevated 17- ketosteroid.

**CYTOGENETIC DISORDERS**

Prof. Mohammed Kamal
Dept. of Pathology, BSMMU
April 2014
Two kinds of sex determination in human

1. Environmental sex determination
2. Genotypic sex determination
   XX  normal female
   XY  normal male

Genetic sex in humans

XX  - normal female
X   - female phenotype -infertile (Turner’s)
XXX - normal female (triplo-X)
   - the X chromosome relates to the female phenotype
   - minimum of XX for normal female
XY  - normal male
XXX  - “normal male” – (Klinefelters)
XXXXY - severe Klinefelters syndrome
   - male phenotype
Y   - monosomy Y - embryonic lethal

What is so different between the X and Y chromosomes?

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  - if testes, then female external sexual anatomy

TRUE HERMAPHRODITISM

- An intersex condition in which an individual is born with ovarian and testicular tissue
- External genitalia are often ambiguous, the degree depending mainly on the amount of testosterone produced by the testicular tissue between 8 and 16 weeks of gestation.
- Sex hormones also variable
- Very rare
TRUE HERMAFRODITISM

- Majority 47XXY, 46XX/46XY, or 46XX/47XXY, and various degrees of mosaicism
- Fertilization of two haploid ovum and fusion of the two zygotes early in development.
- Fertilized of one ovum by two sperms followed by trisomic rescue in one or more daughter cells.
- Fusion of two fertilized ova to form a tetragametic chimera
- Mutation in the SRY gene

Trisomic rescue

- Genetic phenomenon in which a fertilized ovum containing three copies of a chromosome loses one of these chromosomes to form a normal, diploid chromosome complement.
- If both of the retained chromosomes came from the same parent, then uniparental disomy results.

PSEUDO HERMAFRODITISM

- Person with secondary sex characteristics or a phenotype that is different from what would be expected on the basis of the gonadal tissue (ovary or testis).
- In some cases, the external sex organs look intermediate between the typical clitoris or penis.
- In other cases, the external sex organs have an appearance that does not look intermediate, but rather has the appearance that would be expected to be seen with the "opposite" gonadal tissue.
- Because of this, pseudohermaphroditism is sometimes not identified until puberty.
PSEUDO HERMAPHRODITISM

- "male pseudohermaphrodite" when a testis is present
- "female pseudohermaphrodite" an ovary is present
- Various cause (cytogenetic, mendelian, Teratogenic)

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  - Elevated 17-ketosteroid.
CHROMOSOME STRUCTURAL ABERRATIONS

Prof Mohammed Kamal
Dept. of Pathology, BSMMU
April 2014

Deletion

- Begins with a chromosome break.
- Induced by heat, radiation, viruses, chemicals, transposable elements, and recombination errors.
- No reversion; DNA is missing.

DELATIONS

- TWO GROUPS:
  - Large chromosomal deletions
  - Microdeletion
- Deletion of centromere typically results in chromosome loss (usually lethal)
Large chromosomal deletions

- **Terminal**
- **Cri du chat, 5p15**
- **Wolf-Hirschhorn, 4p36**

- **Interstitial**
  - Williams, 7q11.2, microdeletion (FISH)
  - Retinoblastoma, 13q14
  - Prader-Willi, 15q11.2
  - Angelman, 15q11.2
  - DiGeorge, 22q11.2

Cri du chat or Lejeune’s syndrome

de novo deletion of 5p-

Cri-du-Chat syndrome

- Is one of the most common syndromes caused by a chromosomal deletion.
- It affects between 1 in 20,000 and 1 in 50,000 babies.
- French for "cry of the cat," because of distinctive cry of children with this disorder.
- The cry is caused by abnormal larynx development
- Less noticeable as the baby gets older

Cri-du-Chat syndrome

- Cri-du-chat deletion length may vary
- Multiple genes are missing as a result, each may contribute to the symptoms of the disorder.
- **Genes involved are:**
  - **TERT** (telomerase reverse transcriptase): important during cell division. It helps to keep the telomeres intact
  - **CTNND2 gene** (catenin delta 2): associated with severe mental retardation in some cri-du-chat syndrome cases
  - neuronal migration, function of synapses.

Microdeletion

- Loss of tiny fragment of a chromosome.
- Require high resolution prometaphase banding & FISH for visualization

Chromosome pair 15 with microdeletion in 15q11-q13 in one homologue (arrow) (550 bands; G-banding).

Williams Syndrome

- Interstitial deletion in the chromosomal region 7q11.23, encompassing the ELASTIN gene.
- Elastin gives blood vessels the stretchiness and strength. The elastin protein is made only during embryo development and childhood, when blood vessels are formed.
- Clinical manifestations:
  - supravalvar aortic stenosis, mental retardation, elfin facies, impaired visuospatial constructive abilities, and transient hypercalcemia in infancy.
Williams Syndrome

- The chromosomal deletion that causes Williams Syndrome is very small (MICRODELETION)
- It cannot be seen in a classic karyotyping technique.
- However, the deletion can be observed using a special technique

Contiguous gene syndrome

- Syndrome due to abnormalities of 2 or more genes that map next to each other on a chromosome
- Most often caused by a deletion that involves several contiguous genes.
- e.g. DMD with retinitis pigmentosa in the same person

Prader-Willi syndrome: 15q11-13 deletion/ rearrangement
- 1/10,000-25,000 births
- Infancy ⇒ poor feeding, hypotonia, 2nd – 3rd year ⇒ insatiable appetite, obesity, eat to death by age 5 or 6 if not treated, development delay, behavioral problem.
- Other examples: Angelman synd., WAGR synd, Williams Syndrome

WAGR

- W-Wilms’ tumor
- A-Aniridia
- G-Genital and/or urinary tract abnormalities
- R-mental retardation/developmental disabilities
Ring chromosomes

- chromosome breaks in two places and the ends fuse together to form a circular structure.
- A ring chromosome is denoted by the symbol r.
- Radiation, other mutagens / Spontaneously.
- Although ring chromosomes are very rare, they have been found in nearly all human chromosomes.

Disorders due to ring chromosome

- Ring chromosome 20 syndrome associated with epilepsy.
- Ring chromosome 14 and ring chromosome 13 syndrome are associated with mental retardation and dysmorphic facial features.
- Ring chromosome 15 is associated with mental retardation, dwarfism and microcephaly.
- Ring formation of an X-chromosome causes Turner syndrome.

Inversion

- Two break with rearrangement (reversed end to end i.e. the broken piece reintegrates in opposite orientation) involving a single chromosome.
- Generally do no result in lost DNA.
- Two types of inversions:
  - Pericentric = include the centromere
  - Paracentric = do not include the centromere

Consequences of inversion

- Usually do not cause any abnormalities in carriers as long as the rearrangement is balanced with no extra or missing genetic information.
- However, heterozygous individuals for an inversion, have an increased risk of production of abnormal chromatids (this occurs when crossing-over occurs within the span of the inversion).
- This leads to lowered fertility due to production of unbalanced gametes.

Inversion types

a) Pericentric inversion (includes centromere)

b) Paracentric inversion (does not include centromere)

Inversion

- Linked genes often are inverted together, so gene order typically remains the same.
- Homozygous: ABCDEFGH ⇒ no developmental problems
- Heterozygote: ABCDEFGH
- Gamete formation differs, depending on whether it is a paracentric inversion or a pericentric inversion.
Inversion in human

- The most common inversion seen in humans is on chromosome 9, at inv(9)(p11q12).
- This inversion is generally considered to have no deleterious or harmful effects, but there is some evidence it leads to an increased risk for miscarriage for about 30% of affected couples.
- Newfoundland → Carriers of pericentric inversion of long arm of Ch. 3.

Inversion of Ch. 3

Insertion (duplication)

- Insertions can be anywhere in size from one base pair incorrectly inserted into a DNA sequence to a section of one chromosome inserted into another.
- On a chromosome level, an insertion refers to the insertion of a larger sequence into a chromosome. This can happen due to unequal crossover during meiosis.

Insertion (duplication)

Duplication (INSERTION) consequences

- More common but much less harmful than deletions.
- Duplication of whole gene e.g. globin, haptoglobin etc.
- Multigene family

Multigene families

- Groups of genes from the same organism that encode proteins with similar sequences either over their full lengths or limited to a specific domain.
- Examples: gene that encode the hemoglobins, immunoglobulins, histocompatibility antigens, acts, tubulins, keratins, collagens, heat shock proteins, salivary glue proteins, chorion proteins, cuticle proteins, yolk proteins, and phaseolins, as well as histones, ribosomal RNA, and transfer RNA genes.
The theory of molecular drive in 1982 by Gabriel A. Change in location of chromosome segment; no DNA is lost or gained. May change expression = position effect.

- Intrachromosomal
- Interchromosomal
  - Reciprocal - segments are exchanged.
  - Non-reciprocal - no two-way exchange.

- Several human tumors are associated with chromosome translocations; myelogenous leukemia and Burkitt lymphoma.

**TRANSLOCATION**

A chromosomal translocation as revealed by two different karyotyping techniques

Robertsonian translocation

- Long arm fusion in an acrocentric ch. (13, 14, 15, 21, 22) short arm is lost
- Gametes: Normal / Balanced / unbalanced
- Translocation Down’s syndrome, Philadelphia chromosome
In CML, the translocation occurs between chromosomes 9 and 22, the Philadelphia chromosome. It is an acquired mutation — that is, a person is not born with it. It is not passed on to their children. Exactly why the Philadelphia chromosome forms is unknown in most cases, although exposure to ionizing radiations is responsible.

Translocation produces a new, abnormal gene called BCR-ABL. This abnormal gene produces Bcr-Abl protein with tyrosine kinase activity. This protein causes the excess WBCs typical of CML.

Reciprocal translocation

- No loss of genetic material
- Phenotypically normal
- Increased risk of producing abnormal gametes
- Translocation involving q of Ch 11 and 22 is relatively common

Isochromosome

- When a chromosome divides at right angle rather than at its usual longitudinal plane.
- Loss of one arm with duplication the other arm (mirror image)
- 15% Turner’s where the X ch. is composed of two long arms.
- Can happen – in acrocentric Ch. i.e. 45 XX i (21p); in X ch. 46XXi (X)
- 100% risk of trisomic offspring

CHROMOSOME FRAGILE SITES

- Secondary constriction seen in specific cultural conditions are liable to break
- 80 common & 26 rare fragile sites. Most are induced by antifolate agents
- e.g. Folate sensitive fragile site on X chromosome.
- However, most are not associated with clinical abnormality.
- Molecular basis is trinucleotide repeats.
Expansion of the CGG repeating codon to such a degree results in a methylation of that portion of the DNA. Methylation of the FMR1 locus in chromosome band Xq27.3 is believed to result in constriction of the X chromosome, which appears 'fragile' under the microscope at that point.

CHROMOSOME BREAKAGE

Visible breaks in metaphase chromosome inherited as AR trait.

Cause: Faulty DNA repair or synthesis, radiation, chemicals

FANCONI ANEMIA / BLOOM SYNDROME / ATAXIA TELANGECTASIA

MUTATION AND IT'S CONSEQUENCES

Prof. M. Kamal
Pathology, BSMMU
10 May 2014

Mutation

A change of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal genetic element.

Mutations result from unrepaired damage to DNA or to RNA genomes (radiation or chemical mutagens), errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements.

Mutations may or may not produce changes in the phenotype.

Mutations play a part in both normal and abnormal biological processes such as: evolution, cancer and the development of the immune system.
Four classes of mutations

1. Spontaneous mutations (molecular decay),
2. mutations due to error prone replication bypass of naturally occurring DNA damage (also called error prone translesion synthesis),
3. errors introduced during DNA repair
4. induced mutations caused by mutagens.

Classification of mutation types

- By effect on structure
- By effect on function
- By effect on fitness [harmful or beneficial]
- By impact on protein sequence
- By inheritance

Classification of mutation types

By effect on structure

- Small-scale mutations: affect a small gene in one or a few nucleotides,
- Large-scale mutations in chromosomal structure

Gene mutations which affect only one gene

DNA sequence

Transcription ↓ mRNA sequence

Translation ↓ Polypeptide

Normal gene

DNA (antisense strand) GGTCTCCTCAGCCA
mRNA CCAGAGAGUGCGGU

Codons

Polypeptide Pro-Glu-Glu-Cys-Gly

Amino acids

The antisense strand is the DNA strand which acts as the template for mRNA transcription

Small-scale mutations

- Point mutations
  - often caused by chemicals or malfunction of DNA replication,
  - exchange a single nucleotide for another
  - classified as transitions or transversions
    - Transition exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T).
    - Transversion (less common) which exchanges a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G).
- Insertions add one or more extra nucleotides into the DNA.
- Deletions remove one or more nucleotides from the DNA.
Classification according to change in codon

- Point mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon codes for:
  - **Silent mutations**: which code for the same amino acid.
  - **Missense mutations**: which code for a different amino acid.
  - **Nonsense mutations**: which code for a stop and can truncate the protein.

- A point mutation can be reversed by another point mutation, in which the nucleotide is changed back to its original state (true reversion) or by second-site reversion (a complementary mutation elsewhere that results in regained gene functionality).

Classification by effect on function

- **Loss-of-function mutations** are the result of gene product having less or no function.
  - **Amorphic mutation**: When the allele has a complete loss of function (null allele). Phenotypes associated with such mutations are most often recessive.
  - **Haploinsufficiency**: when the reduced dosage of a normal gene product is not enough for a normal phenotype.
  - **Gain-of-function mutations** changes the gene product resulting in gain of new and abnormal function. These mutations usually have dominant phenotypes. Often called a neomorphic mutation.
  - **Dominant negative mutations** (also called antimorphic mutations) have an altered gene product that acts antagonistically to the wild-type allele. These mutations usually result in an altered molecular function (often inactive) and are characterised by a dominant or semi-dominant phenotype. In humans, Example: Marfan syndrome. In this condition, the defective glycoprotein product of the fibrillin gene (FBN1) antagonizes the product of the normal allele.
  - **Lethal mutations** are mutations that lead to the death of the organisms which carry the mutations.
  - A back mutation or reversion is a point mutation that restores the original sequence and hence the original phenotype.

Classification by effect on fitness

- **A harmful mutation** is a mutation that decreases the fitness of the organism.
- **A beneficial mutation** is a mutation that increases fitness of the organism, or which promotes traits that are desirable. Genetic drift is the basis for most variation at the molecular level (neutral theory of molecular evolution).
- **A neutral mutation** has no harmful or beneficial effect on the organism. Such mutations occur at a steady rate.
- **A deleterious mutation** has a negative effect on the phenotype, and thus decreases the fitness of the organism.
- **An advantageous mutation** has a positive effect on the phenotype, and thus increases the fitness of the organism.
- **A nearly neutral mutation** is a mutation that may be slightly deleterious or advantageous, although most nearly neutral mutations are slightly deleterious.

Classification by inheritance

- **Heritable mutation**: in tissue or cells on path to be changed to gametes.
  - By pattern of inheritance the human genome contains two copies of each gene – a paternal and a maternal allele.
  - **A heterozygous mutation** is a mutation of only one allele.
  - **A homozygous mutation** is an identical mutation of both the paternal and maternal alleles.
  - **Compound heterozygous mutations** or a **genetic compound** comprises two different mutations in the paternal and maternal alleles.
  - **Non inheritable somatic** (e.g., carcinogenic mutation)
  - **A wild type or homozygous non-mutated** organism is one in which neither allele is mutated.

By inheritance ability

- **Somatic mutations** (also called acquired mutations) which involve cells outside the dedicated reproductive group and which are not usually transmitted to descendants.
- **Germ line mutations**: which can be passed on to descendants through their reproductive cells. A germline mutation gives rise to a constitutional mutation in the offspring, that is, a mutation that is present in every cell.
- A new mutation that was not inherited from either parent is called a **de novo mutation**.
Classification by impact on protein sequence

- Frameshift mutation
- Nonsense mutation
- Missense mutation
- Neutral mutation
- Silent mutation (synonymous mutation)

A frameshift mutation is a mutation caused by insertion or deletion of a number of nucleotides (not triplet), the insertion or deletion can disrupt the reading frame, or the grouping of the codons, resulting in a completely different translation from the original. The earlier in the sequence the deletion or insertion occurs, the more altered the protein produced is.

In contrast, any insertion or deletion that is evenly divisible by three is termed an in-frame mutation.

Mutations: Additions

A frame shift mutation

Normal gene

\[
\text{GGTCTCCTCAGCCA} \\
\text{CCAGAGGAGUGCGGU}
\]

Codons

Pro-Glu-Glu-Cys-Gly

Amino acids

Addition mutation

\[
\text{GGTCTCCTCAGCCA} \\
\text{CCACGAGGAGUGCGGU}
\]

Codons

Pro-Arg-Gly-Val-Arg

Mutations: Deletions

A frame shift mutation

Normal gene

\[
\text{GGTCTCCTCAGCCA} \\
\text{CCAGAGGAGUGCGGU}
\]

Codons

Pro-Glu-Glu-Cys-Gly

Amino acids

Deletion mutation

\[
\text{GGTC/CCTCAGCCA} \\
\text{CCAGAGGAGUGCGGU}
\]

Codons

Pro-Gly-Ser-Ala-Val

A nonsense mutation is a point mutation in a sequence of DNA that results in a premature stop codon, or a nonsense codon in the transcribed mRNA, and possibly a truncated, and often nonfunctional protein product.

Nonsense mutation: Disaster

Normal gene

\[
\text{GGTCTCCTCAGCCA} \\
\text{CCAGAGGAGUGCGGU}
\]

Codons

Pro-Glu-Glu-Cys-Gly

Amino acids

Substitution mutation

\[
\text{GGTCTCCTA*C} \\
\text{CCAGAGGAGUGAGGU}
\]

Codons

Pro-Glu-Glu-STOP
**Missense mutations** or **nonsynonymous mutations** are types of point mutations where a single nucleotide is changed to cause substitution of a different amino acid. This in turn can render the resulting non-functional protein.

**Mutations: Substitutions**

<table>
<thead>
<tr>
<th>Normal gene</th>
<th>Substitution mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGTCTCTCACGCCA</td>
<td>GTGACCTCACGCCA</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>CCAGAGGAGUCGGU</td>
<td>CCAGUGAGUGCGGU</td>
</tr>
<tr>
<td>Codons</td>
<td>Codons</td>
</tr>
<tr>
<td>Pro-Glu-Glu-Cys-Gly</td>
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</tr>
</tbody>
</table>

Amino acids

Substitutions will only affect a single codon. Their effects may not be serious unless they affect an amino acid that is essential for the structure and function of the finished protein molecule (e.g., sickle cell anaemia).

**Classification by impact on protein sequence**

- **Silent mutations** (synonymous mutation) are mutations that do not result in a change to the amino acid sequence of a protein.
- They may occur in a region that does not code for a protein, or they may occur within a codon in a manner that does not alter the final amino acid sequence.
- synonymous mutations are a subcategory, occurring only within exons.

- **A neutral mutation** is a mutation that occurs in an amino acid codon which results in the use of a different, but chemically similar, amino acid.
- The similarity between the two is enough that little or no change is often rendered in the protein. For example, a change from AAA to AGA will encode arginine, a chemically similar molecule to the intended lysine.

**Loss of heterozygosity (LOH)**

- Loss of heterozygosity (LOH) in a cell is the loss of normal function of one allele of a gene in which the other allele was already inactivated.
- This term is mostly used in the context of oncogenesis; after an inactivating mutation in one allele of a tumor suppressor gene occurs in the parent's germline cell, it is passed on to the zygote resulting in an offspring that is heterozygous for that allele.
- In oncology, loss of heterozygosity occurs when the remaining functional allele in a somatic cell of the offspring becomes inactivated by mutation.
- This could cause a normal tumor suppressor to no longer be produced which could result in tumorigenesis.
Mutations in untranslated regions:

- **Transcribed**: Mutations that occur in transcribed but untranslated regions might still affect the translation system by affecting the recognition signal for binding of ribosomes. They might conceivably affect mRNA stability, attenuation, and, where the gene product is an RNA, mutations might cause a loss of product function or cause improper processing or modification of the product.

- **Untranscribed regions**: Mutations in regions that are neither transcribed nor translated might affect either transcriptional start or stop signals and thus the regulation of the region in question. It is also possible they might affect "structural" regions of the DNA, affecting gene expression indirectly.

Splice site mutations - Introns must be spliced from mRNA to produce the correct protein. This process must be carried out very accurately and it is guided by the nucleotide signals at the splice sites. If a mutation alters these signals, the intron may not be removed and an incorrect protein will be produced.

Biochemical and molecular basis of single-gene disorders

- Enzyme defects and their consequences
- Defects in receptors and transport systems
- Alterations in structure, function or quantity of nonenzyme proteins
- Genetically determined adverse reactions to drugs.

Enzyme Defects and Their Consequences

- Accumulation of the substrate
- Metabolic block and decreased amount of the product (± lack of feedback inhibition)
- Failure to inactivate a tissue damaging substance
**ENZYME DEFECTS**
- Defective enzyme with reduced activity
- Reduced amount of normal enzyme, metabolic block and decreased amount of the product (a lack of feedback inhibition)
- The consequence is a metabolic block, accumulation of the substrate
- **DECREASED END PRODUCTS**
- End product is a feedback inhibitor of the enzyme involved in the early reactions
- Deficiency of the end product → overproduction of the intermediates and their catabolic products
- Some may be injurious at higher concentrations
- **ALBINISM**
- Deficiency of tyrosinase → deficiency of melanin from its precursor tyrosine

**DECREASED END PRODUCTS**
- Accumulation of the substrate
- End product is a feedback inhibitor of the enzyme involved in the early reactions

**INACTIVATION OF TISSUE DAMAGING SUBSTRATE**
- Failure in inactivation of a tissue damaging substrate
- α1-ANTITRYPSIN DEFICIENCY → inability to inactivate neutrophil elastase in the lung → destruction of elastin in the walls of alveoli → pulmonary emphysema

**DEFECTS IN MEMBRANE RECEPTORS IN TRANSPORT SYSTEMS**
- Receptor mediated endocytosis
- Transport protein
- **FAMILIAL HYPERCHOLESTEROLEMIA**
- Reduced synthesis or function of low density lipoproteins (LDL) receptors → defective transport of LDL into the cells → excessive cholesterol synthesis by complex intermediary mechanisms

**Familial Hypercholesterolemia**
- Possibly the most frequent Mendelian disorder, with a gene frequency of 1:500
- Results from a mutation of the gene encoding the low density lipoprotein (LDL) receptor
- Heterozygotes
  - 2-3x elevation of serum cholesterol
  - tendon xanthomas and premature atherosclerosis in early adulthood
- Homozygotes
  - 5-6x elevation of serum cholesterol
  - tendon xanthomas and premature atherosclerosis develop earlier
  - may have myocardial infarction by age 20 years

**Alterations in Structure, Function or Quantity of Nonenzyme Proteins**
- **Hemoglobinopathies**
  - sickle cell disease – abnormal β-chain
- **Thalassemias**
  - decreased synthesis α or β chains of hemoglobin
- **Abnormal Structural Proteins**
  - collagen – Ehlers-Danlos syndrome
  - elastin – Marfan’s syndrome
- **Muscular dystrophies**

**Disorders associated with defects in structural proteins**
- **Marfan syndrome**
  - A disorder of the connective tissues of the body, manifested principally by changes in the skeleton, eyes, and cardiovascular system.
  - 70% to 85% of cases are familial and show autosomal dominant inheritance
  - the remainder are sporadic and arise from new mutations
- **Pathogenesis**
  - defect in extra cellular glycoprotein fibrillin-1, which forms a scaffolding for deposition of elastin fibers
  - more than 500 distinct mutations in FBN1 gene are known, most resulting in an abnormal protein
  - this abnormal protein disrupts assembly of microfibrils – dominant negative.
Mutations resulting in unusual reactions to drugs

- Glucose-6-phosphate dehydrogenase (G6PD)
  - G6PD activity is necessary to protect the red blood cell from oxidative stress
  - Drugs that block G6PD (e.g., primaquine) can cause severe hemolysis in patients who lack this enzyme
- G6PD deficiency → Antimalarial → Severe hemolysis

- Cytochrome P450 enzymes
  - Used by the liver to metabolize many drugs
  - Changes in CYP enzyme levels affect drug metabolism.

PEDIGREE CHART

- Drawing a family history (pedigree) chart is a helpful shorthand method of documenting affected relatives.
- Identifying patterns of inheritance in families, and
- Identifying those at risk for genetic conditions.

Powerful tools in human genetic studies is pedigree analysis.
- Standard symbols for the construction of pedigrees.

POSSIBLE COMBINATION OF GAMETS

<table>
<thead>
<tr>
<th>A</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>AB</td>
</tr>
<tr>
<td>O</td>
<td>AO</td>
</tr>
</tbody>
</table>

Punnet square

Mating diagram
Pedigree chart

and

Punnet square

are NOT SAME

Spontaneous mutation

Spontaneous mutations on the molecular level can be caused by:

- **Tautomerism** — A base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base, resulting in incorrect base pairing during replication.
- **Depurination** — Loss of a purine base (A or G) to form an apurinic site (AP site).
- **Deamination** — Hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include C → U and A → HX (hypoxanthine), which can be corrected by DNA repair mechanisms; and 5MeC (5-Methylcytosine) → T, which is less likely to be detected as a mutation because thymine is a normal DNA base.
- **Slipped strand mispairing** — Denaturation of the new strand from the template during replication, followed by renaturation in a different spot (“slipping”). This can lead to insertions or deletions.

Error prone replication by-pass

There is increasing evidence that the majority of spontaneously arising mutations are due to error prone replication (translesion synthesis) past a DNA damage in the template strand. As described in the article DNA damage (naturally occurring), naturally occurring DNA damages arise about 60,000 to 100,000 times per day per mammalian cell. In mice, the majority of mutations are caused by translesion synthesis. Likewise, in yeast, Kunz et al. found that more than 60% of the spontaneous single base pair substitutions and deletions were caused by translesion synthesis.

Errors introduced during DNA repair

- Although naturally occurring double-strand breaks occur at a relatively low frequency in DNA (see DNA damage (naturally occurring)), their repair often causes mutation. **Non-homologous end joining** (NHEJ) is a major pathway for repairing double-strand breaks. NHEJ involves removal of a few nucleotides to allow somewhat inaccurate alignment of the two ends for rejoining followed by addition of nucleotides to fill in gaps. As a consequence, NHEJ often introduces mutations.

Thank you
Induced mutation

- Induced mutations on the molecular level can be caused by:
  - Chemicals
    - Hydroxylamine: \( \text{NH}_2\text{OH} \)
    - Base analogs (e.g., BrdU)
    - Alkylating agents (e.g., N-ethyl-N-nitrosourea)
  - Agents that form DNA adducts (e.g., ochratoxin A metabolites)
  - DNA intercalating agents (e.g., ethidium bromide)
  - DNA crosslinkers
  - Oxidative damage
    - Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns, which leads to incorrect base pairing during replication.
- Radiation
  - Ultraviolet radiation (nonionizing radiation).
  - Two nucleotide bases in DNA — cytosine and thymine — are most vulnerable to radiation that can change their properties.
  - UV light can induce adjacent pyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer.
  - UV radiation, in particular longer-wave UVA, can also cause oxidative damage to DNA.

Somatic mutations

- Main article: Loss of heterozygosity
- See also: Carcinogenesis
  - A change in the genetic structure that is not inherited from a parent, and also not passed on offspring, is called a somatic cell genetic mutation or acquired mutation.
- Cells with heterozygous mutations (one good copy of gene and one mutated copy) may function normally with the unmutated copy until the good copy has been spontaneously somatically mutated. This kind of mutation happens all the time in living organisms, but it is difficult to measure the rate. Measuring this rate is important in predicting the rate at which people may develop cancer.
- Point mutations may arise from spontaneous mutations that occur during DNA replication. The rate of mutation may be increased by mutagens. Mutagens can be physical, such as radiation from UV rays, X-rays or extreme heat, or chemical (molecules that misplace base pairs or disrupt the helical shape of DNA). Mutagens associated with cancers are often studied to learn about cancer and its prevention.

Mutations: Inversion

Inversion mutations, also, only affect a small part of the gene.

Normal gene

\[
\begin{align*}
\text{GGTCCTCAGCCCA} & \quad \text{GTGCTCTCACGCCA} \\
\text{CCAGAGAGUGCGGU} & \quad \text{CCAGGAGAGUGCGGU}
\end{align*}
\]

Codons

\[
\begin{align*}
\text{Pro-Glu-Glu-Cys-Gly} & \quad \text{Pro-Gly-Glu-Cys-Gly}
\end{align*}
\]

Amino acids

Mutations of haemoglobin

- Haemoglobin is a tetramer = 2 \( \alpha \) and 2 \( \beta \) chains.
- The genes for these polypeptides are found on different chromosomes.
- The \( \beta \) chain gene is found on chromosome 11.
- The \( \alpha \) chain gene is found on chromosome 16.
- The nucleotide sequences have been worked out.
- Several inherited diseases occur on the \( \beta \) chain, which contains 146 amino acids.

\( \beta \) haemoglobin sense strand cDNA sequence

- cDNA (complementary DNA) is obtained by back-transcribing the mRNA used to translate the polypeptide.
- So cDNA has no introns.
- This is done using reverse transcriptase enzyme.

Methionine initiator

\[
\begin{align*}
\text{ATG} & \quad \text{GTG} & \quad \text{CAT} & \quad \text{CTG} & \quad \text{ACT} & \quad \text{CCT} & \quad \text{GAG} & \quad \text{GAG} \\
\text{GAT} & \quad \text{ACC} & \quad \text{CTA} & \quad \text{AGGTAC} & \quad \text{ACTACGTGAT} & \quad \text{CTGAT} & \quad \text{GGCGAGTAC} & \quad \text{ACTGATACCATGTCGCACGCACGACCACTGATCTGAGTGAGGAGGACGGTCTGCTG}
\end{align*}
\]

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<table>
<thead>
<tr>
<th>Mutation</th>
<th>Codon</th>
<th>Change to DNA sense strand</th>
<th>Change in Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (sickle cell anaemia)</td>
<td>6</td>
<td>GAG to GTG</td>
<td>Glu to Val</td>
</tr>
<tr>
<td>C (cooley’s syndrome)</td>
<td>6</td>
<td>GAG to AAG</td>
<td>Glu to Lys</td>
</tr>
<tr>
<td>G_{San Jose}</td>
<td>7</td>
<td>GAG to GGG</td>
<td>Glu to Gly</td>
</tr>
<tr>
<td>E</td>
<td>26</td>
<td>GAG to AAG</td>
<td>Glu to Lys</td>
</tr>
<tr>
<td>M_{Makulwaki}</td>
<td>63</td>
<td>CAT to TAT</td>
<td>His to Tyr</td>
</tr>
<tr>
<td>O_{Arabia}</td>
<td>67</td>
<td>GTG to GAG</td>
<td>Val to Glu</td>
</tr>
<tr>
<td>O_{Miwauki}</td>
<td>121</td>
<td>GAA to GTA</td>
<td>Glu to Val</td>
</tr>
</tbody>
</table>

**Sickle Cell Anaemia**

**Children inherit traits from their parents. The study of the inheritance of these characteristics forms the basis of human genetics.**

**Earlobe attachment**

- If earlobes hang free, they are detached.
- If they connect directly to the sides of the head, they are attached.
- Earlobe attachment is a continuous trait: while most earlobes can be neatly categorized as attached or unattached, some are in-between.

**Observable Human Characteristics**

- We are all UNIQUE.
- Even though we share some characteristics with our peers and our family members, every one of us has a unique combination of traits.
- Some traits are controlled by genes that pass from parent to child.
- Others are acquired through learning.
- But most are influenced by a combination of genes and environmental factors.
- Some examples of variable traits that are easy to observe are shown in next slides:
Tongue Rolling
Dimples
Wet (dominant) or dry (recessive) earwax

PTC tasting (phenylthiocarbamide)
To about 75% of us, PTC (phenylthiocarbamide) tastes very bitter.
For the other 25%, it is tasteless.
The ability to taste PTC is controlled mainly by a single gene that codes for a bitter-taste receptor on the tongue.

Different variations (alleles) of this gene control whether PTC tastes bitter or not.
PTC tasting follows a very predictable pattern of inheritance.
Tasting is dominant, meaning that if you have at least one copy of the tasting version of the gene, you can taste PTC.
Non-tasters have two copies of the non-tasting allele.

PTC tasting (phenylthiocarbamide)

PTC tasting

Dad has all "tasting" receptors. He can taste PTC.
Mom has all "non-tasting" receptors. She cannot taste PTC.
The kids have both "tasting" and "non-tasting" receptors. They can taste PTC.
Gregor Mendel (1822-1844)

My scientific studies have afforded me great gratification; and I am convinced that it will not be long before the whole world acknowledges the results of my work.

Gregor Mendel

MENDELISM

1866 Gregor Mendel ► inheritance of "factors" in pea plants.
- Mendel’s laws
  ► based on mathematical probabilities
  ► Predictions of resulting phenotypes when certain crosses were made in the garden pea
- Mendel postulated dominant and recessive traits in heredity.

Gregor Mendel experiment

Mendel’s laws-
- Unit inheritance (Uniformity): Blending of characters do not occur (character of parents may not be expressed in F1, could reappear in later generations)
- Law of segregation: Members of a single pair of characteristics (genes) always segregate and pass to different gametes.
- Independent assortment: Members of different gene pairs assort to the gametes independently i.e. there is random recombination of the paternal and maternal chromosomes in the gametes.

Relating mendelism with genetic disorders: Alkaptonuria and Inborn Errors of Metabolism

- 1908 Sir Archibald Garrod
  ► Proposed “inborn errors of metabolism” -lack of a specific enzyme.
  ► Recurrence patterns in several families followed an autosomal recessive pattern of inheritance, and
  ► Postulated that it was caused by a mutation in a gene encoding an enzyme involved in the metabolism of alkaptons.
Single gene disorders

A single gene disorder is the result of a single mutated gene.

There are estimated to be over 4000 human diseases caused by single gene defects.

Single gene disorders can be passed on to subsequent generations in several ways.

Certain conditions may affect inheritance patterns.

Single gene disorders can be passed on to subsequent generations in several ways.

Prevalence of some single gene disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal recessive</td>
<td></td>
</tr>
<tr>
<td>Sickle cell anemia</td>
<td>1 in 625</td>
</tr>
<tr>
<td>(African Americans)Cystic fibrosis</td>
<td>1 in 2,000</td>
</tr>
<tr>
<td>(Caucasians)Tay-Sachs disease</td>
<td>1 in 3,000</td>
</tr>
<tr>
<td>(American Jews)Phenylketonuria</td>
<td>1 in 12,000</td>
</tr>
<tr>
<td>Mucopolysaccharidoses</td>
<td>1 in 25,000</td>
</tr>
<tr>
<td>Glycogen storage diseases</td>
<td>1 in 50,000</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>1 in 57,000</td>
</tr>
</tbody>
</table>

Prevalence of some single gene disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal dominant</td>
<td></td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>1 in 500</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>1 in 1250</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>1 in 2,500</td>
</tr>
<tr>
<td>Hereditary spherocytosis</td>
<td>1 in 5,000</td>
</tr>
<tr>
<td>Marfan syndrome</td>
<td>1 in 20,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-linked recessive</td>
<td></td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>1 in 7,000</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>1 in 10,000</td>
</tr>
</tbody>
</table>

A gene codes for a protein

<table>
<thead>
<tr>
<th>DNA</th>
<th>mRNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCTGAGCAACTATTGATGAA</td>
<td>CTGAGCAACTATTGATGAA</td>
<td>PEPTIDE</td>
</tr>
</tbody>
</table>

20 AMINO ACIDS numerous proteins
DNA
Deoxyribonucleic Acid

Nucleotides are the building blocks of DNA. They contain 4 nitrogen-carbon-hydrogen bases that bond to form specific pairs: adenine can only pair with thymine; cytosine can only pair with guanine. The combination of base pairs cannot vary.

Nucleotide

DNA STRUCTURE

hydrogen bonded nucleotides on opposite helices
DNA helices are antiparallel
carbons on sugar define ends... 5’ and 3’
pyrimidines bond with purines
T - A
C - G

DNA REPLICATION

model of replication proposed by Watson & Crick (1953)
parental strand = template
semiconservative model (new double helix has 1 template + 1 new daughter strand)
replication fork

A sequence of three bases (coding for an amino acid is codon):

codon

1 codon = 1 amino acid
20 Amino acids are codified by:

<table>
<thead>
<tr>
<th>DNA sequence</th>
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</thead>
<tbody>
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</tr>
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<td>DNA sequence</td>
</tr>
</tbody>
</table>

**Genes**

- **GENE**: Unique sequence DNA that codes for a protein which give rise to a phenotype.
- The basic unit of genetic information.
- They determine the nature and the function of the cell.
- A **genome** is the full set of genes in each cell of an organism.

**Gene**

- There are two general types of gene in the human genome:
  - **non-coding RNA genes**
    - represent 2-5 per cent
    - encode functional RNA molecules
  - **protein-coding genes**
    - represent the majority
    - expressed in two stages: transcription and translation.
    - show incredible diversity in size and organisation
    - have no typical structure.
    - However, several conserved features.
- The boundaries of a protein-encoding gene are defined as the points at which transcription begins and ends.
- The core of the gene is the coding region.
- The coding region begins with the initiation codon, which is normally ATG.
- It ends with one of three termination codons: TAA, TAG or TGA.
- On either side of the coding region are DNA sequences that are transcribed but are not translated.
Both the coding region and the untranslated regions may be interrupted by introns.

Most human genes are divided into exons and introns.

The exons are the sections that are found in the mature transcript (messenger RNA).

The introns are removed from the primary transcript by a process of splicing.

Definitions:

- **Allele**: Alternative forms of both normal and abnormal genes
  - may be variations of normal e.g., blood group alleles
    - Three alleles in ABO blood group: IA, IB, and IO
  - may result in a medical disorder e.g., cystic fibrosis, hemophilia, Marfan disease

- **Locus**: The physical location of gene on a chromosome
  - It is fixed
    - Since human chromosomes are paired, individuals have two alleles at two loci, one on each chromosome.
    - The ABO locus is located on chromosome 9

- **Genotype**: Genetic constitution of an individual, which is the specific allelic makeup of an individual.

- **Phenotype**: Observed expression of a gene, is the end result of the genetic and environmental factors.
ABO Blood group

- Three alleles, \(I^A\), \(I^B\), and \(I^O\)
- Any individual has one of six possible genotypes (AA, AO, BB, BO, AB, and OO)
- One of four possible phenotypes: "A-B-AB-O"

HOMOZYGUS: Conditions having identical allele at one locus, which can be either normal or abnormal. Both alleles the same \([dd, DD]\)

HETEROZYGUS: Two different alleles at one locus. Usually one normal and one abnormal or mutant allele. Alleles are different \([Dd]\)

Hemizygous: only one copy (genes on the X chromosome in males)

Trait: observed expression of the gene

DOMINANT CONDITION: A single copy of the allele is enough for the condition to be expressed. It is seen both in heterozygote and the homozygote.

RECESSIVE CONDITION: Seen in homozygote. The allele must be present in both chromosomes.

Compound heterozygote: Two different mutant allele at one locus.

Double heterozygote: Two mutant alleles that are each at a different locus.

HETEROGENEITY

Genetic heterogeneity (locus heterogeneity): mutations of different genes causing the same disease
Example: Retinitis pigmentosa has autosomal dominant, autosomal recessive, and X-linked origins.

Clinical heterogeneity (allelic heterogeneity)
- The phenomenon in which different mutations at the same locus causes a similar phenotype.
Example: \(\beta\)-thalassemia may be caused by several different mutations in the \(\beta\)-globin gene.

Phenotypic heterogeneity: a mutation within the same gene causes a different phenotype.
Example: mutations in the \(RET\) gene have been implicated in the etiology of Hirschprung disease as well as multiple endocrine neoplasia (MEN) Type 2.

SINGLE GENE DISORDERS

Prof. M. Kamal
Pathology, BSMMU
May 2014
Objectives

- Distinctions between major patterns of single gene inheritance
  - Autosomal dominant, autosomal recessive, sex-linked recessive, sex-linked dominant
- Factors which complicate inheritance patterns

Wild-type (normal) allele: prevailing version, present in majority of individuals

Mutant allele: usually rare, differ from wild-type allele by mutation

Mutation: permanent change in nucleotide sequence or arrangement of DNA

Polymorphism: Natural variations in a gene, DNA sequence, or chromosome that have no adverse effects on the individual and occur with fairly high frequency in the general population. \( \geq 2 \) relatively common (each > 1% in population) alleles at a locus in the population

Dominant trait - a trait that shows in a heterozygote

Recessive trait - a trait that is hidden in a heterozygote

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Homozygous - Having two identical alleles at a particular locus, usually in reference to two normal alleles or two disease alleles.

Heterozygous - Having two different alleles at a particular locus, usually in reference to one normal allele and one disease allele.

Compound heterozygous - Having two different mutant alleles of the same gene, rather than one normal and one mutant.

Single-gene traits are often called ‘Mendelian’

Like the garden peas studied by Gregor Mendel, they occur in fixed proportions among the offspring of specific types of mating.

Patterns of Single Gene Inheritance depend on 2 factors:
1. Whether the gene is on an autosome or a sex chromosome
2. Whether the phenotype is dominant or recessive

Thus, there are 4 basic patterns of single gene mendelian inheritance:
1. Autosomal Recessive
2. Autosomal Dominant
3. X-linked Recessive
4. X-linked Dominant

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AUTOSOMAL DOMINANT INHERITANCE

- General features:
  - The trait appears in every generation without skipping.
  - Every affected child has an affected parent.
    - Most common scenario in clinical practice: Heterozygote affected mate with normal homozygote person. In this situation 50% of the child will inherit the trait.
  - Unaffected do not transmit the trait.
  - Both sexes are affected equally.
  - The defective product of the gene is usually a structural protein, not an enzyme.

AUTOSOMAL DOMINANT DISORDERS

Autosomal dominant disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency/10,000 births</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant otosclerosis</td>
<td>30</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>20</td>
</tr>
<tr>
<td>von Willebrand disease</td>
<td>10</td>
</tr>
<tr>
<td>Adult polycystic kidney disease</td>
<td>10</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>5</td>
</tr>
<tr>
<td>Neurofibromatosis</td>
<td>4</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
<td>2</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>1</td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>1</td>
</tr>
<tr>
<td>Dominant blindness</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total (of all dominant disorders)</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Factors Which May Alter Presentation of AD Pedigree

- New mutations e.g. Achondroplasia
- Reduced penetrance e.g. polydactyly
- Variable expressivity e.g. Neurofibromatosis
- Genetic heterogeniety e.g. Sensinnuronal deafness
- Phenocopy e.g. Conradi syndrome Vs. Warfarin embryopathy
- Epistasis e.g. Bombay blood group
- Pleiotropy e.g. Marfan Syndrome, Porphyria
- Variation due to sex e.g. Huntington’s disease
Lethal alleles

- Some allele combinations are lethal.
- Mexican hairless dogs result from a mutation in a gene that shows lethality hh, hH, HH
- Achondroplasia

Penetrance

Penetrance: The proportion or percentage of a given genotype that display the expected phenotype under given environmental conditions.

Incomplete penetrance: Failure of a genotype to be expressed with the phenotype normally associated with it.

Examples: Observe 100 individuals of a given genotype/phenotype and 63 exhibit the expected phenotype. The penetrance is 63% and is termed incomplete.

Reduced penetrance

Expressivity

Expressivity: Range of phenotypes that can be expressed by a given genotype under specified environmental conditions.

Variable expressivity: Variation in phenotypic expression. A phenotype that varies in intensity.

Examples: Neurofibromatosis

Variable Expressivity: Neurofibromatosis

Co-dominance: ABO blood gr., HLA genes

Intermediate inheritance: Sickle cell trait

Multiple alleles: An individual has two alleles, but a population can have many alleles within the individual members.

<table>
<thead>
<tr>
<th>Gene Genotype</th>
<th>Phenotype (Blood group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAB OO</td>
<td>O</td>
</tr>
<tr>
<td>AO / AA</td>
<td>A</td>
</tr>
<tr>
<td>BB / BO</td>
<td>B</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
</tr>
</tbody>
</table>
**Epistasis**

- the masking of the action of an allele of one gene by the allelic combinations of another gene.
- the interaction of nonallelic genes in the formation of the phenotype.

Example: Bombay blood group

**Bombay blood group**

H gene is epistatic to the ABO gene.

- H protein attaches the A or B protein to the cell surface.
- hh genotype = no H protein. All ABO genotypes appear as type O.

**Pleiotropy**

Several apparently unrelated phenotypic effects caused by a single gene. Usually means that a genes is involved in multiple processes. Different subset of symptoms in different individuals.

**Examples: Marfan Syndrome**

**Porphyria**

**Phenocopy**

A trait caused by the environment that appears inherited.

- environmental influence cause an effect similar to a phenotype under genetic control.

Exposure to teratogens

- Thalidomide causes limb defects akin to rare inherited phocomelia.

Infection

- Rubella in pregnant mothers causes deafness mimicking inherited forms of deafness.
Blonde Hair Colour

Genetic heterogeneity
Different genes can produce identical phenotypes.

- Individuals with identical phenotypes may reflect different genetic causes.
  - Deafness
  - Albinism
  - Cleft palate
  - Poor blood clotting

Autosomal Recessive Inheritance

- A recessive trait only becomes phenotypically apparent when two copies of a gene (two alleles i.e. homozygous) are present.

Autosomal Recessive Inheritance

- Rare traits appear characteristically in siblings.
- Parents and relatives are normal.
- Commonest clinical scenario: Mating of 2 heterozygotes where segregation frequency is 25-50-25
- Both sexes are affected in equal number
- For rare traits, chance of finding parental consanguinity is increased
- All children of two affected parents are affected
Cystic fibrosis

Phenotype
- production of thick secretions – often block the ducts from which they are extruded
- often malnourished and many respiratory infections
- eventually cysts form in the pancreas and it degenerates
- individuals are often infertile

Glycogen Storage Diseases are genetic enzyme deficiencies associated with excessive glycogen accumulation within cells.

Some enzymes whose deficiency leads to glycogen accumulation are part of the interconnected pathways shown here.

X-linked recessive

- Special features: Sporadic case may be due to new mutation. Heterozygous females - subtle clinical features, int. enzyme levels.
- Heterogeneity: Albinism as AR, Ocular albinism as X linked.
- Example: Duchenne muscular dystrophy, Haemophilia, Becker muscular dystrophy, Lesch-Nyhan syndrome.

X LINKED RECESSIVE INHERITANCE

- Incidence is much higher in males than females
- The trait is passed from an affected man through all his daughter to average half of their sons.
- Trait never transmitted directly from father to son
- Trait may be transmitted through a series of carrier females
- Carries show variable expression of the trait.

X-linked recessive pedigree
Duchenne Muscular Dystrophy

- XLR
- Affects one in 3500 to 5000 newborn males
- 1/3 of these with previous family history
- 2/3 sporadic
- Progressive muscle weakness
- Defects in muscle proteins
- Death of muscle tissue
- Mother carries the recessive gene and passes it to her child
- Trait is usually expressed in males only

X LINKED DOMINANT INHERITANCE

- Affected male have no normal daughter & no affected son.
- Affected heterozygous female transmit the condition to ½ their children of either sex.
- Affected homozygous female transmit to all their children.
- Affected females are more common than affected males.
- Examples: Xg blood group systems, Vit. D resistant rickets, Browning of the enamel of the teeth, Albright’s hereditary osteodystrophy, Taybi Syndrome

XD Pedigree

SEX LIMITED INHERITANCE

- In some X-linked recessive diseases (Duchenne muscular dystrophy) expression of the disease phenotype is limited exclusively to males.
- In some X-linked dominant traits, such as incontinentia pigmenti expression is limited to females, males do not survive to term.
- There are autosomal diseases that are limited to expression in only one sex e.g. Precocious puberty and beard growth (expressed only in males), hereditary form of prolapsed uterus in females

Sex-Influenced Traits

Some traits appear to be specific to one sex, but are not sex-linked: their genes are not on the X chromosome.

- Trait that is dominant in one sex, but recessive in the other is a sex-influenced trait.
- E.g. male pattern baldness.
- Baldness is dominant in males: heterozygotes and homozygotes both become bald.
- In females, baldness is recessive: only homozygotes become bald. Also, a sparse hair pattern rather than completely baldness.
SINGLE GENE DISEASES THAT DO NOT FOLLOW MENDEL’S LAW

Age of Onset and Other Factors Affecting Pedigree Patterns
Age of Onset
- Not all genetic disorders are congenital; many are not expressed until later in life, some at a characteristic age and others at variable ages.
- A genetic disorder is determined by genes, a congenital disease is that present at birth and may or may not be genetic.
  - Many genetic disorders develop prenatally and thus are both genetic and congenital (e.g., osteogenesis imperfecta).
  - Some may be lethal in prenatal life.
  - Others expressed as soon as the infant begins independent life.
  - Others appear later, at a variety of ages (from birth to post-reproductive years).

Other Factors Affecting Pedigree Patterns
- Small family size: the patient may be the only affected member → the inheritance pattern may not be immediately apparent.
- New mutation: is a frequent cause of AD and X-linked disease.
- Diagnostic difficulties: owing to absent or variable expression of the gene.
- Other genes and environmental factors: may affect gene expression.
- Persons of some genotypes may fail to survive to time of birth.
- Accurate info. about presence of disorder in relatives or about family relationships may be lacking.

Genetic Heterogeneity
- Genetic heterogeneity: includes a number of phenotypes that are similar but are actually determined by different genotypes. May be due to allelic heterogeneity, locus heterogeneity, or both.
  - Allelic heterogeneity: different mutations at the same locus.
  - Locus heterogeneity: mutations at different loci.
- Recognition of genetic heterogeneity is an important aspect of clinical diagnosis and genetic counseling.

Locus Heterogeneity
- Pedigree analysis may be sufficient to demonstrate locus heterogeneity.
- Example-1, retinitis pigmentosa
  - A common cause of visual impairment due to photoreceptor degeneration associated with abnormal pigment distribution in retina.
  - Known to occur in AD, AR, and X-linked forms.
- Example-2, Ehndlers-Danlos syndrome,
  - Skin & other connective tissues may be excessively elastic or fragile, defect in collagen structure.
  - May be AD, AR, or X-linked.
  - At least 10 different loci involved.

Allelic Heterogeneity
- An important cause of clinical variation.
- Sometimes, different mutations at same locus → clinically indistinguishable or closely similar disorders.
- In other cases, different mutant alleles at same locus → very different clinical presentations.
- Example-1: RET gene (encodes a receptor tyrosine kinase)
  - Some mutations cause dominantly inherited failure of development of colonic ganglia → defective colonic motility and severe chronic constipation (Hirschsprung disease).
  - Other mutations in same gene → dominantly inherited cancer of thyroid and adrenal gland (multiple endocrine neoplasia).
  - A third group of RET mutations → both Hirschsprung disease and multiple endocrine neoplasia in the same individual.
In fact, unless they have consanguineous parents, most people with autosomal recessive disorders are more likely to have compound rather than truly homozygous genotypes. Because different allelic combinations may have somewhat different clinical consequences, one must be aware of allelic heterogeneity as one possible explanation for variability among patients considered to have same disease.

ALLELIC DISORDERS (Clinical heterogeneity). This is an extreme example of how different mutations in the same gene can cause divergent phenotypes, in which there are actually two different diseases caused by the same gene.

SINGLE GENE DISEASES THAT DO NOT FOLLOW MENDEL’S LAW

- Disorders due to triplet repeat mutation
- MITOCHONDRIAL INHERITANCE
- Uniparental Disomy and Genomic Imprinting
- Gonadal mosaicism

Disorders due to triplet repeat mutation

- Long repeating sequences of three nucleotides, in most cases C and G
- Examples: Fragile X syndrome (CGG), Myotonic dystrophy (CTG), Huntington’s disease (CAG)

FRAGILE SITES

- In the 1940’s, geneticists noticed that more males than females were mentally retarded.
- Among mentally retarded males, there is a subpopulation which shows a peculiar karyotype:
- Their X chromosomes are often broken at a particular site when their cells are cultured in media.

The site at which this happens is called the fragile X site and the gene involved is the FMR-1 gene.

The FMR-1 gene is in the long arm of the X chromosome at position Xq27.3.

This fragile site is associated with the second most common cause of mental retardation (behind Down’s syndrome).
Fragile X syndrome

- Familial mental retardation gene-1 (FMR-1) at Xq27.3 contains tandem repeats of CGG
- CGG repeats in normal persons 6 to 46
- In transmitting male & carrier female 50 to 230 (premutation)
- In affected persons 230 to 4000 (full mutation)

anticipation

is a phenomenon whereby the symptoms of a genetic disorder become apparent at an earlier age as it is passed on to the next generation.
In most cases, an increase of severity of symptoms is also noted.
Anticipation is common in trinucleotide repeat disorders such as Huntington's disease and myotonic dystrophy where a dynamic mutation in DNA occurs.

MITOCHONDRIAL INHERITANCE

- Almost all mitochondrial DNA is maternally inherited
- All children of an affected mother an affected & all children of affected father are normal
- mtDNA encodes enzymes involved in oxidative phosphorylation. Rich tissue are skeletal & cardiac muscle, kidney, CNS.
- Example: Kearns-Sayre synd., Laber's optic neuropathy, mitochondrial myopathy

Mitochondrial inheritance prdigeer
Mitochondrial inheritance

- Expression of disorders is quite variable because of uneven distribution of normal & mutant mtDNA in daughter cells.

Uniparental Disomy and Genomic Imprinting

- Uniparental disomy: Presence of two copies of a chromosome (or part of a chromosome) from one parent and none from the other.
- Discovered in 1988 in a child with cystic fibrosis and short stature who received two copies of the same chromosome 7 with a mutant CF gene from her carrier mother, and none from her noncarrier father.

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Genomic Imprinting

- Differential expression of genetic traits depending on whether it has been inherited from mother or father.
- Most regions of the genome are converted to gene products equally from the maternally and paternally derived members of a chromosome pair.

For a few specific regions, however, this is not true, and the genetic information in a portion of certain chromosomes is inactivated when inherited from one sex parent but not when inherited from the other.
- Only one copy of the genes is transcribed in imprinted regions, the other remains genetically silent (at least in somatic cells).

Prader-Willi syndrome (PWS)

- The firstrecognized example of uniparental disomy of an imprinted part of the genome
- A multiple congenital anomaly/mental retardation syndrome characterized by infantile hypotonia, feeding problems and failure to thrive, dysmorphia and hypogonadism followed by obesity, mental insufficiency and short stature.
- Prader-Willi syndrome results from the absence of the paternal contribution to long arm of chromosome 15 (either by deletion or maternal disomy) which is genetically active and necessary for normal development.

Pedigree of imprinted maternally expressed phenotype.
- The phenotype is expressed only when the mutant allele is inherited from the mother.
- Thus, mutant imprinted alleles can remain masked when they are paternally inherited, but clinically re-appear in one-half of children of carrier daughters.
Prader-Willi syndrome (PWS)

- Approximately 70% of affected individuals have a small deletion of the long arm of chromosome 15, always occurring in the paternally-derived chromosome 15.
- The remaining 30% of patients have maternal uniparental disomy for chromosome 15. That is, they have two otherwise normal copies of maternal chromosome 15 and no paternal 15.
- The paternal contribution is necessary because the homologous maternally derived genes are inactivated or imprinted (perhaps by methylation).

Angelman syndrome

- Angelman syndrome also involves imprinting of the same chromosome region - here the maternal contribution of the critical region is missing.
- The critical genetic region which determines Prader-Willi synd. is *maternally* imprinted (i.e. inactivated when inherited from the mother), whereas the critical region which determines Angelman synd. is *paternally* imprinted (i.e. inactivated when inherited from the father).
- Both disorders result when the expected active genetic contribution from one parent is missing, either by deletion or uniparental disomy.

Gonadal mosaicism

- Mosaicism is in the parent's ovaries or testes.
- Any individual ovum or sperm either has the mutation or not.
- Mutation in early post-zygotic cells can affect only cells destined to become gonads.
- A phenotypically normal parent who has germline or gonadal mosaicism can transmit the disease to the offspring through mutant gametes.
- Therefore, if conception involves one of these mutant sex cells, the resultant child will not be mosaic, but will simply have the genetic disease caused by that particular mutation.

First in the Family: VHL Mosaicism

- Mosaicism may explain why a DNA mutation can not be detected in a person who has VHL tumors and cysts, or why unaffected parents may have one or more affected children.
- VHL is generally inherited as an autosomal dominant trait.
- There are families in which a child with VHL has parents who do not have VHL. Some people with VHL do not have a VHL genetic mutation. And some unaffected parents are known to have more than one affected child.

Somatic Mosaicism. A portion of developing tissue will have the mutated VHL gene. Thus VHL may develop in some, but not all tissue sites.

Germline or Gonadal Mosaicism. Some of the egg or sperm cells have a VHL gene mutation.
SINGLE GENE DISEASES THAT DO NOT FOLLOW MENDEL’S LAW

Prof. Mohammed Kamal
Dept. of Pathology, BSMMU
May 2014

Disorders due to triplet repeat mutation
- Fragile X syndrome (CGG)
- Myotonic dystrophy (CTG)
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only one copy of the genes is transcribed in imprinted regions, the other remain genetically silent.
- The first human clinical syndromes recognized to result from imprinted loci were Prader-Willi syndrome and Angelman syndrome as reported in 1989 (Nicholls et al., 1989).
- These studies revealed that identical genetic deletions as well as uniparental disomy for a domain on 15q resulted in markedly different clinical phenotypes depending on the parental origin of the deletion/disomy.

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Germline or Gonadal Mosaicism. Some of the egg or sperm cells have a VHL gene mutation.
MOSAIC Versus CHIMERA

Mosaics and chimeras are animals that have more than one genetically-distinct population of cells.

- In mosaics, the genetically different cell types all arise from a single zygote.
- Chimeras originate from more than one zygote.

Cytogenetic Mosaics
- More than one cytogenetically-distinct population of cells.
- Example, 46, XX and 47, XXX.
- Turner syndrome mosaics

Chimeras
- More than one genetically-distinct population of cells that originated from more than one zygote.

Chimeric cattle are not all rare. When a cow has twins, it is almost inevitable that anastomoses (areas of joining) develop between the fetal circulatory systems early in gestation. This leads to exchange of blood between the two fetuses. Fetal blood contains hematopoietic stem cells, and each fetus is permanently "seeded" with stem cells from its twin.

Major clinical significance is seen when one fetus is a female and one a male. In such cases, the female fetus is exposed to hormones from the male and is masculinized.
- Such female cattle are called freemartins.
Chimeras are also produced experimentally, and have been a valuable research tool in several biomedical disciplines.

The basic technique is to combine two very early embryos such that their cells intermix and the resulting conceptus has cells from both original embryos.

Experimental Chimera: transgenic mixing of species from a human and a rat

The chimeric animal shown below is a baby "geep", made by combining a goat and sheep embryo.

MULTIFACTORIAL INHERITANCE

Pure environmental phenomenon
MULTIFACTORIAL INHERITANCE

- Many of our inherited characteristics are multigenic or even multifactorial in nature.
- They depend on the interaction between multiple genes or between genes and external factors.
- This means that a disease that is essentially genetic can also be triggered by environmental factors.

POLYGENIC TRAITS

- In a polygenic trait the combined action of many genes produces a continuously varying trait.
- Multiple genes that regulate height and skin color result in continuously varying traits that exhibit a range of possible phenotypes.
POLYGENIC TRAITS

- A polygenic trait is a trait controlled by many (poly) genes.
- Human skin color is an example; it thought to be the controlled by about 12 genes.

POLYGENIC INHERITANCE: 

- The distribution of height in a population if it were determined by one locus with three alleles.
- The distribution of height in a population if were determined by two loci, each with three alleles.

POLYGENIC vs MULTIFACTORIAL TRAITS

- Polygenic traits can also be multifactorial, meaning they have an environmental component.
- Traits like height, skin color, disease and behavior are all multifactorial traits.
- Multifactorial inheritance underlies some of the more clinically important human traits including:
  - Heart disease
  - Stroke
  - Diabetes
  - Schizophrenia

MULTIFACTORIAL INHERITANCE FEATURES

- Most affected children have normal parents. This is true of diseases and quantitative traits.
- Recurrence risk increases with the number of affected children in a family.
- Recurrence risk increases with severity of the defect. A more severely affected parent is more likely to produce an affected child.
- Consanguinity slightly increases the risk for an affected child.

MULTIFACTORIAL INHERITANCE FEATURES

- Risk of affected relatives falls off very quickly with the degree of relationship.
- If the two sexes have a different probability of being affected, the least likely sex, if affected, is the most likely sex to produce an affected offspring.

REGRESSION TO THE MEAN
REGRESSION TO THE MEAN

Galton’s "regression to mediocrity."

- Galton noticed that extremely tall fathers tended to have sons shorter than themselves, and extremely short fathers tended to have sons taller than themselves.
- "Tallness" or "shortness" didn't breed true like they did in Mendel's pea experiments. The offspring seemed to regress to the median.

THRESHOLD MODEL OF DISEASE

- If multifactorial traits are quantitative traits with continuous distribution, how can they control diseases, such as cleft lip or spina bifida? One either has the disease or doesn't. There is no intermediate.
- As the number of multifactorial genes for the trait increases, the liability for the disease increases. When it reaches a threshold, the liability is so great that abnormality, what we call disease, results.

THRESHOLD MODEL OF DISEASE

- The threshold model for multifactorial traits. Below the threshold the trait is not expressed. Individuals above the threshold have the disease.

Repetitive DNA, polymorphism
Genetic Diagnosis

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May 2014
The human genome contains approximately three billion base pairs of DNA. Within this there are between 20,000 and 25,000 genes, which together add up to less than 1-1.5 percent of the entire genome. Most of the rest is made up of several types of non-coding repeated elements. Non-coding RNA molecules, regulatory DNA sequences, LINEs, SINEs, introns, and sequences for which as yet no function has been elucidated (non-coding repeated elements-Junk DNA).

According to Copy Number of base sequences: Three Broad Classes of DNA Sequence

**Highly Repetitive DNA**: tens of thousands to millions of copies of a given sequence.

**Moderately Repetitive DNA**: ~10 - ~1000 copies of a given sequence.

**Unique or Non-Repetitive DNA**: 1 – ~ ≤10 copies of a given sequence.

Most genes lie in unique sequence DNA.

Types of Repetitive Elements

- Repetitive elements differ in their position in the genome, sequence, size, number of copies, and presence or absence of coding regions within them.
- The two major classes of repetitive elements are
  - Interpersed elements and
  - Tandem arrays.
Interspersed repeated elements
- are usually present as single copies and
distributed widely throughout the genome.
- constitute about 45 percent of the
genome.
- "jumping genes"

Jumping Genes And Their Effect On The Kernel Colour Of Indian Corn
Dr. Barbara McClintock the Nobel Prize in Medicine in 1983

INTERSPERSED REPEATS OBSERVED
IN THE HUMAN GENOME
- DNA transposons
- LTR retrotransposons
- Non LTR retrotransposons:
  - LINEs
  - SINEs
  - Others

Repetitive DNA in humans, two main groups
LINE (Long Interspersed Nuclear Element)
- average length of 6500 base-pairs
- A human genome contains about 60,000 to
100,1000 L1 elements.
SINE (Short Interspersed Nuclear Element)
- much shorter in length, 150 to 300 base-pairs in
length.
- make up 5% of the Human DNA.

Tandem Repeats
- Tandem repeats occur in DNA when a pattern of
two or more nucleotide bases is repeated and
the repetitions are directly adjacent to each
other.
- example:
  - in this case sequence A-T-T-C-G is repeated
three times.
- They include three subclasses: satellites,
minisatellites and microsatellites.
- Sequences repeated in tandem are common at
the centromere , and at or near the telomeres
(the chromosome tips).

Satellites
- The size of a satellite DNA ranges from 100 kb to over 1
Mb.
- Most satellites in humans or in other organisms are
located at the centromere
Minisatellites
- The size of a minisatellite ranges from 1 kb to 20 kb.
- One type of minisatellites is called variable number of
tandem repeats (VNTR).
Microsatellites
- Microsatellites are also known as short tandem repeats
(STR), because a repeat unit consists of only 1 to 6 bp
and the whole repetitive region spans less than 150 bp.
Most of our DNA is identical to DNA of others. However, there are inherited regions of our DNA that can vary from person to person. Variations in DNA sequence between individuals are termed "polymorphisms". DNA polymorphism is very useful for DNA analysis.

**DNA Polymorphisms**

- What types of DNA polymorphism exist?
  - RFLP: Restriction fragment-length polymorphism
  - VNTR: Variable number of tandem repeats
    - minisatellite
  - STR: Short tandem repeats
    - microsatellites
  - SNP: Single nucleotide polymorphism
- Although there are many variations in methodology, the basic principal for detection of DNA variability is differences in the size of fragments

**RFLP**

(Restriction Fragment Length Polymorphism)
Restriction Enzymes (endonucleases)
- Cuts DNA from any source at specific sequences in palindromes (DNA sequences that read the same (5’ 3’) on both strands).
- They cut within the molecule, they are often called restriction endonucleases.
- If RE cuts straight across the double helix it will produce "blunt" ends.
- If cuts in offset fashion with overhanging piece of single-stranded DNA called "sticky ends" because they are able to form base-pair with complementary sticky end.

Example: enzyme HaeIII cuts DNA wherever it encounters the sequence □5’GGCC3’ 3’CCGG5’
The cut is made between the adjacent G and C.
Alul and HaeIII produce blunt ends
BamHI, HindIII and EcoRI produce "sticky" ends

RFLP
- Example: enzyme HaeIII cuts DNA wherever it encounters the sequence □5’GGCC3’ 3’CCGG5’
The cut is made between the adjacent G and C.

Application of RFLP
- Screening human DNA for the presence of mutant genes
- DNA “fingerprinting”
Screening for the sickle-cell gene

Application of RFLP

VNTR (Variable Number Tandem Repeat)

Fingerprinting

DNA fingerprinting

A method used to identify multilocus DNA banding patterns that are specific to an individual by exposing a sample of the person's DNA to molecular probes and various analytical techniques such as Southern blot analysis.

DNA fingerprinting is often used to provide evidence in criminal law cases. Also called genetic fingerprinting.
Genetic Fingerprinting

STR (Short Tandem Repeats)
- STRs are repeated sequences of a few (usually four) nucleotides, e.g., TCATTCATTCAT. They often occur in the untranslated parts of known genes.
- The exact number of repeats (6, 7, 8, 9, etc.) varies in different people (and, often, in the gene on each chromosome; that is, people are often heterozygous for the marker).
- In the U.S., where 13 STRs — scattered over different chromosomes — are examined, the chance that two people picked at random have the same pattern is less than 1 in 1 trillion.

Single nucleotide polymorphism or SNP
- Also a change in a single base pair, but does not necessarily cause a change in a restriction enzyme site
- Widely distributed across the genome, occurring approximately once in every 500-1000 bp
- SNPs are identified by sequencing, allele-specific hybridization or allele-specific primer extension
- Variations can affect how humans develop diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents.
- SNPs are also thought to be key enablers in realizing the concept of personalized medicine

DNA LABORATORY TECHNIQUES
Prof. M. Kamal
A brief review of technology

DNA isolation and fragmentation
- Phenol-chloroform, salting-out, columns
- Mechanical shearing, restriction enzymes

DNA labelling and hybridization
- Radioisotopes, fluorescent dye
- Denaturation to single-strand with heat
- Reformation of complementary double-stranded DNA with slow cooling

DNA amplification
- Polymerase chain reaction
- Recombinant technology

Electrophoresis
- Separation of DNA on basis of size
- Column chromatography increasingly replacing electrophoresis for high throughput applications

Identification of base sequence in DNA
- Southern Blotting
- Dot blot

DNA synthesis
- Oligonucleotide synthesis
- Gene synthesis

DNA amplification
- Polymerase Chain Reaction
- Recombinant DNA

DNA probe
- A single-stranded DNA molecule used in laboratory experiments to detect the presence of a complementary sequence among a mixture of other single-stranded DNA molecules.
Oligonucleotide synthesis

- Oligonucleotide synthesis is the non-biological, chemical synthesis of defined short sequences of nucleic acids.
- Synthesized oligonucleotides are single-stranded DNA molecules around 15-20 bases in length up to 160 to 200 bases.
- They are most commonly used as primers for DNA sequencing and amplification, as probes for detecting complementary DNA or RNA.

Gene synthesis

- Gene synthesis is the process of synthesizing an artificially designed gene into a physical DNA sequence.
- First demonstrated by Har Gobind Khorana in 1970 for a short artificial gene.
- It has become an important tool in many fields of recombinant DNA technology including heterologous gene expression, vaccine development, gene therapy and molecular engineering.

PCR (Polymerase Chain Reaction)

- A polymerase is a naturally occurring enzyme that catalyzes the formation and repair of DNA (and RNA).
- A heat-stable DNA polymerase enzyme extracted from the bacterium *Thermus aquaticus* is used.
- What is the chain reaction? Molecular reproduction technology, the target DNA could be exponentially amplified.

Polymerase Chain Reaction

- A technique by which many copies of a specific DNA sequence are produced starting from a few copies of a particular DNA sequence very rapidly.
- At least a portion of the sequence of the DNA molecule should be known.
Principle of the PCR
- The cycling reactions
  - Denaturation at 94°C
  - Annealing at 54°C
  - Extension at 72°C
- Reactants
  - a. DNA
  - b. Primers: small pieces of DNA with base sequence homology to the ends of the DNA to be amplified
  - c. Thermal stable DNA polymerase
  - d. Deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP)

Stages of the PCR cycle

Stages of the PCR cycle
- Strand separation
  - The original DNA (the target DNA) is heated to 95°C for 5 minutes and denatured.
  - It separates into two single strand lengths of DNA.

Stages of the PCR cycle
- Primer binding
  - The solution is rapidly cooled to 54°C to allow the primers to bind to the complimentary base sequences on each of the single strands of DNA.
  - This provides starting point for DNA replication
    

Stages of the PCR cycle
- Strand synthesis
  - Solution is heated to 72°C
  - The DNA polymerase catalyses the synthesis of complimentary strand for each of the single strands of DNA.
  - Result is two identical double strands of DNA

Uses of PCR
- Medical applications
- Infectious disease applications
- Forensic applications
- Research applications
- Others

PCR thermocycler
- Three major steps in a PCR are repeated for 30 or 40 cycles.
- Done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.
- After 30 cycles, single molecule of DNA has been amplified into more than a billion copies ($2^{30} = 1.02 \times 10^9$).
DNA Sequencing

- The most popular method for doing this is called the **dideoxy method**.
- DNA is synthesized from four deoxynucleotide triphosphates. The top formula shows one of them: deoxythymidine triphosphate (dTTP). Each new nucleotide is added to the 3’-OH group of the last nucleotide added.
- The dideoxy method is also called the **chain termination method**.

Recombinant DNA

- Recombinant DNA: New combinations/arrangements of DNA constructed in the laboratory
- It has been created artificially from two or more sources incorporated into a single recombinant molecule.
- Genetic Engineering: The design and construction of new combinations of genes (DNA)

Construction of recombinant DNA molecules

- Gene of interest is isolated from appropriate organism
- Gene is recombined with a vector (carrier) DNA molecule
- Recombinant DNA is introduced into appropriate host cell
- Recombinant DNA is expressed at high levels in host cell
- Gene product may be purified for use in treatments (antibiotics, hormones, etc.)
- Gene may confer new properties on host cell that carries recombinant DNA (herbicide-resistance, pest-resistance, ability to metabolize toxins, etc.)
- Once a gene is cloned, its product may be produced in mass quantity
Harnessing the Power of Recombinant DNA Technology – Human Insulin Production by Bacteria

1) Isolate human cells and grow in tissue culture.
2) Isolate DNA from the human cells.
3) Join the plasmid and human fragment and cut with a restriction enzyme.
4) Mix the recombinant plasmid with bacteria.
5) Allow new bacteria to incorporate the recombinant plasmid into the bacterial cell, then screen bacteria to find the ones that have incorporated the human gene for insulin.
6) Use the same restriction enzyme to cut the plasmid DNA, creating matching sticky ends.
7) Meanwhile, isolate plasmid DNA from a bacterium.
8) Grow trillions of new insulin-producing bacteria.

Route to the Production by Bacteria of Human Insulin

A fermentor used to grow recombinant bacteria.

Then the single cell with many recombinant plasmids produces trillions of like cells with recombinant plasmid – and the human insulin gene.

The final steps are to collect the bacteria, break open the cells, and purify the insulin protein expressed from the recombinant human insulin gene.

Some recombinant DNA products being used in human therapy

- Insulin for diabetics
- Factor VIII for males suffering from hemophilia A
- Factor IX or hemophilia B
- Human Growth factor (GH)
- Erythropoietin (EPO) for treating anemia
- Three types of interferons
- Several interleukins
- (GM-CSF) for stimulating the bone marrow after a bone marrow transplant
- HBsAg to vaccinate against hepatitis B infection
This procedure allows detection of various DNA gene sequences, and is one of the most widely used procedures in molecular biology.

Restriction endonuclease cuts down target DNA into small pieces.

Agarose gel electrophoresis to separate DNA fragments

Transfer of DNA fragments from gel to nitrocellulose membrane

DNA fragments transferred to NCM

Radioactively labeled probe hybridizes to target DNA fragment
Dot blot

- A Dot blot (or Slot blot) is a technique in molecular biology used to detect biomolecules.
- It is a simplification of the northern blot, Southern blot, or western blot methods.
- In a dot blot the biomolecules to be detected are not first separated by chromatography.
- Instead, a mixture containing the molecule to be detected is applied directly on a membrane as a dot.
- This is then followed by detection by either nucleotide probes

Dot blot in sickle cell

- Dot blot results are shown for a normal control (AA), a carrier for sickle (AS), and sickle disease (SS).
- The prenatal sample F has both an A and an S signal, indicating a heterozygote or carrier of the sickle gene.

Dot blot: advantages and disadvantages

- The technique offers significant savings in time
- However, it offers no information on the size of the target biomolecule.
- Furthermore, if two molecules of different sizes are detected, they will still appear as a single dot.
- Dot blots therefore can only confirm the presence or absence of a biomolecule or biomolecules which can be detected by the DNA probes or the antibody.

In Situ Hybridization

- What is it?
  - Labeled nucleic acid probes are used to locate specific nucleic acid sequences in situ

- USE
  - To demonstrate
    • a particular DNA sequence in chromosome/cell/tissue
    • tissue-specific expression of a given mRNA
    • To detect a particular genetic region.
    • Method to “map” genes

In situ hybridization

- Chromosome in situ hybridisation
  - FISH fluorescence label direct or indirect
- Tissue in situ hybridization
**Chromosome in situ hybridization**
- Detection with fluorescence microscopy
- Metaphase spreads → double hybridization spots (sister chromatids)
- Resolution about 1 Megabase

**Tissue in-situ hybridization**
In this procedure a labeled probe is hybridized against RNA/DNA in tissue sections

**Array-Based Comparative Genomic Hybridization (Array CGH)**
- Genomic abnormalities can be detected without prior knowledge of what these aberrations may be, using a global strategy such as array CGH.
- In array CGH the test DNA and a reference (normal) DNA are
- labeled with two different fluorescent dyes (most commonly Cy5 and Cy3, which fluoresce red and green, respectively).
- The differentially labeled samples are then hybridized to a glass slide spotted with DNA probes that span the human genome at regularly spaced intervals, and usually cover all 22 autosomes and the X chromosome.

**EPIGENETIC ALTERATIONS**
- Epigenetics is defined as the study of heritable chemical modification of DNA or chromatin that does not alter the DNA sequence itself.
- Examples of such modification include the methylation of DNA, and the methylation and acetylation of histones.
- Our understanding of these types of molecular alterations is rapidly growing, and it is clear that epigenetic modifications are critical for normal human development including the regulation of tissue-specific gene expression, X chromosome inactivation, and imprinting, as well as for understanding of the cellular perturbations in the aging process and cancer.
WHAT IS GENETIC TESTING?

- Genetic testing is the examination of a person's
  - chromosomes,
  - DNA or
  - the biochemical product of a gene

- Results of these tests may
  - confirm or refute a suspected genetic condition
  - or possible predisposition to a condition.

WHAT IS DIRECT GENE TESTING?

- When a gene has been located precisely on a chromosome
- Where the mutations are known

- The gene is examined directly for the presence or absence of mutation
- The test is very accurate.

The DNA examination

- May involve the analysis of the gene itself (direct gene testing)

- Or of short segments of the DNA close to or within a gene (indirect gene tracking).
LIMITATIONS OF DIRECT GENETIC TESTING
- Locus and mutation(s) may not be known.
- There may be many mutations over different length of the gene.
- Other genes, environmental factors can affect the expression of the gene.

WHAT IS INDIRECT GENE DIAGNOSIS
- Indirect gene diagnosis or gene tracking or linkage analysis is used when
  - mutation(s) in a gene have not yet been defined or
  - where the DNA region containing the gene is known but the gene itself has not been precisely located.

Basis of indirect gene Dx.
- Polymorphic markers are special segments of DNA are located very close to the gene (genetic linkage) on the same chromosome.
- These segments nearly always travel with the gene when it is passed from parent to child.
- These markers are different in different families.
- The marker may travel with either the correct copy or the mutated gene copy.

Principle of linkage analysis
- The top diagram shows paternal (blue) and maternal (red) chromosomes aligned in a germ cell.
- Three DNA sequences, labeled A, B and C. Capital letters-paternal alleles and lower case letters maternal alleles.
- The middle panel shows the physical process of recombination, which involves crossing over of DNA strands between the paired chromosomes.
- The bottom panel shows what happens when the crossover is resolved. The paternal and maternal alleles are mixed (recombined) and these mixed chromosomes are passed to the sperm or ovum.
- If A is the disease gene and B and C are genetic markers, recombination is likely to occur much more frequently between A and C than it is between A and B. This allows the disease gene to be mapped relative to the markers B and C.

The markers that are linked to the faulty or correct gene copies are
- special to each family,
- so this method of genetic testing can only be done within families.
- Indirect gene tracking is a “family test.”
Gene tracking in a family with Duchenne muscular dystrophy where no mutation has been found in the affected proband III4. Analysis of markers A, B and C has enabled the construction of haplotypes: the affected haplotype is shown by an orange box.

### Prenatal diagnosis of genetic diseases

### Purpose of prenatal diagnosis
- To detect abnormalities in fetal life and allow termination.
- Provide a range of informed choice to the couples at risk of having a child with abnormality.
- Provide reassurance and reduce anxiety, especially among high-risk groups.

### Indications for prenatal diagnosis
- Advanced maternal age
- Previous child with a chromosome abnormality
- Family history of a chromosome abnormality
- Family history of single gene disorder
- Family history of a neural tube defect or other congenital abnormalities
- Abnormalities identified in pregnancy
- Other risk factors (consanguinity, poor obst., history, maternal illnesses)

### Methods of prenatal diagnosis
- **Invasive:**
  - Amniocentesis
  - Chorionic villus sampling
  - Cordocentesis
  - Preimplantation genetic diagnosis
  - Fetoscopy
- **Non-invasive testing:**
  - Maternal serum AFP
  - Maternal serum screen
  - Ultrasonography
  - Isolation of fetal cells from maternal circulation
Invasive methods of prenatal diagnosis

Amniocentesis
- Aspiration of 10-20 ml of amniotic fluid through the abdominal wall under ultrasound guidance around the 16 weeks of gestation.
- In about 14 days there will be enough cells for chromosome analysis or biochemical or DNA studies some time a longer time is needed to grow more cells.
- Couples should be informed of the risk of abortions (0.5-1%) and the possibility of termination if wished.

Chorionic villus sampling
- It enables diagnosis in first trimester (10-11 week of gest.) under ultrasound guidance by transcervical or transabdominal aspiration of chorionic villi.
- These are fetal cells derived from the outer layer of trophoblast.
- Disadvantage:
  - Higher risk of abortion (2-3%)
  - Limb abnormalities if carried before the 9 weeks of gestation.

Cordocentesis
- Visualisation of the umbilical vessels by transabdominal ultrasound and enabling fetal blood sampling.
- It is usually used in the management of Rhesus isoimmunization and in some cases to solve the problem of mozaicism.
Non-invasive methods of prenatal diagnosis

Maternal serum AFP

- Mostly done around the 16 weeks of gestation.
- More specific for the diagnosis of NTD (95% of NTD can occur with out a history)
- Amniocentesis was used to confirm the diagnosis but with a good detailed ultrasound first and second degree can be diagnosed
- It has been found that by periconceptional supplementation with folic acid decrease the rate of occurrence of NTD and other abnormalities

Maternal screening test

- It is now a standard practice to offer screening for NTD, Down's synd. and Edward synd.
- Using a blood sample obtained from the mother at the 16 (15-20) weeks of gestation
- It can diagnose up to 75% of NTD and 60-70% of Down's sy.

Ultrasonography

- It is used for obst. diagnosis as placental localisation and multiple preg. As well as for prenatal diagnosis of structural abnormalities which are not associated with known chromosome, biochemical, or molecular defects.
- It is a non invasive with no risk to the foetus or mother
- It is offered to those with a history of genetic disease

Problems in prenatal diagnosis

- Failure to obtain a sample or culture failure
- An ambiguous chromosome result
- An unexpected chromosome result
The Human Genome Project aimed to sequence the human genome in order to track down the genes responsible for inherited disease in humans.

- There are six main objectives/areas of work of the Human Genome Project.
  1. Human gene maps and mapping of human inherited diseases
  2. Development of new DNA technologies
  3. Sequencing of the human genome
  4. Development of bioinformatics
  5. Comparative genomics
  6. Functional genomics

Prenatal treatment

- In most situations the diagnosis of prenatal abnormalities has a subsequent option of termination of the pregnancy.
- While this applies in most situations, there is cautious optimism that with the advent of gene therapy prenatal diagnosis will, in time, lead to effective treatment in utero.
  1. Treatment of genetic disease by conventional means requires identification of the gene products and an understanding of the pathophysiology of the disease process.
  2. Gene therapy can be defined as the replacement of a deficient gene product or correction of abnormal gene. Gene therapy can be carried out either ex vivo by treatment of cells or tissue from an affected individual in culture, with reintroduction into affected individual or in vivo.

Treatment of genetic diseases

- Treatment of the autosomal recessive disorder - congenital adrenal hyperplasia (CAH).
- Affected female are borne with virilisation of the external genitalia.
- There is an evidence that this can be prevented by powerful steroid therapy at early gestational age.

Gene therapy

- Use of DNA as a pharmaceutical agent to treat disease.
- The most common form is DNA that encodes a functional, therapeutic gene to replace a mutated gene.
- Other forms involve directly correcting a mutation, or using DNA that encodes a therapeutic protein drug (rather than a natural human gene) to provide treatment.
- In gene therapy, DNA that encodes a therapeutic protein is packaged within a "vector", which is used to get the DNA inside cells within the body. Once inside, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein, which in turn treats the patient's disease.
**Examples of gene therapy**

- **Combined immunodeficiency**
  - deficiency of the adenosine deaminase
  - bone marrow retrovirus

- **Cystic fibrosis**
  - deficiency of the transmembrane reg. gene
  - liposomes fusing with epithelial cells

- **Haemophilia A**
  - gene for factor VIII
  - liver tissue application into portal vein

- **Lung carcinoma**
  - K-ras (oncogene) at 30-40% adenocarcinomas
  - instillation of the mirror gene coding transfer of RNA
  - block of the decoding p53 tum. suppressor gene at 50-70% of all carcinomas
  - instillation of good work
  - gene's copy retrovirus into tumour deposit

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**Genetic Counseling**

**What is Genetic Counselling?**

- Genetic counseling is the process by which patients or relatives, at risk of an inherited disorder, are advised of:
  - the consequences/nature of the disorder
  - the probability of developing or transmitting the disorder
  - the options open in management and family planning in order to prevent, avoid or accommodate it.

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**Genetic counseling involves**

- evaluating family history and medical records
- genetic tests
- evaluating the results of this investigation
- helping parents understand and reach decisions about what to do next

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**Role of the counsellor**

- Assess the risk of a genetic disorder by researching a family’s history and evaluating medical records.
- Weigh the medical, social and ethical decisions surrounding genetic testing.
- Provide support and information to help a person make a decision about testing.

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**Successful treatment of patients**

- Leber's Congenital Amaurosis,
- X-linked SCID
- ADA-SCID
- Adrenoleukodystrophy and Parkinson's disease.
Role of the counsellor

- Interpret the results of genetic tests and medical data.
- Provide counseling or refer individuals and families to support services.
- Explain possible treatments or preventive measures.
- Discuss reproductive options.