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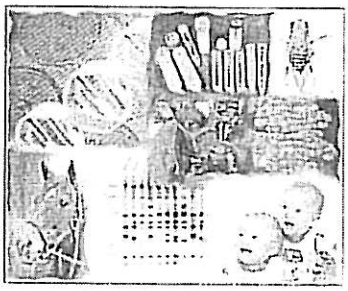
2250

Genetic Testing



Dr. Ebrahim Sakhinia

Tabriz University of Medical Sciences

Types of Genetic Tests

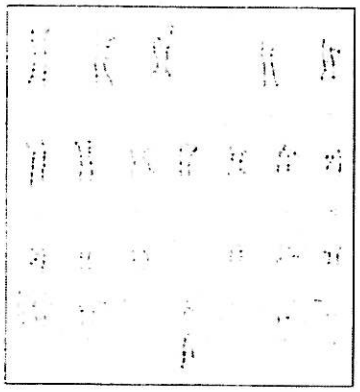
- Cytogenetic
- DNA
- Metabolic

Cytogenetic Test Methods

Routine karyotype

FISH

Routine banded karyotype



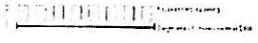
Routine banded karyotype detects

- Abnormal number of chromosomes
- Large duplications and deletions
- Balanced rearrangements (translocations, inversions)

FISH

fluorescent in situ hybridization: (FISH) A technique used to identify the presence of specific chromosomes or chromosomal regions through hybridization (attachment) of fluorescently-labeled DNA probes to denatured chromosomal DNA

Step 1. Preparation of probe. A probe is a fluorescently-labeled segment of DNA complementary to a chromosomal region of interest.



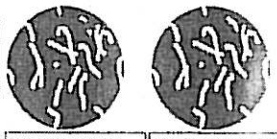
Step 2. Hybridization. Denatured chromosomes fixed on a microscope slide are exposed to the fluorescently-labeled probe. Hybridization (attachment) occurs between the probe and complementary (i.e., matching) chromosomal DNA.

FISH

Step 3 Visualization. Following hybridization, the slide is examined under a microscope using fluorescent lighting. Fluorescent signals indicate the presence of complementary chromosomal DNA; absence of fluorescent signals indicate absence of complementary chromosomal DNA.

Green signal =Normal control

Pink signal =Chromosome region of interest



Normal control:	Patient with deletion:
Two green signals	Two green signals
Two pink signals	One pink signal

FISH detects small (submicroscopic) chromosome

- Deletions
  - 15q11.2 deletion in Prader-Willi syndrome and Angelman syndrome
  - 22q11.2 deletion in velocardiofacial syndrome
- Duplications
  - *PMP22*– CMT1A
  - *PLP1*– Pelizeus-Merzbacher syndrome

International Laboratory Directory

575 Clinical and research laboratories

1115 Inherited diseases

800 clinical tests

315 research tests only

DNA testing

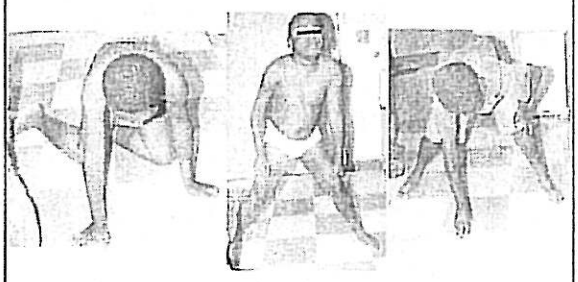
Why?

- Confirm/refute clinical diagnosis
  - Not usually
  - eg fragile X, neurodegenerative disorders
- Assess carrier status
  - Individuals or couples
- Prenatal diagnosis
- Predictive testing
  - Adult onset inherited disorders
  - Huntington's, cancer predisposition

DNA testing

For whom?

- Family history or other clinical indication
- Typical Region:
  - *BRCA1* mutation carriers: about 5,000-10,000
  - *CFTR* mutation carriers 125,000 – 250,000
- Screening may be justifiable
  - If intervention available
- Risk-modifying gene mutations will be more common, but more controversial to test for and may need to be handled differently



A 10-year-old boy with DMD showing Gower sign maneuver. He walked before 16 months of age and had trouble getting up.

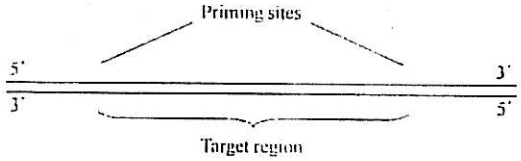
DMD

A male with DMD at 4-, 10-, and 29-year-old showing the progression of the disease. He has deletion of exons 49–54 of the dystrophin gene.

Methods for direct testing for a specific mutation

Polymerase chain reaction

- *In vitro* synthesis of large amounts of DNA by copying from small starting quantities
- Small synthetic primers define the boundaries of synthesis
- Primers can be tagged e.g. with radioisotope or fluorescent dyes to produce labelled products



1. Heat denaturation 94°C

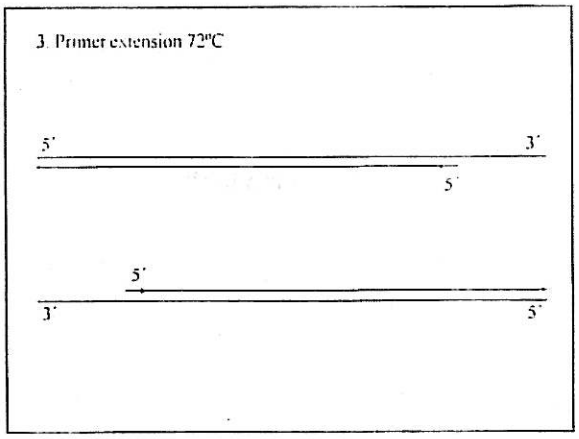
5' 3'

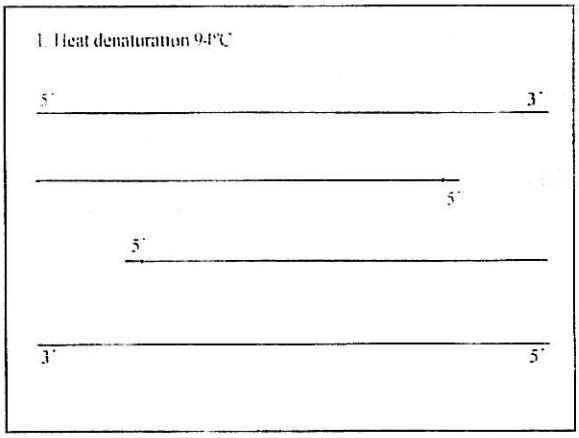
3' 5'

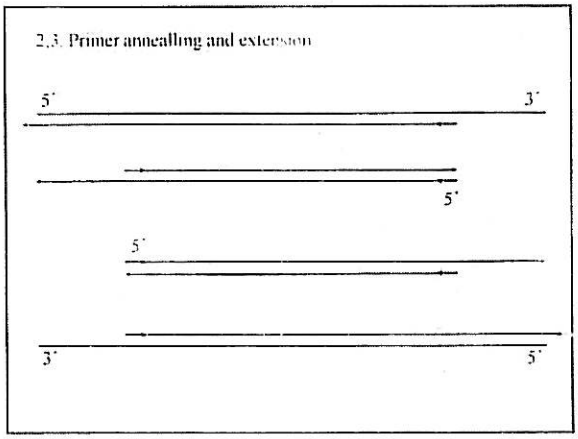
2. Primer annealling 55°C

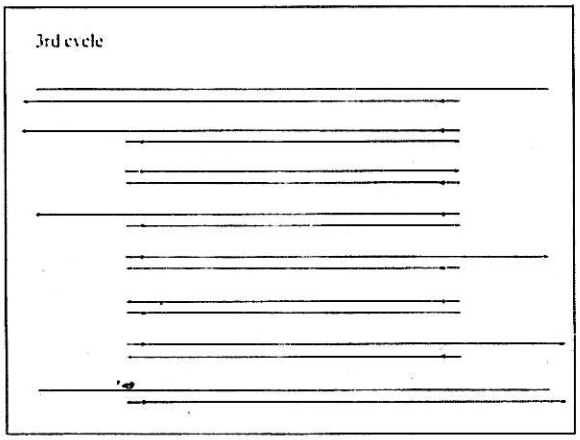
5' 3'

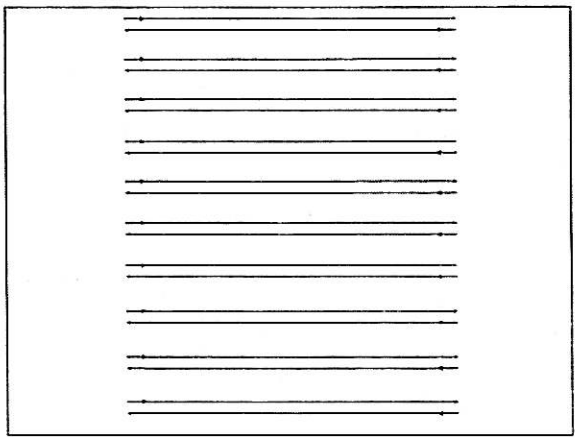
3' 5'

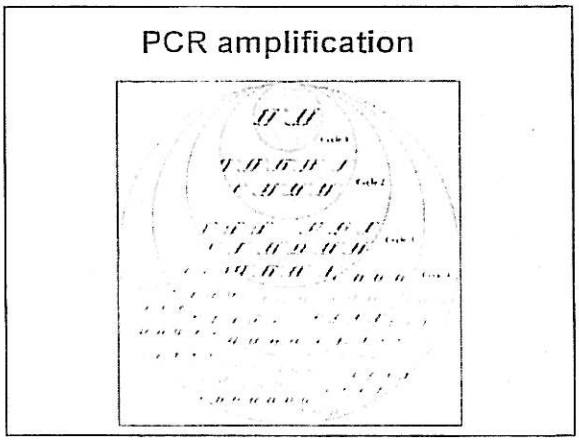












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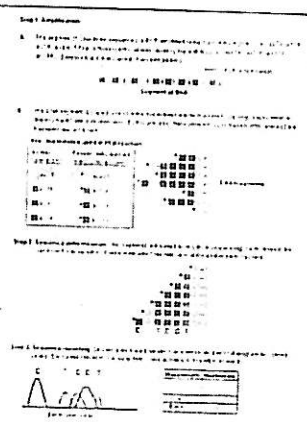
DNA-based test methods

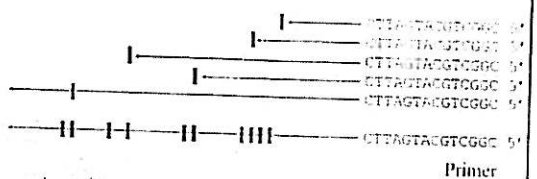
Sequence analysis
Mutation scanning
Targeted mutation analysis
Trinucleotide repeat analysis
Southern blot analysis

Sequence analysis



DNA sequencing

Template

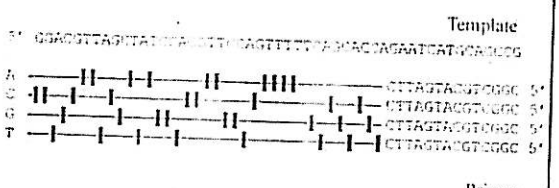


Primer

4 reactions, *e.g.*
A reaction contains dATP, dCTP, dGTP, dTTP, ddATP

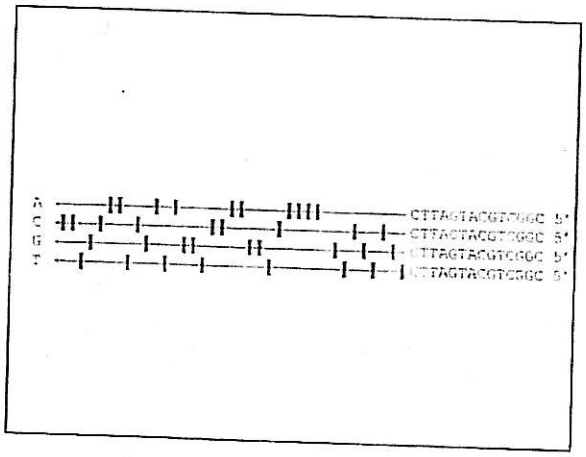
DNA sequencing

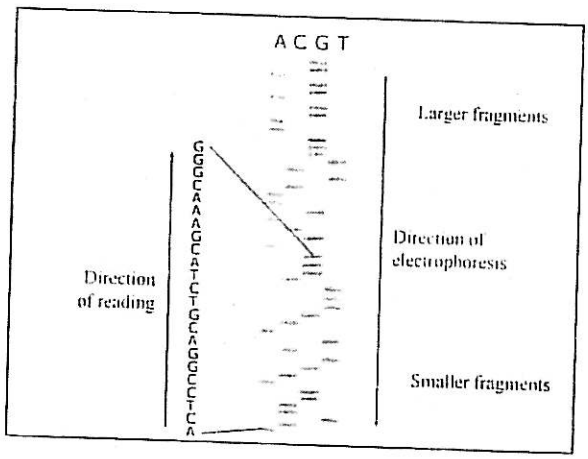
Template



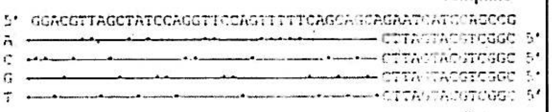
Primer

4 reactions, *e.g.*
A reaction contains dATP, dCTP, dGTP, dTTP, ddATP
C reaction contains dATP, dCTP, dGTP, dTTP, ddCTP

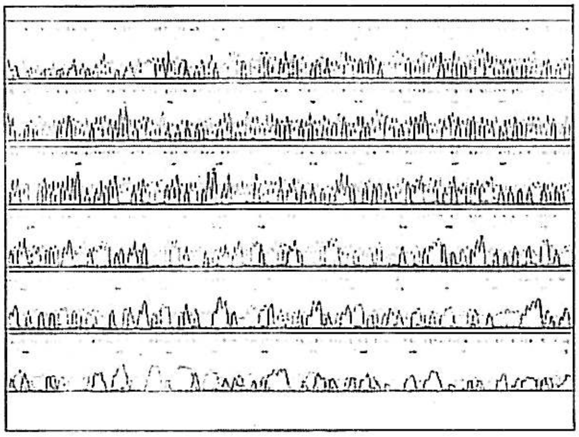




DNA sequencing

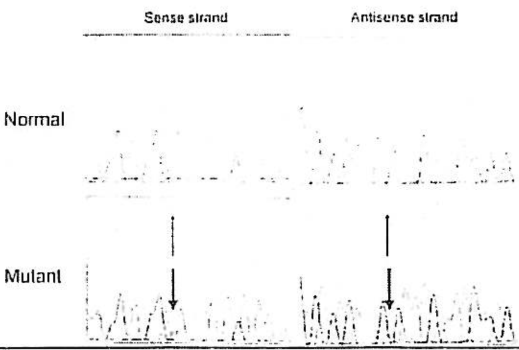


4 reactions, e.g.
A reaction contains dATP, dCTP, dGTP, dTTP, ddATP
C reaction contains dATP, dCTP, dGTP, dTTP, ddCTP



Heterozygous mutation detection by sequencing

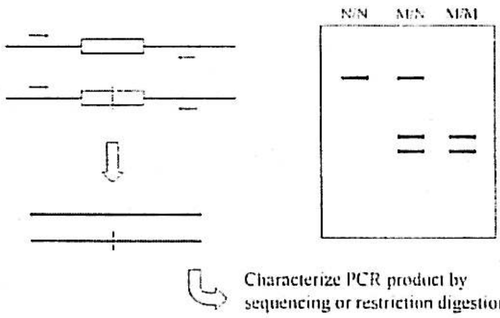
An imperfect technology



DNA-based test methods

- Sequence analysis
- Mutation scanning
- Targeted mutation analysis
  - Trinucleotide repeat analysis
  - Southern blot analysis

PCR - mutation detection



Characterize PCR product by sequencing or restriction digestion

Polymerase chain reaction

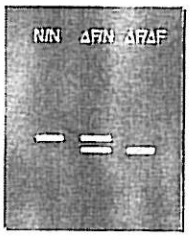
Analysis of products

- Gel electrophoresis
  - Size of fragment
- Structural analysis by –
  - Restriction digestion
  - Direct sequencing
  - Special mutation detection methods
    - SSCP, DGGE, CCM, DHPLC etc

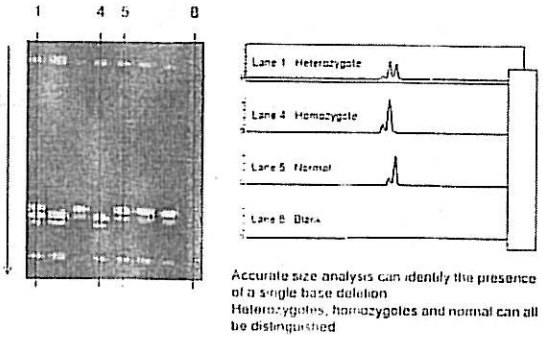
Polymerase chain reaction

CF mutation ΔF508

- 3 bp deletion eliminates single aa from CFTR
- Analysis by PCR & gel electrophoresis: altered product size
  - Or by other method
- 50% of CF patients will be homozygous



Connexin 26 (*GJB2*) Δ35G assay

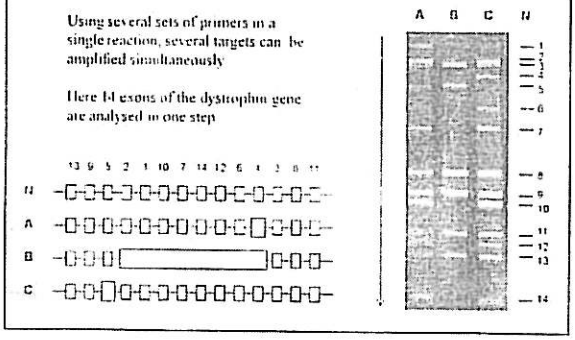


Accurate size analysis can identify the presence of a single base deletion
Heterozygotes, homozygotes and normal can all be distinguished

Multiplex PCR

Using several sets of primers in a single reaction, several targets can be amplified simultaneously

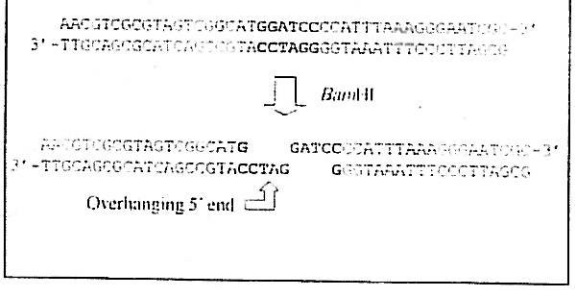
Here 14 exons of the dystrophin gene are analysed in one step



Restriction endonucleases

- Derived from bacteria
  - eg *Eco*RI from *Escherichia coli*
- Many different recognition sequences
  - Generate specific fragments from given DNA source
- Different overhanging ends at cut site
  - Important for designing cloning experiments

Restriction endonucleases



Restriction endonucleases

Sickle-cell disease

Detection of known point mutation detection by restriction enzyme cleavage

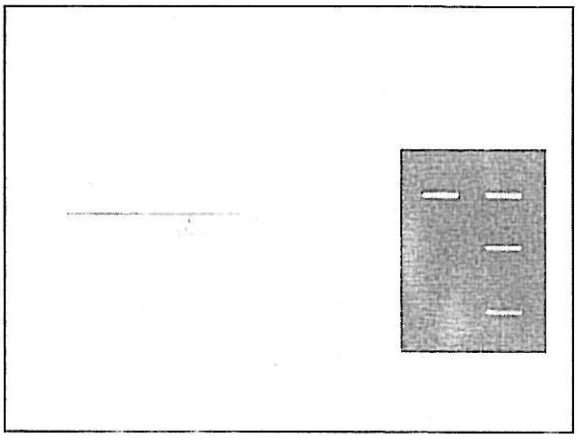
β_A CCT GAG GAG
Pro Glu Glu
5 6 7

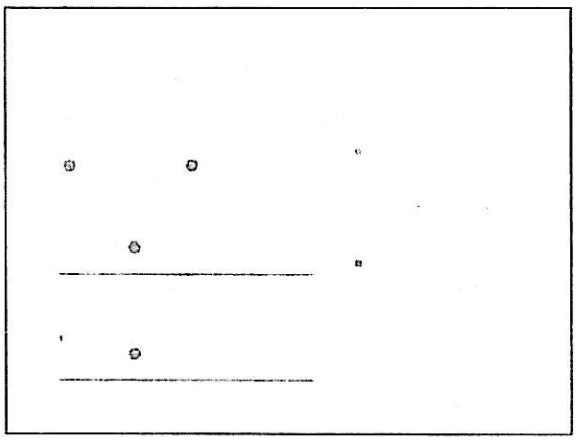
CCT GAG GAG
GGA CTC CTC

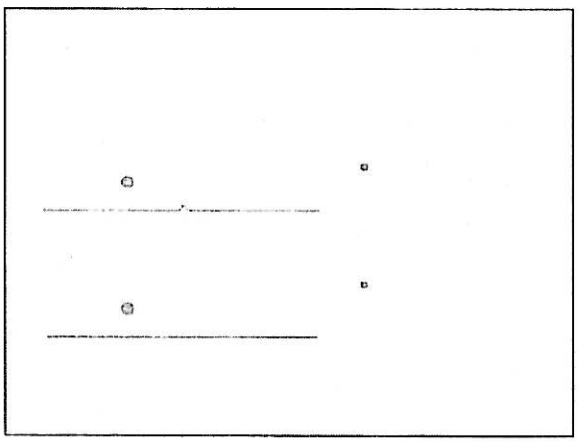
β_S CCT GTG GAG
Pro Val Glu
5 6 7

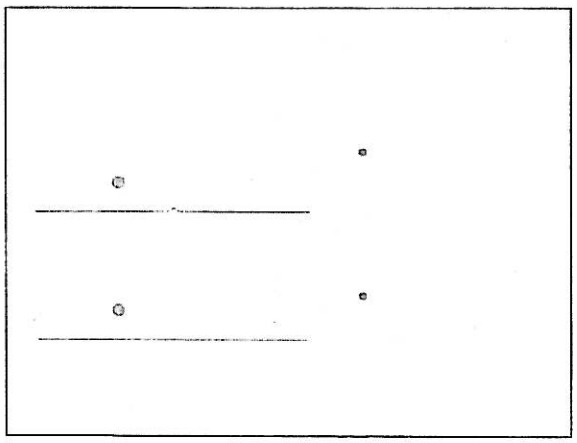
CCTNAGG
GGANTCC

*Msl*I recognition site

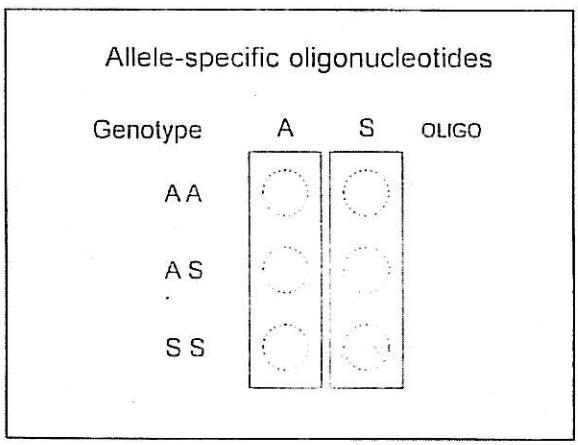








Allele-specific oligonucleotides



Detection of unknown mutations

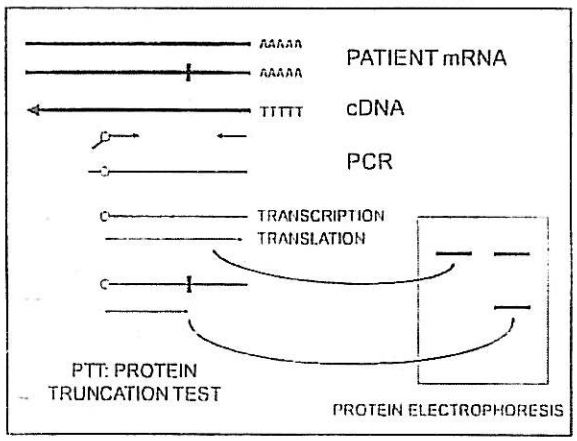
Some common methods

- Direct sequencing
- SSCP
  - Single strand conformational polymorphism
- PTT
  - Protein truncation test
- CCM
  - Chemical cleavage of mismatches
- DHPLC
  - Denaturing high performance liquid chromatography

Point mutation
-nonsense

```
GACTTTCGGCGAATG
AspPheArgArgMet
        ⇩
GACTTTCGGTGAATG
AspPheArg***Met
```

- Truncated protein produced
- May exploit this for mutation detection



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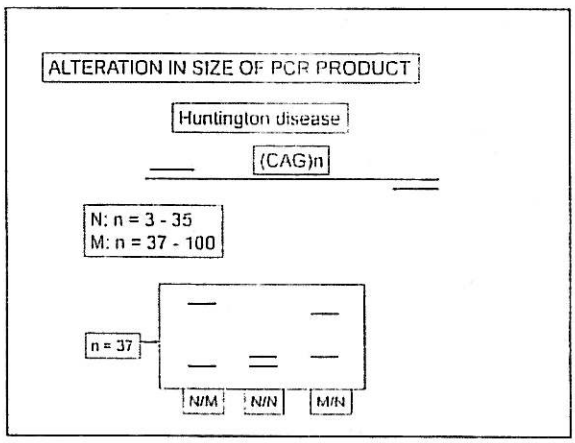


Metabolic Tests

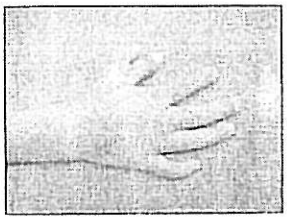
Analyte
Enzyme assay

Patterns of Inheritance

Dr. Ebrahim Sakhinia
Medical Genetic, Tabriz University of Medical Sciences

Overview

- Relevance
- Family Trees
- Inheritance Patterns
  - How to recognise them
  - How they work
  - In clinical practice



Relevance

- Inheritance patterns = major principle in genetics
- Diagnosis – spotting genetic disorders
  - For all clinicians. Why you ask for a family history!
- Risk to relatives

Pedigree drawing: Basic symbols

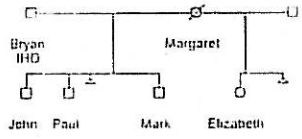
Male □ Female ○

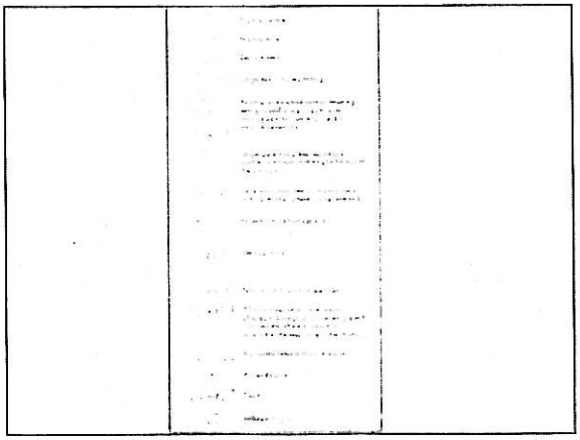
Unaffected □ Affected ■

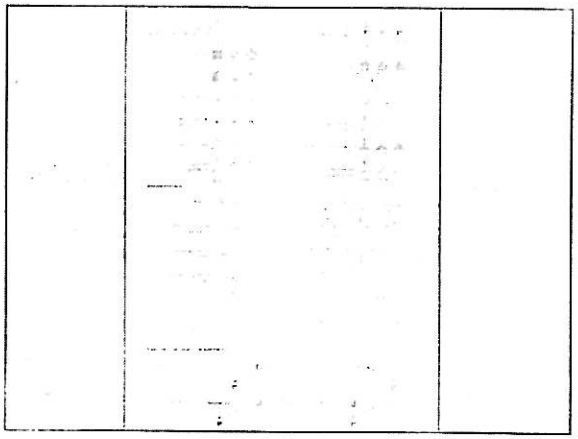
Miscarriage △ Dead ⊠

Family trees and pedigrees

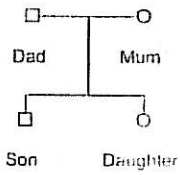
- Pedigree = short hand to record a family tree
- Which is better: text or picture?
  - John has two brothers Paul and Mark, and one half sister Elizabeth. His mother Margaret died last year, had 2 miscarriages, and his father Bryan has ischemic heart disease
  - OR:







Pedigree Drawing: relationships

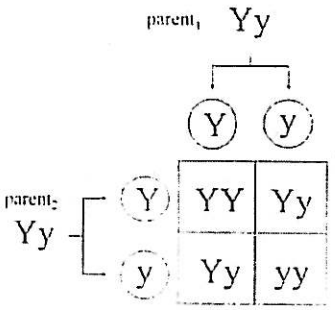


How are traits transmitted?

A. The particulate theory of inheritance

- Characters are distinct and hereditary determinants [genes] are particulate in nature
- Each adult has two genes for each character; different forms of the genes are called alleles
- Members of the gene pair segregate equally into gametes
- Fusion of the gametes at fertilisation restores the pair of genes and is random
- Different genes assort independently in gametes

Monohybrid crosses and Law of Segregation



Gene for seed colour has two alleles:
Y results in yellow seeds
y results in green seeds

Meiosis results in the formation of two types of gametes in equal numbers

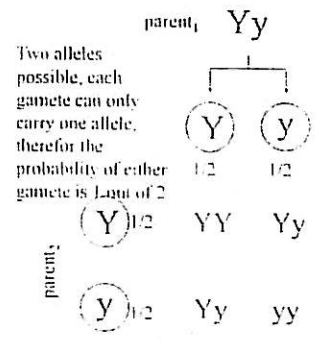
Fertilisation restores the two copies of the gene to the offspring

1 B 1 Monohybrid crosses and Law of Segregation

The offspring genotype is the specific alleles that they carry, but what do the offspring look like [what is their phenotype]?

One allele may be dominant over another, in this case the Y allele is dominant and the y allele is recessive

Transmission of alleles and probability



Two alleles possible, each gamete can only carry one allele, therefor the probability of either gamete is 1 out of 2

p(YY) = p(Y gamete) from parent1 *and* p(Y gamete) from parent 2 = 1/2 X 1/2 = 1/4

P(Yy) = p(Y gamete) from parent1 *and* (y gamete) from parent2 OR (y gamete) from parent1 and p(Y gamete) from parent2 = [1/2 X 1/2] + [1/2 X 1/2] = 1/4 + 1/4 = 1/2

Transmission of alleles and probability

parent$_1$ Yy

	Y (1/2)	y (1/2)
Y (1/2)	YY	Yy
y (1/2)	Yy	yy

parent$_2$

Genotypic ratio:
1 YY : 2 Yy : 1yy

Phenotypic ratio:
3 yellow : 1 green

Terms to describe transmission genetics

- **Gene** = basic unit of biological information. specific segment of DNA that encodes a protein
- **Allele** = alternative forms of a gene
- Genotype = alleles at a locus
- **Phenotype** = observable characteristics
- Homozygote = identical alleles at a locus
- Heterozygote = different alleles at a locus

Patterns of Inheritance

- Single gene (Mendelian)
  - Inheritance based on very simple rules…
- Not single gene (non-mendelian)

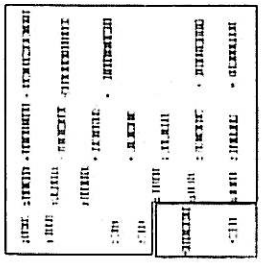
Gregor Mendel

Categories of Genetic Disorders

Single Gene :Patterns
- Autosomal dominant - Huntington's
- Autosomal recessive - Cystic fibrosis
- X-linked recessive - Duchenne MD
- X-linked dominant - Rare

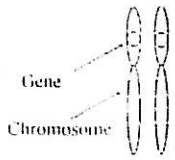
Mitochondrial Disorders - ?
- Neurological disorders, deafness

Autosome vs Sex chromosomes

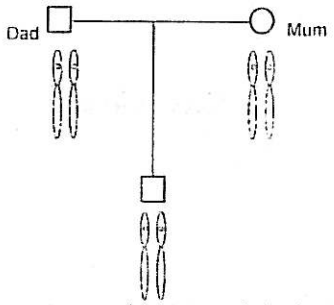


Dominant versus recessive

- Chromosomes and genes are in pairs
- Each gene in a pair is called an allele



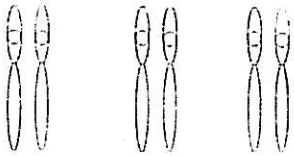
Dominant versus recessive



- Chromosomes and genes are inherited – one from dad, one from mum

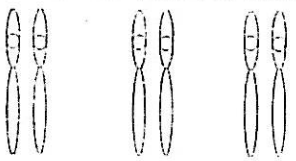
Dominant versus recessive

- Sometimes alleles are different,
- eg hair colour, BLACK vs red
- Phenotype outcome depends on the nature of the allele: DOMINANT or recessive



Dominant versus recessive

- Sometimes alleles are different,
- eg hair colour, BLACK vs red
- Phenotype outcome depends on the nature of the allele: DOMINANT or recessive

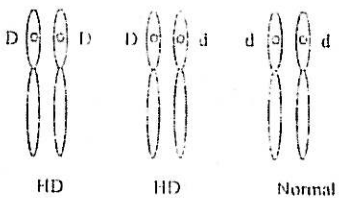


Dominant versus recessive

- For disease genes, gene mutations can also be DOMINANT or recessive alleles.
- For the rest of this talk, 'd' or 'D' are used to represent the alleles of a disease causing gene. The one in red is the disease causing allele
- So D means a DOMINANT disease gene allele, and d is a recessive one

Autosomal Dominant

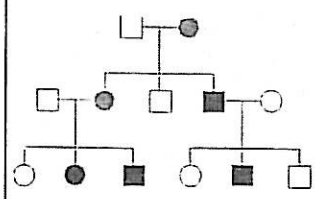
- So, if autosomal dominant (eg. Huntington's disease) then:-
- D, disease allele; d, normal allele



Categories of Genetic Disorders

Single gene

Autosomal dominant



Males & Females

M:F ≈ 1:1

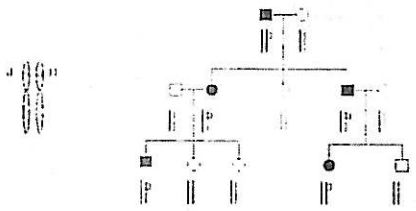
Male to male

Multiple generations

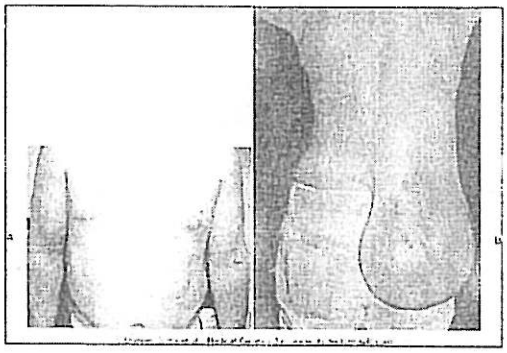
"Vertical transmission"

Modes of Inheritance

Autosomal Dominant



Neurofibromas
Café-au-lait patches



Categories of Genetic Disorders

Single gene

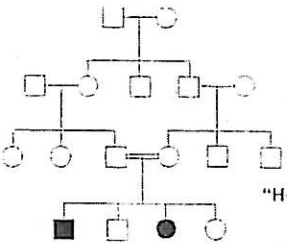
Autosomal dominant:

- New mutation
- Pleiotropy
- Reduced penetrance
  - Age dependent
- Variable expressivity
- Anticipation
  - Unstable/dynamic mutations

Categories of Genetic Disorders

Single gene

Autosomal recessive

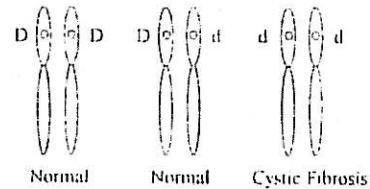


- Males ≅ Females
- M:F = 1:1
- Single generation
- Consanguinity

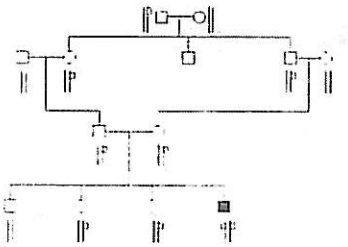
"Horizontal transmission"

Autosomal Recessive

- If, Autosomal recessive (eg. Cystic fibrosis) then:-
- d, disease allele, D normal allele



Modes of Inheritance
Autosomal Recessive



Categories of Genetic Disorders
Single gene

Autosomal recessive :

Genetic heterogeneity

Ethnic groups / populations

Hardy-Weinberg formula

$(p + q) = 1$

$(p^2 + 2pq + q^2) = 1$

Hardy-Weinberg principle

This is often the case for recessive diseases such as cystic fibrosis. Only the affected homozygotes, with genotype *aa*, are distinguishable. The Hardy-Weinberg principle tells us that the frequency of *aa* should be q^2.

$q^2 = 1/2,500 = 0.0004$

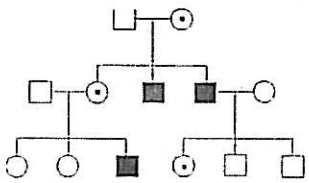
For cystic fibrosis in the Caucasian population, $q^2 = 1/2,500$. To estimate q, we take the square root of both sides of this equation. Since $p + q = 1$, $p = 0.98$. We can then estimate the genotype frequencies of *AA* and *Aa*.

$(p + q) = 1, (p^2 + 2pq + q^2) = 1$

The frequency of heterozygotes is $2pq = 2q = 2/50 = 1/25$.

Categories of Genetic Disorders
Single gene

X-linked recessive :



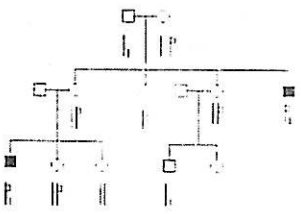
Males affected

Females carrier

"Diagonal inheritance"

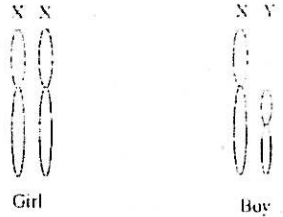
No male to male transmission

Modes of Inheritance
X-Linked



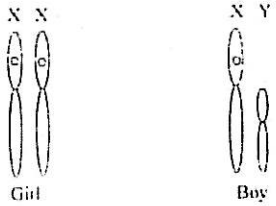
X linked recessive

- Involves the SEX chromosomes so the chromosomes are different:-



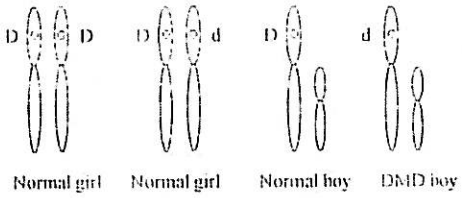
X linked recessive

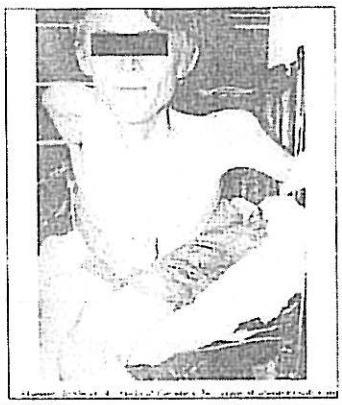
- And so are the genes. for x chromosome genes, boys have one copy, girls have 2
- In terms of disease genes, the Y chromosome is almost irrelevant

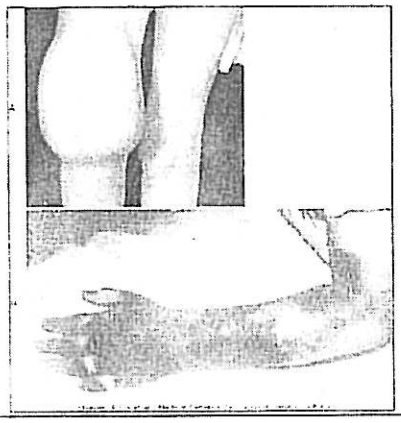


X linked recessive

- eg. Duchenne Muscular Dystrophy
- d, disease allele; D, normal allele







Categories of Genetic Disorders

Single gene

X-linked recessive :

X-inactivation / Lyonization

Carrier testing

Categories of Genetic Disorders

Single gene:

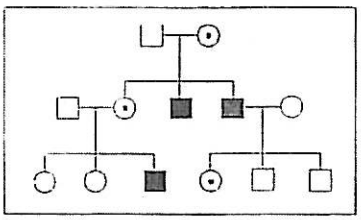
X-linked dominant

Hemizygous males

Lethality

Mosaicism in females

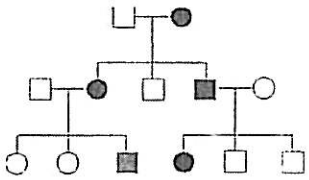
Carriers are sometimes drawn in family trees



Categories of Genetic Disorders

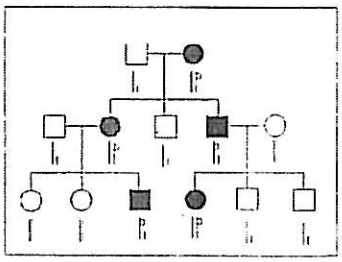
Single gene:

X-linked dominant :



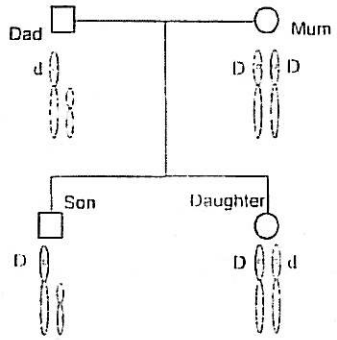
Males & females
Males > females
No male to male transmission

X linked dominant



-Males & females
-Males > females
-No male to male transmission

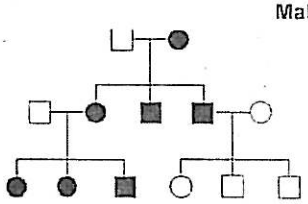
X linked: why not father to son?



Categories of Genetic Disorders:

Non-Mendelian

Mitochondrial



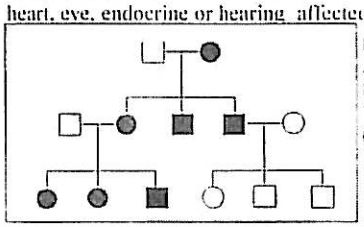
Males & females through females only

NonMendelian inheritance

- Mitochondrial inheritance
- Chromosomal translocations
- Multifactorial
  - Complex gene disorders

Mitochondrial inheritance

- Key to pattern: always in the maternal lineage
- Why? Mitochondria are inherited from the egg and have their own DNA.
- Think about in conditions where muscle, brain, heart, eye, endocrine or hearing affected



M F=1
Always from Mum
Never from Dad
Variable severity
(heteroplasmy)

Categories of Genetic Disorders

Non-Mendelian

Mitochondrial

Variable severity

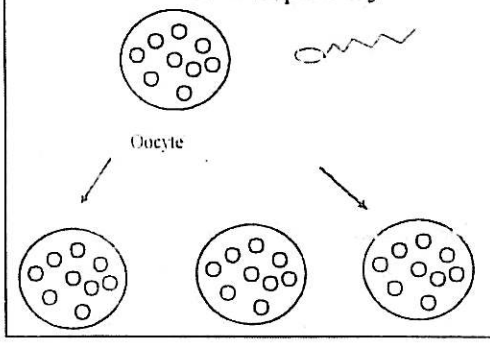
Homoplasmy

(mtDNA are all identical may be found in human tumours)

Heteroplasmy

(a mixture of more than one type mtDNA)

Heteroplasmy



Categories of Genetic Disorders

Multifactorial:

Childhood - Common Disorders

- Cleft lip +/- palate
- Congenital heart disease
- Neural tube defects

Adult - Common Diseases

- Cancer
- Diabetes
- Coronary artery disease

Categories of Genetic Disorders

Multifactorial

Factors affecting

- Sex
- Ethnic differences
- Increasing risk with severity
- Increasing risk with recurrence

Multifactorial conditions

- Many common conditions occur as a consequence of many factors, genes + environment
- IHD: FH, smoking, diet, Diabetes, BP
- Instead of just one gene 'monogenic', many genes 'polygenic' act together, each confering small increases in risk
- Inheritance patterns are not clear cut

What causes cancer ?

no single cause

Introduction

- Most congenital malformations are not caused by single genes or chromosome defects.
- Many common adult diseases, such as cancer, heart disease, and diabetes, have genetic components, but again they usually are not caused by single genes or by chromosome abnormalities.
- These diseases are the result of a complex interplay of multiple genetic and environmental factors.

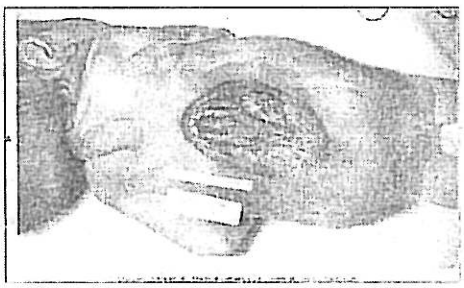
Multifactorial Inheritance and Common Diseases

Dr. Ebrahim Sakhinia
Tabriz University of Medical Sciences

Neural tube defects (NTDs).

- NTDs are thought to arise from a combination of genetic and environmental factors.
- recurrence risks for siblings of affected individuals range from 2% to 5%.
- NTDs can usually be diagnosed prenatally, sometimes by ultrasound and usually by an elevation in α-fetoprotein (AFP) in the maternal serum or amniotic fluid. Fetuses with open spina bifida are more likely to be detected by AFP assays.
- A major epidemiological finding is that mothers who supplement their diet with folic acid at the time of conception are less likely to produce children with NTDs.

Neural tube defects (NTDs).



Congenital Malformations

- Approximately 2% of newborns present with a congenital malformation.
- In general, sibling recurrence risks for most of these disorders range from 1% to 5%.
- Some congenital malformations, such as cleft lip/palate and pyloric stenosis, are relatively easy to repair.
- Others, such as the neural tube defects, usually have more severe consequences.

THE GENETICS OF COMMON DISEASES

- Some of these disorders, the congenital malformations, are by definition present at birth.
- Others, including heart disease, cancer, diabetes, and most psychiatric disorders, are seen primarily in teenager and adults.

Club Foot

Congenital Malformations

- While some cases of congenital malformations may occur in the absence of any other problems.
- For example, hydrocephaly and club foot are often seen secondary to spina bifida, cleft lip/palate is often seen in babies with trisomy 13, and congenital heart defects are seen in many syndromes, including trisomy of chromosomes 13, 18, and 21.

Environmental factors

- Environmental factors have also been shown to cause some congenital malformations.
- An example is thalidomide , a sedative used during pregnancy in the early 1960s.
- When ingested during early pregnancy, this drug often caused phocomelia (severely shortened limbs) in babies.
- Maternal exposure to retinoic acid, which is used to treat acne, can cause congenital defects of the heart, ear, and central nervous system.
- Maternal rubella infection can cause congenital heart defects.

Congenital Malformations

- Single genes that can cause congenital malformations are including the HOX, PAX, and TBX families of genes.
- Another example is the *RET* proto-oncogene, which is responsible for some cases of Hirschsprung (congenital aganglionic megacolon) disease.
- The genetic factors contributing to many important congenital malformations are as yet unidentified.

Multifactorial Disorders in the Adult Population

Summary of Congenital Diseases

- Congenital malformations are seen in roughly 1 of every 50 live births. Most of them are considered to be multifactorial disorders.
- Specific genes and environmental causes have been detected for some congenital malformations, but the causes of most congenital malformations remain largely unknown.

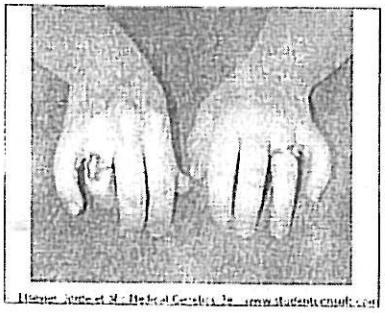
Heart Disease

- Generally, these studies also show that the risk is higher
- (1) if there are more affected relatives,
- (2) if the affected relative is female (the less commonly affected sex) rather than male,
- and (3) if the age of onset in the affected relative is early (before 55 years of age).

Heart Disease

- is the leading killer of Americans, accounting for approximately 25% of all deaths in this country.
- The most common underlying cause of heart disease is coronary artery disease (CAD), which is caused by atherosclerosis.
- A number of risk factors for CAD have been identified, including obesity, cigarette smoking, hypertension, elevated cholesterol level, and positive family history.
- The role of family history in CAD, and they show that an individual with a positive family history is 2 to 7 times

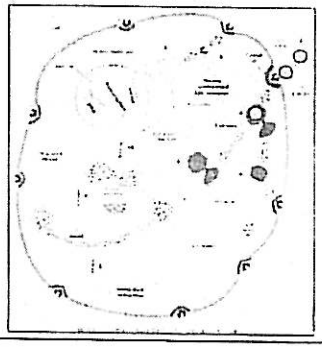
Xanthomas



Familial Hypercholesterolemia

- Autosomal dominant (FH) is an important cause of heart disease, accounting for approximately 5% of myocardial infarctions (MIs) in persons younger than 60 years of age.
- FH is one of the most common autosomal dominant disorders: in most populations surveyed to date, about 1 in 500 persons is a heterozygote.
- Plasma cholesterol levels are approximately twice as high as normal , resulting in substantially accelerated atherosclerosis and the occurrence of distinctive cholesterol deposits in skin and tendons, called xanthomas.

The process of receptor-mediated endocytosis



Familial Hypercholesterolemia

- Homozygotes are much more severely affected, Most homozygotes experience MIs before 20 years of age,
- Without treatment, most FH homozygotes will die before the age of 30 years.
- In a process known as *endocytosis*, LDL-bound cholesterol is taken into the cell via LDL receptors on the cell's surface. FH is caused by a reduction in the number of functional LDL receptors on cell surfaces. Because the individual lacks the normal number of LDL receptors, cellular cholesterol uptake is reduced, and circulating cholesterol levels increase.

Cardiomyopathy

- is a common cause of heart failure.
- About half of hypertrophic cardiomyopathy cases are familial and are caused by autosomal dominant mutations in any of 10 genes that encode various components of the cardiac sarcomere.
- The most commonly mutated genes are those that encode the β-myosin heavy chain (35% of familial cases), myosin-binding protein C (20% of cases), and troponin T (15% of cases).

common genetic cause of elevated LDL cholesterol

- Heterozygosity for a mutation in gene that encodes the low-density lipoprotein (LDL) receptor is seen in approximately 1 in 500 individuals; in these individuals, LDL cholesterol levels are approximately doubled.
- Mutations in the gene encoding apolipoprotein B, which are seen in about 1 in 1,000 individuals.
- other genes involved in lipid metabolism and transport, including those genes that encode various apolipoproteins.

Summary of Heart Disease

- Heart disease aggregates in families. This aggregation is especially strong if there is early age of onset and if there are multiple affected relatives. Specific genes have been identified for some subsets of families with heart disease, and lifestyle changes (exercise, diet, avoidance of tobacco) can modify heart disease risks appreciably.

The long QT (LQT) syndrome

- This disorder, which can be caused either by inherited mutations or by exposure to drugs that block potassium channels, predisposes affected individuals to potentially fatal cardiac arrhythmia.
- An autosomal dominant form, known as Romano-Ward syndrome, can be caused by mutations in any of six genes, four of which (*KCNQ1*, *HERG*, *KCNE1*, and *KCNE2*) encode potassium channel subunits, one of which (*SCN5A*) encodes a sodium channel, and one of which (*ANK2*) encodes ankyrin B, an anchoring protein that interacts with ion channels.

Hypertension

- Systemic hypertension, which is seen in approximately 25% of the adult populations of most developed countries, is a key risk factor for heart disease, stroke, and kidney disease.
- Most important environmental risk factors for hypertension are increased sodium intake, decreased physical activity, psychosocial stress, and obesity.

Stroke

- As with heart disease, strokes cluster in families; one's risk of having a stroke increases by two- to three-fold if a parent has had a stroke
- Stroke is a well-known consequence of several single-gene disorders, including sickle cell disease, MELAS (a mitochondrial disorder), and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy .
- Inherited deficiencies of protein C and protein S, both of which are coagulation inhibitors, are associated with an increased risk of stroke, especially in children.
- A specific mutation in clotting factor V, termed the factor V Leiden allele. Heterozygosity for this allele, produces a seven-fold increase in the risk of venous thrombosis (clots). In homozygotes, the risk increases to 100-fold.

Cancer

- Although numerous cancer genes have been isolated, environmental factors also play an important role in causing cancer by inducing somatic mutations. (tobacco). Diet (i.e., carcinogenic substances and the lack of "anticancer" components such as fiber, fruits, and vegetables) is another leading cause of cancer and may also account for as much as one third of cancer cases.
- It is estimated that approximately 15% of worldwide cancer cases are caused primarily by infectious agents (e.g., human papilloma virus for cervical cancer, hepatitis B and C for liver cancer).

Hypertension

- A small proportion of hypertension cases are the result of rare, single-gene disorders, such as Liddle syndrome (low plasma aldosterone and hypertension caused by mutations that alter the ENaC epithelial sodium channel) and Gordon syndrome (hypertension, high serum potassium level, and increased renal salt reabsorption caused by mutations in the *WNK1* or *WNK4* kinase genes).
- At least eight genes have now been identified that can lead to rare forms of hypertension, and all of them affect the reabsorption of water and salt by the kidney, which in turn affects blood volume and blood pressure.

Breast Cancer

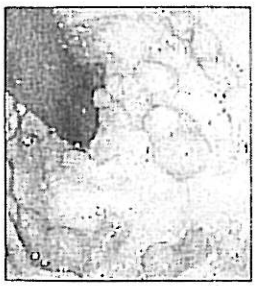
- Several genes are now known to predispose women to developing hereditary breast cancer. Most important among these are *BRCA1* and *BRCA2*, two genes involved in DNA repair.
- Germline mutations in the *TP53* and *CHK2* genes can cause Li-Fraumeni syndrome, which also predisposes to breast cancer.
- Cowden disease, a rare autosomal dominant condition that includes multiple hamartomas and breast cancer, is caused by mutations in the *PTEN* tumor suppressor gene.
- Ataxia telangiectasia, an autosomal recessive disorder caused by defective DNA repair, includes breast cancer in its presentation.
- Mutations in the *MSH2* and *MLH1* DNA repair genes, which lead to hereditary nonpolyposis colorectal cancer (HNPCC).
- Despite the significance of these genes, it should be emphasized that more than 90% of breast cancer cases are not inherited as Mendelian diseases.

Breast Cancer

- Breast cancer is the second most commonly diagnosed cancer (after skin cancer) among women.
- Breast cancer can also occur in men, with a lifetime prevalence that is roughly 100 times lower than that of women.

- If a woman has one affected first-degree relative, her risk of developing breast cancer doubles. The risk increases further with additional affected relatives, and it increases if those relatives developed cancer at a relatively early age (before 45 years of age).

Colorectal Cancer



Colorectal Cancer

- It is estimated that 1 in 20 Americans will develop colorectal cancer.
- The risk of colorectal cancer in people with one affected first-degree relative is two to three times higher than that of the general population.
- Familial colon cancer can be the result of mutations in the *APC* tumor suppressor gene or in one of six DNA mismatch repair genes (HNPCC).
- Most colon cancer cases (more than 90%) are not clearly inherited and are likely to be caused by a complex interaction of somatic gene alterations and environmental factors.
- The latter risk factors include a lack of physical activity and a high-fat, low-fiber diet.

Diabetes Mellitus

- The etiology of diabetes mellitus is complex and not fully understood.
- Nevertheless, progress is being made in understanding the genetic basis of this disorder, which is the leading cause of adult blindness, kidney failure, and lower-limb amputation and a major cause of heart disease and stroke.
- The three major types of diabetes,
- type 1 (formerly termed insulin-dependent diabetes mellitus, or IDDM),
- type 2 (formerly termed non-insulin-dependent diabetes mellitus, or NIDDM),
- and maturity-onset diabetes of the young (MODY).

Prostate Cancer

- Having an affected first-degree relative increases the risk of developing prostate cancer by a factor of two to three.
- It is estimated that about 5% to 10% of prostate cancer cases are the result of inherited mutations.
- Genome scans have indicated that several chromosome regions may contain prostate cancer susceptibility genes. One of the most promising is a region on chromosome 1q that contains the *RNASEL* gene.
- Mutations in *RNASEL* account for a small percentage of familial prostate cancer cases.
- Nongenetic risk factors for prostate cancer may include a high-fat diet.

Type 1 Diabetes

- Siblings of individuals with type 1 diabetes face a substantial elevation in risk: approximately 6%, as opposed to a risk of about 0.3% to 0.5% in the general population.
- The risk for offspring of diabetic mothers is only 1% to 3%, while it is 4% to 6% for the offspring of diabetic fathers.
- Genetic factors are not only responsible for the disorder. There is good evidence that specific viral infections contribute to the causation of type 1 diabetes in at least some individuals, possibly by activating an autoimmune response.
- It is estimated that the HLA system accounts for about 40% of the familial clustering of type 1 diabetes.

Type 1 Diabetes

- This form of diabetes, which is characterized by T-cell infiltration of the pancreas and destruction of the insulin-producing beta cells, usually manifests before 40 years of age.
- Patients with type 1 diabetes must receive exogenous insulin to survive
- In addition to T-cell infiltration of the pancreas, autoantibodies are formed against pancreatic cells. These findings, along with a strong association between type 1 diabetes and the presence of several human leukocyte antigen (HLA) class II alleles, indicate that this is an autoimmune disease.

Type 2 Diabetes

- Type 2 diabetes accounts for more than 90% of all diabetes cases, and it affects approximately 10% to 20% of the adult populations of many developed countries.
- There is nearly always some endogenous insulin production in persons with type 2 diabetes, and the disease can often be treated successfully with dietary modification, oral drugs, or both.
- Type 2 diabetes patients suffer from insulin resistance.
- This disease typically occurs among people older than 40 years of age, and, in contrast to type 1 diabetes, it is seen more commonly among the obese.
- Neither HLA associations nor autoantibodies are seen commonly in this form of diabetes.

Type 1 Diabetes

- The insulin gene, which is located on the short arm of chromosome 11, is another logical candidate for type 1 diabetes susceptibility.
- a strong risk association is seen with allelic variation in a VNTR polymorphism located just 5' of the insulin gene.
- It is estimated that inherited genetic variation in the insulin region accounts for approximately 10% of the familial clustering of type 1 diabetes.
- Other studies have identified at least 20 additional candidate regions that may contain type 1 diabetes susceptibility genes. One of these regions, 2q33, contains the *CTLA4* (cytotoxic lymphocyte associated-4) gene, which is involved in T cell proliferation.

Maturity-Onset Diabetes of the Young

- This form of diabetes, which accounts for 1% to 5% of all diabetes cases, typically occurs before 25 years of age and follows an autosomal dominant mode of inheritance.
- In contrast to type 2 diabetes, it is not associated with obesity.
- About 50% of cases are caused by mutations in the gene that encodes glucokinase, a rate-limiting enzyme in the conversion of glucose to glucose-6-phosphate in the pancreas.
- MODY can also be caused by mutations in any of five genes that encode transcription factors involved in pancreatic development or insulin regulation: hepatocyte nuclear factor 1-α (*HNF1α*), hepatocyte nuclear factor 1-β (*HNF1β*), hepatocyte nuclear factor 4-α (*HNF4α*), insulin promoter factor 1 (*IPF1*), and neurogenic differentiation 1 (*NEUROD1*).
- Mutations in these genes, all of which are expressed in pancreatic beta cells, lead to beta-cell abnormalities and thus to diabetes.

Type 2 Diabetes

- The two most important risk factors for type 2 diabetes are a positive family history and obesity; the latter increases insulin resistance.
  - Even in the absence of weight loss, exercise increases insulin sensitivity and improves glucose tolerance.
  - A region on chromosome 2q and mutations in a gene that encodes calpain-10 (a cysteine protease) are associated with type 2 diabetes susceptibility.
  - A significant association has also been observed between type 2 diabetes and a common allele of the gene that encodes peroxisome proliferator-activated receptor-γ (PPAR-γ), a transcription factor that is involved in adipocyte differentiation and glucose metabolism.

Alzheimer Disease

- Alzheimer disease (AD), which is responsible for 60% to 70% of cases of progressive cognitive impairment among the elderly, affects approximately 10% of the population older than 65 years of age and 40% of the population older than 85 years of age.
- Approximately 10% of AD cases are caused by autosomal dominant genes. Early-onset cases cluster more strongly in families and are more likely to follow an autosomal dominant inheritance pattern. This disease is genetically heterogeneous; at least four AD susceptibility genes have been identified. Three of the genes (encoding presenilin 1, presenilin 2, and amyloid-β precursor protein) cause early-onset AD and affect the cleavage and processing of the amyloid precursor protein. A fourth encodes the apolipoprotein E protein and is strongly associated with late-age onset of AD.

Obesity

- Obesity is most commonly defined as a body mass index (BMI) greater than 30.
- Although obesity itself is not a "disease," it is an important risk factor for several common diseases, including heart disease, stroke, hypertension, and type 2 diabetes.
- There is a strong correlation between obesity in parents and obesity in their children.
- Adoption and twin studies indicate that at least half of the population variation in obesity may be caused by genes. Specific genes and gene products involved in appetite control and susceptibility to obesity, including leptin and its receptor, are now being studied.

Psychiatric Disorders

Alcoholism

- Twin and adoption studies show that alcoholism clusters quite strongly in families, reflecting a possible genetic contribution to this disease. Familial clustering is particularly strong for type II alcoholism (early-onset form primarily affecting males).

Schizophrenia

- Schizophrenia is a severe emotional disorder characterized by delusions, hallucinations, retreat from reality, and bizarre, withdrawn, or inappropriate behavior.
- The lifetime recurrence risk for schizophrenia among the offspring of one affected parent is approximately 8% to 10%, which is about 10 times higher than the risk in the general population.
- The risks decrease when the affected family member is a second- or third-degree relative.
- More than 20 genome scans have been performed in an effort to locate schizophrenia genes.
- These include dysbindin (chromosome 6p), neuregulin 1 (chromosome 8p), and D-amino-acid oxidase activator (chromosome 13q). However, the mechanisms through which mutations in these genes contribute to schizophrenia susceptibility are not yet known.

Screens can detect mutations that affect an animal's behavior

(A) Wild-type *C. elegans* engage in social feeding. The worms swim around until they encounter their neighbors and commence feeding. (B) Mutant animals feed by themselves.

Other Complex Disorders

- In some cases specific susceptibility genes have been identified. These include, for example, Parkinson disease, hearing loss, multiple sclerosis, amyotrophic lateral sclerosis, epilepsy, asthma, inflammatory bowel disease, and some forms of blindness.

Bipolar Disorder

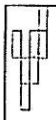
- also known as manic-depressive disorder, is a form of psychosis in which extreme mood swings and emotional instability are seen.
- The prevalence of the disorder in the general population is approximately 0.5% to 1%, but it rises to 5% to 10% among those with an affected first-degree relative.
- Bipolar disorder is more strongly influenced by genetic factors than is unipolar disorder (major depression).
- Examples of these genes include those that encode monoamine oxidase A (MAOA), the serotonin transporter (5HTT), and catechol-O-methyltransferase (COMT), a gene that has also been associated with schizophrenia susceptibility.

Some General Principles and Conclusions

- There is a tendency,"If it's genetic, you can't change it").
- This is incorrect.
- Most of the diseases have both genetic and environmental components. Thus, environmental modification (e.g., diet, exercise, stress reduction) can often reduce risk significantly.
- Such modifications may be especially important for individuals who have a family history of a disease, because they are likely to develop the disease earlier in life.
- Those with a family history of heart disease, for example, can often add many years of productive living with relatively minor lifestyle alterations. By targeting those who can benefit most from intervention, genetics helps to serve the goal of preventive medicine.

Some General Principles and Conclusions

- First, the more strongly inherited forms of complex disorders generally have an earlier age of onset. Often, these represent subsets of cases in which there is single-gene inheritance.
- Second, when there is laterality, bilateral forms sometimes cluster more strongly in families (e.g., cleft lip/palate).
- Third, while the sex-specific threshold model fits some of the complex disorders (e.g., pyloric stenosis, cleft lip/palate, autism, heart disease), it fails to fit others (e.g., type 1 diabetes).



Some General Principles and Conclusions

- While the genetics of common disorders is complex and often confusing, the public health impact of these diseases and the evidence for hereditary factors in their etiology demand that genetic studies be pursued.
- Substantial progress is already being made. The next decade will undoubtedly witness many advancements in our understanding and treatment of these disorders

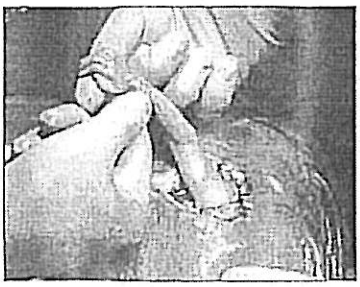
Some General Principles and Conclusions

- In addition, it should be stressed that the identification of a specific genetic alteration can lead to more effective prevention and treatment of the disease.
- Identification of mutations causing familial colon cancer may enable early screening and prevention of metastasis.
- Pinpointing a gene responsible for a neurotransmitter defect in a behavioral disorder such as schizophrenia could lead to the development of more effective drug treatments.
- In some cases, such as familial hypercholesterolemia, gene therapy may be useful. It is important for health care practitioners to make their patients aware of these facts.

Is Cancer a genetic disease ?

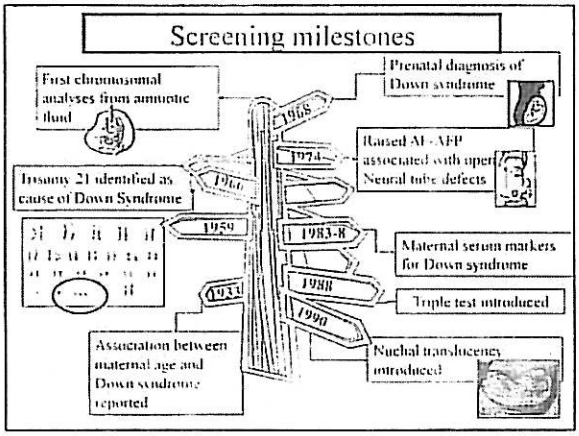
1914 - Boveri

روشهای نوین تشخیص در طب جنین



Ebrahim Sakhinia, DMT, PhD
Department of Medical Genetic
Tabriz University of Medical Sciences

Screening milestones

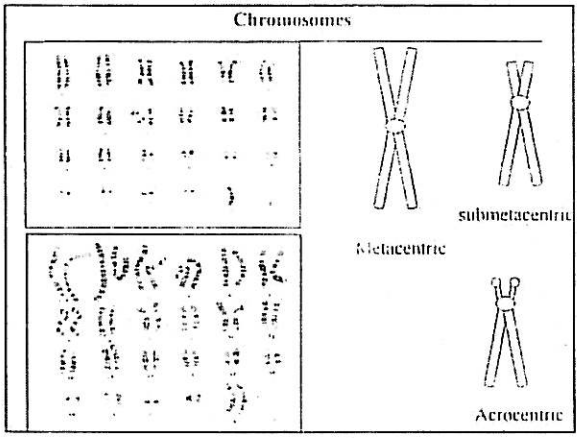


Genetic Screening

Purpose - to detect

- Chromosomal Abnormalities
- DNA changes
- Protein/biochemical changes

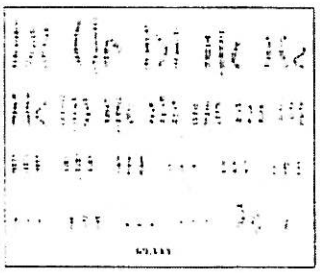
Chromosomes



Why Cytogenetics?

- 0.7% livebirths
- 5% stillbirths
- 50% miscarriages
- Up to 100% cancers
- >60 known syndromes
- *Major contribution to learning difficulties*

Triploidy



2% of Conceptions

Intrauterine growth retardation: Large head to body ratio

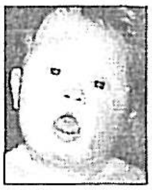
Can occasionally survive to birth but usually die in the newborn period.

Almost always miscarry

Syndactyly (fingers joined together)

Aneuploidy

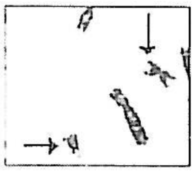
Down syndrome, trisomy 21



47,XX,+21 or 47,XY,+21 Incidence at birth 1/700

Robertsonian translocation

1. 46,XX,+21,der(21;21)(q10;q10)
parent carrier - 100% recurrence risk



2. 46,XX,+21,der(14;21)(q10;q10)
- father carrier = 2% recurrence risk
- mother carrier = 12% recurrence risk

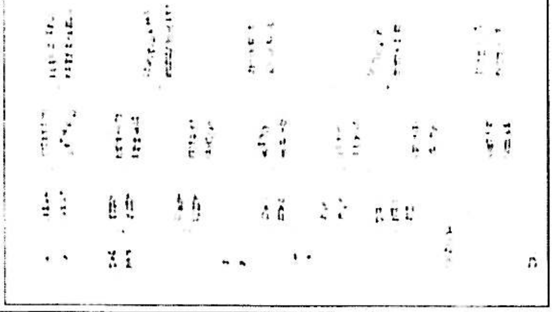
Risk of Baby with Chromosomal Abnormality by Maternal Age		
Mother's Age at Expected Date of Delivery (years)	Chance of Baby with Down's Syndrome *At 10-20 wks	Chance of live-born baby with a chromosomal abnormality
20 - 24	1:1490	1:500
25 - 30	1:1250	1:480
31 - 34	1:1140	1:420
35	1:355*	1:179
36	1:280*	1:149
37	1:220*	1:124
38	1:165*	1:105
39	1:125*	1:81
40	1:90*	1:64
41	1:70*	1:49
42	1:50*	1:39
43	1:40*	1:31
44	1:35*	1:24
45	1:25*	1:21
46	1:20*	1:16
47	1:16*	1:13
48	1:14*	1:10
49	1:11*	1:8

Edwards syndrome, trisomy 18

47,XX,+18 or 47,XY,+18

Incidence at birth 1/5,000-1/6,000

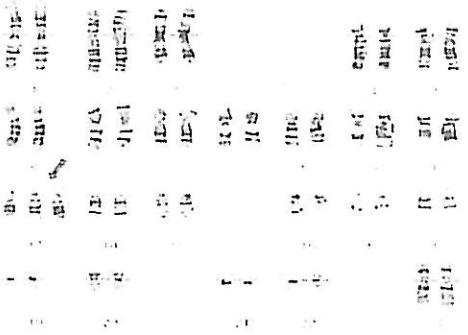
Edwards syndrome, trisomy 18



Trisomy 13 Patau syndrome; 47,XY,+13

- 1/12,000 concept; 1/20,000 livebirths
- midline defects: oro-facial clefts; microphthalmia; postaxial polydactly; CHD; renal defects; omphalocele; cutis aplasia; MR
- 95% die within 1st yr
- ~80% nondisjunction; ~20% trans
- >95% spontaneously aborted

Patau syndrome, trisomy 13

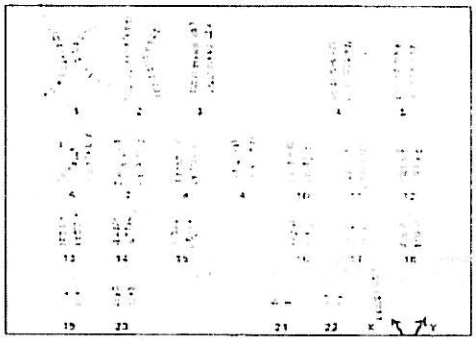


Sex

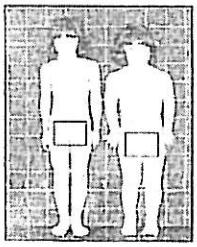
Chromosome

Aneuploidy

Turner Syndrome 45,XO



Turner's syndrome

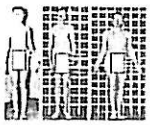


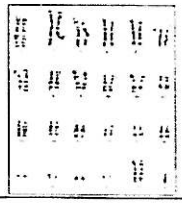
- 2% of conceptions
- 99% miscarry
- Infertility
- Webbing of neck
- Short Stature (limbs) - Growth hormones
- NORMAL INTELLIGENCE

Female does not have the usual pair of two complete X chromosomes
Most commonly, female has only one X chromosome
Others may have two X chromosomes, but one of them is incomplete
Sometimes, a female has some cells with two X chromosomes, but other cells have only one (mosaic)

Kleinfelters syndrome

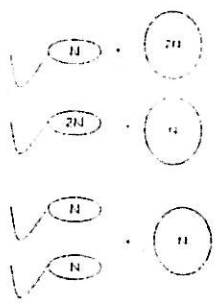
47,XXY or XXY syndrome - caused by a chromosome aneuploidy



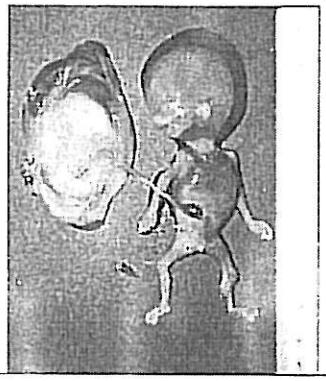


- Affected males have an extra X sex chromosome.
- Second-most common aneuploidy condition
- 1 1000 males
- Abnormal testicular development
- Infertility
- Tall,
- Long limbs
- Lower IQ

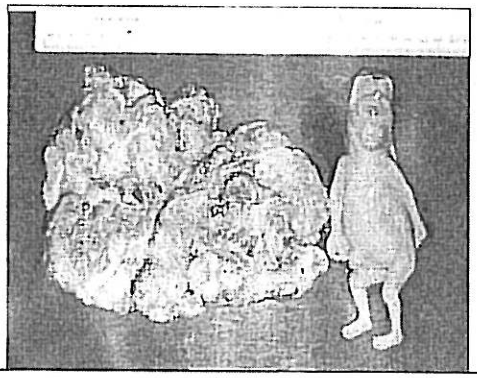
Origin of triploidy



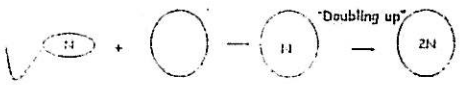
Double maternal



Double paternal



Molar pregnancy



Haploid sperm + Empty egg → Haploid zygote → Diploid zygote

Consequences: Double paternal genome
"Conceptus without an embryo"
Massive cystic placenta

Techniques

- Non-invasive
- Minimally invasive
- Invasive

Non-invasive techniques

- Ultrasound
- Magnetic Resonance Imaging (MRI)
- Maternal serum screening

Double Test

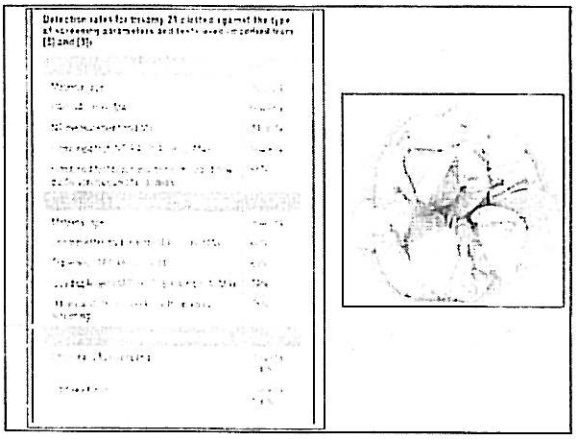
- Low pregnancy associated plasma proteins-A (PAPP-A) level (Decreased with trisomies).
- Raised serum Beta-hCG (Human chorionic gonadotropin) (Increased with 21 and 18, decreased with 13).
- Double test + maternal age diagnose.
  - 60% sensitive DS, 5% false positive rate

Triple Test

- - Used for Down Synd. Screening. It comprises
  - . AFP
  - . hCG
  - . uE3 (unconjugated oestriol)
- Best carried at 15-18 weeks. In DS AFP & uE3 are low while hCG is raised
- Triple test+ maternal age diagnose 60% DS
- In trisomy 18 all above components are low

Quadruple test

- Triple test+ Inhibin A estimation
- This test + maternal age detects 76% DS



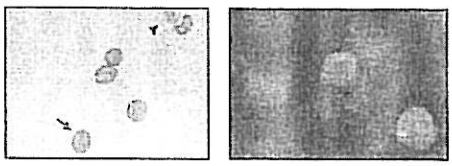
Minimally Invasive Techniques

- Cell free fetal DNA (cffDNA)
- Pre-implantation genetic diagnosis (PGD)

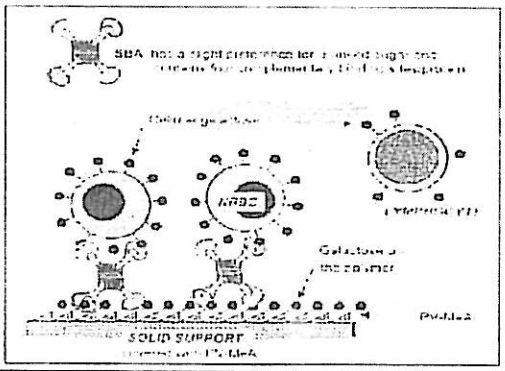
Fetal cells in maternal circulation

- Nested primer PCR for Y-specific DNA
- Fetal trophoblasts, lymphocytes, granulocytes, and nucleated red blood cells (NRBCs)
- Rarity of fetal cells in maternal blood ($1:10^7$)
- Detection rate for aneuploidy: 40-59%

Fetal cells in maternal circulation

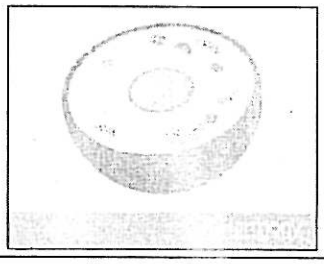


Fetal cells in maternal circulation

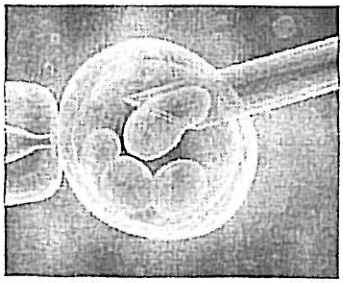


Cell-free DNA

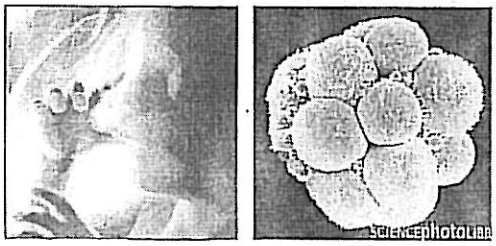
- As cells turnover, chromosomes fragment releasing DNA into the blood
- Cell-free DNA (cfDNA) are short DNA fragments (50-300 base pairs)



Preimplantation Genetic Diagnosis (PGD)

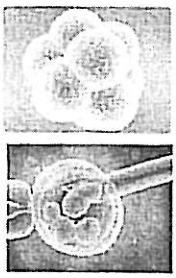


Pre-natal vs Pre-implantation diagnosis



Pre-implantation Diagnosis

- Introduced initially in 1990
- Biopsy of a single cell per embryo, followed by its genetic diagnosis through different techniques (FISH, PCR, aCGH), and the subsequent replacement to the patient of those embryos classified by genetic diagnosis as normal.



PGD Indications

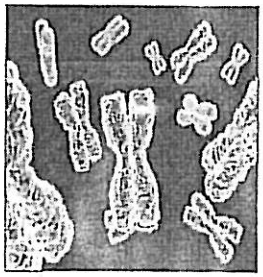
Procedure is offered to couples:

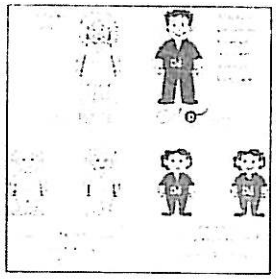
- With known ***single gene disorders*** that can be detected by PGD
- With known *chromosomal abnormalities* that can be detected by PGD
- Requesting sex selection for *X-linked disorders*

PGD Indications

- The procedure has also been offered to couples undergoing IVF *at risk for aneuploidy*
  - maternal age > 35 y
  - Prior trisomic conception
- with *recurrent pregnancy losses*
- Prior *failed IVF cycles (>3 prior embryo transfers with high quality, morphologically normal embryos)*
- Requesting PGD for *HLA-typing (to allow selection of embryos that are histocompatible with live siblings)*
- Requesting *sex selection* for "family balancing"

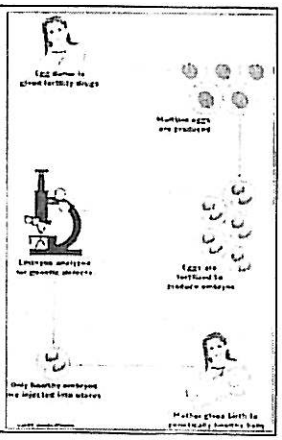
Single Gene Disorders



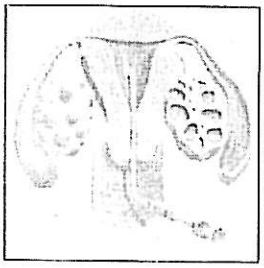


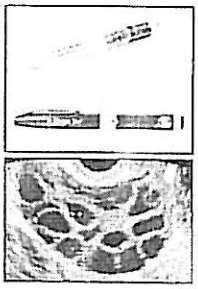
PGD Process

- Ovulation Induction
- Retrieval
- Fertilization
- Embryo Bx on Day-3
- Genetic Analysis
- Embryo Transfer

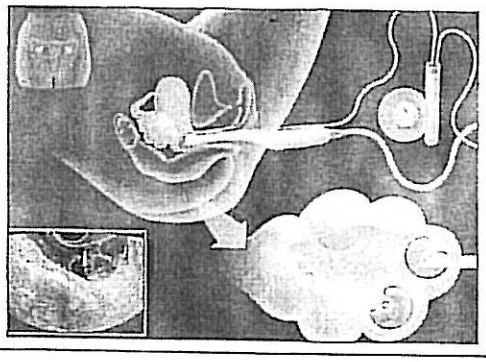


Ovulation induction

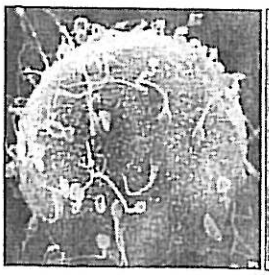


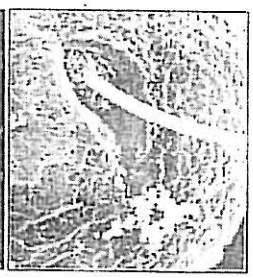


Oocyte Retrieval



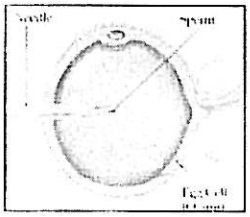
Fertilization



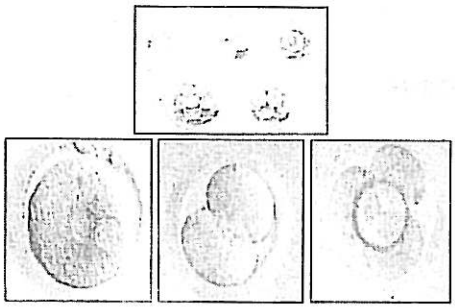


Fertilization

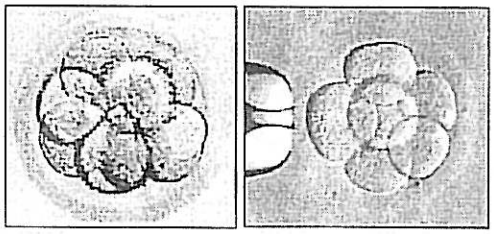
- Conventional Insemination
- Intracytoplasmic Sperm Injection (ICSI)



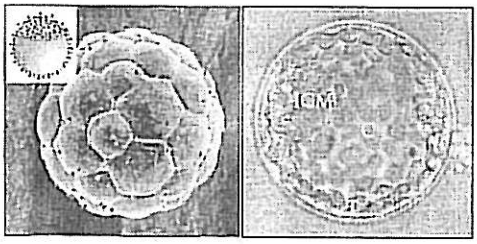
Embryo Culture



Day 3/Cleavage Stage Embryo



Day 5 / Blastocyst



Cleavage Stage Biopsy

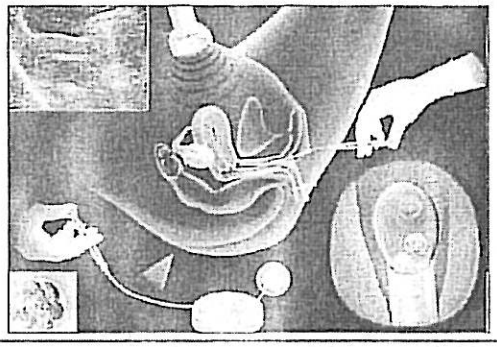
- Most widely used technique
- Day 3, 6-8 cell stage

Cleavage Stage Biopsy

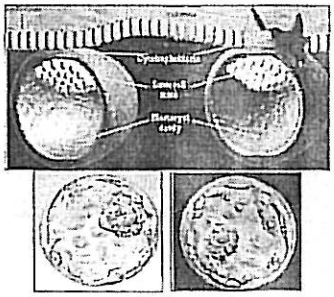
Genetic Analysis/PCR

- DNA amplification
  - sequence harboring the mutation)
  - billions of copies in several hrs
- Mutation Characterization
  - (by using mutation specific primers
  - by digestion with restriction enzymes
  - by heteroduplex analysis

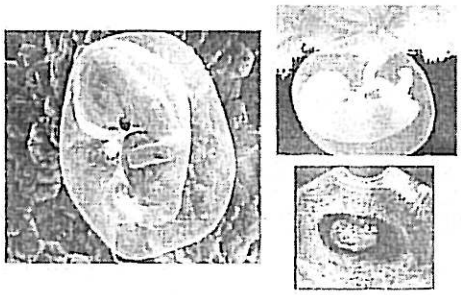
Embryo Transfer



Embryo Implantation



Early Pregnancy

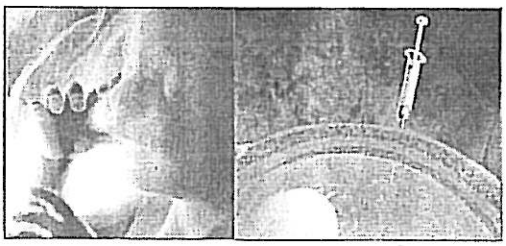


Can Mistakes Happen?

Causes of Misdiagnosis

- Human Error
  - Unprotected sex
  - mislabeling, misidentification, misinterpretation
  - wrong embryo transfer
  - incorrect probes or primers
- Technical
  - Probe or primer failure
  - contamination (maternal, paternal, operator, carry-over)
- Intrinsic (embryo)
  - Mosaicism
  - Allele drop out
  - Uniparental Disomy

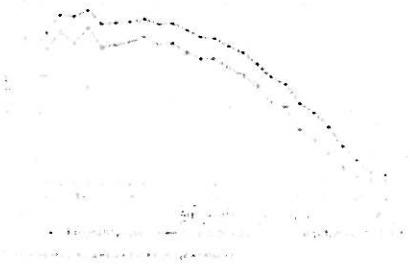
Pre-natal Diagnosis



Single Gene Disorders

- Most common autosomal recessive disorder
  - β-thalassemia/sickle cell anemia, CF, SMA
- Most common autosomal dominant disorder
  - Myotonic Dystrophy, Huntington Disease, NF-1, Charcot-Marie-Tooth
- Most common X-linked disorder
  - Fragile X, DMD, and Becker Muscular Dystrophy
  - Hemophilia A and B

PGD and Age

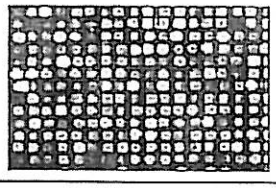


Conclusions

- Before PGD is performed, genetic counseling must be provided to ensure that patients fully understand the
  - risk for having an affected child
  - the impact of the disease
  - the available options
  - the multiple technical limitations including the possibility of an erroneous result
- Prenatal diagnostic testing is strongly encouraged to confirm the results of PGD

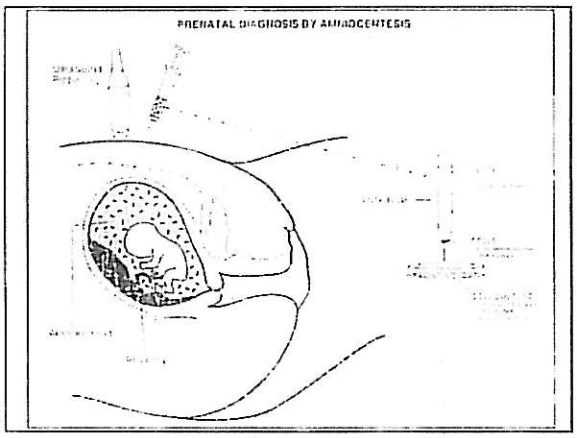
What's in the Future?

- With the advent of the microarray techniques for the analysis of the genome, transcripts of thousands of genes can be tested at one time, and the combination of both might dramatically change our future



Invasive Techniques

- Amniocentesis
- Chorionic villus sampling (CVS)
- Percutaneous umbilical blood sampling (cordocentesis)

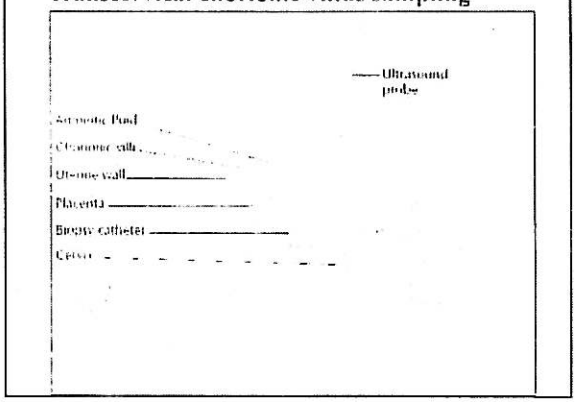


Amniocentesis

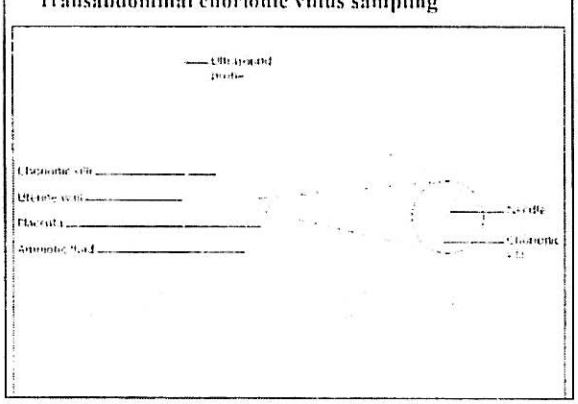
- Done between 15 and 20 wks; amniotic fluid contains fetal cells shed from the gut and skin.

1. Chromosomal analysis: most commonly Down syndrome testing for maternal age, high risk serum tests and ultrasound markers.
2. DNA analysis for genetic disease.
3. Enzyme assays for inborn errors of metabolism.
4. Investigation of fetal lung maturity (lecithin/sphingomyelin ratio or presence of phyophatidyl glycerol)
5. Bilirubin (for rhesus iso-immunization)

Transcervical chorionic villus sampling



Transabdominal chorionic villus sampling



Chorionic villus sampling

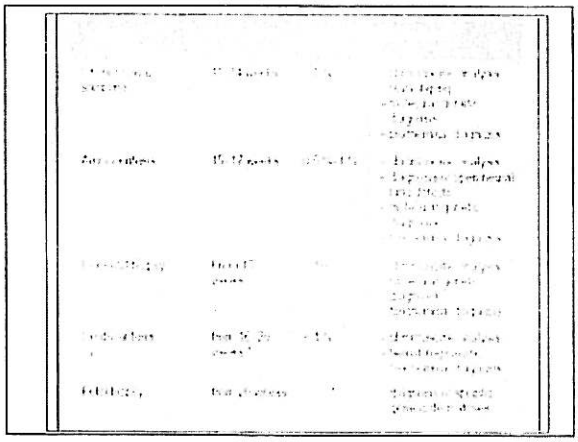
- CVS is performed at 10 to 13 wks; allows earlier and safer methods of pregnancy termination when they are abnormal.

- Indications:

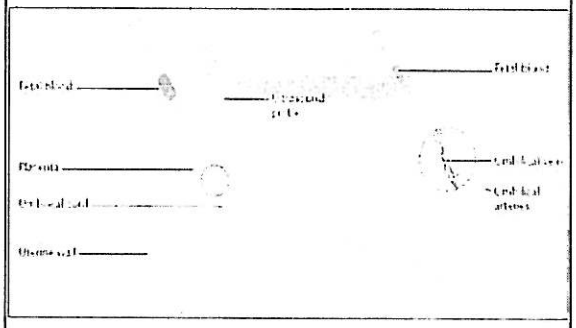
1. Karyotyping when ultrasound findings suggest aneuploidy.
2. DNA analysis, particularly for haemoglobinopathies and recessive or X-linked disorders

- Complications:

1. Pregnancy loss- 2-3%
2. Maternal cell contamination lead to false negative results



Percutaneous umbilical blood sampling



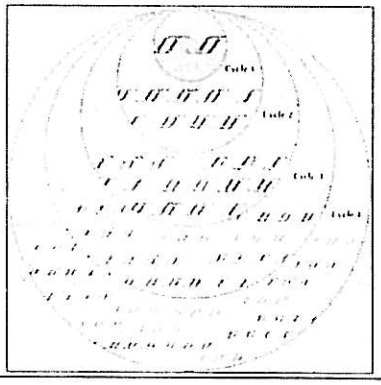
EMBRYOSCOPY & FETOSCOPY

- Direct visualization of embryo and fetus.
- Limited field of vision.
- Provide information only about external fetal structures .

NEW MOLECULAR ANALYTIC TECHNIQUES

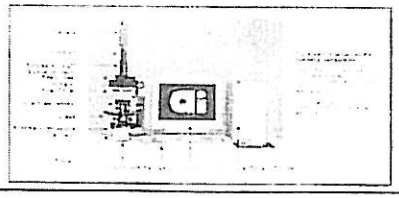
- Fetal cell obtained by CVS and Amniocentesis can be used for prenatal Dx. For congenital anomalies by following new techniques
  1- Southern blotting:
  Cleavage of chromosomal DNA at specific sites and used for tests
  2- PCR
  3- FISH

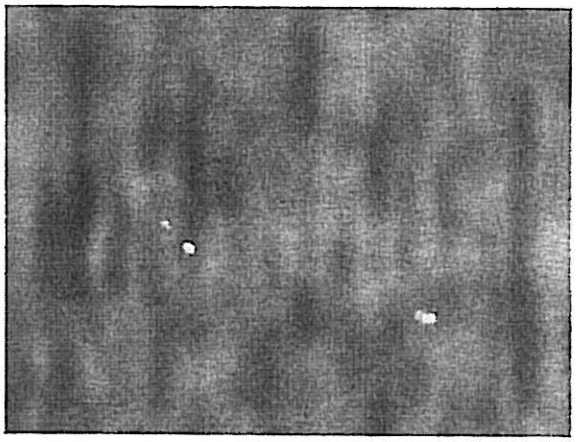
Molecular Cytogenetics



Fluorescent in situ hybridisation (FISH)

Detection of DNA material on slides using fluorescent dyes & UV light





روشهای نوین و سریع ژنتیک مولکولی در غربالگری آنومالیهای جنینی

Molecular Cytogenetics

- FISH:
  - Use DNA probes for specific chromosomes
  - Can paint metaphase
  - Useful for quick result and identifying small areas
  - Eg deletions, ESACs
- QF-PCR:
  - Quantitative fluorescent PCR
  - Use polymorphic sites to define number of copies present
  - Useful for quick result in prenatal diagnosis

Quick result from Amniocentesis

- FISH:
  - Use probes for 13,21 and X, Y, 18 on two different slides
  - takes 24 hours
- QF-PCR:
  - Use polymorphic markers for chromosomes 13, 18, 21
  - Results in 24 hours
  - Becoming more common
  - Can only detect abnormalities for these chromosomes
  - Usually go on and do full karyotype - ???

Quantitative fluorescent PCR aneuploidy screen (QF-PCR)

Principle:

- Test STR markers on chromosomes
  - 4-5 on autosomes of choice (13, 18, 21)
  - Fewer on X and Y
- Used to detect numerical chromosome abnormalities
- Can test blood, amniotic fluid, CVS, post-mortem tissue etc.

QF-PCR

Advantages:

- Rapid result (48 – 72 hours)
- 99% accuracy
- No live cells required

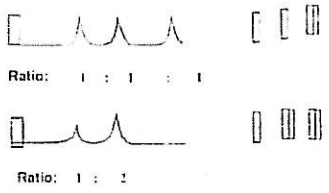
Disadvantages:

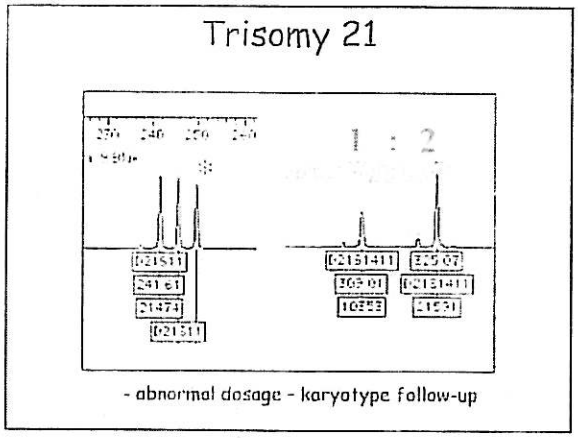
- Won't detect mosaicism (<30%)
- Won't detect other chromosome abnormalities
- Mechanism of aneuploidy remains unknown
- Blood-stained amnio may make testing impossible
- Risk of maternal contamination if CVS not carefully prepared/dissected

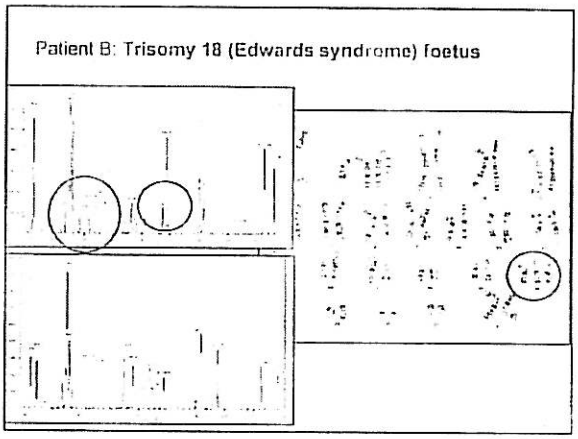
Quantitative Fluorescent PCR

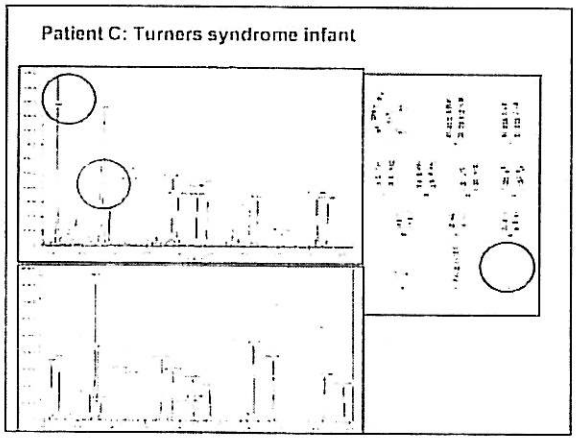
Trisomy detection in prenatal samples

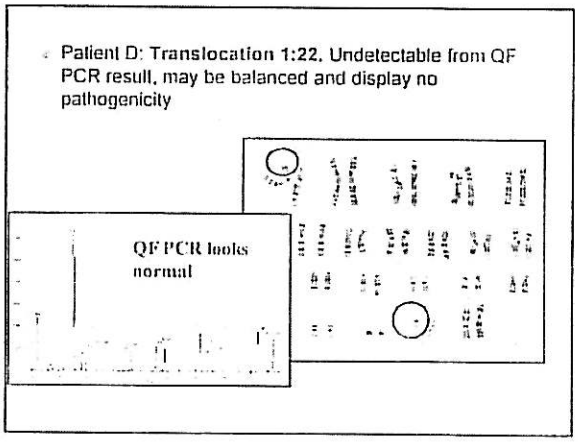
Hypervariable region on chromosome 21 amplified by F-PCR



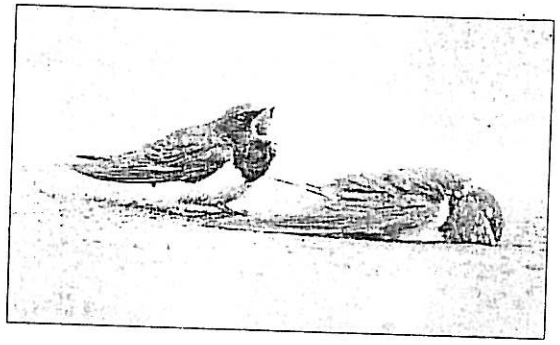








سرطان یک بیماری ژنتیکی است!؟



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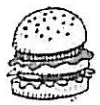
Overview

- Is Cancer a genetic disease ?
- What kinds of genes control cancer?
- A successful cancer cell must
- Three classes of genes involved in cancer
- Multistep process of Cancer

- Hereditary Breast and Ovarian Cancer: What's New and Necessary?
- What Are Risk Factors

What causes cancer ?

no single cause

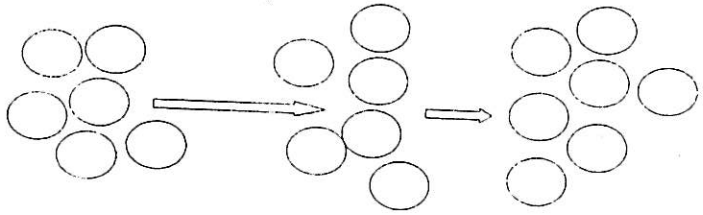



Is Cancer a genetic disease ?

1914 - Boveri

1. Tumours are monoclonal in origin

the cells of a tumour all show the genetic characteristics of the original transformed cell



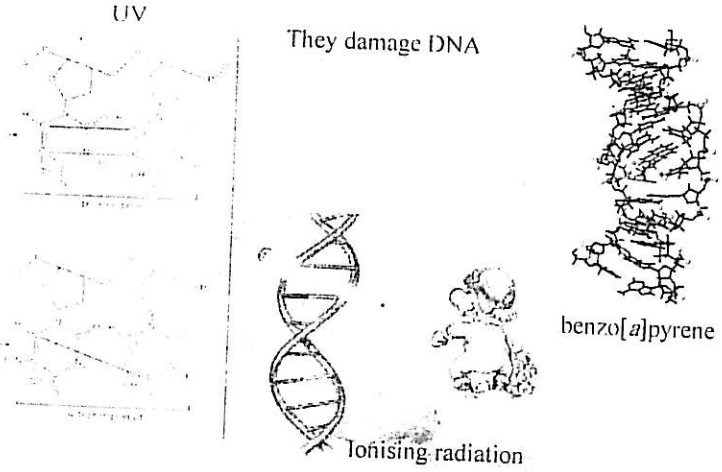
2. Cancer, or risk of cancer can be inherited

Evidence comes from:-

1. Epidemiological study of monozygotic and dizygotic twins (Lichtenstein et al (2000) N Engl J Med 343,78-85).
2. Cancer induction in mice is influenced by the genetic background.(see review Balmain (2002) Cell 108,145-152).
3. Chromosomal abnormalities are associated with cancer eg/Downs syndrome (trisomy 21) and leukaemia.

Study of 44,000 pairs of twins estimated the risk of cancer for person with twin having colorectal, prostate or breast cancer :-	Monozygotic twins	11-18%
	Dizygotic twins	3-9%

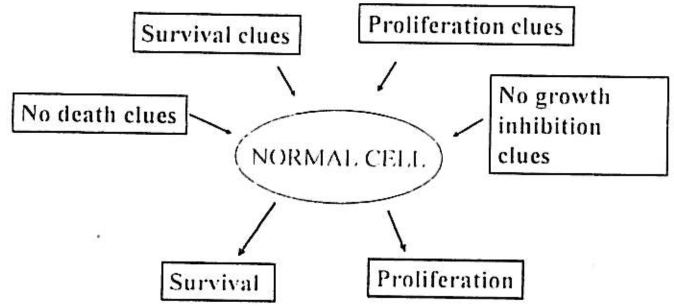
3. Mutagens influence the development of Cancer



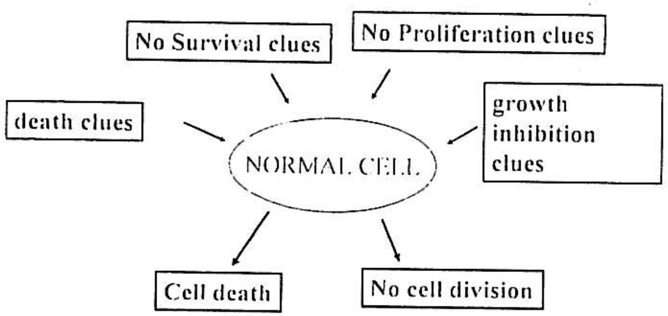
Is cancer a genetic disease ?

- Cancer is a genetic disease in that it is cased by alterations in DNA.
- Most human cancers are due to a number of somatic mutations.
- There are some rare human diseases that ARE Hereditary and MAY predispose the affected person to developing cancer

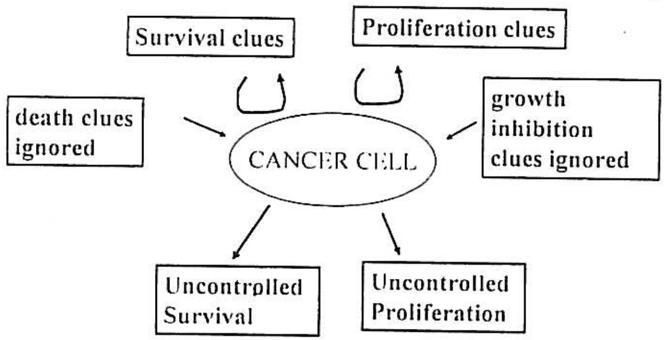
What kinds of genes control cancer?



What kinds of genes control cancer?



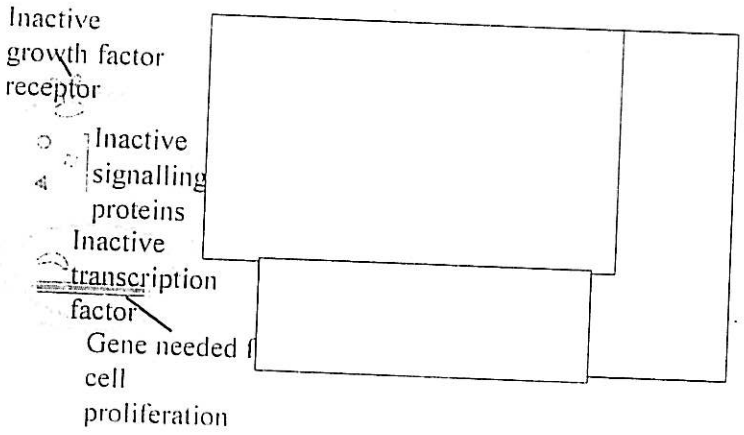
What kinds of genes control cancer?

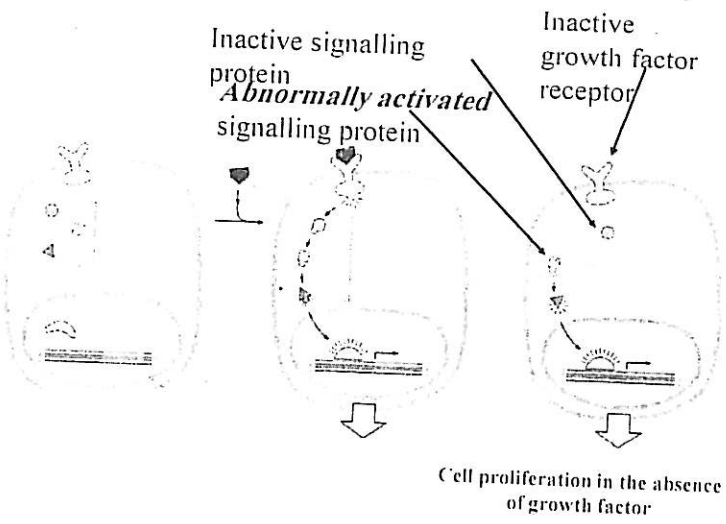


A successful cancer cell must:

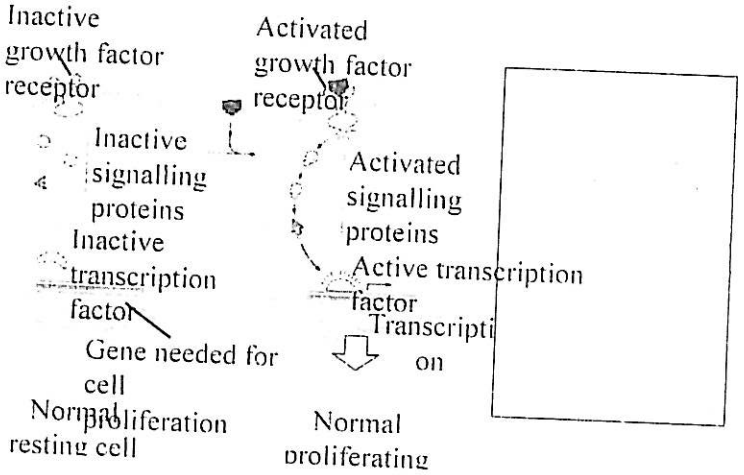
- become independent of external growth signals
- become insensitive to external anti-growth signals
- become able to avoid apoptosis
- become capable of indefinite replication
- become capable of sustained angiogenesis
- become capable of tissue invasion and metastasis

Growth factors and cell proliferation





Growth factors and cell proliferation

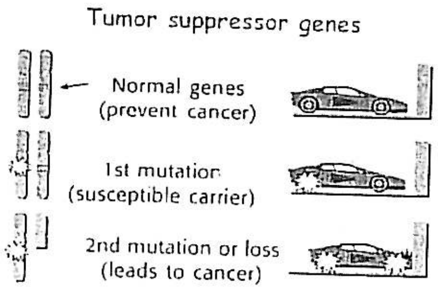


Three classes of genes involved in cancer

- Tumour suppressor genes. – genes that prevent cancer (e.g., *p53*, *APC*).
- Oncogenes :– genes that can themselves cause cancer (e.g., *bcl-2* t(14,18)(q32;q21) or *ras*^{D} mutation, Large T antigen).
- PLUS DNA repair genes-loss of function increases spontaneous and environmentally induced mutation rates (cause cancer suseptability syndromes).
- Any combination of changes may be found in individual tumours.

Tumour Suppressor genes

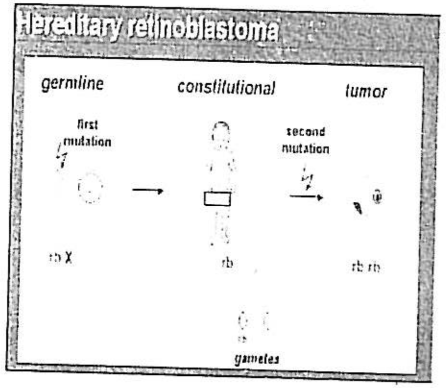
Recessive, loss of function mutations promote cell transformation



Often act as regulators of growth/differentiation
- Affect differentiation
- Control cell contact eg. DCC
- Growth inhibitory factors eg. TGF-beta
- Interact and control oncogenes
- Regulate transcription of cell cycle factors eg. Rb, p53, wilms gene

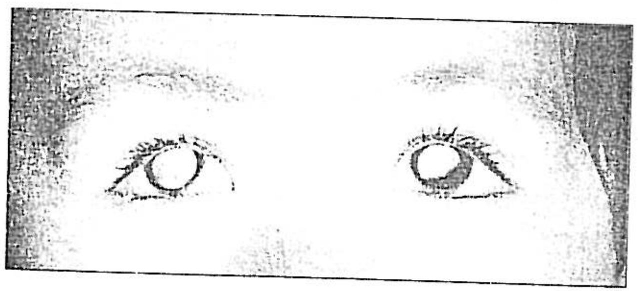
Hereditary Rb

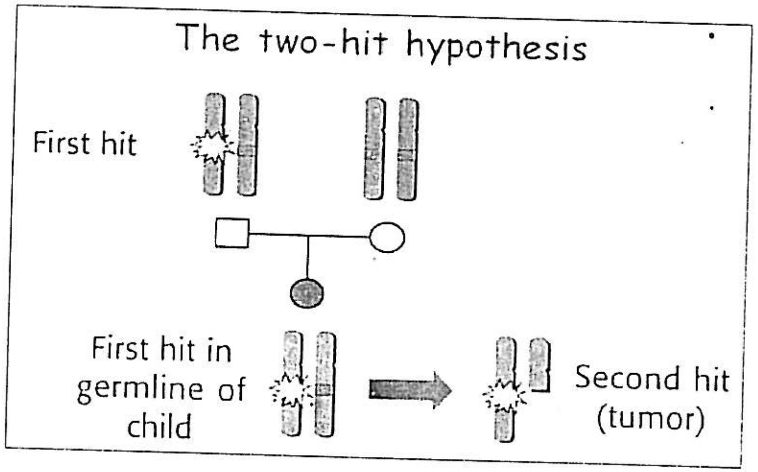
- Often carried by paternal allele (de novo mutation)
- All tissues heterozygote, 50% chance of transmitting to offspring
- Early onset
- Single second hit required



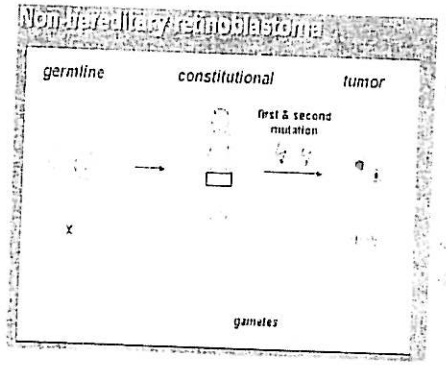
The RB1 gene and Retinoblastoma
A Paradigm for Tumour Suppressors

Knudsen's Two Hit Hypothesis





Aquired Rb



- First genetic event is somatic
- Two somatic hits within the same cell required and loss of both Rb alleles required for tumour development.
- No risk to offspring
- Chance of 2 hits in the same cell low - rare

p53

>50% of tumours have mutated p53

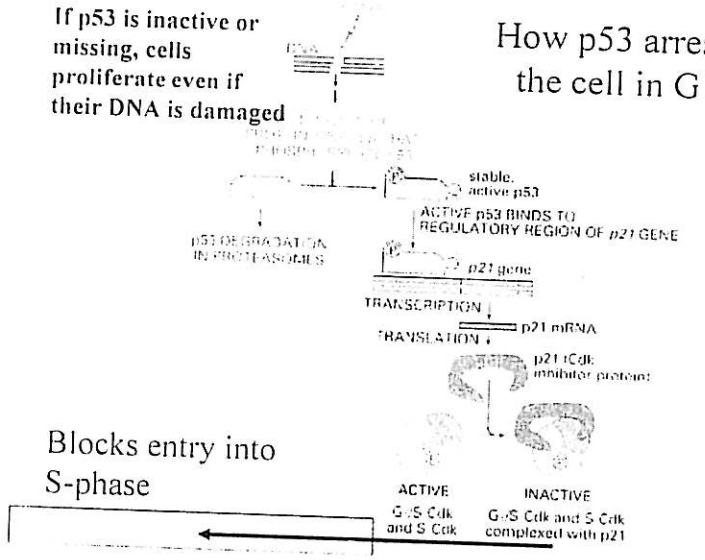
p53 expression is induced by DNA damage

Cell cycle arrest Cell death

If p53 is inactive or missing, cells proliferate even if their DNA is damaged

If p53 is inactive or missing, cells proliferate even if their DNA is damaged

How p53 arrests the cell in G1



Blocks entry into S-phase

Oncogenes

=Proliferation genes

Over-expression or activation by mutation

↓

Excessive cell proliferation

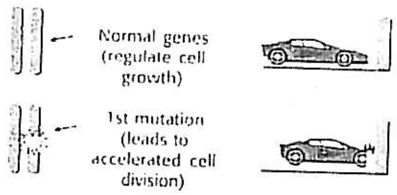
Mutation of only **one copy** of the gene can be enough

Unmutated (normal) gene = proto-oncogene

oncogenes

Dominant gain of function mutation promotes cell transformation

Oncogenes



One mutation sufficient for role in cancer development

- Involved in basic functions of the cell, growth and differentiation
- Growth factors eg SIS (homologous to PDGF)
- Growth factor receptors eg ERBB (homologous to EGFR)
- Transcription eg. Jun, Myc
- Signal transducers eg. ABL, MOS, RAS, SRC

Sequence of mutations underlying colon cancer

Normal epithelium

↓ Tumour suppressor gene *(APC)* lost

Excessive epithelial proliferation

↓ Oncgene *(ras)* activated

Small benign tumour

↓ Tumour suppressor gene *(DCC)* lost

Large tumour

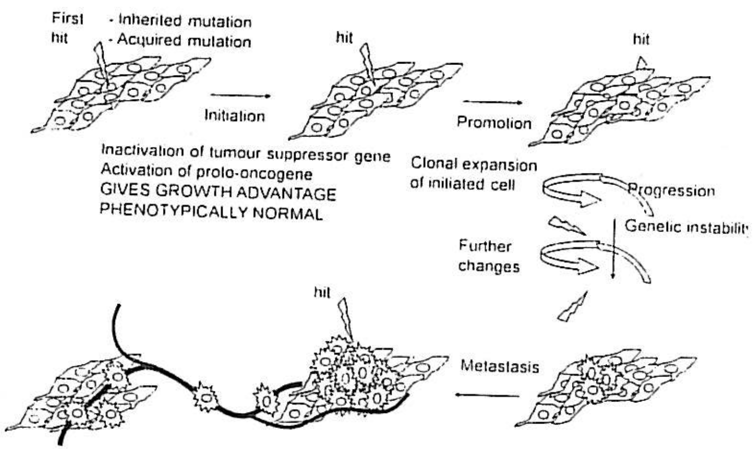
↓ Tumour suppressor gene *(p53)* lost

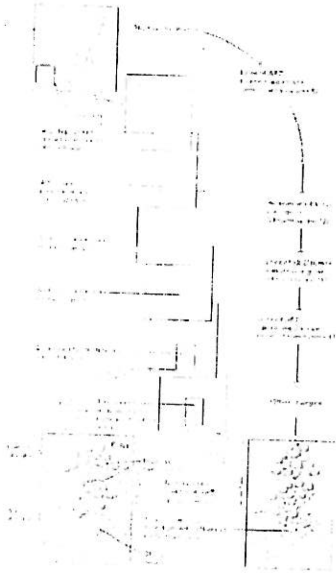
Tumour becomes invasive

↓ Rapid accumulation of mutations

Metastasis **Cancer**

Multistep process of Cancer





Normal Epithelium

A genetic model for colorectal tumorigenesis

↓ APC – structural protein (tumour suppressor)

Hyper proliferative Endothelium

↓

Early Adenoma

↓ Ras – GTPase (oncogene)

Intermediate Adenoma

↓ DCC – Cell differentiation (tumour suppressor)

Late Adenoma

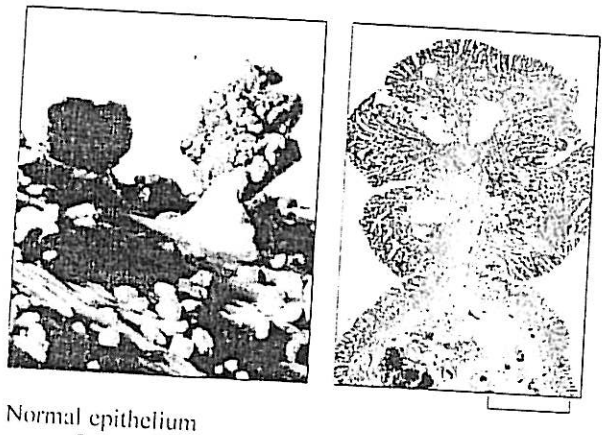
↓ p53 – DNA damage response (tumour suppres:

Carcinoma

↓ other

Metastasis

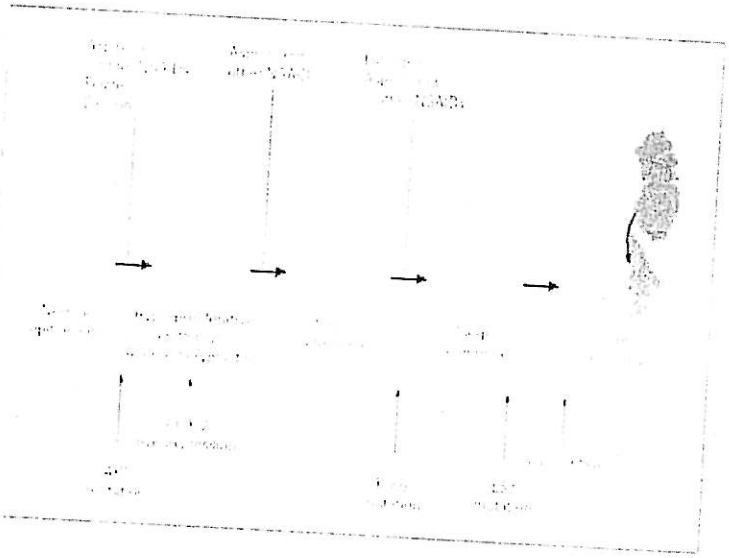
Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759-67



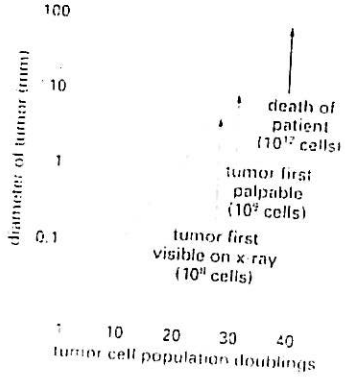
Normal epithelium

↓ Tumour suppressor gene *(APC)* lost

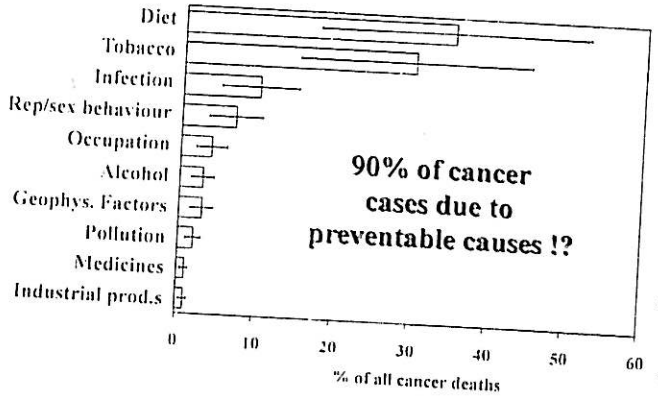
Excessive epithelial proliferation



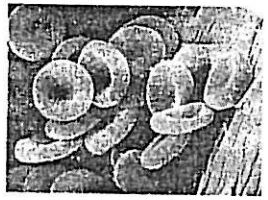
Cancer Diagnosis



Estimates of proportion of cancer deaths attributable to various factors in USA



Loss of Function: Thalassaemias and other haemoglobinopathies



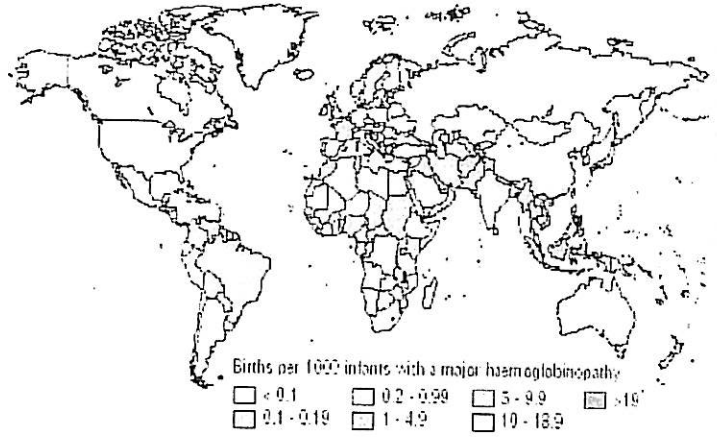
Dr. Ebrahim Sakhinia
Tabriz University of Medical Sciences

This lecture:

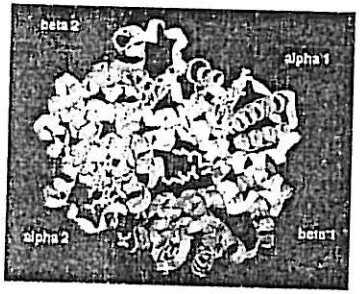
- Background
- Structural Hb Variants
  - Sickle Cell Disease
- Thalassaemias
  - α
  - β: molecular genetics, clinical features, diagnosis and screening
- Summary and questions

Background

- Haemoglobinopathies: heterogeneous group of inherited disorders characterised by the absent, reduced or altered expression of one or more of the globin chains of haemoglobin
- Most common serious Mendelian diseases worldwide
- Approx. 7% of world's population are carriers for an inherited disorder of haemoglobin
- Clear relation between molecular and clinical events



Haemoglobin (Hb)



Hb consists of 2 α-globin like chains (α or ζ) and 2 β-globin like chains (β, δ, γ, or ε)

Embryonic Hb ($\zeta\gamma$ /$\zeta\varepsilon$ /$\alpha\varepsilon$) replaced by HbF ($\alpha_2\gamma_2$) before birth. After birth, HbF is mainly replaced by HbA ($\alpha_2\beta_2$) and HbA_2 ($\alpha_2\delta_2$) over first years of life; HbF constitutes ~1% of total Hb in adults

Globin Alleles

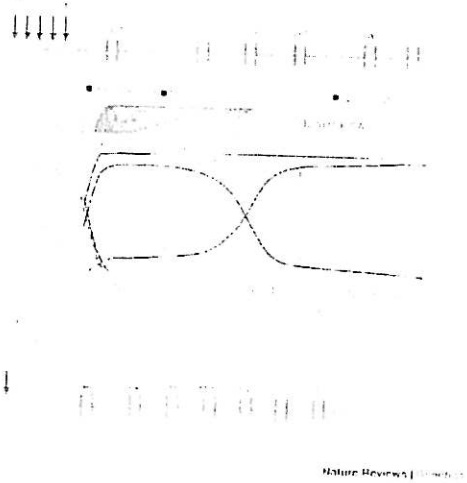
- Normal HbA = $\alpha_2\beta_2$
- There are 2 α-globin genes (HBA1, HBA2) on each Chr 16, therefore there are 4 α-globin alleles (2 from each parent) in each individual: Genotype αα/αα
- There is only one β-globin gene (HBB) on Chr 11, therefore there are 2 β-globin alleles (1 from each parent) in each individual: Genotype β/β

α-globin and β-globin gene clusters

α-gene cluster: chromosome 16

β-gene cluster: chromosome 11

- Promoter, enhancer and **upstream regulatory region** (α: HS-40 β: β-LCR)
- Sequential activation of genes: 5' > 3'
- Sequential activation of embryonic, fetal and adult Hbs



Haemoglobinopathies

- Structurally abnormal haemoglobins
  - **Hb S: Sickle Cell Disease**
  - HbC
  - HbE
- ↓Rate of synthesis: Thalassaemias
  - **α-thalassaemia**
  - **β-thalassaemia**
- Hereditary Persistence of Fetal Haemoglobin (HPFH)
  - Defect in normal switch from fetal to adult Hb, may modify clinical effects of above disorders

Structural Hb Variants

- Over 700 described, most not pathogenic as do not interfere with normal function of Hb
- Clinically important variants include HbS (Sickle Cell Disease), HbE
- Unstable Haemoglobins precipitate inside red blood cells, forming aggregates (Heinz bodies) resulting in membrane damage and haemolytic anaemia

Human Haemoglobins

Hb	Stage of Development	Structure	% in adults	Conditions in which increased
A	Adult	$\alpha_2\beta_2$	92	
A_{1c}	Adult	$\alpha_2(\beta$-N-glucose$)_2$	5	Diabetes mellitus
A_2	Adult	$\alpha_2\delta_2$	2-3	β-thalassaemia
H	Adult	β_4	0	Some α-thalassaemias
F	Fetal	$\alpha_2\gamma_2$	<1	Newborn, δ-β- and β-thalassaemia, HPFH
Barts	Fetal	γ_4	0	Some α-thalassaemias
Gower I	Embryonic	$\zeta_2\varepsilon_2$	0	Embryo < 8wks
Gower II	Embryonic	$\alpha_2\varepsilon_2$	0	Embryo < 8wks
Portland	Embryonic	$\zeta_2\gamma_2$	0	< 8wks and α^0-thalassaemia (hydrops fetalis)

Adapted from Old, 2003

Variant Haemoglobins and their manifestations

Type	Examples	Mutation(s)	Clinical Manifestation
Sickling	HbS	Glu>Val, β codon 6	Sickling due to decreased solubility
	HbC-Harlem	Glu>Val, β codon 6 and Asp>Asn, β codon 73	Sickling due to decreased solubility
Unstable	Hb Koln	Val>Met, β codon 98	Anaemia with Heinz body formation
Decreased oxygen affinity	Hb Kansas	Asn>Thr, β codon 102	Mild anaemia
Increased oxygen affinity	[illegible]	His>Leu, α1 codon 89	Polycythaemia due to decreased oxygen transport
M Haemoglobin	HbM Boston	His>Tyr, α1 or α2 codon 58	Cyanosis due to ferric Hb
Decreased synthesis	Hb Lepore-Hollandia	δ Codon 1-22 / β codon 50-146	Thalassaemia
	HbE	Glu>Lys, β codon 26	Thalassaemia
	Hb Constant Spring	α2 codon 142	Thalassaemia

Adapted from Old, 2003

Molecular defects in variant haemoglobins

- Number of different molecular mechanisms
- Majority have one amino acid replacement resulting from single base change, leading to synthesis of unstable protein and/or one with difference in charge (allows detection by electrophoresis, e.g. HbS)
- Shortened or elongated chains (e.g. Hb Wayne, elongated)
- Unequal crossing over (fusion haemoglobins resulting from hybrid gene)

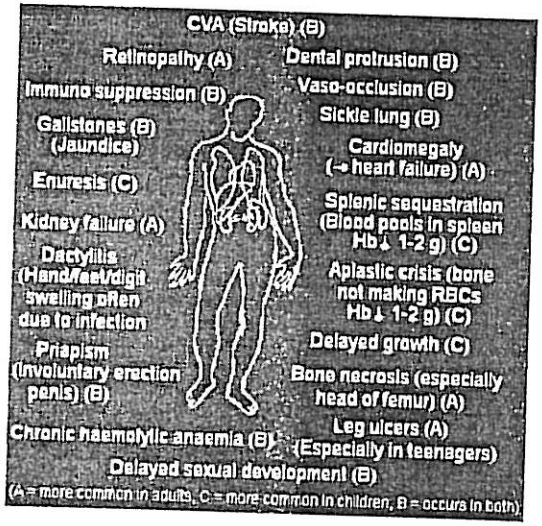
Numbers of Hb variants for each type of molecular defect

Type of defect	Example	α	β	δ	γ
Single point mutation: one AA replacement	Hb S	229	716	86	70
2 point mutations: 2 AA replacements	Hb C (Baltimore)	1	21		
Elongated α chains	Hb Constant Hill	4	12		
Shortened α chains	Hb Wayne	4	2		
Deletion insertion	Hb Hasharon		3		
Extended polypeptide chain (termination codon mutant)	Hb Constant Spring (Hb Icaria)	5	3		
Frameshift		1			
Unstable	Hb Hasharon	1	3		
Fusion haemoglobins					
Aγ	Hb Lepore				3
βγ	Hb Miyada			3	
αβγ	Hb Rochester				1
Aβ	Hb Kenya				1

Adapted from Olo, 2003

Sickle Cell Disease

- Autosomal recessive inheritance
- Homozygous Hb SS ($\beta^S\beta^S$), single base change in amino acid 6 of β globin (Glu>Val)
- Compound heterozygotes:
  - Hb SC ($\beta^S\beta^C$)
  - Hb SD ($\beta^S\beta^D$)
- Hb S/β^- or β^0 thalassaemia
- All produce significant symptoms; Hb SS most severe)
- High frequency due to conferred resistance to malaria in heterozygotes (Hb AS, sickle cell trait)



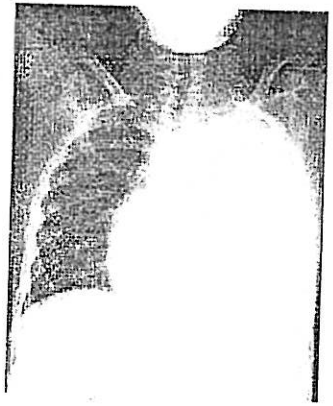
Clinical Features: SCD

- Sickling of RBCs precipitated by deoxygenation/infection/dehydration/ physiological stress
- Sickling > haemolysis > non-deformable RBCs > vaso-occlusion > hypoxia > ischaemia > pain ("Sickle Crisis")

Avascular Necrosis of Head of Femur

Dactylitis (painful swelling of hands and feet)

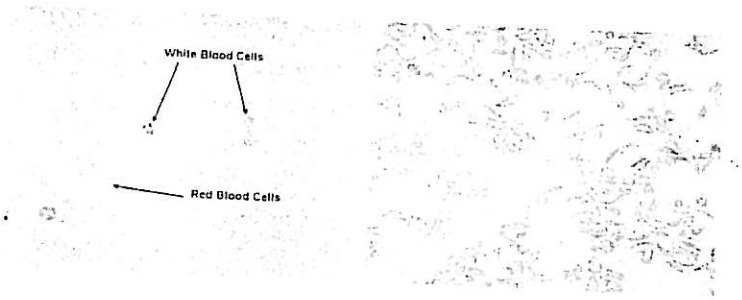
Acute Chest Syndrome



Diagnosis: SCD

- Clinical features and FH (familial Hypercholesterolemia)
- Anaemia (marked in Hb SS), but normal MCV and MCH (unless also thalassaemia trait-up to 25% cases)
- Increased Reticulocyte count (bone marrow production of RBCs)
- Typical changes on blood film
- Hb electrophoresis (no normal Hb A)

Diagnosis: Blood Film



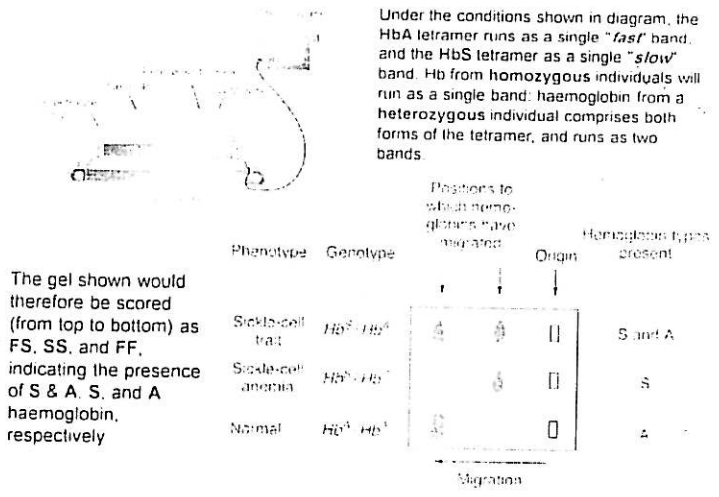
Blood films of normal (left) and sickle cell anaemia (right). Latter shows characteristic sickling of red blood cells (RBCs) and marked variation in size of RBCs

Sickle Cell Trait

- Heterozygous Hb AS
- No abnormal clinical features
- Sickling rare unless O_2 saturation falls <40% (anaesthesia, unpressurised aircraft etc.)
- Unable to use blood film or FBC (Biomedical Technology Corporation) for diagnostic purposes: need Hb electrophoresis
- Genetic counseling (AR (Autosomal recessive) inheritance, testing of partner etc.)

Hb Electrophoresis

- **Sickle Cell Hb:** replacement in the **sixth position of the β-chain** of the negatively-charged **glu** in the standard **HbA** by neutral **val** in **HbS** results in a protein with a slightly *reduced negative charge*.



Screening: SCD

- Prevalence (UK) 1:2380
- All babies born in UK offered screening for SCD via blood spot test
- Antenatal screening for haemoglobinopathies offered to all pregnant women

Defective globin chain synthesis: Thalassaemias

- Absence or reduction in synthesis of one or more globin chains causing an imbalance in the ratio of products of the 2 globin gene clusters, normally expressed in equal amounts
- Heterogeneous
- Classified according to particular chain synthesised in reduced amounts
- Precipitation of the chains produced in excess causes microcytic and hypochromic RBCs and variable anaemia

α-thalassaemia

- 2 α globin genes: normal $\alpha^{HBA1}\alpha^{HBA2}/\alpha^{HBA1}\alpha^{HBA2}$
- Defective gene expression in **one** α-globin gene (α^+ thalassaemia: gene deletions and point mutations, reduced levels of α-globin)
- Defective expression in **both** α-globin genes (α^0 thalassaemia: gene deletions, absence of α-globin)

Molecular basis of α-thalassaemias

Type of mutation	Type	Total	Example
Deletion of one α gene	α	1	$-\alpha^{}$
Deletion of two α genes	α	2	$--^{}$
Deletion of the HS-40 with intact α genes	α	1	$---^{}$
RNA processing mutations			
splice junction changes	α	1	α2 IVS-I-1(G→C)
polyadenylation site mutations	α	1	α2 (-AATAAA→-AATAAG)
RNA modification mutations			
nonsense	α	1	α2 codon 116
frameshift	α	2	α2 codon 59 (-T)
frameshift	α	1	α2 codon 30/31 (+G)
initiation codon	α	1	α2
initiation codon	α	2	α^{-}
termination codon	α	25	α2 codon 142 (TAA→CAA) Hb Constant Spring
highly unstable α-chain variants	α	22	α2 codon 14 (-TGG) Hb Quong Sze
highly unstable α-chain variants	α	2	α2 codon 14 (-TGG) Hb Icaria

Adapted from Old 2003

α^{+} thalassaemia

- $-\alpha/\alpha\alpha$ or $\alpha^{T}\alpha/\alpha\alpha$
- 2 common deletions: 3.7 kb deletion ($-\alpha3.7$) and 4.2kb deletion ($-\alpha4.2$)
- Created by unequal crossing over between homologous sequences in α-globin gene cluster
- Non-deletion effects: mutations affecting RNA processing, translation or result in highly unstable α-globin chain

α^{0}-thalassaemia

- Deletions involve both α-globin genes in gene cluster
- Homozygous state (--/--) results in Hb Barts Hydrops Fetalis syndrome (death *in utero* or soon after birth due to complete lack of α-globin chain synthesis)
- Silent α-thalassaemia: $-\alpha/\alpha\alpha$, asymptomatic
- α-thalassaemia trait: $\alpha\alpha/--$ or $-\alpha/-\alpha$, asymptomatic +/- mild anaemia
- HbH disease: $--/-\alpha$, moderate anaemia, hepatosplenomegaly, leg ulcers, jaundice. Blood film HbH inclusions (HbH = β_4)
- At least 14 deletions identified
- α-globin locus control region (HS-40) deletions; point mutations affecting remaining globin gene of α^{+} thalassaemia deletion mutation – both rare

Summary: α-thalassaemia phenotypes

$\alpha\alpha/\alpha\alpha$	Normal phenotype
$-\alpha/\alpha\alpha$ or $\alpha^{T}\alpha/\alpha\alpha$	α^{+}-thalassaemia trait
$-\alpha/-\alpha$ or $\alpha^{T}\alpha/\alpha^{T}\alpha$	Homozygous α^{+}-thalassaemia
$--/\alpha\alpha$	α^{0}-thalassaemia trait
$--/-\alpha$ or $--/\alpha^{T}\alpha$	Hb H disease
--/--	Hb Bart's Hydrops Fetalis
In combination with other Hb'opathies (eg Hb E)	Interaction are complex

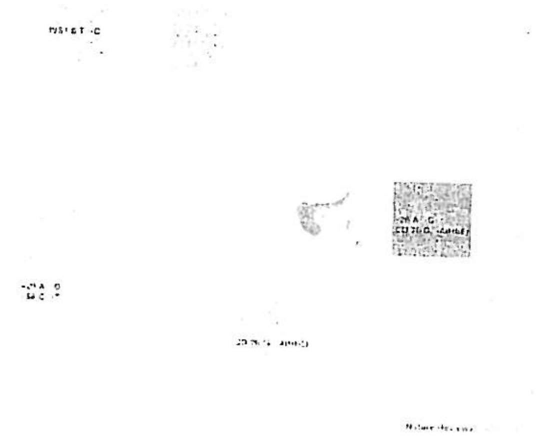
β-thalassaemia

- Deficiency of β-globin chain synthesis (**reduced** - β^+: severe($^+$) or mild ($^{++}$); **absent** - β^0)
- Most due to point mutations (c.f. α-thalassaemia deletions)
- Mutations affect globin gene transcription, RNA processing or translation, RNA cleavage and polyadenylation, or result in a highly unstable globin chain
- Severity related to degree of globin chain imbalance
- Extremely diverse clinical phenotypes

Clinical Manifestations of β-thalassaemia

- Related to degree of anaemia
- Spectrum of phenotypes can be difficult to classify between mild and severe
- β-thalassaemia trait: Usually asymptomatic carrier state, +/- mild anaemia
- β-thalassaemia intermedia (thalassaemia major not requiring blood Tx): anaemia, hepatosplenomegaly, iron overload, may have features of β-thalassaemia major without need for regular blood Tx

Regional Specificity: Global distribution of the β-thalassaemia mutations: common mild mutations are shown in bold.



β-thalassaemia Major

- Mutations in **both** β-globin genes
- Children: failure to thrive, dev delay, hepatosplenomegaly, facial abnormalities (bones of skull attempt to produce RBCs)
- Severe anaemia requiring regular blood transfusions (lifelong, every 2-4 wks)
- Hb electrophoresis: mainly HbF ($\alpha_2\gamma_2$)
- Complications of iron overload (incl. Heart failure)
- Splenectomy (but increased infection risk)
- Bone Marrow Transplant (numerous associated risks and not always possible)

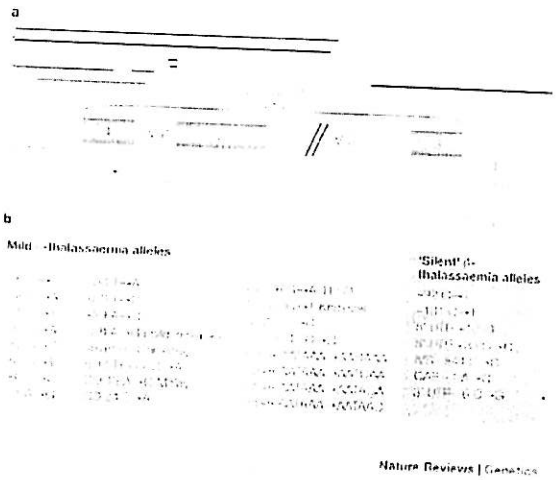
Phenotypic diversity of β-thalassaemia

- Genetic and Environmental factors
- Primary/Secondary/Tertiary
- Acquired complications as a result of primary defect in globin synthesis
- Environmental/social factors may modify individual responses

Primary Modifiers: β-thalassaemia alleles

- >200 mutations identified (most point mutations)
- Differing severity of particular alleles
- Most "mild" alleles result from mutations in promoter elements or poly(A) cleavage sites; cause mild phenotype in heterozygotes, intermediate phenotype in homozygotes, severe/major phenotype in compound heterozygote with severe allele

Main classes of mutations causing β-thalassaemia

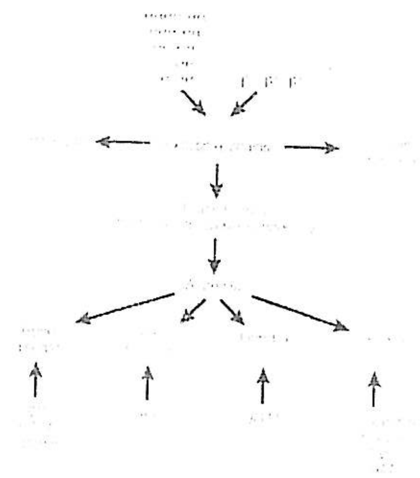


Secondary Modifiers: other loci involved in globin synthesis

- **Wide diversity** even among individuals with same β-thalassaemia genotype
- **HbE thalassaemia**: marked phenotypic variability (silent>Tx-dependent)
- **Modifiers of excess α-globin chains** (incl. α- and γ- globin loci) affect phenotype
  - Coinheritance of α-thalassaemia (may ameliorate β-thalassaemia severity-less imbalance): complex interactions
  - Variation in fetal Hb production
  - Triplicated/quadruplicated α-globin gene arrangements (increase severity of β-thalassaemia phenotype-more imbalance; no effect in normals)
    - HPFH
    - $^{G}\gamma$-gene polymorphism (HbF at times of stress)
- Other loci

Tertiary Modifiers: modifying loci not involved in globin chain production

- **Variability at unrelated loci** may have phenolypic effects esp. relating to complications of β-thalassaemia
- **Bilirubin metabolism**: additional TA repeats in UGT1 gene associated with jaundice and gallstones
- **Iron metabolism**: HFE alleles C282Y and H63D (latter more common in populations with increased frequency of β-thalassaemia)
- **Bone disease**: osteoporosis and fractures modified by polymorphisms at bone metabolism loci (Vit D, collagen, Oe receptor)
- **Co-selection**: P. falciparum malaria and heterozygote advantage, differing responses to infection and regional variability



Nature Reviews | Genetics

Acquired and environmental factors

- Acquired complications (splenomegaly, folate deficiency, recurrent infection)
- Pattern of infectious illnesses
- Body temperature
- Ethnological and cultural factors affecting patients' response to disease

Screening: thalassaemias

- Antenatal screening for haemoglobinopathies offered to all pregnant women in UK
- If found to be affected/carrier, partner offered screening; if both parents affected/carriers, then offered PND if possible.

Summary

- The haemoglobinopathies exemplify many of the mechanisms causing loss of function of a gene product
- They are common and have significant global public health implications
- Good correlation between molecular and clinical findings
- Easy to study tissues involved (blood)

Change	Example
Delete	
(a) the entire gene	Most α-thalassaemia mutations
(b) part of the gene	60% of DMD
Insert sequence into gene	Insertion of LINE-1 repetitive sequence into F8 gene (haemophilia A)
Disrupt gene structure	
(a) by a translocation	X-autosome translocations in women with DMD
(b) by an inversion	Inversion in F8 gene
Prevent the promoter working	
(a) by mutation	β-globin g.-29A>G mutation
(b) by methylation	CDKN2A gene in many tumours
Destabilise the mRNA	
(a) polyadenylation site mutation	α-globin g.AATAAA>AATAAG mutation
(b) by nonsense-mediated RNA decay	β-globin p.Q39X
Prevent correct splicing	
(a) inactivate donor splice site	PAX3
(b) inactivate acceptor splice site	PAX3
(c) alter an exonic splicing enhancer	SMN2
(d) activate a cryptic splice site	β-globin IVS1-110G>A mutation
Introduce a frameshift in translation	PAX3
Convert a codon to a stop codon	PAX3
Replace an essential amino acid	PAX3
Prevent post-translational processing	EDS VII
Prevent correct cellular localisation of product	ΔF508 mutation CFTR

From Strachan and Read 2004

Further Reading

- Strachan T, Read AP. Human Molecular Genetics 3, 2004. Chapter 16 p462-480 *(Textbook)*
- Weatherall DJ. Nature Reviews Genetics, 2001 2(4): 245-255 *(Review paper)*
- Old M. in Encyclopedia of the Human Genome, 2003. (Globin Genes: Polymorphic Variants and Mutations) *(Review chapter)*
- Weatherall DJ, Clegg JB. The Thalassaemia Syndromes (4^{th} Edn), 2001. *(Authoritative monograph)*