Chapter 2

Mutation and Polymorphism

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Q 1. Definitions

Ans.

1. **Genotype** is the genetic composition of an individual which describes the state of two copies (alleles) of a gene at a given locus on the chromosome.
   Example: If the eye color of an individual is green, then the two different alleles for the gene will be: G-Green color and g-Black color.
   The three genotypes will be:
   a. GG: Homozygote
   b. Gg: Heterozygote
   c. G or g: Hemizygote.
2. **Phenotype** is the external/observable expression of a trait. The phenotype of an individual depends upon the genotype and its environmental interaction.
   Examples: Eye color, skin color, height of an individual, etc.
3. **Locus** is the specific reference position where a gene is located on the chromosome. If it gives the information of many genes, it is called loci.
4. **Allele** is one of the two possible genetic sequences for a given gene at a particular locus on the chromosome.
   Example: For describing the height of a tree, the two alternate sequences/alleles can be represented as T and t for the trait.
   - ***Wild type allele*** is the allele of a particular gene which remained unchanged from our ancestors or through the evolutionary process and is seen in majority of healthy individuals.
   - ***Variant or mutant allele*** is the alternate sequence of the DNA instead of wild type, present in the gene due to change in the DNA nucleotide sequence.
5. **Mutations** are changes in the genetic material of an organism, which affects the normal organization (sequence, structure or function) of gene or the entire chromosome.
6. **Novel mutation** is the alternate sequence of the DNA different from the wild type which was not reported earlier and is identified in the genomic sequence during a research or a diagnostic test.
7. **Polymorphism** is the frequency of two or more variants in a given population greater than 1%, indicating that it is present due to normal evolution and has no major effect on phenotype.

Q 2. What is mutation? Explain how mutations arise in genetic material (or explain the basic pathogenesis of mutation).

Ans. **Mutation** is the inheritable change in the genetic material of an organism, which affects the normal organization (sequence, structure or function) of gene or the entire chromosome. Mutations that cause changes in one sequence or that is limited to a small locus in the gene can be termed small gene mutations and those

that cause big alterations at chromosomal level are called large chromosomal mutations. These changes in the DNA subsequently affect the RNA and protein. The mutations that cause a positive effect are beneficial are likely cause of evolution and those which cause a disease are called pathogenic mutations.

Mutations can arise in genetic material due to:

1. **Natural factors:** Defect in DNA replication, DNA repair mechanism and chromosomal anomalies during cell division can lead to mutations which can be pathogenic or benign. The frequency of these mutations in a specific population depends upon the process of natural selection, genetic drift, gene flow and the rate of mutation. The rate of mutation depends upon the size and site of gene, parental gender and age.
   Example: Achondroplasia *de novo* mutation rate is more in advanced aged fathers.
2. **Environmental factors:**
   - ***Mutagens*** like chemicals, dyes, form aldehydes, benzene compounds, food adulterants, tobacco, etc. can cause both chromosomal and DNA mutations.
   - ***Radiations:*** Natural and artificial ionizing radiations like X-rays, gamma rays, alpha, beta and neutron particles from diagnostic and therapeutic radioactive interventions and occupational exposure can lead to somatic and germline mutations. It depends upon the dose and time of exposure to radiations. More the dose and exposure, more will be the rate of mutation but, small doses can also lead to variations in DNA. One chest X-ray exposure dose during diagnostic intervention is 0.1 mSv (unit of radiation average dose per year) and recommended dose exposure is 15 mSv per year. So, during diagnostic intervention or occupational exposure, risk and benefit of the procedure should be balanced to prevent occurrence of mutations.

Q 3. Write about different types of mutations.

Ans.

I. **Mutations based on tissue involved:**
   - ***Somatic mutations*** happen during mitotic division in somatic tissues after differentiation of the zygote in somatic and gonadal tissues. It does not transmit to future generation and can be diagnosed from somatic tissue by molecular test.
     Example: Segmental neurofibromatosis or cancer mutations.
   - ***Germline mutations*** get transmitted from the parental germ cells and present both in somatic and germ cells. It can be diagnosed in the peripheral blood by molecular tests and can be further transmitted to next generation.
     Example: Sickle cell anemia.

II. **Mutations based on the level and mechanism observed in a cell's DNA:**

1. GENE LEVEL

a. **Mutations in coding regions:** Point mutations or small-scale mutations caused due to base substitutions where one base sequence is substituted with another resulting in the change in one codon in the RNA sequence.

Mechanisms for point mutations: (Figure 2.1 A to C)

i. **Base substitutions:** At gene level:
   - **Transitions:** Adenine (A) is substituted by Guanine (G) (A-G), switch between two purines or a substitution of Cytosine (C) to Thymine (T) (C–T), switch between two pyrimidines.
   - **Transversion:** Either A/G are substituted by C/T, i.e. either a purine is replaced by pyrimidine or a pyrimidine is replaced by a purine.
   - **Mispairing:** Normally in DNA, A pairs with T and G pairs with C. During mispairing, non-Watson-Crick pairing occurs wherein A pairs with C and T pairs G.

ii. **Frameshift mutations:** The reading frame (three bases forming an amino acid) in the RNA shifts due to insertions or deletion of one or two bases leads to many different changes in the codons that code for the protein.
Example: Hereditary motor and sensory neuropathy occurs due to insertion and Duchenne muscular dystrophy due to deletion of exon.

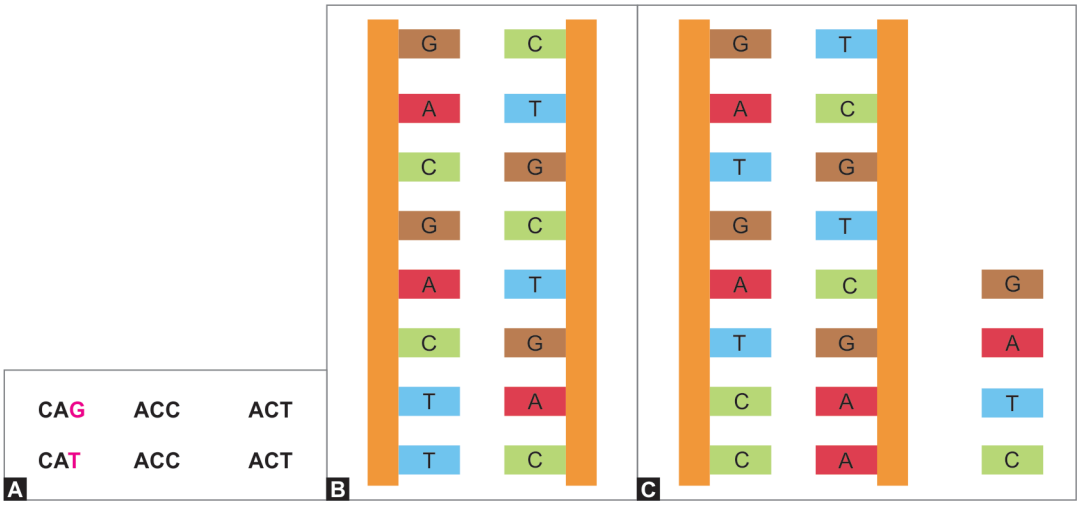

Figure 2.1: (A) Point mutation; (B) Normal nucleotide base pairing; (C) Mispairing of nucleotide base pairs (A–C, G–T)

b. **Mutations in non-coding regions** like promoter region, splice sites, miRNA, siRNA and various regulatory sites also lead to severe phenotypic manifestations.

2. TRIPLET REPEAT DISORDERS (*See* Chapter 16 for detail)

These occur due to expansion and instability of triplet repeat sequence (CAG CAGCAG).
Example: Huntington disease.

3. CHROMOSOMAL LEVEL (LARGE SCALE) ABERRATIONS (*See* Chapter 3 for detail)

Various structural or numerical aberrations may affect more than one gene and can cause severe disease.

a. **Structural aberrations:**

i. **Deletions:** Deletions of large parts of chromosomes are usually caused due to heat or radiation, viruses, chemicals or even due to errors in recombination, etc. These cannot be reverted as the segment is lost permanently.

ii. **Duplications:** These result from doubling of chromosomal segments.

iii. **Insertions:** These can cause the increase in the genetic information.

iv. **Inversions:** The genetic information is not changed in amount, but it is inverted and rearranged and affects gene expression.

v. **Translocations:** They occur when a segment of one chromosome transfers to another chromosome and attaches there.

b. **Numerical aberrations: Aneuploidy and polyploidy** cause the change in the number of the chromosomes.

Q 4. What are structural effects of mutation on the protein products?

Ans. Structural effects of mutations on the proteins: There can be minimal to lethal consequences of mutations on the structure of the protein products by two ways (Figure 2.2):

- **Synonymous or silent mutations:** A single base pair substitution preferably at the third nucleotide in the triplet sequences wherein the final amino acid is not changed and there is no alteration in the protein features.
- **Nonsynonymous mutations:** When the substitution of a nucleotide(s) results in change in the amino acid or its sequence from the original triplet which it was supposed to code for, it leads to nonsynonymous mutation. It can result in abnormal protein function and can lead to disease.

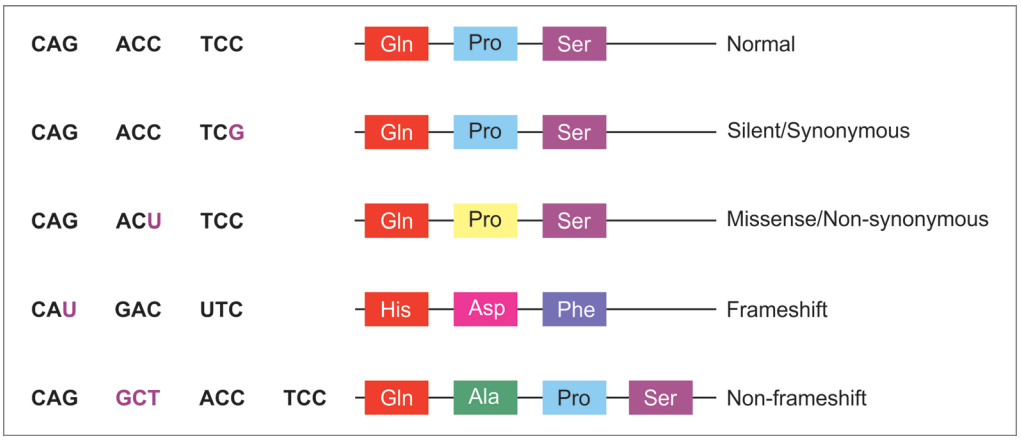

Figure 2.2: Structural effects of mutations on the protein products

- ***Missense mutations:*** Alteration of one single amino acid sequence that leads to abnormal structure of the protein.
  Example: Most of the structural hemoglobinopathies
- ***Frameshift:*** Complete downstream amino acid sequence of the polypeptide changes leading to frame out mutation.
  Example: Duchenne muscular dystrophy.
- ***Nonsense mutation:*** An introduction to stop codon may abruptly stop the polypeptide synthesis leading to incomplete protein product.

Q 5. What are functional effects of mutation on the protein products?

Ans. Functional effects of mutations on the proteins: There can be minimal to lethal consequences of mutations on the function of the protein products. Loss or gain of the function of the protein product is the ultimate functional effect due to mutations.

1. **Loss of function mutations** is the changes due to which the protein activity can get reduced or is completely lost. When there is decreased protein activity, it is called hypomorph (the change) and the complete loss of the protein activity is caused due to null allele or amorph. Loss of function mutations in metabolic disorders show autosomal recessive or X-linked recessive pattern of inheritance as there is one normal allele to compensate the protein activity.
2. **Haploinsufficiency** is the phenomenon leading to reduced enzyme production due to heterozygous genotype. It results in disease symptoms due to presence of only one normal copy of the gene.
   Example: Familial hypercholesterolemia.
   When there is homozygous mutation leading to the complete loss of function of protein, there is a much severe kind of phenotypic presentation.
3. **Gain of function mutations:** Either there will be an increase in the gene expression, or a completely new function is exhibited due to gain of function mutations. This can happen due to activation of a point mutation or increased gene dosage effect.
   Example: The increase in the number of triplet repeats causing Huntington's disease and HER-2 overexpression leading to HER-2 positive breast cancer.
4. **Dominant negative effect:** When a mutation present in heterozygous state produces a protein product which interferes with the protein product of normal allele and causes complete loss of protein activity, it is called dominant negative effect.
   Example: Osteogenesis imperfect.

Q 6. Describe polymorphisms, different types of polymorphism and its role in human genetics.

Ans. Polymorphism: The genomic sequences of different individuals when compared with

each other, will be same at many loci. They are monomorphic. But some loci will differ in their nucleotide sequence, which are called polymorphic sections or polymorphisms. The factor which distinguishes these variants being referred to as mutants or polymorphism is their frequency in the population. If the frequency of the variant allele is less than 1% in the population, it is called mutant allele and if its frequency is higher than 1%, it is called a polymorphism.

The different types of DNA polymorphisms are:

1. **Single nucleotide polymorphisms (SNPs):** It is a sequence of DNA on which humans vary by one nucleotide. They can be synonymous or nonsynonymous.
2. **Tandem repeat polymorphisms:** It consists of a series of nucleotides that are repeated in tandem (i.e. one time after another).
   Example: Microsatellite, simple sequence repeat (SSR), or short tandem repeat (STR), minisatellite, variable number of tandem repeats (VNTR) and mobile repeats like Alu and long interspersed elements (LINE).
3. **Structural polymorphisms:** Structural variants involve deletions or insertions of a nucleotide sequence (also called a copy number variant), inversions, and translocations.

Polymorphic variants can be non-pathogenic and can be used for various purposes in human genetics:

- For gene mapping by linkage analysis
- Forensic medicine—for crime detection and paternity confirmation
- HLA matching of tissues.

Q 7. What do you mean by genotype-phenotype correlation? Explain with example.

Ans. In some **single gene disorders** like sickle cell disease, fragile X syndrome, muscular dystrophy, genetic change (genotype) responsible for a specific disease can be identified based on the clinical examination.

But for the complex diseases and due to genetic heterogeneity sometimes it becomes difficult to identify the etiological mutation on clinical basis. The variants found on whole genome sequence of an individual or whole exome sequencing gives a lot of genetic data and the exact sequence change to find out which one is actual causative or is associated with the disease is a challenging task. For identifying the actual association, different phenotypic details of the proband are taken and are correlated with the obtained genetic changes one by one based on their function, pathogenicity and mode of inheritance. This association between the genotype and responsible phenotype of an individual is called genotype–phenotype correlation.

Example: Larsen syndrome is a rare genetic disorder that has been associated with a wide variety of different symptoms caused by mutations of the FLNB gene.

Q 8. Explain genetic and clinical heterogeneity.

Ans. **A. Genetic heterogeneity** means different variations at genomic sequence / different genetic mechanism which will result in similar phenotypes. There are two types of genetic heterogeneity:

1. **Allelic heterogeneity:** Different variations at the same locus in the same gene resulting in similar phenotype is called allelic heterogeneity.
   Allelic diversity is also an important consideration in disorders, such as retinitis pigmentosa, cystic fibrosis, hemophilia, and β-thalassemia. Direct mutation analysis in these disorders for only a subset of specific mutations may fail to detect a different disease-causing mutation in a particular family, who may have critical genetic counseling implications for many individuals within that pedigree.
2. **Locus heterogeneity:** This can be defined as mutations at two or more genetic loci that produce similar phenotypes (either biochemical or clinical). This is relevant since genetic heterogeneity can present problems for heterozygote detection. Example: Hearing loss can occur with

different homozygous mutations in genes MYO6 and SCL26A5.

- ***Double heterozygotes:*** Hearing loss due to autosomal recessive homozygous mutations in two different genes cause parents to be affected but their child who is heterozygous for each mutation does not manifest the disease.

B. Clinical heterogeneity: It is defined as very different biochemical or clinical phenotypes due to the presence of more than one mutation within the same gene. *Example:* Different mutations of RET proto-oncogene cause different human diseases like papillary thyroid carcinomas, multiple endocrine neoplasia type 2, Hirschsprung's disease and congenital disorder of enteric nervous system.

Q 9. What are different DNA repair pathways? Write about their mechanism and different disorders associated with it.

Ans. DNA repair mechanism occurs in every organism from bacteria to humans. If the DNA got damaged, it gets repaired by various mechanisms involving various enzymes like

- DNA photolyase for thymine dimer formation
- DNA repair endonucleases for cleavage
- Endonuclease excise the nucleotides
- Glycosylases to cleave glycosidic bonds
- DNA polymerase for replication
- DNA ligase for sealing the gaps.

Various DNA repair mechanisms are given in Table 2.1.

TABLE 2.1: Various DNA repair mechanisms

Type of DNA repair	*Mechanism*	*Disorders*
Light	Light dependent repair or photo reactivation cleaves thymine dimers, cytosine dimers and cytosine-thymine dimers in prokaryotes	—
Nucleotide	Excision repair thymine dimers, damaged base (S)	○ Xeroderma pigmentosum (XP) ○ Cockayne syndrome (CS) ○ Trichothiodystrophy (TTD)
Base excision repair	Replaces the abnormal base	○ Cancer predisposition ○ Colorectal cancer ○ Immunological defects
Mismatch repair	Excise and replace the mismatches in nucleotide after replication	Mismatches in hMLH1 and hMSH2 genes cause sporadic colorectal carcinomas
Post-replication repair	Rec A protein binds to the single strand at the gap in the template strand and mediate pairing with homologous and non-homologous segment of the sister double helix	○ Bloom syndrome due to BLM gene ○ Breast cancer (BRCA1, 2)

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