MEDICAL GENETICS- INTRODUCTION

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Recommended Books

- Elements of Medical Genetics: P Turnpenny & S Ellard, 14th Ed. Churchill Livingstone
- Medical Genetics: Jorde,Carey, Bamshad & White, 3rd Ed., Elsevier.
- Robbin's Pathologicas Basis of Diseases, Kumar, Abbas Fausto & Aster, 8th Ed., Elsevier.

Most of the diseases have either major or minor genetic contribution

Thus diseases may be divided in to:

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 (1/2500 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)

Medical Genetics

Application of genetic principles to medical practice.
Includes studies of :

- ***Inheritance***
- ***mapping***
- ***disease genes,***
- ***diagnosis and treatment,***
- ***genetic counseling***
- **Prevention and treatment**

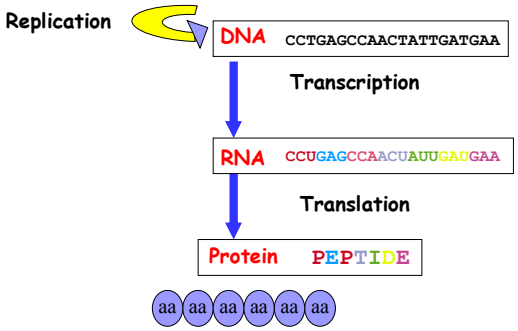
Long Long
history of Genetics

Type of genetic disease

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

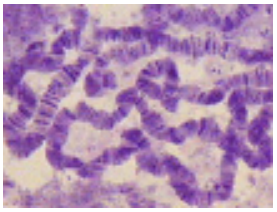
CHROMOSOME FEATURES

Central Dogma of Genetics

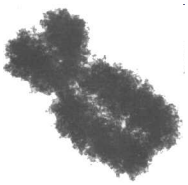


- Chromosomes are long coiled pieces of DNA, with supporting proteins.
- Genes are short regions of this DNA that hold the information needed to build proteins

Chromosome



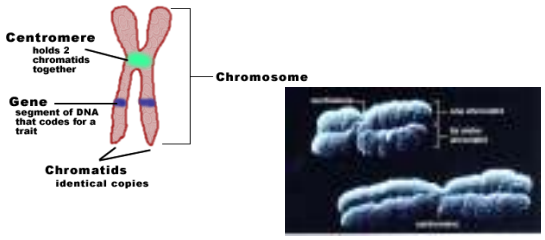
Interphase



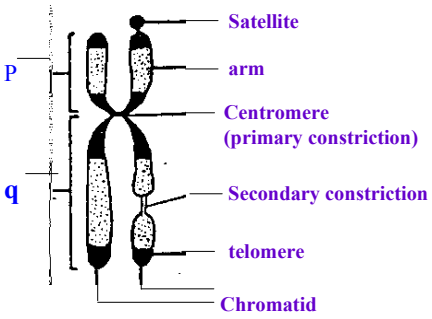
Metaphase

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.



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 - Allow the ends of the chromosome to be replicated

Protect the DNA from digestion by nucleases

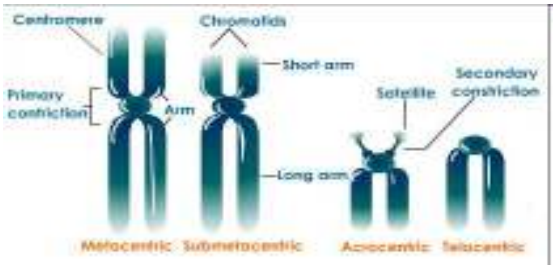
DNA sequence (thousands of copies of TTAGGG) at the end of all chromosomes

•**Satellite:** Small segments of chromatin distal to the secondary constriction on the 'p' of the acrocentric

•**Ch. 13, 14, 15, 21 & 22.**

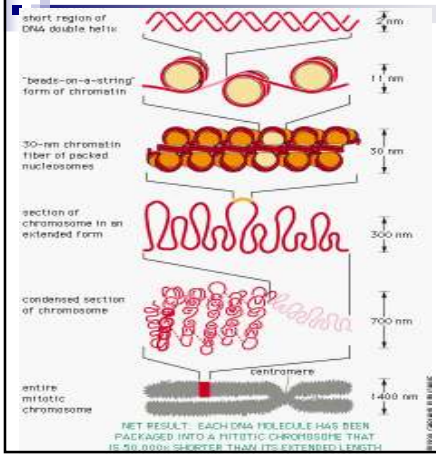
Chromosome classification

- 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

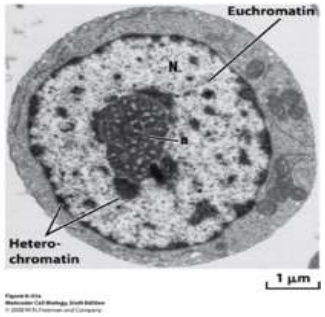
- **The haploid human genome contains approximately 3 billion base pairs of DNA packaged into 23 chromosomes.**
- **6 billion base pairs of DNA per cell.**
- **Each base pair is around 0.34 nanometers long**
- **Each diploid cell therefore contains about 2 meters of DNA**
- **Moreover, the human body 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?***



Levels of Chromosome packing

On staining pattern- 2 types of areas on chromosomes seen in nucleus

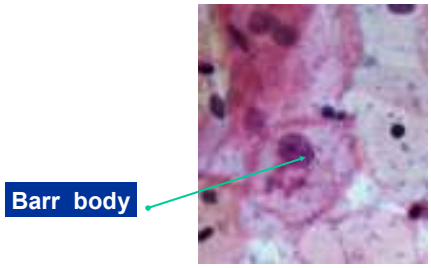
- **EUCHROMATIN**
  - When DNA is in it's least condensed form
  - Transcribed
  - Replicated early
- **HETERO CHROMATIN**
  - When DNA is in it's most condensed form
  - **Devoid of genes or has inactive genes**
  - Not transcribed
  - Replicated late



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

Common Name	Genus and Species	Diploid Chromosome Number
Cat	*Felis catus*	38
Human	*Homo sapiens*	46
Donkey	*E. asinus*	62
Pig	*Sus scrofa*	38

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 and 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

CYTOGENETIC DISORDERS

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Genetic mutation

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

Hiroshima and Nagasaki aftermath

Types of chromosomal disorders

- Abnormalities in number
- Abnormalities in structure

NUMERICAL CHROMOSOME MUTATIONS

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 (2*n*) Two gametes form a diploid zygote with twice this number (2*n*)

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

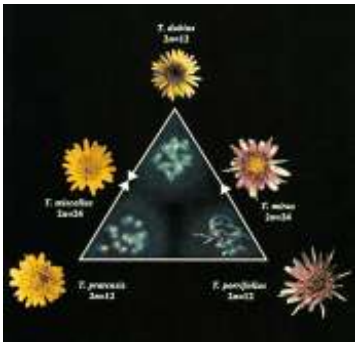
Variation involving entire sets of chromosomes

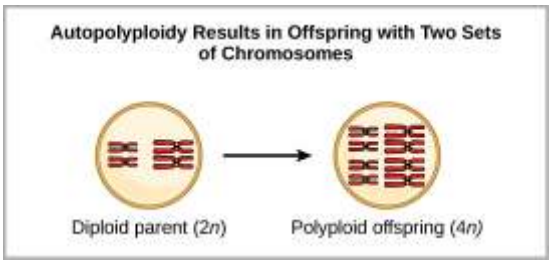
Euploid Type	n	Chromosome example
Haploid or monoploid	One (n)	1 2 3……
Diploid	Two (2n)	1,1 2,2 3,3……
Polypoid	More than two	
--Triploid	Three (3n)	1,1,1 2,2,2 3,3,3……
--Tetraploid	Four (4n)	1,1,1,1 2,2,2,2 3,3,3,3……

Two types of polyploidy

- **Autopolyploidy:** all of the chromosome sets come from the same species.
  - **Failure of cell division (2N --> 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



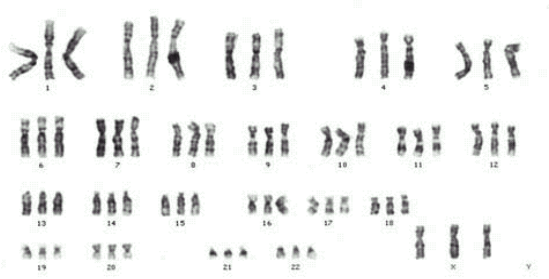


TRIPLOIDY 69+XXX or XXY or XYY

- 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.

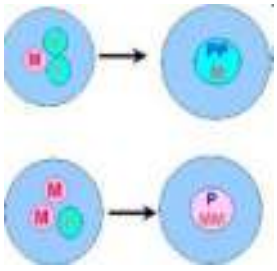
Triploidy

- TRIPLOIDY
- 69+XXX or XXY or XYY



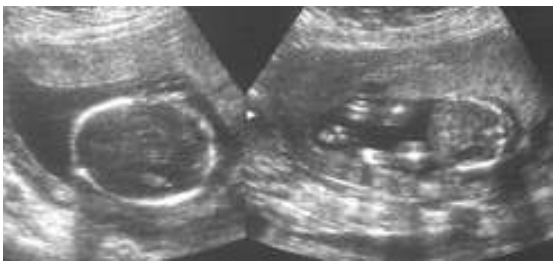
TRIPLOIDY 69+XXX or XXY or XYY

- **Pathogenesis**
  - Fertilization error e.g. dispermy
  - Failure of meiosis in germ cells i.e. fertilization of a deploid 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

NO RECURRENCE RISK

Unless you have bad……

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***

Aneuploidy

exact multiple of the haploid number

Type	No. of chromosomes	Chromosome example
Disomic (normal diploid)	2n	1,1 2,2 3,3 4,4….
Monosomic	2n – 1	1,1 2,2 3,0 4,4….
Nullisomic	2n – 2	1,1 2,2 0,0 4,4….
Polysomic		
--Trisomic	2n+1	1,1 2,2 3,3,3 4,4….
--Double trisomic	2n+1+1	1,1 2,2,2 3,3,3 4,4….
--Tetrasomic	2n+2	1,1 2,2 3,3 4,4,4,4….

Frequency of common chromosomal numerical disorders among live born infants

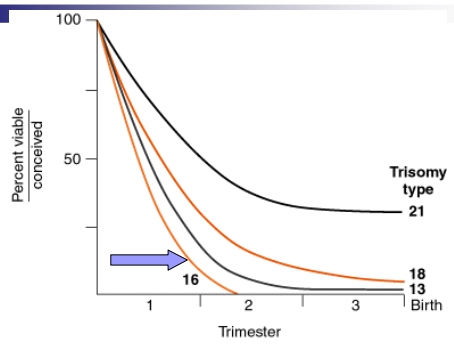
Autosomal	
Trisomy 13	1:15,000
Trisomy 18	1:5,000
Trisomy 21 (Down syndrome)	1:800
Sex chromosome	
Klinefelter syndrome (47,XXY)	1:700 M
Triple X syndrome (47,XXX)	1:1,000 F
Turner syndrome (45,X; other)	1:1,500 F
XYY Syndrome (47,XYY)	1 in 800 M

Aneuploidy is almost always harmful.

Imbalanced gene dosage causes the negative effects of aneupoidy.

Aneuploidy can affect any chromosome

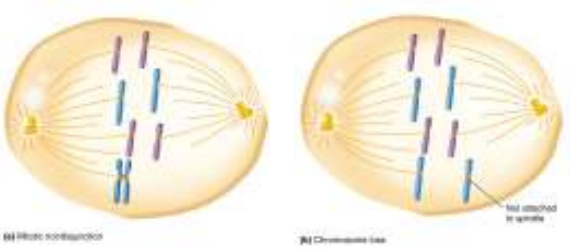
The reason only trisomy 13, 18 or 21 is seen in live births is because other autosomal aneuploides are embryonic lethal conditions.



Spontaneous fetal loss for autosomal trisomies

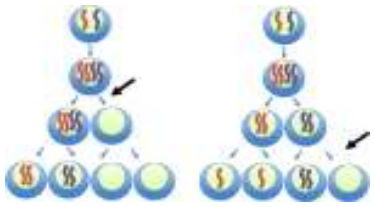
most chromosomal aneuploidies do not survive to birth

Mitotic Nondisjunction and Chromosome Loss Lead to Somatic Cell Aneuploidy



Aneuploidy is usually due to nondisjunction

Non-disjunction during meiosis



Trisomy 21 Down Syndrome

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)
- 10 to 20 fold increased risk of Acute lukaemias.
- Presenile dementia (changes like Alzheimer's)
- Abnormal immune response (serious infections, thyroid autoimmunity)

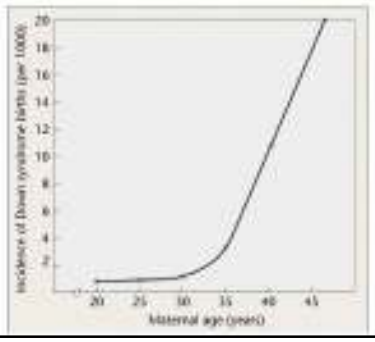
Incidence of Some Associated Medical Complications in Persons with Down Syndrome

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

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

Mother's age	Incidence
20	1/1550
25	1/1050
30	1/1200
35	1/350
40	1/70
45	1/25
48	1/9

Incidence of Down Syndrome Increases with Maternal Age



- 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 nack of the fetal neck, called nuchal transluceny, and the risk of Down syndrome
- Several other items that can be found during an ultrasound exam {echogenic bowel, echogenic intracardiac focus, and dilitation of the kidneys (pyelctasis)}
- 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

Diagnostic procedure	Gestational age when test is done (weeks)	Risk of fetal loss (%)
Chorionic villus sampling	10 to 12	0.5 to 1.5
Early amniocentesis	12 to 15	1.0 to 2.0
Second-trimester amniocentesis	15 to 20	0.5 to 1.0

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

Situation	Oocyte	Sperm	Consequence
Normal	X	Y	46, XY normal male
	X	X	46, XX normal female
Female Nondisjunction	XX	Y	47, XXY Klinefelter syndrome
	XX	X	47, XXX triplo-X
		Y	45, Y nonviable
		X	45, X Turner syndrome
Male Nondisjunction (meiosis I)	X		45, X Turner syndrome
	X	XX	47, XXX triplo-X
Male nondisjunction (meiosis II)	X	YY	47, XYY Jacobs syndrome
	X		45, X Turner syndrome

Summary: Aneuploidy of the sex chromosomes

1. Need at least one X chromosome
   (44, --) and (45, -Y) not even seen in spontaneous abortions

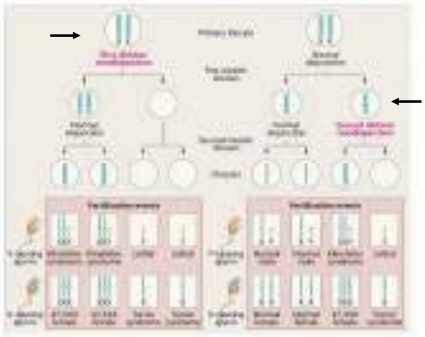
2. Single X (45, X) – viable, but not fertile
   - need at least 2X for normal female

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 monosomy 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 45 X0
- Deletion of small arm 46Xi (Xq)
- Deletion of portions of small or long arm
- Mosaics 45X/46XY, 45X/47XXX
- Only 1% fetuses with 45 X0 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 amenorrhoea.
- 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 XXYY, 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.

SEX CHROMATIN BODY

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

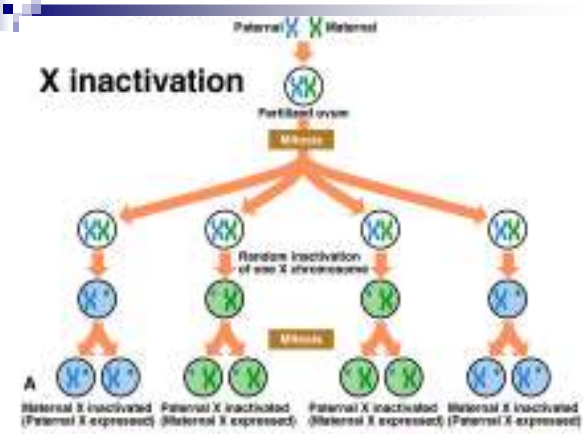
In 1961 Mary Francis Lyon - British geneticist

- studied color coat in mice
- knew that coat color was X-linked

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



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

Inconsistencies between syndromes and X inactivation

If normal XX female has one X inactivated, why is a X Turner female not normal?

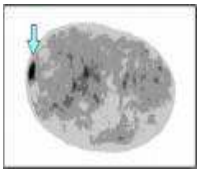
Similarly, if XXY male has one X inactivated, why does he have Klinefelter syndrome?

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

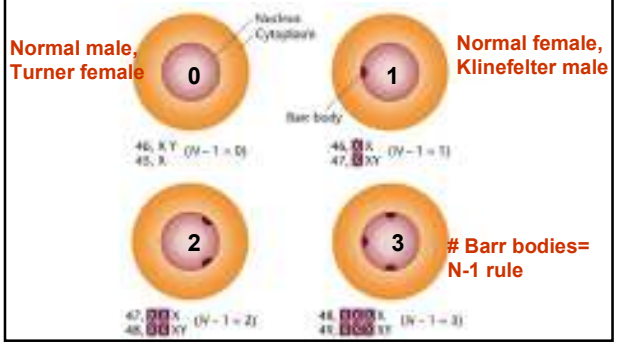
- Many of the genes on X escape inactivation
- eg. MIC-2
- Genes inactivated are DMD, G 6PD, HPRT etc.
-
- Molecular basis of X- inactivation: X- inactive specific transcript gene XIST X 13.

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



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
XXY - "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

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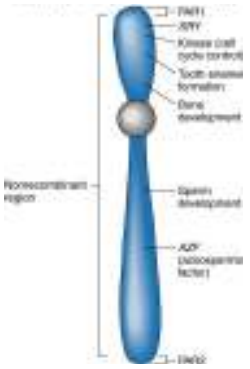
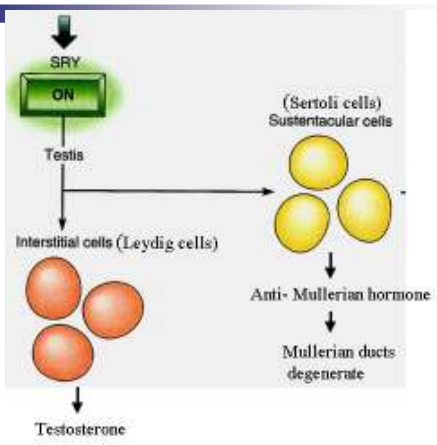
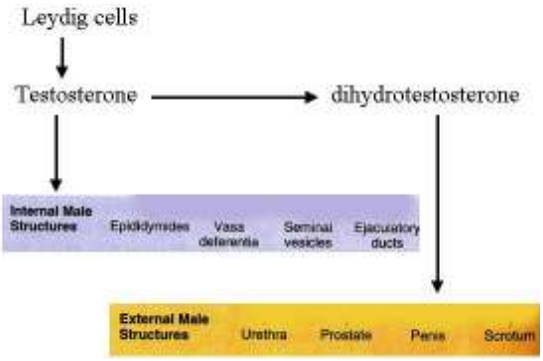


SRY (Sex-determining Region Y) is a sex-determining gene on the Y chromosome in humans

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

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- Y chromosome (SRY region;TDF gene) is not present.
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- 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

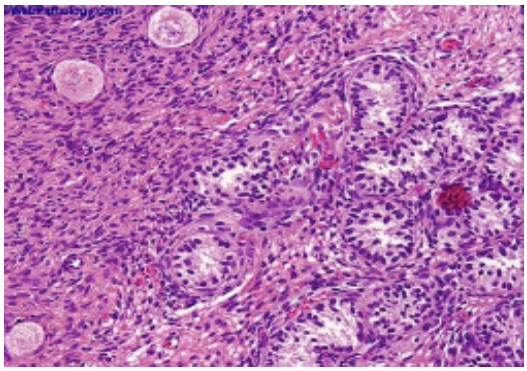
Abnormal Development

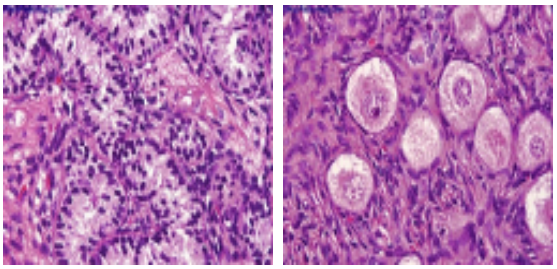
Hermaphroditism

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

TRUE HERMAFHRODITISM

- 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 genetalia
- Various cause (cytogenetic, mendelian, Teratoganic)

MALE PSEUDO HERMAPHRODITISM

- Hetergenous 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
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• No uterus, Fallopian tube or ovary
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-Unlike 5-α reductase deficiency
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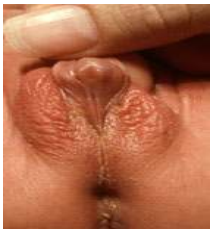
FEMALE PSEDOHERMAPHRODITISM

- **CONGENITAL ADRENAL NYPERPLASIA (Adrenogenital synd.)**



- Several genetic & clinical forms, all are AR
- Block in a specific step in cortisol biosynthesis
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CONGENITAL ADRENAL NYPERPLASIA

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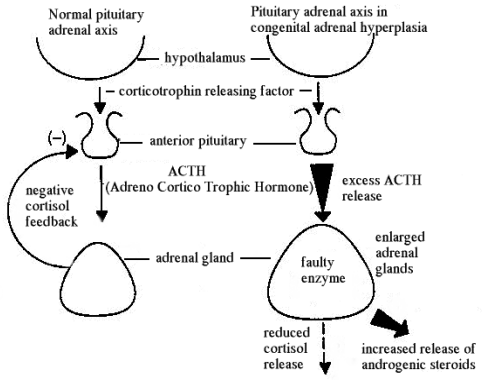

- End of cytogenetics april 2014

CYTOGENETIC DISORDERS

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

♂ HERMAPHRODITISM ♀

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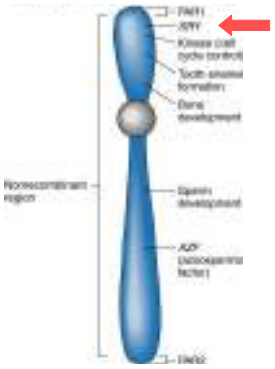

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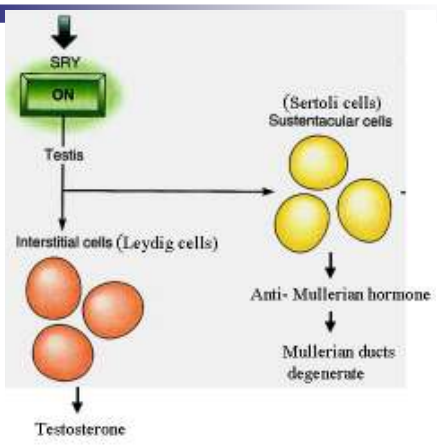

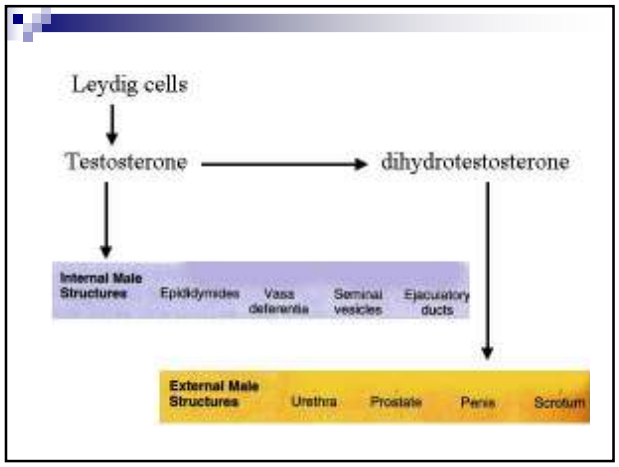

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  - if ovaries, then male external sexual anatomy
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TRUE HERMAFHRODITISM

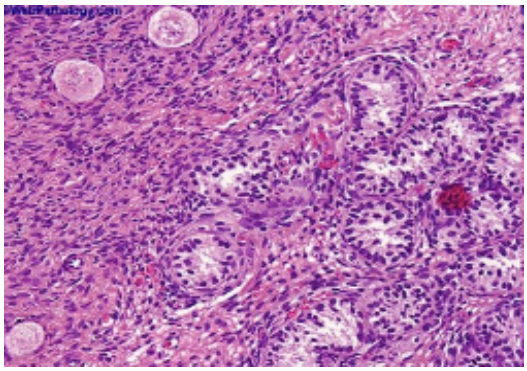
- An intersex condition in which an individual is born with ovarian and testicular tissue.
- External genitalia are often ambiguous, the degree depending mainly on the amount of testosterone produced by the testicular tissue between 8 and 16 weeks of gestation.
- Sex hormones also variable
- Very rare

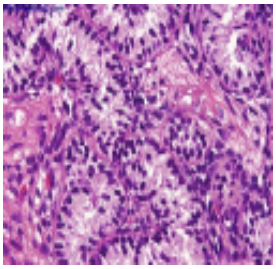
TRUE HERMAFHRODITISM

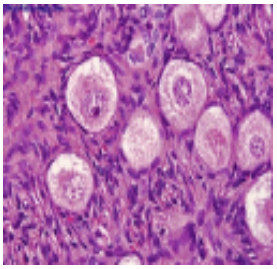
- Majority 47XXY, 46XX/46XY, or 46XX/47XXY, and various degrees of mosaicism
- Fertilization of two haploid ovum and fusion of the two zygotes early in development.
- Fertilized of one ovum by two sperms followed by trisomic rescue in one or more daughter cells.
- Fusion of two fertilized ova to form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

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- 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, Teratoganic)

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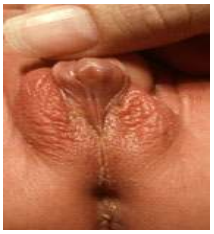

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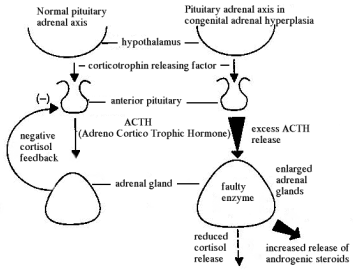


CHROMOSOME STRUCTURAL ABERRATIONS

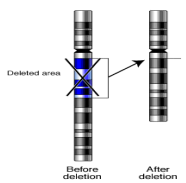
Prof Mohammed Kamal
Dept. of Pathology, BSMMU
April 2014

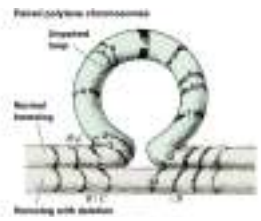
CHROMOSOME STRUCTURAL ABERRATIONS

- ►Changes in structure of chromosome (Breakage → rearrangement)
- ►Large amount of DNA (> 4 million bp) should be involved to demonstrate the change
- ►Occur spontaneously, increased by environmental mutagens

Deletion

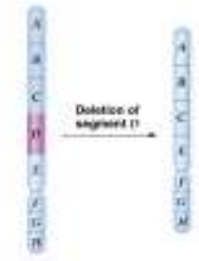
- **Begins with a chromosome break.**
- **Induced by heat, radiation, viruses, chemicals, transposable elements, and recombination errors.**
- **No reversion; DNA is missing.**





DELATIONS

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

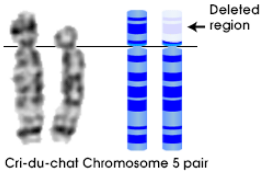


Large chromosomal deletions

- **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 Chromosome 5 pair

Cri-du-Chat syndrome

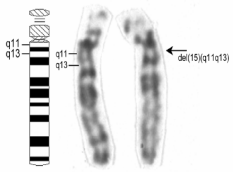
- Is one of the most common syndromes caused by a chromosomal deletion.
- It affects between 1 in 20,000 and 1 in 50,000 babies.
- French for "cry of the cat," because of distinctive cry of children with this disorder.
- The cry is caused by abnormal larynx development
- Less noticeable as the baby gets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

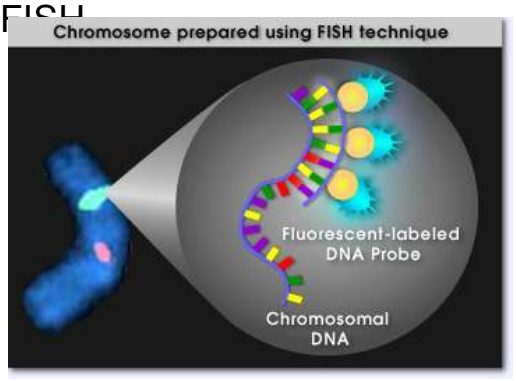
Williams Syndrome

- 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:
- supravalvular 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 (MICRODELATION)
- It cannot be seen in a classic karyotyping technique.
- However, the deletion can be observed using a special technique

FISH



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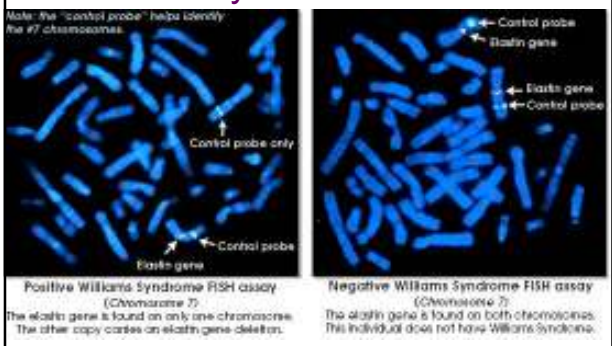


Contiguous gene syndrome

- 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

Contiguous gene syndrome

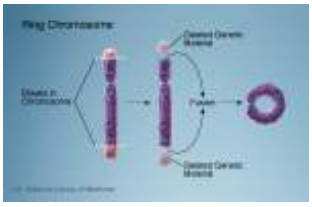
- **Prader- Willi syndrome** : 15q 11 – 13 deletion/ rearrangement
- **1/10,000-25,000 births**
- Infancy ⇒ poor feeding, hypotonia, 2nd – 3rd year ⇒ insatiable apatite, obesity, eat to death by age 5 or 6 if not treated, development delay, behavioral problem.
- **Other examples: Angleman synd., WAGR 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.



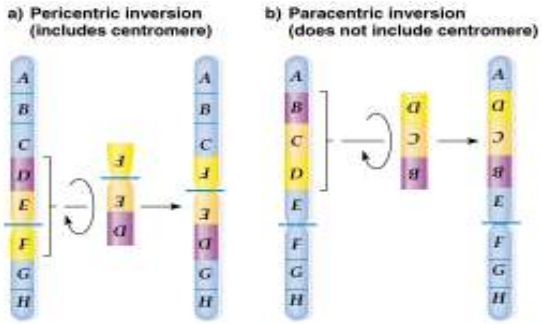
Disorders due to ring chromosome

- 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**

Inversion types



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.

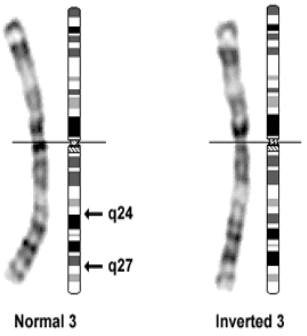
Inversion

- **Linked genes often are inverted together, so gene order typically remains the same.**
- **Homozygous:ADCBEFGH ⇒ no developmental problems**
  ADCBEFGH
- **Heterozygote: ABCDEFGH ⇒ unequal-crossing**
  ADCBEFGH
- **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.

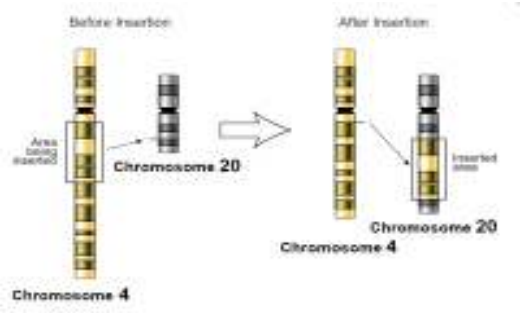
Inversion of Ch. 3



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)



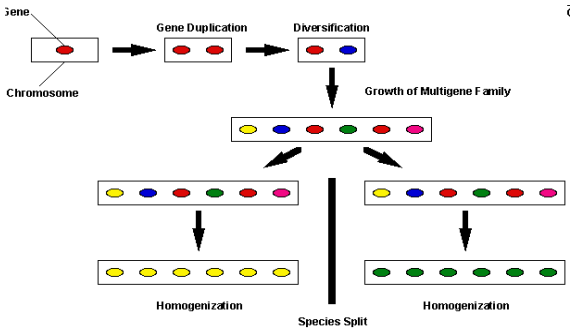
Duplication (INSERTION) consequences

- 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, actins, 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.



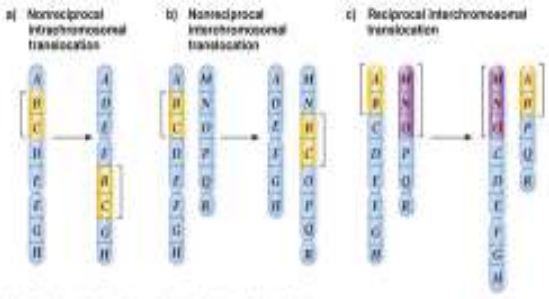
TRANSLOCATION

Change in location of chromosome segment; no DNA is lost or gained.
May change expression = position effect.

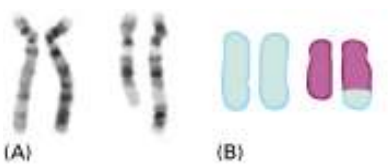
- Intrachomosomal
- Interchromosomal
  - Reciprocal - segments are exchanged.
  - Non-reciprocal - no two-way exchange.

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

TRANSLOCATION



A chromosomal translocation as revealed by two different karyotyping techniques

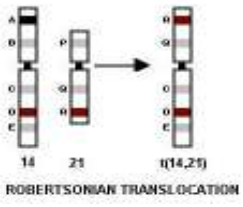


Chromosome banding

Chromosome painting

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



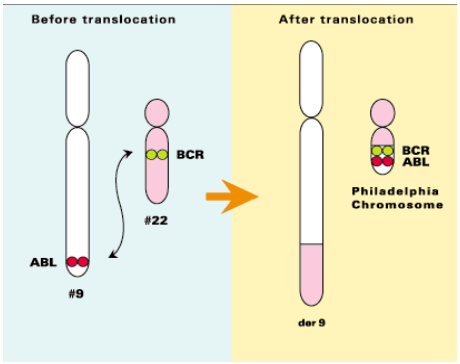
Philadelphia chromosome

- 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.

Philadelphia chromosome

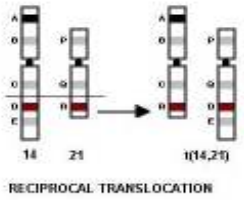
- Translocation produces a new, abnormal gene called BCR-ABL.
- This abnormal gene produces Bcr-Abl protein with tyrosine kinase activity
- This protein causes the excess WBCs typical of CML.

Philadelphia chromosome



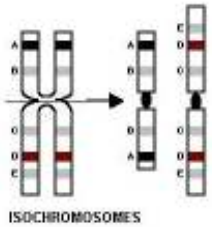
Reciprocal translocation

- 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

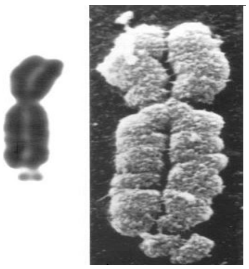
- When a chromosome divides at right angle rather than at its usual longitudinal plane.
- Loss of one arm with duplication the other arm (mirror image)
- 15% Turner's where the X ch. is composed of two long arms.
- Can happen – in acrocentric Ch. i.e. 45 XX i (21p); in X ch. 46XXi (X)
- 100% risk of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



CHROMOSOME FRAGILE SITES

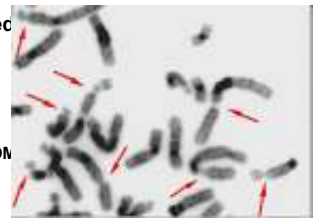
- Secondary constriction seen in specific cultural conditions are liable to break
- 80 common & 26 rare fragile sites. Most are induced by antifolate agents
- e.g. Folate sensitive fragile site on X chromosome.
- However, most are not associated with clinical abnormality.
- Molecular basis is trinucleotide 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



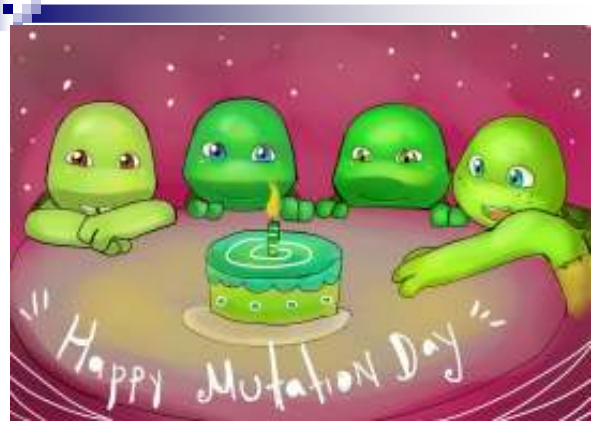
CHROMOSOME BREAKAGE

- **Visible breaks in metaphase chromosome inherited as AR trait.**
- **Cause : Faulty DNA repair or synthesis, radiation, chemicals**
- **FANCONI ANEMIA /BLOOM SYNDROME /ATAXIA TELANGECTASIA**



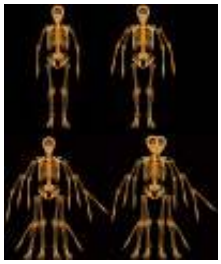
MUTATION
AND
IT'S CONSEQUENCES

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 by-pass of naturally occurring DNA damage (also called error prone translesion synthesis),
3. errors introduced during DNA repair
4. induced mutations caused by mutagens.

Mutation classes

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)	GGTCTCCTCACGCCA
	↓
mRNA	CCAGAGGAGUGCGGU
	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

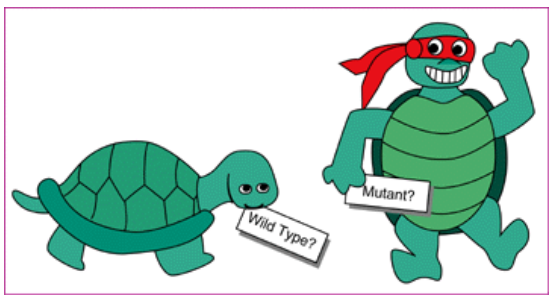
- **Loss-of-function mutations** are the result of gene product having less or no function.
  - **Amorphic mutation:** When the allele has a complete loss of function (null allele). Phenotypes associated with such mutations are most often recessive.
- **Haploinsufficiency:** when the reduced dosage of 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

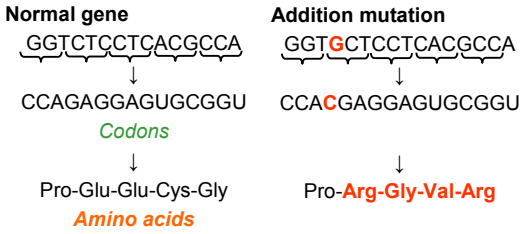
- **Frameshift mutation**
- **Nonsense mutation**
- **Missense mutations**
- **Neutral mutation**
- **Silent mutations (*synonymous mutation)***

Classification by impact on protein sequence

- 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*

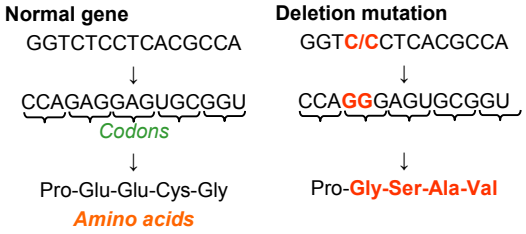
Mutations: Additions

A frame shift mutation



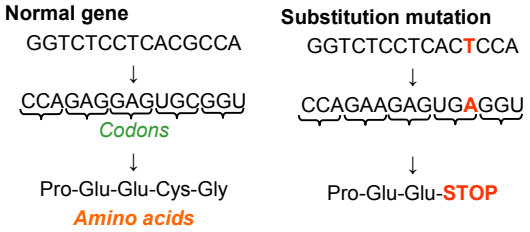
Mutations: Deletions

A frame shift mutation



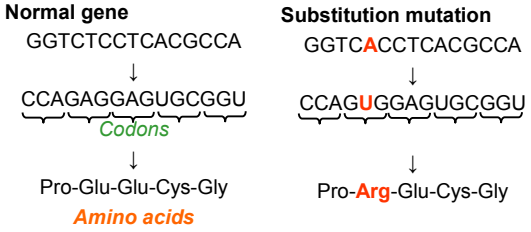
A **nonsense mutation** is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and possibly a truncated, and often nonfunctional protein product.

Nonsense mutation: Disaster



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

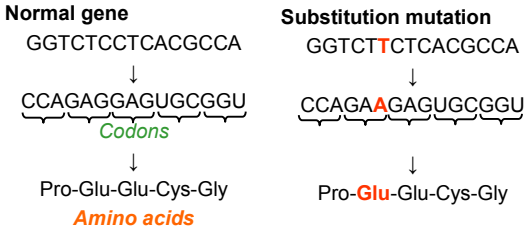


Substitutions will only affect a single codon
Their effects may not be serious unless they affect an amino acid that is essential for the structure and function of the finished protein molecule (e.g. sickle cell anaemia)

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.

No change



Classification by impact on protein sequence

- 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 BASIS OF MENDELIAN DISEASE

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 (± 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

- **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 Hypercholestoleremia**
- 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.

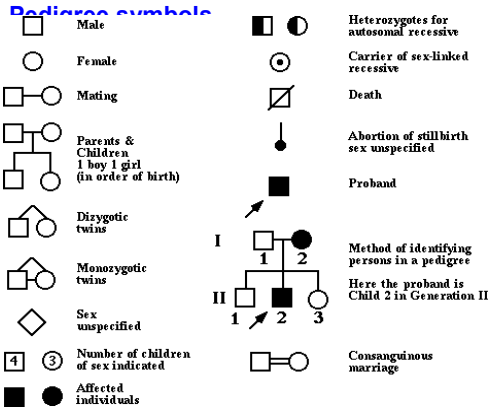
Mutations resulting in unusual reactions to drugs

- Glucose -6-phoshpate 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 → Antimalerial → Severe hemolysis
- Cytochrome P450 enzymes
  - used by the liver to metabolize many drugs
  - changes in CYP enzyme levels affect drug metabolism.

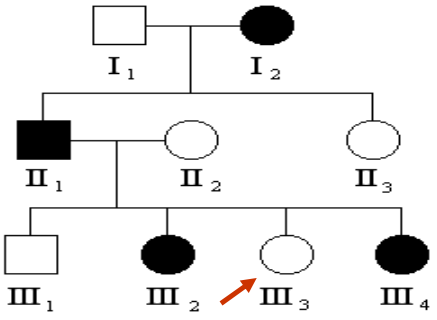
PEDIGREE CHART

- Drawing a family history (pedigree) chart is a helpful shorthand method of in 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.**



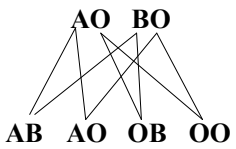
Pedigree chart construction



POSSIBLE COMBINATION OF GAMETS

	A	O
B	AB	BO
O	AO	OO

Punnet square



Mating diagram

- *Pedigree chart*
- *and*
- *Punnet square*

are NOT SAME

- Thank you

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.

Mutation classes

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.[22] Likewise, in yeast, Kunz et al.[23]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

Induced mutation

- *Induced mutations* on the molecular level can be caused by:-
- Chemicals
  - Hydroxylamine NH 2OH
  - Base analogs (e.g., BrdU)
  - Alkylating agents (e.g., *N*-ethyl-*N*-nitrosourea) These agents can mutate both replicating and non-replicating DNA. In contrast, a base analog can mutate the DNA only when the analog is incorporated in replicating the DNA. Each of these classes of chemical mutagens has certain effects that then lead to transitions, transversions, or deletions.
  - Agents that form DNA adducts (e.g., ochratoxin A metabolites)[26]
  - 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 adjacentpyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer. UV radiation, in particular longer-wave UVA, can also causeoxidative 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 to offspring, is called a*somatic cell genetic mutation* or *acquired mutation*.[76]
- 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.[77]
- 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

GGTCTCCTCACGCCA

↓

CCAGAGGAGUGCGGU

Codons

↓

Pro-Glu-Glu-Cys-Gly

Amino acids

Inversion mutation

GGTCCTCTCACGCCA

↓

CCAGGAGAGUGCGGU

↓

Pro-**Gly**-Glu-Cys-Gly

Mutations of haemoglobin

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

β 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.

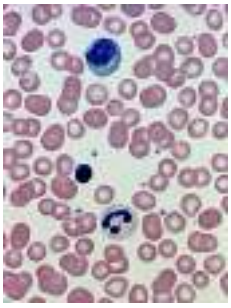
Methionine initiator

ATG GTG CAT CTG ACT CCT **GAG GAG** AAG TCT GCC
GTT ACT GCC CTG TGG GGC AAG GTG AAC GTG GAT
GAA GTT GGT GGT **GAG** GCC CTG GGC AGG CTG CTG
GTG GTC TAC CCT TGG ACC CAG AGG TTC TTT GAG
TCC TTT GGG GAT CTG TCC ACT CCT GAT GCT GTT
ATG GGC AAC CCT AAG GTG AAG GCT **CAT** GGC AAG
AAA **GTG** CTC GGT GCC TTT AGT GAT GGC CTG GCT
CAC CTG GAC AAC CTC AAG GGC ACC TTT GCC ACA
CTG AGT GAG CTG CAC TGT GAC AAG CTG CAC GTG
GAT CCT GAG AAC TTC AGG CTC CTG GGC AAC GTG
CTG GTC TGT GTG CTG GCC CAT CAC TTT GGC AAA
GAA TTC ACC CCA CCA GTG CAG GCT GCC TAT CAG
AAA GTG GTG GCT GGT GTG GCT AAT GCC CTG GCC
CAC AAG TAT CAC **TAA**

Nonsense terminator

Mutation	Codon	Change to DNA sense strand	Change in Amino Acid
S (sickle cell anaemia)	**6**	**GAG to GTG**	**Glu to Val**
C (cooley's syndrome)	6	GAG to AAG	Glu to Lys
$G_{San\ Jose}$	7	GAG to GGG	Glu to Gly
E	26	GAG to AAG	Glu to Lys
$M_{Saskatoon}$	63	CAT to TAT	His to Tyr
$M_{Milwauki}$	67	GTG to GAG	Val to Glu
O_{Arabia}	121	GAA to GTA	Glu to Val

Sickle Cell Anaemia



Blood smear (normal)
Image Credit: http://lifesci.rutgers.edu/~babiarz/

Sickle cell anemia
Image Credit: http://explore.ecb.org/

SINGLE GENE DISORDERS: Basic concepts

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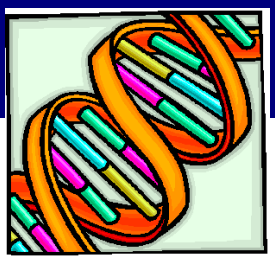
April 2014



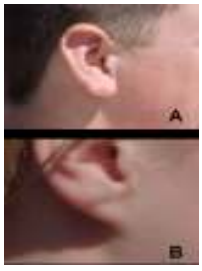
Children inherit traits from their parents. The study of the inheritance of these characteristics forms the basis of human genetics.

Observable Human Characteristics

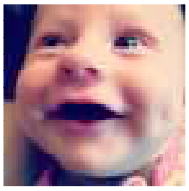
- We are all UNIQUE.
- Even though we share some characteristics with our peers and our family members, every one of us has a unique combination of traits.
- Some traits are controlled by genes that pass from parent to child.
- Others are acquired through learning.
- But most are influenced by a combination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

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.



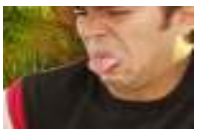
- Tongue Rolling
- Dimples
- Wet (dominant) or dry (recessive) earwax
-



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.

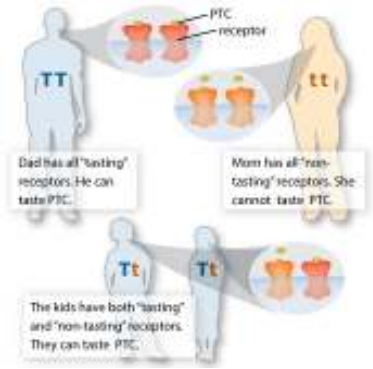


PTC tasting

(phenylthiocarbamide)

- 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



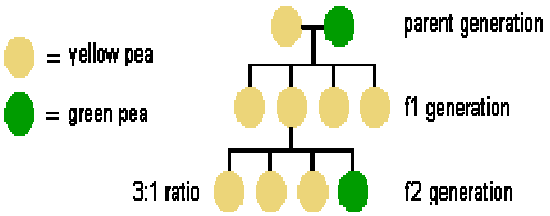
Gregor Mendel (1822-1844)

- 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 mendilism 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 alkaptans.

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

- **Autosomal dominant**

Disorder	**Prevalence**
Familial hypercholesterolemia	1 in 500
Polycystic kidney disease	1 in 1250
Huntington disease	1 in 2,500
Hereditary spherocytosis	1 in 5,000
Marfan syndrome	1 in 20,000

Prevalence of some single gene disorders

- **Autosomal recessive**

Disorder	**Prevalence**
Sickle cell anemia	1 in 625
(African Americans)Cystic fibrosis	1 in 2,000
(Caucasians)Tay-Sachs disease	1 in 3,000
(American Jews)Phenylketonuria	1 in 12,000
Mucopolysaccharidoses	1 in 25,000
Glycogen storage diseases	1 in 50,000
Galactosemia	1 in 57,000

Prevalence of some single gene disorders

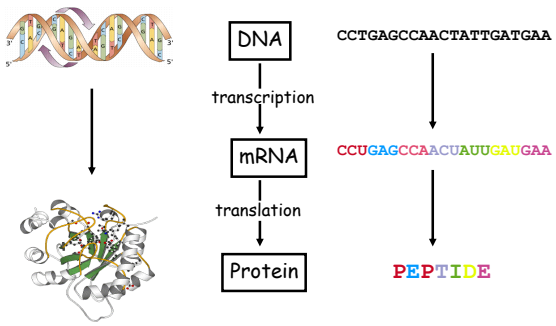
- **X-linked recessive**

Disorder	**Prevalence**
Duchenne muscular dystrophy	1 in 7,000
Hemophilia	1 in 10,000

single-letter code	*abbreviation*	*full name*
A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

20 AMINO ACIDS numerous proteins

A gene codes for a protein



DNA
Deoxyribonucleic Acid

Nucleotides are the building blocks of DNA

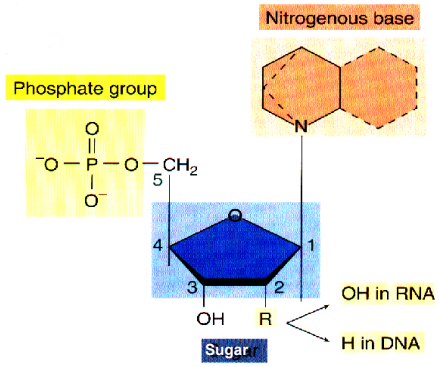
They contain 4 nitrogen-carbon-hydrogen bases that bond to form specific pairs:

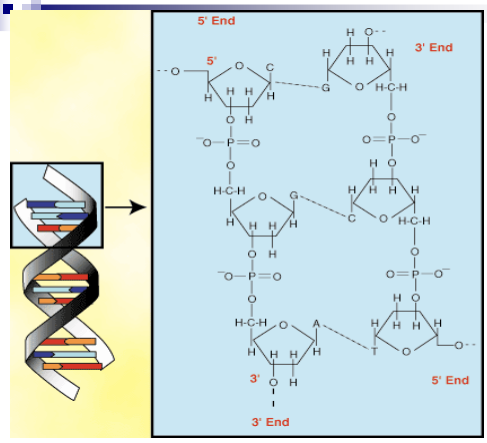
adenine can only pair with **thymine**

cytosine can only pair with **guanine**

The combination of **base pairs** *cannot vary*

Nucleotide





D N A

DNA STRUCTURE

hydrogen bonded nucleotides on opposite helices

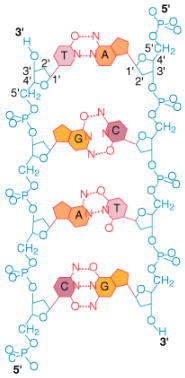
DNA helices are antiparallel

carbons on sugar define ends... 5' and 3'

pyrimidines bond with purines

T - A

C - G



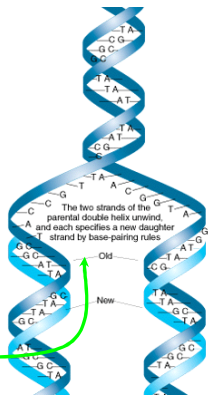
DNA REPLICATION

model of replication proposed by Watson & Crick (1953)

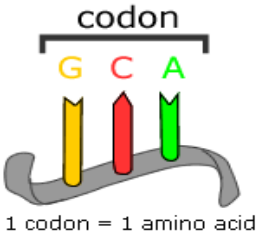
parental strand = template

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)



20 Amino acids are codified by:

First letter	Second letter U	Second letter C	Second letter A	Second letter G	Third letter
U	UUU, UUC Phenylalanine	UCU, UCC, UCA, UCG Serine	UAU, UAC Tyrosine	UGU, UGC Cysteine	U C A G
	UUA, UUG Leucine		UAA, UAG Stop codon	UGA Stop codon; UGG Tryptophan	
C	CUU, CUC, CUA, CUG Leucine	CCU, CCC, CCA, CCG Proline	CAU, CAC Histidine	CGU, CGC, CGA, CGG Arginine	U C A G
			CAA, CAG Glutamine		
A	AUU, AUC, AUA Isoleucine	ACU, ACC, ACA, ACG Threonine	AAU, AAC Asparagine	AGU, AGC Serine	U C A G
	AUG Methionine; start codon		AAA, AAG Lysine	AGA, AGG Arginine	
G	GUU, GUC, GUA, GUG Valine	GCU, GCC, GCA, GCG Alanine	GAU, GAC Aspartic acid	GGU, GGC, GGA, GGG Glycine	U C A G
			GAA, GAG Glutamic acid		

DNA sequence

```
.....acctc ctgtgcaaga acatgaaaca cctgtggttc ttccttctcc
tggtggcagc tcccagatgg gtcctgtccc aggtgcacct gcaggagtcg
ggcccaggac tggggaagcc tccagagctc aaaaccccac ttggtgacac
aactcacaca tgcccacggt gcccagagcc caaatcttgt gacacacctc
ccccgtgccc acggtgccca gagcccaaat cttgtgacac acctccccca
tgcccacggt gcccagagcc caaatcttgt gacacacctc ccccgtgccc
ccggtgccca gcacctgaac tcttgggagg accgtcagtc ttcctcttcc
ccccaaaacc caaggatacc cttatgattt cccggacccc tgaggtcacg
tgcgtggtgg tggacgtgag ccacgaagac cccgaggtcc agttcaagtg
gtacgtggac ggcgtggagg tgcataatgc caagacaaag ctgcgggagg
agcagtacaa cagcacgttc cgtgtggtca gcgtcctcac cgtcctgcac
caggactggc tgaacggcaa ggagtacaag tgcaaggtct ccaacaaagc
aaccaagtca gcctgacctg cctggtcaaa ggcttctacc ccagcgacat
cgccgtggag tgggagagca atgggcagcc ggagaacaac tacaacacca
cgcctcccat gctggactcc gacggctcct tcttcctcta cagcaagctc
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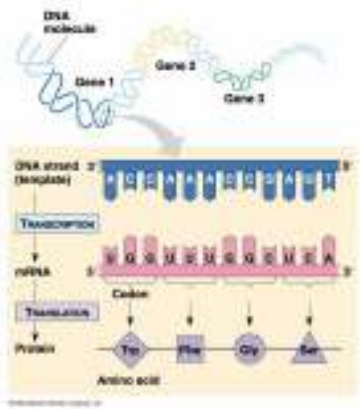


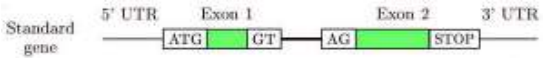
Genes

- **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

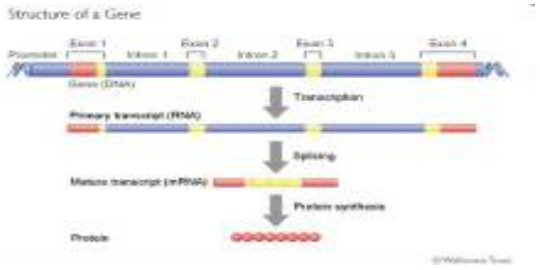
- **There are two general types of gene in the human genome:**
- **non-coding RNA genes**
  - **represent 2-5 per cent**
  - **encode functional RNA molecules**
- **protein-coding genes.**
  - **represent the majority**
  - **expressed in two stages: transcription and translation.**
  - **show incredible 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.



- The main characteristics are:
  - Coding and non coding exons
  - Introns
  - Translation start site (ATG)
  - Splice sites (GT, donor and AG, acceptor)
  - Translation termination site (STOPs: TAG, TGA and TAA)

- 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

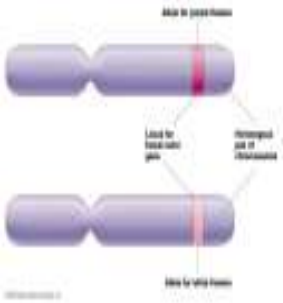


Definitions:

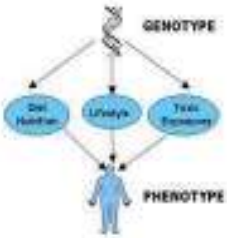
- **ALLELE: Alternative forms of both normal and abnormal genes**
- may be variations of normal
  e.g., blood group alleles
  Three alleles in ABO blood group:
  IA, IB, and IO
- may result in a medical disorder
  e.g., cystic fibrosis, hemophilia, Marfan disease

- **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***

LOCUS



Genotype & phenotype



- **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)

-is the phenomenon in which different mutations at the **same locus** causes a **similar phenotype.**

Example: β-thalassemia may be caused by several different mutations in the β-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 Hirshprung disease as well as multiple endocrine neoplasia (MEN) Type 2.

End of single gene basic concepts

SINGLE GENE DISORDERS

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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. [≥ 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

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**'

because

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

Single-gene disorders are primarily disorders of the pediatric age range

greater than 90% manifest before puberty

only 1% occur after the end of the reproductive period

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

AUTOSOMAL DOMINANT INHERITANCE

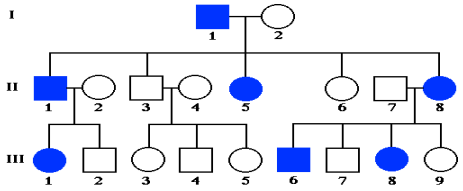
- **General features:**
- The trait appears in **every generation** without skipping
- Every affected child **has an affected parent**
  - Most common scenario in clinical practice: Heterozygote affected mate with normal homozygote person. In this situation 50% of the child will inherit the trait.
- **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

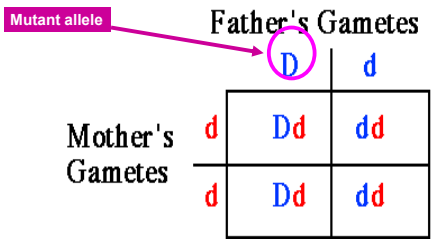
Disease	*Frequency/10,000 births*
Dominant otosclerosis	30
Familial hypercholesterolaemia	20
von Willebrand disease	10
Adult polycystic kidney disease	10
Huntington disease	5
Neurofibromatosis	4
Myotonic dystrophy	2
Tuberous sclerosis	1
Familial adenomatous polyposis	1
Dominant blindness	1
Total (of all dominant disorders)	100

AD pedigree



Pedigree 1. An idealized pedigree of a family with hypercholesterolemia, an autosomal dominant disease where the heterozygote has a reduced number of functional low density lipoprotein receptors.

Father affecred, mother normal- possibilities for offspring



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

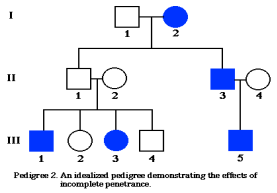
Penetrance

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



Pedigree 2. An idealized pedigree demonstrating the effects of incomplete penetrance.

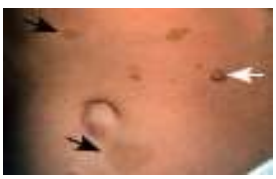
Expressivity

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.

Gene Genotype	Phenotype (Blood group.)
OAB OO	O
AO / AA	A
BB / BO	B
AB	AB

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: ??????????????????

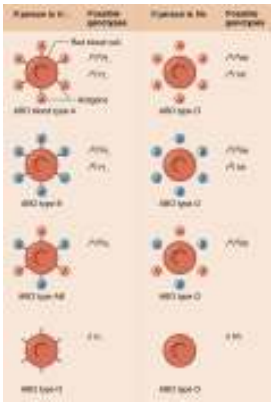
Bombay blood group

Epistasis: Bombay blood group

H gene is epistatic to the ABO gene.

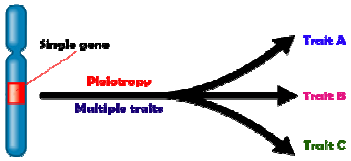
• H protein attaches the A or B protein to the cell surface.

- 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

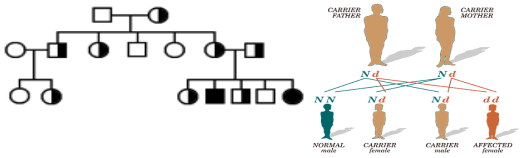
AUTOSOMAL RECESSIVE INHERITANCE

- 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

AR

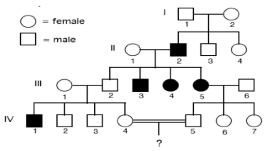


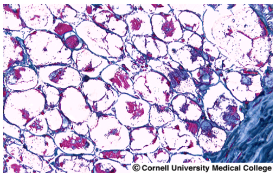
Cystic fibrosis

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**

Consanguinity





Hepatocytes in Lipid storage disease

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 inter-connected pathways shown here.

glycogen
⇅
glucose-1-P
⇅ Glucose-6-Phosphatase
glucose-6-P → glucose + P_i
⇅
fructose-6-P
↓ Phosphofructokinase
fructose-1,6-bisP
⇅
Glycolysis continued

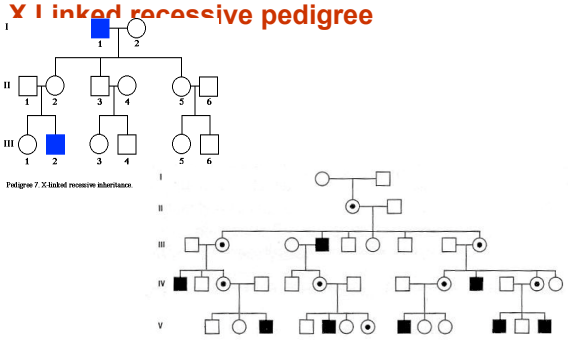
X LINKED RECESSIVE INHERITANCE

- 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

- Special feature: 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: Duchanne muscular dystrophy, Haemophilia, Becker muscular dystrophy, Lesch-Nyhan syndrome

X Linked recessive pedigree



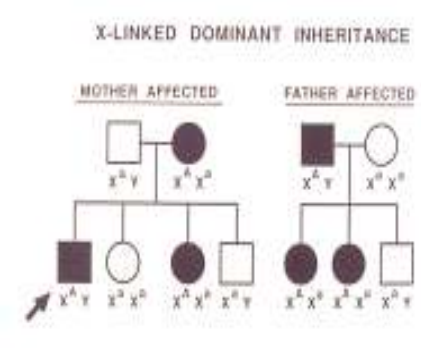
Duchenne Muscular Dystrophy

- 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.

	BB	Bb	bb
male	bald	bald	hair
female	bald	hair	hair

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 genetical
  - 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**

- **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

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)

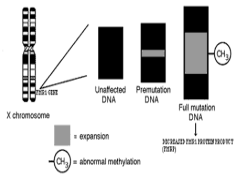
The site at which this happens is called the fragile X site and the gene involved is the *FMR-1* gene

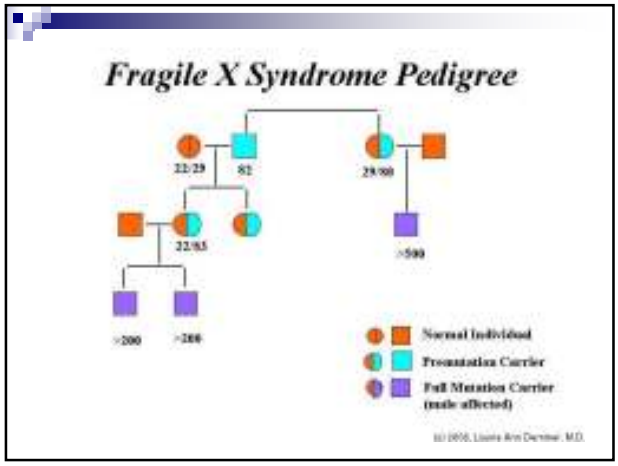
The *FMR-1* gene is in the long arm of the X chromosome at position Xq27.3

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.

Features of the disorder

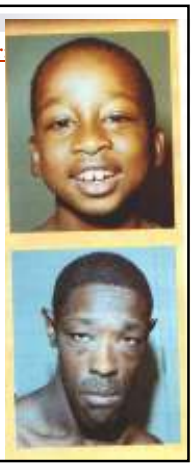
Mental Retardation

Average IQ of affected males is about 40

Behavior changes resembling autism

Delayed language skills

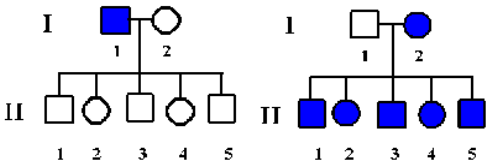
Poor coordination
Coarse facial features
Malformed, large ears
Long, narrow faces
Very large testicles



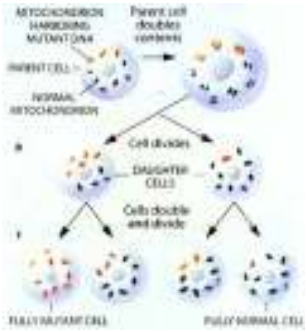
MITOCHONDRIAL INHERITANCE

- 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 oxydative phosphorylation. Rich tissue are skeletal & cardiac muscle, kidney, CNS.
- Example:_ Kearns- Sayre synd., Laber's optic neuropathy, mitochondrial myopathy

Mitochondrial inheritance prdigree



Mitochondrial inheritance



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

Uniparental Disomy and Genomic Imprinting

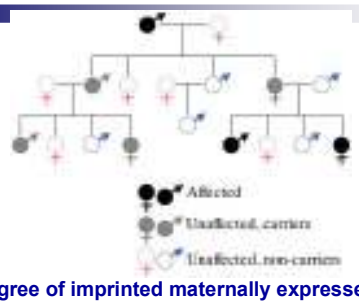
- Uniparental disomy: Presence of two copies of a chromosome (or part of a chromosome) from one parent and none from the other.
- **Discovered in 1988 in a child with cystic fibrosis and short stature who received two copies of the same chromosome 7 with a mutant CF gene from her carrier mother, and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

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.**

GENOMIC IMPRINTING

- **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 remain genetically silent (at least in somatic cells).**



- **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)

- The first recognized 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

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.

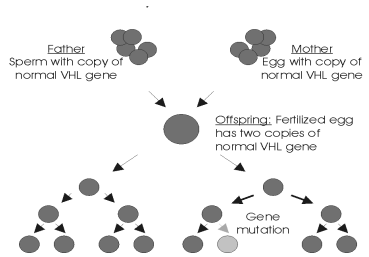
Gonadal mosaicism

- 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 mosaicsm 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.

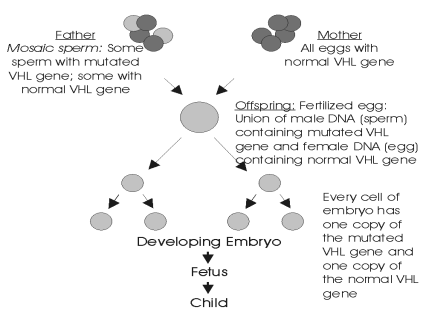
First in the Family: VHL Mosaicism

- 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

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Dept. of Pathology, BSMMU
May 2014

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

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 lacking folic acid.

Disorders due to triplet repeat mutation

- Long repeating sequences of three nucleotides, in most cases C and G

CGG CGG CGG CGG CGG .....

Examples:

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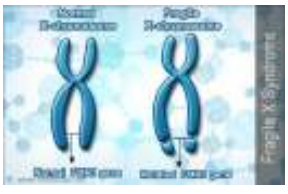

- X chromosomes are often appear broken at fragile site when cultured in media lacking folic acid.
- The site at which this happens is called the fragile X site and the gene involved is the *FMR-1* gene
- The *FMR-1* gene is in the long arm of the X chromosome at position Xq27.3

Familial mental retardation gene-1 (FMR-1) at Xq27.3 contains tandem repeats of CGG

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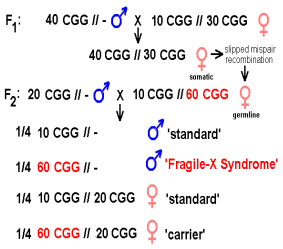

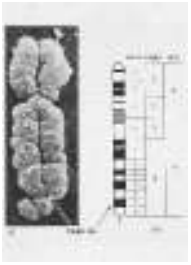

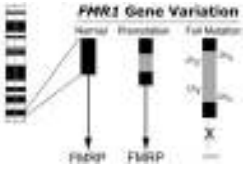

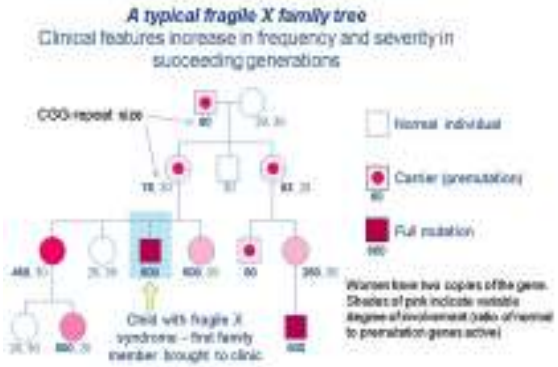

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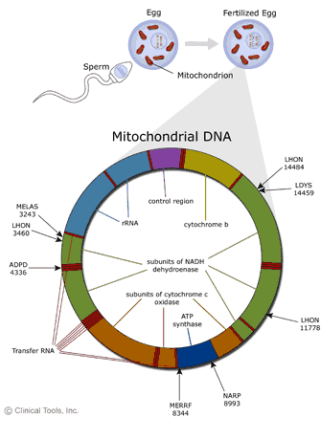
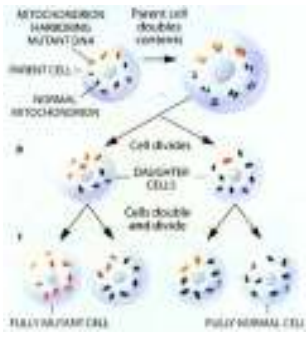
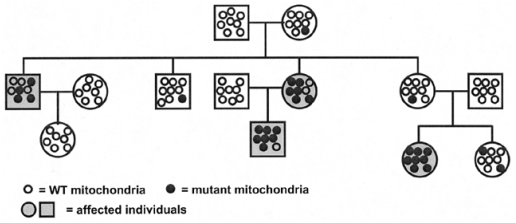


Mitochondrial inheritance



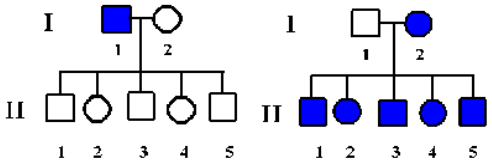
- Expression of disorders is quite variable because of uneven distribution of normal & mutant mtDNA in daughter cells after cell division.

Mitochondrial inheritance



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

Mitochondrial inheritance prdigree



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.

GENOMIC IMPRINTING

- Differential expression of genetic traits depending on whether it has been inherited from mother or father.

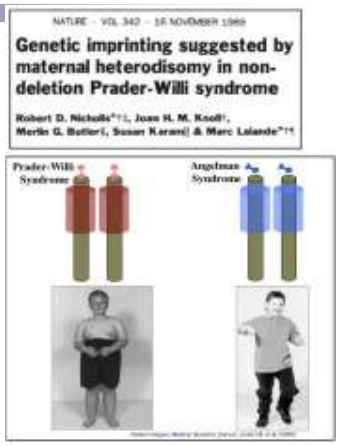
GENOMIC IMPRINTING

- Most regions of the genome are converted to gene products equally from the maternally and paternally derived members of a chromosome pair.
- 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 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.

Recognition of imprinted inheritance of Prader-Willi and Angelman syndromes.

Nicholls et al. reasoned that parentally imprinted gene(s) reside in human 15q11-13.


NATURE · VOL 342 · 16 NOVEMBER 1989

Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome

Robert D. Nicholls*†‡, Joan H. M. Knoll†, Merlin G. Butler§, Susan Karam‖ & Marc Lalande*†‡

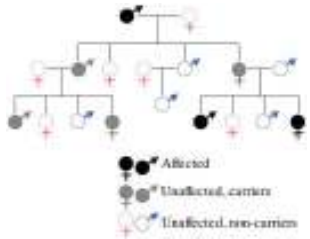
Prader-Willi syndrome (PWS)

- **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.**

Pedigree of an 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.

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 mosaicsm 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.

Gonadal mosaicism

- The clinical situation when a person has two populations of cells in the gonads (testes or ovaries), one population of cells containing the usual genetic complement whilst the other contains a DNA mutation or chromosome anomaly.
- The genetic change is confined solely to the germline (the cells which produce the gametes) of the parent so that the other cells in the person's body have the usual genetic complement.

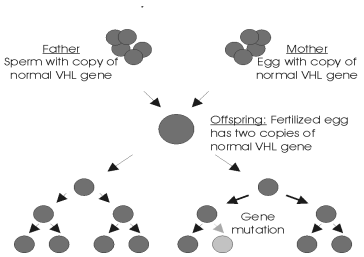
Gonadal mosaicism

- If a sperm or an egg produced from the cells in the parent's gonads containing the DNA mutation or chromosome anomaly is used to form a fetus, the child will have the genetic condition. Although the parent is healthy, he or she could have another child with the same genetic condition if the child is formed from a sperm or egg from the patch of cells in the gonad which contains the genetic change. A child would not have the condition if formed from the cells in the gonad with the usual genetic pattern.
- Gonadal/germline mosaicism is a likely explanation of the rare situations where a person without a dominant condition can have two children with the same autosomal dominant condition.

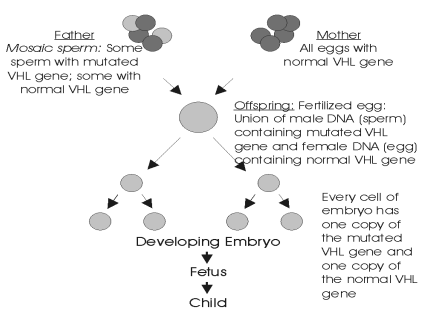
First in the Family: VHL Mosaicism

- 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.
- Why unaffected parents may have one or more affected children.

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.



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.**

In mythology, a chimera is a fire-breathing monster composed with a lion's head, a goat's body and a serpent's tail.

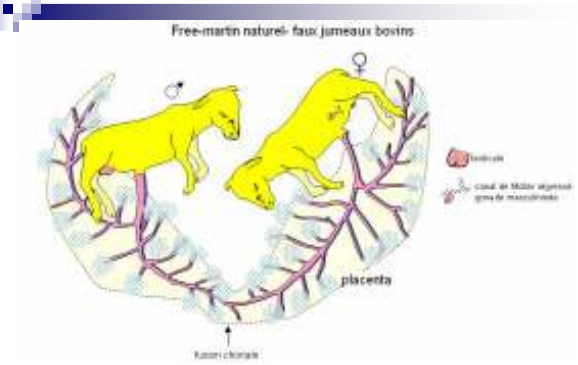
- **Cytogenetic Mosaics**
- **more than one cytogenetically-distinct population of cells.**
- example, 46, XX and 47, XXX.
- Turner syndrome mosaics

Chimeras

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

- **Chimeric cattle are not at 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 signifcance 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.**



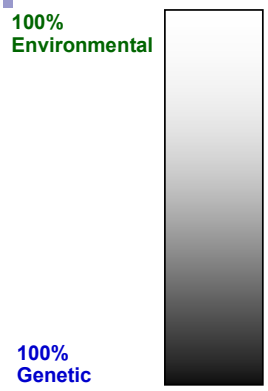
- **Chimeras are also produced experimentally, and have been a valuable research tool in several biomedical disciplines.**
- The basic technique is to combine two very early embryos such that their cells intermix and the resulting conceptus has cells from both original embryos.

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

Purely genetic 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.

MULTIFACTORIAL INHERITANCE

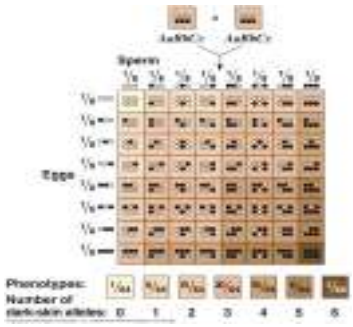
- If multiple elements are responsible for a characteristic or disorder this is known as multifactorial inheritance.
- Physical build and intelligence are examples of 'normal' multifactorial characteristics.
- Known multifactorial conditions include spina bifida, cleft lip and palate, club foot, congenital cardiac disorders and Crohn's disease.

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

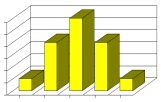
POLYGENIC TRAITS

- 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.

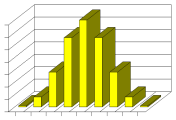


POLYGENIC INHERITANCE: *contribution of genes involved*

- 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**
  - **Schiozphrenia**

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

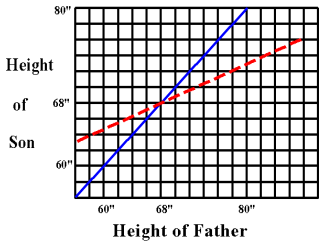
REGRESSION TO THE MEAN

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.

REGRESSION TO THE MEAN



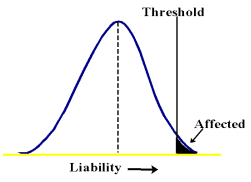
- **A representation of Galton's studies on the inheritance of height.**
- **If the son's height were determined only by the father's height, the correlation should be that of the solid line. The dashed line is what is observed. Galton called this "regression to mediocrity."**

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

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

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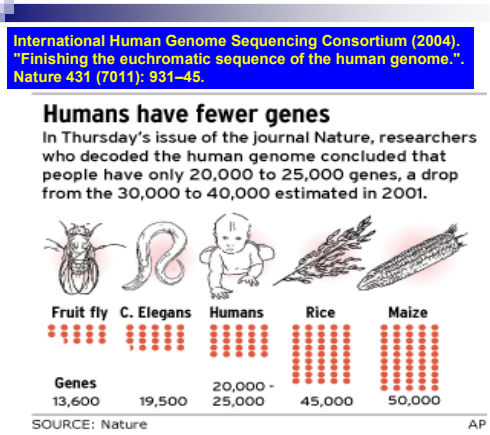
DNA base sequence

International Human Genome Sequencing Consortium (2004). "Finishing the euchromatic sequence of the human genome.". Nature 431 (7011): 931–45.



- 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 noncoding repeated elements.
- non-coding RNA molecules, regulatory DNA sequences, LINEs, SINEs, introns, and sequences for which as yet no function has been elucidated (noncoding 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 .

Repetitive or 'Junk' DNA

Despite its majority, scientists still do not know much about these "junk" DNA they are necessary and important to DNA synthesis.

These repetitive DNA can exists anywhere along a DNA strand

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
  - ***interspersed 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:
  - **A-T-T-C-G**-**A-T-T-C-G**-**A-T-T-C-G**
  - 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.

DNA POLYMORPHISM

Prof. M. Kamal

DNA POLYMORPHISM

- **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

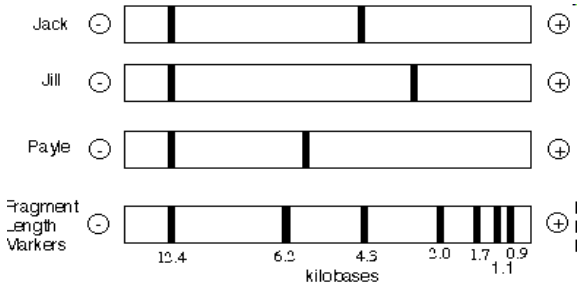
RFLP

(Restriction Fragment Length Polymorphism)

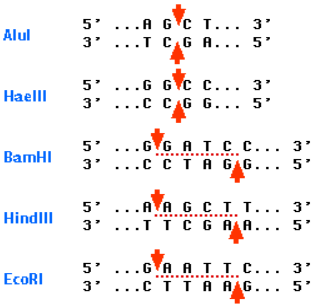
Restriction Enzymes (endonucleases)

- 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.

RFLP

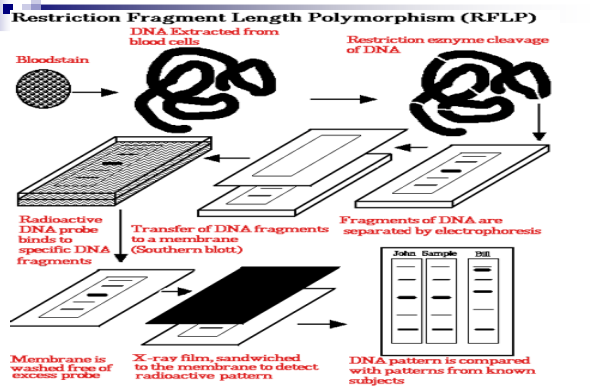


- Example: enzyme HaeIII cuts DNA wherever it encounters the sequence
  - 5'GGCC3'
    3'CCGG5'
- The cut is made between the adjacent G and C.



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

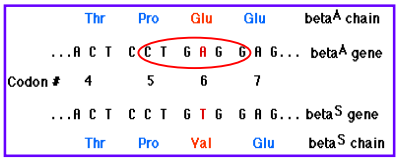


Application of RFLP

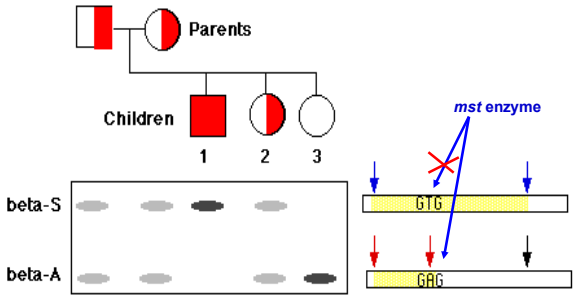
- Screening human DNA for the presence of mutant genes
- DNA "fingerprinting"

Screening for the sickle-cell gene

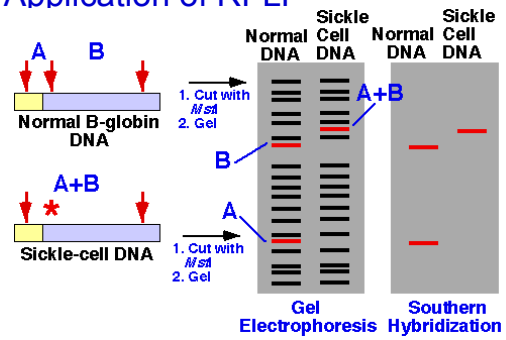
- "Normal" beta chains (betaA) ► **glutamic acid** at 6th position
  Patient's beta chain (betaS) ► **valine**
- The only difference between the two genes is the substitution of a T for an A in the middle position of codon 6.
- This mutation CHANGES sequence **CTGAGG**
- It is recognized and cut by ***mst* enzyme**



Screening for the sickle-cell gene

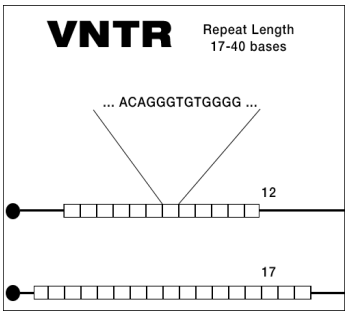


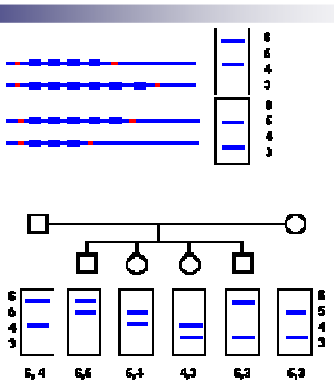
Application of RFLP



VNTR (Variable Number Tandem Repeat)

- Hypervariable sections of DNA consist of a short sequence of bases known as a motif, repeated at a variable number of times
- example, the top chromosome includes 12 motifs and the lower chromosome 17 motifs. The number of repeats varies by individual from as few as 7 to more than 40.
- *Sequence **motifs** are short, recurring patterns in DNA that are presumed to have a biological function.*



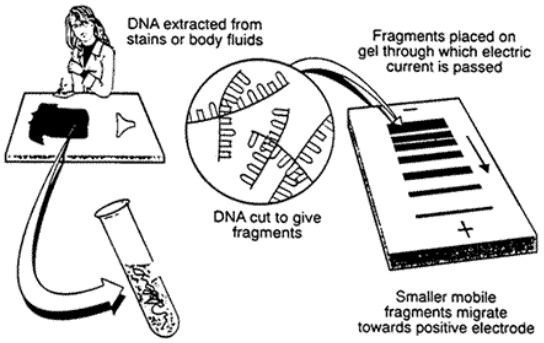


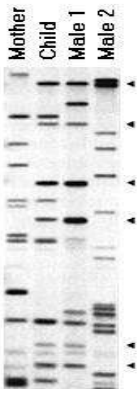
Fingerprinting

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)

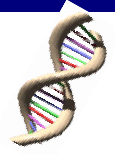
- STRs are repeated sequences of a few (usually four) nucleotides, e.g., TCATTCATTCATTCAT. They often occur in the untranslated parts of known genes
- The exact number of repeats (6, 7, 8, 9, etc.) varies in different people (and, often, in the gene on each chromosome; that is, people are often heterozygous 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
  - SNP's 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



Technology

- DNA isolation and fragmentation
- DNA labeling and hybridization
- DNA amplification:
- Separation of DNA on basis of size (Electrophoresis/chromatography)
- Identification of base sequence in DNA
- DNA synthesis

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

A brief review of technology

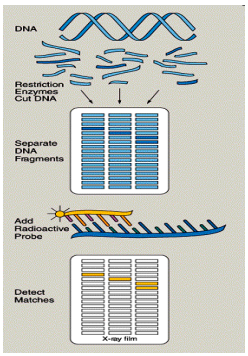
- DNA amplification:
  - Polymerase chain reaction
  - Recombinant technology
- Electrophoresis
  - Separation of DNA on basis of size
  - Column chromatography increasingly replacing electrophoresis for high throughput applications
- Identification of base sequence in DNA
  - Southern Blotting
  - Dot blot

DNA labelling and hybridization

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

DNA probe

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



DNA synthesis

- Oligonucleotide synthesis
- Gene synthesis

DNA amplification

- Polymerase Chain Reaction
- Recombinant DNA

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.**

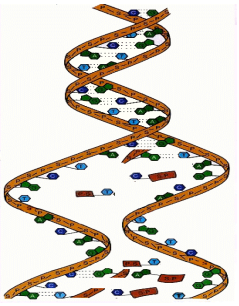
Principle of the PCR

- **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

Strand separation

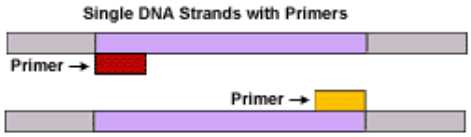
- The original DNA (the **target** DNA) is heated to 95^0C for 5 minutes and denatured.
- It separates into two single strand lengths of DNA.



Stages of the PCR cycle

Primer binding

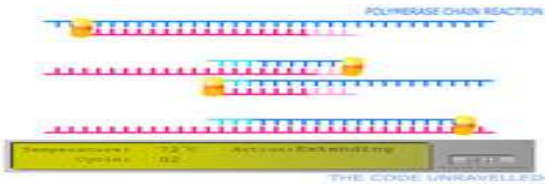
- The solution is rapidly cooled to 54^0C to allow the primers to bind to the complimentary base sequences on each of the single strands of DNA.
- This provides starting point for DNA replication



Stages of the PCR cycle

Strand synthesis

- **Solution is heated to 72^0C**
- **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**

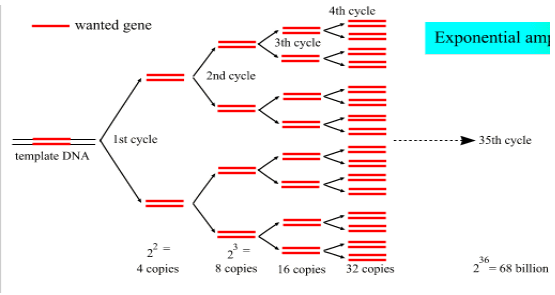


Uses of PCR

- **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 x 10^9).

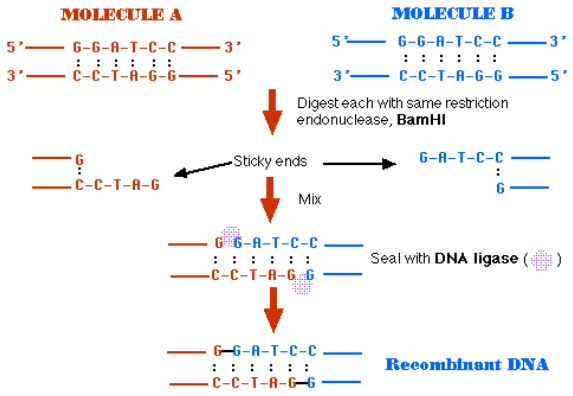


DNA Sequencing

- The most popular method for doing this is called the **dideoxy method**.
- DNA is synthesized from four deoxynucleotide triphosphate. 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

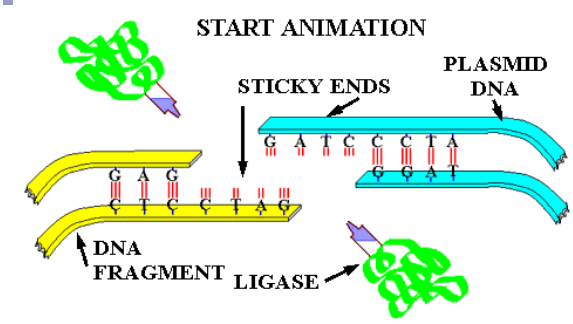
- **Recombinant DNA: New combinations/arrangements of DNA constructed in the laboratory**
- **It has been created artificially from two or more sources incorporated into a single recombinant molecule.**
- **Genetic Engineering: The design and construction of new combinations of genes (DNA)**



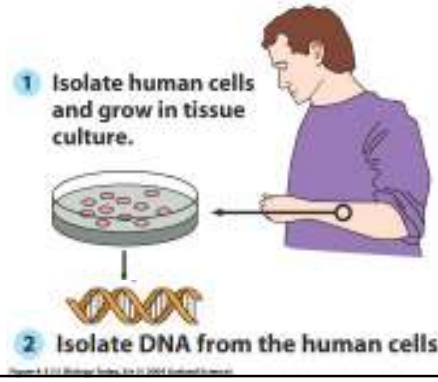
Construction of recombinant DNA molecules

- 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.)

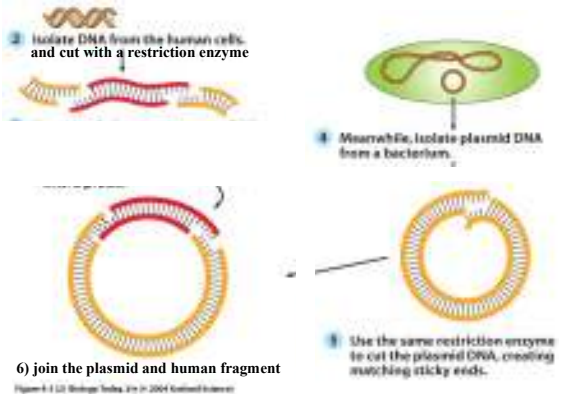
- Once a gene is cloned, its product may be produced in mass quantity



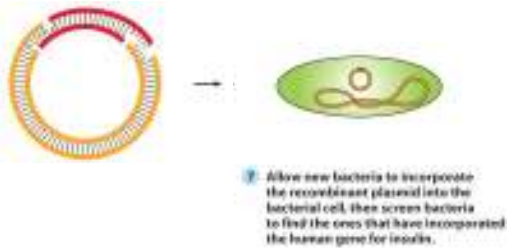
Harnessing the Power of Recombinant DNA Technology – Human Insulin Production by Bacteria



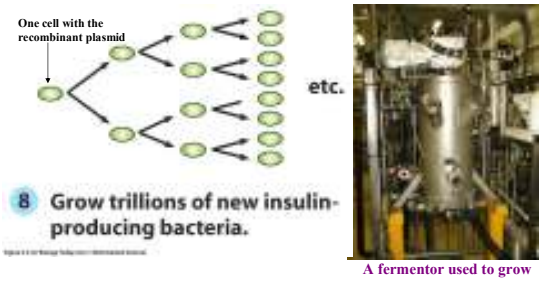
Human Insulin Production by Bacteria



Human Insulin Production by 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.

Route to the Production by Bacteria of Human Insulin

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

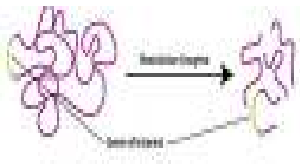
- Insulin for diabetics
- Factor VIII for males suffering from hemophilia A
- Factor IXor hemophilia B
- Human Growth factor**GH**)
- Erythropoietin (**EPO**) for treating anemia
- three types of interferons
- several interleukins
- (**GM-CSF**) for stimulating the bone marrow after a bone marrow transplant
- **HBsAg** to vaccinate against hepatitis B infection

SOUTHERN BLOTTING

- This procedure allows detection of various DNA gene sequences, and is one of the most widely used procedures in molecular biology.

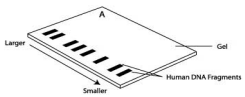
SOUTHERN BLOTTING

- Restriction endonuclease cuts down target DNA into small pieces.



SOUTHERN BLOTTING

- Agarose gel electrophoresis to separate DNA fragments

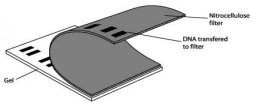


SOUTHERN BLOTTING

- Transfer of DNA fragments from gel to nitrocellulose membrane

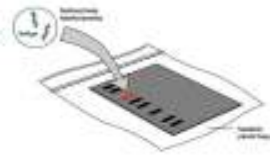
SOUTHERN BLOTTING

- DNA fragments transferred to NCM



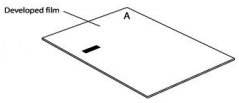
SOUTHERN BLOTTING

- Radioactively labeled probe hybridizes to target DNA fragment



SOUTHERN BLOTTING

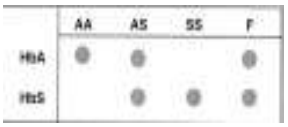
- Exposed X-ray film shows position of the probe binding.



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**

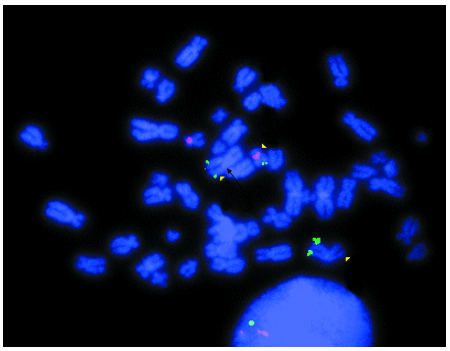
In situ hybridization

- 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

Chromosome in situ hybridization



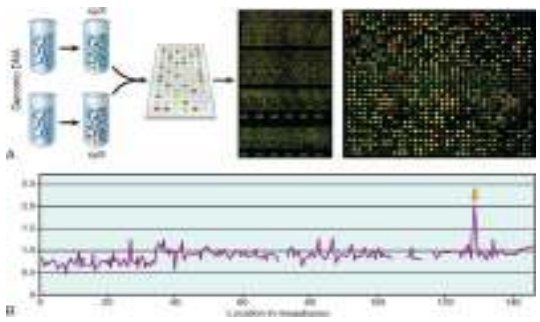
Tissue in-situ hybridization

In this procedure a labeled probe is hybridized agianst RNA/DNA in tissue sections

Tissue *in situ* hybridization to show HPV DNA in cervical epithelium

Array-Based Comparative Genomic Hybridization (Array CGH)

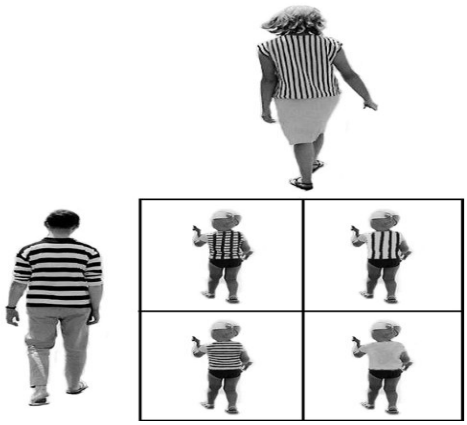
- Genomic abnormalities can be detected without prior knowledge of what these aberrations may be, using a global strategy such as array CGH.
- In array CGH the test DNA and a reference (normal) DNA are
- labeled with two different fluorescent dyes (most commonly Cy5 and Cy3, which fluoresce red and
- 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 coverall 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 andcancer.

GENETIC TESTING



WHAT IS GENETIC TESTING?

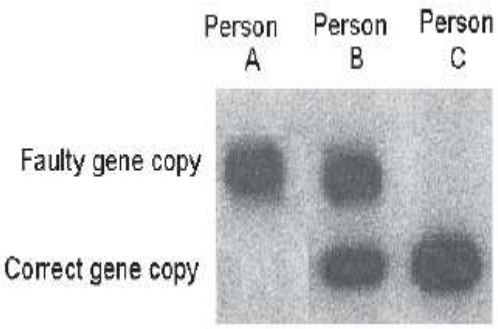
- Genetic testing is the examination of a person's
  - chromosomes,
  - DNA or
  - the biochemical product of a gene
- Results of these tests may
  - confirm or refute a suspected genetic condition
  - or possible predisposition to a condition.

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).

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.**



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.

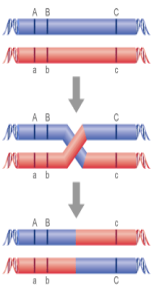
Tracking

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.

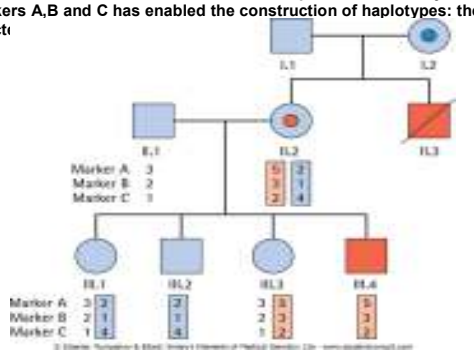
- The markers that are linked to the faulty or correct gene copies are
  - special to each family,
  - so this method of genetic testing can only be done within families.
- Indirect gene tracking is a "family test".

Principle of linkage analysis



- The top diagram shows paternal (blue) and maternal (red) chromosomes aligned in a germ cell
- Three DNA sequences, labelled 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 maternal and paternal alleles are mixed (recombined) and these mixed chromosomes are passed to the sperms 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.

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 affecte



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

Purpose of prenatal diagnosis

- Allow couples at high risk to know that the presence or absence of the disorder could be confirmed by testing
- Allow the couples the option of appropriate management (psychological, pregnancy/delivery, postnatal)
- To enable prenatal treatment of the affected foetus

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

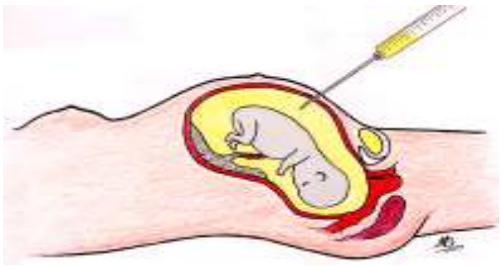
- ***Invasive:***
- Amniocentesis
- Chorionic villus sampling
- Cordocentesis
- Preimplatation genetic diagnosis
- Fetoscopy

- ***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 for 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.

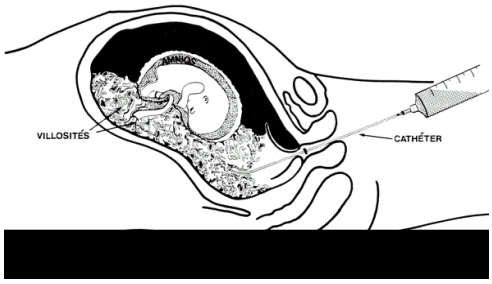
Amniocentesis procedure



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 drived from the outer layer of trophoblast.
- Disadvantage:
  - higher risk of abortion (2-3%)
  - limb abnormalities if carried before the 9 weeks of gestation.

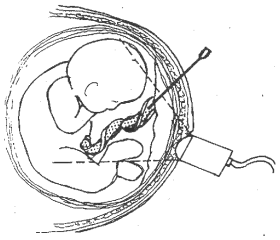
Chorionic villus sampling procedure



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.

Cordocentesis



Non-invasive methods of prenatal diagnosis

Maternal serum AFP

- Mostly done around the16 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

Non-invasive methods of prenatal diagnosis
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.

Maternal screening test

Incresed risk of	AFP	UE3	HCG
Down's syn.	Dec.	Dec.	Inc.
Trisomy 18	Dec.	Dec.	Dec.
NTD	Inc.	Not applicable	Not applicable

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

Ultrasonography

- Detailed fetal anomaly scanning is offered also to all pregnant women around the 18 weeks of gest. as a screening procedure for structural anomalies (NTD and cardiac anomalies)
- It can identify features which suggest underlying chromosomal abnormality indicating amniocentesis.

Problems in prenatal diagnosis

- Failure to obtain a sample or culture failure
- An ambiguous chromosome result
- An unexpected chromosome result

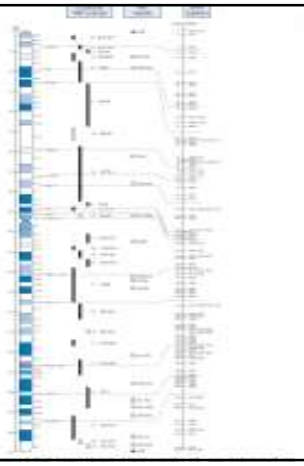
The Human Genome Project aimed to sequence the human genome in order to track down the genes responsible for inherited disease in humans.

- 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

Human Chromosor No.3

- A summary map



Prenatal treatment

- In the 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 .

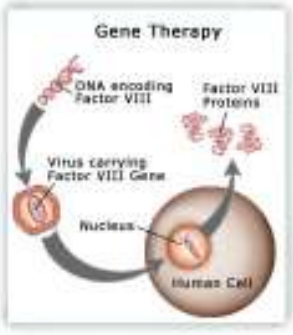
Treatment of genetic diseases

- 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 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.

process of gene therapy



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 (onkogene) 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

- Successful treatment of patients
  - Leber's Congenital Amaurosis,
  - X-linked SCID
  - ADA-SCID
  - Adrenoleukodystrophy and
  - Parkinson's disease.

Genetic Counselling

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.

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

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.

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.

Thank you and good luck

Thank you and good luck