Genetic Disorders

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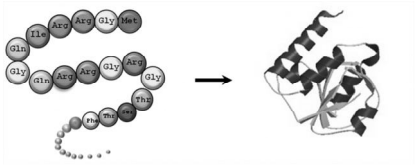
Commonly Used terms

- **Hereditary disorders** are derived from one's parents and transmitted in the germ line through the generations and therefore are **familia**l.
- **Congenital** implies "born with".
- All genetic diseases are not congenital.
- Some congenital diseases are not genetic.
- **Mutation** may be defined as a permanent change in the DNA

Amino Acid	3-Letter Code	1-Letter Code
Alanine	Ala	A
Cysteine	Cys	C
Aspartic acid or aspartate	Asp	D
Glutamic acid or glutamate	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y



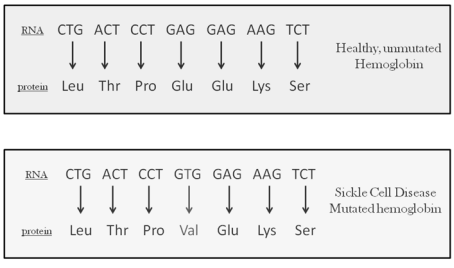


Figure 52.1: The RNA and protein sequences for healthy hemoglobin and the mutated form that causes sickle cell disease.

Types of mutation

- Genome mutation
- Chromosome mutation
- Gene mutation

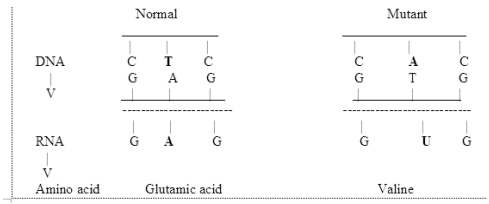
- **Genome mutation** results from loss or gain of whole chromosomes, giving rise to monosomy or triosomy.
- **Chromosome mutation** results from rearrangement of genetic material and give rise to visible structural change in the chromosome.
- **Gene mutation** results from partial or complete deletion of a gene or more often affect a single base.

- **Effect of mutation** depends on:
  - Types of mutation
  - Sites of mutation

- **Effect of gene mutation**
  - **Mutation within coding sequences (exon) causes**
    - Missense mutation
    - Non-sense mutation
- **Mutation within non-coding sequences (intron)**
  - Reduction of transcription
  - Lack of transcription
  - Defective transcription

- **Missense mutation:** A point mutation (single base substitution) may alter the code in triplet of bases and lead to the replacement of one amino acid by another in the gene product. As the mutations alter the meaning of the genetic code, they are termed "Missense mutation".

- **Example:** Sickle mutation affecting the β-globin chain of haemoglobin. Here change in amino acid of the normal β-globin chain (β A), converts to sickle β-globin (βS).



Here, the nucleotide triplet CTC, which codes for glutamic acid, is changed to CAC, which codes for valine. This single amino acid substitution alters the physicochemical properties of the haemoglobin, giving rise to sickle cell anaemia.

- **Non-sense mutation:** A point mutation may change an amino acid codon to a chain terminator or stop codon termed "non-sense mutation".
- Example: In β-thalassaemia, a point mutation affecting the codon for glutamic acid (CAG), creates a stop codon (UAG) if U is substituted for C. These change leads to premature termination of β-globin gene translation, the resulting short peptide is rapidly degraded. The affected individual lack β-globin chain and develop severe form of anaemia called βo - thalassaemia.

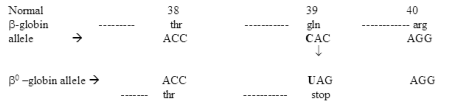


Fig: *A point mutation leading to premature chain termination. Partial mRNA sequence of the β-globin chain of haemoglobin showing codons for amino acid 38 to 40. A point mutation (C → U) in codon 39 changes glutamine (gln) codon to a stop codon and hence protein synthesis stops at the 38 th amino acid.*

Transcription of DNA is initiated and regulated by promoter and enhencer sequences that are found downstream or upstream of the gene. Point mutation or deletion involving these regulatory sequences may interfere with binding of transcriptional factors and leads to marked reduction of transcription, lack of transcription or defective transcription. Defective splicing of initial mRNA transcripts results failure to form mature mRNA transcript. Therefore, translation can not take place and the gene product is not synthesised.

- **Deletion and Insertion:** Small deletion or insertions involving the coding sequences lead to alteration in the reading frame of DNA strand, hence, they are termed **frame shift mutation.**

- If the number of base pair involved is three or multiple of three, the frame shift doses not occur; instead, an abnormal protein missing or more amino acid is synthesised.
- Mutation occurs spontaneously during the process of DNA replication. Certain environmental influences increase the rate of spontaneous mutation, such as radiation, chemicals and virus.

Classification of Genetic disorders

- **Disease related to mutant gene of large effect or Mendalian disorder**
  - Storage disorders
  - Inborn error of metabolism
- **Diseases with multifactorial inheritance (polygenic)**
  - Hypertension
  - Diabetes mellitus
- **Chromosomal disorders**
  - Diseases resulting from genomic or chromosomal mutation associated with numerical or structural changes in chromosome**.**

Transmission patterns of single gene disorders:

- Autosomal dominant
- Autosomal recessive
- X-linked disorder

Autosomal Dominant Disorders

Basic features:

- Manifest in heterozygous state
- At least one parent of an index case is usually affected
- Both male and female are affected
- Both can transmit disease
- When an affected person marries an unaffected one, every child has one chance in two of having the disease.

Additional features:

- Some patients do not have affected parent due to new mutation involving either the egg or sperm. Their siblings are neither affected nor at increased risk
- Clinical features can be modified by reduced penetration and variable expressivity. Some individual inherit the mutant gene but phenotypically normal.
- In many occasion the age at onset is delayed.
- Two major categories of non-enzyme proteins are usually affected
  - Those involved in regulation of metabolic pathway
  - Key structural proteins
  - Mutations of gene that encode enzyme proteins do not usually manifest, because upto 50% loss of enzyme activity can usually be compensated.

Why loss of one normal allele give rise to severe phenotypic effects are not fully understood. When the gene encodes one sub-unit of multimeric protein the product of the mutant allele can interfere with the function of normal protein.

Example of autosomal dominant disorders:

- Neurofibroma
- Polycystic disease of kidney
- Marfan's syndrome
- Osteogenesis imperfecta
- Achondroplasia
- vonWIlebrand's disease
- Familial polyposis coli
- Hereditary spherocytosis
- Familial hypercholestolaemia

Autosomal Recessive Disorders

Features:

- Manifest in homozygous state
- Parents are not usually affected.
- Both males and females are affected.
- Both males and females can transmit the disease
- Siblings may show the disease
- Siblings have one chance in four of being affected.

Other features of autosomal recessive disorders in contrast to autosomal dominant disorders:

- The expression of the defect tends to be more uniform than autosomal dominant disorders.
- Complete penetrance is common.
- The onset is frequently early in life.
- In many cases, enzyme proteins are affected by the mutation. In heterozygotes, equal amount of normal and defective enzymes are synthesised. Usually natural "margin of safety" ensures that cells with half their complement of the enzyme function normally.
- Disorders includes all inborn errors of metabolism.

Examples of autosomal recessive disorders:

- Thalassaemia
- Sickle cell anaemia
- Cystic fibrosis
- Wilson's disease
- α1–antitrypsin deficiency
- Haemochromatosis
- Lysosomal storage disease
- Glycogen storage disease

X-linked Disorders

- All sex-linked disorders are X-linked. Almost all X-linked disorders are recessive. The only assigned with certainty to the Y-chromosome is the determinant for testes.
- The Y-chromosome, for the most part, is not homologous to the X, so mutant gene on the X are not paired with alleles on the Y. Thus male is said to be hemizygous for X-linked mutant genes, so these disorders are expressed in the male.
- Heterozygous female usually does not express the full phenotypic changes because of paired normal allele become active by the phenomena of Lionisation but become carrier.
- Manifest in hemizygous male.
- Affected male does not transmit the disorder to his son, but all daughters are carriers.
- Sons of heterozygous women have one chance in two of receiving the mutant gene.
- X-linked dominant conditions are only a few. These disorders are transmitted by an affected heterozygous female to half her sons and half her daughters and by an affected male to all his daughters, but none of his sons, if the female parent is unaffected.

Examples:

- Duchenne muscular dystrophy
- Haemophilia A and B
- G-6-P-dehydrogenase deficiency
- Agamma globinaemia
- Diabetes insipidus

Biochemical and Molecular Basis of Single Gene Disorders

- The genetic defect may lead to formation of an abnormal protein or reduction in the output of the gene product.
- Mutation may affect protein synthesis by affecting transcription, mRNA processing or translation.
- Phenotypic effect of mutation may result directly from abnormalities in the proteins encoded by the mutant gene or indirectly owing to interaction of the mutant proteins with other normal proteins.

Mechanism involved in single gene disorders:

- Enzyme defect and their consequences
- Defect in membrane transport system
- Alteration in the structure, function or quality of non-enzyme protein.
- Mutation results unusual reaction to drugs

Biochemical consequences of enzyme defects:

- Accumulation of substrates
- Decreased amount of end-product due to metabolic block
- Failure to inactivate a tissue-damaging substrate.

Disorders with Multifactorial Inheritances

- Results from combined environmental influences and two or more mutant genes having additive effects.
- A number of normal phenotypic characteristics are governed by multifactorial inheritance, such as hair colour, eye colour, skin colour, height and intelligence.

Examples of multifactorial disorders:

- Cleft-lip and cleft-palate
- Congenital heart diseases
- Coronary artery disease
- Hypertension
- Gout
- Diabetes mellitus
- Congenital hypertrophic pyloric stenosis

Cytogenetic Disorders (Chromosome Mutation)

- **Types of Cytogenetic disorders:**
  - **Due to an abnormal number of chromosome**
    - Aneuploidy
    - Mosaicism
      - Mosaicism of sex chromosome – 45,X/47,XXX
      - Mosaicism of autosome – 46,XY/47XY+21
  - **Due to alteration in the structure of one or more chromosome**
    - Deletion
    - Translocation
      - Balanced reciprocal translocation
      - Robertsonian translocation (centric fusion)
    - Isochromosome
    - Ring chromosome
    - Inversion

- **Euploid –** Any exact multiple of haploid number
- **Aneuploidy**- Chromosome complements that is not an exact multiple of 23. Aneuploidy results from non-disjunction and anaphase lag.

- **Non-disjunction**- When a homologous pair of chromosome fails to disjoin at the first meiotic division or the two chromatids fail to separate either at the second meiotic division or in somatic cell division.
- **Anaphase lag-** One homologous chromosome in meiosis or one chromatid in mitosis lags behind and is left out of the cell nucleus, resulting one normal cells and one cell with monosomy.

- **Mosaicism-** When two or more populations of cells are present in the same individual it is call mosaicism. Mosaicism results from mitotic errors in early development.
- *Structural changes in chromosome result from chromosome breakage followed by loss or rearrangement of genetic materials.*

- **Deletion-** loss of portion of chromosome
  - Terminal deletion – due to single break
  - Interstitial deletion – Due to two breaks
- **Translocation –** A segment of one chromosome is transferred to another.
- **Balanced reciprocal translocation –** single break in each of two chromosome with exchange of material e.g. 46,XX, t(2;5)(q32;p14).

- **Robertosian translocation –** A reciprocal translocation between two acrocentric chromosome
- **Isochromosome-** When one arm of a chromosome is lost and remaining arm is duplicated a chromosome is formed consisting of two short arms only or two long arms.
- **Ring chromosome-** When a deletion occurs at both ends of a chromosome with fusion of damaged end.
- **Inversion-** Two breaks within a single chromosome with reincorporating the inverted segments.
  - **Paracentric –** Involve only one arm of the chromosome
  - **Pericentric-** If the breaks are on the opposite side of the centromere.

Examples of Cytogenetic disorders:

- Down's syndrome or Triosomy 21
- Cri du chat syndrome or 5p-
- Klienfelter's syndrome
- Turner's syndrome
- XYY syndrome
- Multi X syndrome

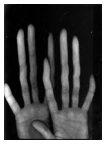
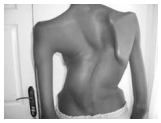
Some Common genetic Disorders

Marfen's Syndrome:

- Is a disorder of connective tissue of the body manifested principally by changes in the skeleton, eyes and cardiovascular system.
- The individual have unusually long, slender extremities, particularly elongation in the fingers, termed 'spider finger'.
- Approximately 70-85 % are familial and transmitted by autosomal dominant pattern.
- The remainders are sporadic and arise from new mutation.
- The disorder results from mutation in the fibrilin gene. Fibrilin is a glycoprotein secreted by fibroblasts that aggregate either alone or in conjugation with other proteins to form a microfibrilar network in the extracellular matrix. Microfibrilary fibre forms scaffolding for deposition of elastin.

Marfen Syndrome



Familial Hypercholesterolaemia:

- It is a 'receptor disease' resulting from mutation in the gene encoding the receptors for low-density lipoprotein (LDP), which is involved in the transport and metabolism of cholesterol.
- There is loss of feedback control and elevated levels of cholesterol induce premature atherosclerosis leading to greatly increased risk of myocardial infarction.
- Transmitted by autosomal dominant pattern.

Neurofibromatosis:

- **Two type:**
  - Type-1; von-Recklinghausen's disease
  - Type-2; Acoustic neurofibromatosis

von-Recklinghausen's disease:

- Relatively common
- 50% have family history
- 50% results from new mutation
- Extremely variable expressivity but 100% penetrance
- Three major feature:
- Multiple neural tumours dispersed anywhere on or in the body
- Numerous pigmented skin lesions some of which are "cafe au lait spot".
- Pigmented iris hamartoma (lish nodule).

Acoustic Neurofibromatosis:

- Less common
- Bilateral acoustic nerve tumours are invariable with or without skin tumour
- "Cafe au lait spot" may present
- Lish nodules are absent

Down's Syndrome:

- Is the most common chromosomal disorder.
- Is the major cause of mental retardation.
- Incidence: 1 in 800 births.
- Karyotype:
  - Triosomy 21 type; 47XX +21 (95%)
  - Translocation: 46XY, -14, +t(14q,21q), (4%)
  - Mosaic: 46XY/47XX+21 (1%)
- Most common cause is meiotic non-disjunction.
- Parents are normal karyotype
- More commonly occurs in mother over 45 years of age.

Clinical features:

- Flat facial profile
- Oblique palpabral fissure
- Epicanthic fold
- Mental retardation
- Gentle shy manner
- Congenital heart diseases (in 40%)
- Risk of acute leukaemia (10 to 20 fold)
- Senile dementia after 40 years
- Abnormal immune response

Cri du Chat Syndrome:

- It is so named because the affected infants have characteristic cry of a cat upto the age of one year.
- Results from deletion of arm of chromosome 5 (5p-)
- Common clinical features:
  - Severe mental retardation
  - Microcephaly
  - Round facies

Common features of all chromosomal disorders:

- In general they induce chronic problem relating to sexual development and fertility
- They are often difficult to diagnose at birth and many are first recognised at the time of puberty.
- In general, higher the number of X chromosome in the male and female, the greater the likelihood of mental retardation.

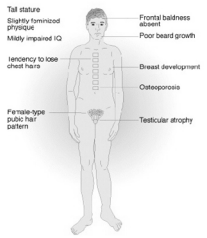
Klienfelter's Syndrome:

- Is defined as male hypogonadism that occurs when there are two or more X-chromosome and one or more Y-chromosome.
- Incidence: 1 in 850 live births.

- Karyotype:
  - Classic patter: 47XXY 82% resulting from non-disjunction
  - Mosaic: 46 XY/47 XXY 15%
  - Others: 47 XXY/48 XXXY, 48 XXXY or 49 XXXXY
- Rarely diagnose before puberty, because 3% testicular abnormality does not develop before early puberty
- Most patients have increase in length between the sole and the pubic bone
- Eunachoid body habitus:
  - Abnormally long leg
  - Small atrophic testes
  - Small penis
  - Lack of secondary male characteristic (deep voice, beard, male distribution of pubic hair)

- Elevated FSH
- Reduced level of testosterone
- Elevated estradiol level
- The ratio of oestrogen and testosterone level determines the degree of feminisation in individual cases.
- It is the principal cause of male infertility
- Spermatogenesis is reduced due to changes in the testes. Tubules are totally atrophied and replaced by pink, hyaline, collagenous ghost. Some patients have gonadal dysgenesis, which include apparently normal tubules interspersed with atrophic tubules. Leydig cell appear prominent

Klinefelter syndrome phenotype



Turner's Syndrome:

- Results from complete or partial monosomy of X chromosome and is characterised by hypogonadism in phenotypically female.
- Incidence: 1 in 3,000 female birth
- Karyotype:
  - Classic: 45X
  - Defective second X chromosome: 46 X, r(X) or 46X, i(Xq)
  - Mosaic: 45X/46XX

- Clinical features:
  - Short stature
  - Low posterior hair line
  - Webbing of neck
  - Broad chest and wide spaced nipple
  - Coarctation of aorta
  - Cubitus vulgaris
  - Streak ovaries, infertility, amenorrhea
  - Pigmented nevi
  - Peripheral lymphedema at birth
- Normal mental status
- Reduced oestrogen level
- Elevated pituitary gonadotropin level

Different types of Sex

- Genetic Sex
- Gonadal Sex
- Ductal Sex
- Phenotypic Sex

- **Genetic sex** is defined by presence or absence of Y chromosome
- **Gonadal sex** is based on the histologic characteristics of gonads.
- **Ductal sex** depends on presence of derivatives of mullarian or Wolfian ducts.
- **Phenotypic Sex or genital sex** is based on the appearance of the external genitalia.

- **Sexual Ambiguity or hermaphroditism:** Whenever there is disagreement among various criteria for determining sex.
- **Types of sexual ambiguities:**
  - True hermaphrodite
  - Psuedohermaphrodite
    - Male pseudohermaphrodite
    - Female pseudohermaphrodite

- **True hermaphrodite –** Presence of both ovarian and testicular tissue.
- **Psuedohermaphrodites –** Disagreement between phenotypic and gonadal sex
- **Male pseudohermaphrodite –** has testicular tissue but female type genitalia.
- **Female pseudohermaphrodite –** has ovaries but male external genitalia.

Hermaphrodites




Michel Jackson

Molecular Diagnosis

Application of Molecular Diagnosis:

- Detection of mutation that underlie the development of genetic disease either prenatally or after birth.
- Detection of acquired mutation that underlie the development of neoplasm.
- Accurate diagnosis and classification of neoplasm especially those that originate in the haemopoietic system.
- Diagnosis of infectious diseases, including HIV.
- Determination of relatedness and identity in transplantation, paternity test and forensic medicine.

Molecular Diagnosis of Genetic Diseases:

- **Types of Approach:**
  - Direct gene diagnosis involving detection of the mutation.
  - Gene tracking, an indirect method based on linkage of disease gene with a harmless "marker gene".

Direct Gene Diagnosis:

- **Principle:**
  - Detection of some important qualitative differences from normal in the DNA sequence of the gene in question.
  - Some mutations alter or destroy certain restriction sites on the normal DNA. Restriction enzyme recognises and cleaves the normal sequences but not the altered sequences; hence mutant gene loses one of the enzyme cutting sites. When DNA from a normal individual is digested with restriction enzyme and hybridised with radioactive cDNA probe specific for given gene a single band of known Kb length that react with the probe is detected on Southern blot analysis. A similar analysis of DNA from patients' cells leads to formation of a single band of larger Kb length owing to loss of enzyme sites.

- If mutation does not alter the cutting site of any known restriction enzyme, another technique is applied which is known as allele-specific oligonucleotides. Single base change may produce altered allele containing oligonucleotides. Such allele specific oligonucletids can be radio-labelled and used in Southern blot analysis
  The oligonucleotides containing the sequence or normal gene hybridises with both normal and mutant DNA but hybridisation to mutant DNA is unstable, owing to single base pair mismatch. So, labelled normal probe produces a strong auto-radiographic signal from DNA extracted from patient homozygous for mutant gene and faint signal with DNA from heterozygotes. Probe containing mutant sequence, the pattern of hybridisation is reverse.

Indirect DNA diagnosis: Gene Tracking

- Indirect gene diagnosis is possible only if structure of mutant gene and that of its normal counterpart are known. In large number of genetic disease information about the gene sequence or some times chromosomal location of the gene is lacking. In these instances, "gene tracking" is applied. This is accomplished by exploiting naturally occurring variation in DNA sequences i.e., polymorphism. Two types of DNA polymorphism are useful in these regards: (1) Restriction fragment length polymorphism (RFLP) and (2) Variable number of tandem repeats (VNTR).

- **RFLP** refers to variation in fragment length between individuals that results from DNA sequence polymorphism.
- **VNTR** results from presence of short sequences of DNA that are arranged in a head to tail fashion and repeated several times in a tandem array**.** Because the number of repeats varies from one chromosome to another, VNTR can be used to distinguish different chromosomes. VNTR polymorphism is also used for identification of individuals.