Genetics in Dentistry

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Dedicated to

My Wife

Pushpa

For her support,
understanding and patience

(GPP)

The memory of my nephew

Dr Abhijit Mahato (1979–2008)

B Tech (Jadavpur University), M Tech (IIT, Kanpur), PhD
(Pratt School of Engineering, Duke University, Durham, USA)

……who left us all, too early.

(NKM)

Preface

Genetics is an all-pervading biological science with applications in every branch of medicine. Forays in genetics research have helped us understanding the details of several ailments in human beings. Dentistry, as we all know, is one of the major streams of medical science. The discipline of dentistry has grown tremendously and has contributed valuably and immensely to the development of modern medicine. Though understanding of dental diseases in molecular terms is in its nascent stage, the relevance of such a perspective is unquestionable. Though adequate literature is available on genetic correlations of dental disorders, it is hard to get a compilation containing details of all major dental disorders and their genetic associations at one place.

Now, everyone has begun to realize the importance of genetic interpretation of dental disorders for the purpose of understanding the dynamics of disease processes and simultaneously designing treatment at a more fundamental level.

In our humble effort we have tried to deal with the basic concepts of genetics and their implications with relation to dental ailments in a separate section of the book. Latest advancements in genetic techniques and therapy are dealt with separately in the final section of the book. The language of the book has been kept as simple as possible, comprehensible for any interested undergraduate or postgraduate reader.

'Genetics in Dentistry' is the first of its kind of effort that would enable students as well as practitioners to understand the nuances of genetics involved in dental practice. All dentistry related chapters have been extensively researched and referenced for better orientation of recent scientific advancements and would be immensely helpful for postgraduate students writing their thesis.

As it is our first attempt towards this new perspective and since genetics as well as dentistry are ever-growing disciplines, we would be grateful to readers for any feedback regarding deficiencies in the content or presentation of the text.

GP Pal
Niladri Kumar Mahato

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Essentials of Human Genetics

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DEFINITION OF GENETICS

We all have observed that children of same parents, more often than not, resemble each other as well as resemble their parents. We also try to guess the proximity of this resemblance towards one of the two parents. It is quite interesting to observe that the resemblances are not just confined to their physical appearances (facial features, height, color of skin and hair, etc.), but are often perceptible in their mental attributes (intelligence, tastes, attitudes, etc.) also. This is because the characteristics of parents are passed on to the children through the gametes furnished by each parent (sperm and ovum). The process of transmission of characters from one generation to the next (parents to children) is called **inheritance** or **heredity**.

The question that crops in our mind is about what are the substrates that actually determine the characters in an individual. The characters, in fact, are determined by certain factors called **genes;** the fundamental units of inheritance. For details about genes refer to Chapter 4. The genes determining specific characters in an individual are transmitted to them physically through gametes of the parents. The individual in turn passes these traits onto its offsprings through his or her gametes. An individual is either short or tall in stature or with black or blond hair entirely due to the presence or absence of specific genes responsible for a particular character or trait. Since an individual receives genes from parents (through sperm and ovum), he or she inherits characters both from the father and the mother. An important fact about gene transmission is that when they are transmitted from one generation to the next, the transmission of a trait is not random but it follows some discreet statistical laws depending upon the type of the character and of course, the behavior of the gene during gamete formation. Therefore the science of **genetics** can be defined as ***the study of genes and of the principles that govern the passage of genes from one generation to the next.***

DIVISIONS OF GENETICS

Human genetics can now be divided into several branches. Few important subdivisions of genetics are as under.

Molecular genetics: Includes the study of chemical structure of gene at molecular level. This branch also includes the study of function of gene and regulation of its activity (Chapter 4).

Cytogenetics: Deals with the study of chromosomes (Chapter 2). Cytogenetics provides the cytological explanation of different genetic principles.

Biochemical genetics: Concerns with the study of genes and their products, the enzymes, which control important stages of various metabolic processes. This branch deals with the **inborn errors of metabolism.**

Cancer genetics: The cell cycle is under genetic surveillance and control. The cycle progresses from one stage to the next through several stages called the checkpoints. The structure of all genes is scrutinized at these periodic intervals for allowing only the healthy genes to proceed to the next stage. Cancer genetics

studies the abnormalities related to these checkpoints to find the reasons that cause cancer (Chapter 16).

Immunogenetics: The immunological make- up of an individual is under strict control of certain genes. Immunogenetics deals with the genetics of production of different types of antibodies (Chapter 7).

Developmental genetics: Deals with the genetic control of development of an embryo (Chapter 8).

Population genetics: This branch deals with frequencies and distributions of genes in human population and the rates of their mutation.

CLASSIFICATION OF GENETIC DISEASES

Diverse genetic mechanisms are involved in different hereditary diseases. The cause of a genetic disorder may have its base in the abnormality of the structure of a single gene or multiple genes. Genetic diseases may be also due to a gross abnormality in the structure of an entire chromosome. Thus genetic diseases may be classified as under:

1. ***Disorders due to mutation in single gene***: Single gene mutations are responsible for these disorders and they follow laws of Mendelian inheritance. These disorders may be autosomal dominant, autosomal recessive or X-linked (Chapter 6). Thousands of disorders can be categorized in this group. Some examples related to dentistry are given below.

Autosomal Dominant

- Achondroplasia
- Dentinogenesis imperfecta type 1
- Amelogenesis imperfecta hypoplastic type 2 (AIH2)
- Amelogenesis imperfecta hypocalcification type
- Hypodontia
- Osteogenesis imperfecta.

Autosomal Recessive

- Cystic fibrosis
- Amelogenesis imperfecta (local hypoplastic type)
- Amelogenesis imperfecta (pigmented hypomaturation type)
- Neonatal osseous displasia 1.

X-Linked Dominant

- Amelogenesis imperfecta (Hypoplastic)
- Vit. D resistant rickets.

X-Linked Recessive

- Hemophilia
- Ectodermal dysplasia type 4
- Amelogenesis imperfecta hypomaturation type (AIH)
- Chondrodysplasia punctata -1

2. ***Multifactorial disorders:*** Cumulative or additive effects of multiple genes are implicated in these disorders. The normal characters like height, color of skin, intelligence and physique are determined by the interaction of many genes. Common congenital malformations like cleft lip and palate and diseases like hypertension and diabetes mellitus are multifactorial disorders. Some kind of oral conditions like dental caries, periodontitis and malocclusion have strong genetic susceptibility. These kinds of disorders are results of interplay between gene expression and environmental factors.

   The multifactorial disorders follow different pattern of inheritance as compared to single gene disorders (Chapter 6).

3. ***Disorders due to chromosomal abnormality:*** This group includes gross structural anomalies that give rise to alterations in the number of chromosomes (absence of a chromosome or presence of an extra chromosome), i.e. **Trisomy 21** (Down's syndrome) or **Turner's syndrome** (XO). This class also includes disorders, which result due to abnormality in the structure of chromosomes such as deletions and translocations. The invention of **banding** and **FISH** (fluorescent *in situ* hybridization) techniques has helped to detect even minor abnormalities in chromosomes (Chapter 2). Subtle or point chromosomal abnormalities are included in the single gene disorders.

4. ***Somatic genetic disease:*** Cell divisions (mitosis in somatic and meiosis in germ cells) constantly occur during the lifetime of an individual. During each cell division there are chances that a change in the structure of a gene (gene mutation) may

take place due to an error in DNA replication. It may also happen that at the end of a cell division (mitosis or meiosis) one of the daughter cells might receive an unequal number of chromosomes (due to error in chromosomal separation). These kinds of mutation or mistakes in chromosomal distribution are accountable for numerous somatic and germ line diseases.

GENETICS IN DENTISTRY

It was first observed by the French biologist **Maupertius (1689-1759)** that the conditions like **polydactyly** and **albinism** were inherited in human beings. Likewise **John Dalton** (1766-1844) observed that **color blindness** and **hemophilia** were inherited diseases. However, human genetics was recognized as a science only after rediscovery of Mendel's Laws of Inheritance in early 1900. From the mid 20th century onwards, oral health care professionals had started realizing that many diseases related to the oral cavity were in fact inheritable. Information from the Human Genome Project (2001) and recent genetic researches has clearly indicated that many diseases with the dental, oral and craniofacial manifestations have a genetic basis both in terms of heritability (disease running in families) as well as arising from structural mutation in a particular individual.

Tooth Agenesis

The etiology of ***tooth agenesis*** was largely unknown till the recent past. But today we know that the development of tooth is strictly under the control of many genes. Several mutations in the developmental genes could result into failure of tooth development. Familial tooth agenesis may be transmitted as an autosomal dominant, recessive or an X-linked condition. Similarly, most cases of ***hypodontia*** exhibit polygenic inheritance pattern. Hypodontia is associated with syndromes like Down's syndrome, ectodermal dysplasia and the Ellis-van Creveld syndrome. This demonstrates that the development of other organs and tissues of the body is closely related to the development of dentition and perhaps regulated by common genes (Refer Chapter 10 for genetics of tooth agenesis).

Dental Caries

Certain microorganisms have been incriminated as the causal factors for two major diseases of oral cavity, i.e. ***dental caries*** and ***periodontal diseases***. Recent research data have pointed that these conditions have a strong genetic predisposition. Different people have different susceptibility risk for developing periodontitis. Studies have shown that the increase vulnerability to severe adult periodontitis is due to variation in the ***interlukin-1*** (IL-1) gene cluster that is situated on chromosome number 2 (Refer Chapter 13 for genetics of dental caries).

Craniofacial Syndromes

The development of craniofacial region during the early stages of development is genetically determined in terms of migration of definite ***neural crest cell*** and through this to the expression of certain sequential ***homeobox*** genes (Chapter 8). Epithelial-mesenchymal interaction during embryogenesis is regulated by growth factors and the retinoic acid superfamilies. Conditions like ***hemifacial microsomia*** and ***craniosynostosis*** have their origin in neural crest cell disorders. The mutation in ***fibroblast growth factor receptor*** genes are responsible for abnormal suture development and found to occur in ***Apert***, ***Crouzon*** and ***Pfeiffer*** syndromes. ***Cleidocranial dysplasia*** is characterized by defects in the membranous bones of the cranial vault and clavicle. The mutations responsible for this deformity have been found to occur in the ***core binding factor 1*** gene (CBFA1). The gene responsible for a well known craniofacial abnormality the ***Treacher Collins syndrome*** is situated on the long arm of chromosome 5 (Refer Chapter 11 for craniofacial syndromes). Many craniofacial abnormalities are due to interaction between environmental and genetic factors.

Cleft Lip, Cleft Palate and Cancers

Among the commonly occurring malformations of the oral cavity ***Cleft lip*** and ***cleft palate*** (Chapter 12) are amongst the top in the list. These congenital malformations are inherited as multifactorial traits. The same is true for ***malocclusion*** (Chapter 15).

The head and neck region are very common sites for carcinomas in general and oral cancer are the ones

seen quite frequently. The dynamics of cancer involves changes in the genome that result in uncontrolled cellular proliferation and metastasis. The ***Growth factor*** and ***growth factor receptor*** genes regulate the proliferation of cells. Genes responsible for cancer are known as ***oncogenes.*** These genes function normally in regulating cellular activity. A mutation in these genes may trigger them to acquire oncogenic properties. Cell division is strictly under genetic control and each of the steps is under constant surveillance of cellular mechanisms. ***Cell cycle checkpoints*** exist at appropriate transition points of the cell cycle. The activities at these checkpoints are executed by special proteins that are synthesized by specific genes like the p54 gene. Anomalies in these genes lead to abnormal cell division and subsequently to tumor formation. Structural integrity of the DNA is determined and checked at the checkpoints before allowing it to proceed to subsequent stages of cell division. Mutations of the checkpoint controlling genes and proteins cause several cancers. The **tumor suppressor genes** constitute another important cell cycle controlling element. These genes apply brakes to the events in a cell division in case of detection of an abnormality at any stage. These genes are constitutively active or in simple terms, active by default in all normal cells. A mutation causing abnormal activity of any tumor suppressor gene may lead to cancer. Researchers have also identified several tumor-forming genes that occur in normal cells but remain inactive by themselves. Such ***proto-oncogenes*** trigger unwarranted cell division if they are activated by any means or when any normally occurring inhibition acting on them is withdrawn (Refer Chapter 16 for Genetics of Cancer).

It is hoped that the near future will witness a lot of exciting advances in: Use of primary teeth as source of stem cells, tissue engineering in dentistry, use of saliva as a diagnostic fluid in detecting genetic dental disorders and salivary gland gene transfer. It is quite imperative that dental practitioners now will increasingly require knowledge of human genetics and the awareness of the applications of new molecular-based diagnostic and therapeutic technologies. Thus a sound knowledge of genetics will definitely improve the ability of dentists to diagnose and treat patients suffering from inherited and genetically caused dental diseases.

Since recent past more and more diseases are being recognized as having something related to genes and genetics. This is perhaps due to interpretations based on our new and expanding knowledge at the molecular level and progress in modern diagnostic techniques. On other hand this may also be due to the fact that owing to the overall improvement in hygiene and health care, the incidence of communicable diseases and nutritional deficiency has reduced thereby shifting our attention to diseases resulting from gene-related etiology. Genetic disorders are now considered significant causes for disease in all age groups.

MENDEL AND HIS LAWS OF INHERITANCE

Johann Gregor Mendel

Johann Gregor Mendel was born in Austria on July 22, 1822. He had to face relentless difficulties in his childhood and youth due to poverty and ill health. It was to the credit of his young man that he remained steadfast in the face of all the adversities for the pursuit of knowledge. It took him almost eight years to complete his initial experiments on pea plants. Mendel published his reports in the proceedings of the Brunn Natural Science Society in 1866. Mendel's work remained unappreciated and unnoticed till the turn of the century when the postulates of Mendel were rediscovered and revisited by three independently working scientists, Erich Von Tshermak, Hugo de Varies and Carl Correns in the beginning of the 20th century.

Mendel's work did not get recognition during his lifetime. He passed away in 1884, much before his monumental work immortalized him as the *'father of modern genetics'*.

THE PEA PLANTS EXPERIMENTS

Mendel's experiments were designed to find out the mechanisms responsible of inheritance of traits in the pea plants. His experiments basically involved two types of crosses. One between plants differing in a single pair of contrasting characters such as cross between a pure tall and a pure dwarf plant called the **Monohybrid cross** and subsequent crosses within the offsprings in each generation obtained from the

monohybrid crosses. The other type of experiment called the **Dihybrid cross** was carried out between plants differing in two pairs of contrasting characters; a cross, for example, done between plants having yellow and round seeds and plants having green and wrinkled seeds. The contrasting pairs of characters in the dihybrid cross were represented by the color and the texture of the seeds in the two different varieties of plants.

Explaining Certain Terms

Self-pollination: Pollens of a flower pollinating the stigma of the same flower is called as **self-pollination** (Self-fertilization).

Cross-pollination: Pollens of a flower pollinating different flower stigma is called **cross-pollination**. (Cross-fertilization).The offspring which result from cross breeding between pure strains is called a **hybrid.**

Monohybrid cross: The cross between the plants or animals differing in single pair of contrasting characters is called **monohybrid cross**, e.g. cross between tall and dwarf plants.

Dihybrid cross: The cross between plants or animals differing in two pairs of contrasting characters is called **dihybrid cross**, e.g. plants with yellow and round seeds are crossed with plants having green and wrinkled seeds.

Monohybrid Crosses Experiments

Mendel crossed plants that differed only in a single pair of contrasting character or trait. He crossed between pure tall plants and pure dwarf plants. The character or trait in case of these plants was the height of the stem and the pair of contrast was the tallness and dwarfness of the respective plants. The purity was verified with repeated self-pollination where the tall plants always inbreeded tall and the dwarf plants yielded dwarf offsprings on repeated inbreeding for several generations.

And when these two varieties of plants (tall and short) were crossed, Mendel observed that:

(a) All the hybrid members of the first generation, called the **First filial generation** (F1), were tall plants.

(b) When the F1 population was allowed to self-pollinate, the plants of the F1 generation gave rise to the **Second filial generation** (F2) with the following features.
- The character of dwarfness that had disappeared in the F1 reappeared in F2.
- 75% of the F2 plants were tall and 25% were dwarfs.

(c) After self-pollination the F2 generation gave the following results:
- The dwarf plants of F2 when pollinated with dwarfs in the F2, always yielded dwarfs.
- The tall plants when pollinated with the other tall plants within the F2, only 1/3rd of plants always yielded tall offsprings.
- Remaining 2/3rd of the tall plants yielded tall and dwarf plants in the ratio of 3:1.

Similar to the experiments in monohybrid crosses related to the height of the stem of plants, Mendel conducted experiments with other contrasting characters such as the shape and color of the seeds and pods, etc. (Table 1.1) and remarkably got statistically comparable results.

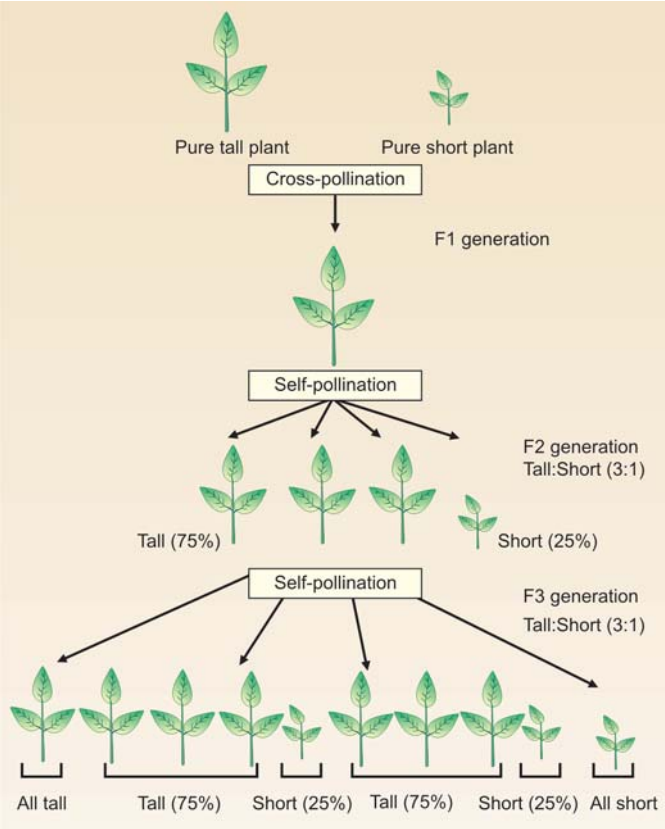

FIGURE 1.1: Cross between tall and short plants. Note the proportion of short and tall plants in each generation

TABLE 1.1: Contrasting characters in Mendel's experiments

Characters	Variety of the character	
Height of stem	short	tall
Shape of seed	round	wrinkled
Color of seed	gray	white
Color of pod	green	yellow
Form of pod	inflated	constricted
Position of flower	axial	terminal
Cotyledon color	yellow	green

Mendel was not only able to put forward the principles of heredity; he could also predict many of the outcomes of his experiments. He derived several conclusions related to the governance of hereditary traits that hold well till today.

1. Inheritable characters are transferred with the help of factors through generations. These factors were later identified as genes.
2. The heritable factors are transmitted through gametes (sperms and ova).
3. **The factors** for each character or trait **exist as a pair**.

   Each of the factors (genes, as we know them today) is responsible for a trait and is located at identical positions on each chromosome of a particular pair of chromosomes (homologous chromosomes). The fixed position on a particular chromosome for a definite gene is called the **locus** (plural loci) for the gene.

   The same positions (loci) on two homologous chromosomes contain genes responsible for the same character. For example the loci representing the height of a plant may contain genes responsible for tallness (T) or dwarfness (t) in different combinations as (TT, Tt or tt). The alternative form of genes, e.g. 'T' and 't' present at the same locus are called **allelomorphs** or **alleles**. The alleles define a particular character depending upon their dominance with respect to each other. Homologous chromosomes carrying identical alleles (same genes) are termed **homozygous**. A situation with different genes at the loci defining a particular trait is called **heterozygous (Fig. 1.2)**.
4. The members of the homologous pair of chromosomes separate from each other at the time of gametogenesis.

   Each of the gametes carries only a single chromosome out of the homologous pair. A single gamete will carry a single locus containing either a dominant or a recessive gene depending on the chromosomal constitution of the individual parent (Fig. 1.3).
5. Crossing between plants of a pure variety differing in a single pair of contrasting character yields only the dominant character in first generation (Fig. 1.4 and 1.5), whereas both the characters are expressed in second generation (Fig. 1.6).

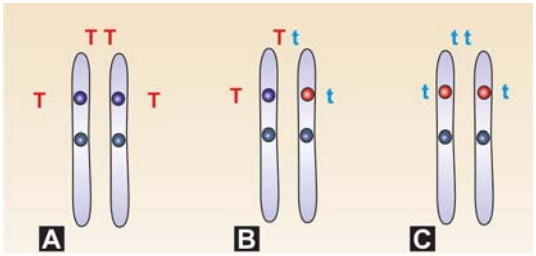

FIGURE 1.2: Homologous pair of chromosomes (A, B and C) showing various combinations of genes (TT, Tt and tt)

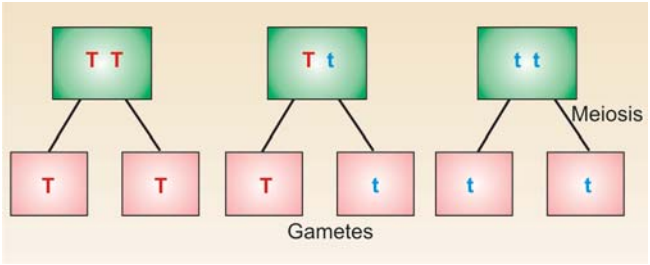

FIGURE 1.3: Diagram showing the separation of genes present in pair, during gamet formation

Punnett squares are grids that are extensively used to compute the genetic constitution of an individual by entering the constitution of the gametes on the top and side squares of the grid. Analyzing the above shown cross that is same as the first cross in Mendel's

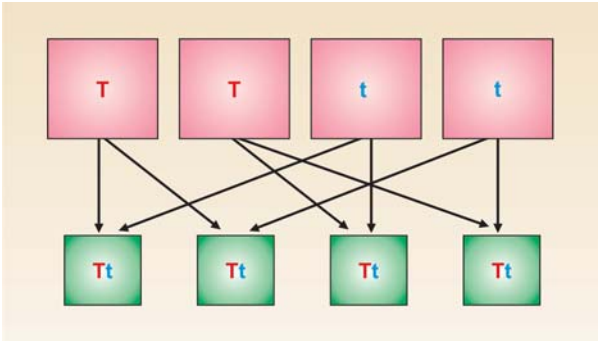

FIGURE 1.4: Genotype of F1 generation after crossing between pure short and pure tall plants. Phenotypically they were all tall due to the presence of dominant gene for tallness (T) in their genome

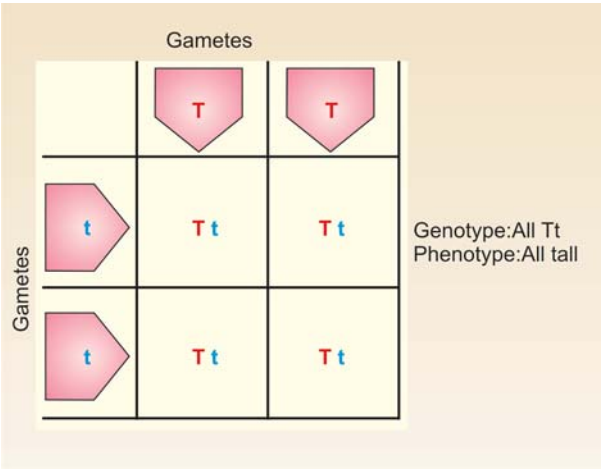

FIGURE 1.5: The Punnett' square showing offspring resulting due to mating between the pure tall and pure short plants

monohybrid cross experiment to yield **the first filial generation** in the Punnett square, looks like as below.

The **genotype** of an individual is defined as its genetic constitution for any particular trait. The term **phenotype** denotes the physical appearance for a particular trait. A gene is considered to be a **dominant** if it is able to express itself in the phenotype even when it is present in a heterozygous condition, e.g. the gene for tallness 'T' in (Tt). The gene for shortness't' is only expressed when it is present in a homozygous state (tt) in an individual and is called a **recessive** gene.

It is thus evident that all the progeny tall plants of the F1 generation (Tt) have different genotypes than the pure bred parent tall plants (TT) though they are same in their phenotypes.

Self-pollination of F1 plants can be analyzed in the F2 generation with the Punnett square as shown in Figure 1.6.

Self-pollination in the F1 generation yielded the F2 progeny. The F2 progeny constituted two varieties of phenotype with the reappearance of short stature in the plants. The genotype of the tall plants showed two varieties; the homozygous tall and the heterozygous tall plants. The concepts of dominance and recessive perspectives are also clear from the results in the grid squares. Characters are transmitted from one generation to next following statistical laws. When the plants of F1 generation were self-pollinated both tall and short plants appeared in the ratio of 3:1. When the plants of F2 generation were self-pollinated the tall and short plants always appeared in the fixed ratio (Figs 1.1 and 1.7).

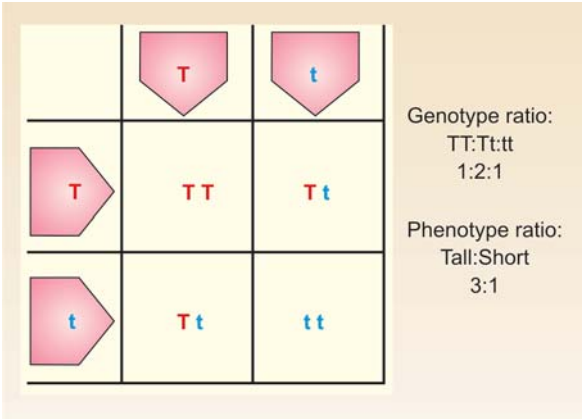

FIGURE 1.6: Offspring resulting due to self-pollination of F1 generation

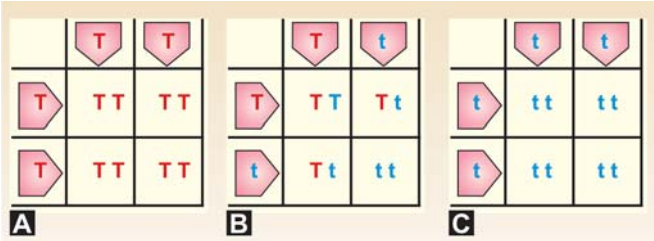

FIGURE 1.7: Offspring resulting due to self-pollination of F2 generation. In F3 generation we get all tall (A), tall and short (in ratio of 3:1) (B) and all short (C)

The results of the F3 generation in the Punnett square show the genotype and the phenotype of the individual progeny.

Dihybrid Crosses Experiments

For his dihybrid cross experiments Mendel selected two varieties of pea plants that differed in two pairs of contrasting characters. He selected, for example, pure variety of plants having yellow and rounded seeds and crossed them with another pure variety of plants having green and wrinkled seeds (dihybrid cross). These crosses were conducted to study the inheritance of a pair of contrasting characters with relation to the inheritance of the other coexisting pair of contrasting character in successive generations.

Mendel found that in F1 generation all plants were yellow with round seeds indicating that the yellow color and the round shape as were **dominant** over the green color and the wrinkled shape that were **recessive** in nature.

On self-pollination, the F1 plants yielded the F2 progeny. These offsprings were of four different phenotypes in the ratio 9:3:3:1 with 9 yellow and round, 3 yellow and wrinkled, 3 green and round and 1 green and wrinkled types of seeds.

It was thus observed that the two pairs of contrasting characters actually were transmitted independent of each other. The offsprings even demonstrated the new combination of characters in the form of yellow and wrinkled and green and round seeds in the F2 generation. The details of the experiments and their discussion are not discussed here.

Carl Correns, one of the rediscoverers of Mendel's work in 1900, promoted the ideas of Mendel as the "laws of inheritance". Following three concepts are recognized as Mendel's Laws.

MENDEL'S LAWS

It was earlier believed that traits or characters of parents become blended, diluted and lost in the offsprings of subsequent generations. Mendel's experiments have shown that these parental characters are determining by certain 'factors' (genes) and do not "mix" or "contaminate each other" and express in the progeny at a later stage (see Fig. 1.1). Mendel's first law of inheritance was based on this evidence.

The Law of Uniformity

Plants with two contrasting (one tall and the other short) characters when crossed, the characters do not blend. If any character is not expressed in the first generation it may reappear without change in a subsequent generation.

The Law of Segregation

An individual possesses two factors (genes) for a particular character with each of these factors situated on one of the chromosomes of a homologous pair. At the time of formation of gametes each member of the pair of chromosome separate independently from one another so that each gamete carries only one chromosome of the pair and as such only one of the two factors (gene) responsible for the determination of a character (see Fig. 1.3). In Mendel's words, neither of the factors has "*taken over anything from the other*". The genes of a pair are separated completely unaltered on a chromosome that migrates to a gamete during gametogenesis.

The Law of Independent Assortment

Members of different gene pairs (determining different sets of characters) that exist on the same chromosome, assort independent of each other during gametogenesis to migrate into a gamete. Because of such independent assortment new combinations between different sets of characters are produced in an offspring. For explanation refer to dihybrid cross.

The dihybrid cross experiments yielded four different phenotypes in the F2 generation with yellow-round, yellow-wrinkled, green-round and green-wrinkled seeds. This implied that the genes responsible for yellow and green colors and round and wrinkled shapes of seeds separated out independently that resulted in four different phenotypes and 9 different genotypes in the F2 generation.

SUMMARY

(a) Conclusions from hybridization experiments:

(i) The factors responsible for inheritance of character are basically the genes.

(ii) The factors or genes for each character occur in pair.

(iii) These genes are transmitted from one generation to next through gametes.

(iv) Members of a pair of genes separate from each other at the time of gametogenesis so that each gamete carries only one gene.

(v) Only one character (dominant) is expressed in first generation and both characters (dominant and recessive) are expressed in second generation when pure bred plants differing in pair of contrasting character is crossed.

(vi) Statistical laws are followed in transmission of characters.

(vii) Inheritance of one pair of factors is independent to other pair of factors in case of dihybrid cross experiments.

(b) ***Hybrid***

Offspring of cross (mating) between two genetically different organisms.

(c) ***Monohybrid crosses***

Cross (mating) between individuals or plants differing in a single pair of contrasting characters. Such cross yields monohybrids which are genetically heterozygous for the particular trait and factor.

(d) ***Dihybrid crosses***

Cross (mating) between individuals or plants differing in two pairs of contrasting characters.

(e) ***Locus***

The position of a gene on a chromosome is called locus.

(f) ***Allele***

Alternative form of a gene present at any particular locus.

(g) ***Homologous***

Chromosomes come in pairs in autosomes and as sex-chromosomes in the females. The members of the pair are identical to each other in their morphology. These chromosomes of a pair are called homologous.

(h) ***Homozygous***

A condition of having same allele at a given loci on homologous pair of chromosomes.

(i) ***Heterozygous***

A condition of having different alleles at a given loci on a homologous pair of chromosomes.

(j) ***Genotype***

The genetic constitution or makeup of an individual.

(k) ***Phenotype***

It is the physical, mental or biochemical manifestation of an individual in relation to a particular character resulting from the expression of associated genes. Phenotype may be influences by environmental factors.

(l) ***Dominant***

Is a trait that can express itself even in heterozygous state of a particular gene (single dose) e.g. tallness.

(m) ***Recessive***

It is a trait which is expressed only in homozygous condition (double dose) e.g. shortness of a gene.

(n) ***Mendel's laws of inheritance***

(i) The law of uniformity.
(ii) The law of segregation.
(iii) The law of independent assortment.

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Chapter

2 Chromosomes and their Classification

- Introduction to human chromosomes
- Classification of chromosomes and analysis
- Chromosomal analysis
- Sex chromatin
- Lyon's hypothesis

It was the beginning of the 20th century that the importance of Mendel's findings was beginning to get appreciated. This was due to simultaneous understanding of several aspects of cell division and the structure of the chromosome. The first account of mitosis was accounted by A Scheider in 1873 followed by W Flemming in 1879 who described the migration of individual chromosomes into the daughter cells after the detachment of the sister chromatids. Subsequently Benden showed haploid (half) number of chromosomes in the gametes and restoration of the diploid number of chromosomes in the somatic cells after fertilization. It was 1902 when Walter S Sutton and Theodore Boveri came up with the '**chromosome theory of heredity**' that claimed that Mendel's pair of 'hereditary factors', were in fact, physically located on the chromosomes. According to Mendel each trait was represented by a pair of factors. The presence or the absence of one or both the factors determined the expression of that particular trait in an individual. The emerging concepts of gametogenesis and fertilization further explained Mendel's observations, calculations and foresight.

INTRODUCTION TO HUMAN CHROMOSOMES

The human chromosomes are nuclear structures. They look like a net that is spread across the nucleus in a nondividing cell (interphase). The strands of the net are called chromatin and are chiefly made up of Deoxyribonucleic acid (DNA) and histone proteins; stained dark with basic dyes. Certain areas in the net look thick, coiled and condensed and are called **heterochromatin** whereas certain other areas resemble thin and lightly stained threads termed the **euchromatin**. The euchromatin is active during the functioning of the cell. The chromosomes get fully coiled and look like separate and individual thick rod like entities only during cell division. The chromatin net structure is restored once the cell has completed its mitotic or meiotic phases of cell division.

The Number of Human Chromosomes

- The number of chromosomes is always specific and constant for each species.
- In each of human somatic cell there are 46 chromosomes referred to as the diploid set and designated as **2n**.
- The 46 chromosomes can be grouped into 23 pairs of chromosome; each pair different from the other. The constituent partners in a group are very similar to each other.
- During the process of formation of sperm or ovum (gametogenesis), the number of chromosomes is reduced to half, i.e. to 23 or to haploid **(n)** state with one chromosome from each pair of the diploid set migrating to the gametes.
- With fertilization the haploid sets (n/23) of the sperm and the ova fuse to restore the diploid set (2n/46) in the first cell of the embryo.

Chromosomal Classification into Autosomes and Sex Chromosomes

The complement of 46 chromosomes in each human cell is classified into 44 autosomes (22 pairs) and 2 sex chromosomes (1 pair).

One member of each pair of autosomes and sex chromosome is contributed by either the father (paternal) or the mother (maternal).

The sex chromosomes are of two different types; the X and Y chromosomes.

The chromosomal constitution of the females of the human race is 44 autosomes and two X chromosomes (44 + XX), forming a homomorphic pair of sex chromosomes.

The chromosomal organization in human males is 44 autosomes and a pair of dissimilar sex chromosome, (44+ XY), i.e. one X and one Y chromosome, forming a heteromorphic pair of sex chromosomes.

The Y chromosome is always contributed by the male parent via its Y chromosome-containing-gamete.

Chromosomal Size and Shape

The chromosomes remain extended and uncoiled in the interphase stage of cell cycle with the length of the chromatin, if measured, extending a few meters. The chromosomes coil and condense maximally during the metaphase stage of cell division when the average size of the chromosomes is about 5 µm. It is during the cell division that we can, in fact, visualize individual chromosomes.

Chromosomes look like an entangled mesh of chromatin thread when in the interphase. Prior to the onset of cell division and progressive thickening of individual chromosome, each chromosome undergoes duplication of its DNA content and appears like two closely placed free strands attached together roughly near their waists. This event is called the phase of DNA replication. Subsequently during the later stages of cell division, as the chromosomes get condensed further; each of them looking like a thick rod (in metaphase) or like the letters J or V (in anaphase).

Chromosomal Structure

Each metaphase chromosome comprises of two identical components (after DNA replication). These two symmetrical halves are called sister **chromatids** and they are attached together at a constricted region that stains lightly and is called the **centromere**. The centromere defines the **primary constriction** of the chromosome and divides the chromosome into a **short arm (p)** and a **long arm (q)** (Fig. 2.1). **Centromeres** play a pivotal role during the movement of chromosomes during cell division.

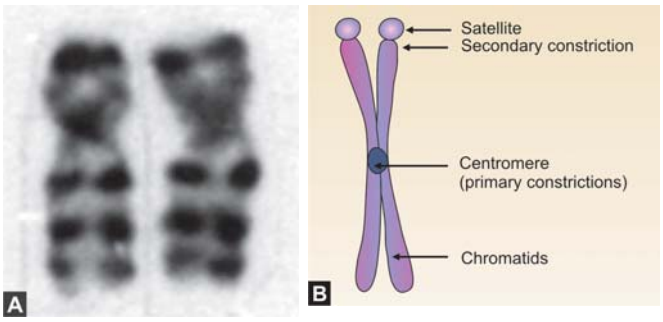


FIGURE 2.1A and B: Structure of a chromosome as seen under the microscope during metaphase. A. Photograph of a pair of homologus chromosomes; B. Schematic diagram

Certain chromosomes usually carry an additional **secondary constriction** in one or both the chromatids. These constrictions are linked to the formation of the nucleolus and hence referred to as the **nucleolar organizing region**. The secondary constriction may lie at the distal end of a chromatid giving rise to a small fragment of chromosome at the extreme end of the chromosome called the **satellite**.

The centromere (primary constriction) is situated anywhere along the length of the chromosome. The level of the constriction and consequently the lengths of the p and q arms are different for different chromosomes but specific for a particular chromosome. The location of the centromere, the length of the chromosome and the existence of satellites are taken as parameters to classify as well as to identify chromosomes.

CLASSIFICATION OF CHROMOSOMES AND ANALYSIS

Classification

Classifications are used to identify chromosomes.

- *Standard (Denver) classification:*
  Chromosomes are classified into **seven groups** in an arrangement in descending order of their lengths. Groups are designated alphabetically from **groups A to G**. The longer female sex

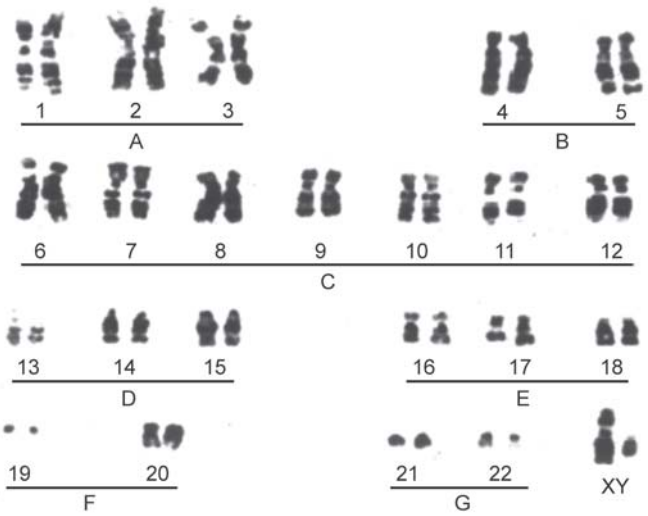


FIGURE 2.2: Photograph of karyotype. The chromosomes are arranged as per denvor classification. (*Courtesy* of Dr Dilip C Master, Professor of Anatomy and Head, Human Molecular and Cytogenetics, Medical College, Vadodara, India)

chromosome X is included in the group C and the smaller male sex chromosome Y is included in group G (Fig. 2.2).

- ***Classification based on the position of the centromere:***

  Metacentric: Centromere located near the middle of the chromosome; the length of **p = q**.

  Submetacentric: Centromere located slightly away from the middle; the length of **p < q**.

  Acrocentric: Centromere located very near to the end; the length of **p<< q**.

  Telocentric: Centromere located at one end of the chromosome; effectively having only a **single arm** (Fig. 2.3).

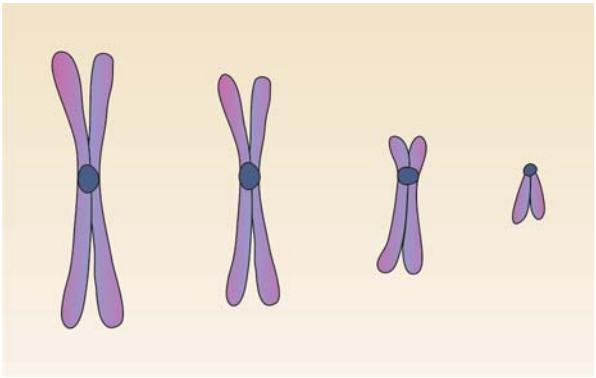


FIGURE 2.3: Classification of chromosomes. This classification is based on the position of the centromere

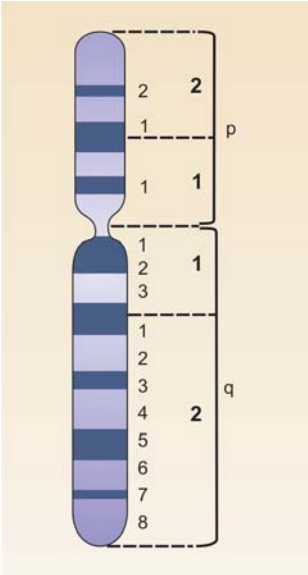


FIGURE 2.4: Diagram showing an X-chromosome with long (q) and short (p) arms. The chromosome is subdivided into regions and bands

- ***The Paris nomenclature:*** This classification entails banding techniques (special staining) and therefore is more accurate in identification of chromosomes. The arms of the chromosomes are divided into short segments and designated numbers 1, 2 and 3 beginning from the centromere and proceeding distally. Each of these small segments or regions is subdivided into *Z banded regions* (Fig. 2.4). Thus not only a particular chromosome and a segment in its arm can be accurately identified in this classification; small structural anomalies can also be detected within small regions in the segments.

CHROMOSOMAL ANALYSIS

Chromosomal analysis is an accurate tool to investigate several clinical conditions to arrive at a precise diagnosis. It may be indicated in cases of congenital malformation, mental retardation, repeated abortion, sex determination, prenatal diagnosis and other analytical purposes.

The chromosomal make-up of an individual is called as his or her **karyotype.** Karyotype is essentially a photomicrograph of an individual's chromosomes arranged according to the standard classification (Fig. 2.2). Diagrammatic representation of karyotype is called as **ideogram**. A karyotype is done to:

a. Identify and number the chromosomes
b. Detect numerical and structural anomalies of chromosomes.

Technique of Karyotyping (Chromosomal Preparation)

The procedure (Fig. 2.5) to obtain a karyotype of an individual is called Karyotyping. The metaphase chromosomes from somatic cell are prepared and photographed. Photographs of individual chromosomes are cut and arranged as per the Standard Classification.

Rapidly dividing cells are used to yield the chromosomes. The cells are usually obtained from sources like peripheral blood lymphocytes (most commonly used), skin fibroblasts, bone marrow cells, chorionic villi and amniotic fluid cells. The sequential steps followed are described below.

About 5 ml of venous blood is collected in a heparinized vial under sterile conditions and then the Lymphocytes are separated from the red cell population with the help of a centrifuge.

A culture vial is prepared that contains **culture media** and **fetal calf serum** for nourishment of the lymphocytes. **Phytohemagglutinin** in the vial stimulates cell division. Antibiotics are added to the medium to prevent infection.

The white cell suspension is then put in the culture vial.The vial is incubated for three days at 37° C.

Colchicin is added to the culture vial after 72 hours. Colchicin stops the formation of mitotic spindles and arrests cell division in metaphase. The chromosomes are maximally condensed and easily visible at this stage.

The dividing lymphocytes are separated off with a centrifuge 2 hours after the colchicin is added.

The cells are subsequently treated with hypotonic saline. This causes the cells to swell and become turgid.

The cells are then fixed by adding a mixture of glacial acetic acid and methanol.

When the cells get suspended in the fixative, they are dropped on chilled slides from a height. This causes the cell wall to disintegrate thereby allowing the chromosomes to spread in a limited area of cell rupture. This is called the **metaphase spread**.

These slides are stained and microphotographed. The karyotype of an individual is obtained after the images of chromosomes are cut from the photograph and arranged. Karyotypes of male and female sexes are shown in Figures 2.6 and 2.7 respectively.

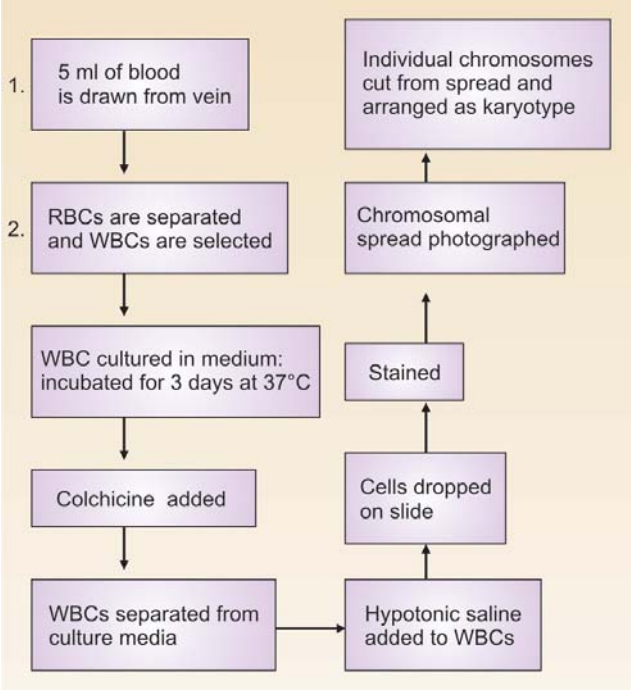


FIGURE 2.5: Flow chart of steps in the preparation of a karyotype

Banding of Chromosomes

Banding techniques allow precise analysis of chromosomes. Bands are obtained with the help of several staining methods.

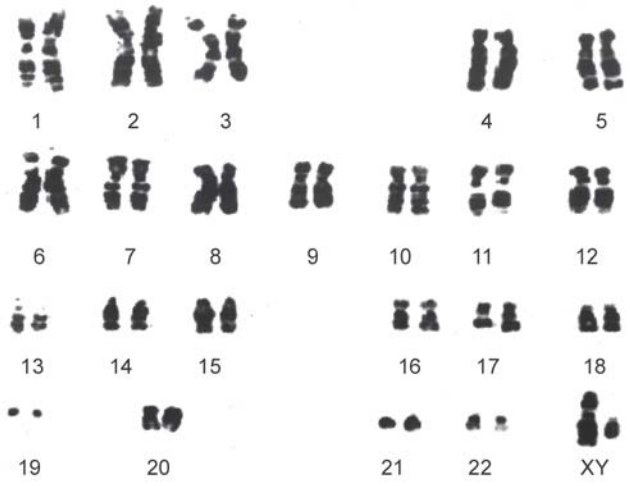


FIGURE 2.6: Male karyotype. (*Courtesy* of Dr Dilip C Master, Professor of Anatomy and Head, Human Molecular and Cytogenetics, Medical College, Vadodara, India)

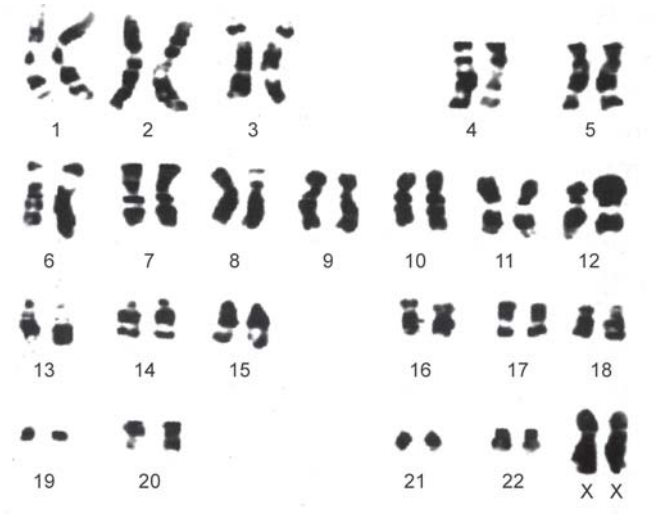


FIGURE 2.7: Female karyotype. (*Courtesy* of Dr Dilip C Master, Professor of Anatomy and Head, Human Molecular and Cytogenetics, Medical College, Vadodara, India)

G-banding

Unique pattern of light and dark bands are obtained on the chromosomes after treating the slides with trypsin that denatures the chromosome proteins and then staining the cells with Giemsa solution.

Q-banding

The method involves staining of chromosomes with quinacrine mustard. The pattern of banding is similar to the G-banding but the slides can only be visualized under ultraviolet fluorescent microscope.

R-banding

R-bandings are the reverse banding as seen in G-banding. The slides are preheated before staining with the Giemsa solution.

C-banding

Both the primary as well as the secondary constrictions are stained with this method.

Fluorescent *in situ* Hybridization (FISH)

This technique is based on the principle of DNA hybridization. A radiolabelled single stranded DNA probe is manufactured having a known and desired sequence of nucleotides. This probe gets annealed to the complementary target sequence on the interphase or metaphase chromosomes. These probes can be localized on a nitrocellulose filter by autoradiography. The technique is now widely used as it is accurate and rapid.

Various types of FISH available are:

Centromeric Probe

These probes are helpful for identification of chromosomes. Each chromosome has highly repetitive and specific DNA sequences in and around the centromere. The probes are designed to identify a particular chromosome accurately.

Chromosome Specific Unique Probe

These probes are designed to anneal onto very precise segments of the chromosome that bears unique sequences of DNA. They are useful even to detect submicroscopic deletions or duplication.

Whole Chromosome Paint Probe

Entire chromosomes are visualized with this technique.

Multicolor Spectral Karyotyping

This technique allows observing all the chromosomes simultaneously. A multicolor spread is obtained after all the chromosomes are painted or fluoresced to get a multicolor karyotype. Spectral Karyotyping (SKY) detects chromosomal deletions and translocations (Fig. 2.8).

SEX CHROMATIN

Interphase nuclei in the female exhibits a dark stained mass of heterochromatin just beneath the nuclear membrane (Fig. 2.9). This mass of chromatin material is called the **sex chromatin** or **Barr body**. Sex chromatin is observed only in females and is absent in males. It is thus a tool for determination of sex in humans.

The identification of Barr body can be done rapidly after isolating epithelial cells from the skin, vagina and oral cavity or from blood cells. Typically, the buccal mucosa is scraped and put on a slide and evenly spread. The cells are then fixed in alcohol. The slides are observed under high magnification after staining

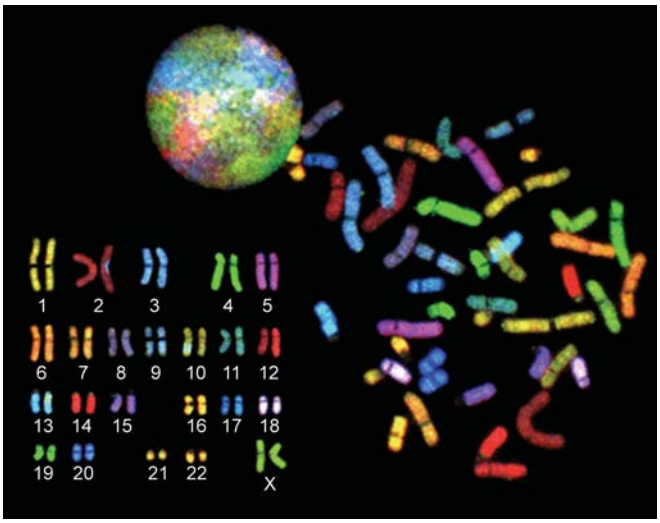


FIGURE 2.8: Spectral karyotyping (SKY) of a normal female (*Courtesy* of National Human Genome Research Institute, NIH, Bethesda, USA)

with any basic dye. Chromatin positive cells usually denote a female sex.

Human female polymorphonuclear white cells also show a small **drumstick** like structure at one end of the nucleus (Fig. 2.9)**.** This drumstick body is absent in males. The Barr body technique is not a very suitable method for determination of sex. Karyotyping is a more acceptable and accurate method for the purpose.

Barr Body and the Drumstick are Features of the Female Nuclei

The distinct relation between sex chromatin and sex chromosomes was worked out by Ohano, Kaplan and Kinosita in 1959. They observed that the sex chromatin was derived from one of the two X chromosomes in females. In females one of the X chromosomes became the condensed and inactive heterochromatin (Barr body) whereas the other turned into euchromatin, active in cellular metabolism.

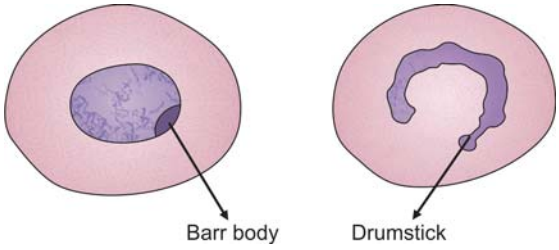


FIGURE 2.9: Two different cells from a female showing Barr body in a squamous cell and a drumstick in a polymorphonuclear cell

LYON'S HYPOTHESIS

The process of inactivation of one X chromosome is called Lyonization after Mary F Lyon. In 1962 she demonstrated that during the early stages of embryogenesis at about 15th or 16th day of development, one of the X chromosomes convert into a coiled and inactive heterochromatin structure; the Barr body.

Features of Lyonization

- One of the two X chromosomes becomes inactive.
- The inactivation occurs at about 5000 cell stage in early embryonic life.
- The X chromosome in a female cell is randomly selected for inactivation. Thus in some cells the maternally derived X chromosomes are inactivated whereas in the rest the inactivated X chromosomes are of paternal origin.
- Therefore the cell populations in a female represents a mosaic pattern with respect to having a cluster of cells with active paternally derived X chromosomal genes and also a set of active X chromosomal genes of maternal origin, in the same individual.
- During cell division the Barr body uncoils and participates in the division and shows late replication.
- After cell division the same X chromosome gets inactivated again. This pattern continues in all subsequent cell divisions.
- Barr bodies may number more than one but the number of Barr bodies is always one less than the total number of X chromosomes in the cell.

Thus Barr bodies in the following situations are as follows:

Normal male (XY) exhibit no Barr body, normal female (XX)—One Barr body, Turner syndrome (X0) no Barr body, klinefelter syndrome (XXY)—One Barr body and triple X syndrome (XXX)—two Barr bodies.

Thus at any time point a somatic cell contains only a single active X chromosome and the other X chromosome (s), if present, shows up as Barr body/ bodies.

The Importance of X Inactivation

At its onset embryogenesis in the females requires active participation of both the X chromosomes. Thereafter one of the X chromosomes is randomly inactivated in subsequent course of development. The presence of only a single active X chromosome in either a male or a female cell is sufficient to maintain the protein levels expressed by the genes on the X chromosome. The presence of an extra active X chromosome causes the dose of the gene products to be double and are eventually deleterious or fatal. Nature has thus evolved a mechanism of inactivation of an X-chromosome for the regulation of dose of its genes. This mechanism is called **dosage compensation**.

The Y chromosomes never form Barr bodies though at times they may be more than one in number in certain abnormal situations. This is because the Y chromosome has very few genes and has very negligible influence on the phenotype. Hence the Y chromosomes are not subjected to dosage compensation.

SUMMARY

(a) The human chromosomes are 46 in number comprising 22 pairs of autosomes and a pair of female (XX) and male (XY) sex chromosomes.

(b) Chromosomes are visualized during cell division. Metaphase chromosomes are thick rod like. The centromere forms the primary constriction. Chromosomes are classified according to the location of the centromere into metacentric, sub-metacentric, acrocentric and telocentric.

(c) Karyotype denotes the chromosomal make-up of an individual.

(d) Chromosomal spreads are prepared by arresting cell division in metaphase. Special staining is used for banding the segments of chromosomes in a pattern to identify and detect structural alterations in chromosomes.

(e) Specific and precise detection of microdeletions, translocation and identification is done by using modern techniques like SKY and FISH.

(f) Barr body is formed by the inactivation of one X chromosome if they are more than one in number in a cell. Barr bodies and drumsticks are used to determine the sex of an individual.

Chapter

3 Structure of DNA and RNA

- Structure and packaging of DNA
- Replication of DNA
- Mitochondrial DNA
- Structure of Ribonucleic acid (RNA)

We have discussed in the previous chapters that the factors which determine heritable characters of an individual are the genes. The genes form the units of heredity and are transmitted from one generation to the next being physically located on chromosomes. The chromosomes are principally constituted of nucleic acids and are structurally supported by protein molecules. However, it is only the nucleic acids that build-up the genes and are linked to the transmission of a trait.

There are two types of nucleic acids.

(a) Deoxyribonucleic acid (DNA): The bulk of the cellular DNA is found in the nucleus as chromosomes. Circular strands of DNA are also found in the mitochondria. Human genes are made up only of DNA.

(b) Ribonucleic acid (RNA): RNA is largely found in the nucleolus within the nucleus. Other locations where RNA is found are the ribosomes and the cytoplasm. RNAs work as functional intermediaries between genes and their final products, the proteins.

STRUCTURE AND PACKAGING OF DNA

A. Structure of DNA (Chemical):
Three different types of chemical compounds compose the DNA.

- *Sugar molecule*—It is called deoxyribose and is a 5 carbon pentose sugar (Fig. 3.1).
- *Phosphoric acid* (Fig. 3.2).
- *Nitrogenous bases* (Fig. 3.3).

These are of 4 types:
Adenine - (A)
Thymine - (T)
Cytosine - (C)
Guanine - (G)

Adenine and Guanine are classified as Purines while Cytosine and Thymine as Pyramidines.

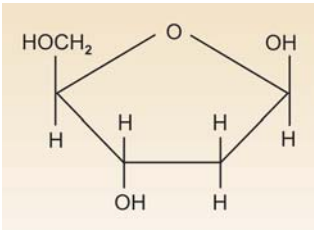


FIGURE 3.1: Deoxyribose molecule

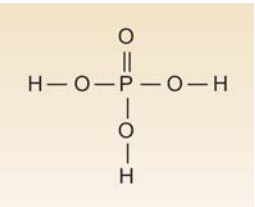


FIGURE 3.2: Phosphoric acid

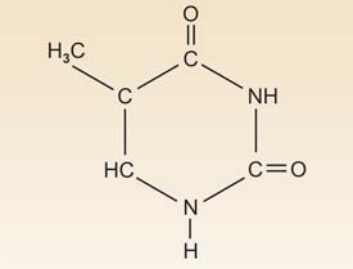
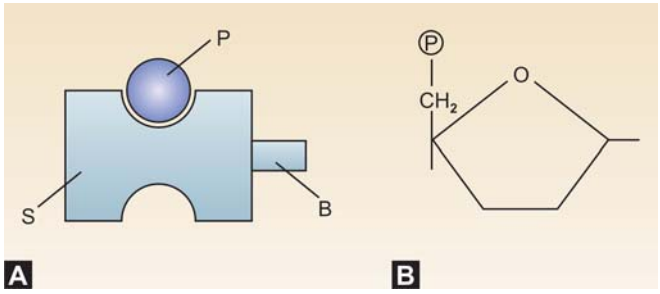


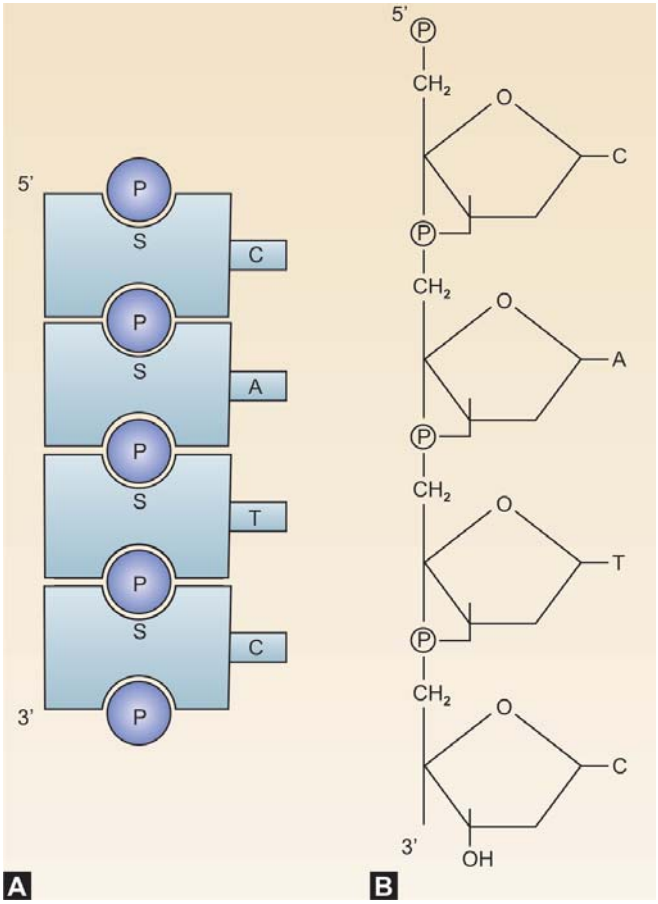
FIGURE 3.3: Molecular structure of thymine

B. ***Structure of DNA (Molecular):***

- DNA exists in the form of a long polymer which is formed by linkage of a series of nucleotide molecules like in a chain.
- A nucleotide molecule is formed of one molecule of deoxyribose sugar, one molecule of phosphoric acid and one nitrogenous base attached on the sides of the deoxyribose. As there are four varieties of nitrogenous bases, there are four types of nucleotides in the DNA.
- The phosphate molecule in a nucleotide is attached to the fifth carbon atom of the sugar (deoxyribose) and the nitrogenous base to the first carbon atom of the sugar molecule (Figs 3.4A and B).
- The third carbon atom of the deoxyribose of the next nucleotide is attached to the phosphate molecule of a nucleotide. Hence, the sugar and the phosphate molecules are arranged in a linear fashion to form a polynucleotide chain. The nitrogenous base attached to sugar molecule is directed at right angle to the long axis of a single polynucleotide chain (Figs 3.5A and B).
- All polynucleotide chains have marked ends. It can be noticed (Figs 3.5A and B) that at the upper end of the chain the 5th carbon atom of the sugar molecule of the last nucleotide just terminates in a phosphate. This end is called as 5′ or 5′ P terminus.
- The other end of the chain ends in sugar molecule or a nucleotide whose 3rd carbon atom is free and not linked to the phosphate of any nucleotide and bears an OH group (hydroxyl group) instead. This end of polynucleotide chain is called 3′ end or 3′ OH terminus.
- Watson and Crick in 1953 worked out the DNA helix model as been made up of two such polynucleotide chains which lie side by side but run in opposite directions (antiparallel). One chain runs from its 5′-3′ direction whereas the other in 3′ - 5′ direction (Fig. 3.6).



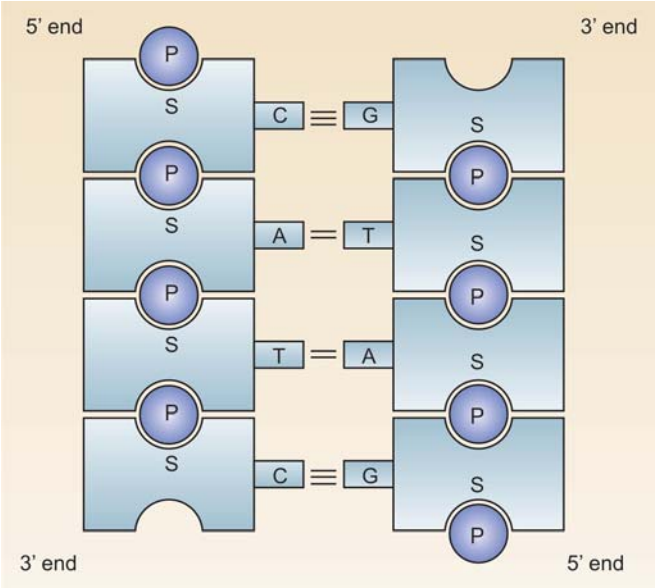
FIGURES 3.4A and B: Schematic diagram (A) of a molecule of nucleotide (P = Phosphoric acid, S = Sugar and B = Nitrogenous base), (B) = Structural diagram of nucleotide



FIGURES 3.5A and B: The schematic diagram of a part of polynucleotide chain (A) Structural diagram (B) C = Cytosine, A = Adenine and T = Thymine

The nitrogen bases face towards the inside of the skeleton formed by the two strands of the nucleotide chain.

- The 3′ end of the DNA strand is called the "head" end and the 5′end its "tail".
- The two chains are held together by two types of hydrogen bonds between the nitrogenous bases (Fig. 3.6).



FIGURES 3.6: Schematic diagram of antiparallel polypeptide chains showing sugar-phosphate backbone and nitrogenous base pairing

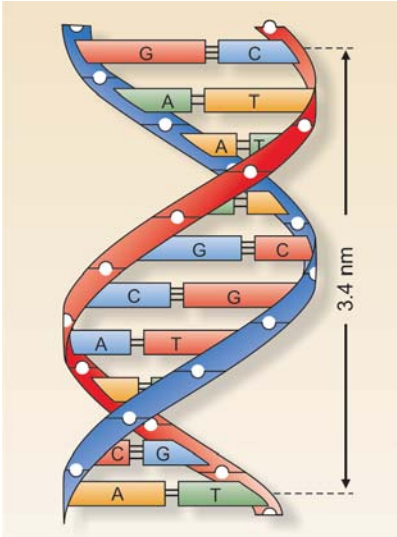


FIGURE 3.7: The DNA double helix. Note that one complete turn of helix measures about 3.4 nm and contains 10 pairs of nucleotides

- Pairing between two nitrogenous bases is predetermined and constant, i.e. Adenine (A) always pairs with Thymine (T) and Cytosine (C) with Guanine (G). This specific pairing is due to the fact that these molecules are complementary and the combination of the specific bases facilitates stable hydrogen bonds between them. Nucleotides A and T share two hydrogen bonds while C and G are joined by three bonds.
- As a consequence of specific base pairing, two strands of DNA are complementary to each other. It means that if the sequence of bases on one chain is A T G C A, then correspondingly, the exactly opposite region on other chain will have the sequence T A C G T that can thus anneal together.
- The **double helix** of a DNA molecule is formed as the two complementary chains (polynucleotide chains) twist around each other.

A single and complete 360° turn of the helix measures about 3.4 nm along the long axis and contains 10 pairs of nucleotides. The distance between two adjacent nucleotides is 0.34 nm. The diameter of helix is about 2 nm (Fig. 3.7)

One turn of helix measure about 3.4 nm and contains 10 pairs of nucleotides.

Packaging of DNA in a Chromosome

A chromosome is composed of a double helix of DNA and histone proteins. The average length of the DNA filament of a single chromosome can extend upto 50 mm but the chromosome is only 5 microns in length when maximally condensed in the metaphase. Thus there is about 10,000 times reduction in length. This is due to the fact that in a metaphase chromosome filament of DNA undergoes several orders of coiling (Fig. 3.8) or condensation.

- The primary or first order coiling is due to turning of the DNA double helix on itself.
- These primarily coiled DNA double helix then wind around histone complexes (histone beads). This secondary coiling of DNA filaments around histone beads forms structures called **nucleosomes**. The DNA filaments wind twice around each histone bead and contain approximately 146 nucleotide pairs. Nucleosomes are attached to one another forming long chains.
- The nucleosomes arrange in a spiral to form a closely stacked thick structure; the **chromatin filament**.
- Chromatin filament coil again to form chromatin loops.

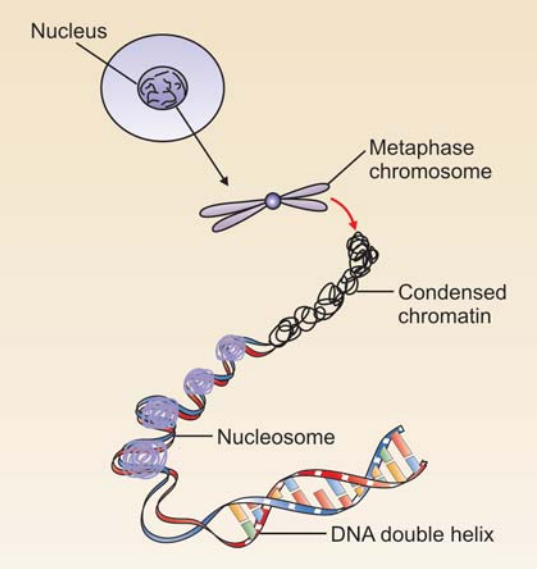
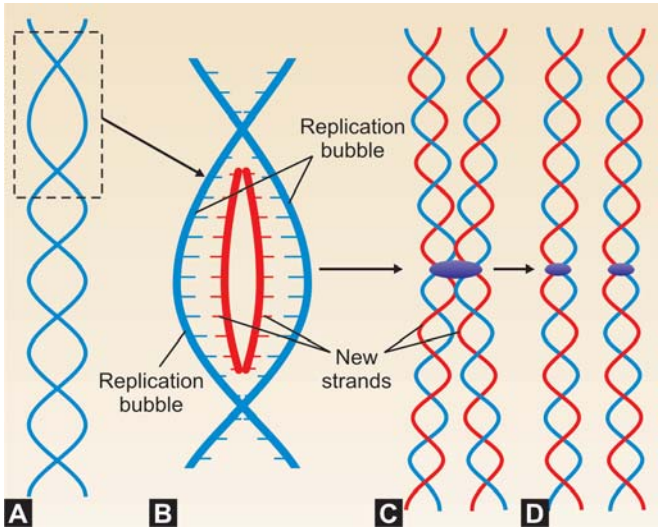


FIGURE 3.8: DNA packs tightly into metaphase chromosomes



FIGURES 3.9A to D: The process of replication. The figure (B) is enlarged view of box in Fig. (A). The sister chromatids (C) splits to form two individual chromosomes (D). Each newly formed chromosome now consists of an old and a new strand

- Additional coiling of the loops on themselves to give the shape of a chromosome as visible during the metaphase of cell division.

These successive degrees of coiling gives rise to the **solenoid model** of chromosomal structure.

On straightening a strand of DNA taken from a typical human chromosome, it measures about 5 cm in length. It may have about half a billion to 3 billion nucleotides. If we arrange all the molecules of DNA present in the 46 human chromosomes end-to-end, they would measure about 2 meters or 6 ft in length. Human body consists of approximately 10^{14} cells and if all the DNA of an individual is joined end-to-end, the total length of DNA would measure approximately 2×10^{14} m or 2×10^{11} km. This length would be good enough to go from the earth to the sun and back for about 500 times.

REPLICATION OF DNA

Nondividing cells remain in the interphase stage of the cell cycle. Cell division begins with the doubling or duplication of the DNA content of each chromosome. This event of DNA replication is also called the synthetic phase and results in the formation of two sister chromatids in each chromosome. DNA replication is followed by the prophase, metaphase, anaphase and telophases of mitosis or meiosis that include distribution of chromosomes and cytoplasm to the daughter cells. The double helix model of Watson and Crick ideally explains the events during replication.

- The tightly coiled DNA filament gets uncoiled during S (synthesis) phase of cell division. The two strands of DNA molecules are separated (denatured) by specific enzymes on breaking the hydrogen bonds between nitrogenous bases (Figs 3.9A to D). The two separated strands of polynucleotide chains are complementary to each other.
- **Origin of replication (ori)** are sites along a DNA strand at which replications begin. The double stranded DNA gets denatured at these sites and the replication begins from the 3′ to the 5′ direction on both the strands. Replication begins on both the strands but in opposite directions. Due to replication, bubble-shaped structures pop up long the chromosome at multiple points simultaneously, called **replication bubbles**.
- The human genome doubles in approximately 9 hours in a cell with about 100 bubbles being active in each chromosome, each bubble apart by about 40000 nucleotide pairs.
- The region in each bubble at which parental DNA strand is progressively separated with the help

of enzymes looks like the alphabet Y. The stem of the Y is formed by the double stranded DNA whereas the two arms of the Y are made-up of the denatured single strands. This region of on the chromosome is called the **replication fork**.

The total replication time is reduced as each chromosome replicates by many thousands origin sites.

At each replication fork about 10 to 100 nucleotide pairs are added per second. A chromosome usually takes 15 to 30 minutes to replicate. Because all the chromosomes of a cell do not replicate simultaneously, complete replication of all chromosomes of a cell takes 8 to 10 hours.

- As specified by the rules of base pairing, each nucleotide of an old chain attracts its complementary nucleotide that attach through hydrogen bonds with their complementary nucleotides on the old chain (Fig. 3.9).

The growing end of a replicating new DNA strand elongate with the addition of one nucleotide at a time.

- The phosphate components link the sugar radicals of neighboring nucleotides to each other. Thus a new chain is formed opposite to the old polynucleotide chain. The new chain grows only at its 3'end.
- *The genetic information is conserved and transmitted unchanged to each daughter cell* as the new strand is identical to the old template strand.
- As the newly synthesized DNA double helix contains an original or old strand (that is said to be conserved as it comes from the parent) and a newly constituted complementary strand, this method of DNA replication is described as **semiconservative** (Fig. 3.9).

MITOCHONDRIAL DNA

In addition to the nucleus, the mitochondria also contain DNA. Mitochondrial DNA, similar to the nuclear DNA, is double-stranded but arranged as circular structures. It consists of about 16.6 kb nucleotide base pairs and codes for 37 genes with 22 genes for tRNAs, 2 for rRNA and 13 genes for enzymes responsible for oxidative phosphorylation.

Oxidative phosphorylation enzymes are involved in energy production. Therefore mitochondrial abnormalities are associated with the loss of coupling between oxidation and phosphorylation. Presentations of mitochondrial disorders are variable because of the phenomenon of *heteroplasmy* (see Chapter 6). The characteristics and examples of mitochondrial disorders are given in Chapter 6.

Mitochondria of sperm are not transmitted into the oocyte during fertilization and the entire mitochondrial complement in the zygote is derived exclusively from the mother. Thus mitochondrial DNA abnormalities are transmitted only through females and follow maternal pattern of inheritance. Both sexes are equally affected (see Chapter 6).

mtDNA act as excellent genetic markers for tracing human ancestry as they do not undergo genetic recombinations during gametogenesis, similar to what happens with the Y-chromosomes. It is established that about 1 change per mitochondria lineage occurs in every 3800 years at a constant rate. This fact helps us to estimate that modern human population originated somewhere in the Sub-Saharan Africa approximately 130,000 years ago and migrated to various parts of the world. They first moved out of Africa to the Middle-East about 100,000 years ago and from there to the east and south Asia (67,000 years ago). The journey continued to Australia and to Europe about 40,000 years ago. From East Asia migration went on further to North America (about 20,000 years back) and from there to South America about 13,000 years ago.

STRUCTURE OF RIBONUCLEIC ACID (RNA)

Both the nucleolus and cytoplasm contain RNA molecules. RNAs work as functional intermediaries between genes and their final products, the proteins. RNA is not concerned with inheritance in human beings. It is synthesized by reading DNA template molecules with the help of ribosomes.

There are three types of RNAs.

(a) Messenger RNA (mRNA)
(b) Ribosomal RNA (rRNA)
(c) Transfer RNA (tRNA).

Messenger RNA (mRNA)

The nucleus is the site for messenger RNA (mRNA) synthesis. It is single stranded product of transcription.

mRNA is formed at transcription bubbles with arrangement of nucleotides on the template strand that is read from its 3′ to 5′ end. mRNA itself, though, is synthesized from its 5′ to the 3′ end. It thus carries all the genetic information present on a particular segment of the DNA strand in the form of sequence of base arrangements. However, there is no thymine in mRNA and has a uracil molecule instead. Several hundred to several thousand nucleotides arranged in a single strand compose a messenger RNA molecule. mRNA comes out through nuclear pores into the cytoplasm after its formation in the nucleus. Soon it gets attached to ribosomes outside the nuclear envelope. The protein synthesizing apparatus of the cell utilizes the genetic information on the mRNA for translation of proteins. mRNA population constitutes about 10% of the total RNA present in a cell. The life span of mRNA varies from few hours to few days.

Ribosomal RNA (rRNA)

About 80% of the total RNA present in the cell is contributed by rRNA. As implied, rRNAs occur in ribosomes. The part of the DNA which codes for rRNA is associated with formation of the nucleolus and is called the nucleolar organizer. DNA loops of chromosomes 13, 14, 15, 21 and 22 contain genes for ribosomal RNA and constitute the nucleolus. rRNA is produced inside the nucleus.

Two subunits, a large and a small, make-up the ribosome. The rRNA molecule occurs as three different dimensions; the 28s, 18s and 5s units. The large ribosome subunit contains the 28s and 5s molecules. The 18s molecules are present in small ribosomal subunits.

Ribosomal RNAs in the ribosome initiate as well as maintain the process of protein formation (translation) by interaction with the mRNA strands as they pass through the ribosomes.

Transfer RNA (tRNA)

Consisting of about 75 to 80 nucleotides, a tRNA molecule is single stranded and is synthesized at particular regions of the genome. The tRNA molecule is bent in the middle of the polynucleotide chain and forms two arms on its sides named **clover leaf model** for obvious similarity with the structure.

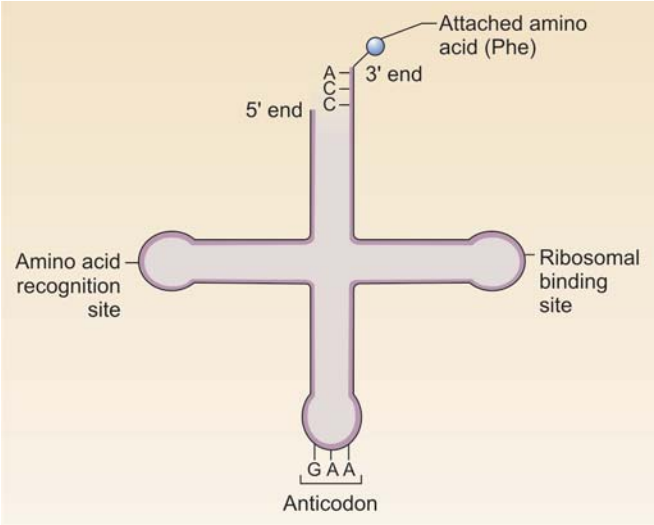


FIGURE 3.10: Clover leaf model of tRNA

A specific amino acid is designated to each tRNA molecule and hence 20 types of tRNA exist in the cytoplasm. A tRNA with its amino acid (amino-acyl tRNA) is transported to the ribosome where it docks and pairs on the mRNA molecule after being correctly recognized for such a base pairing. Protein synthesis and chain elongation occur with the sequential assembly of the amino acids by the tRNA on the mRNA molecule. Four different special sites are present in the tRNA molecule (Fig. 3.10).

- *Recognition site*—Recognizes the appropriate amino acids to be attached with the help of specific amino- acid sequences.
- *Codon recognition site*—A 3 base sequence site that is complementary to a sequence of three bases (codon) on the mRNA molecule. Base pairing between tRNA and mRNA happens at these sites after the tRNA molecule lands on the mRNA.
- *Amino acid attachment site*—This sites attach specific amino acids after their correct identification.
- *Ribosomal recognition site*—This site facilitates tRNA to recognize their specific positions inside the ribosome.

Following are the differences between DNA and RNA molecules.

TABLE 3.1: Differences between DNA and RNA molecules

DNA	*RNA*
DNA is present in chromosomes.	RNA is present in nucleolus and cytoplasm.
The sugar molecule in DNA is deoxyribose.	The sugar molecule in RNA is ribose.
DNA is a double-stranded, helical structure formed by nucleotides arranged in a linear sequence.	RNA is single stranded which is formed by nucleotides arranged in a linear sequence.
Four nitrogenous bases are found in DNA (A, T, C and G).	It also contains 4 bases but thymine is replaced by uracil (A, U, C and G).
DNA is the hereditary material and information for life process are encoded in DNA molecules	It is nonhereditary in nature and helps in protein synthesis.

SUMMARY

(a) DNA

(i) Eukaryotic genes are composed of DNA molecules and are responsible for inheritance of characters. DNA is present in nucleus (chromosomes) and mitochondria.

(ii) DNA is in the form of a long sequence that is formed by adding up of nucleotide molecules as in a chain. (A nucleotide molecule itself is formed of a single molecule of deoxyribose sugar, a single molecule of phosphate and single nitrogenous base).

(iii) The DNA molecule is made up of two highly coiled and condensed polynucleotide chains (double helix) which lie side by side but runs in opposite directions (antiparallel).

(iv) There is strict and definite pattern of pairing between the bases of the two parallel running DNA strands.

(v) During cell division chromosomes (and the DNA) duplicate themselves by the process of replication.

(vi) The process of replication generates a new strand of DNA (semiconservative) against each old and complementary template strand.

(b) RNA

(i) RNA does not constitute eukaryotic genes and therefore is not a hereditary material.

(ii) RNA is abundant in the nucleolus as well as in the cytoplasm.

(iii) The sugar molecule in RNA is ribose and nitrogenous bases are A, G, C and U.

(iv) There are three different types of RNAs (mRNA, rRNA and tRNA) which play an important role in synthesis of proteins.

Chapter

4 Structure and Function of Genes

- Molecular structure of genes
- Genetic code
- Transcription
- Alternative splicing
- Translation
- Gene expression and its regulation
- Genetic mutation, its types and Mutagens
- Mechanism of DNA repair

It is estimated that there are about 30,000 genes located on 23 human chromosomes (as per Human Genome Project, 2001). Genes are arranged in a single linear order in the chromosome similar to arrangement of beads on a string. Genes are responsible for the determination of inheritable characters as well as characters that arise fresh in an individual *(de novo)* due to alteration in the structure or the function of a gene. Two different kinds of genes exist in chromosomes, i.e., the **structural genes** and the **control genes (regulatory genes)**. Function of structural genes is to synthesize specific proteins molecules, whilst the control genes regulate the synthesis and activity of structural genes. The function of regulatory genes is to promote or to inhibit the steps of transcription of a structural gene and later its translation into protein.

MOLECULAR STRUCTURE OF GENES

DNA molecules are the main constituents of a gene. A structural gene can be defined as "*a segment of DNA which contains the information (code) for synthesis of one complete and functional polypeptide chain (or an enzyme)*." Thus genes are nothing but blue prints or directories for protein synthesis.

The sequences of DNA in a structural gene should logically exist, and as was actually found, in a contiguous arrangement one after the other similar to the sequence for amino acids one after the other as they are situated in a polypeptide chain. It was further observed that there were many noncoding sequences which are called "**introns**" interposed, in addition to and in between the coding sequences termed "**exons**". The number of introns in various genes is variable and sometimes it may so happen that introns may exist in more numbers than exons (coding sequences). Though introns are transcribed (vide infra), they are not included in mature mRNAs for translation in the ribosome.

The makeup of a structural gene not only contains the sequences of exons and introns but also possesses certain **flanking regions** at their ends. These flanking regions are important for regulation of gene expression (Fig. 4.1).

The sequence of DNA that is transcribed into a single mRNA starting at a promoter and ending at a terminator is called **a transcription unit** of the DNA.

Sequences which control transcription constitute the flanking region at 5′ end of a gene. This region is called the promoter region and contains "TATA" box and "CAT" box. TATA boxes are stretches of DNA within promoter regions that contain repetitive base pairings between Adenine and Thiamine. The presence of TATA box is essential for transcription initiation. Downstream into the gene after the promoter region there is code for initiation of transcription or the ***start points*** of transcription. The sequences of start points are followed by the first codon representing an amino acid and this codon is always the same irrespective of the gene and codes for the amino acid methionine (ATG). This codon is followed by subsequent sequences of exons and introns of the gene. The 3′end of the gene bears any of the UAA, UGA or UAG codons. These terminal codons or stop codons are transcribed onto the mRNA and are essential for terminating the polypeptide chain synthesis during the process of translation at a later stage.

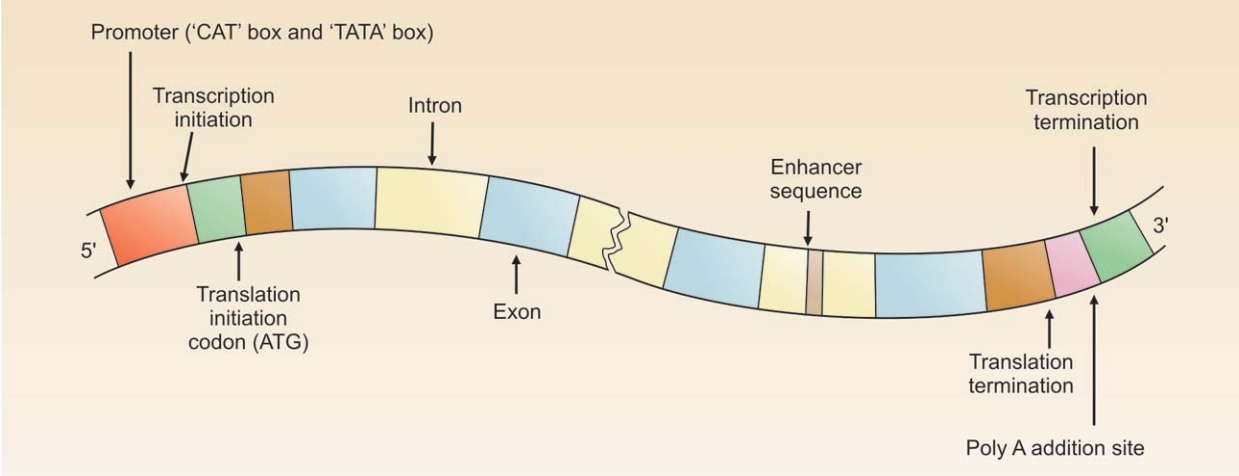


FIGURE 4.1: Structure of a gene. (*Courtesy* of Prof Inderbir Singh, Human Embryology, 8th Edition, MacMillan)

The TATA box consists of GGGCGGG sequences and CAT box CCAAT. These regions are indispensible for initiation of transcription as they bind to the transcription factors. At 3′ end of a gene, the flanking region consists of translation termination codon (TAA) which is followed by poly (A) cap codon (see transcription later). The DNA transcription starts at 5′ end and ends at 3′ end of the coding strand or the sense strand of the gene. For initiation of transcription it is necessary that the promoter region should bind to the enzyme ***RNA polymerase***. However, in order to bind to this site the polymerase requires additional proteins called ***transcription factors***. The transcription factors and related proteins bind to specific promoter regions and activate gene expression.

Some details of DNA Sequences

Both the nucleus and mitochondria contain DNA molecules. The count of total number of human genes is estimated at 30,000 genes implying that only a minor percentage of chromosomal DNA constitutes functional genes. The rest of vast amount of DNA sequences are transcriptionally inactive and called **Junk DNA** having unknown functions. Several repetitive DNA sequences make up the junk DNA. DNA is divided into two classes, the **genic DNA** and **extragenic DNA**, for convenience of understanding.

Genic DNA

- Functional genes are usually present below the telomere with varying distribution of genes in different chromosomes, e.g. number 19 and 22 are gene rich while chromosome number 4 and 18 contain very few genes.
- Genes are small (single exon) or very large (79 exons). A single exon may contain many nucleotide base pairs.
- Most of human genes are single (single-copy genes that code most of the hormones, receptors, structural and regulatory proteins).
- In situations there may be more than one gene for the same function, e.g. many Alpha-globin genes are present on chromosome number 16 and many Beta globin genes are present in groups on chromosome number 11. Ribosomal RNA genes present on the short arms of various acrocentric chromosomes represent multiple genes for same functional output included in **multigene families** produced by *gene duplication*.

Extragenic DNA

These represent repetitive DNA sequences that are not transcribed (nongenic or extragenic) and called the Junk DNA. Their functions are not yet defined and may be of profound significance. The **tandemly repeated DNA sequences** and **interspersed repetitive DNA sequences** are two varieties of the DNA.

Tandemly Repeated Sequences are noncoding and can be found as **satellite DNA**, **minisatellite DNA** and **microsatellite DNA**. The satellite DNA is present near the centromere. **The minisatellite DNA** mainly consists of telomeric DNA of TTAGGG sequences that are 3 to 20 kilo-bases in length that

protects the ends of chromosomes. The hypervariability of these tandem repeats of sequences forms the basis of **DNA finger- printing** (Chapter 18). The microsatellite DNA is formed by tandem repeats of a few (one to four) base pair sequences. These repeat base pair sequences are present throughout the genome.

Though the functions of these stretches of DNA are not clear, the hypervariability of minisatellite DNA forms the basis of finger printing. The telomeric minisatellite DNA plays a role in the stability of chromosomes and is lost with each cell division resulting in the senility and programmed death of the cell.

GENETIC CODE

Basically the genes are the blueprints or directories that instruct the synthesis of proteins. Proteins are made up of polypeptide chains which in turn are made up of amino acids. The amino acids are supposed to be linked together in a particular sequence in a polypeptide chain to be effective.

The numbers, types and arrangement of amino acids in a protein molecule determines the structure and function of that protein. Proteins are made up of various combinations of 20 amino acids.

A sequence of three bases on a DNA strand codes for one amino acid. There are four different types of nitrogenous bases in DNA (A, C, T and G).

In a situation if a single base codes for one amino acid, 4 bases would code for just 4 amino acids ($4 \times 1 = 4$). If now two bases are allowed to code for one amino acid in various combination, we get codes for only 16 amino acids ($4^2 = 16$) which is not enough for coding all the amino acids. However if 3 bases are used in permutations to code for one amino acid then we get more than the adequate number of codes ($4^3 = 64$) with each amino acid designated more than a single code of three nucleotides (degenerative code). Thus genetic information (codes) is piled up in the form of the genes (DNA molecule) represented by sequential arrangement of three bases that determine the make-up of proteins. This arrangement of three nucleotides is called the **triplet code sequence**. Table 4.1 enumerates the 20 amino acids and their DNA codes.

"Transcription" (vide infra) causes the transfer of these triplet codes from DNA to mRNA. The triplets of nucleotide bases in the mRNA molecule which code for a particular amino acid is called a **codon**.

Table 4.1: Names of amino acids, their DNA codes and mRNA codons.

S.No.	Name of amino acid	DNA codes	RNA codons
1.	Alanine	CGA, CGG	GCU, GCC
2.	Arginine	GCA, GCG	CGU, CGC
3.	Asparagine	TTA, TTG	AAU, AAC
4.	Aspartic acid	CTA, CTG	GAU, GAC
5.	Cysteine	ACA, ACG	UGU, UGC
6.	Glutamine	GTT, GTC	GAA, GAG
7.	Glutamic acid	CTT, CTC	CAA, CAG
8.	Glycine	CCA, CCG	GGU, GGC
9.	Histidine	GTA, GTG	CAU, CAC
10.	Isolucine	TAA, TAG	AUU, AUC
11.	Leucine	GAA, GAG	CUU, CUC
12.	Lysine	TTT, TTC	AAA, AAG
13.	Methionine	TAC	AUG
14.	Phenylalanine	AAA	UUU
15.	Proline	GGA, GGG	CCU, CCC
16.	Serine	AGA	UCU
17.	Threonine	TGA	ACU
18.	Tryptophan	ACC	UGG
19.	Tyrosine	ATA, ATG	UAU, UAC
20.	Valine	CAA	GUU

The mRNA strand also contains **chain initiation** and **chain termination** codons. The initiation codon is present at the start of the mRNA and its sequence usually is AUG that marks the beginning of translation (polypeptide synthesis) in the ribosomal apparatus. The termination codon is present at the end of mRNA with sequences UAA or UAG. The synthesis of a polypeptide chain is terminated when a ribosomal apparatus reads through the stop codon.

All genetic codes as well as the protein manufacturing mechanisms are universal and found in all organisms synthesizing proteins. As such a cell can read a genetic code and translate it into the relevant protein irrespective of the source of the code. Human insulin can be produced in a large scale by bacteria that carry the human insulin coding gene put into the bacterial genome by genetic engineering. On the other hand viruses use host cell mechanisms for replication, used to our disadvantage.

The sequential arrangement of bases of a codon, if disturbed or changed, may lead to the defective formation of protein causing disorders of metabolism, etc.

TRANSCRIPTION

Transcription is a process of synthesis of messengers RNAs where genetic information stored in the DNA of a gene is transmitted to the mRNA (Fig. 4.2). This is the first step towards the formation of proteins.

Process of Transcription in Brief

Two strands of DNA double helix are separated (denatured) from each other forming **transcription bubbles** (cf replication bubbles). This is achieved by the activation of transcription factors and attachment of RNA polymerase at the promoter region of the gene. This kind of separation in DNA double strands can occur at more than one site throughout the genome during protein synthesis (interphase) or DNA replication (before mitosis).

One of the strands is called the **coding strand** (sense strand) and an mRNA is always synthesized identical to the coding strand. An important thing to remember is that the mRNA can only be identical in sequence to the coding strand if the mRNA is assembled on the opposite DNA strand that is complementary to the coding strand and called the **template strand** (anti-sense).

An mRNA transcript is always formed **on** the template strand and a template strand is always 'read' from its 3′ to the 5′ ends.

The separation of strands takes place at the location of a particular gene that is to be transcribed. We just understood that the two DNA strands of a gene can be designated as a coding and a template strand. In context of a particular gene (DNA), the 5′ of the coding strand contains specific sequences called the promoter region. The process of transcription begins with the activation and binding of **transcription factors** and the release of **RNA polymerase** at the promoter region of a gene.

Just a single strand of the DNA double helix is used for synthesis of mRNA molecule. The mRNA molecule is single stranded and synthesized by the enzyme RNA polymerase. With the help of this enzyme appropriate ribonucleotides are added to the mRNA chain sequentially.

The transcription of mRNA begins at its 5′ end and ends at the 3′ end of the molecule. Every base in a newly synthesized mRNA molecule is complementary to a corresponding base in the DNA of the gene (template strand). The cytosine (C) pairs with guanine (G), thymine (T) with adenine (A) but adenine pairs with uracil (U). Thus the information of a particular

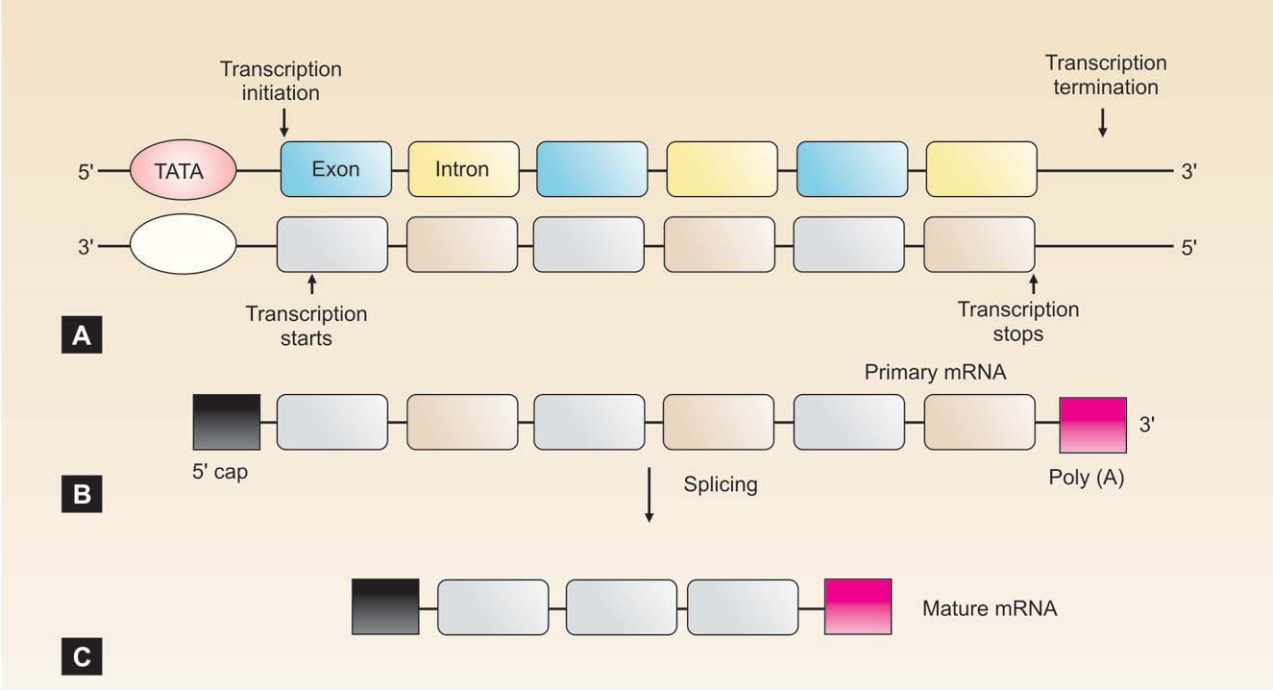


FIGURE 4.2: Transcription of mRNA from DNA (A). 5' capping and 3' end polyadenylation (B), and splicing of mRNA to get mature RNA (C). (*Courtesy* of Prof. Inderbir Singh, Human Embryology, 8th Edition, MacMillan)

gene (coding DNA strand) is transferred to the mRNA unchanged.

All the sequences of a structural gene are transcribed on to the mRNA molecule including extrons and introns; ones that do not make to the final transcriptory product.

Transcription is terminated by intrinsic and extrinsic mechanisms.

The G-C rich regions on the DNA give rise to hairpin bends on the RNA molecule as they are transcribed. This is called the intrinsic mechanism that causes a physical separation of the RNA from the DNA. The extrinsic mechanisms cause chain termination with Rho –factor enzymes that interact and inactivate the RNA polymerase at the RNA –DNA junctions.

Steps in the Process of Transcription

- Transcription begins at 5′ end and ends at 3′ end of the gene (the coding strand).
- The mRNA synthesis or so as to say, the assembly of its nucleotides begin on the template strand from the 3′ towards the 5′ end of the template strand.
- The mRNA itself is assembled from its 5′ end to the 3′ end.
- Each base in the newly synthesized mRNA molecule is complementary to a corresponding base in the DNA of the template strand and thus exactly has the same nucleotide sequence as that of the coding strand. Therefore information of a particular gene (DNA sequence) is transferred to the mRNA unchanged.
- There are discrete mechanisms to terminate transcription like the hairpin bend inducing intrinsic sequences on the DNA template or enzyme mediated termination such as the Rho-factor, etc. These events cause the mRNA to detach from the template strand.

Some special events occur to the nascent mRNA molecule after its synthesis. Entire transcribed mRNA doesn't participate in translation or protein synthesis. mRNA molecules are edited and pruned according to the requirement of protein synthesis in the cell during or after transcription.

- The intervening non-coding sequences (introns) are excised (Fig. 4.2) from the mRNA molecule. The exons are **spliced** together to form a mature RNA molecule which is relatively shorter in length. This process is known as splicing (removal of introns by cutting them off and joining the ends of extrons).
- Molecule(s) of GTP gets attached to the 5′ end of the mRNA. Phosphate of the GTP is added to the terminal base of mRNA. This added Guanine structure is methylated and is called a methylguanine cap. This 5′ cap protects the mRNA from degradation and facilitates the transport of mRNA to the cytoplasm. Similarly the 3′ end of mRNA is provided with a stretch of about 200 bases of adenylic acid called poly (A) tail which also protects mRNA from degradation and facilitates the transport of mRNA to cytoplasm.
- The mRNA then migrates from nucleus to cytoplasm where it attaches to ribosomes for synthesis of protein (translation).

ALTERNATIVE SPLICING

The postulate of "one gene one protein or enzyme" theory can be doubted after demonstration of a far greater number of proteins (>100,000) than the existing number of genes (30,000 – 35,000). This observation can logically be achieved by the process called **alternative splicing**. The exons (protein coding areas) can be rearranged within the mRNA in different patterns (Fig. 4.3). The provision to leave out one or a few exons in between can drastically alter the translation product of the manipulated exon by changing the sequence of amino acids present in the resultant polypeptide chain. In this way different protein molecules are formed by a single gene. Similarly the function of a protein can be modified after translation by phosphorylation or combination with other proteins. This process can experimentally be verified. Thus alternative splicing can explain the discrepancy of the number of genes *vis a vis* that of the proteins (Fig. 4.3).

TRANSLATION

"Transcription" brings the genetic information for the synthesis of polypeptide chain from the nucleus (DNA) to the mRNA molecule. The cytoplasmic protein synthesizing machinery utilizes information

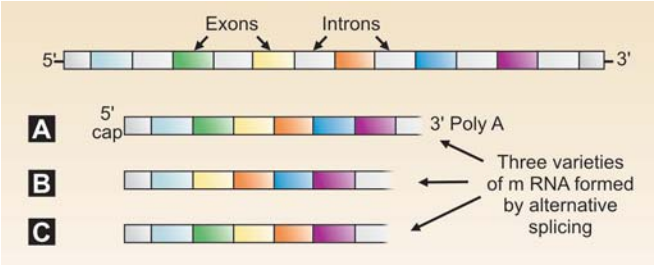


FIGURE 4.3: Diagram showing the process of alternative splicing. Transcription of a structural gene may form a mature mRNA in which all exons are present (A); where one exon is excluded in the process of splicing (B and C). Thus a single gene can form three different kinds of proteins. (*Courtesy* of Prof. Inderbir Singh, Human Embryology, 8th Edition, MacMillan)

on the mRNA to produce a protein molecule (polypeptide chain) in a cell. The translation apparatus consists of the following components:

Messenger RNA

Forms an important component of the translation machinery as it provides the coding sequence of bases determining the sequential arrangement of amino acids in the polypeptide chain. mRNA is translated from the 5′ to the 3′ end of the molecule.

Ribosome

Consists of a small and large subunit that come together on the mRNA strand to form a mature ribosome. The small unit reads the code on mRNA while the large unit aligns successive tRNA molecules and helps in the attachment of amino acids one upon the other by peptide bonds.

Transfer RNA

A given tRNA is attached to a specific amino acid by the enzyme **aminoacyl-tRNA synthetase**. A tRNA attached to its amino acid is called **charged tRNA.**

The codon on the mRNA binds to an anticodon on tRNA. This brings the attached amino acid into line for elongation of the growing polypeptide chain.

Translation consists of three stages, **initiation**, **elongation** and **termination (Fig. 4.4)**.

Initiation

- Begins at 5′ cap on the mRNA.
- The mRNA **initiation complex** is formed at the beginning of the molecule. This is fashioned by giving attachment to initiation factors (eIF4, eIF2, eIF3 and eIF5), small subunit of ribosome (40 S) and an initiator tRNA (with the UAC nucleotide sequence as the anticodon).

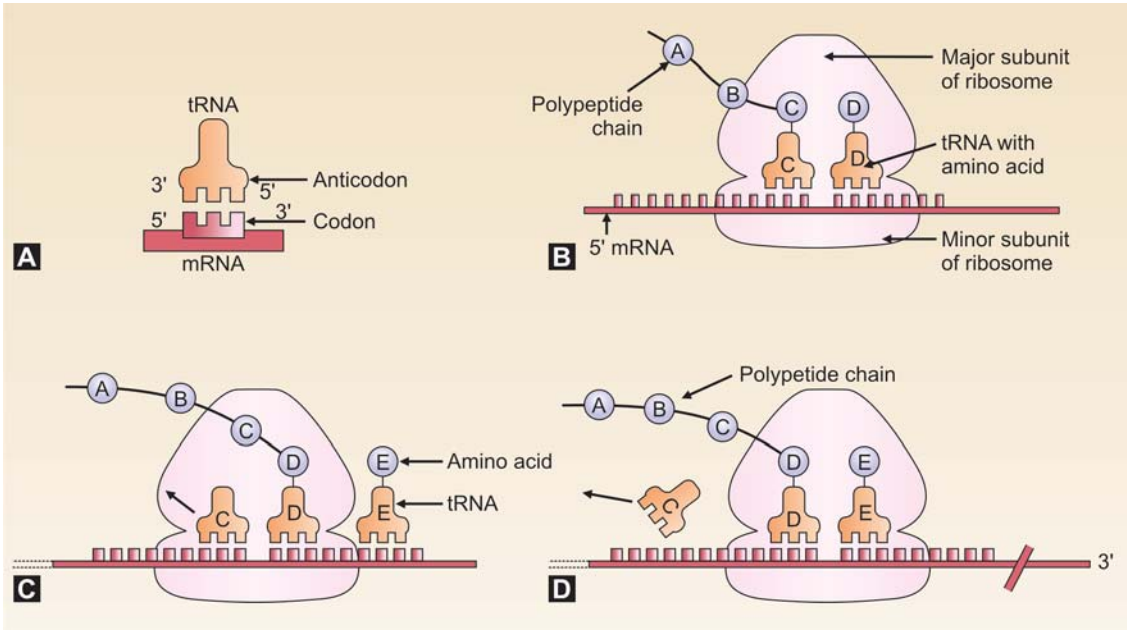


FIGURE 4.4: Diagram showing the process of translation. The ribosome moves along the molecule of mRNA. While it is moving it captures the tRNA molecules that match the codon in mRNA. The amino acid molecules which are brought by tRNA join with each other to form polypeptide chains (protein)

- The initiation complex moves along the mRNA towards its 3′ direction soon after the initiation complex is formed. It moves up till the first AUG nucleotide sequence is encountered. The AUG nucleotide sequence acts as **start codon** that signals the start of polypeptide synthesis.
- At this time point the large subunit (60 S) of ribosome gets attached on top of the small subunit. All other initiation factors are now released from the initiation complex.
- The AUG codon in the mRNA gets attached to the initiator tRNA (with UAC anticodon sequence) that moves in along with its amino acid methionine and occupies a domain inside the large subunit called the Peptidyl tRNA or simply the P site. The bonding is done with the help of hydrogen bonds.

Elongation

The immediate next three codons on the mRNA and the corresponding large subunit domain form the A site or aminoacyl site. Charged tRNAs called aminoacyl tRNAs land with their anticodons to attach with the corresponding codons at the A site. The methionine molecule is shifted from the tRNA of the peptidyl site (deacylated) to the aminoacyl site and bonded to the amino acid on the aminoacyl tRNA.

- The larger subunit of ribosome moves or translocates relative to the small subunit one codon further up towards the 3′ on the mRNA as the first step in elongation. This movement causes the A site become empty, the tRNA at the A site to shift to the P site and the deacylated tRNA dissociate from the ribosome.
- Next, the 30 S small subunit of ribosome moves along the mRNA to align perfectly with the large subunit and activates the next triplet of the mRNA at the new A site.
- The next aminoacyl tRNA enters the A site.
- Polypeptide is transferred from the P site to the tRNA at the A site.
- Translocation moves the ribosome one codon at a time, releases the deacylated tRNA from the P site, places the tRNA from the A site (the peptidyl RNA) to the P site and keeps A site ready for the next aa-tRNA.
- The process continues and elongation takes place codon by codon.

Termination

Polypeptide synthesis is terminated when the ribosomal unit reads through the stop codon.

- On coming to the stop codon on the mRNA strand, the ribosome binds to a **release factor (RF)**.
- The ribosome is unable to bind to any new tRNA now.
- The tRNA releases the polypeptide chain for further processing. This release is affected by the RF that recognizes the stop or termination codon and releases the chain form the ribosome.
- RF (the release factor) is consequently removed from the ribosome.
- The ribosomal subunits, 40 S and 60 S are separated from each other and are recycled.
- Folding of the polypeptide chains follows with their release from the ribosome.

Several ribosomes can move in tandem and at equal speeds on an mRNA strand placed at distances of about 80 nucleotides from each other (with a difference of about 25 amino acids between their polypeptide chains). The complex formed by aggregates of such ribosomes attached to a single mRNA is called a polysome or **polyribosome**.

As stated earlier a single gene can synthesize more than one protein by the process of ***alternative splicing***. Further diversity in protein synthsis is effected commonly by chemical events such as phosphorylation, N-acylation, glycosylation, etc. or by its combination with other proteins— processes known as posttranslational modification.

GENE EXPRESSION AND ITS REGULATION

The cells of the body are all not of a single type in structure or function though all of them are derived from a single cell, the zygote. It is quite interesting to find that at a given points of development there is spatial and temporal difference in the profiles of gene expression in each cell or a group of cells although all the cells contain equal and the same number of genes. Even after complete development and differentiation, diverse population of cells show differential expression of genes, e.g. skin cells synthesizing keratin, neurons producing neurotransmitters, etc.

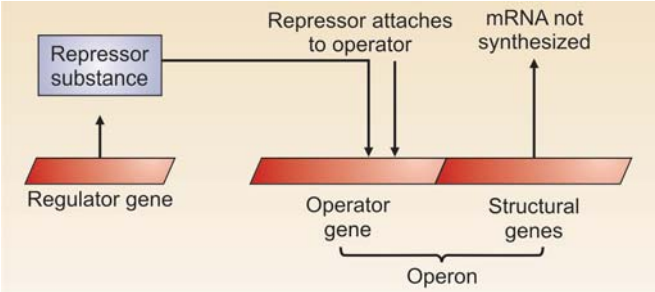


FIGURE 4.5: The operator gene is inhibited by the repressor substance produced by the regulator gene. This leads to inactivation of structural genes (i.e. they are unable to produce mRNA and thus, protein)

Basic Control of Gene Expression

Regulator gene and **operator gene** are the two different kinds of genes that govern the expression of other structural genes.

- The structural genes are under the control of operator genes which induce their transcription and are situated adjacent to the structural genes in a chromosome. The unit of the operator gene and structural genes is referred to as **operon** (Fig. 4.5).
- The operator genes are further controlled by the regulator genes that are situated usually away from the operon. A **repressor** substance is synthesized by the regulator gene which inhibits or represses the operator gene that further inhibits transcription of the structural gene. Activation of a regulator gene suppresses proteins synthesis from the structural genes.
- A repressor substance may combine with certain enzymes or metabolites that prevent its action on the operator gene. Thus an operator gene comes out of the inhibition of the repressor and stimulates transcription in a structural gene to start protein synthesis.
- Transcription is more complex in the higher organisms in terms of its regulation by **transcription factors** specific to certain DNA elements in the **promoter regions** that include the TATA, CAT boxes etc. (Fig. 4.1). DNA sequences, the **"enhancers"**, are known to increase the level of transcription. "**Silencers**" are regions on the DNA fragment that inhibit transcription.

GENETIC MUTATION, ITS TYPES AND MUTAGENS

Mutations are *changes that occur newly in the genetic material of an individual and may be heritable*. The 'changes' may range from an alteration in the smallest unit of a gene, i.e. in a nucleotide (in the coding and noncoding regions) to change in the gross morphology or number of the chromosomes.

- **Gene mutation** or a **point mutation** is a heritable change occurring in the structure of a gene.
- **Chromosomal mutations** are changes occurring at the level of chromosomes (gross structural or numerical changes).
- Mutation generally means a gene mutation and is seen across all living organisms. It is also the ultimate source of all genetic variations and accounts for species evolution.
- Mutations are essential for the long-term survival of any species and a species cannot acquire new genes without mutations. New traits that are necessary for adapting to the changing environment originate from mutations. Thus *mutation provides raw material for evolution* in a species. However, most mutations are damaging to the organism.

Some mutations are discussed below. An account of chromosomal anomalies are given in the next chapter.

Somatic and Germinal Mutation

- Mutations are called somatic mutations if they occur in somatic cells. Germinal mutations are mutations occurring in germ cells (egg or sperm).
- Somatic mutations can arise at any stage in the life of an individual.
- Somatic mutations cannot be transmitted to offsprings while germinal mutations are transmitted to the next generation as they occur in the gamete producing germ cells in the parents.
- Somatic mutations produce phenotypical changes in the particular affected individual while germinal mutations show-up in the subsequent generations.
- Somatic mutations give rise to genetically two different types of cell lines in the individual. Germinal mutations on the other hand don't

produce mosaic offspring as all the cells of the child would contain the anomaly received through a defective gamete.

- Mutations of the germ line are heritable but somatic mutations are not.

A gene usually loses its function as a result of a mutation. At times a mutation may also lead to acquisition of a new function or increased level of gene expression.

"Loss of Function Mutation"

A complete inactivation of gene (elimination of the function of gene) or reduced activity of the gene can result from a mutation. This event or mutation is called a "**loss of function mutation".** This is also known as "**knockout**" or **"null**" mutation. Most of the loss of function mutations is recessive in the sense that these mutations need to be present in a homozygous state to exert the effects of the 'loss' (e.g. loss of an enzyme).

"Gain of Function Mutation"

Over expression of a gene (increase in gene product) or activation of a gene in a tissue where the gene in question is normally inactive, may result from a mutation. These mutations are called "gain of function mutation" and are dominant (a heterozygous state of the mutation can manifest the effects). Presence of such mutations in homozygous state manifest severe forms of disorder e.g. homozygous achondroplasia. A majority of such mutations lead to over expression of genes sometimes resulting in cancer (Chapter 16).

Molecular Basis of Gene Mutation (Point mutation)

Alterations may happen in the arrangement of nucleotides in a DNA molecule even if the process of replication of DNA is very precise and stringently controlled. These changes are invisible through the microscope yet may have profound phenotypic effects in an individual. These smallest changes may involve an addition, deletion or substitution of a single nucleotide pair in the DNA molecule.

Point mutations (gene mutations) are of following types, namely (a) substitution mutations and (b) frame shift mutations with deletion or insertion.

Substitution

It is a common kind of mutation where a nitrogenous base of a triplet code of DNA is replaced by another nitrogenous base. The alteration of the codon now codes for a different amino acid. Substitution of the base A in the GAG triplet code (coding glutamic acid) by U alters it to a GUG codon (coding Valine) in the mRNA during transcription of Beta-globin chains of Hemoglobin leads to sickle cell anemia. The resultant defective B-globin polypeptide chains deform the RBCs by forming needle-like crystal aggregates in the hemoglobin.

A substitution mutation may not always be lethal as seen in the sickle cell disease. Nondeleterious mutations are known as **silent mutations**. However a gene mutation may be beneficial at times as seen in sickle cell mutation that imparts resistance to malaria.

Frame Shift Mutation

An insertion or deletion of a nitrogenous base in between the sequences in DNA or mRNA results in a frame shift mutation. The mutation leads to shifting of the reading frame or apparatus that reads the sequences of the codons. This shifting is caused by the insertion or removal of bases in the sequence. Such mutations may occur during transcription (DNA mutation) or during translation (mRNA mutation). (Fig. 4.6).

Frame shift mutations are often lethal because all the triplet codes beyond the point of mutation are misread by the apparatus as the system can read only a set of 3 bases at a time. Highly altered proteins are

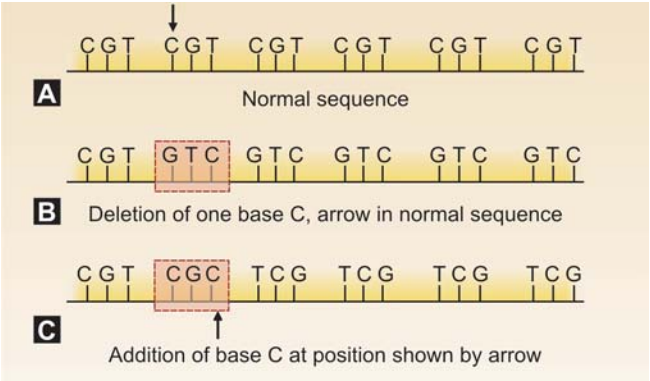


FIGURE 4.6: Various types of frame shift mutation

synthesised. Single mutations are more lethal than a triple contiguous mutation as a triple substitution omits a single amino acid whereas a single base mutation disturbs the reading frame usually resulting in termination of the process.

Mutagens

Majority of mutations usually are unprompted and called **spontaneous** mutations. These mutations hardly have a detectable cause but are attributed to errors in steps during DNA replication. Mutations can rise also due to exposure to certain environmental agents. These agents are known as **mutagens**.

Mutagens can be classified into two groups:

1. **Physical and Chemical mutagens**: Physical agents such as high temperature is are known mutagenic agents in animals. Many chemicals like mustard gas, formaldehyde, benzene, thalidomide and LSD are considered mutagenic in animals.
2. **Radiations:** Known causes of mutations include both natural and artificial ionizing radiations. Cosmic rays of the sun are sources of natural ultraviolet radiations. The other sources of natural radiation are the radioactive elements like thorium, radium and uranium present in the earth.

   X-rays, gamma rays, alpha and beta rays (particles) and neutrons are artificial sources of radiation. Radiations may cause breaks in chromosomes and chromatids. These breaks involve sugar phosphate backbone of the polynucleotide strands resulting in severe anomalies (Chapter 5).

MECHANISM OF DNA REPAIR

The frequency of DNA damage by chemicals and radiations are quite high evaluated at the rate of about 10,000 throughout the genome in 24 hours. The damages are automatically repaired by specific and precise molecular mechanisms without any residual effects.

- The enzyme **DNA ligase** repairs small nicks in the DNA strand.
- Repair is executed with the help of the enzyme **AP endonuclease** at places of a base-pair loss.
- Repair is executed in steps at sites with a large damage in the DNA strand. The damaged area is first cleaved by the enzyme **endonuclease**. Next the damaged portion is removed by the enzyme **exonuclease**. A newly synthesized DNA strand is then inserted with the help of the enzyme **DNA polymerase**. The break is finally sealed by **DNA ligase** enzyme.
- DNA fragmented by ultraviolet light is repaired by the products of at least eight genes.

SUMMARY

(a) A gene is defined as *"a segment of DNA which contains the information (code) for synthesis of one complete polypeptide chain"*. Thus a gene provides instructions for building a specific protein.
(b) The control genes regulate the activity of structural genes.
(c) The DNA portion of a structural gene not only contains coding sequences for amino acids **(exons)** but also contains noncoding sequences **(introns)**.
(d) A structural gene contains flanking regions at their ends. These regions are important for regulating the affairs of the gene.
(e) Proteins are made up of polypeptide chains. Polypeptide chains are constructed of specific sequences of amino acids.
(f) A sequence of three bases on DNA strand codes for one amino acid. The sequence is called the triplet sequence.
(g) A DNA strand constituting a structural gene contains all the sequentially arranged codes for amino acids that combine to form a complete polypeptide chain.
(h) Transcription defines the transferring of the blueprint in the DNA (coding and noncoding sequences) of a gene to messenger RNA.
(i) As the base pairing is unique in selectivity, the information of DNA strand is transferred to mRNA unchanged identical to the coding strand.
(j) Control genes regulate the activation of structural genes.
(k) Control genes are of two different kinds, i.e. the regulator genes and the operator gene.
(l) The unit of an operator gene and a structural gene is called an operon.

(m) Repressor substances are synthesized by regulator genes. These substances inhibit the operator gene which further inhibits structural genes.

(n) Certain metabolites may combine with a repressor substance to inactivate them. The operator gene is thus released of inhibition and gets activated.

(o) Regulation of transcription is more complex in higher organisms

(p) C*hanges occurring in the genetic material of an individual* is defined as mutation. Mutations may be heritable.

(q) Mutation may occur in a gene (point mutation or gene mutation) or it may occur in a chromosome (chromosomal mutation).

(r) Point mutations are either **substitution** mutations or **frame shift** mutations.

(s) Point mutations are due to an addition, deletion or substitution of a single nucleotide in the DNA sequence of a chromosome.

(t) Mutations in somatic cell are called **somatic mutations** and in germ cell are called **germinal mutations**.

(u) Environmental agents causing mutations are called **mutagens**.

Chapter

5 Chromosomal Anomalies

- Numerical anomalies
- Numerical abnormalities of autosomes
- Structural anomalies
- Cri-du-chat syndrome
- Existence of different cell lines (mosaicism/ chimerism)

In order to initiate a normal process of embryonic development and subsequent growth in an individual, it is necessary to have a complete set of all the 46 chromosomes that are both structurally and functionally normal. The genes that constitute the chromosomes function with precision and with a subtle balance only when the entire complement of the genome participates in a close interaction. This delicate balance of the genes can be disturbed by several chromosomal abnormalities which are of the following types. Anomalies may be in the form of:

A. Numerical anomalies
B. Structural anomalies
C. Existence of different cell lines (Mosaicism/ Chimaerism).

Abnormalities of the chromosomes account for a significant proportion of morbidity and mortality in humans. These defects are responsible for more than 50% of all spontaneous abortions, up to 1% of all congenital disabilities and a number of malignancies.

NUMERICAL ANOMALIES

There are situations where an individual may contain more (47, 48 .... etc.) or less (45) number of total chromosomes in all its cells instead of the normal count of 46. This increase or decrease in the chromosomal count can be accounted for by the presence of one or more than one chromosome to be present in one or more extra copies or the absence of one or more than one chromosomes in a cell. These anomalies when described in terms of the particular chromosome/ chromosomes involved in the defect are called 'somies'. For example a cell with a missing chromosome would contain a set of 45 chromosomes and the condition is called as a **monosomy** (as one of the pairs of chromosomes would be single, without the other of the pair mono = one, soma = body) for that chromosome. As in the Turner's syndrome (XO), the female has only one X chromosome of the pair (XO) instead of the usual XX. Thus it is a monosomy of the X chromosome. Similarly in conditions where a cell has three copies of a chromosome instead of two (the pair), the condition is called a **trisomy**. A commonly found trisomy is the Down's syndrome (21st chromosome). Likewise an individual having four copies of the same chromosome it is designated as **tetrasomic** for that chromosome. Somies may involve both the autosomes and the sex chromosomes.

A dissimilar kind of numerical abnormality of the chromosome number is called **polyploidy**. The condition is manifested by the increase in the number of chromosomes of a cell exactly in the multiple of its **haploid** number. A haploid is half of the normal set of chromosomes in the individual. In humans the complete set of chromosomes in a cell consists of 46 chromosomes called the diploid set. Hence, haploid set in humans comprise 23 chromosomes. Therefore the cells that have three haploid sets of chromosomes (69 chromosomes) instead of the normal two sets (46 chromosomes) are called **triploidy** and those with four haploid sets (92) are called **tetraploidy** or just polyploidy.

Anomalies like 'somies' (related to individual chromosomes) or 'ploidies' (related to sets of chromosomes) involve abnormal chromosomal numbers and are called **aneuploidies**.

Mechanisms of Numerical Anomalies

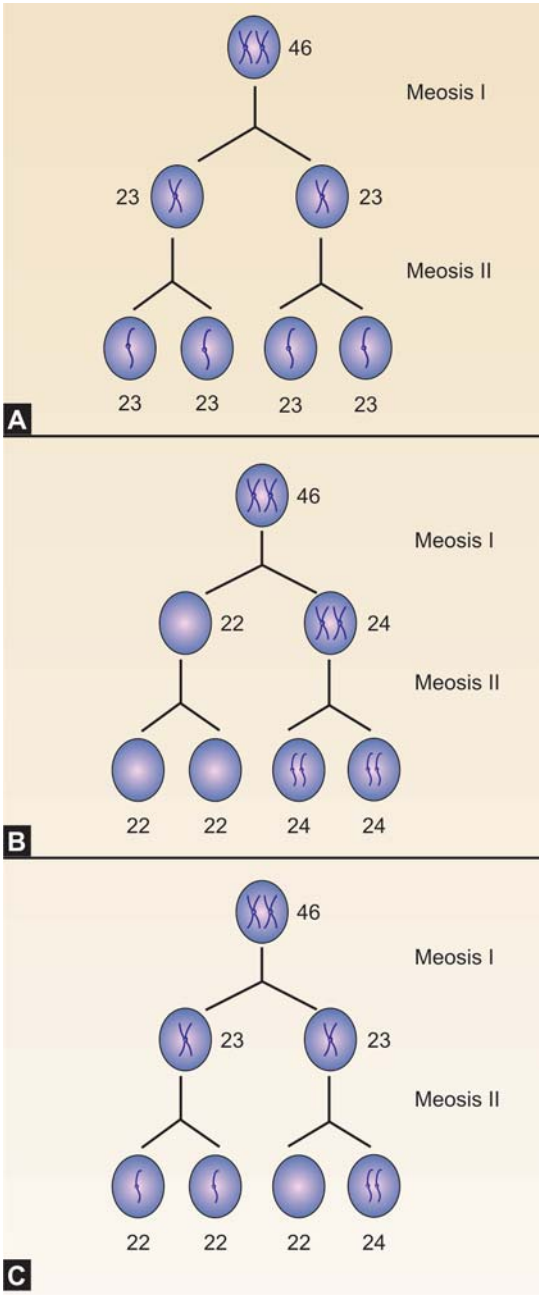
During the events of spermatogenesis or oogenesis it may so happen that an egg or a sperm may receive more or less than its share of chromosomes due to a faulty separation of the chromosomes or chromatids during the process. These defective gametes may hand over aneuploid chromosomes into the zygote and the resultant embryo would manifest chromosomal numerical anomalies.

The failure of homologous pair of chromosomes to separate during meiosis I or meiosis II is called **non-disjunction**. Both these types of nondisjunctions are shown in Figures 5.1A to C.

Consequences of Nondisjunction (trisomy and monosomy)

During the first meiotic division two chromosomes (with sister chromatids) of a pair may migrate to the same pole instead of two different poles of the dividing cell (Fig. 5.1 B). This causes both the chromosomes to aggregate in one of the daughter cells and the other cell receives none of the chromosomes. Similarly during meiosis II a pair of sister chromatids may fail to segregate to go to two opposite poles of the dividing cell and migrate to a single pole. There is an equal distribution of chromosomes in both the situations described above (meiosis I and II) where the two chromosomes or the sister chromatids go to same pole (Figs 5.1B and C). As seen in the figure, all the resultant gametes in the first situation are defective (Fig 5.1B). Two of the gametes carry an extra number of a chromosome while the other two are devoid of the chromosome. In the second situation (Fig 5.1C) two of the resultant gametes are normal whereas the other two resemble situation I. All the defective gametes are aneuploids. Fertilization between the defective gametes with a normal one (23 chromosomes) will result in two types of zygotes. The one formed with an extra chromosome (24 chromosomes) in the gamete would contain three chromosome of the type instead of two. This would thus result in a **trisomy** of the chromosome.

The other type of zygote formed by the union of a gamete devoid of a particular chromosome (22 chromosomes) with a normal one (23 chromosomes) will contain 45 chromosomes only. This condition of absence of a chromosome of the pair is called **monosomy** of the said chromosome.



FIGURES 5.1A to C: A, B and C showing gametogenesis, A = normal gametogenesis, B = shows the failure of separation of a homologous chromosome during meiosis I. Out of four gametes that are formed, two have an extra chromosome while in the remaining two gametes, the particular chromosome is missing. C = Failure of separation of sister chromatids during meiosis II

Developing zygotes where cells divide by mitosis may also exhibit nondisjunction. As a result a non-disjunction in the developing zygote gives rise to two or more different cell types having different number

of chromosomes in them. This phenomenon is known as **mosaicism** (see below). The multiple of the haploid number of chromosomes (triploidy, 69 or tetraploidy, 92) are unknown in human except for some liver cells. Most of the polyploidies result in spontaneous abortion; a few may survive till birth.

Causes of Nondisjunction

Advancing maternal age and chromosomal abnormalities have been closely associated with each other. Disorders like trisomy 21 (Down's syndrome), trisomy 13 and 18 have well-established association with advanced maternal age.

Meiosis I begins in primary oocytes before birth of a female and is completed at the time of an ovulation. Thus a primary oocyte remains in an extended and suspended stage of activity from a period well before birth till any of the ovulatory cycle in the female (from menarche till menopause). Further, meiosis II is eventually completed only after fertilization of the secondary oocyte. The state of this suspended meiotic activity, which is further delayed by a late motherhood, probably predisposes a gamete to non-disjunctions because of abnormalities in spindle formation. Though defective oocytes have been implicated in many cases of fetal chromosomal anomalies, nondisjuncted sperms can also cause numerical anomalies.

The other causes implicated for nondisjunctions are radiations from radioactive sources, delayed fertilization after ovulation, smoking, alcohol consumption, oral contraceptives, drugs, pesticides or some inherent genetic mechanism.

NUMERICAL ABNORMALITIES OF AUTOSOMES

Trisomies related to the following chromosomes are commonly observed in live-born children with congenital anomalies. Most other autosomal trisomies and monosomies are very severe and incompatible with life.

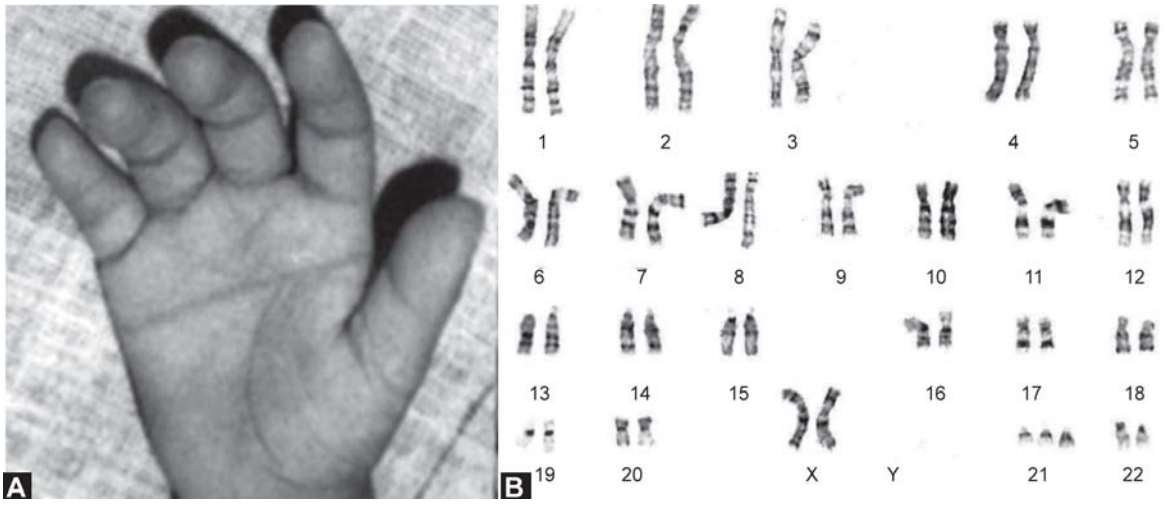
Trisomy 21 (Down's Syndrome or Mongolism)

Characteristics of the Disease

Occurs in 1 out of 700 live-births. Incidence increases with the increasing maternal age. The risk at 45 years of age for its incidence is as high as 1 in 16. Males are more commonly affected than females.

Children are mentally retarded with low I.Q. scores (range from 25–75) showing poor growth, short stature and reduced muscle tone. About 40% of children suffer from major heart defects. Facial features typically are small head circumferences, epicanthic folds, protruding tongue, small ears and sloping palpebral fissures.

The hands are short and broad. There may be a single transverse palmar crease resulting from the fusion of the proximal and distal transverse palmar creases (Simian crease, Fig. 5.2A). The mean survival age is about 16 years though it varies from few weeks to decades. Most affected adults tend to develop Alzheimer's disease in later life for specific metabolic defects.



FIGURES 5.2: (A) Palmar Simian crease in a child with Down's syndrome. (*Courtesy* of Dr Dilip C Master, Professor of Anatomy and Head, Human Molecular and Cytogenetics, Medical College, Vadodara, India). (B) The karyotype of Down's syndrome (*Courtesy* of Dr Steven M Carr, Professor of Biology, Memorial University, Newfoundland, Canada).

Mechanisms that may cause Down's Syndrome:

- Trisomy
- Translocation
- Mosaicism.

The trisomy of the 21st chromosome most commonly causes (95%) of Down's syndrome (Fig. 5.2B). The karyotype in a trisomic Down's is either 47+XY or 47+XX. The source of the extra 21st chromosome is mostly from a nondisjunction in maternal meiosis I. Robertsonian translocations (see below) may be the cause of Down's syndrome in 3% cases. The genotype in a Robertsonian Down's is 46 + XX or 46 + XY. Children with mosaicism contain two cell populations (one normal and the other trisomic). These Down's are due to early life disjunctions. Due to coexistence a set of normal cells in the individual, the person is less severely affected.

Counseling

Counseling may be prenatal or postnatal. Prenatal counseling can be advised in high risk pregnancies. Following the delivery of a Down's child, the risk of begetting a Down's child in subsequent pregnancies increases significantly in elderly ladies. Also the risk of getting a Down's child is three folds in a previously affected lady when compared to a woman of the same age not affected with such a child.

In cases of translocations, the recurrence risk is 1 to 3% in case of the father bearing a translocation but risk increases substantially to 15% in case of a carrier mother.

Postnatal counseling is about the management of the child towards normal life and appropriate therapy.

Prenatal diagnosis: The prenatal diagnosis of Down's syndrome can be done in cases with a prior history of such an event or on clinical suspicion. It can be done by amniocentesis for chromosomal analysis.

The triple test is an important diagnostic tool that estimates three specific biochemical markers (α-fetoprotein, estriol and chorionic gonadotrophin) present in the maternal serum at 16 weeks of gestation. In Down's pregnancies the level of α-fetoprotein and estriol levels tend to be reduced as compared to the normal, while the level of human chorionic gonadotrophin is increased. Estimating the increasing levels of **inhibin-A** in the maternal serum also helps in diagnosis.

Trisomy 13: Patau's Syndrome

Usually the pregnancies with this anomaly terminate spontaneously within days of conception. However, the incidence of Turner's syndrome in all live-births is 1 in 5000. Most of the affected children die within a month while a few of them may survive for a few months.

Characteristics of the Disease

Children are severely retarded both physically and mentally and possess small skulls and eyes. The child may have cleft lip and cleft palate, extra finger or a malformed thumb. The CVS, CNS and excretory systems are susceptible to infections.

Trisomy of chromosome 13 is responsible for this syndrome that rarely develops also due to certain Mosaicism and Robertsonian translocation patterns. The risk of recurrence is very small but the incidence increases with advanced maternal age

Numerical Abnormalities of the Sex Chromosomes

The sex chromosomes are commonly affected by the following numerical chromosomal anomalies:

- Trisomies like XXX or aneuploidies such as XXY, XYY
- Monosomy like 45, XO
- Mosaicism, 46, XY/46, XX or 46 XX/45 XO.

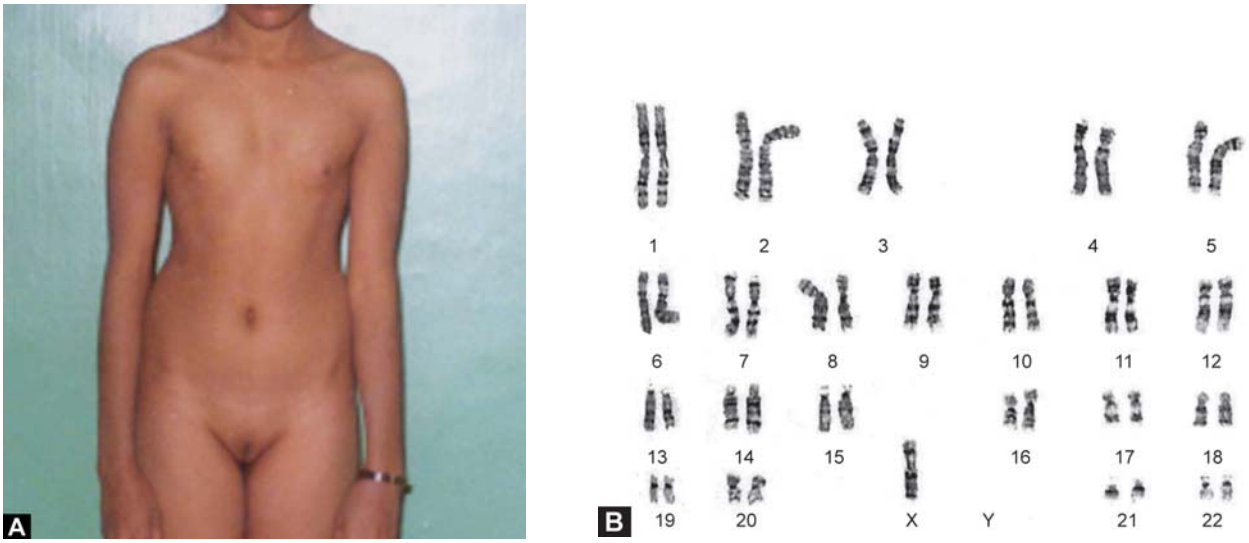
An extra X or Y chromosome usually has a relatively mild effect. This is because the Y chromosome contains relatively few functional genes and in each somatic cell all except one functional X chromosome form Barr bodies. Developing zygotes tolerate sex chromosomal anomalies better than those of the autosomes.

Turner Syndrome, 45, XO

This syndrome is actually a monosomy of the sex chromosome and is a common cause of spontaneous abortions. About 20% of spontaneously aborted fetuses have 45, XO genotype. Incidence in live-born infants varies from 1 in 5000 to 1 in 10,000. They survive to develop phenotypically into a female.

Characteristics of the Disease

The affected individuals are females possessing normal intelligence or might be slightly retarded. Usually



FIGURES 5.3A and B: (A) Photograph of an 18 year old female with turner's syndrome. Note the broad, flat (shield) chest with widely spaced nipples; scanty pubic hair and webbed neck. (*Courtesy* of Dr Dilip C Master, Professor of Anatomy and Head, Human Molecular and Cytogenetics, Medical College, Vadodara, India). (B) Karyotype of Turner syndrome (*Courtesy* of Prof Steven M Carr, Professor of Biology, Memorial University, Newfoundland, Canada)

show a webbed neck, low posterior hair line, cubitus valgus and a broad chest with widely spaced nipples. They have a short stature (Fig. 5.3A).

Individuals are poor in arithmetical skills, reading maps and drawing diagrams.

Patients are mostly infertile which is due to a primary developmental failure of the ovaries. This also leads to the absence of menstruation. Breasts fail to develop and pubic hair is scanty as a lack of secondary sexual character. Individuals characteristically possess ill-developed or rudimentary gonads (ovaries) called the **streak gonads**. Ventricular septal defects or coarctation of aorta may exist. Sex-chromatin or Barr body examination is always negative as there is only one X-chromosome.

Genetic Characteristics and Counseling

The most frequent cause of Turner's syndrome (45, XO) is the monosomy of the X chromosome (Fig. 5.3B). Mosaicism - 45, XO/46, XX, Isochromosome - 46,X,i(Xq) and Ring chromosome - 46, X, r(X) may also account for certain percentage of the anomaly.

Estrogen replacement therapy (HRT) should be tried at adolescence for development of secondary sexual characteristics. Though affected females are sterile but can bear child with the help of "***In vitro fertilization***". Genital tract reconstructions with surgery may be attempted to obtain a normal conjugal union.

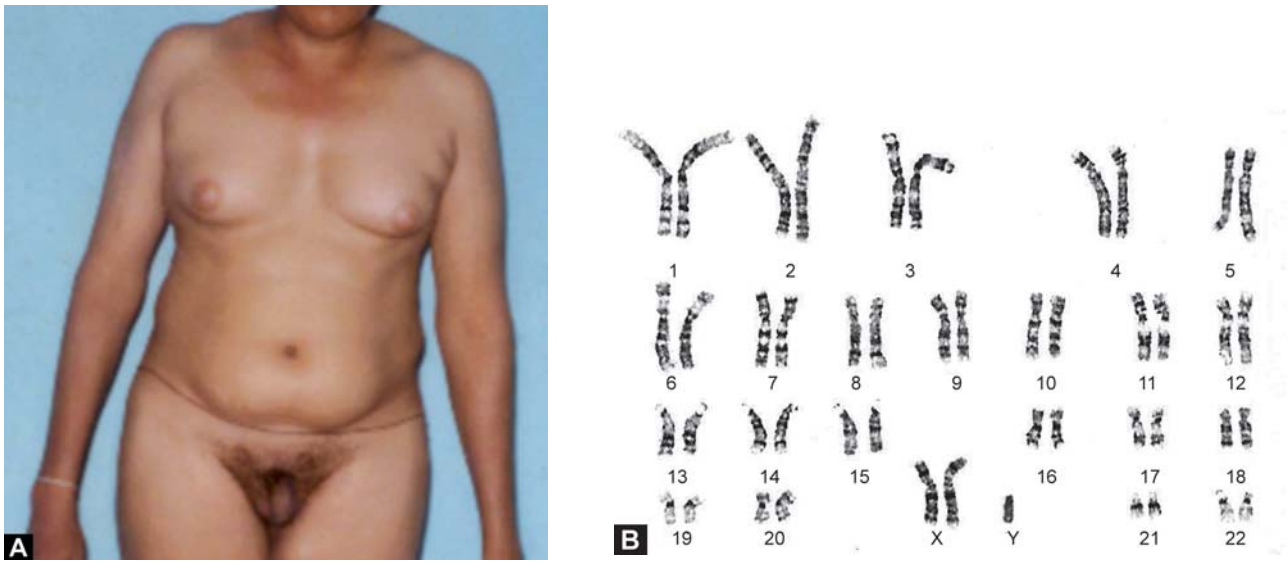
47, XXY: Klinefelter Syndrome

Characteristics of the Disease

Occurrence rate is about in 1 per 1000 newborn males. Affected individual is phenotypically a male. Syndrome usually remains undetected until adolescence. Patients are diagnosed when they attend infertility clinics for treatment of infertility. Appearance of an affected person is normal though he might be quite tall with a mild degree of mental retardation. Puberty is usually delayed and individuals have very small pair of testes but a normal penis and scrotum. Secondary sexual characters don't develop fully and the pubic and facial hair is scanty. Gynecomastia (enlarged breast) may be a prominent feature in some (Fig. 5.4A).

Genetic Characteristics and Counseling

Usual Klinefelter cases have karyotype of 47, XXY (Fig. 5.4B) with some individuals showing a mosaic pattern. Mosaic (46XY/47, XXY) individuals show Barr



FIGURES 5.4A and B: (A) Klinefelter's syndrome characterized by gynecomastia, underdeveloped genitalia, feminine pattern of distribution of pubic hair and lack of moustache and beard. (*Courtesy* of Dr Dilip C Master, Professor of Anatomy and Head, Human Molecular and Cytogenetics, Medical College, Vadodara, India). (B) Karyotype showing 47, XXY (*Courtesy* of Dr Steven M Carr, Professor of Biology, Memorial University, Newfoundland, Canada)

body. Treatment with testosterone (HRT) at puberty may expedite the onset and development of secondary sexual characteristics.

47, XYY Males

Characteristics of the Disease

The incidence is rated approximately at 1 per 1000 newborns. The males are phenotypically normal but usually tall built and almost always associated with slight mental retardation. Contrary to common belief they are not overtly criminals but may be hyperactive and restless individuals. These individuals may show emotional immaturity, impulsive behaviors or delinquent tendencies. Karyotype represents - 47, XYY. The additional Y chromosome is usually due to non-disjunction in meiosis II during spermatogenesis in the father.

STRUCTURAL ANOMALIES

Structural abnormalities of chromosomes commonly refer to defects in the form of a missing portion or a portion being represented twice or more in a chromosome. The abnormalities in structure of chromosomes are often secondary to events of **chromosome breakage**. Chromosomes are extremely fragile and they may at times, break spontaneously. Chromosomal breakages may be induced by certain external agents like **X-rays**, **chemicals**, and **viral infections**. Fragmentation in chromosomes occurs usually at the ends that are away from the centromere (telomere). These small regions are lost during cell division and they are unable to move to the poles in daughter cells. In fact chromosomal breakages are detected by specific mechanism and are not allowed to transmit to daughter cells in either mitosis or meiosis. Some of these anomalies, especially near the centromere, may remain undetected. Following are the important structural abnormalities.

(a) Deletion
(b) Inversion
(c) Ring chromosome
(d) Isochromosome
(e) Translocation.

Structural abnormalities cause loss of genes (as in deletion), gain of genes (as in duplication) or a change in the normal position of the genes (as in inversion and translocation) and result in genetic disorders.

Karyotype Symbols

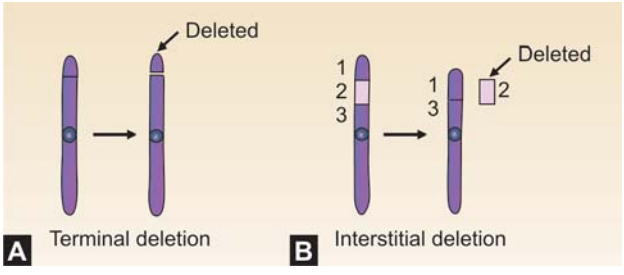
p	Short arm of chromosome
q	Long arm of chromosome

ter	Terminal portion
qter	Terminal portion of long arm
pter	Terminal portion of short arm
+	Before a chromosome number indicates that the chromosome is extra e.g. +21.
–	A chromosome is missing. A plus or minus sign after the number of a chromosome indicates the addition or deletion respectively of a region in that chromosome.
Mos	Mosaic
/	Separates karyotypes in mosaics, e.g. 47, XXX/45, X
del	Deletion
inv	Inversion
r	Ring chromosome
i	Isochromosome
rep	Reciprocal translocation
rob	Robertsonian translocation

(a) Chromosomal Deletion

Deletion denotes breakage that occurs in a part of the chromosome away from the centromere. The broken part is subsequently lost.

A part of a chromosome may get deleted near its terminal end by a single break. Sometimes a short intervening portion of a chromosome may break off by two separate breaks (Figs 5.5A and B). There may be several genes within the broken off portion of the chromosome that eventually is deleted or lost. An individual cannot survive if a large piece of chromosome is deleted. A loss of more than 2% of genome will be lethal. Deletions are classified into two kinds.



FIGURES 5.5A and B: Types of chromosomal deletion. A = terminal deletion, B = interstitial deletion

(i) Microscopic or Chromosomal Deletion

This can be visualized microscopically by using usual karyotyping methods in cases of sufficiently large deletions, e.g. deletion of the short arm of chromosome 5 (cri-du-chat syndrome) and of the short arm of chromosome 4 (Wolf-Hirschhorn syndrome) are some of the well known examples.

CRI-DU-CHAT SYNDROME

Characteristics of the Disease

The incidence is about 1 in 50,000 births. This syndrome is due to deletion of short arm of chromosome 5 (5p-). New-borns with this syndrome bear underdeveloped larynx which make them produce the characteristic cat like cry. Other features include microcephaly with physical and mental retardations. Infants suffering from Wolf-Hirschhorn (4p-) are severally mentally retarded and may also show the stunted physical growth.

(ii) Submicroscopic Microdeletions

These defects cannot be visualized by usual karyotype methods and hence needs molecular techniques for their detection, e.g. FISH.

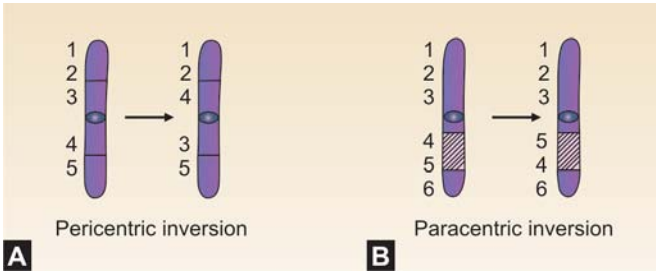
Following a few of the common microdeletion syndromes.

Syndromes	Affected chromosome
Prader-willi	15
Angelman	15
Wilms tumor	11

(b) Chromosomal Inversion

The abnormality involves only a single chromosome which breaks at two points. The broken segment rearranges itself back into the chromosome by inverting its position. Inversions may be of two different types, i.e. pericentric (involving the centromere) and paracentric (not involving the centromere), as shown in Figures 5.6A and B.

Inversion abnormalities do not result in clinical problems in individuals as inversions usually do not cause a loss in chromosomal material; the breaks



FIGURES 5.6A and B: Types of chromosomal inversions
A = pericentric, B = paracentric inversion

generally occur at noncoding sites. However, if the disruption or break is caused at the site of an important functional gene, it may result in an abnormality in a person. A person with a chromosomal inversion in his or her germ cells furnishes abnormal gametes. It is a fact that genes undergo recombination and rearrangements during 'crossing over' phases in meiosis I. Inversions existing in chromosomes undergoing recombination produce unequal distribution of genes and hence defective gametes. Fertilization with these gametes commonly results in spontaneous abortions.

(c) Ring Chromosomes

Rarely chromosomes form a closed circle (ring) structure. Formation of ring chromosomes start with breaks near the tips (ends) of each arm of a chromosome. These broken and sticky ends then fuse with each other (Fig. 5.7). The two distal fragments are lost during cell division.

DNA replicates in these chromosomes before cell division. But migration of the sister chromatids is abnormal due to the anomalous configuration of the chromosome. This results in the loss of the entire

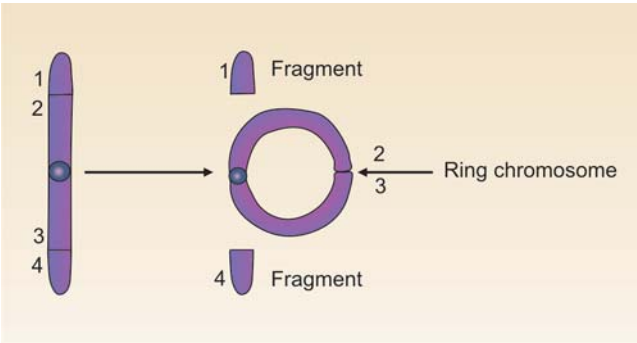


FIGURE 5.7: Formation of ring chromosome

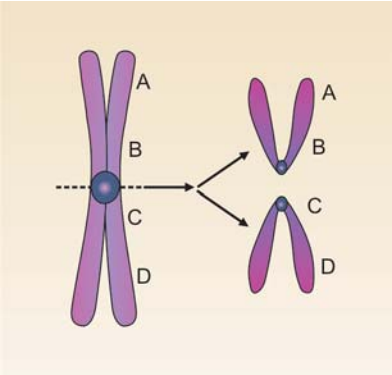


FIGURE 5.8: Isochromosomes are formed due to abnormal splitting of centromere

chromosome from the cell. As such individuals with ring chromosomes tend to develop a mosaic cell population.

(d) Isochromosomes

Isochromosomes result as a consequence of incorrect splitting of chromosomes at their centromeres (Fig. 5.8) during cell division. This faulty splitting results in the formation of two isochromosomes. Each defective splitting produces two types of chromosomes. Each of them contain two arms that are identical to each other. One of the chromosomes has two short arms whereas the other possesses two long arms. Cells having, for example an isochromosome with two short arms, will contain an extra short arm as well as the set of genes located on the short arm (in fact the total dose of those short arm and its genes would exist in triplicate or in thrice dose in the cell; the normal chromosome providing the third short arm and the homologous genes. This cell is devoid of one long arm that is lost and migrates to the other daughter cell with the formation of the long arm isochromosome.

(e) Chromosomal Translocation

The exchange of genetic material between chromosomes is called translocation. Micro and macro segments of chromosomes are found to get fragmented and exchanged between homologous as well as between heterologous chromosomes. Translocation can be either balanced or unbalanced. A translocation is balanced when the event does not result in any loss

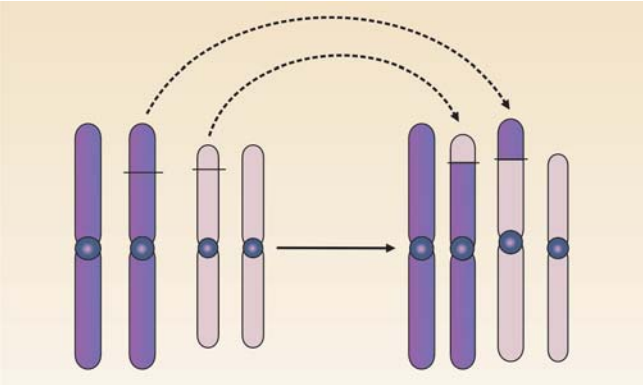


FIGURE 5.9: Reciprocal translocation

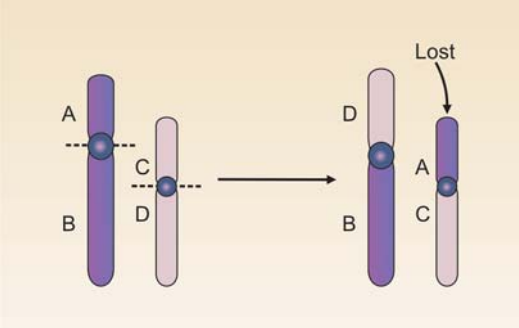


FIGURE 5.10: Robertsonian translocation, showing fusion of two long arms and loss of the fused short arms of two chromosomes

of genetic material. The entire complement of genes gets appropriately expressed in an individual. Unbalanced translocations accompany loss of genes. People with balanced translocations are normal, but may have affected children if translocations involve their germ cells.

Two types of Translocations usually observed:

(i) Reciprocal Translocations

Incidence: Approximately1 in 500 people.

This translocation occurs between two non-homologous chromosomes after breakage and exchange of the fragments (Fig. 5.9).

Reciprocal translocations are usually balanced rearrangements that more often than not have no detectable phenotypic effects in the individual. The reciprocal translocation between chromosome 11 and 22 (between groups D and G) are relatively common.

Gametogenesis is defective as the chromosomes with translocations are unable to align properly to form the bivalents during meiosis I. As a consequence asymmetrical distribution of chromosomes results in abnormal gametes.

(ii) Robertsonian Translocation

Incidence: About 1 in 1000 people.

Acrocentric chromosomes (groups D and G) may break at their centromeres giving rise to a long arm and a short arm with the satellite body. The long arm from one group may fuse with the long arm of the other group (D/G). This fusion may also occur between long arms within the same group, e.g. 21/22 or 21/21. The short arms thus generated by the breaks fuse together and are lost (Fig. 5.10).

This is functionally a balanced translocation though the total chromosome number is reduced to 45 (loss of the fused short arms and satellites that don't carry significant genetic material).

A 21/21 translocated individual is normal with a total of 45 chromosomes in all his somatic as well as germ cells. During meiosis, however, one group of gametes receive the translocated 21/21 chromosome and the other group gets none of the 21 st chromosome. On fertilization with normal gametes, the 21/21 containing gamete begets a Down's child and the gamete with no 21st chromosome produces a monosomic (45 XX/XY,-21) child that is incompatible with life.

On the other hand, individuals bearing a D/G translocation involving the 21st chromosome (45 XX/XY), produce 4 types of gametes. On fertilization with a normal gamete the 4 varieties of gametes may probably yield a normal child (46 XX/XY), one normal child that receives the translocated 21st chromosome (45 XX/XY), one Down's affected child resulting from inheriting two normal chromosomes 21 (one each from the mother and the father and the additional one from the translocated chromosome involving the 21st chromosome, e.g. 14q /21q) or an incompatible with life child that receives only one normal 21st chromosome from the normal gamete and none from the translocated germ cell.

Individuals having translocations involving the D and G (21st chromosome) groups may conceive a Down's child for every four pregnancies. 21/21 translocations will have an absolute risk of 100% for Down's syndrome progenies.

Almost about 4% cases of all Down's syndromes are due to Robertsonian translocations. The others are accounted for by nondisjunctions at gametogenesis.

EXISTENCE OF DIFFERENT CELL LINES (MOSAICISM / CHIMERISM)

Chromosomal Mosaic or Mosaicism

Majority of we people have identical chromosomal constitution in all our cells and tissues. Chromosomal mosaicism is a condition where two genetically different cell populations coexist in the same individual. The difference is generally observed in the number of chromosomes in the cells. Mosaicism may affect both the autosomes and the sex chromosomes. Whatever the differences though, the types of different cell lines in a mosaic pattern are derived from a single zygote as the source of the cells. In mosaics a group of cells may be normal (46, XX / XY) whereas others may have extra or less number of chromosomes. Mosaicisim involving sex chromosomes may denote a full range of anomalies, e.g. true hermaphrodites (46XX/46XY), a variant of Turners (46XX/X0), etc.

Causes of Mosaicism

The most usual cause of chromosomal mosaicism is a nondisjunction occurring in an early embryonic mitotic division.

Mosaicism not only occurs at the chromosomal level but also at the level of the gene. If a gene mutates at a very early stage of embryonic cell division, the fetus possesses two different cell lines, i.e. some cells with the normal gene and others with the mutant gene. Mosaics may result from isochromosomal splitting and delay in chromosomal migration called the 'Anaphase lag' also resulting in unequal distribution of the chromosomes.

Chimeras

If undifferentiated and early life cells from two or more embryos of different mouse are mixed together and introduced into the uterus of a foster mother, the resultant mass of cells can give rise to a normal mouse at term. However, this mouse would constitute different cell population in its body. The cells will have identical number of chromosomes yet each different cell line would be unique is its parent source. This individual is called a chimera. Thus a chimera is an individual with two or more genetically distinct cell lines derived from more than one zygote or life source.

Chimerism may be induced by the fusion of two zygotes obtained by fertilization of two different ova from two different sperms. Chimerism may evolve with sharing of placenta between dizygotic twins, sequestration of very minute maternal tissue into the fetus from the fetoplacental unit. Chimeras exhibiting different sex chromosomes may represent a distinct bisexual karyotype with 46, XY/46, XX genotype (true hermaphrodites; see also mosaicism).

The fundamental difference between mosaicism and chimerism lies in the multiplicity of sources of the cells in chimeras and a solitary source in the mosaics.

SUMMARY

(a) Numerical Anomalies

(i) Abnormalities in chromosome numbers may arise due to an increase (47, 48, etc.) or decrease (45) in the total number of chromosomes instead of normal complement of 46.

(ii) Anomalies in chromosome numbers usually occur during gametogenesis when homologous pair of chromosomes fail to separate from each other during meiosis I. This phenomenon is called **non-disjunction**.

(iii) The most commonly observed numerical chromosomal abnormalities are.

- Down's syndrome (trisomy 21)
- Patau's syndrome (trisomy 13)
- Edward's syndrome (trisomy 18)
- Turner syndrome (45, X)
- Klinefelter syndrome (47, XXY)
- XXX females
- XYY males.

(b) Structural Anomalies

(i) Aberration in chromosomal structure may result either from missing (deletion) or getting represented twice (duplication) of a piece of chromosome.

(ii) Structural abnormalities occur due to abnormal rearrangement of the chromosome.

Following are the most commonly observed structural abnormalities:

- Deletion
- Inversion
- Ring chromosome
- Isochromosome
- Translocation.

(c) Mosaicism

(i) An individual with two or more different cell populations is called a chimera.

(ii) The chromosomal constitutions of the cell lines vary in autosomal or the sex chromosomal number.

(iii) Chromosomal mosaicism is due to a non-disjunction occurring during an early embryonic mitotic division, isochromosomal splitting, anaphase lag, ring chromosome, etc. that cause unequal chromosomal distribution.

(d) Chimera

(i) An individual having two or more genetically distinct cell populations derived from more than one zygote or life source is called a chimera.

Chapter

6

Patterns of Inheritance and Disorders of Genes

- Single gene (Mendelian/Monogenic) inheritance
  - *Autosomal dominant inheritance*
  - *Autosomal recessive inheritance*
  - *Sex-linked inheritance*
- Mitochondrial inheritance
- Multiple genes (Polygenic/Multifactorial) inheritance
- Some important terms commonly used in relation to genetic inheritance

It is imperative to be acquainted with the patterns or the genetics of inheritance (dominant, recessive, sex-linked, etc.) before we can actually understand important aspects of a genetic disorder (disorders caused by abnormalities in a gene). In this chapter we shall learn about the patterns in which a defective gene is passed onto new generations and how these methods determine the degree of severity in the outcome of the diseases in the progeny. This understanding is helpful for an accurate diagnosis of genetic disorders and evaluating the risk of acquiring the genetic disease in a newborn.

Knowledge of the exact pattern of inheritance aids in suggesting measures that may prevent the inheritance of genetic disorders.

Common traits or disorders follow the following patterns of inheritance:

- **Single gene** (Mendelian/Monogenic) **inheritance**.
- **Multiple genes** (Polygenic/Multifactorial) **inheritance**.

SINGLE GENE (MENDELIAN/MONOGENIC) INHERITANCE

The traits in this type of inheritance are carried by single genes and the pattern obeys the Mendel's laws of inheritance (Chapter 1). The single gene inheritance is further classified into the autosomal and sex-linked inheritance types.

The patterns of transfer of genes present on autosomes determine Autosomal while sex-linked inheritance is established by the mode of transmission of genes present on sex chromosome (X or Y). Autosomal inheritance is further classified into **autosomal dominant** and **autosomal recessive** types. In case of an autosomal dominant inheritance, a dominant gene that is present in the genome of a person (somatic cell) expresses itself even if it is present in a single dose (heterozygous state). If such a mutation arises in the germ cells (gametes) of a person in the gonads, the trait is carried to the offspring and expresses itself clinically (in phenotype) even when the gene it is present in a single dose (heterozygous state). However, an autosomal recessive trait is manifested clinically only when a gene is present in double dose (homozygous state) on a pair of chromosome.

Genes present on sex chromosome (X or Y) determine the characteristics of sex-linked inheritance whether the trait is transmitted to the next generation by X-linked or Y-linked genes.

Formulation of a Pedigree Chart

A pedigree chart is a diagram showing the ancestral history of a group of relatives with relation to a particular disease in question in an individual. To investigate a genetic disorder it is important to record the family history of relatives in relation to the occurrence of that particular genetic disorder in them. The information about the health of the whole family is recorded in the form of a pedigree chart. Following are certain conventions used in the preparation of pedigree chart. (Fig. 6.1)

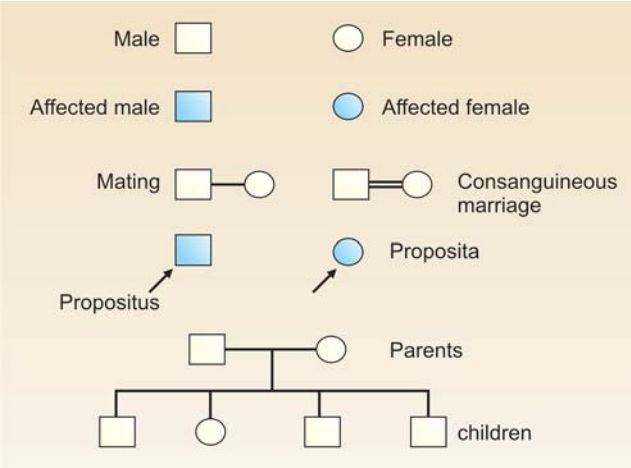


FIGURE 6.1: Symbols used in a pedigree chart

- Squares in the diagram represent males and circles represent females.
- Solid squares or solid circles symbolize affected persons.
- The individual being investigated and for whom the pedigree is prepared is termed as the proband; propositus (male) or proposita (female). The position of proband in the chart is indicated by an arrow.
- A horizontal line which connects a male and a female represents mating.
- The offsprings from a mating are represented in order of their birth from the left to the right.
- Roman numerals in the pedigree represent successive generations, e.g. I, II, III, etc. designated to each of the horizontal rows in the chart.

Autosomal Dominant Inheritance

An autosomal dominant trait or disease can be manifested in an individual if the gene responsible for this kind of trait or disorder is present on an autosome (nonsex chromosome). The expression of the trait or the disorder occurs even if the gene is present in a single dose, i.e. a normal dominant gene or a mutated yet dominantly expressing gene produces its effect even when it is present in only one of the chromosomes of a particular pair (heterozygous state). On the other hand such genes or traits that express in the heterozygous state are called dominant genes. These genes are transmitted following the laws of autosomal dominant inheritance. Some common examples of dominant traits and disorders in medicine and dentistry are:

- Achondroplasia
- Dentinogenesis imperfecta type 1
- Amelogenesis imperfecta hypoplastic type 2 (AIH2)
- Amelogenesis imperfecta hypocalcification type
- Hypodontia
- Osteogenesis imperfecta
- Huntington's disease
- Myotonic dystrophy
- Polycystic kidney disease
- Congenital cataract
- Polydactyly.

Distinctive Features of an Autosomal Dominant (AD) Trait

- An autosomal dominant trait or disorder is seen in every generation without skipping.
- The disorder may appear for the first time (*de-novo*) in an individual due to a new or fresh mutation occurring either at the time of gametogenesis in the parents or in the early period of embryogenesis in the individual. The new mutation obeys the laws of autosomal dominant inheritance.
- If not arising due to a new mutation, an affected person will always have an affected parent.
- Transmission is observed from male-to-male; from female-to-female; from female-to-male and from male-to-female, i.e. between all the sexes. The traits or disorders equally affect the males and the females. This signifies that the gene responsible for this type of inheritance is located on an autosome. Figure 6.2 shows equal distribution of traits in both the sexes.
- Autosomally dominant genes present in a heterozygous state have 50% chance to get transmitted and affect half of offsprings.
- The disorder cannot be transmitted further to next generations by the normal offsprings as they do not have the abnormal gene (Fig. 6.2).
- Theoretically, normal and affected children in a generation should be equal in number.

All the observations discussed earlier are explained in the **Punnett squares** below. We know that two genes present on a pair of homologous

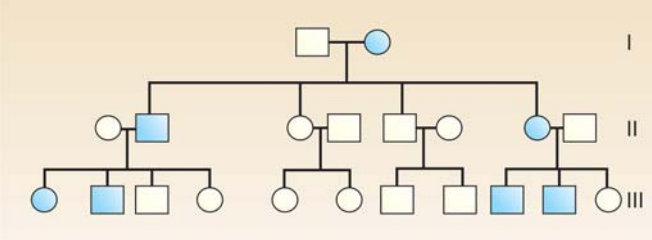


FIGURE 6.2: Pedigree in an autosomal dominant inheritance

chromosomes at the same locus are responsible for determining a particular trait. We also know that the expression of a trait will also depend on the 'dominance' or the 'recessiveness' of a gene. Certain normal genes as well as the effects of certain mutations in a gene always express in a dominant fashion. If we represent a normal gene as "**d**" and a dominant mutant gene as "**D**" then the genotype of one parent who is homozygous for the normal allele will be "**dd**" and will be normal. The other person or parent bearing the dominant mutated gene (in heterozygous state) will have a "**Dd**" genotype. The genetic risk of outcome in such mating can be calculated as shown in Figure 6.3.

In another situation a mating between both affected parents carrying a normal and a dominantly mutated gene (**Dd)** produces a different set of affected offsprings (Fig. 6.4).

Offsprings with homozygous (**DD**) genotype would be severely affected due to presence of the mutant genes in double doses (Fig. 6.4).

Common Autosomal Dominant Disorders

Some of the common medical disorders showing Autosomal dominant inheritance are enumerated

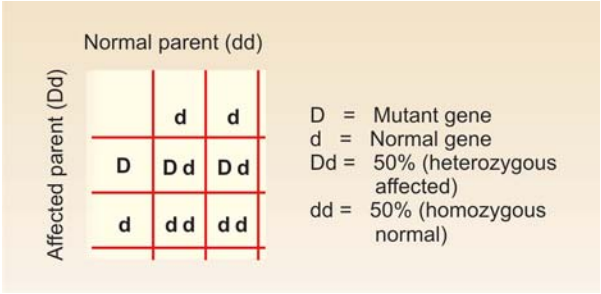


FIGURE 6.3: Mating between affected (Dd) and normal (dd) parents, (Dd × dd)

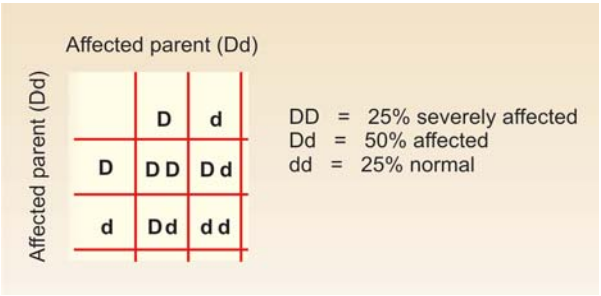


FIGURE 6.4: Mating between two affected (Dd) parents, (Dd × Dd)

below with their brief characteristics. The Autosomal dominant dental disorders are dealt in the appropriate sections of the book later.

Achondroplasia

Achondroplasia is an autosomal dominant trait. It is a class of skeletal growth syndromes characterized by short stature due to slow development of the middle portions of the long bones in the arms and legs. The most common form of *achondroplasia* is due to a defect of *the Fibroblast Growth Factor Receptor* (**FGFR**), and is recognized by exaggerated cranial growth and bossing (depression) at the bridge of the nose. A second form is pseudoachrondroplasia, which is due to a defect of Cartilage Oligomeric Matrix Protein (**COMP**) in the joints, and is characterized by more typical development of cranial proportions. The COMP gene is situated on chromosome 19 (19q13.1). Both forms of achondroplasia are described as sporadic, meaning that they occur in different families due to independent mutations. Thus, most affected children are born to parents of ordinary stature, one of whom has a germline mutation. In the children of two parents with achondroplasia (**Dd** × **Dd**), most affected offspring are heterozygous (**Dd**), which suggests that the homozygous dominant genotype (**DD**) is lethal.

Shown in the accompanying photograph are seven pseudoachondroplasic members of the **Ovitz family**, a family of Romanian Jews who toured Eastern Europe as a musical troupe before World War II (their taller siblings working backstage), survived imprisonment at Auschwitz, and finally immigrated to Israel. They were photographed on arrival in Haifa in 1949. Their father was (apparently) of ordinary

(Photo and text of Ovitz family is produced here with the kind permission of Dr. Steven M Carr, Professor of Biology, Memorial University, Newfoundland, Canada, http://www.mun.ca/biology/scarr/Achondroplasia.html).

height and was twice married, both times to women of ordinary height. With his first wife, he had two affected daughters [possibly the two older women in flowered dresses], and with his second five affected children (three girls and two boys) shown here, as well as three children of ordinary height. This suggests the father had a germ line mutation.

Huntington's Chorea

Characteristics of the disease: Incidence is approximately 1 in 15,000 people. This is a fatal disorder that usually begins with disorders of movement. Occurs in the middle ages though 10% of disorders are seen before the age of 20 years (juvenile variety). Shows phenomenon of anticipation (explained later). Characterized by involuntary movements like facial grimacing, limb movements followed by unsteady gait and slurred and unclear speech. Intellectual impairment and dementia precede death.

There is a progressive loss of neurons due to cell death in the CNS. This autosomal dominant disease shows complete penetrance. The mutation is located on the short arm of chromosome number 4 that codes for an abnormal protein called **Huntingtin** which has been implicated for cell death or apoptosis in the central nervous system.

Autosomal Recessive Inheritance

An autosomal recessive trait is only expressed when the responsible gene or the mutation exists in a homozygous state and as such in double dose. Following is a list of some common autosomal recessive disorders and traits.

- Cystic fibrosis
- Amelogenesis imperfecta (local hypoplastic type)
- Amelogenesis imperfecta (pigmented hypomaturation type)
- Neonatal osseous displasia 1
- Sickle cell anemia
- Phenylketonuria
- Schizophrenia
- Alkaptonuria
- Spinal muscular atrophy
- Albinism.

Distinctive Features of an Autosomal Recessive (AR) Inheritance

Autosomal recessive inheritance is characterized by the expression of gene or traits only when they are present in a homozygous form in the genome.

The presence of an autosomal recessive trait or a mutation in a heterozygous state does not express the disorder and the individual is perfectly healthy. An individual who is heterozygous for an autosomal recessive trait is called **carrier**.

Autosomal recessive mode of inheritance can be distinguished by the following features:

- Brothers and sisters (siblings) in the same generation manifest the trait. The trait is not seen in previous (parents) or in subsequent generations (offsprings). The disorder is seen only in the IVth generation and not in either of the IIIrd or Vth generation as depicted in Figure 6.5.
- Members of both the sexes are equally affected.
- The proband usually have closely related parents.

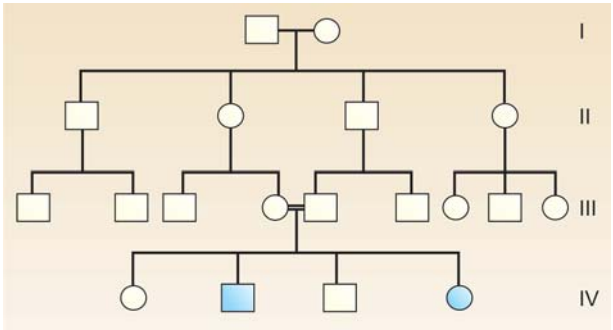


FIGURE 6.5: Pedigree in a typical autosomal recessive inheritance

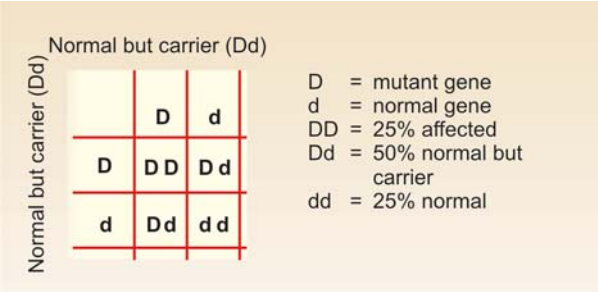


FIGURE 6.6: Mating between two heterozygous (carrier) parents, (Dd × Dd)

(Fig. 6.5) Such an offspring is said to arise from a consanguineous marriage or union. Each of the parents is a carrier (heterozygote) for the particular trait or disease.

- The results of mating between two carriers of an autosomal recessive trait (**Dd**) are shown in Figure 6.6.
- In case of mating between an affected individual (homozygous, **DD**) and a carrier (heterozygous, **Dd**), 50% of the offsprings get affected (homozygous) and 50% obtain a carrier status (heterozygous). This pattern may be easily confused for a dominant inheritance (Fig. 6.7). This kind of inheritance is thus known as **pseudo-dominant inheritance**.

Some Common Autosomal Recessive Disorders

Many of the disorders in medicine, especially those involving errors of metabolism exhibit autosomal recessive type of inheritance. Dental diseases that inherit in this pattern are discussed later in appropriate sections. Some of the medical disorders are mentioned below.

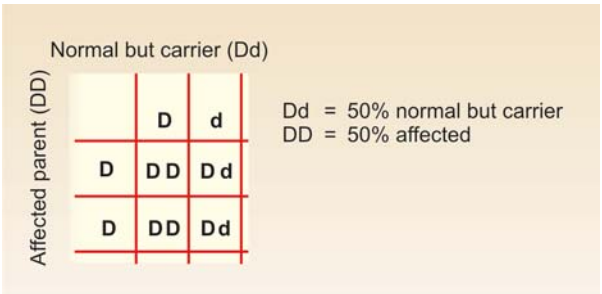


FIGURE 6.7: Mating between one carrier (Dd) and one affected (DD) parent, (DD × Dd)

Spinal Muscular Atrophy

Characteristics of the disease: Affected individuals exhibit progressive weakness of muscles resulting from degeneration of the spinal motor neurons. Type I of the disease is severest characterizing hypotonia and lack of spontaneous movement in infants. Type II and III are milder form of the disease with a later age of onset. Death usually occurs within first two years of life due to impairment of swallowing and respiratory functions. The milder form of disease results in recurrent respiratory failures but death is often delayed. The gene responsible for SMA disorder is situated on the long arm of chromosome number 5.

Cystic Fibrosis (mucoviscidosis)

Characteristics of the disease: Occurs in 1 out of 2500 people in the population. It is a common and fatal disorder seen in the children of white populations of Europe. Cystic fibrosis (CF) is characterized by the accumulation of thick, sticky, honey like mucous fluid which leads to blockage of airways, intestines and other viscera causing secondary infections. Death results mostly due to the obstruction of the respiratory tract. The lung tissue gets fibrosed leading to cardiac failure (corpulmonale). Blockage of the pancreatic ducts, malnourishment, cirrhosis of the liver, occasional congenital bilateral absence of vas deferens in males (making them sterile) are some of the features associated with the disease.

Patients usually die in childhood or adolescence. But life can be prolonged up to 30 years of age with effective treatment. The gene implicated for CF is present on the long arm of chromosome number 7. The CF gene was cloned in 1989 and was named cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR protein contains 1480 amino acids which act as a chloride channel. The mutation occurs at the 508th codon resulting in the loss of the amino acid phenylalanine. The defective chloride channel causes increase in the level of extracellular chloride and accumulation of intracellular sodium. The chloride content of secretions makes them viscid and sticky. Prenatal diagnosis should be advised to the parents of an affected child for future pregnancies.

Sex-linked Inheritance

Meaning and it Variance

Sex-linked inheritances are defined as inheritances linked to inheritance of genes on the sex chromosomes. This pattern of inheritance shows two variances; the Y-linked and the X-linked types of sex-linked inheritances.

Y-linked Inheritance: Only a very few traits transmit as **Y-linked** inheritances. The H-Y histocompatibility antigen gene, the testes differentiating gene and gene related to spermatogenesis and the hairy ear gene are carried on the Y-chromosome.

Y-linked traits are transmitted from an affected male to all his sons but not to his daughters (female offsprings do not receive any Y- chromosome). Males are the exclusively affected sex by a Y-linked disorder. All sons of affected males inherit the trait and females never transmit or receive the trait.

X-linked Inheritance: The term X-linked inheritance denotes all types of sex chromosome linked inheritance. Given the paucity of genes on the Y chromosomes, inheritances linked to the Y chromosome are only a very few. Thus all sex linked inheritances are grouped in X-linked nomenclature. Recessive or dominant are the two varieties of X-linked inheritances.

X-linked Recessive Inheritance: All genes on the X-chromosomes are not involved in the determination of sex. Some of them are functionally similar to the structural genes present on the autosomes. These genes have functions other than determination of sexual phenotypes, e.g. the gene for color perception or the gene coding for blood clotting factors. Following are few X-linked recessive disorders:

- Diabetes mellitus
- Hemophilia
- Ectodermal dysplasia type 4
- Amelogenesis imperfecta hypomaturation type (AIH).
- Chondrodysplasia punctata -1
- Duchenne muscular dystrophy.

Males have only one X-chromosome while females have two of them. Recessive traits manifest only when the responsible genes are present in double doses (homozygous state). Hence it is quite rare to find females with recessive traits linked to the X chromosome. A female who is heterozygous for a particular gene or trait or mutation on the X chromosome does not manifest the trait. The normal allele present on the locus of the other homologous X-chromosome compensates for recessive mutation. However, a heterozygous female can transmit the gene to the next generation. Hence heterozygous females for recessive trait are called **carriers**. On the contrary, as the males have only one X chromosome, even a single recessive mutant gene present on the solitary X chromosome would produce the diseased phenotype (compensation is not possible for the mutant gene as they do not have a normal allele). A male with a mutant gene on his X chromosome is called **hemizygous** for that particular allele. The affected male transmits the gene to all his daughters who will become carriers and further transmit the disease to 50% of his grandsons through the daughter.

Distinctive Features of X-linked Recessive Inheritance

- Males are affected predominantly. The mutated gene only when present in double doses (homozygous situation) in the female is capable of causing the disorder, in them.
- Traits are transmitted to the sons through unaffected but carrier females.
- The affected males can never transmit the disorder to their sons as the concerned gene is not present on the paternal Y-chromosome transmitted to the son (Figs 6.8 and 6.10).

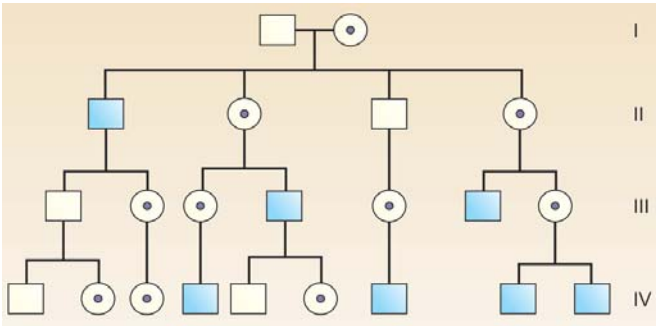


FIGURE 6.8: Pedigree of X-linked recessive inheritance

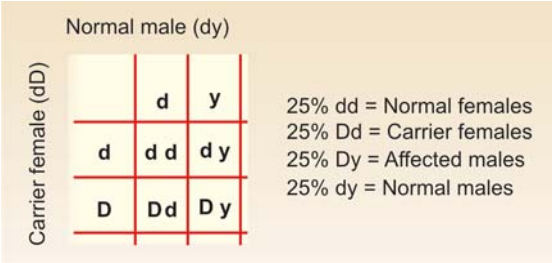


FIGURE 6.9: Mating between normal male (dy) and carrier female (dD)

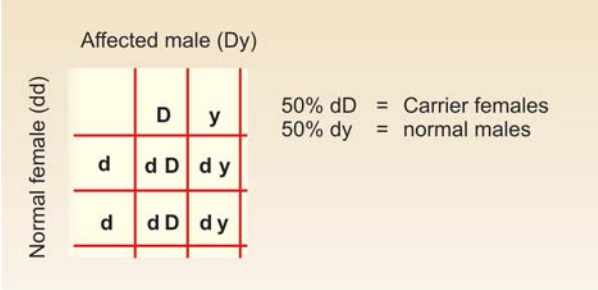


FIGURE 6.10: Mating between affected male (Dy) and normal female (dd). Male offspring are always normal

- As the mutant gene on X-chromosome is received from a normal but carrier mother, affected males usually have normal parents (Figs 6.8 and 6.9).
- Females may show minor effects of a sex-linked recessive trait in cells where the normal X-chromosomes get randomly inactivated (Lyon's hypothesis).

Risks involved in inheritance of ***X-linked*** recessive traits are calculated below in the Punnet squares (Figs 6.9 and 6.10). Symbol "d" represents a normal allele and "D" denotes its mutated form. Therefore the genotype of a carrier female would be "**Dd**" and the genotype of normal male will thus denoted as "**dy**" with **"d"** representing the normal gene present on the single X-chromosome. The small 'y' represents the Y- chromosome that lacks in the presence of a homologous gene.

Some Common X-linked Recessive Disorders

Some of the common medical conditions transmitted as X-linked recessive disorders are enumerated below.

Duchenne Muscular Dystrophy (DMD)

Characteristics of the disease: The incidence of the disease is rated at 1 in 3500 males. DMD is the commonest and most severe form of muscular dystrophy seen in males. Slow and progressive muscle weakness start presenting soon after the age of 3 years. Proximal muscles of lower limbs are severely affected with weakness and wasting. Pseudohypertrophy occurs in the calf muscles as they get replaced by fat and connective tissue. Patients cannot walk by the age of 11 years and the boys get wheel chair bound early. Joint contractures and respiratory failure leads to death around 18 years of age.

The gene is located on the short arm of X-chromosome (Xp21). DMD gene encodes a protein known as dystrophin. Dystrophin acts a link protein between muscle fibers and surrounding cell membrane and helps in transmission of the power of muscle contraction. Absence of which is responsible for muscle cell degeneration. This is also associated with an increased permeability of muscle membranes that leads to the escape of muscle enzymes into the blood. Increased levels of serum creatine kinase (ck) are indicative of the disease along with clinical assessment. Physiotherapy is helpful.

Hemophilia: This is a disorder of blood coagulation exhibiting an X-linked recessive trait of inheritance. Factors VIII and IX are absolutely necessary for blood coagulation. These factors convert prothrombin to thrombin. Thrombin further converts fibrinogen to fibrin eventually forming the structural framework for blood to clot. Hemophilia A and B are the two different kinds of the disease encountered commonly. Hemophilia A (royal hemophilia) is caused by deficiency of factor VIII with an incidence of 1 in 5000 males and several royal families of Europe have suffered this kind of hemophilia (Fig. 6.8).

Hemophilia B, also known as Christmas disease, is caused by the deficiency of factor IX and has an incidence of 1 in 40000 males.

Characteristics of the disease: Minor trauma or surgery causes prolonged bleeding from the wound. The disease is noted for bouts of spontaneous bleeding that occur into muscles and joint cavities. The genes for hemophilia A and B are located on the long arm of X- chromosome near its distal end. Transfusion of plasma-derived factors VIII or IX often used as replacement therapy. Gene therapy may be available for more effective treatment in future.

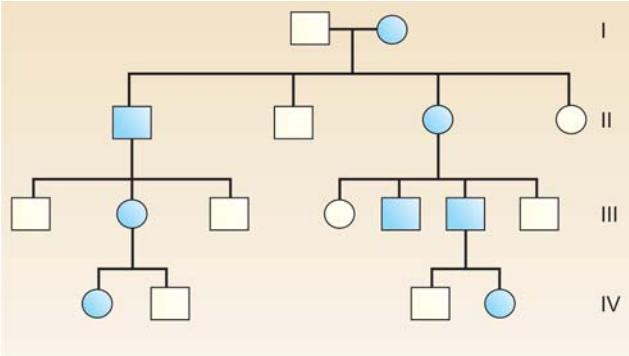


FIGURE 6.11: A pedigree of four generations showing X-linked dominant inheritance. Affected male transmits this disease to none of his sons

X-linked Dominant Inheritance

This type of inheritance is related to the transmission of a dominant or mutant gene located on the X chromosome. As the gene or the mutation is dominant it expresses even when it is present in the heterozygous state. As a result females as well as males manifest the trait when the gene is present in a single X-chromosome. The X-linked dominant inheritance resembles an autosomal dominant inheritance. It can however be differentiated as an affected male having an X-linked dominant trait will transmit this trait to all his daughters but to none of his sons. Therefore to distinguish this trait from autosomal trait one has to follow the progeny of affected male (Fig. 6.11).

Disorders that show X-linked dominant characteristics:

- Vitamin D-resistant rickets
- Xg-blood groups
- Amelogenesis imperfecta (Hypoplastic).

MITOCHONDRIAL INHERITANCE

All mitochondria and hence entire complement of mitochondrial DNA (mtDNA) is inherited from the maternal gamete, the oocyte. Mitochondrial inheritance is also known **cytoplasmic inheritance** as the entire cytoplasm of the zygote is derived from the maternal gamete (oocyte).

The mitochondrial disorders are only transmitted through the mother to all her sons and daughters. Affected males cannot transmit the disease to their offsprings.

- The mtDNA shows high rates of mutation as compared to the nuclear DNA. Mitochondrial mutations in DNA may cause diseases. Most of these conditions decrease the ATP generating capacity of a cell or tissue. Common tissues to be affected thereby are muscles and the nervous tissue that normally use large amounts of energy for functioning. Hence mutations in mtDNA lead to a wide range of clinical features including hypotonia of skeletal muscles, cardiomyopathy, neuropathy, seizures, dementia, encephalopathy, ataxia, stroke, dystonia and acidosis. Some of the mtDNA disorders are named as Leber's Hereditary Optic Neuropathy; Neurodegeneration Ataxia and Retinitis Pigmentosa (NARP), Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke like Episodes (MELAS), Myoclonic Epilepsy and Rugged Red Fiber Disease (MERRF), etc.

At the onset of a mitochondrial disease only a few mitochondria get mutated while others remain normal. Therefore the expression of the disease varies depending upon the number of mutant or affected mitochondria in the cell. As and when the proportion of mutant mitochondria increases with time, the clinical severity of the disease also increases from "mild" to "severe" degrees. **Heteroplasmy** is the simultaneous existence of two populations (normal and mutant) of mitochondria within a cell.

MULTIPLE GENES (POLYGENIC/ MULTIFACTORIAL) INHERITANCE

Quite a few and fairly common traits and disorders do not follow the pattern of simple Mendelian (single gene) inheritance. Several common traits like intelligence, blood pressure, height, weight, hair color, color of the eye, facial appearance, etc. have a far more complex genetic basis. Only two different types of phenotypic individuals would have resulted in humans (one short and the other tall) if the trait of height were to be determined by just a pair of genes as in case of Mendelian experiments with garden peas. If we assume **'T'** to represent the trait of tallness and 't' to denote shortness in an individual, the genotype in the tall would either be 'TT 'or 'Tt'. The short would have 'tt' as the genetic composition for the trait. However we encounter individuals whose heights show remarkable quantitative variations from one

extreme to the other even within a single family. These are called **Continuous traits** or **quantitative traits** and cannot be clearly classified into distinct groups but are measured quantitatively.

Just as we are unable to predict height or blood pressure, a number of disorders also do not follow Mendelian (monogenic) laws of inheritance and hence cannot be measured. But these diseases have no intermediate forms even though they are governed by multiple factors. These disorders are either present or absent in a person and are called threshold traits, e.g. a person is either a diabetic or a nondiabetic. A few such examples are:

Congenital Malformation	Adult Onset Disease
Neural tube defects	Diabetes mellitus
Cleft lip	Hypertension
Cleft palate	Ischemic heart disease

The cumulative or additive effects of several genes which are situated at different loci on chromosomes determine these threshold traits. This kind of inheritance is called **polygenic inheritance**. Genes in polygenic inheritance do not behave as dominant or recessive but exert a collective effect on the outcome of the trait.

It is as a result of interactions between genetic and environmental factors that almost all common physical traits, disorders or congenital malformations are expressed. They don't depend entirely on a single gene for their existence. Factors like diet, sunlight, diseases, chemical exposure, radiation, etc. may influence activity in genes and its final outcome. Polygenic inheritance is therefore also called **Multifactorial inheritance**.

SOME IMPORTANT TERMS COMMONLY USED IN RELATION TO GENETIC INHERITANCE

- **Pleiotropy**
  Autosomal dominant gene generally results in a single effect and by and large involves a particular organ or part of the body. However, when a single gene disorder produces multiple phenotypic effects, it is termed as pleiotropy. In cases of the collagen disorder osteogenesis imperfecta, the causative mutation produces defective collagen. However, this diseased collagen leads to many other effects like osteosclerosis, blue sclera and brittle bone etc. giving rise to pleiotropy.
- **Variable expressivity of gene:** Autosomal dominant genes can vary in expression from person-to-person. In clinical terms the spectrum of the expression of a gene may range from its mild, moderate to the severest of forms of the trait. A common example is that of polydactyly (extra finger). The occurrence of this dominant trait in individuals may vary from having a rudimentary small digit-like structure to a fully developed extra finger. This is explained by the degree of variability in expression of a gene.
- **Reduced or incomplete penetrance:** It is a variant of variable expressivity. Reduced or incomplete penetrance signifies variable expression of a dominant gene that is present in the heterozygous condition. Individuals with this condition fail to manifest the disorder clinically. Thus a dominant trait may appear to have skipped a generation in spite of the gene being inherited in the subject. Penetrance of a gene in any generation is expressed in terms of percentage (%) calculated from the number of offspring showing the trait as compared to expected number of affected individuals.

  Reduced penetrance or the variation in the expression of gene may be due to influence of the activity of other genes at different loci. It may be also due to difference in environmental factors.
- **Sex-limited traits:** Sometimes the expression of a trait is limited to one sex. The expression of sex- limited traits in humans is represented by the growth of facial hair normally in the males and the development of breast in females.
- **Sex-influenced traits:** The expression of the trait of common baldness shows sex influence. The trait of baldness behaves as an autosomal dominant trait in males and hence is quite common in men while it acts as an autosomal recessive trait in females; a bald female is seen very rarely. Thus a trait or a characteristic is said to be sex-influenced when it expresses differently in males and females.

- **Codominance:** Situations where both the alleles representing a trait are expressed fully despite existing in the heterozygous state they are called codominant. For example, a person with the blood group AB possesses both the allelic genes A and B at the loci that are related to blood groups in humans (near the tip of long arm of chromosomal 9). The genes for the antigen A and antigen B are therefore codominant as both get expressed on the red blood cells.
- **Intermediate inheritance:** A recessive trait or an abnormal recessive (mutant) allele is unable to express itself in a heterozygous state. It only manifests itself in a homozygous situation. However in certain conditions a recessive gene exhibits intermediate levels of expression even in the heterozygous condition. The degree of expression remains somewhere between the levels of an abnormal homozygous and that seen in a normal homozygous state for that recessive trait. In sickle cell anemia individuals having the mutation in a heterozygous state possess both abnormal as well as normal hemoglobin in blood.
- **Anticipation:** If a genetic disease manifests in one generation at an earlier age than the age of occurrence in the previous generation, the disease is said to exhibit 'anticipation'. This advancement of the age of, occurrence in seen in every successive. Generation e.g. as seen in Huntington's disease.
- **Genetic imprinting:** Sometimes there is a differential expression of a mutated gene in an individual depending on whether the gene is derived from the father or the mother, e.g. Prader Willi syndrome (PWS) and Angleman Syndrome (AS). Both these disorders are caused by micro-deletions of chromosome 15. If the mutated 15th chromosome is derived from the father it results in PWS whereas, if the mutated chromosome is maternal in origin it causes AS; both diseases have different clinical manifestations.
- **Uniparental disomy:** Sometimes both chromosomes of a pair of chromosome may be derived from a single parent instead of the usual one maternal and one paternal copy in a pair. This may give rise to syndrome like the PWS.
- **Compound heterozygote**: An individual is said to be a compound heterozygote if both the genes located at the respective loci are mutated. Thus the individual is a heterozygote for a particular trait where both the genes are defective or mutated.

SUMMARY

(a) Common traits or disorders follow either **monogenic** (single gene) or **polygenic** (multiple genes) patterns of inheritance.

(b) Single gene or mutation present on an autosome (**autosomal inheritance**) or sex-chromosome (**sex-linked inheritance**) is regulated by monogenic or mendelian inheritance.

(c) **Autosomal dominant inheritance** expresses itself even if the dominant gene or mutation is present in a heterozygous state (single dose).

(d) **Autosomal recessive inheritance** is manifested only when the responsible gene is present in a homozygous condition (double dose).

(e) Features of **autosomal dominant** inheritance.
- As the gene is present on an autosome, both sexes are equally affected.
- Half of offsprings are affected in each generation and the normal offsprings do not transmit the disorder to the next generation.

(f) **Pleiotropy –** The condition where a single gene disorder produces multiple phenotypic effects is called pleiotropy.

(g) Features of **autosomal recessive** inheritance.
- The disorder is seen only the siblings in one generation.
- Both sexes are equally affected.
- Parents of affected offspring are closely related (Consanguineous).

(h) Features of **X-linked recessive** inheritance.
- Primarily only the males are affected.
- Carrier females are unaffected and transmit the disease to their sons and gets half daughters as carriers.

- Affected males cannot transmit the disease to their sons as the gene is present on the X and not the Y-chromosome.

(i) Features of **X-linked dominant inheritance**.
- Males and females are equally affected.
- The trait or the mutation expresses itself even when it is present in a heterozygous condition.
- Affected males transmit the trait to all his daughters but not to any of his sons. Affected females pass the trait to 50% of all her offsprings.
- Resembles autosomal dominant inheritance (pseudo autosomal dominant) except that transmission is skipped in case of male-to-male inheritance.

(j) Features of **mtDNA inheritance.**
- Only the mother is responsible for transmitting disease to all her sons as well as daughters.
- Diseased males cannot transmit the disease to their offsprings.

(k) **Polygenic** inheritance concerns with the expression of a trait or disorder that is determined by additive effects of interplay between activities of many genes situated at different loci on various chromosomes.

(l) These traits are also not entirely defined by interaction between genes but also are influenced by diverse environmental factors. **Multifactorial inheritance** is synonymous with polygenic inheritance.

Chapter

7

Genetics of Immunity

- Concept of immune mechanisms
- Structure of immunoglobulins
- Immunodeficiency disorders
- Transplantation of tissues

CONCEPT OF IMMUNE MECHANISMS

System of Immunity

Viruses, bacteria, parasites and several other pathogens surround us at all times. Though some of these organisms reside normally in the body (commensals), some of them can breach the defense barricades and mechanisms of the body and cause diseases in humans. The body has, in fact, a well-developed system that fights such an attempt of invasion and thus prevents diseases that would perhaps be fatal had not the system been in existence. The capability of an organism to prevent or modulate the occurrence of diseases both from within and without is called **immunity**. The system not only recognizes and destroys viruses, bacteria and other pathogens that attack the body from outside but also eliminates cancerous cells and toxins that arise within the body. The system is called the **immune system** for the function it serves. The immune system has the unique ability to differentiate between cells and tissues that belong to the same body (self) and those derived from sources other than the same body (nonself) and decide selectively to preserve (self) or to attack and destroy (nonself) cells or tissues.

Components of Immunity

Immune mechanisms operate chiefly through cells that circulate in the blood and mediate the actions of the immune system. The cells are the white blood cells called the lymphocytes. The formation of lymphocytes is initiated in bone marrow from the resident bone marrow precursor cells that are known as the **stem cells**. The undifferentiated cells gradually mature and differentiate into functional lymphocytes. The functional lymphocytes are of two different kinds, the **T** and **B** lymphocytes. The T lymphocytes are differentiated and activated in thymus hence, also called as thymus-dependent cells. The B lymphocytes probably undergo differentiation as well as maturation in bone marrow itself and hence known as bone marrow derived cells. Lymphoid organs (lymph nodes, spleen, tonsils, Peyer's patches, etc.) act as stores for most of the B cells in the body. The T cells however are always actively circulating in blood stream. The other equally important component of the immune system is derived from the reticuloendothelial system called the **macrophages**.

Microorganisms and pathogens constantly attack our body and try to invade through wounds, discontinuous epithelial surfaces, the respiratory and the GI tracts, etc. and access the blood or any suitable place for them to multiply.

The immune system is activated in several steps by such an incursion by pathogens from outside or from developments within.

(1) T-cell Immunity or Cell Mediated Immunity

A large number of T lymphocytes are activated that are involved in executing this type of immunity. T lymphocytes are specifically activated to identify signals on the pathogens and to destroy them. The action of these cells is also facilitated by the action of other immune cells. There are several types of T cells

(e.g. killer T cells, suppressor T cells and helper T cells) that serve different functions.

(2) B-cell Immunity or Humoral Immunity

Humoral immunity is mediated by the B lymphocytes. These lymphocytes are transformed to plasma cells under the influence of several factors. These plasma cells synthesize antibodies or immunoglobulins. Several varieties of immunoglobulins attack and destroy invading organisms.

Functioning Components of the B-cell Immunity

Antibody

Antibodies are defined as protein molecules synthesized by organisms in response to the presence of a foreign substance, in order to neutralize its effects. An antibody has a specific affinity for identification of and binding to the foreign material against which it is synthesized by a plasma cell. Antibodies are produced by plasma cells which are derived from activated and transformed B lymphocytes. A single plasma cell produces and secretes about 2000 identical copies of antibody molecules every second and secretion continues unabated for approximately 4 to 5 days till the plasma cell survives.

Antigen

Antigens are defined as immunity stimulating substances (a foreign macromolecule) that are capable of inducing antibody formation from B lymphocytes. Many substances are antigenic in nature (anything which is as big as or bigger than a protein molecule may act antigenic). Antibodies are produced against *epitopes*, the minimum sized protein molecules that can excite the formation of an antibody.

Basics of Immune Response

Specific and characteristic identifiable features are located on the surface of antigens like viruses, bacteria and cells of higher organisms that are called **antigenic determinants (epitopes)**. A large molecule or cell may contain hundreds of different antigenic determinants or epitopes. The immune system in an individual gets activated against a cell or a substance inside the body if the system detects even a single epitope on that cell that is different or absent in its own system. A typical antigen includes several different epitopes and therefore induces the production of many different antibodies. Antibodies are produced in response to all varieties of antigenic determinants in an antigen.

B or T cells themselves are capable of detection of antigenic determinants of an antigen. They are facilitated by certain other immune cells for such an activity. Each B cell or T cell is capable of recognizing only one antigenic determinant on an antigen. Certain receptors on the surface of the T or B cells are configured exactly to match and bind with a specific antigenic determinant on the surface of an antigen (like a lock and key). A determinant specific binding leads to cell division in the B lymphocyte. Mitosis is stimulated and the resultant daughter cells formed are of the same genetic constitution. These cells are called **clones**. Some of these clones differentiate to become antibody secreting plasma cells. A particular clone is capable and destined to secrete just a single kind of antibody that is directed against the epitope of the antigen that initiated the B cell proliferation into a plasma cell. The subsequent combination of an antigen with its specific antibody is termed as **antigen-antibody complex** (Fig. 7.1).

Complement System

The complement system comprises of about 20 plasma proteins that are triggered in a cascade on activation. This 'activation' is triggered with the formation of the antigen-antibody complex. The components of the complement system are activated sequentially and attack the antigen-antibody complex. The component of the complement system are basically certain activated plasma proteins capable of destroying trapped microorganisms and toxins in the system.

The complement system is a component of the immune system and hence acts either directly or indirectly (with activation of mast cells and macrophages) to destroy the offending pathogens in the body.

Diversity of Immune Response

There is a large diversity of antigens in the nature that interact with the immune system of our bodies' day

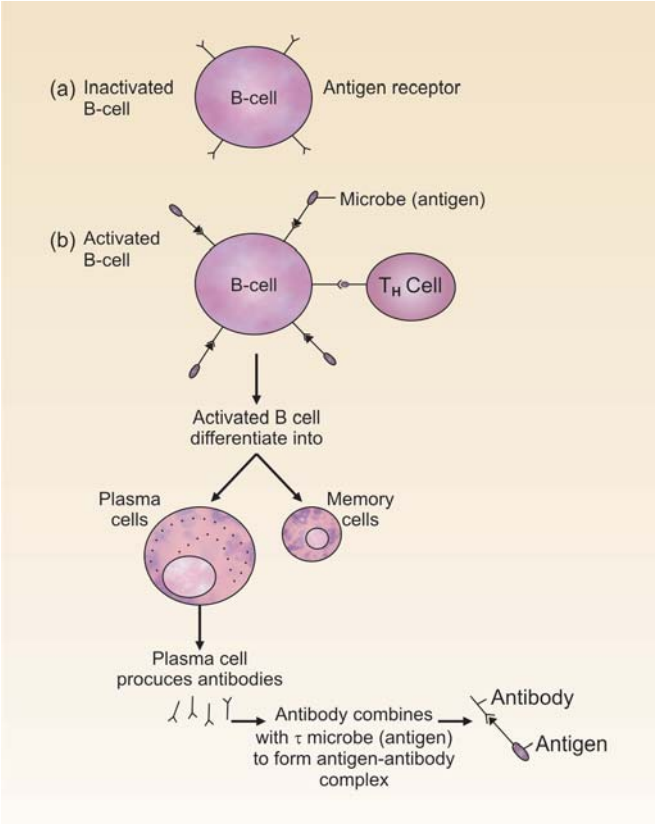


FIGURE 7.1: Schematic diagram showing the activation of a B cell to form a plasma cell and subsequent formation of antibodies and an antigen-antibody complex

in and day out. As a consequence, there are a multitude of receptors on the surfaces of the immune T and B cells that bind to the different antigenic determinants. Since there are millions of antigens and antigens receptors, there are millions of varieties of antibodies. It is a fact that antibodies are proteins and their synthesis is under genetic control. The obvious question comes up in mind that how a million of different antigen receptors as well as antibodies can possibly be generated from the available number of genes in the human genome.

To understand the mechanism of production of such enormous and diverse spectrum of antibodies we should first understand the structure of antibody (immunoglobulin).

STRUCTURE OF IMMUNOGLOBULINS

Chemically immunoglobulins are glycoprotein in nature. Most antibodies contain four polypeptide chains. Two of these chains are long (each consists of about 450 amino acids) and are called **heavy (H)** polypeptide **chains**. These two chains are identical to each other. Short carbohydrate chains are attached to each heavy chain (Fig. 7.2).

The two other chains are called **light (L)** chains or short polypeptide chains. They are also identical to each other and consist of approximately 220 amino acids.

Each light chain holds to the respective heavy chain with a disulfide bond. Two heavy chains are attached to each other approximately at their middle with the help of a disulfide bond. The region where the two heavy chains are connected is flexible and is called the hinge region. Because of this flexibility an antibody can assume a configuration that resembles the letter T or Y shape.

A heavy and light chain can be divided into two distinct regions. The tips of the H and L chains consist of the **variable (V) region** while the remaining region is called as **constant (C) region**.

The antigen binding region of the antibody is the variable (V) site. The antigen binding site of an antibody is typically very similar in structure to that of an epitope of an antigen to which it binds. The variable regions are different in each kind of antibody but are specific to the antigen it binds. The variable region is responsible for the detection and attachment to a particular epitope in a particular antigen. Most antibodies have two antigen binding sites.

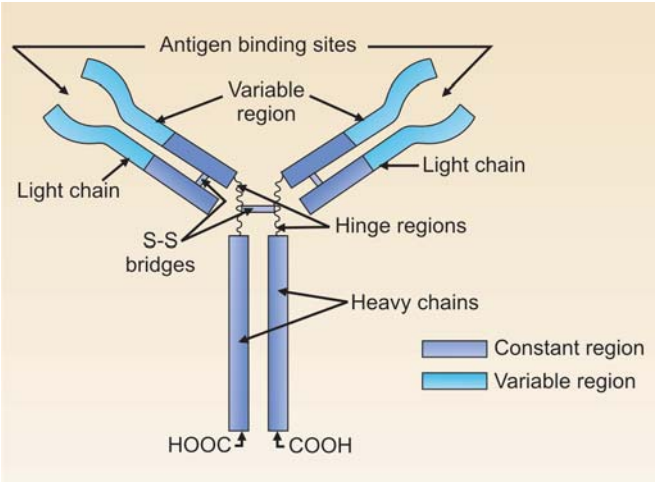


FIGURE 7.2: Schematic diagram of a typical antibody molecule. It is composed of four polypeptide chains (two heavy and two light) that are held together by S-S bridges. The area of the antigen binding site consists of regions with variable amino acid sequences

For all antibodies of a single class the constant (C) regions of H and L chains are nearly identical in structure.

The heavy chains are grouped into five classes of, γ, μ, α, δ and ε. The five different classes of immunoglobulins (antibodies) are determined by the chemical constitution of the five heavy chains. These different classes are IgG, IgA, IgM, IgD and IgE types. Table 7.1 denotes the distinctiveness and utility of various immunoglobulins.

Two different kinds of L chains exist in a given antibody namely the κ (Kappa) or the λ (lambda) chains. Thus the molecular formula of IgG is δ2λ2 or δ2κ2.

Approximately, one million antibodies are present in an individual and each of them differs in their antigen-binding specificity and affinity. It can thus be concluded that the variable region of an immunoglobulin molecule shows a wide range of variability; each with a different configuration. This structural variability is brought about by different combination of arrangements in the amino acid sequences at the variable end of the antibodies.

Determination of Diversity in an Antibody

- Chromosome number 2 and 22 bear the genes responsible for syntheses of the κ and λ light chains respectively and a gene on the chromosome 14 codes for heavy chains.
- The amino terminal ends (variable regions) of both the heavy and the light chains contain amino acid sequences of about 115 amino acids. Different types of antibodies constitute different sequences in the variable regions of both the heavy and the light chains. The carboxyl terminal end is made up of about 110 amino acids in the light chains kappa and lambda and forms the constant region C. The heavy chain has a constant region that is three to four times longer than that of the light chain.
- The DNA segments coding for the V region are separate from those that code for the C regions. This fact is established from restriction map study of the DNA segments that are responsible for coding of the C and V regions of κ or λ light chains. The joining (J) regions that join the variable (V) and constant (C) portions of the antibody molecules are coded by the intermediate portions of DNA segments between the V and C coding regions.
- Placed between the V and J regions, the heavy chain possesses even a fourth region known as the diversity or D region (Fig. 7.3). Noncoding DNA sequences separate each coding region in the DNA segments (V, D, J and C coding sequences).
- Further, the variable regions of a given chain are coded by a large number of DNA segments. The D, J and C regions of the chain, in comparison, are coded by relatively few number of DNA segments (Fig. 7.3).

TABLE 7.1: Various antibodies and their functions

Name	*Molecular formula*	*Percentage of antibody and serum concentration (mg/ml)*	*Function of antibody*
Ig G	$\gamma_2\lambda_2$ $\gamma_2\kappa_2$	80% of all antibodies 8 - 16	Protect against bacteria and viruses. Crosses placenta to provide immune protection in new born
Ig M	$\mu_2\lambda_2$ $\mu_2\kappa_2$	5-10% of all antibodies 0.5 - 2	Serves as antigen receptors. Antibodies of ABO blood groups
Ig A	$\alpha_2\lambda_2$ $\alpha_2\kappa_2$	10-15% of all antibodies 1.4 - 4.2	Localized protection on mucous membrane
Ig D	$\delta_2\lambda_2$ $\delta_2\kappa_2$	0.2% of all antibodies < 0.04	Involves in activation of B cells
Ig E	$\varepsilon_2\lambda_2$ $\varepsilon_2\kappa_2$	0.1% of all antibodies < 0.007	In allergic reaction, provides protection against parasite and worms

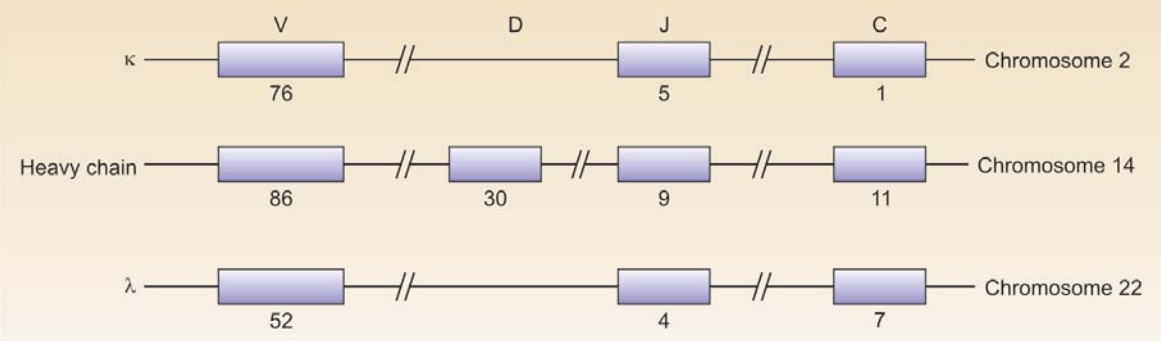


FIGURE 7.3: Schematic diagram showing various DNA segments coding for light and heavy chains (V = Variable region; D = Diversity region; J = Junctional region and C = Constant region)

- A particular antibody molecule is assembled out of specific light and heavy chains. Each region (V, D, J and C) of a chain is again formed by unique sequencing of amino acids.
  For example, the variable region (V) of a heavy chain is synthesized from one out of 86 possible genes coding for the variable region; the D region is expressed from 1 out of 30 genes; 1 out of 9 genes form the J region and 1 out of 11 shape the C region. Thus a single heavy chain is formed by 4 different genes taken from different available options. This recombination of several genes available for expressing different regions in the immunoglobulin imparts the diversity in the number and types of antibodies produced by the immune system.
- Any specific variable (V) region gene of a heavy chain can be **spliced** on to any one of the (D) region genes. This combination can further be spliced on to any J region gene. This splicing process is called V-D-J joining that gives the genetic representation of the V-D-J segment of the antibody heavy chain (Fig. 7.4). The constant portion gene of heavy chains (obtained from any one type of gene out of Cμ, Cδ, and Cα, etc.) is now attached to the V-D-J segment to complete the heavy chain genetic sequence.
- After the splicing of V, D, J and C genes is over, it is followed by transcription. Transcription is followed by RNA processing. All the intervening sequences are removed during RNA processing.
  The processed mRNA represents a gene containing all the four adjacent coding regions (V, D, J and C) of the heavy chain. This messenger RNA in a given B cell will thus produce a heavy chain with a specific variable (VDJ) and constant (C) regions, i.e. a specific type of antibody. All the progeny or clones of the same plasma cell will continue to produce identical antibodies.

Variability in antibody types results from:

- The possible combinations between large numbers of available genes that code for variable regions of heavy as well as light chains.
- Splicing of genes can also create altered codons at splice junctions. These codons generate new configurations in the molecule and hence an additional source of variation.
- Somatic mutation of antibody producing genes.

IMMUNODEFICIENCY DISORDERS

Individuals are said to become immunodeficient when they exhibit lack of function of the cells of the immune system. The T cells, B cells or both may show a decline in their counts or effectiveness. Patients with T cell dysfunction suffer from viral illness but can accept mismatched skin transplants. Lack of B-cell function enhances susceptibility to bacterial infections. These individuals may accept mismatched blood transfusions due to the absence of proper humoral immunity. Disorders of immunity may be inherited as genetic diseases or may be acquired from outside. A few of these diseases are summarized below.

Severe Combined Immunodeficiency (Swiss-type Autosomal Recessive Agammaglobulinemia)

Cellular and humoral immunities both are severely affected. Individuals are highly susceptible to both viral and bacterial infections.

Characteristics of the Disease

The disease has been identified as an autosomal recessive disorder. The absence or ineffectiveness of the enzyme **adenosine deaminase** is implicated in the disease. Mutation occurring in some T cell receptors and certain other T cell related proteins are responsible for syndromes similar to SCIDS. Affected children also suffer from the deficiency of granulocytes. Patients typically have low IgA and IgM levels in the serum. The thymus is usually absent or reduced in size in such cases.

Antibiotics are helpful in combating infections whereas bone marrow transplantation revives the WBC population.

Thymic Agenesis (Di George Syndrome)

Characteristics of the Disease

The absence of the thymus gland and subsequent maldevelopment of T cells result in this syndrome. Abnormalities in the development of the IIIrd and the IVth pharyngeal pouches bring about the absence of thymus and also are associated with nondevelopment of the parathyroid gland. The disease is distinguished by severely depleted levels or absence of T lymphocytes. Diseased children suffer from recurrent viral infections. Certain congenital heart diseases and tetany are also encountered in the syndrome.

Deletion of a particular region of the long arm of chromosome 22 is associated with the disease. Transplantation of fetal thymus in the patient may help prolong life.

X-linked severe combined immunodeficiency (swiss type X-linked agammaglobulinemia) and acquired immunodeficiency syndromes are examples of immunodeficient states.

TRANSPLANTATION OF TISSUES

It is seen that our immune system does not react against self antigens but mounts a severe response against nonself or 'foreign' antigens when they are introduced or transplanted into the host body. This unique character of the immune system to reject transplanted 'foreign' tissues and antigens is achieved by priming the immune cells to various self antigens during the early period of development of the fetus. Cells reacting against the one's own antigens during fetal life are discarded automatically by cellular events. T and B cells that do not express receptors against the 'self' and hence do not produce reactions (antibodies) against one's own tissues are preserved for action in future against 'nonself' entities including pathogens. Thus when any tissue or organ from a person (donor) is transplanted to an unrelated recipient, the lymphocytes of the recipient immediately recognizes the foreign tissue or organ. These tissues act as an assembly of nonself antigens. Immune responses are mobilized against the transplanted tissue and the tissue is rejected. Transplants between identical twins and transplantation of tissues from one part of an individual to any other part of the same person can be done without rejection as the cells and tissues bear identical antigens that behave as self antigens and do not evoke immune responses. Except for identical twins, tissue transplantation between individuals entails a detailed comparison between specific antigens between the individuals. Special tests predict the compatibility and success of such a transplant. The term **histocompatibility** denotes the evaluation of such a similarity between individuals. **Tissue typing** is a process where the major and minor histocompatibility antigens (vide infra) of the donor and recipient tissues are examined and matched for their likeness (histocompatibility). Antigens on the donor cells, if not present on the cells of the recipient, results in the rejection of transplants.

Genetics of Histocompatibility

Specific proteins (antigens) called *antigenic determinants* are present on the surface of a cell. The expression of these antigens are genetically determined by alleles located at different loci (more than a dozen) known as **histocompatibility loci**. Each of the several loci produces a specific antigen, and each histocompatibility loci may contain one out of many available alleles. Therefore in whole of the human population each individual has a different combination of epitopes (antigenic determinants) on the cells due to unique combinations of alleles at those loci. Thus an individual builds its unique genetic identity. As observed, stronger immune response is exerted by some of the histocompatibility loci and their alleles when compared to the others, i.e. some of the loci are more important than the others in context of HLA vide

infracompatibility. The **major histocompatibility complex (MHC)** loci are the most significant of all loci is in the human race. There are of course many other minor loci in the genome. In cases of transplantation between individuals incompatible for minor histocompatibility loci, some of the undesirable effects of transplantation can be treated with **immunosuppressive drugs**. As these expressed antigens were first observed on white blood cells, human MHC locus is also called **HLA** (human leuokyte antigen) and it is situated on chromosome 6; the minor loci being scattered throughout the genome.

The HLA-A, HLA-B, HLA-C and HLA-D are the four different regions on the chromosome 6 that comprise the HLA loci. Each region of a locus again may be composed out of an 'allele' selected out of a series of available alternative "alleles" (Table 7.2).

TABLE 7.2: Number of alleles available for various types of HLA locus regions

No. of Alleles	*HLA Locus*
57	A
111	B
34	C
228	D

One allele for each of the A, B, C and D regions is present on each of the 6th chromosome. A **haplotype** is the make-up of the HLA alleles carried on each of the two chromosomes 6 in an individual. As obvious, different combinations of the HLA alleles on both the chromosome 6 provide innumerable possible HLA genotypes. It is because of this that two unrelated individuals (belonging to different families, clans, countries, races, etc.) show a range of differences in their HLA geno- and phenotypes. As expected though, the HLA make-up in siblings and in closer relatives are relatively similar to each other antigenically at their HLA loci. HLA constitutions in monozygotic or identical twins are exactly the same.

It is mandatory to conduct HLA typing tests in the donor and the recipient before any tissue transplantation is contemplated. The test is usually done by using a PCR based technique.

HLA and Disease

Curiously enough it has been found that the occurrence of as well as susceptibility to certain diseases are closely linked to the presence of a particular HLA type in an individual. An explanation for this might be a very close association or proximity of the disease producing gene to a particular HLA complex that segregate together during meiosis (gamete formation). HLA associations of dental disorders are discussed later in the book (Chapter 14).

SUMMARY

(a) The ability to resist the invasion of pathogens is brought about by the immune system.
(b) T-cell (Cell mediated) and B-cell (Humoral) immunity are the two subsets of the immune system.
(c) Cell mediated immunity involves several T-cells (killer, suppressor and helper) capable of destroying invading microorganisms.
(d) Humoral immunity involves B lymphocytes that are transformed to plasma cells which produces antibodies (immunoglobulins).
(e) **Antigens** are foreign macromolecules which are capable of inducing antibody formation.
(f) **Antigenic determinants (epitopes)** are identifiable features on the surface of antigens which can be recognized by B or T cell. Antibodies are always formed against an epitope.
(g) Antibodies are made up of four polypeptide chains. Two of the chains are **heavy (H)** and the other two are **light (L).** Disulfide bonds connect these chains with each other.
(h) The **variable (V) region** of the antibody is its antigen binding site. **Variable (V) regions** are different yet specific for each kind of antibody and thus can identify and attach to a particular antigen.
(i) Five different classes of antibodies (immunoglobulins) exist; IgG, IgA, IgM, IgD and IgE.
(j) Both the heavy and the light chains differ in their amino acid sequences in the variable regions. These sequences are different in each type of antibody. Thus a perfect antibody can be synthesized for a particular type of antigen.
(k) The variability in the structure of an antibody is achieved through different possible arrangement in the genes that code for the regions in the chains.

(l) Antigenic determinants (epitopes) of a cell or a substance are present on its surface. Before tissue or organ transplantation, similarities in certain antigens are assessed between the donor and the recipient. This is called **histocompatibility** testing.

(m) Antigenic determinants of great importance are expressed by genes located at **histocompatibility loci** (for both major and minor antigens).

(n) The composition of genes at the histocompatibility loci in an individual is unique due to specific combination of alleles at them. Thus each human carries a particular haplotype.

(o) The most significant histocompatibility loci in humans located on chromosome 6 is called the **Major Histocompatibility Complex (MHC)**. The matching at the MHC determines the outcome of a transplant.

Chapter

8 Molecular Control of Development

- Molecular processes in development
  - *Growth and differentiation signaling molecules*
  - *Growth factor receptors*
  - *Signal transduction*
  - *Transcription factors*
- A brief account of the molecular control of early embryonic development
  - *Establishment of the axes of embryo*
  - *Segmentation*
  - *Determination of regional characteristics*

It is amazing to realize the degree of precision and detailing involved in the process of the development of a full-grown individual starting from the first cell of life, the zygote. The intricate mechanisms that control each of the steps in the process are nothing short of miracles of molecular engineering given the thousands of stages at which the process can veer towards undesired destination. Thus though it may seem normal it is actually a matter of chance, so marvelously sustained as the 'usual', that majority of the population walks around in normal physical and mental formats. The following sections of the chapter discuss the molecular mechanisms that guide and control the various processes of growth and differentiation in the developing embryo. As detailing of each of the steps in molecular control of development is beyond the scope of this book, the description of the events would nevertheless enable the students of dentistry to understand the fundamental concepts of developmental genetics.

Embryonic development in humans or for that matter any kind of regulation imposed on the functioning of a cell, tissue or organ is enforced by expression of definite protein molecules. Eventually these are the genes that exert their influence on cellular functions by synthesis of a specific proteins needed for a particular function. The synthesis of proteins differs from cell-to-cell and within the same cell at different points of time. This provides the basic mechanism for control of any cellular process.

The process of ***Growth*** in an organism is achieved by cell division through mitosis that multiplies the number of existing cells and also by increasing the amount intercellular matrix. The process of ***differentiation*** is the creation of new types of cells or tissues, which were not previously present. The differentiated cell possesses new morphological and functional characteristics, which distinguish it from other cells. As described below, we now know that these characteristics result from the formation of new enzymes and proteins. Earlier workers tried to study the mechanism of differentiation by experiments on embryos of amphibia and chicks. Their work has produced many interesting results some of which are as follows:

It has been observed that certain regions of the embryo have the ability to influence the differentiation of neighboring regions. Interesting experiments have shown that these areas can induce formation of the same and specific tissues if they are implanted at areas outside their normal site of occurrences.

The ***primary organizer*** (identified at the dorsal lip of the blastopore) is the first organizer that is recognizable in the embryo. The failure of development of the primary organizer results in absolute failure of embryonic development. On the other hand if the dorsal lip of the blastopore is grafted on to a different site of another embryo, it induces the development of an entire embryo at the implanted site. Thus the signals that determine the initial organization in an embryo come from the ***primary organizer***.

The effects of these organizers are brought about by enzymes or signaling proteins that are basically the product of gene transcription and translation. These signals may be in the form of (**a**) ***inductors*** which stimulate the tissue to differentiate in a particular

manner; or ***(b) inhibitors*** which have a restraining influence on differentiation.

Therefore the study of the controlling mechanisms can be termed ***Genetic control of development*** or described as ***Molecular control of development*** as it is now well-documented that the final control of the mechanisms of control rests with the genes involved in such control.

Though all the cells in the body contain the same complement of genes and other nuclear molecules, specialization of the structure and functions of a cell is determined by activation of only a certain number of genes in a particular type of cell. The process of protein synthesis involves two of the fundamental processes in cell biology; transcription and translation.

The basic process of transfer of genetic information begins with transcription of the mRNA molecule from DNA that occurs inside the nucleus followed by extrusion of mRNA outside the nucleus.

The sequential arrangement of codons on the mRNA is used for synthesis of proteins by translation occurring in the cytoplasm and involves protein synthesizing machinery in the cytoplasm, e.g. ribosomes, tRNA, etc.

In any given cell, at any given time (interphase), only a few of its genes are active and others are resting. Cells are said to be differentiated structurally and functionally because of expression of a small number of developmental regulatory genes (***master genes***) in them during specific time of embryonic development. The expression of such master genes initiate cascade of events in different cells imparting the cell and subsequently the tissues of the embryo their unique structural and functional identity. Every differentiated cell contains two types of genes; the ***housekeeping genes*** and the specialty ***genes.*** The majority of genes (80–90%) in a cell are housekeeping genes which are required for basic cellular metabolic functions. These common genes are also widely expressed in other cell types of the body. The specialty genes are expressed to define the unique features of different cell types.

In higher organisms regulation of gene expression is quite complex and is brought about by the action of specialized molecules such as hormones or growth factors on the target cells. Regulation (either facilitation or suppression) of gene expression is effected through binding of a transcription factor to specific DNA segments in the promoter region of a gene (vide infra). There are stretches of DNA sequences called ***enhancers*** that may be located within the noncoding sequences of the gene, or located upstream or downstream of the gene. These regions on the DNA can bind transcription factors and increase the rate of transcription. There are also similar regions which inhibit transcription and are called ***silencers***. Transcription factors may bind to these specific regions on the DNA activating or inhibiting (turn on or turn off) gene expression.

MOLECULAR PROCESSES IN DEVELOPMENT

It is well known now that several genes and gene families play important role in the development of the embryo. Most of these genes produce transcription factors which control RNA transcription from the DNA template in the target cells. The transcription factors thus play an important role in gene expression as it can switch specific genes on and off by activating or repressing it. It is believed that several transcription factors control gene expression, which in turn, regulates the fundamental embryological processes like ***induction, segmentation, migration, differentiation and apoptosis (programmed cell death)*** in embryonic cells till permanent cell lines are established in tissues. The above fundamental embryological processes are mediated by growth and differentiation factors, growth factor receptors and various cytoplasmic proteins.

Our existing knowledge regarding the molecular basis for embryonic development is mainly based on the Drosophila (fruit fly). However, evidences are now being gathered indicating that the basic body plan of mammalian embryo is under the control of many such similar genes as those in the fly that have been identified for controlling morphogenesis in Drosophila.

At the molecular level signaling is effected by protein molecules that act from outside the cells and can act locally or from a distance as intercellular **signaling molecules**. Many signaling molecules are called **growth factors.** Signaling molecules need to bind to **receptor molecules** that usually exist as transmembrane proteins in the plasma membrane of the cells. Attachment of the signaling molecules to the receptor molecules cascade a series of events through which a molecular signal is relayed from the cell

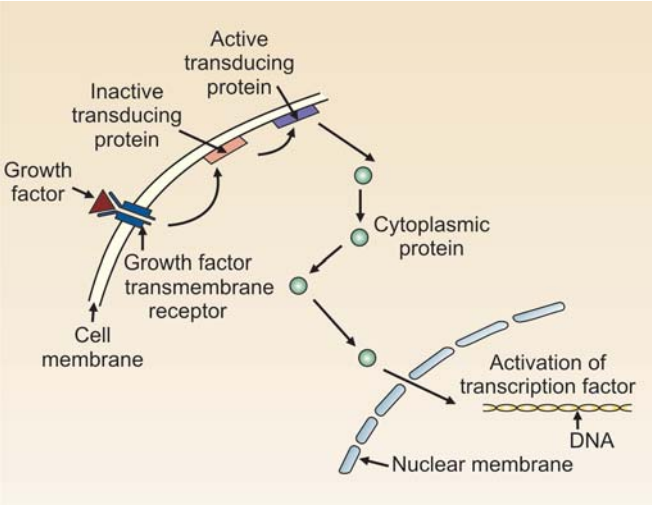


FIGURE 8.1: Signal transduction pathway**.** When in a normal cell a growth factor binds to a growth factor receptor, it gets stimulated. The inactive transducing protein gets activated and sends a signal to the nucleus by activating a series of cytoplasmic kinases. The signal reaching the nucleus in the form of a transcription factor begins transcription of the gene. (*Courtesy* of Prof Inderbir Singh, Human Embryology, 8th Edition, MacMillan)

membrane to nucleus (**signal transduction pathway**) using related molecules. Signal transduction is used by the cell to activate several mechanisms including generation of **transcription factors** which initiates gene expression in the nucleus (Fig.8.1).Transcription factors binds to the DNA at promoter or enhancer region of the specific gene and initiate the process of transcription. The transcription factors are important molecules that guide embryological development.

It is expected that mutations and disturbed expression of genes related to growth factors, receptors, or the transcription factors would be associated with various kinds of growth anomalies and cancers. The details of this phenomenon are discussed in appropriate sections of the book.

1. Growth and Differentiation Signaling Molecules

Proteins capable of stimulating cellular proliferation and cellular differentiation occur naturally and are termed ***growth factors***. The epidermal growth factor (***EGF***), fibroblast growth factor (***FGF***) and the platelet derived growth factor (***PDGF***), stimulate the proliferation of epidermal cells, fibroblasts and the connective tissues, etc.

Growth factors typically act between cells in embryos through attachment to specific cell membrane receptors as intercellular signaling molecules. Several methods are adopted for execution of the effects of the signaling molecules. Growth factors can modify the expression or the effects of one another.

The signals are called hormones, which travel through blood to reach a distant place in the body. This system constitutes the ***Endocrine system.***

Paracrine system act by signaling targets cells situated in the near vicinity of the signal executing cell.

The ***Juxtacrine*** mode of action requires that the effecter as well as the effected cells remain in cell-to-cell physical contact. The "gap junction" and "notch signaling are well known examples of juxtacrine system model of signaling. The notch signaling is described later in this chapter.

A few common growth and differentiation factor groups and their role in development are described below.

Growth Factors and their Functions

- **Epidermal Growth Factor (EGF)**
  Determines growth and proliferation of cells of ectodermal and mesodermal origin.
- **Transforming Growth Factors (TGFs)**
  TGF –B1 to TGF –B5 Forms the extracellular matrix, induces epithelial branching, myoblast proliferation.
  Bone Morphogenetic Factors (BMP 1 to 9) Helps bone formation, cell division, cell migration and apoptosis.
  Müllerian Inhibiting Factor (MIF) Regression of paramesonephric duct.
  Nodal Formation of primitive streak, right-left axial fixation formation of mesoderm.
  Lefty Determination of body asymmetry
  Activin Proliferation of granulosa cells
  Inhibin Inhibition of gonadotrophin
- **Hedgehog proteins**
  Sonic Hedgehog, Desert, and Indian. Shh control neural tube formation, somite differentiation, gut formation, limb development, and growth of genital tubercle.

- **WNT Protein**
  Midbrain development, somite and urogenital differentiation, limb patterning.
- **Fibroblast Growth Factors (FGFs)**
  Mesoderm differentiation, angiogenesis, axon growth, limb development, development of various parts of brain, liver induction, mesenchymal proliferation in jaw, induction of prostate gland, outgrowth of genital tubercle.,
- **Insulin-like Growth Factors (IGFs)**
  IGF-1 act as factor for bone growth, IGF-2 is a fetal growth factor.
- **Nerve Growth Factor (NGFs)**
  Stimulate the growth of sensory and sympathetic neurons.

Abnormalities in the growth factor signaling pathway may lead to abnormal growth or cancer. The over expression of growth factors can lead to non-cancerous disorder like psoriasis. Mutation and over expression of ***PDGF gene*** may also cause cancers like osteosarcoma and astrocytoma. Mutation in growth factor receptors can lead to insulin-resistant diabetes (insulin receptor) and dwarfism (fibroblast growth factor receptor). Mutation and overexpression of these receptors are responsible for variety of cancers.

HEDGEHOG PROTEINS

Embryological development is most influenced by Hedgehog proteins of signaling at places like the notochord, neuroectoderm, primitive node, zone of polarizing activity in limb, genital tubercle, retina, hair buds and lung buds, etc. Some of the members of the family are the Sonic hedgehog, Indian hedgehog, desert hedgehog and tiggy winkle hedgehog proteins. Mutations in these protein genes cause spiky outgrowths in the belly of the Drosophila and hence the gene is named hedgehog. This molecule is very active throughout the process of embryogenesis.

The hedgehog molecule is active at its N-end and binds to cholesterol molecule on the cell surface whereas the C-end of the molecule is relatively inactive. Sonic hedgehog along with cholesterol molecules bind to a transmembrane receptor called as **Patched**, located on the plasma membrane.

The normal action of patched is to inhibit another transmembrane protein called **smoothened.** The binding of sonic hedgehog to the patched inactivates the action of the patched. This leads to the activity of the smoothened to allow signaling. Such cellular signaling ultimately activates a zinc finger transcription factor, **Gli**. As expected of a transcription factor, the Gli goes inside the nucleus to bind at specific locations on the DNA molecule and initiates transcription of gene.

Some of the effects of mutations in the above-mentioned genes are as follows:

MUTATION	EFFECTS
Mutation of sonic hedgehog gene (Situated on chromosome number 7q36)	Causes incomplete cleavage of developing brain into right and left cerebral hemispheres and cyclopia (holoprosencephaly
Mutation of patched receptor (9q22)	Causes multiple basal cell carcinomas, bifid ribs, ovarian fibromata
Mutation of smoothened protein (7q31),	Causes basal cell carcinoma and medulloblastomas
Mutation of Gli (7p13)	Causes genital anomalies and syndactyly.

2. Growth Factor Receptors

Receptors are specialized protein molecules that recognize and bind specific signal molecules (ligands) such as growth factors and hormones. The ***transmembrane receptors*** are proteins situated across the plasma membrane of the cell. Receptors bind to the specific signaling molecules on the outer side of the membrane and activate certain molecules (G proteins, etc.) on the inner side of the membrane. This is followed by a series of activation, mainly by phosphorylation, in some cytoplasmic proteins known as protein kinases, e.g. ***Tyrosine kinase, Protein kinase C***, etc.

Other kinds of surface receptors also exist beside the transmembrane receptors. The ***notch receptor*** plays an important role in embryonic development. In this kind of signaling (juxtacrine signaling), a protein on one cell surface interacts with a receptor on an adjacent cell surface. Notch is a cell surface receptor, which has a long extracellular part and a smaller intracellular part. Contact with the specific protein (***delta*** *or* ***jagged***) present on the surface of a nearby dominant cell activates the notch receptor. Attachment to one of these proteins causes the notch receptor to

be broken in its intracytoplasmic domain. This broken portion acts as a transcription factor that regulates gene expression in that cell. Thus one cell (called dominant) can influence transcription of an adjacent cell. Such example is visible in a developing neuron (dominant) that inhibits its surrounding cells to develop into glial cells. This phenomenon is termed as lateral inhibition.

Other growth factor receptors are responsive to molecules secreted by cells of the extracellular matrix (ECM) like glycoproteins, collagen, proteoglycans, etc. Receptors for fibronectin and laminin are called ***integrins***.

For cell-to-cell communication, the gap junction channels are made up of connexin proteins.

3. Signal Transduction

The process by which a cell converts one kind of signal into another is called signal transduction. Binding of extracellular signaling molecules to receptors triggering a sequence of biochemical reactions inside the cell marks a signal transduction. These reactions are carried out by different enzymes as a chain of reactions and hence referred to as a "signal cascade". Extracellular growth factors regulate cell growth and differentiation by a complex pathway through signal transduction. Each sequential step in the pathway is genetically determined (Fig. 8.1). The steps are sequenced as:

- Growth factor binding.
- Activation of the receptor.
- Activation of cytoplasmic proteins called ***signal transducing proteins***; many of such proteins are situated on the inner surface of plasma membrane.
- Activation of one or more of the several cytoplasmic protein (kinase) systems.
- Formation of transcription factor.
- Effect of transcription factor: activation or inhibition the expression of a growth or a differentiation related gene.

We have already the idea that the processes in embryogenesis involve structures like 'organizers' that 'induce' the formation of specific types of cells from a common precursor. This process of induction is in fact carried out by organizers with the help of synthesis of several factors. The synthesis of these important factors is under the control of certain well known genes that are involved with development of the embryo. One such gene is the ***PAX-6 gene*** which encodes for a transcription factor regulating several important events in embryogenesis including development of the eye.

4. Transcription Factors

A large number of transcription factors are common and found in all types of cells and across several organisms. However, few transcription factors are found only in certain types of cells or are active only during specific stages of development. The transcription factors regulate gene expression by acting on promoter or enhancer regions of specific genes. These transcription factors are transcription regulatory protein molecules that bind to specific sites on the DNA. These proteins have 'typical' structural configurations at their binding sites with DNA. These sites are called 'motifs'. Some of these 'typical' configurations are what we know as basic helix-loop–helix protein, zinc finger protein, etc.

Abnormalities in transcription pathways may lead to abnormal growth or cancer. The mutation of signal transducing proteins (e.g. ras gene) is responsible for almost 30% of human tumors. Mutations of genes which code for certain transcription factors are responsible for colon cancer, neuroblastoma, Burkitt's lymphoma and lung cancer.

Specific abnormalities related to transcription factors are referred to in the section of the text dealing with molecular control of some important events in dental development.

Some of the important Transcription factors and their functions are discussed below:

- **Basic helix-loop-helix protein** is involved in myogenesis, neurogenesis, hematogenesis and the development of pancreas. This kind of transcription factors contain a short length of amino acids in which two alpha-helices are separated by an amino acid loop.
- **Zinc Finger Proteins** regulate expression of genes. The DNA binding domain in this protein is the zinc finger motif. The transcription protein is constituted by zinc ions binding to regularly placed cysteine and histidine units of the

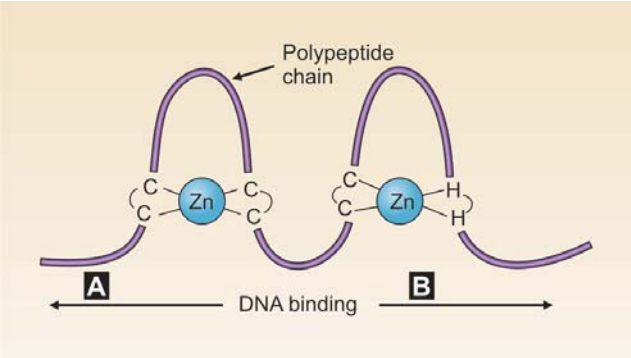


FIGURE 8.2: Schematic diagram showing the Zinc finger motifs. A = cys-cys zinc finger, B = cys-his zinc finger

polypeptide chain. This results in puckering of the chain into finger-like structures (Fig. 8.2). These fingers configure to specific sites of the desired DNA helix. The kidney, gonads, hindbrain and white blood cells are some of the diverse examples of structures influenced by this transcription factor.

Anomalies in genes like GLI 3, WT1 and ZIC2 located in chromosomes 7p13, 11p13 and 13q32 respectively cause head, hand and foot abnormalities, Wilms' tumor, ambiguous external genitalia and Holoprosencephaly. Mutations in the gene ZIC3 located at Xq26 may result in abnormal position of heart, liver and spleen.

- **HOX genes** regulate segmentation, patterning of the hind brain and formation of the axis of the embryo.

The HOX genes in humans encode a special class of transcription factors that regulate the sequential development of different body segments. Originally discovered in Drosophila this class of genes are called **homeotic** genes because mutations in these genes are capable of transforming one part of the body into another (e.g. growth of legs in place of antennae). The regional morphogenic characterization of individual segments of Drosophila embryo is brought about by the expression of a group of ***homeotic genes***. These genes determine which embryonic segment should bear antennae, wings or legs. These 8 homeotic genes are situated on chromosome number 3 and are arranged in two groups or clusters (***Antennapedia*** and ***Bithorax)***. These genes are collectively called the ***homeotic complex*** or ***HOM-C***. Each of the 8 genes contains a highly conserved coding sequence of 180 base pair region of DNA (usually near their 3′ end) called the ***homeobox*** (Fig.8.3). The homeobox codes for a 60 amino acid protein called ***homeodomain.*** These homeodomains, thus, remain constitutively integrated within the bigger polypeptides coded by the homeotic genes. These polypeptides synthesized by homeotic genes are transcription regulating factors. Homeodomains within these polypeptides recognize and bind to specific DNA sequences of target genes.

The eight genes present in the homeotic complex express themselves in a selective sequence. That means the genes, which are cranial in position in the cluster or so as to say, located towards the 3′ end of the entire DNA material of the fly if put together in a 3′ to 5′ sequence, are expressed in the cranial segments or cranial regions of the developing embryo. Successive downstream genes are expressed in the caudal structures of embryo giving each of the regions its structural identity in the craniocaudal axis. As stated before, the homeotic genes express polypeptide transcription factors. The products of a preceding or cranial gene regulate transcription in a succeeding or caudal homeotic gene.

Experimental mutation, suppression, or expression of the homeotic genes at different regions causes abnormal regional patterns in the embryo.

Such homeotic genes (clustered genes, each having a homeobox) similar to Drosophila are found in mammals and in humans. The human genes (called ***HOX genes***) have same clustered organization, follow

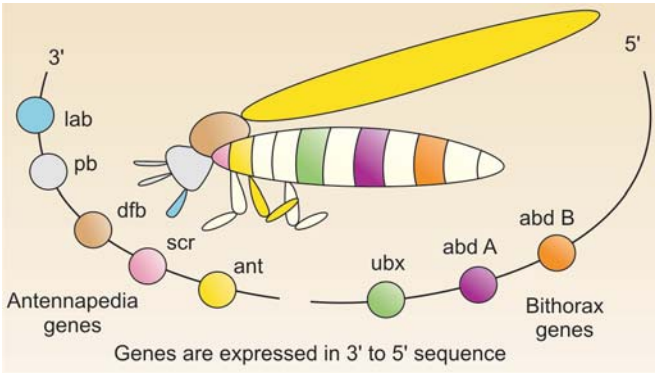


FIGURE 8.3: Diagram showing the arrangement of homeotic genes of Drosophila on chromosome number 3. These eight genes are arranged in two clusters—Antennapedia and Bithorax. These genes are expressed in a craniocaudal sequence. (*Courtesy* of Prof Inderbir Singh, Human Embryology, 8th Edition, MacMillan).

same order of gene arrangement within the cluster, their expression and functions are also in sequences as observed in Drosophila. Quite interestingly, when the sequences within the homedomain and some short regions of the homeobox were compared, striking similarities were noticed across species. These sequences and their protein products (called 'motifs') have been ***strictly conserved*** through evolution. The amino acid sequences of homeodomains of Drosophila are up to 90% similar when compared with that of humans. During hundreds of millions of years of evolution these genes have duplicated twice in man and hence human chromosomes have four copies of the clusters of homeobox genes. The genes (***HOXA, HOXB, HOXC, and HOXD***) arranged on four different chromosomes (Chromosome number 7, 17, 12 and 2). Genes in each group are numbered from 1 to 13 corresponding to the fly genes and each group can be placed in a vertical alignment (Fig. 8.4). Genes with same number but present on different chromosomes in the vertical alignment form a ***paralogous group***. In humans, the HOXA and its paralogs are expressed in the cranial segments, the HOXB and its paralogs are expressed in the next caudal segment, the HOXC and its paralogs in the next segment and the pattern follows. The products of paralogs interact for a final result of expression within a segment. There are 39 genes in all and each gene contains a homeobox region, which encodes for homeodomain protein. Similar to fruit fly, homeobox genes of humans are also expressed sequentially in craniocaudal direction during axis formation. The sequential expression of HOX genes correlates with the development of structures in craniocaudal sequence.

The HOX genes are responsible for cranial to caudal patterning of the derivatives of ectoderm, mesoderm and endoderm germ layers. HOX genes regulate the differentiation of somites, vertebrae and hindbrain segmentation. The expression of individual HOX gene may also occur in places like hair, blood cells and developing sperm cells. This indicates that though the main function of HOX genes is to set up structures along the main axis of the embryo, but the individual gene may also guide the formation of specific structure, which may not lie along the body axis.

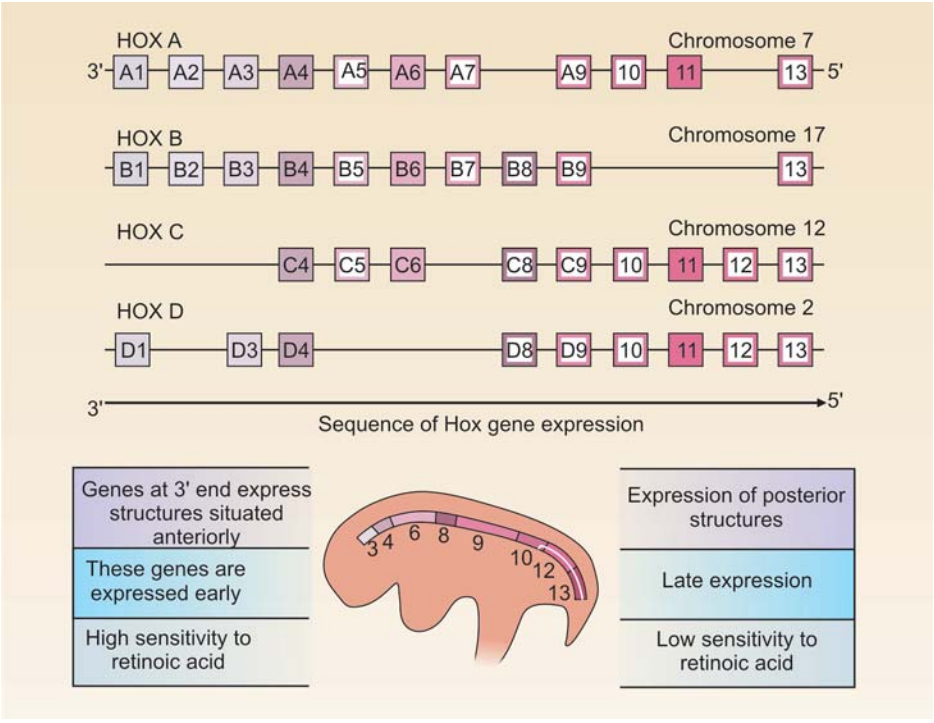


FIGURE 8.4: Alignment of four human HOX complexes. Expression of paralogous groups in the hindbrain and spinal cord, in cranial to caudal direction, are indicated by their numbers. (*Courtesy* of Prof Inderbir Singh, Human Embryology, 8th Edition, Macmillan)

- **PAX Genes (paired box genes) shape the development of s**ense organs (eye and ear) and the nervous system. These genes regulate cellular differentiation at the time of epithelial-mesenchymal transition.

The paired box gene is DNA sequence that encodes a 128 amino acid protein. This protein transcription regulating factor binds to the DNA at sites (domains) for activation of transcription. In humans the Pax gene family consists of 9 genes (Pax-1 to Pax-9).Details of the importance of these genes in dentistry is discussed later in the book.

Some of the developmental abnormalities associated with Pax genes are discussed below.

- The Pax-2 gene located on the 10th chromosome (10p25) when mutated results in renal malformation and malformation of retina and optic nerve *(renal-coloboma syndrome).* Mutations in the Pax-3 (chromosome 2q35), Pax- 6 (chromosome11p13) cause loss of hearing, areas of depigmentation in hair and skin and abnormal pigmentation of iris along with the absence of iris and sarcoma.
- **SOX Genes (LEF-1, SRY type HMG)** are expressed in many structures during development. Sox genes consist of over 20 members in the family. The Sox genes contain a 79 amino acid domain that is known as HMG (high mobility group) box. These genes show homology with Y-linked SRY gene. SRY gene plays a major role in male sex determination. The name of this group (SOX) was derived from SRY HMG box. The HMG domain activates transcription by bending DNA (hence, also called DNA bending protein) in such a way that other regulatory factors can also bind with promoter region of genes. The skeletal tissue and type II collagen development is linked to the Sox-9 gene. Mutation of Sox-9 on chromosome 17 result in bowing of long bones. A mutation of the Sox-10 on chromosome 22 is incriminated in Hirschsprung disease.
- **POU Genes (Pit-1, Oct) Play a vital** role in cleavage of the early embryonic cells. A Pou transcription factor is constituted by a homeodomain region and a second site on the factor that binds to the target DNA segment. The Pou gene family is named by the fist alphabets of few of the first genes identified *i.e.*, **P**it-1, **O**ct-1 and **U**nc-86.

The development of anterior pituitary gland is related to the expression of the **P**it-1 gene. The Oct-2 gene is expressed in the B-cell activating immunoglobulin synthesizing genes. The Unc-86 gene is involved in the development of nematode neuronal cells.

- **Lim proteins** regulate muscle differentiation. These genes constitute a large family and are associated with the development of all parts of body. Absence of Lim-1 protein results in headless embryo.
- **T-BOX (TBX) Genes** initiate the induction of mesoderm germ layer and specification of hind v/s forelimbs. Notochord differentiation is related to T-Box expression.

Also called *Brachyury* T-box genes, encode transcription factors that play important roles in development of mammary glands, upper limb and heart. Mutation of TBX-3 on chromosome 12 causes hypoplasia of mammary gland and abnormalities in upper limbs. Mutation in TBX-5 may cause arterial septal defects and absence of forearm.

- **Dlx Genes (Dlx-1 to Dlx-7)** are involved in morphogenesis of jaw and inner ear. The Dlx gene family consists of 6 members and is closely associated with HOX genes.

A BRIEF ACCOUNT OF THE MOLECULAR CONTROL OF EARLY EMBRYONIC DEVELOPMENT

In the preceding part of this chapter we have seen that the development of human body is regulated by sequential gene expression. In this process specific genes are expressed in sequence, one after the other, at different regions of the body resulting in development of dissimilar structures at different regions of the same body. Finally the entire process of development results in structural and functional differentiation of highly specialized tissues and organs endowed with definite roles. These cascades of gene expression and the resultant sequence of embryological events are well-studied in the fly Drosophila that is very briefly discussed now.

1. Establishment of the Axes of Embryo

The process of axes differentiation and early embryonic development in Drosophila is one of the earliest

events and under strict genetic control but in human this part of development occurs under lesser rigid genetic scrutiny.

In Drosophila the development of the anteroposterior, dorsoventral and right/left axes are under the control of a group of maternal genes, which are called ***maternal effect genes***. These genes are expressed outside the egg (within the mother fly) even before fertilization. The products of expression of these genes are transcription factors called ***morphogens*** that subsequently act on future zygotic targets. These products are carried into the egg where they diffuse unequally in the oocyte cytoplasm to establish gradients across the future anteroposterior axis of the egg. Due to the presence of such gradients proteins are differentially distributed in the common cytoplasm of the egg. These gradients determine the synthesis of specific proteins in the different segments of the embryo.

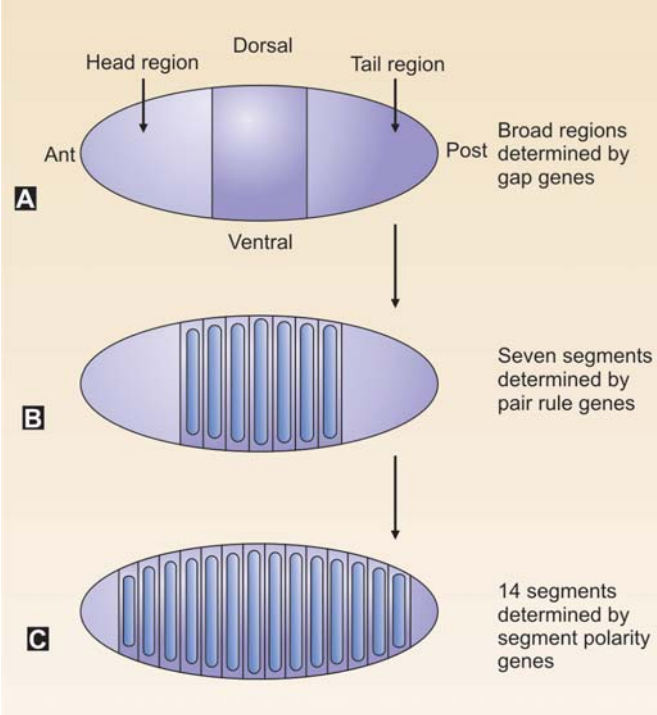


FIGURE 8.5: The process of establishment of the axes in a Drosophila embryo is governed by maternal effect genes. The segmentation of the embryo is controlled by segmentation genes (zygotic genes). Broad regions are determined by gap genes (A), seven segments by "pair rule genes" (B), and (C) 14 segments by "segment polarity genes". (*Courtesy* of Prof. Inderbir Singh, Human Embryology, 8th Edition, Macmillan)

In the Drosophila body axes are established even before fertilization. In mammals body axes do not become fixed until the end of cleavage or early gastrulation. The formation of anteroposterior axis in human embryo is initiated by the cells of future anterior margin of the embryonic disk. This area of disk expresses the genes (***OTX2, LIM1***, *and* ***HESX1***) which are necessary for formation of the head even before gastrulation. The ***B-Catenin, BMP-4*** *and* ***activin*** genes lead to the formation of primitive streak. These genes are first expressed in the cranial region of embryo. Once the primitive streak is formed the embryonic axes (craniocaudal, dorsoventral and right/left) are soon established.

2. Segmentation

The Drosophila embryo next divides into identical segments (Fig. 8.5). This is achieved by ***segmentation genes***, which are subclass of genes called **zygotic *genes***. The diffused 'morphogens' control the expression of ***segmentation genes***. The segmentation is completed in three steps in the Drosophila embryo.

The segmentation ***gap genes*** control the first step of segmentation that divides the embryo into broad regions. Gap genes are controlled by *maternal* bicoid proteins.

The ***pair rule genes*** regulate the subdivision of the embryo in 7 segments along craniocaudal axis in the second step of segmentation. The pair rule genes are regulated by the products of genes regulating the previous step, the *Gap genes.*

Segmentation enters the third stage where the ***segment polarity genes*** like the ***Gooseberry, hedgehog, patched, wingless*** genes divide the embryo further into 14 segments. These segment polarity genes are controlled by the genes of the previous step, the *pair rule genes*. Similar segmentation genes used in developing humans have been identified.

3. Determination of Regional Characteristics

The process of segmentation is followed by development of regional characteristics in the newly formed segments of embryo. As discussed earlier, activation of the ***homeotic genes*** brings about specific characterization of individual segments of the developing embryo. These genes determine the growth of antennae, wings or legs in the appropriate segments of the fly.

The 8 ***Homeobox*** genes contains a highly conserved coding sequence of 180 base pair region of DNA (usually near their 3′ end) called the ***homeobox*** (Fig. 8.4).

Similar to fruit fly, homeobox genes of humans are also expressed sequentially in craniocaudal direction during axis formation. The sequential expression of HOX genes correlates with the development of structures in craniocaudal sequence.

A number of other gene families that regulate development also contain similar homeobox domains synthesizing homeodomains but with different sequences in their genes, *e.g.* ***Paired, Pax (Pax-4 and Pax-6), POU, LIM***, etc.

There is a direct relationship between vitamin A (***retinol***) and expression of *HOX* gene. Either too much or too little of retinoic acid (metabolite of Vitamin A) causes misexpression of ***HOXB-1***. This may lead to abnormal development of legs, hindbrain and pharyngeal neural crest cells. The retinoic acid may cause extra pair of limb in frogs at the site of tail. This is an example of homeotic shift similar to formation of extra pair of wings in fruit fly.

SUMMARY

(a) The process of ***Growth*** in an organism is achieved by cell division.

(b) The process of ***Differentiation*** is the creation of new types of cells or tissues.

(c) Thus the signals that determine the initial organization in an embryo come from the ***primary organizer***.

(d) Molecular processes in development are governed by fundamental embryological processes like ***induction, segmentation, migration, differentiation and apoptosis*** (programmed cell death) in the embryonic cells.

(e) Cellular signals are relayed from the cell membrane to nucleus in sequential steps (**signal transduction pathway**) for the initiation as well as control of cellular processes.

(f) Several molecules are involved in the process of signal transduction like growth and differentiation signaling molecules, e.g. Epidermal Growth Factor (EGFs), Transforming Growth Factors (TGFs), Hedgehog proteins, the WNT proteins, etc.

(g) These Growth factors bind to their receptors to execute specific signal transduction activities.

(h) Transcription factors are the molecules that interact directly or indirectly with the genomic DNA to carry out final effects of cell signaling. Proteins like the **Basic helix-loop–helix protein, Zinc Finger Proteins, HOX gene and Sox Gene** proteins, etc. are important transcription factors.

(i) The process of early embryonic development includes the steps of establishment of the axes of embryo followed by segmentation of the embryo and determination of its regional characteristics.

Section

Genetics in Dentistry

Chapter

9

Methods of Genetic Analysis

- Identification of heritable dental pathology
- Segregation analysis
- Twin studies
- Linkage analysis
- Association studies

How Scientists Conclude if a Disease is Genetically Determined or not?

At times it becomes difficult for a clinician to determine the cause of a disease, especially if the disease is heritable and its symptoms are a part of the presentation put forward by genetically determined traits of a disorder. The presence of many affected members in a family suggests its genetic etiology. However, to determine that a disease has a definite genetic basis, it should exhibit a specific pattern of inheritance (dominant, recessive or X-linked, etc.). When the gene responsible for disease is transmitted from one generation to the next it follows specific laws of mendelian inheritance. Though this is true for the diseases which are due to single gene (monogenic) defects, genetic diseases are also determined due to the action of many genes (polygenic) acting together. Not only this, some diseases result out of interactions between many genes and environmental factors (multifactorial). The genetic basis for multifactorial diseases is difficult to work out. Scientists use many techniques to demonstrate the genetic basis of disease. The following methods are used for evaluation of genetic diseases. Some of them are described very superficially here.

IDENTIFICATION OF HERITABLE DENTAL PATHOLOGY

In case of a dentist noticing a disease, the etiological basis of which is not known or clearly defined, he should carefully identify its unique characteristics. He should take a careful family history. If the family history reveals that many other family members are also affected with same disease he should become suspicious about the genetic predisposition of the disease. Thus familial aggregation of a disease is the first step in the identification of a genetic correlation of the disease.

SEGREGATION ANALYSIS

The next step leads the investigator to determine the pattern of inheritance of the disease. This begins with a necessary step of drawing the family pedigree. Members of the family spread in many generations (both on maternal and paternal sides) are interviewed. In the pedigree chart the affected and nonaffected members are symbolically represented. On the basis of pedigree one can define the mode of inheritance of the disease, i.e. whether the disease is autosomal dominant, recessive, X-linked, polygenetic or multifactorial (Refer Chapter 6 for mode of inheritance) in its pattern of inheritance. This kind of study is known as segregation analysis. Thus segregation analysis is the method to determine the mode of inheritance of a particular phenotype from the family data. The aim of the segregation analysis is to find out the effect of a single gene or so-called major gene in the pedigree.

Segregation analysis is the statistical method for determining the mode to inheritance of a particular trait from family data particularly those traits that are determined by a single gene (major gene) (Townsend et al, 1998). Segregation analysis tells us whether the

gene responsible for disease is a dominant or recessive in character and whether it is present on an autosome or a sex chromosome. This kind of analysis holds good for single gene inheritance (Mendelian inheritance) but not ideal for the interpretation of multifactorial disease inheritance because the analysis fails to discriminate between effects exerted by the genetic causes and those by the environmental sources that together cause the disease (Diehl et al, 1999 and Elston, 1981). Segregation analysis does not find the gene responsible for the disease.

The characteristic of multifactorial inheritance is that the proportion of affected persons who are near relatives of each other (in an extended family tree) is greater than the incidence of the multifactorial disease, in isolation, in the general population. However, the incidence of persons affected with multifactorial diseases is much less when compared to single gene inheritance. In this kind of inheritance the dosage of polygenes differs amongst the individual affected persons or between families. Polygenic inheritance shows continuous phenotypic variation of the disease (as in case of periodontitis variation may range from mild to sever disease). While on other hand monogenic inheritance show either the presence or absence of the disease in absolute terms.

The analyses of multifactorial traits in human populations have been confined to the determination of the observed variation into genetic and environmental components based on comparisons between relatives (i.e. parents and offspring, siblings, half sibs and twins). For the phenotype variability (V_p) of a trait, the variability between individuals is considered to be the result of a combination between genetic variance (V_g) and environmental variance (V_e), i.e. $V_p = V_e + V_g$. The heritable estimate can be calculated as the ratio of V_g/V_p and is represented in terms of percentage, i.e. 0 to 100 % (Townsend et al, 1998). With the increasing computer usage models have been developed to detect the contribution of individual genetic locus as compared to polygenic and environmental effects.

TWIN STUDIES

In case of multifactorial diseases where genetic and environmental factors play important role in the causation of the disease (Refer Chapter 7), twin studies are useful. Human twins are of two basic categories: **monozygotic** or identical twins resulting from a single ovum fertilized by a single sperm and **dizygotic** where two ova are fertilized by two sperms. Monozygotic twins are genetically identical (they have same genes) while dizygotic twins share 50% of their genes. Thus monozygotic twins should show the same phenotype, as their genotype is identical. If there is some difference in the phenotype of these twins it may be due to the influence imposed by different environmental factors only. On the other hand, differences between dizygotic twins are both due to genetic and environmental variables.

Presence or absence of the trait or disease (in a large number) in the two categories of twins is calculated in percentage. The genetic component involved in the causation of a disease is confirmed if the percentage of the twin pairs in which both the twins are affected is greater for monozygotic twins as compared to dizygotic twins. If the percentage of disease occurring in both the monozygotic twins is 42% and in dizygotic twins only 6%, it indicates that genetics plays an important role in the development of the disease. A very large number of twin pairs are needed for twin studies, which are reared together in the same environment. The development of new-sophisticated genetic modeling methods has made it possible to estimate the genetic and environmental parameters and specify interactions between them.

The genetic basis of a disease can also be tested in monozygotic twins who are separated after birth and reared in two different environments (Bouchard et al, 1990). In these twins all the similarity will be due to common genes and all the dissimilarity will be due to environmental factors. So if both the twins of a pair are suffering from the same disease while living apart, it is due to gene linked effects and if only one of them suffers from a disease it may be due to environmental concerns. Thus this kind of a study overcomes the problem of twins displaying similarities because of their common environment.

The genetic and environmental effects can also be studied in the offsprings of monozygotic twins (Porter, 1990). The offsprings of monozygotic twins can be considered as half-sibs though they are socially first cousins. This monozygous half-sib model offers a powerful tool to estimate the genetic and environmental disease risk in families. This kind of a study also tells us about maternal effects on the

progeny (as mothers are different, though fathers are also different but they have same genetic constitution).

LINKAGE ANALYSIS

Once evidence of the effects of a major gene(s) has been detected and established, the next logical step is to identify the location of gene(s) within the genome. Linkage analysis is used to map a disease (mutant) gene to its specific location on a chromosome. This mode of analysis takes the help of many families containing multiple diseased individuals. Genotypes are determined for affected and unaffected individuals of the family. The linkage analysis is usually made between two genes out of which one is a mutant gene causing disease and other gene acting as a marker gene. The marker gene is characterized by detectable polymorphism. It is important that these two genes, i.e. marker gene and disease gene (mutant gene) should be linked as a result of being in close physical proximity. In the method of linkage analysis segregation of the disease with the polymorphic marker is studied for each chromosome. Eventually a marker is identified which co-segregates with the disease gene more often than would be expected by chance. This proves that disease gene is linked, i.e. present on the same chromosome on which the specific marker is situated. However, application of these methods to identify the genetic basis of dental disorders has been limited because of difficulties in obtaining large family pedigrees and also in identifying polymorphic marker genes (Conneally et al, 1980 and White and Lalouel, 1987) in relation to dental diseases.

Next step is to determine the linkage distance between the two genes. This can be achieved by calculating the ***recombination frequency*** (*see box*). For example if a pedigree shows only one recombinant offspring and seven nonrecombinant (linked) out of eight offspring, then the recombination frequency is calculated to be 0.125 (12.5 %) and the distance between two genes is equal to 12.5 cM (centi Morgan).

Lod (*logarithm of odd*) score method is used to calculate the linkage and map the distance between two linked genes (*see box*). With a high LOD score and a low recombination fraction the researcher can be fairly certain that the gene responsible for the disease has been localized.

Once the location of the disease causing gene is mapped on the chromosome, one can sequence this area and can identify the gene. Investigators also come to identify the type of mutation involved in the disease. Next step in the final identification of this gene is to study the gene in both the affected and nonaffected individuals of the family. If the mutation is found in all the affected members but absent in all nonaffected individuals it can be made sure that the gene responsible for the disease is identified.

Linkage analysis has been extremely useful in the identification of genes responsible for diseases with simple mendelian inheritance such as hypodontia. The application of linkage analysis to complex disorders (multifactorial diseases) without obvious patterns of Mendelian inheritance has been much less successful because complex diseases are most likely influenced by genetic heterogeneity (multiple genetic causes leading to the same disease) and also by environmental factors. In spite of this many multifactorial diseases have been identified by linkage analysis. A gene that influences a multifactorial trait (quantitative trait) is termed quantitative trait locus (QTL). As several genes determine the traits in a multifactorial disease, many QTLs will be involved together with various environmental effects. If the genes are linked to well-designed genetic markers (RFLPs, microsatellites or SNPs), these genes related to multifactorial traits can be mapped.

Further Details

Genetic Markers

It has been estimated that the 99.9% of DNA sequences of one individual are identical with that of another person. It means the phenotypic and genotypic differences between two individuals are due to 0.1% sequences, which are not same across individuals and are unique to a person. These different sequences may act as genetic markers.

Variation in sequences at a locus leads to a phenomenon called as polymorphism. Polymorphism is the change in the DNA sequence, or repeat element at a specific location. These points on the genome are called genetic markers. The first generation of these markers were termed *restriction fragment length polymorphism* (RFLPs), *random amplification*

of polymorphic DNA (RAPD), *amplified fragment length polymorphism* (AFLP) and the second generation markers are designated terms like *microsatellites* and *single nucleotide polymorphisms* (SNPs). These markers are usually spanned all over the genome and they are useful in mapping human disease gene as they are situated very close to the disease gene. Most commonly applied markers these days are the microsatellites and the SNPs. Microsatellites are DNA regions with variable number of short tandem repeat sequences flanked by unique sequences. The tandem repeats are usually dinucleotides and are in variable numbers. RFLPs and microsatellites are commonly used for linkage study of multifactorial traits in humans.

The SNPs are now considered to be superior to microsatellites for detection of linkages. This is due to the fact that a genome wide scan with microsatellites result in about 350 markers, with one microsatellite marker with every 5-10 cM length of the genome whereas the use of one SNPs yields approximately 3000-10000 markers every 5 Kb length of the genome.

Single Nucleotide Polymorphism (SNP)

A **single nucleotide polymorphism** (**SNP**, pronounced *snip*) is a variation occurring in the DNA sequence. In this variation a single nucleotide - A, T, C, or G - in the DNA sequence differs between members of a species or between paired chromosomes in an individual. For example two sequenced DNA fragments from identical regions in the genomes of two different individuals are AGGC**C**AA to AGGC**T**AA. The analysis shows that the two sequences contain a difference in a single nucleotide (C toT). In this case we say that there are two variation of a single nucleotide which are referred as ***alleles***: C and T. Most of the SNPs have two alleles. For a variation in the DNA sequence to be considered a SNP, it must occur in at least 1% of the population.

It is interesting to note that the most common type of mutation found in DNA is a single nucleotide polymorphism, which is mostly silent in terms that it does not produce any ill effect on the phenotype. The silent mutation causes none or at the most insignificant change in protein synthesis by a gene and without any change in the phenotype. These kind of silent SNPs are present throughout the genome (there are about 10 million). The SNPs can act as markers and are important tools in linkage analysis (gene mapping). They help to follow the movement of the maternal and paternal chromosomes through each generation in each member of the family. If a particular marker(s) travel with the disease gene then the marker is said to ***cosegregate*** with the disease gene. This also means that the marker and the disease gene are placed very close to each other on the chromosome. Once the positions of the cosegregating markers are identified, the DNA sequencing of neighboring areas (around the marker) identifies the gene for the disease.

The SNP has the answer to the question as to why different persons response differently to the same disease, i.e. why some people are more susceptible to periodontitis as compared to others. Because of the presence of genetic polymorphism (SNPs) the proteins produced by the different alleles of a gene are different. These proteins function differently and the functioning of the different proteins can be enhanced or modified by modulating certain environmental factors, e.g. diet, smoking, microbes, etc. Thus the susceptibility to the disease varies from person-to-person depending upon the individual risks involved.

What is the HapMap?

The HapMap is a catalog of common genetic variants (SNPs) that occur in human beings. There are more than 10 million SNPs present in humans. This catalog describes what these variants are, where they occur in our DNA and how they are distributed among people within populations and among populations in different parts of the world. The International HapMap Project is designed to provide information that other researchers can use to link genetic variants to the risk for developing specific illnesses that will lead to new methods of preventing, diagnosing and treating disease.

Genetic Linkage

During the formation of gametes reshuffling or redistribution of genes occur by the process of ***crossing over*** between a pair of homologous chromosomes. (*During prophase I of meiosis pairs*

of DNA duplicated homologous chromosomes unite in the formation of synapses. The nonsister chromatids then exchange chromosomal segments during crossing over). This is in accordance of Mendel's third law of inheritance "the principle of independent assortment" which states that members of the different gene pairs assort to gametes independent of one another (refer chapter 1). This mechanism of assortment is mostly true for genes present on different chromosomes but not always true for genes present on the same chromosome.

If two genes are present very close to each other on the same chromosome, there are good chances that both the genes would segregate and pass together (inherited) in the same gamete. These types of genes are called the ***linked genes.*** However, if two genes are situated at a great distance on the same chromosome then there are fair chances that they would segregate to different gametes as separate entities during crossover. This will result in the formation of new combination of genes in a fresh gamete. Thus when genes are situated close to each other on the same chromosome ***recombination*** is rare and when they lie farther apart, recombination is more common. Therefore the recombination frequency is more when the genes are found on different chromosomes or separated by a great enough distance on the same chromosome. If recombination occurs for at least half (50%) of the time or at rates more than this in all events of meiosis taken together, the related genes in question are said to be ***not-linked.***

The frequency of recombination may be used conversely, as a measure for determination of the distance between two locations on a genes, i.e. more the distance between two genes on the same chromosome, more the frequency of recombination; closer the distance, less is the frequency of recombination.

What is Recombination Frequency?

The distance between two gene loci on a chromosome can be measured and expressed in terms of the amount of the recombination between the loci (i.e. frequency of genetic recombination). This indirectly measures the chances of separation of two genes during crossover in meiosis. The genetic distance between two genes is expressed in terms of ***map units.*** For genes, which are close to each other on a chromosome, the distance of 1 map unit equals to 1% recombination between the two genes. This unit is also represents one ***centinmorgan (cM)*** unit. 1cM = 1% recombination frequency signifying that if two genes are placed 1cM apart on a chromosome, they are separated from each other only once in 100 meiosis and for the remaining 99 events of meiosis they remain linked to one another and segregate together to a gamete. The recombination frequency is represented as (theta or θ). Therefore, for 1% recombination, the value of $\theta = 1$ (0.01). If $\theta = 5$ (0.05) this means that two closely placed genes will separate from each other during 5% of all meiosis (i.e. they will remain linked during 95% of all meiosis or 19 times out of 20 meiosis).

Although the frequency of recombination increases as the physical distance between the genes increases, the upper limit of recombination frequency is 50% ($\theta = 0.5$). Thus genes on the same chromosome with more than 50% chances of recombination are said to be nonlinked. The recombination frequencies thus vary from $\theta = 0$ to $\theta = 50$. The recombination frequency is not only used to determine the distance between two genes. The comparison of the same parameters applied to recombination frequencies of more than two genes, the order of their locations and their relative distances from each other can also be determined.

LOD Score Method

The Lod score method is a new method to calculate the linkage distance between genes. In this method a series of likelihood ratios are calculated for different values of recombinant fractions ($\theta = 0$ to $\theta = 0.5$). The likelihood of a linkage as opposed to nonlinkage for different recombination fractions are computed and expressed as ***logarithm of the odd*** (Lod score). This likelihood ratio for a given value of θ is calculated as under:

$$\frac{\text{Likelihood of the observed data if genes are linked at a value of } \theta}{\text{Likelihood of the observed data if genes are not linked } (\theta = 0.5)}$$

The logarithm to the base 10 of this ratio is known as Lod score (Z).

Thus Lod score $\mathbf{Z = \log 10 \times [L\ \theta / L\ (0.5)]}$ In practice the value of Lod scores are found from a

table, which lists Lod scores for various standard pedigrees and various values of recombination frequency. Lod scores are advantageous to be used because they allow the pooling of scores from different families. A lod score of 3.0 and above indicate linkage between two genes. More the Lod score more closely placed are the genes. A score of 3.0 means the likelihood of observing the given pedigree is more than 1000 to 1 in case of two linked loci. On the other hand a lod score of less than –2.0 is considered as a nonlinked score. A Lod score between –2.0 and 3.0 is considered inconclusive. Once the linkage of a mutant gene to a particular location on a chromosome has been established, identification of the mutant gene and the type of mutation becomes possible.

ASSOCIATION STUDIES

Association studies have been widely employed to attempt to identify genetic basis of complex (multifactorial) diseases.

Two general approaches have been used to investigate the molecular genetics of complex diseases: candidate gene approaches and whole genome screens (genome-wide association studies).

In the candidate gene approach method, association analysis of genetic polymorphisms has been mostly performed in a case-control setting with unrelated affected subjects compared with unrelated unaffected subjects. Significant differences in allele frequencies between cases and controls are taken as evidence for involvement of an allele in disease susceptibility.

Genome-wide Association Studies

A genome-wide association study is an approach that involves scanning markers rapidly across the complete sets of DNA, or genomes, across a large number of people to find genetic variations associated with a particular disease (Morley et al, 2004). Once new genetic associations are identified, researchers can use the information to develop better strategies to detect, treat and prevent diseases. Such studies are particularly useful in finding genetic variations that contribute to common yet complex diseases such as asthma, cancer, diabetes, heart disease and mental illnesses.

Genome-wide association studies are relatively new ways to identify genes involved in human disease. This method searches the genome by scanning for small variations called single nucleotide polymorphisms or SNPs (see box), which occur more frequently in people with a particular disease than in people without the disease. Each study can look at hundreds or thousands of SNPs at the same time. Researchers use data from this type of study to pinpoint genes that may contribute to a person's risk of developing a certain disease. In case a positive association is found between a particular disease and particular markers, the SNPs, and if the same markers are also detected in a healthy person, he or she may be predicted to be at risk of developing the disease in future. Genome-wide association studies examine SNPs across the genome; they represent a promising way to study complex and yet common diseases in which several genetic variations are attributed to the risk of development of the disease in a person. This approach has already identified SNPs related to several complex conditions including diabetes, heart abnormalities, *Parkinson's disease*, and *Crohn's disease*. Researchers hope that future genome-wide association studies will identify variations that affect a person's response to certain drugs and influence interactions between a person's genes and the environment.

Method to Carry out the Genome-wide Association Studies

To carry out a genome-wide association study, researchers use two groups of participants: people with the disease being studied and similar people without the disease. Researchers obtain DNA from each participant by drawing a blood sample.

The complete set of DNA (or genome) is then purified from the blood, placed on tiny chips and scanned on automated laboratory machines. The machines quickly survey each participant's genome for strategically selected markers of genetic variation, which are called single nucleotide polymorphisms, or SNPs. If certain genetic variations are found to be significantly more frequent in people with the disease compared to people without disease, the variations are said to be "associated" with the disease. The associated genetic variations can serve as powerful

Chapter

10 Genetics of Developmental Disorders of Teeth

- Molecular (Genetic) control of development of tooth
- Tooth agenesis
- Supernumerary teeth or Hyperdontia
- Taurodontism
- Amelogenesis imperfecta
- Dentinogenesis imperfecta
- Dentine dysplasia
- Hypophosphatasia

MOLECULAR (GENETIC) CONTROL OF DEVELOPMENT OF TOOTH

Each tooth has a distinct morphology and a specific location and determination of both the factors is under strict genetic control. Following description discusses the sequential molecular events that control the development of tooth and bring forth definite morphological stages during such development.

Our knowledge of molecular control of tooth development is mainly based on the studies conducted in mouse embryos. Most of the investigations and experiments on these embryos were carried out between embryonic age 9.0 and 17.0 days (E9.0 – E17.0). These studies dealt with the expression of a number of transcription factors, signaling molecules, growth factor receptors and extracellular matrix molecules.

Teeth develop on the mandibular and maxillary arches through a series of interactions between the oral epithelium and underlying mesenchyme (i.e., the ectomesenchyme of first arch which is derived from the neural crest). Following are the main steps in the formation of a tooth.

- A thickening of the oral epithelium appears at E11.5 in mice. This is the first morphological sign of tooth development. The thickened oral epithelium is now called as dental lamina.
- The dental lamina now grows into the underlying mesenchyme of the first branchial arch and forms the epithelial bud at E13.5. During the formation of epithelial bud the mesenchymal cells get condensed around the developing bud and form dental papilla. The dental papilla later forms tooth pulp and odontoblasts. The dentine is formed by these odontoblasts.
- On the embryonic day 14.5 (E14.5) the epithelial bud changes to a cap shaped structure. This stage is called as the cap stage. The cap undergoes further folding and forms a bell shaped structure (bell stage, E15.5). The epithelium of the bell eventually gives rise to ameloblasts, which form enamel.
- The central portion of the epithelium of cap forms a special signaling center called as enamel knot. The enamel knot is involved in the formation of tooth cusps.

As stated earlier the formation of tooth begins with the interaction between the oral epithelium and the mesenchyme of the first branchial arch. It is believed that the oral epithelium of the first branchial arch sends signals to underlying mesenchyme. Under the influence of these signals the mesenchyme starts responding by expressing various regulatory genes.

How is the Oral-Aboral Axis Formed?

The oral epithelium of the first branchial arch from the day E9 starts expressing the gene Fgf-8 that secretes the fibroblast growth factor (FGF). This growth factor leads to the expression of Lhx-6 and Lhx-7 genes on the ectomesenchyme just beneath the oral epithelium. Lhx genes are the Lim-homeobox domain genes which are expressed as transcription factors and control the pattern of tooth formation. The expression of Fgf-8 is

restricted to the oral epithelium and matches very closely to the expression domain of Lhx-6/7 in the ectomesenchyme of the first branchial arch. The expression of these genes (Fgf-8, Lhx-6/7) thus establishes the oral-aboral axis.

How are the Sites of Formation of Tooth Germ Decided?

Many genes are involved in the formation of tooth germs. These are mentioned as under:

- The fibroblast growth factor encoded by the Fgf-8 gene (Fig. 10.1) that acts on the underlying mesenchyme of the first branchial arch and induces the expression of Pax-9 gene (Carlson, 2004). The Pax-9 gene is the member of Pax gene family and encodes a paired domain containing transcription factor. This gene defines the localization of tooth germs. Thus the exact site of appearance of the tooth germ is decided by the expression of Pax-9 in the mesenchyme region at the prospective sites of all teeth.

TABLE 10.1: Some important genes involved in tooth development

Genes	*Function*
Fgf-4	Induces expression of Msx-1 in dental mesenchyme / responsible for growth of dental epithelium and dental mesenchyme.
Fgf-8	Induces expression of Msx-1, Dlx-1/2, Pax-9, Lhx-6/7, Barx-1, Activin-A in the dental mesenchyme.
Fgf-9	Same as Fgf-4 / Fgf-8
Pax-9	It maintains Bmp-4 expression in dental mesenchyme. Mutation results in failure of development of all teeth.
Pax-6	Mutation develops additional upper incisors.
Msx-1	Required for the expression of Bmp-4, Fgf-3, and Dlx-2 in the dental mesenchyme. Mutations result in failure to develop all teeth.
Msx-2	The Msx-1/2 mutants result in the arrest of teeth development.
Dlx-1/ Dlx-2	Double mutant mice fail to develop maxillary molars. However single mutants have normal teeth.
Dlx-3	Mutation in humans results in enamel hypoplasia and taurodontism.
Shh	Stimulates proliferation of dental epithelium. Determines morphology of tooth.
Lef-1	Mediator of BMP-4 signaling. Mutation leads to arrest of development of teeth at bud stage.

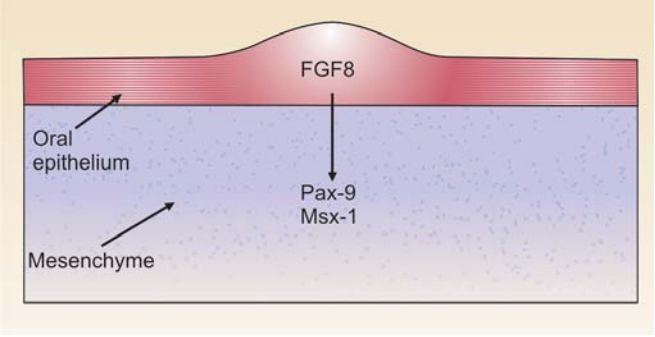


FIGURE 10.1: Initiation of development of tooth

- Pax-9 gene is expressed first in the prospective molar region (at E10) and then in the prospective incisor sites.
- The expression of Fgf-8 is widespread (not limited to only those areas where future tooth will form). However, the expression of Pax-9 is limited only to those sites, in the mesenchyme, where the future teeth germs would form. It means that there should be some mechanism by which the expression of Pax-9 is inhibited in mesenchyme where tooth is not destined to be formed.
- Both BMP-4 and BMP-2 molecules (Bone morphogenetic proteins) are expressed by Bmp-4 and Bmp-2 genes. BMP-4 and BMP-2 proteins are able to inhibit the 'Pax-9 inducing activity' of Fgf-8 in the tooth mesenchyme (Fig.10.2). At quite early stages, Bmp-4 is expressed only in the epithelium but at later periods is expressed in mesenchyme also.
- The expression of Pax-9 is inhibited in those areas where teeth are not designed to develop.

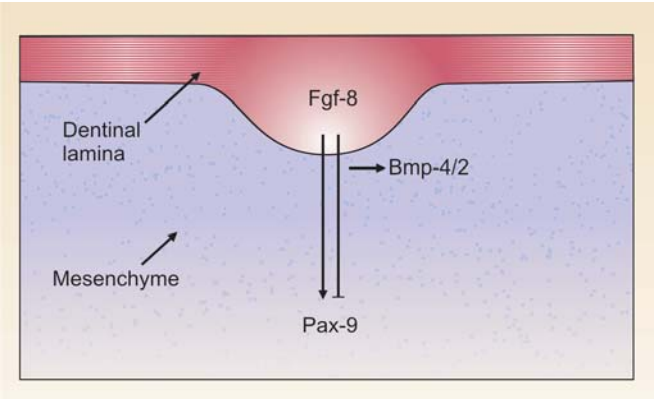


FIGURE 10.2: Fgf-8 activates the Pax-9 while Bmp-4/2 inhibits it. (→ = activates,—| = inhibits)

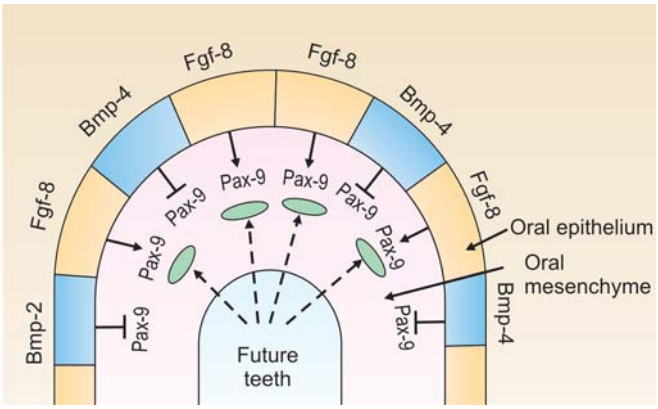


FIGURE 10.3: Future teeth are formed at sites where Fgf-8 activates Pax-9 expression. No teeth develop at sites where Pax-9 is inhibited by Bmp-4/2

- Therefore the tooth germs develop only in those areas where Pax-9 expression is induced in the mesenchyme by the Fgf-8 expression in the overlying epithelium. Tooth germ does not develop in those areas where Bmp-4 signaling inhibits the 'Pax-9 inducing activity' of Fgf-8 (Fig.10.3).
- Bmp-4 signaling also stimulates mesenchymal expression of Msx-1 and Bmp-4 itself. The Msx-1 (muscle specific homeobox like gene) expression is also induced by Fgf-8. Consequently, both Msx-1 and Pax-9 have similar functions i.e., both are essential in the mesenchyme for tooth morphogenesis. Thus, whereas Fgfs and Bmps have opposite effects on the expression of Pax-9, they both stimulate the expression of Msx-1.
- At E 11.5 the potential to direct the tooth development shifts from epithelium to mesenchyme. The expression of Bmp-4 also shifts now from the epithelium to mesenchyme. The Pax-9 and Msx-1 genes are co-expressed in the mesenchyme where the function of both the genes is required for the expression of Bmp-4.
- After E11.5 Pax-9 expression is not under the control of epithelium signals. Similarly, Bmps are no longer able to inhibit Pax-9 expression in mesenchyme.
- The Pax-9 and Msx-1 are essential for the establishment of the odontogenic potential of the mesenchyme.
- Lef-1 is first expressed in the dental epithelium (during dental lamina stage). Expression of this gene then shifts to mesenchyme during the bud stage. The mutation of Lef-1 gene (or Lef-1 knockout mice) shows the arrest of all dental development at its bud stage.
- The expression of Shh is localized in the dental epithelium at E11 and thus Shh is considered to be a good signaling candidate for tooth initiation. Shh stimulates epithelial cell proliferation at the sites of tooth development. Gli-2 and Gli-3 genes are two downstream mediators of Shh action. In case of Gli-2 and Gli-3 double mutation, embryos fail to produce any recognizable tooth bud.

How do Genes Control the Formation and Function of the Enamel Knot?

The enamel knot is the transient signaling center of the epithelium and directs the next phase of tooth development. The enamel knot is responsible for the formation of the tooth cusps that later give each individual teeth its characteristics surface.

- The development of the enamel knot is regulated by signals originating in the mesenchyme. One of the important functions of Pax-9 and Msx-1 is the maintenance of mesenchymal Bmp-4 expression. The Bmp-4 signaling is involved in the formation of the enamel knot.
- Bmp-4 induces the expression of p21, Bmp-2 and Msx-2 in the epithelium. These genes are associated with programmed cell death. The enamel knot is formed at the cap stage of dental development and secretes factors responsible for the apoptosis in the knot itself.
- At the same time the enamel knot secretes Fgf4 and Fgf9 which stimulate proliferation of certain neighboring cell compartments (enamel epithelium and dental papilla) (Fig. 10.4). As Fgf receptors are not present on the enamel knot cells, they do not respond to the Fgfs and thus fail to show proliferation.
- It is believed that Lef-1 gene (Hmg box containing transcription factor) mediates Fgf/BMP signaling in the epithelium at the late bud and early cap stage of tooth development. In the absence of Lef-1 tooth formation is arrested at the bud stage. The expression of Lef-1 has been shown to be

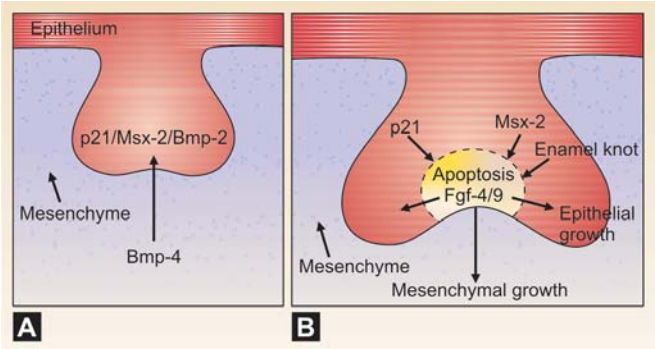


FIGURE 10.4: Mesenchymal Bmp-4 activates p21/ Msx-2/ Bmp-2 in the epithelium. (B) p21/ Msx-2/ Bmp-2 trigger apoptosis in the enamel knot while Fgf-4/9 activates surrounding epithelial and mesenchymal growth

inducible by Bmp-4. Thus Lef-1 is the mediator of Bmp-4 signaling in the epithelial tooth bud and is necessary for the establishment of enamel knot.

- Besides the above-mentioned genes, many other genes (i.e., Bmp-2, Bmp-7, Fgf-9, Slit-1 and Shh) are also expressed by cells of enamel knot. Shh expression is needed for the determination of bud morphology at E 13.

How are the Tooth Type and their Correct Position Determined?

Most of the genes for dental patterning are expressed in the dental mesenchyme before E11. It has been suggested that there exists an odontogenic homeobox gene code that might determine the identity of each individual teeth. These homeobox genes include Msx-1, Msx-2, Dlx-1, 2, 3, 5, 6, and 7, Barx-1, Otlx-2, Lhx-6 and 7. These genes are expressed in specific spatial pattern in the mesenchyme of the first branchial arch which is derived from the neural crest cells of the mid-brain region.

- The instructions for tooth type formation are due to temporal regulation of homeobox genes expression in the ectomesenchyme, which is induced by ectodermal signals before E11.
- The release of Fgf-8 from the epithelium induces the expression of the homeobox gene Barx-1 in the mesenchyme of the proximal part (postrior part) of the first branchial arch. Presumptive molars develop in the proximal part of the first arch. Dlx-1 and Dlx-2 genes are also expressed in this region of the branchial arch and along with Barx-1 they are also involved in the formation of molar teeth. Though the Dlx-1 and Dlx-2 are expressed in both mandibular and maxillary arch but Dlx-5 and Dlx-6 are only expressed in mandibular arch. The mutation of both Dlx-1 and Dlx-2 leads to the absence of maxillary molars only. This is because the expression of Dlx-5/6 is responsible for the formation of mandibular molars. Thus Dlx-5/6 compensate for the loss of Dlx-1/2.
- The release of BMP-4 from the epithelium induces the expression of Msx genes in the distal part (anterior part) of the mesenchyme of first arch where presumptive incisor forms. Thus the expression of Msx-1 and Msx-2 in the distal mesenchyme is induced by BMP-4 and leads to the formation of the incisor teeth.
- It is believed that the formation of canine and premolars in human results due to overlapping domains of gene expression, i.e. overlapping expression of Msx and Dlx genes (Fig.10.5).
- The Msx genes are considered as incisor genes and Barx-1 as molar genes. The formation of canine and premolars in humans may be under the control of overlapping expression of Msx and Dlx genes.

TOOTH AGENESIS

Tooth agenesis is defined as deficiency in tooth number and it is one of the most common

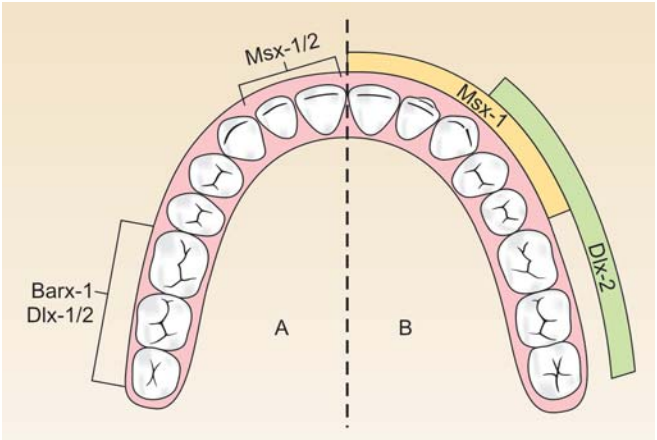


FIGURE 10.5: (A) Msx-1 gene is responsible for formation of incisors, while Dlx and Barx-I for molars. (B) The formation of canine and pre-molars are due to overlapping expression of Msx and Dlx genes

developmental anomalies in humans. The non-syndromic tooth agenesis is observed in various forms, i.e., partial or total (generalized). In mild form only one or few teeth are absent. While in its severe forms, many teeth are absent. It should be noted that agenesis of third molars is much more common than the absence of other teeth (one or more third molars fail to develop in up to 35% of people in various population groups of the world). On the other hand the agenesis of deciduous teeth is quite rare; (below 1%) in various populations. If we exclude the agenesis of the third molars, the absence of more than two teeth is observed in only about 1% of population. The absence of more than six teeth is very rare (0.1–0.3 % of population).

Tooth agenesis is usually an isolated anomaly (non-syndromic) in which tooth agenesis itself is the primary condition. However, it is also observed in association with oral clefts (Boogaard et al, 2000) and various malformation syndromes. When tooth agenesis is associated with syndromes it is usually severe in form. Following is the list of syndromes and other congenital malformations in which tooth agenesis is found in conjunction with other developmental anomalies:

- Rieger's syndrome
- Wolf-Hirschhorn syndrome
- Williams syndrome
- Kabuki make up syndrome
- Cleft lip / cleft palate
- Ectodermal dysplasia
- Chondroectodermal dyspalsia
- Achondroplasia
- Holoprosencephaly.

The following description of tooth agenesis is confined to isolated or nonsyndromic agenesis.

Classification of Tooth Agenesis

Tooth agenesis is observed in following three forms:

Hypodontia refers to the developmental lack of a few teeth (Fig. 10.6). The population frequency is over 5% (missing of wisdom teeth excluded).

Oligodontia refers to the developmental lack of more than six teeth (wisdom teeth not included) (Figs 10.7 and 10.8). The population frequency is low especially for cases when the absence of teeth is the only malformation ("isolated" cases). Most often oligodontia appears as part of a congenital syndrome that affects several organ systems (ectodermal dysplasia, achondroplasia, chondroectodermal dysplasia, Rieger syndrome, etc.).

Anodontia refers to complete lack of teeth, which is very rare in occurrence.

Shapes and positions of existing teeth may also be abnormal in association with missing teeth. The

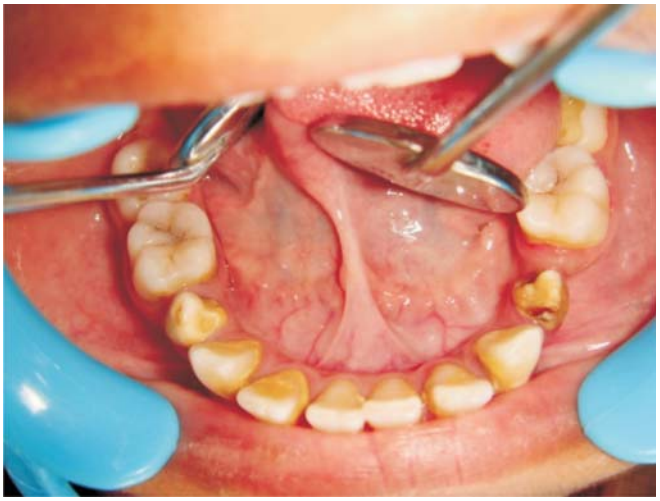


FIGURE 10.6: A patient of hypodontia. *Courtesy* Dr Priyanka Airen Dept. of Pediatric Dentistry, Modern Dental College and Research Center (RC), Indore, India

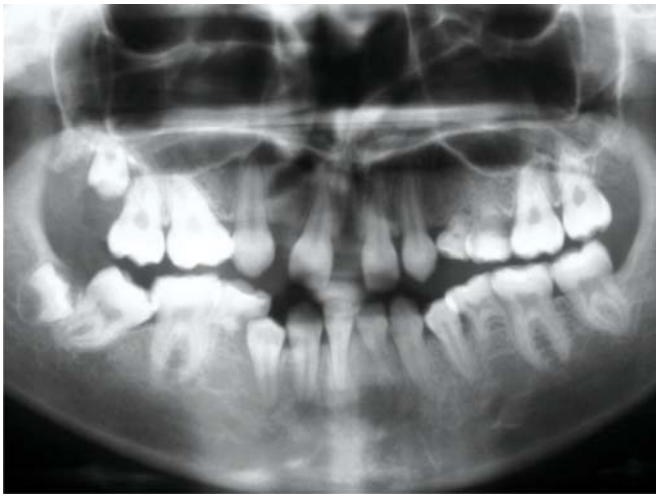


FIGURE 10.7: Congenital partial oligodontia. *Courtesy* Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

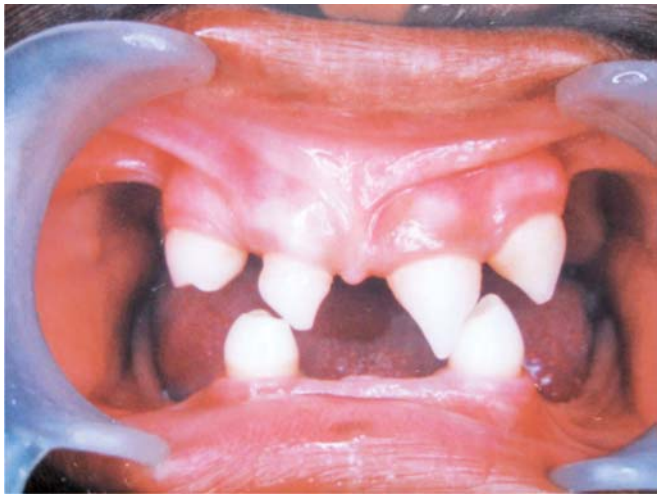


FIGURE 10.8: Patient of ectodermal dysplasia showing developmental lack of teeth (oligodontia). Note the presence of peg-shaped teeth. *Courtesy* Prof. AL Shigli, Head of the Dept. of Pedodontics, Modern Dental College and RC, Indore, India.

features often seen include "peg-shaped" maxillary lateral incisors, "taurodontism" of molars and "malpositions".

Etiology of Tooth Agenesis

Though many environmental etiological factors for tooth agenesis have been identified, there is definitive proof that genetic factors play a major role in their etiology.

Environmental factors that are implicated are: maternal systemic diseases (maternal diabetes, hypothyroidism, rubella infection during pregnancy), anticancer treatment during childhood (radiotherapy and chemotherapy).

Genetics of Tooth Agenesis

Pax-9 and Msx-1 are two key genes involved in the embryological development of teeth and their mutation leads to tooth agenesis (Mostowska et al., 2003: Matalova et al., 2008 and Kapadia, et al. 2007). Pax-9 is situated on chromosome 14 (14q21) and belongs to the Pax gene family that encodes a group of transcription factors playing a major role in early development. Pax proteins are defined by the presence of a DNA-binding domain, the 'paired domain', which makes sequence-specific contact with the target DNA region. Msx-1 is a homeobox gene involved in numerous epithelial-mesenchymal interactions during vertebrate embryogenesis and appears to be incredibly significant during early tooth development. It is situated on the short arm of chromosome 4.

Pax-9 and Msx-1 encode transcription factors that are known to be essential for the switch in the odontogenic potential of developing tisssues in the epithelium and the mesenchyme. These molecules play an important role in the maintenance of mesenchymal Bmp4 expression responsible for the formation of the dental organ. Pax-9 is able to regulate Msx1 expression directly and interact with Msx-1 at the protein level to enhance its ability to transactivate Msx1 and Bmp4 expression during tooth development. Pax-9 and Msx1 act as partners in a signaling pathway that involves Bmp4. Furthermore, the regulation of Bmp4 expression by the interaction of Pax-9 with Msx-1 at the level of transcription and through formation of a protein complex determines the fate of the transition from the bud to cap stage during tooth development.

Till date seven Msx-1 mutations as well as some whole gene deletions have been discovered in tooth agenesis patients. Msx-1 frameshift mutation is responsible for autosomal-dominant oligodontia without clefting or nail dysplasia. The mutation involves duplication of the guanine nucleotide at position 62 in exon 1 of the Msx-1 gene. This mutation in Msx1 is usually associated with the absence of multiple permanent teeth including all second bicuspids and mandibular central incisors.

A number of mutations (upto 15) have been identified in the Pax genes that include nonsense, missense, frameshift and deletion types of defects. Mutation in the initiation codon of Pax-9 causes severe or complete inhibition of Pax9 translation at one allele resulting in a reduced amount of Pax-9 transcription factor, representing a haploinsufficiency for Pax-9. This functional insufficiency or absence of Pax-9 protein produced from Pax-9 gene ultimately results in tooth agenesis.

The *Msx1* and *Pax9* kindred's have a high but equal probability of missing the third molars and hence the absence of third molars is not a useful indicator of the particular gene (*Msx1* or *Pax9*) that is likely to be

affected in a given kindred. Mutations in Msx-1 and Pax-9 genes may cause different types of oligodontia (different sets of teeth are missing in different gene mutations). For example all individuals with a mutation in Msx-1 lack all second premolars and third molars (and a variable number of other permanent teeth). Typically mutations in the Pax-9 cause agenesis of most permanent molars (and again, a variable number of other permanent teeth). These differences presumably reflect different functions of these genes during development.

Very recently it has been shown that oligodontia and predisposition to cancer are caused by a nonsense mutation in the Axin-2 gene. The Axin-2 gene is located on the chromosome 17. The Axin-2 is a Wnt-signaling regulator. Wnt signaling regulates embryonic pattern formation and morphogenesis of most of the organs. Wnt-signal activity is necessary for normal tooth development. During tooth development Axin-2 is expressed in the dental mesenchyme, the odontoblasts and the enamel knot. Aberrations of regulation of Wnt signaling may lead to cancer. The nonsense mutation of Axin-2 is not only associated with tooth agenesis but also with colorectal cancer.

Dlx1 and Dlx2 genes play an important role in odontogenic patterning. These genes are important in the development of maxillary molars in mice. But these genes are not required for development of incisors and mandibular molars. The mutation of Dlx1 and Dlx2 in mice leads to failure of development of maxillary molars.

Mode of Inheritance of Tooth Agenesis

The heritability of developmental missing teeth has been shown in many studies. The responsible genetic factors may be of dominant, recessive or multifactorial (genetic and environment) patterns in terms of inheritance.

Both hypodontia and oligodontia due to mutation in Pax9 and Msx1 genes have autosomal dominant mode of inheritance. However in both the cases the degree and identity of missing teeth may vary between relatives. The variability is probably caused by other genetic and environmental factors and in some cases the etiology is similar to multifactorial traits. Many studies have suggested that most cases of hypodontia have a polygenic inheritance pattern.

In some cases of hypodontia autosomal recessive and X-linked inheritance have also been reported.

SUPERNUMERARY TEETH OR HYPERDONTIA

Hyperdontia is the condition of having **supernumerary teeth** or teeth which appear in addition to the regular number of teeth.

The most common supernumerary tooth is a **mesiodens** which is a malformed, peg-like tooth that occurs between the maxillary central incisors (Fig. 10.9). Fourth and fifth molars that form behind the third molars are another kind of supernumerary teeth. Another rare type of supernumerary teeth is a "third set of teeth" that forms underneath and pushes out the second set of teeth, much like the second set that is formed underneath and pushes out the first set of teeth.

Hyperdontia can be syndromic (i.e. associated with Gardner's syndrome, cleft lip and cleft palate and cleidocranial dysostosis) or nonsyndromic (isolated). Supernumerary teeth in deciduous dentition are less common than seen in permanent dentition.

The etiology of supernumerary teeth is not completely understood. It is thought that the supernumerary tooth is created as a result of a branching of the tooth bud or from fragmentation of the dental lamina. Genetics may also play a defining role in the occurrence of this anomaly (D'souza et al, 2007) as supernumeraries are more commonly found in the relatives of affected children than in the general population (Kawashima et al., 2006). Many studies have indicated that the anomaly follows a simple mendelian pattern of inheritance (autosomal dominanat) (Batra et al, 2005) while some other studies indicate that no such definite pattern of inheritance exist.

TAURODONTISM

Taurodontism is a condition found in teeth where the body of the tooth and pulp chamber is enlarged at the expense of the root (Fig. 10.10). The changes of taurodontism are usually most striking in the molars.

Taurodontism was a frequent finding in early man and is found today in races such as the Eskimos who use their teeth for cutting hides. The mode of inheritance of the condition is not very specific as it is likely to be polygenic. Few studies have suggested it to be a dominant, others as recessive and some others as an X-linked trait. This condition is also associated with various syndromes, e.g.

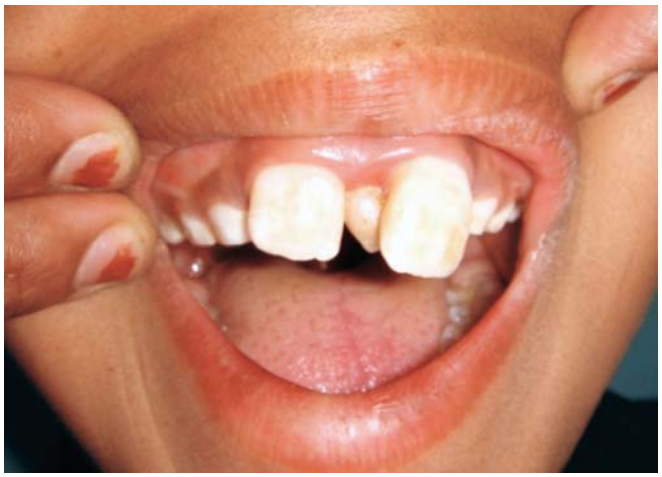


FIGURE 10.9: Supernumerary teeth. *Courtesy* Dr Priyanka Airen, Dept. of Pedodontics, Modern Dental College and RC, Indore, India

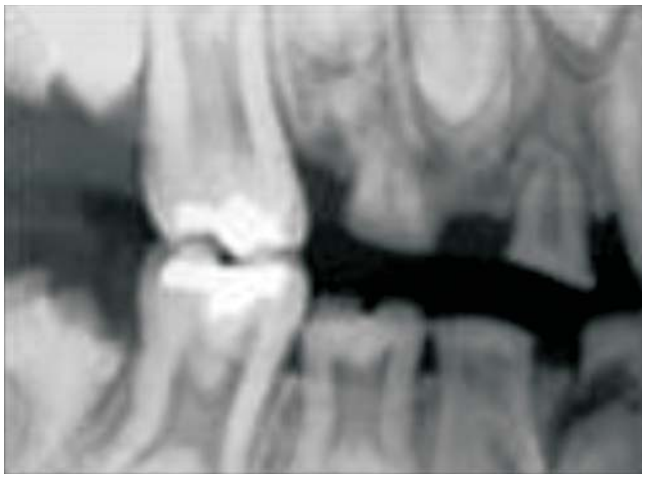


FIGURE 10.10: Teeth showing taurodontism. *Courtesy* Dr Priyanka Airen, Dept. of Pedodontics, Modern Dental College and RC, Indore, India

the trichodento-osseous syndrome, otodental dysplasia *and* Klinefelter syndrome.

AMELOGENESIS IMPERFECTA

When a person's teeth are covered by thin or malformed enamel, the condition is known as amelogenesis imperfecta (AI). The condition results due to abnormal formation of the enamel. This condition affects both the deciduous and permanent teeth.

Amelogenesis imperfecta is an inheritable (genetic) condition caused by mutation in genes which encode for enamel matrix proteins (Santos et al, 2005). These enamel proteins are needed for formation of normal enamel. As there are many genes (proteins) involved in the formation of enamel, AI presents in many phenotypic forms.

AI is usually classified in four major categories (Crawford et al., 1992) and 14 subtypes (Table 10.1).

Characteristics of Various Types of AI
(Figs 10.11 and 10.12)

Hypoplastic type of AI

- Enamel formed is hard and well-calcified but its amount is insufficient (incomplete formation of the organic enamel matrix of teeth).
- Phenotypically enamel defect is seen in various forms, *viz.* as generalized defects (affecting complete enamel thus enamel is thin and translucent) and as localized defects (pits and grooves are seen in the specific areas of the enamel), etc.
- When the enamel is thin the teeth are of small size and as such they may not contact each other mesodistally. As enamel is very thin, teeth are sensitive to thermal stimuli.
- The irregular formation of enamel (absence of enamel in some areas) is due to the absence of ameloblast cells in some areas of the enamel organ.

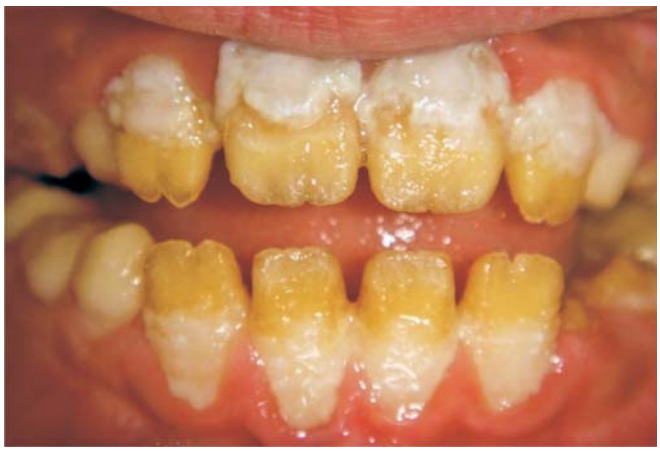


FIGURE 10.11: Amelogenesis imperfecta. *Courtesy* Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

TABLE 10.2: Classification of AI, modes of inheritance, genes and their location

Main category	*Subtype*	*Mode of inheritance*	*Gene*	*Location*
Type 1 Hypoplastic				
	A- Pitted type	AD	—	—
	B-Local type	AD	ENAM	4q13.3
	C–Local type	AR	—	—
	D-Smooth type	AD	ENAM	4q13.3
	E- Smooth type	XLD	AMELX	Xp22.3 – 22.1
	F- Rough type	AD	—	—
	G- Agenesis type	AR	—	—
Type 2 Hypomaturation				
	A- Pigmented type	AR	KLK-4	19q13
			MMP20	11q22.3-23
	B- XLR type	XLR	AMELX	Xp22.3 –22.1
	C- Snow capped type	AD	—	—
Type 3 Hypocalcified				
	A-AD type	AD	—	—
	B-AR type	AR	—	—
Type 4 Hypomaturation / hypoplastic/ Taurodontism				
	A-AD type	AD	DLX3	17q21-q22
	B-AD type	AD	—	—

AD = autosomal dominant, AR = autosomal recessive, XLD = X-linked dominant and XLR = X-linked recessive (— = not known).

Hypomaturation Type of AI

- Enamel is of normal thickness (not hypoplastic).
- It is relatively of normal hardness (slightly hypocalcified). Shows reduced radiographic density.
- Enamel is opaque and has a porous surface that becomes stained (white to brownish yellow).
- Teeth are soft and vulnerable to attrition.

Hypocalcified Type of AI

- Enamel matrix is formed but poorly calcified.
- Enamel is of normal thickness, very soft and has a cheesy consistency.
- It is opaque, fragile and chalky in appearance. Teeth tend to become stained.
- It gets chipped away easily during mastication.
- Many teeth may fail to erupt.

Hypomaturation/Hypoplastic/Taurodontism Type of AI

- The enamel appears mottled.
- Teeth may be pitted on facial surface and are yellowish brown color.
- Molar teeth may show taurodontism.
- Pulp chamber are enlarged in molar teeth.

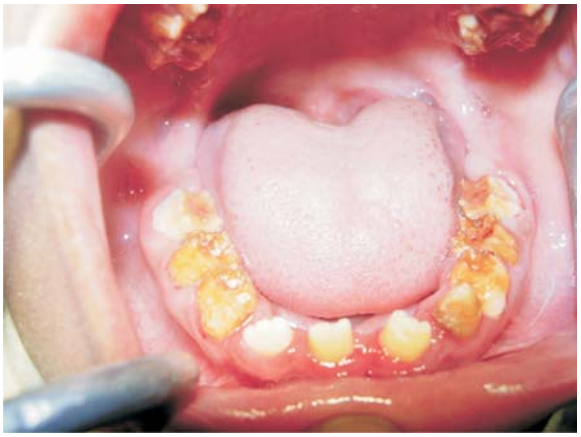
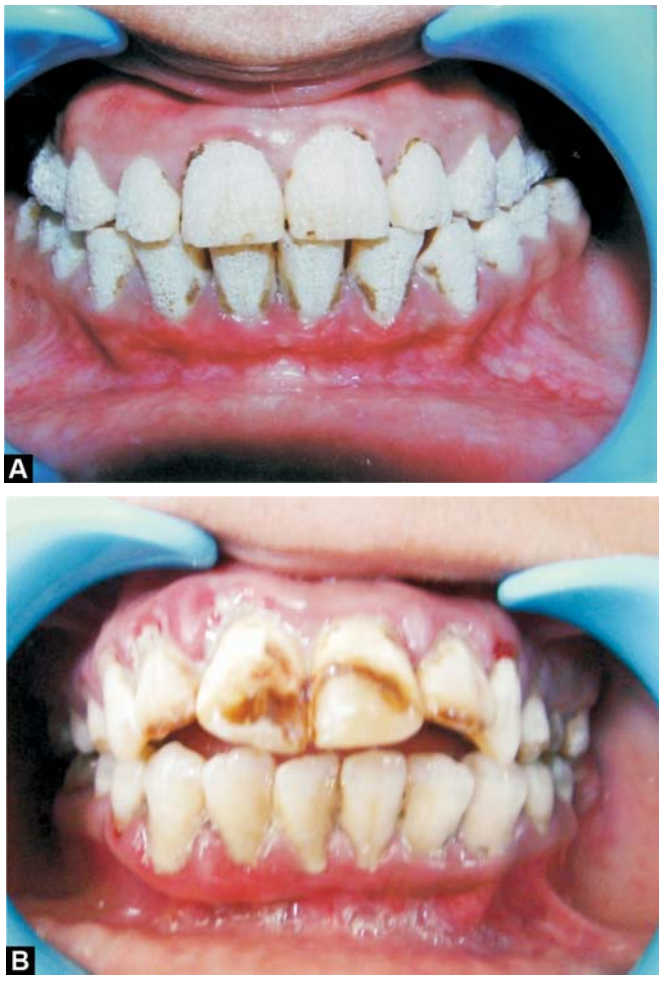


FIGURE 10.12: Amelogenesis imperfecta. *Courtesy* Dr Rajesh Kumar, Dept. of Periodontology, Sri Aurobindo Institute of Medical Sciences, Dental College, Indore, India



FIGURES 10.13A and B: Various types of enamel hypoplasia. A–Enamel showing motteling, B–Enamel showing stains. *Courtesy*, Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

Genetics of AI

How is AI Produced?

The enamel is mostly composed of a mineral (calcium hydroxyapetite) that is formed and regulated by proteins in it. Major proteins that help in the formation of enamel are ***ameloblastin, enamelin, tuftelins*** and ***amelogenin***. Protein *amelogenin* is the most abundant protein in enamel (about 90% of all the enamel proteins). It helps to separate (produces spacing) and support the ribbon-like enamel crystals as they grow. It also regulates the thickness of enamel. Proteins *enamelin* and *ameloblastins* are needed to shape and organize the mineral containing crystals in the developing enamel. Ameloblastins are believed to guide the enamel mineralization process by controlling elongation of enamel crystals and to form junctional complexes between enamel crystals. In the developing enamel *ameloblastin* consists of 5% and *enamelin* 2% of total enamel proteins. Tuftelins are located near the dentoenamel junction. They help in the nucleation of enamel crystals. Tuftelins is present in the enamel tufts.

Once the functions of *amelogenins* and *ameloblstins* are over in the developing enamel they are cleaved and removed during the maturation stage of the enamel. Two different proteolytic enzymes cleave these proteins namely ***enamelysin*** and ***kallikrein-4***. *Kallikrein* is responsible for cleavage of the *amelogenin* protein and *enamelysin* cleaves *amelogenin, ameloblastin* and *enamelins* enamel proteins. Thus after maturation very little protein remains in the enamel (products of cleaved *enamelins* and *tuftelins*).

The malformation of these proteins (either due to their absence or altered structure) leads to the formation of abnormal enamel (amelogenesis imperfecta—AI). As genes code all these proteins, mutations in these genes cause AI. Genes like AMELX, ENAM, KLK4, MMP20 and DLX3 code for the major protein components involved in the formation of enamel. Protein tuftelins is produced by the gene TUFT located on the long arm of chromosome number 1 at position 21st (1q21). The following table gives the details about the genes and proteins coded by them.

Various known genes responsible for AI:

TABLE 10.3: Various types of AI due to mutation in gene

Gene	*Location*	*Protein*	*Type of AI due to mutation*
ENAM	4q13.3	Enamelins	Hypoplastic types of AI
AMELX	Xp22.3 - 22.1	Amelogenins	Hypoplastic type of AI (XLD) Hypomaturation type of AI (XLR)
MMP20	11q22.3 - 23	Enamelysins	Hypomaturation type of AI
KLK4	19q13	Kallikrein-4	Hypomaturation type of AI
DLX3	17q21.3	Transcription factor(Dlx3 protein)	Hypomaturation/ hypoplastic/ taurodontism type of AI

ENAM

This gene codes for the protein *enamelin* needed for normal development of enamel. The gene is located on the long arm of chromosome number 4 at position 13.3 (4q13.3). Various mutations are identified in the ENAM gene that leads to the production of altered *enamelins* protein (Hart et al, 2003 and Rajpor et al, 2001). Sometimes the mutation is significant enough to stop the synthesis of *enamelins* absolutely. The absence or altered structure of *enamelins* leads to abnormal development of enamel. It either produces severe defects in the enamel (completely absent enamel or thin enamel) or milder defects (pits, ridges or grooves in the enamel).

The mutations in ENAM gene are inherited as autosomal dominant (AD) or autosomal recessive (AR) in association with hypoplastic type of AI (Kida et al, 2002).

The AMBN gene codes for the protein *ameloblastin* and is situated close to the ENAM gene. Both genes are located together on the long arm of chromosome 4 at 13th position (4q13). The AMBN gene is also said to be located at the 4q21 position and its mutation leads to AIH2 type of hypoplastic amelogenesis imperfecta.

AMELX

The AMELX gene codes for production of the *amelogenin* protein. Similar to the ENAM gene many mutations of AMELX gene have been identified. The mutation may lead to a nonproduction or altered production of *amelogenin*. This interferes with the formation and organization of enamel crystals. Enamel cannot form without adequate amount of *amelogenin*.

The gene is located on the short arm of X-chromosome between positions 22.31 and 22.1 (Xp22.3 – 22.1). The mutation is inherited in an X-linked dominant (XLD) and X-linked recessive (XLR) pattern. In case of the XLD disease the males are severely affected owing to complete lack of amelogenin. They develop no enamel to cover their teeth. However in the case of females (due to the mechanism of Lyonization of X-chromosome) some enamel is always formed in cells where the normal X-chromosome is not inactivated. But this enamel is phenotypically abnormal as it shows structural defects (vertical grooves) in them. The XLD inheritance is observed in hypoplastic type of AI while XLR inheritance is observed in hypomaturation type of AI (Ravassipour et al., 2000 and Hart et al, 2002).

Similar to the AMELX gene on X chromosome, amelogenin producing gene is present on the Y chromosome. It is known as AMELY. This gene is not similar to amelogenin gene on the X-chromosome because it has a different sequence of amino acids. This gene (AMELY) is not important in enamel formation. Only the AMELX gene is critical in enamel development.

The AMELY is situated on the short arm of Y-chromosome at 11th position (Yp11).

MMP20

The MMP20 (***Matrix Metallopeptidase 20***) gene codes for the protein ***enamelysin.*** *Enamelysin* is needed to cleave other proteins like *ameloblastin* and *amelogenin* during the maturation of the enamel. After the cleavage by *enamelysin* these proteins are easily removed from the enamel. In case of mutations of MMP20 genes enamelysin is not produced. Thus ameloblastin and amelogenin and other enamel forming proteins are not cleaved and remain present in the developing enamel resulting in soft enamel having an abnormal crystal structure. Hypomature teeth are formed as a consequence.

The gene is located on the long arm of chromosome number 11 at position 22.3 (11q 22.3). The mutation of MMP20 gene is inherited in an autosomal recessive pattern and leads to hypomaturation type (pigmented type) of AI (Li W, et al., 2001).

KLK-4

KLK-4 gene codes for the protein ***kallikrein,*** a proteolytic enzyme belonging to the tissue kallikrein family of serine proteases. This enzyme is responsible for the degradation of enamel protein during the maturation stage. The gene is present on the long arm of chromosome 19 at 13th position (19 q 13). The mutation of this gene leads to hypomaturation (pigmented) type of AI which is inherited as an autosomal recessive trait (Hart et al, 2004).

DLX3

The DLX3 (Distal-less homeobox 3) is a transcription factor gene which codes for the Dlx3 protein. It is a

highly penetrant gene whose mutation leads to hypomaturation / hypoplastic / taurodontinism type of AI. The DLX3 gene is located on the long arm of chromosome 17 at position 21.3 (17 q 21.3).

It should be noted that hypocalcified type of AI has not been associated with any specific gene till date.

DENTINOGENESIS IMPERFECTA

The term dentinogenesis imperfecta (DGI) is defined as a genetic disease, which leads in the formation of defective dentine. The dentin is poorly formed with an abnormal low mineral content. Here the enamel is normal but the pulp chamber and pulp canal are obliterated. This condition is also associated with discoloration of teeth (dusky blue to brownish). Teeth usually wear down rapidly leaving short and brown stumps. This problem affects both the primary and permanent teeth.

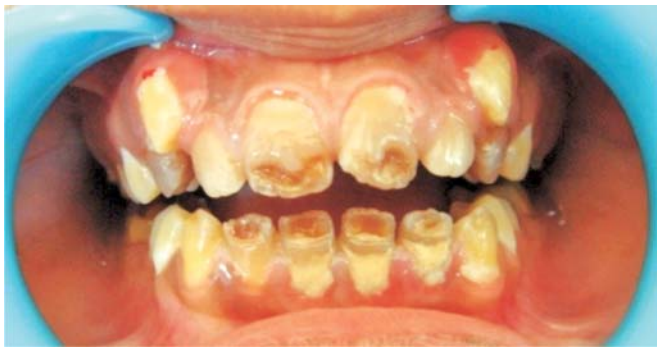
Incidence: The incidence varies from 1 in 6000 to 1 in 8000 births.

Types of DGI

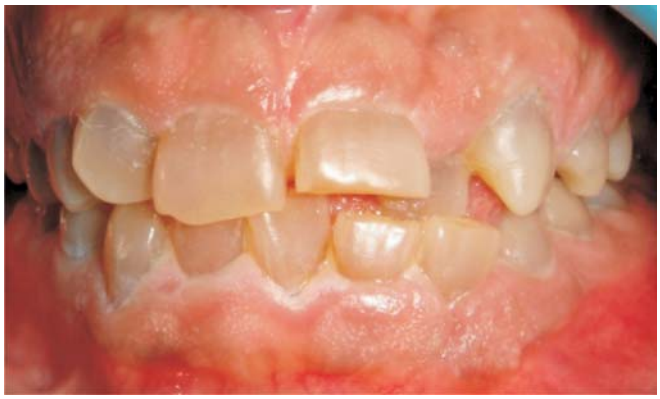
Shields has described three different types of dentinogenesis imperfecta:

1. ***Shields Type I DGI*** – This type is associated with osteogenesis imperfecta (OI), a condition where bones are congenitally brittle and easily broken. This is an inherited defect of collagen formation which results in weak and brittle bones, bowing of limbs and blue sclera. The milk teeth are more severely affected in this condition. Teeth may show an amber translucent color. Crowns are bulbous and pulp chambers show obliteration in radiographs.
2. ***Shields Type II DGI*** –Dentinogenesis imperfecta is an entity clearly distinct from osteogenesis imperfecta and manifests with opalescent teeth (Fig.10.14) and the teeth are the only structure affected in the body. There is no incidence of increased frequency of bone fractures in this disorder. In this type of DGI only the dentin is affected. Witkop and Rao (1971) preferred the term ***hereditary opalescent dentin*** for this condition as an isolated trait and reserving the term dentinogenesis imperfecta for the trait combined with osteogenesis imperfecta. The teeth are blue-gray or amber brown and opalescent. On dental radiographs, the teeth have bulbous crowns, roots that are narrower than normal, and pulp chambers and root canals that are smaller than normal or completely obliterated (Fig.10.15).

   This type of DGI is sometimes associated with progressive loss of hearing.
3. ***Shields Type III DGI***—It is called the brandywine form after the city of Brandywine in Maryland where a large population of patients were affected with this disorder. Similar to the features in type II of the disorder, this particular variant affects only the dentine without any involvement of the bones. Type III tends to be less severe than type II disease. Whether type III should be considered a distinct phenotype or a variation of DGI II is debatable. Witkop (1975) indicated that the type II and type III variants might be one and the same because of their clinical similarities. However, unlike the type II and type I chracteristic, type III



Mother



Son

FIGURE 10.14: Dentinogenesis imperfecta in a mother and her son. *Courtesy*, Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

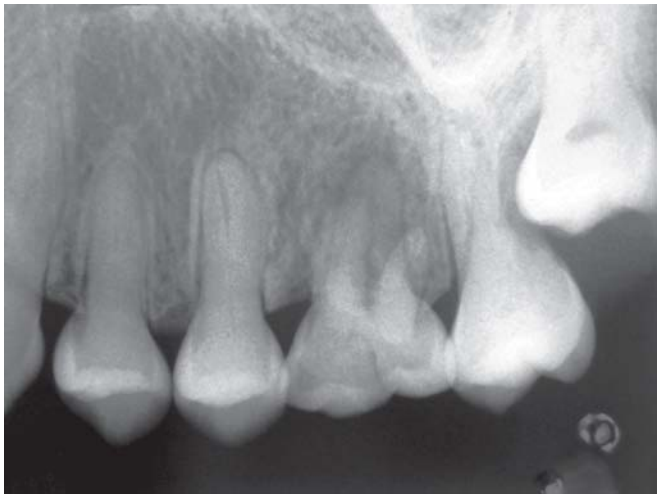


FIGURE 10.15: Dentinogenesis imperfecta. Note that roots are slender and short; crowns are bulbous and pulp chambers show obliteration in radiographs. The radiograph is of the same patient (son) of Fig. 10.14. *Courtesy* of Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

is associated with ***shell-like teeth*** having multiple pulp exposures.

Genetics of DGI

The gene **DSPP** is responsible for coding ***dental sialophosphoprotein***. Soon after the production of this protein it is cleaved into three smaller proteins namely, the *dentin sialoprotein (DSP), dentin glycoprotein* and *dentin phosphoprotein (DPP)* needed for the formation of dentin. The first two proteins are involved in the normal hardening of collagen. All the three proteins help in the deposition of mineral crystals among collagen fibers (mineralization).

Dentinogenesis imperfecta type II is caused due to the mutation in Gene DSPP (Zhang et al, 2001) that codes for *dental sialophosphoprotein*. Thus the deficiency of *dentin sialophosphoprotein* causes DGI. The formation of dentine is defective due to less hardening of collagen, i.e. due to the imperfectly formed matrix. The mineral content in the tissue is less than normal dentine and contains more water (hence soft). This results in discolored teeth which are weak and likely to decay and break.

The DSPP gene is located on the long arm of chromosome number 4 at position 21.3 (4q21.3). About 10 different mutations have been identified in people with DGI. This leads to two forms of DGI, *viz.* type II and type III types (Mac Douqall et al, 1999). The mutation in the DSPP gene is also responsible for causing ***dentin dysplasia type II*** where there is a substitution in a single amino acid (tyrosine with aspartic acid at protein position 6). As the mutation in DSPP gene gives rise to DGI types II and III and dentin dysplasia, it indicates that all the three variants are different allelic forms of the same disease.

DSPP gene is also active at low levels in the inner ear and may play a role in normal hearing. Therefore the DGI type II is associated with progressive loss of hearing (Xiao et al, 2001).

Dentinogenesis imperfecta is inherited as an autosomal dominant trait and an affected person has one affected parent with DGI.

Treatment

The main aim of treatment is to prevent the loss of enamel. This will further prevent the loss of dentin by attrition. Cast metal crowns on posterior teeth and jacket crown on anterior teeth may achieve this purpose.

DENTINE DYSPLASIA

Dentin dysplasia (DD) is a genetic disorder of dentin formation with abnormal pulpal morphology. It affects approximately 1 in 100,000 people. Two varieties of dentin dysplasia, type I and type II, have been recognized. Both are inherited in an autosomal dominant manner.

Type I DD disorder is also known as radicular dentin dysplasia since the underdeveloped roots and abnormal pulp tissues are predominately located in the roots of the teeth. The deciduous teeth lack pulp chambers or have half-moon shaped pulp chambers in short or abnormally shaped roots. The condition may affect primary as well as adult teeth. Since the roots are abnormally short, blunt and conical it usually leads to premature loss of teeth. The color of the teeth is generally normal or with slightly amber translucency. The gene or genes responsible for this condition is not known.

Dentin dysplasia type II appears virtually identical to dentinogenesis imperfecta type II in the primary dentition with yellow-brown to blue-gray discoloration of the teeth and pulpal obliteration. However,

unlike dentinogenesis imperfecta, the permanent teeth in dentin dysplasia type II are normal in color and on radiographs have a thistle-tube pulp chamber configuration with pulp stones (Figs 10.16 and 10.17).

Genetics of DD type II

Dentin dysplasia type II is due to mutations in the gene DSPP. Due to the similar phenotype of the primary teeth and known similar gene loci (gene DSPP, 4q21.3) for DD type II and DGI type II, it was speculated that DD type II could be an allelic variant of mutation in the gene responsible for causing DGI Shields type II (Beattie et al, 2006). Studies have now proved in at least some families that DD type II is caused by mutations in the DSPP gene which is associated also with DI type II.

Management of dentin dysplasia comprises preventive oral health care with meticulous oral hygiene.

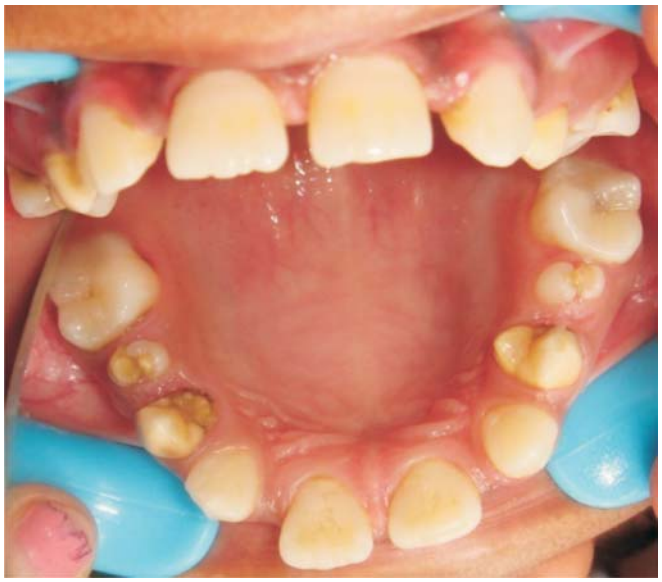


FIGURE 10.16: Dentine dysplasia. *Courtesy* of Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

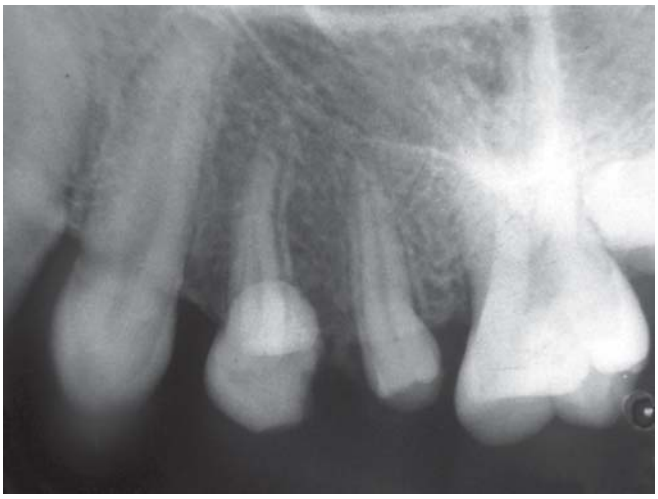


FIGURE 10.17: Dentine dysplasia**.** Roots are abnormally short and have thistle-tube pulp chamber. *Courtesy* of Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

HYPOPHOSPHATASIA

It is an inherited disease affecting development of bones and teeth. This condition results due to the faulty mineralization (calcium and phosphorus) of bones and teeth. This condition occurs due to the deficiency of the enzyme alkaline phosphatase. This enzyme plays a role is the mineralization of bones and teeth. This enzyme is encoded by the **ALPL** gene (*alkaline phosphates, liver/kidney/bone).* The gene is located on the short arm of chromosome number 1 between the p36.1 to p34 positions.

Persons affected with the severe form of disease may show early loss of primary teeth. Affected child has a short stature with bowed legs or knock-knees, abnormal shape of the skull, enlarged ankle and wrist joints. Affected individuals may also lose their adult teeth permanently.

The mildest form of the disease is called **odonto-hypophosphatasia.** In this form teeth are the only structures affected and skeletal abnormalities are not observed. People with this condition have abnormal tooth development and premature tooth loss.

Mutation in ALPL gene produces abnormal alkaline phosphatase that results in poor mineralization. In most cases the mutation is mild and actually is a change in a single amino acid. However in cases of severe mutations there may be complete absence of the enzyme.

Mode of Inheritance

The severe form of hypophosphatasia is inherited as an autosomal recessive trait and the milder form by an autosomal dominant pattern of inheritance.

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Chapter

11 Genetics of Craniofacial Disorders and Syndromes

- Molecular regulation of the development of face
- Ectodermal dysplasia
- Holoprosencephaly
- Mandibulofacial dysostosis (Treacher Collins–Franceschetti syndrome)
- Cleidocranial dysplasia
- Apert syndrome (Acrocephalosyndactyly)
- Crouzon syndrome (Craniofacial dysostosis)
- Pfeiffer syndrome
- Cherubism
- van der Woude syndrome
- Gorlin-Goltz syndrome
- Waardenburg syndrome (WS)
- Osteogenesis imperfecta
- Down's syndrome
- Achondroplasia

MOLECULAR REGULATION OF THE DEVELOPMENT OF FACE

During the beginning of the 4th week of embryonic development the face is represented by stomatodeum situated beneath the developing brain. The stomatodeum is separated from primitive gut by buccopharyngeal membrane. This membrane breaks down at the end of 4th week. Surrounding structures of stomatodeum ultimately form the face. These structures are the frontonasal process, the medial and lateral nasal processes, the maxillary and mandibular processes. All these facial processes are covered by the ectoderm beneath which lies the mesoderm (mesenchyme). The mesenchyme of upper face comes from neural crest cells from the forebrain and midbrain areas while that of the mandible come from neural crest cells of midbrain and hindbrain region. *(BMP signaling is necessary to form the edge of neural crest. This then regulates the expression of WNT, which help in the migration of neural crest cells to the first arch).*

The genetic control of the early development of the face is guided as per sequential events given below.

- The frontonasal process is formed by the synthesis of ***retinoid acid*** in the ectodermal cells covering the forebrain. Retinoic acid is responsible for the maintenance of the fibroblast growth factor –8 (***FGF-8***) signals and sonic hedgehog (Shh) signals (in the forebrain and in frontonasal ectoderm) (Carlson, 2004).
- Shh and FGF-8 molecules stimulate neural crest cells to proliferate in the frontonasal process (Fig. 11.1).
- After the 5th week of development the proliferation of frontonasal process slows down and maxillary, mandibular, medial and lateral nasal processes start growing rapidly (Fig. 11.2). Growth of all these processes results from interactions between the overlying ectoderm and underlying mesoderm. Here again the active signaling molecules in the ectoderm are FGF-8 and Shh. These signals stimulate growth of the mesenchyme.

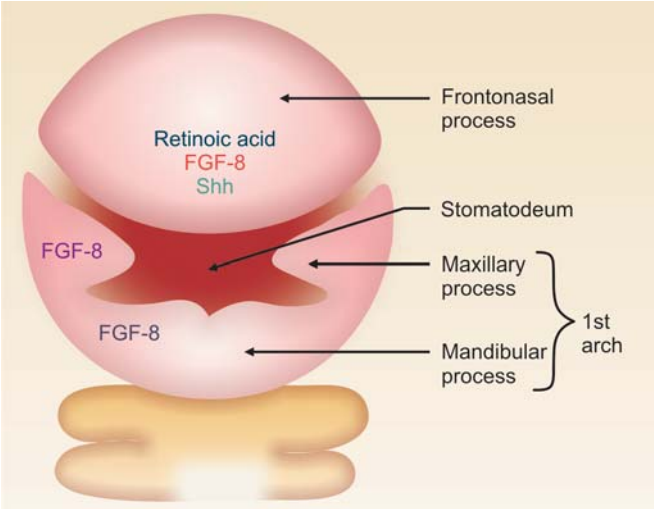


FIGURE 11.1: Formation of face, early stage. Showing expression of various signals

- The growth of the maxillary process is due to establishment of a signaling center in the mandibular arch. FGF- 8 is the molecular signal for maxillary process formation.
- Next the homeobox containing ***Msx-1*** gene is expressed in the mesenchyme of all the facial processes.
- The transcription factor ***Otx-2*** is expressed in the first arch (maxillary and mandibular processes). This gene characterizes the precursors of the first arch. (*It should be noted that the Hox genes are not expressed in the first arch. However, they are expressed in all the other pharyngeal arches).*
- Further development of the mandibular process is strictly under genetic control. The medial region of the mandibular process responds to ***FGF-2*** and ***FGF-4*** local epithelial signals and stimulates growth of the underlying mesenchyme. These signals are mediated through Msx-1 factors. Growth of lateral region of mandibular process is due to FGF-8 signals. These signals are mediated by bone morphogenetic proteins- ***Bmp-4*** and ***Bmp-7*** which are produced in the lateral regions of the mandibular process (Fig. 11.3).
- The development of the mandibular arch in the proximal to distal direction depends upon the expression on ***Dlx*** group of transcription factors (Fig. 11.4).
- Dlx-1 and Dlx-2 are expressed most proximally in the mandibular process; Dlx-5 and Dlx-6 are expressed more proximally and Dlx-3 and Dlx-7 are expressed most distally.
- Dlx-1 and Dlx-2 are also expressed in the maxillary process.

During the 7th week the maxillary processes increase in size and move medially. The maxillary process now fuses with the medial nasal process on each side (Fig. 11.4). Hence, the upper lip is formed by the fusion of two maxillary and two medial nasal processes. Stomatodeum is now bound above by the upper lip.

The two mandibular processes grow medially and fuse in the midline to form the lower lip and lower jaw. The fused mandibular processes now form the lower margin of stomatodeum.

To begin with, the lateral nasal process and maxillary process are separated by a deep furrow, the ***nasolacrimal groove***. This groove extends upto

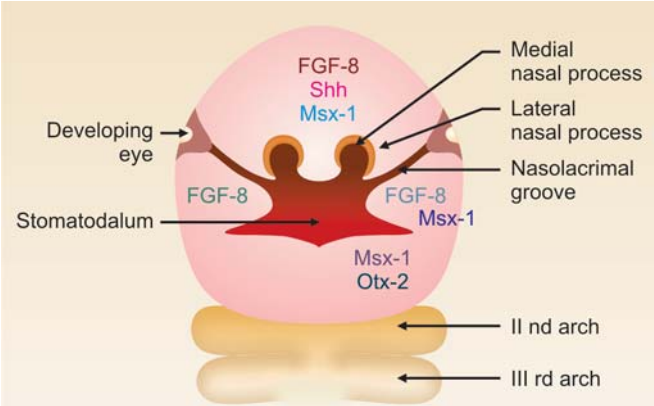


FIGURE 11.2: Formation of medial and lateral nasal processes

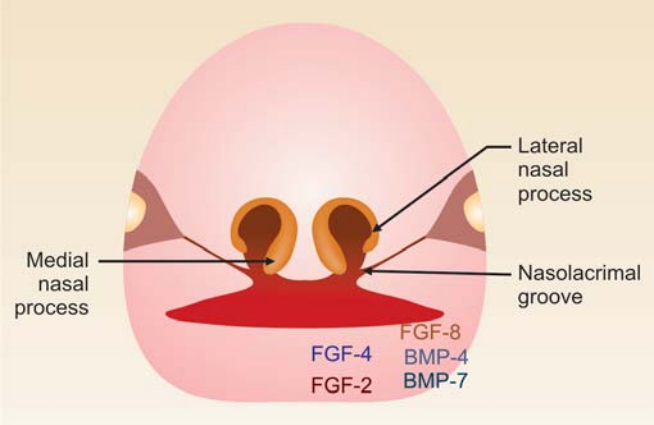


FIGURE 11.3: Signals expressed in medial and lateral regions of the mandibular process

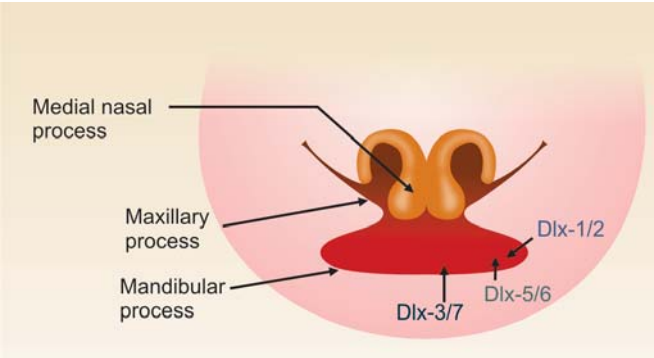


FIGURE 11.4: Fusion of medial nasal process and maxillary process. Note the expression of Dlx genes in mandibular process

the developing eye. The ectoderm of the floor of nasolacrimal groove forms a solid epithelial cord. The cord gets detached from the surface epithelium and gets canalized to form ***lacrimal sac*** and ***nasolacrimal duct*** at a later stage.

After formation of the basic facial structures by the end of 7th embryonic week, these structures are invaded by mesenchymal cells of the 1st and 2nd pharyngeal arches. The first arch mesenchymal cells form muscles of mastication and are innervated by the trigeminal (V) nerve. The mesenchymal cells of second arch form the muscles of facial expression and are thus innervated by the facial (VII) nerve.

ECTODERMAL DYSPLASIA

Ectodermal dysplasias (EDs) are heritable conditions characterized by abnormal development of two or more ectodermal structures such as the hair, teeth, nails and sweat glands. Besides these defects related to embryonic ectoderm, an affected person may also show defects of cranial and facial structures, digits and some other parts of the body.

The disease is caused due to defects in the cutaneous and oral embryonic ectoderm and as such it may simultaneously affect many structures that are derived from the ectoderm. Therefore, each person with ectodermal dysplasia may have a different combination of ectodermal defects. One may have hair and nails affected while another may involve sweat glands and teeth. Each combination is considered a distinct type of ectodermal dysplasia. More than 192 distinct disorders have been defined till date.

Incidence: 1 case per 10,000 births to 1 in 17,000 births.

Pathophysiology

Sweat gland defects—People with ectodermal dysplasia may not sweat or may have decreased sweating due to the lack of sweat gland development. Children with the disease may have defective body mechanisms that control fevers because the skin cannot sweat and control temperature properly. Affected adults are unable to tolerate a warm environment.

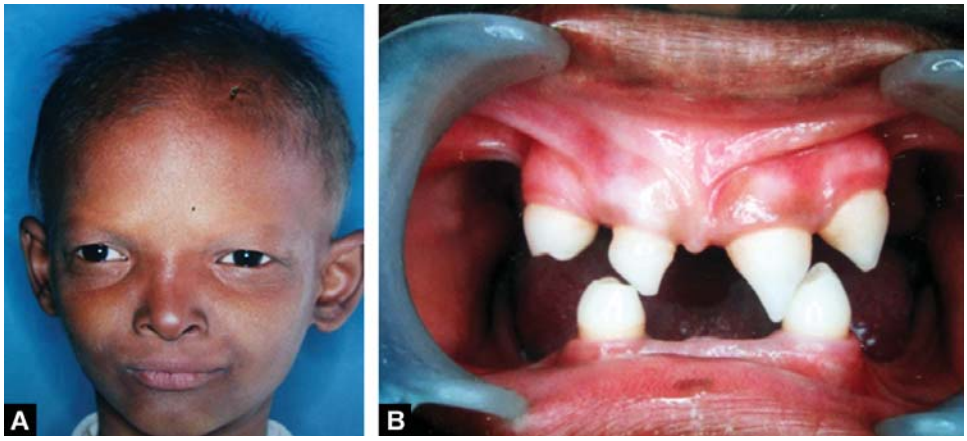
Defects of other glands derived from ectoderm—Hypoplasia of the salivary and lacrimal glands may occur. In some patients mucous glands may be absent in the upper respiratory tract and in the bronchi, esophagus, and duodenum. These defects may result in oral dryness, absence of tears and difficulty in swallowing.

Hair defects: Affected individuals tend to have sparse scalp and body hair (hypotrichosis). The hair is often light-colored, brittle and slow-growing.

Abnormal nails—Abnormal nail plate formation may result in brittle, thin, ridged or grossly deformed nails.

Abnormal teeth—Abnormal morphogenesis or absence of multiple teeth may occur.

Additional features—This includes a prominent forehead, thick lips and a flattened bridge of the nose.



FIGURES 11.5A and B: Patient of anhidrotic (hypohidrotic) ectodermal dysplasia (A) Showing clinical features, (B) Note the oligodontia and peg-shaped teeth. *Courtesy* of Prof. AL Shigli, Head, Dept. of Pediatric dentistry, Modern Dental College and RC, Indore, India

Sometimes affected person may also show thin, wrinkled and dark-colored skin around the eyes; chronic skin problems such as eczema and a bad-smelling discharge from the nose (ozena).

Following are few very common EDs (Table 11.1):

Hypohidrotic ED

This is also known as EDA (anhidrotic ED) or Christ-Siemens-Touraine syndrome. It is the most common form of ED. The condition is mostly (in 95% of cases) inherited as an X-linked recessive trait. As a consequence only males are affected and females are carriers. However, due to lyonization and mosaic expression of the abnormal X-chromosome, females may show mild features of EDA.

Less commonly (in 5% cases) the condition is inherited as an autosomal dominant and autosomal recessive disoder.

Clinical Features

The affected person shows typical facial features represented by a prominent forehead, thick lips, broad nose, sunken cheeks, low set ears and wrinkled and dark eyelids. Teeth are either less in number or completely absent. If present, teeth are conical or pegged-shaped. Patients may have very less, dry and light hair. The skin is dry due to lack of sweat gland formation and these patients may acquire eczematous conditions of the skin. Tears are often absent (Fig. 11.5).

Genetics of Hypohidrotic ED

The **EDA, EDAR**, and **EDARADD** genes provide instructions for manufacturing proteins that work together during embryonic development. These proteins form the parts of a signaling pathway that is critical for interaction between the two cell layers, the ectoderm and the mesoderm. In the early embryo these cell layers form the basis for the genesis of many of the body's organs and tissues. Ectoderm-mesoderm interactions are essential for the formation of several structures that arise from the ectoderm including the skin, hair, nails, teeth, and sweat glands.

The **EDA** gene provides instructions for making a protein called ***ectodysplasin A*** (Bayes, et al 1998 and Monreal, et al 1998). This protein is part of a signaling pathway that plays an important role in the development of ectodermal appendages (hair, teeth and sweat glands) before birth. The ectodysplasin-A has an important role to play in ectodermal-mesodermal interactions during embryonic development. Defects in the molecular structure of this protein inhibit the action of enzymes necessary for normal development of ectoderm (Chen, et al 2001). More than 60 mutations have been identified in the EDA gene. The gene is located on the long (q) arm of the X-chromosome between positions 12 and 13.1 (Xq12-q13.1). The hypohydrotic ED is inherited as an X-linked recessive trait.

The **EDAR** gene provides instructions for making a receptor protein called the ***ectodysplasin A receptor***. This protein is a part of the signaling pathway that plays an important role in development before birth. The *ectodysplasin A receptor* interacts with a protein called *ectodysplasin A* (produced from the EDA gene). *Ectodysplasin A* attaches to this receptor on the cell surface like a key in its lock. When these two proteins are connected they trigger a series of chemical signals that affect cell activities such as division, growth, and maturation. Gene EDR is located on long arm of

TABLE 11.1: Genetics of common ectodermal dysplasia (ED)

Type of ED	*Mode of inheritance*	*Gene*	*Locus of gene*	*Name of protein*
Hypohidrotic ED	XR	EDA	Xq12- q13.1	Ectodysplasin-A
Hypohidrotic ED	AR/ AD	EDAR	2q11-q13	Ectodysplasin-A receptor
Hypohidrotic ED	AR/ AD	EDARADD	1q42.2-q43	EDAR associated death domain protein
Hidrotic ED	AD	GJB6	13q pericentromere	Gap junction protein
AEC (Hay-Wells) syndrome	AD	p63	3q27.	p63 protein
ECC syndrome	AD	p63	3q27	p63 protein

chromosome number 2 between 11 and 13 positions. Mutations of this gene cause hypohidrotic ectodermal dysplasia which is inherited as autosomal recessive trait (Shimomura, et al 2004).

The **EDARADD** gene provides instructions for making a protein called the ***EDAR-associated death domain protein*** (Headon, et al 2001)**.** This protein is part of a signaling pathway active in developmental events before birth. The EDARADD protein interacts with another protein, called the *ectodysplasin A receptor* which is produced from the EDAR gene. This interaction occurs at a region called the death domain. This domain configuration is present in both the proteins. The EDARADD protein acts as an adapter, which means it assists the *ectodysplasin A receptor* in triggering chemical signals within cells. These signals affect cell activities such as division, growth and maturation. Before birth this signaling pathway controls the formation of ectodermal structures such as hair follicles, sweat glands, and teeth. The EDARADD gene is located on the long arm of chromosome number 1 at 43th position (1q43). Mutation of this gene causes hypohidrotic ED, which is inherited as autosomal recessive inheritance.

Mutations in the EDA, EDAR, or EDARADD gene prevent normal interactions between the ectoderm and the mesoderm and thus impair the normal development of hair, sweat glands and teeth. The improper formation of these ectodermal structures leads to the characteristic features of hypohidrotic ectodermal dysplasia. Some mutations in the EDA gene represent alterations or substitutions in single DNA building blocks (base pair parameters) whereas other mutations insert or delete genetic material in the gene. These changes lead to the production of a nonfunctional version of the *ectodysplasin A protein*. This abnormal protein cannot trigger chemical signals needed for normal interactions between the ectoderm and the mesoderm. Without these signals hair follicles, teeth, sweat glands and other ectodermal structures do not form properly leading to the characteristic features of hypohidrotic ectodermal dysplasia.

Hidrotic ED

This condition is also known as the ***Clouston syndrome***. It is inherited as an autosomal dominant disease. The gap junction proteins help in the communication and interaction between cells. The hidrotic ED mutation is present in the GJB6 gene located in the long arm of chromosome number 13 in its pericentriolar region (13q). This gene encodes the gap junction protein *connexin 30* (Cx30) (Jerome, et al 2000 and Guilherme, et al 2004).

Clinical features

Scalp hair is very sparse, fine and brittle. Alopecia is common. Eyebrows are thinned or absent. Nail dystrophy is commonly seen. Persistent paronychial infections are frequent. Fingers and toes are abnormal, i.e. they are either more in number or fused with each other. Bulbous fingertips may be present.

Patients may have normal face, normal teeth and normal sweating.

AEC (Hay-Wells) Syndrome

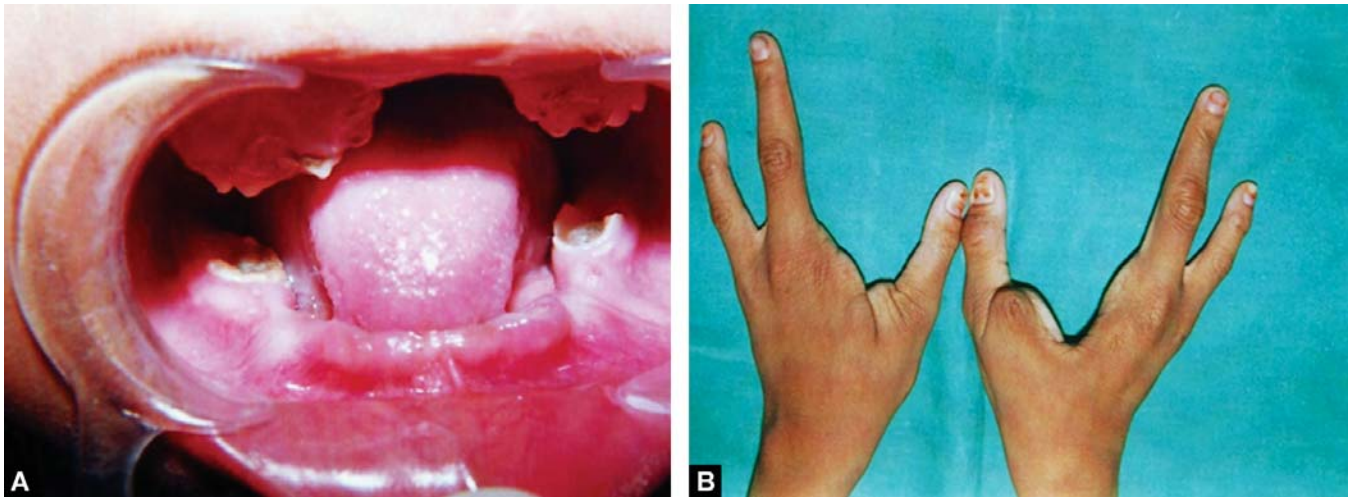
Its full name is *ankyloblepharon-ectodermal dysplasia-clefting*. It is inherited as an autosomal dominant trait of variable expressivity. It is due to a mutation in the p63 gene, which is located on the long arm of chromosome number 3 at position 27. The syndrome is caused by heterogenous missense mutation in p63 (McGrath, et al 2001).

Clinical Features

Patients exhibit characteristic facial features like ankyloblepharon (congenital adhesion of the upper and lower eyelids by fibrous bands), sunken maxilla, broad nasal bridge and cleft palate. Absence or sparse hair in the scalp, absence or malformation of nails and pegged teeth are presented in the disease. Mild hypohydrosis is also common.

EEC Syndrome

EEC is an acronym of ectrodactyly-ectodermal dysplasia-cleft lip/cleft palate syndrome. It is inherited as an autosomal dominant trait of low penetrance and variable expressivity. The disease is due to a mutation in the gene **p63** located on long arm of the chromosome number 3 at position 27 (Bokhoven, et al 2001).



FIGURES 11.6A and B: EEC syndrome. Patient showing the cleft palate (A) and lobster claw deformity of hands, (B) *Courtesy* of Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

Clinical Features

Affected persons may show severely malformed hands and feet (lobster claw deformity).
Some may show hypoplastic metacarpals and metatarsals. Cleft lip and palate are very common (Fig. 11.6).

Other features include dry and coarse hair; abnormal nails, mild hypohydrosis, enamel hypoplasia and microdontia.

Associated defects include blepharophimosis, deafness and abnormalities of the genitourinary tract.

Treatment

- Wigs can be used or special hair care taken for hair loss.
- Patient should always remain in a cool environment to prevent dryness of skin. Skin care products should be used to prevent dryness of the skin and for prevention from exposure to heat.
- Dental evaluation should be conducted every 6 to 12 months. Dental treatment like simple restoration of dentures or dental implants may become necessary in many patients.

HOLOPROSENCEPHALY

The face and the forebrain (prosencephalon) of an embryo normally begin to develop in the 5th and 6th weeks of pregnancy. The development of face is related to the development of the forebrain. If the forebrain fails to segment into normal right and left hemispheres, it may result in deformities of the face. The abnormal development of brain and face is known as ***holoprosencephaly*** **(HPE)**.

Following three types of the HPEs are described in the literature:

Alobar—This type of HPE is features complete failure of separation of right and left cerebral hemispheres. There is single lobe and a single ventricle. This is a very severe form of HPE and the affected usually die during intrauterine life or in their early infancy. This variety is associated with severe craniofacial abnormalities.

Semilobar—This type of HPE is characterized by incomplete separation of cerebral hemispheres. This is associated with milder facial abnormalities.

Lobar—It is marked by substantial but still incomplete separation of the hemispheres. Separation is seen posteriorly but part of frontal lobe may remain fused together. Sometimes patients may have a nearly normal brain.

The diagnosis of the various types of HPE can be easily made with the help of a CAT scan or an MRI.

Signs and Symptoms

The most severe forms of holoprosencephaly produce seizures and mental retardation. Midline structures of

brain like the corpus callosum and septum pellucidum are not developed. The two thalamic lobes may be fused into one. Olfactory tracts and bulbs may remain absent in some cases. The circle of Willis is not well developed usually.

Typical facial defects involve the eyes, nose and upper lip. In some cases the nose may be entirely missing. However, there is no strict association between the types of HPE and the degree of severity of the facial defects in each of the types. Yet some associations are commonly seen:

- ***Facial defects associated with alobar type of HEP***—The most severe of the facial defects is ***cyclopia***; an abnormality characterized by the development of a single eye located in the area normally occupied by the root of the nose and a missing nose or a nose in the form of a proboscis (a tubular appendage) located above the eye. Premaxillary agenesis with median cleft lip, ocular hypotelorism, flat nose and sometimes bilateral cleft lip may be present with ***cyclopia***.
- ***Facial defects associated with semilobar type of HPE***—Bilateral cleft lip with the median process representing the philtrum-premaxilla anlage. There may be midline cleft lip and/or palate. Nose may show a flat nasal tip, absence of nasal septum and/or a flat nasal bridge. Ocular hypotelorism is very common. Microcephaly is also seen in many affected persons. Sometimes a person affected with semilobar type of HPE may have relatively normal facial appearance.
- ***Facial defects associated with lobar type of HPE***—The least severe presentation in the spectrum of facial anomalies is the median cleft lip which is also called premaxillary agenesis. Bilateral cleft lip with a median process, ocular hypotelorism and a flat nose may be the only facial abnormalities. Person may also present with relatively normal facial appearance.

Microforms of HPE that can be observed in relatives of probands with HPE include the following:

- Microcephaly/Single central maxillary incisor/Ocular hypotelorism/Anosmia/hyposmia (resulting from absence of olfactory tracts and bulbs)/Iris coloboma/Absent superior labial frenulum/Midface hypoplasia.

Single Maxillary Median Central Incisor

The incidence of a solitary maxillary median central incisor tooth in the general population is low either in the primary or in secondary dentition. It is a well-recognized genetic anomaly where the affected individuals might present with potentially a more serious condition affecting the midline development of the brain and face called holoprosencephaly (HPE). The presence of single median incisor of unknown etiology is therefore considered a risk factor for HPE even in the absence of any neurological or other clinical signs. Due consideration should be given in these subjects for referral to appropriate genetic testing and counseling.

Causes of HPE

Etiology of HPE is heterogeneous. It is due to the fact that both environmental and genetic factors have been identified as causative agents.

Environmental Causes

- Maternal diabetic mellitus during pregnancy
- Alcohol
- Retinoic acid
- Hypocholesterol.

Genetic Causes

a. Chromosomal Abnormalities

- Trisomy13 (Patau's syndrome) and Trisomy18 (Edward's syndrome)
- Deletion and duplication of various chromosomes.

b. Single Gene Abnormalities

The mutation in a single gene causes two types of HPE, viz. *Syndromic HPE* (associated with various syndromes) and *nonsyndromic HPE.*

Nonsyndromic HPE

The nonsyndromic gene mutations responsible for HPE are usually inherited as autosomal dominant traits. The following table (Table 11.2) indicates

genetics of autosomal dominant nonsyndromic HPEs (Nanni, et al 2000).

SHH—The human sonic hedgehog gene (SHH) encodes a secreted protein (*sonic hedgehog protein)*. This protein is involved in establishing cell fates at several points during development. It is expressed in the Hensen's node and floor plate of the neural tube. Thus it is one of the primary inducers of the ventral neural tube.

Various types of mutations of the SHH gene are known (frame shift, heterozygous deletion, miss-sense mutations, etc). Mutation of sonic hedgehog gene leads to faulty production of sonic hedgehog protein that ultimately results in abnormal development of the forebrain and face (Roessler, et al 1996).

ZIC2—This gene encodes *zinc finger protein 2*. It plays a major role in mediating the response to sonic hedgehog protein signaling. Mutation of ZIC2 causes holoprosencephaly (Brown, et al 1998).

SIX3—This gene encodes the homeobox protein SIX3. It participates in midline forebrain structuring and in formation of eyes. It is present in the anterior region of the neural plate and midline ventral forebrain. Its mutation interferes with the development of brain and eye (Wallis, et al 1999).

TGIF—It modulates the TGIF beta pathways. Thus TGIF links the NODAL signaling pathway leading to the bifurcation of the human forebrain and the establishment of ventral midline structures (Gripp, et al 2000).

TABLE 11.2: Genetics of HPE

HPE loci	*Gene*	*Chromosomal location*
HPE 1	Lanosterol synthase	21q 22.3
HPE 2	SIX3	2p 21
HPE 3	SHH (Sonic hedgehog)	7q 36
HPE 4	TGIF (Homeobox gene)	18p 11.3
HPE 5	ZIC2 (Zinc finger protein 2)	13q 32
HPE 6	ZIC1 (Zinc finger protein 1)	3p 24
HPE 7	PTCH1 (Patched 1)	9q 22.3
HPE 8	TTF1 (Thyroid transcription factor 1)	14q 13
HPE 9	GLI2 (Gli 2)	2q 14

PTCH 1 – This gene encodes for the patched 1 receptor protein. This protein is the receptor for SHH.

Treatment of HPE

The treatment of persons suffering from HPE is basically the management of its symptoms.

- Hormonal replacement in case of pituitary dysfunction.
- Antiepileptic drugs for seizures.
- Surgical repair of abnormalities associated with face such as cleft lip/palate.

MANDIBULOFACIAL DYSOSTOSIS (TREACHER COLLINS-FRANCESCHETTI SYNDROME)

The syndrome is characterized by disorders of craniofacial developmental events. The disease is hereditary in nature.

Incidence: From 1 in 40,000 to 1 in 70,000 live births.

Clinical Features

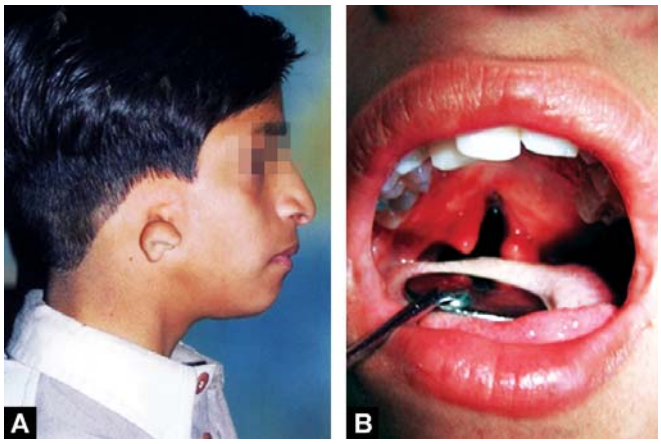
Some of the important clinical features are as under:

- Down slanting palpebral fissure, coloboma of lower eyelids.
- Hypoplasia of malar bones and occasional absence of palatine bones.
- Hypoplasia or sometimes agenesis of mandible. Microstomia.
- Cleft palate and malocclusion of teeth.
- Malformation of the external ear (Fig. 11.7). Sometimes middle and internal ears (auditory ossicles, cochlear and vestibular apparatus) are also affected. This leads to deafness.
- Occasional heart defects.
- Patients are of normal intelligence and have normal reproductive life.

Genetics

Inheritance: The inheritance shows an autosomal dominant (AD) pattern with complete penetrance and variable expressivities.

The syndrome is due to the mutation in the gene called the *Treacher Collins-Franceschetti syndrome 1*



FIGURES 11.7A and B: Mandibulofacial dysostosis. A—Patient showing hypoplasia of mandible and malformation of external ear and B—cleft palate. *Courtesy* of Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

(**TCOF1**) gene. It is located on the long arm of chromosome number 5 (5q31.3-q33.3). Approximately about 150 mutations have been identified in the TCOF 1 gene.

The TCOF1 gene codes for a protein called ***treacle*** which is required for normal craniofacial development. Mutation of gene leads to the absence or reduced production of treacle in the cells (Edwards, et al 1997). Researchers believe that a loss of this protein triggers certain apoptotic signals in cells important for the development of facial bones that self-destruct themselves (undergo apoptosis). This abnormal cell death leads to Treacher Collins syndrome. As per other views towards the syndrome, it occurs due to the failure of migration of neural crest cells in the 1st and 2nd branchial arches. This leads to dysplasia of musculoskeletal derivatives of these arches.

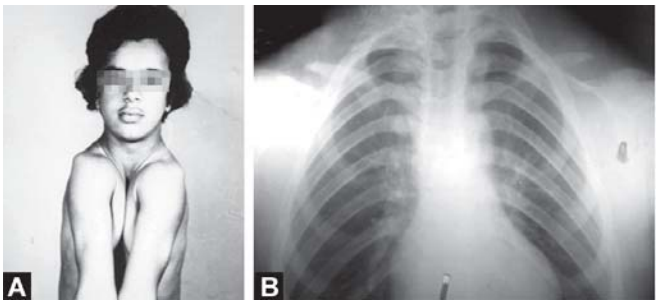
CLEIDOCRANIAL DYSPLASIA

Cleidocranial dysplasia (CCD) is a disorder exhibiting defective endochondral and intramembranous bone formation.

Incidence: 1 per million individuals worldwide.

Clinical Features

Following abnormalities of bone formation are observed.



FIGURES 11.8A and B: A 14 years old patient of cleidocranial dysostosis. (A) This patient can approximate the shoulders in front of the chest. (B) The clavicles on both the sides are partially absent. *Courtesy* of Prof. PG Makhija, Dept of Orthodontics, Modern Dental College and RC, Indore, India

Skull

Closure of the fontanels is delayed with the presence of many sutural bones are in the cranial sutures. Though head is brachycephalic in shape, bossing of frontal, parietal and occipital regions gives the skull a large globular shape.

Shoulder Girdle

Clavicles are either completely or partially absent. The patient can approximate the two shoulders in front of the chest (Fig. 11.8). Sometimes anomalies in other bones of the body may also be seen, e.g. in the pelvis, vertebral column, etc. with shortening of metatarsals, metacarpals and long bones.

Face

Maxilla, lacrimal and zygomatic bones are underdeveloped. Cleft palate is occasionally present. Paranasal air sinuses are underdeveloped. Sometimes the patients may have enlarged mandible as compared to the normal.

CCD patients may show a delayed eruption of permanent teeth. They may have supernumerary or impacted teeth. Patients are of normal intelligence but slightly short in height as compared to other normal members in the family.

Genetics

Inheritance: The CCD is an autosomal dominant (AD) disorder.

Narahara et al (1995) observed CCD in association with a translocation defect involving chromosomes 6 and 18. It is now believed that CCD is due to a mutation of the gene called *core binding factor alpha-1* (**CBFA1**). This gene belongs to the ***RUNT*** transcription factor family and hence the other name of this gene is also *runt related transcription factor 2* (**RUNX2**).

The gene CBFA1 is located on the short arm of chromosome number 6 (6p21) (Mundlos, et al 1995 and Mundlos, et al 1997). CBFA1 gene controls differentiation of precursor cells into osteoblasts and is thus essential for membranous as well as endochondral bone formation. Researchers believe that the CBFA1 protein acts as a "master switch" regulating a number of other genes involved in the development of cells that build bones (osteoblasts). Thus CBFA1 gene plays an important role in osteoblastic differentiation.

Some mutations result in change of just a single building block (amino acid) of the CBFA1 protein (nucleotide alteration). Other mutations introduce a premature stop signal that result in an abnormally short protein. Occasionally the entire gene may be missing due to an anomaly. This leads to the shortage of the functional protein (haploinsufficiency of CBFA1) that interferes with normal bone and cartilage development resulting in the signs and symptoms of cleidocranial dysplasia. Thus it may be concluded that CCD is caused due to mutations in the CBFA1 gene and that a heterozygous loss of function (autosomal dominance) is sufficient to cause the disease.

APERT SYNDROME (ACROCEPHALOSYNDACTYLY)

It is a congenital genetic defect, which affects the first branchial arch (the precursor of maxilla and mandible). This syndrome is characterized by:

- Craniosynostosis (premature fused cranial suture).
- Craniofacial anomalies like midface hypoplasia (Fig. 11.9)
- Syndactyly (fused fingers and toes).

Incidence: 1 per 2, 00,000 live births.

Clinical Features

- Craniosynostosis involves the coronal suture resulting in a prominent forehead, flat occiput and brachycephaly.

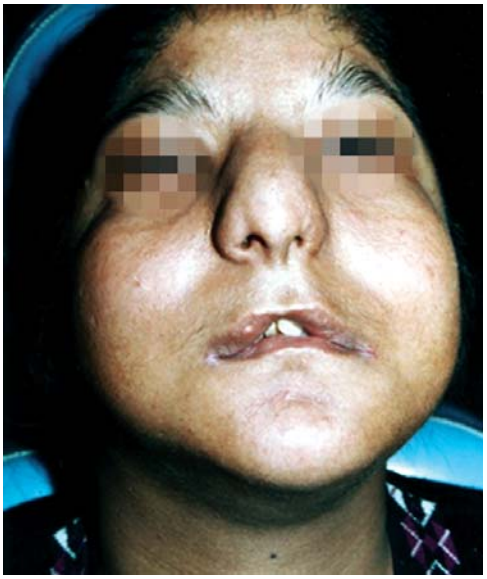


FIGURE 11.9: Apert's syndrome. Patient shows brachycephaly, short wide nose, hypertelorism and maxillary hypoplasia. *Courtesy* of Dept. of Oral Diagnosis, Modern Dental College and RC Indore, India

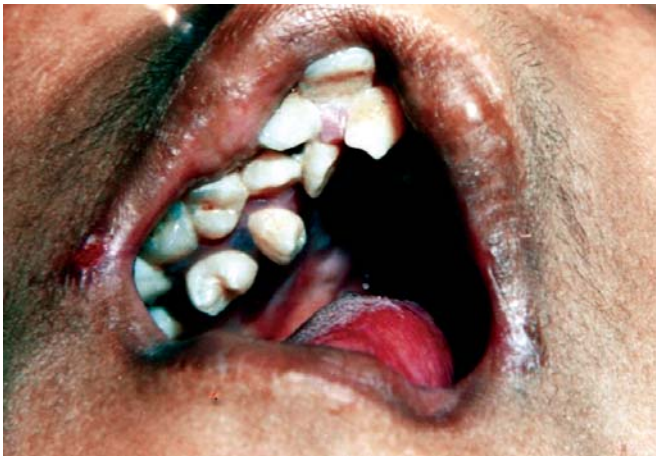


FIGURE 11.10: Supernumerary teeth in Apert syndrome. Note, microstomia. *Courtesy* of Prof. S. Wanjari, Head Dept. of Oral Pathology, Modern Dental College and RC, Indore, India

- Down slanting palpebral fissure.
- Low set ears with occasional loss of hearing.
- Hypertelorism and exophthalmos.
- Depressed nasal bridge and a short wide nose.
- Maxilla is not well-developed. High arched palate. V-shaped maxillary dental arch, malocclusion, supernumerary teeth (Fig.11.10), cleft palate or bifid uvula.
- The digits of hands and feet are either webbed or fused (syndactyly). The severity of the fusion

varies with a minimum 3 of digits on each hand and foot fused.
- Cardiovascular and GIT anomalies are common.
- Patients may be of normal intelligence but usually retarded.

Genetics

The inheritance of the syndrome is autosomal dominant (AD) in nature or the disorder arises fresh due to new mutations in an individual.

Apert syndrome results from mutations in the *fibroblast growth factor receptor 2* (**FGFR2**) genes. This gene is located on the long arm of chromosome number 10 (10q 26). 98% of mutations of FGFR2 genes are substitution mutations such as Ser 252 Trp (between Serine and Tryptophan), Pro 253 Arg (Proline and Arginine) (Wilkie, et al 1995). Such fibroblast growth factor receptor 2 mutations cause an increase in the number of precursor cells of osteogenic pathways. This again leads to increased subperiosteal bone formation and premature ossification of the calvaria ending in early closure of the cranial sutures.

Syndactyly of Apert syndrome may be due to *keratinocyte growth factor receptor* (KGFR) mediated effect. The optic disk pallor is more severe in Pro 253 Arg mutation while cleft palate and visual impairment is seen more in the Ser 252 Trp substitution.

Sometimes Apert syndrome may also be caused by deletion/translocation of the short arm of chromosome 2 to long arm of chromosome 11 or 12.

Some other syndromes like ***Pfeiffer syndrome*** and ***Crouzon syndrome*** (craniofacial dysostosis) also show features of craniosynostosis. These are allelic disorders with overlapping features between the related disorders (Wilkie, et al 1995).

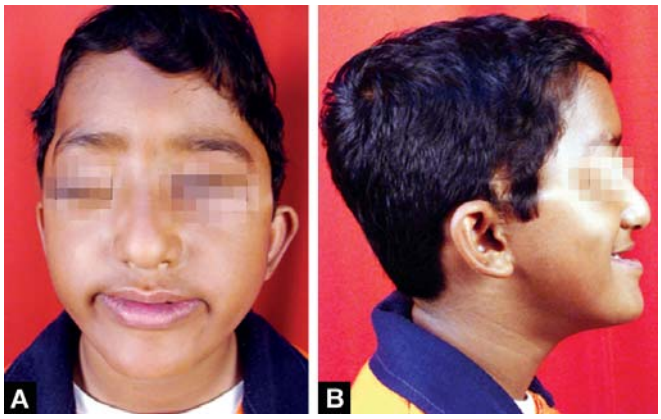
CROUZON SYNDROME (CRANIOFACIAL DYSOSTOSIS)

Crouzon syndrome or craniofacial dysostosis is similar to the Apert syndrome except that it is not associated with syndactyly. Crouzon syndrome is a genetic disorder also known as branchial arch syndrome. Specifically this syndrome affects the first branchial (or pharyngeal) arch; structures that are precursors of the maxilla and mandible. The main feature of this syndrome is its craniosynostosis or the premature fusion of the coronal, sagittal and occasionally the lambdoid sutures. The pathological fusion begins in the first year of life and is completed by the second or third year. The order and rate of fusion in the sutures determine the degree of deformity and disability.

Incidence: 1 in 25000 persons.

Clinical Features (Fig. 11.11)

- Signs of the disease originate from the early closure of cranial sutures.
- Coronal and sagittal sutures are obliterated.
- Short and broad head (brachycephaly).
- Bulging eyes (exophthalmos) due to shallow eye sockets after early fusion of surrounding bones of the orbit.
- Hypertelorism (greater than normal distance between the eyes), divergent squint.
- Progressive optic nerve atrophy results from raised intracranial tension which leads to subsequent visual impairment.
- Under-development of maxilla (insufficient growth of the midface results in protrution of the chin despite normal growth of the mandible). Due to maxillary hypoplasia Crouzon patients generally have a considerable and permanent underbite and subsequently cannot chew using their incisors (they do not use their incisors to take a bite from a sandwitch). For this reason the Crouzon patients eat in an unusual way.



FIGURES 11.11A and B: Crouzon syndrome. (A) Note the frontal bossing, hypertelorism, maxillary hypoplasia, (B) Mandibular hyperganthism. *Courtesy* Dr. S. Dilliwal of Dept of Orthodontics, Modern Dental College and RC, Indore, India

- They may show a narrow/high-arched palate, posterior bilateral crossbite, hypodontia (missing teeth), malocclusion and an increased spacing between the teeth.
- Most Crouzon patients also have noticeably shorter humerus and femur bones in proportion to the rest of their bodies when compared to members of the general population.
- Deafness.
- Mental retardation is a frequent feature.

Genetics

Inheritance- ***Autosomal*** dominant (AD). Sometimes the disorder arises due to new mutations.

The syndrome is due to a mutation in the fibroblast growth factor receptor genes (***FGFR-2***) which is mapped to the chromosome locus 10q25-10q26. This gene provides instructions for furnishing a protein called the fibroblast growth factor receptor 2. Among its multiple functions this protein signals transformation of immature cells into bone cells during embryonic development. Mutations in the FGFR2 gene probably over-stimulate signaling by the FGFR2 protein and result in the bones of the skull fusing prematurely (Rutland, et al 1995).

Crouzon syndrome with *acanthosis nigricans* is always due to an Ala 391Glu mutation within the transmembrane region of the ***FGFR3* gene**. *Acanthosis nigricans* is a brown to black, poorly defined, velvety hyperpigmentation of the skin.

Crouzon syndrome exhibits locus heterogeneity with causal mutations in *FGFR2* and *FGFR3* in different affected individuals.

Treatment

Surgery is typically used to stop the closure of sutures and prevent damage to the growing brain by compression exerted by the nonexpansible skull. Blindness and mental retardation are typical outcomes in neglected cases. Once treated for the cranial vault symptoms, Crouzon patients generally live a normal lifespan.

PFEIFFER SYNDROME

Pfeiffer syndrome is a genetic disorder characterized by premature fusion of certain skull bones (craniosynostosis). This early fusion prevents the skull from growing normally and affects the shape of the head and face. Pfeiffer syndrome also affects bones in the hands and feet.

Incidence: 1 in 100,000 individuals.

Clinical Features

- Pfeiffer syndrome result from the premature fusion of the skull bones.
- High forehead.
- Wide set eyes.
- Underdeveloped upper jaw (maxillae).
- Dental problems due to crowded teeth and often a high palate.
- A beaked nose.
- Poor vision.
- Hearing loss in about 50% of children.
- The thumbs and great toes are wide (broad) and bend away from the other digits.
- Unusually short fingers and toes (brachydactyly) are also common and there may be some webbing or fusion between the digits (syndactyly).

Pfeiffer syndrome is divided into three subtypes as type 1, type 2 and type 3. Type 1 or "classic" Pfeiffer syndrome includes individuals with mild manifestations including brachycephaly, midface hypoplasia and finger and toe abnormalities. Most individuals with type 1 have normal intelligence and a normal lifespan.

Types 2 and 3 are more severe forms of Pfeiffer syndrome often involving problems with the nervous system. Type 2 consists of a cloverleaf-shaped skull, extreme proptosis, finger and toe abnormalities, elbow ankylosis or synostosis, developmental delay and neurological complications.

Type 3 is similar to type 2 but without a cloverleaf skull. Clinical overlap between the three types may occur.

Genetics

Inheritance: It is inherited as an Autosomal Dominant (AD) entity.

Mutations in the FGFR1 and FGFR2 genes cause Pfeiffer syndrome.

FGFR1 gene is located on the short arm of chromosome number 8 (8p11.2-p11.1) and FGFR2 is located on the long arm of chromosome number 10 (10q26) (Lajeunie, et al 1995).

The FGFR1 and FGFR2 genes play an important role in signaling the cell to respond to its environment perhaps by division or cell maturation. A mutation in either of the genes causes a prolonged signaling promoting an early maturation of bone cells in a developing embryo and premature fusion of bones in the skull, hands and feet.

Type 1 Pfeiffer syndrome is caused by mutations in either the *FGFR1* or *FGFR2* genes. Types 2 and 3 are affected by mutations in the *FGFR2* gene. Mutations in the *FGFR1* usually present a milder phenotype of the disease.

Treatment

Treatment includes multiple-staged surgery of craniosynostosis. Midfacial surgery is performed to reduce the exophthalmos and the midfacial hypoplasia.

Differential Diagnosis

The main differential diagnoses include syndromes that are characterized by craniosynostosis. Mutations in the same *FGFR* (*FGFR1*, *FGFR2* or *FGFR3*) can result in different variants of craniosynostosis syndromes thereby implicating a common pathological mechanism with a common FGFR gain of function mechanism resulting in *Pfeiffer, Apert, Muenke,* and *Beare-Stevenson syndromes.*

- Pfeiffer and Apert syndromes are noteworthy for some similarities between them but the two disorders essentially are genetically distinct entities.
- Crouzon syndrome has similar phenotype as the Pfeiffer syndrome but lack the hand and foot anomalies.
- Phenotypic overlap occurs with Muenke syndrome which is caused by a specific *FGFR3* mutation.

CHERUBISM

Cherubism is a rare genetic disorder characterized by abnormal bone tissue in the lower part of the face.

Incidence: The condition is very rare.

Clinical Features

The disease begins in early childhood. Swelling of the lower face starts around the third or fourth year of life and progresses until the late teens. The lower jaw (mandible) and upper jaw (maxilla) become enlarged as the bone is replaced by painless cyst like growths. These growths give the cheeks a swollen and rounded appearance. X-ray reveals multilocular cystic changes in the mandible and maxilla. The abnormal growths are gradually replaced with normal bone in early adulthood. As a result many affected adults have a normal facial appearance. The deciduous dentition may be shed spontaneously and prematurely. The permanent teeth are also defective in their number that may be more or less than the normal. Most people with cherubism have almost no signs and symptoms affecting other parts of the body.

Genetics

Inheritance: The mode of inheritance in cherubism is autosomal dominant (AD). Usually the affect person has only a single affected parent or some time the disease is due to new (*de novo*) mutation.

Cherubism is due to mutation in the **SH3BP2** (SH3-*domain binding protein 2*) gene located on the short arm of chromosome number 4 at the 16.3 position (4p16.3). About 11 different mutations are known in the SH3BP2 gene which give rise to cherubism (Ueki, et al 2001).

Mutations in the *SH3BP2* gene lead to the production of an overactive protein. The overactive protein likely causes inflammation in the jaw bones and triggers the production of ***osteoclasts***, which are bone eating cells that breakdown bone tissue during bone remodeling. An excess of these osteoclasts contributes to the destruction of bone in the upper and lower jaws. A combination of bone loss and inflammation likely underlies the cyst-like growths so characteristic of cherubism.

Treatment

Treatment of cherubism is not well-established. It is usually a self limiting disease. Surgical treatment should be designed on individual basis depending on the functional and esthetic need of the patient.

VAN DER WOUDE SYNDROME

This syndrome is sometimes also known as ***lip-pit syndrome***.

Clinical Features

- People with this disorder are born with a cleft lip, a cleft palate or both.
- Affected individuals usually have depressions (pits) near the center of the lower lip which may appear moist due to the presence of salivary and mucous glands in the pits (Fig. 11.12).
- Some individuals may show mucous cysts of lower lip.
- It is a common form of syndromic cleft lip and palate and accounts for 2% of all cleft lip and palate cases.
- Affected individuals may have hypodontia and usually possess normal intelligence.

Incidence: The incidence of this syndrome is 1 in 35,000 to 1,00,000 individuals worldwide.

Genetics

Inheritance: Van Der Woude syndrome (VDWS) is inherited as an autosomal dominant (AD) trait or sometimes of sporadic inheritance. The syndrome has 80% penetrance and variable expression.

It is caused by mutations of the **IRF6** (*interferon regulatory factor 6*) gene located on the long arm of chromosome 1 (1q32-q41). About 60 different mutations of IRF6 gene are known currently. The IRF6 protein is active in cells that give rise to tissues in the head and face. It is also involved in the development of other parts of the body including the skin and genitals. Mutations in the IRF6 gene that cause van der Woude syndrome prevent one copy of the gene in each cell from producing the functional protein. A shortage of the IRF6 protein affects the development and maturation of tissues in the skull and face. These abnormalities underlie the signs and symptoms of van der Woude syndrome that include cleft lip, cleft palate (an opening in the roof of the mouth) and pits or mounds in the lower lip. The marked phenotypic variation may be caused by the action of certain modifier genes on *IRF6* function. Intriguing linkage studies have suggested that a second modifying gene mapped to chromosome 17p11.2-p11.1 may influence the degree of phenotypic expression of a gene defect at this locus.

Most reported cases of VWS have been linked to chromosome 1q32-q41 (VWS1) but a second VWS locus (VWS2) has been mapped to 1p34 (Koillinen, et al 2001). Direct sequence analyses of genes in this region (1q32-q41) have identified specific mutations in the gene encoding interferon regulatory factor-6 (IRF6). Mutation analysis demonstrated that ***popliteal pterygium syndrome*** can be caused by mutations in the same gene and is therefore allelic to van der Woude syndrome. Many scientists have also reported that VDWS can also be caused due to a microdeletion involving 1q32-q41 in families with this syndrome.

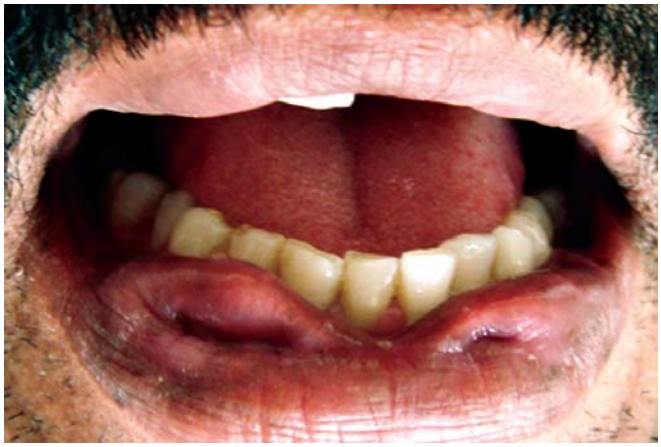


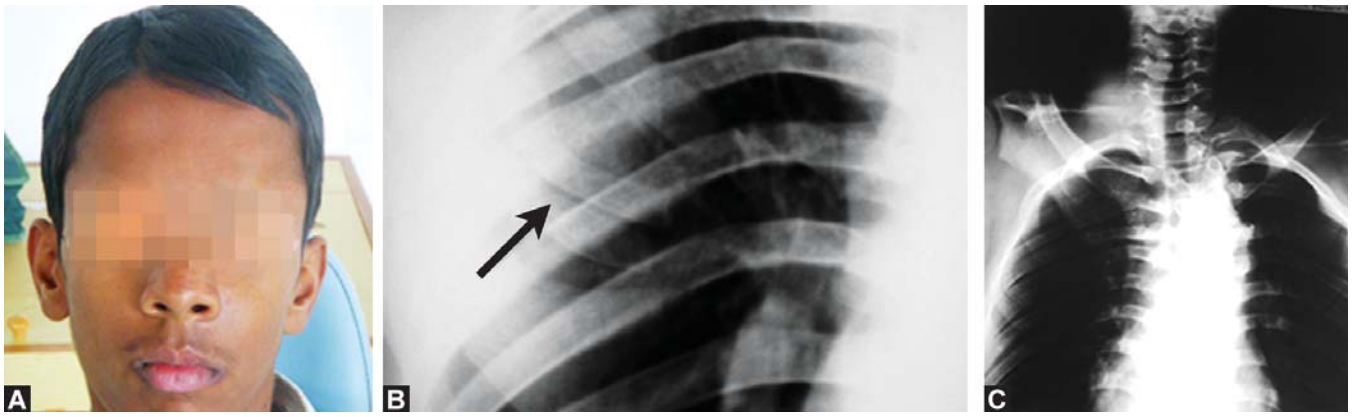
FIGURE 11.12: Van Der Woude Syndrome. Note the presence of pits in the lower lip. *Courtesy* of Dept. of Oral Diagnosis and Radiology, Modern Dental College and RC, Indore, India

Treatment

- Examination and genetic counseling by a pediatric geneticist (dysmorphologist) should be suggested.
- Surgical repair of cleft lip and cleft palate or other anomalies may be required.
- Surgical excision of lip pits is often performed for cosmetic reasons even in less severely affected individuals.

GORLIN-GOLTZ SYNDROME

Jarisch and White first reported the Gorlin syndrome in 1894 that was later described in detail by Gorlin in 1960.



FIGURES 11.13A to C: Gorlin syndrome patient (A) showing befid rib (B) and scoliosis (C) *Courtesy* of Dr Kalpana Patidar, Department of Oral Diagnosis, Modern Dental College and RC, Indore, India

Clinical Features

The clinical features that are considered for diagnosing (Fig. 11.13) Gorlin syndrome are classified into two groups of criteria; major criteria and minor criteria.

Major Criteria

- Two or more basal cell carcinomas in persons younger than 20 years.
- Odontogenic keratocysts of the jaw.
- Bifid, fused ribs.
- Calcification of the falx cerebri.
- Palmar or plantar kits.

Minor Criteria

- Syndactyly of digits.
- Macrocephaly.
- Hypertelorism, frontal bossing, cleft lip or palate.
- Scoliosis, vertebral anomalies.

Incidence: The prevalence of Gorlin syndrome is estimated to be 1 in 57,000 people.

Genetics

Mutations in the PTCH1 gene cause Gorlin syndrome. This gene encodes for a protein called Patched-1 which functions as a receptor protein. The Patched-1 receptor proteins have specific sites into which certain other proteins called ligands (e.g. Sonic Hedgehog) fit like keys into locks. Ligands and their receptors together trigger signals that affect cell development and function. Patched-1 prevents cell growth and division (proliferation) until Sonic Hedgehog is attached. The PTCH1 gene acts as a tumor suppressor gene which means it keeps cells from proliferating too rapidly or in an uncontrolled way. Mutations in this gene prevent the production of Patched-1 or lead to the production of an abnormal version of the receptor. An altered or missing Patched-1 receptor cannot effectively suppress cell growth and division. As a result cells proliferate uncontrollably to form tumors that are characteristic of Gorlin syndrome.

The genetic locus for Gorlin syndrome is located on chromosome bands and subbands 9q22.3-q31. More than 50 germline mutations in *PTCH* are described. About 40% of cases of Gorlin syndrome represent new mutations in affected individuals.

Inheritance: Gorlin syndrome is inherited in an autosomal dominant trait. Usually one of the parents is affected with Gorlin syndrome.

WAARDENBURG SYNDROME (WS)

It is an inherited disorder often characterized by varying degrees of hearing loss and changes in skin and hair pigmentation.

Clinical Features

- Eyes are of two different colors. One eye is usually brown and the other blue. Sometimes one eye has two different colors.
- Difference in eye color is associated with hearing impairment.

- People with WS may also have distinctive hair coloring such as a patch of white hair or premature gray hair as early as age 12.
- Some individuals may show white patches on the skin.
- Cleft lip and cleft palate may also be associated with WS.
- Some individuals may show intestinal or spinal disorders.

Incidence: About 1 in 10,000 to 1 in 20,000 individuals.

Types of WS

Though there are about four different types of Waardenburg syndrome but most common types of WS identified by scientists are Type I and Type II. The type of WS is determined by the distinctive features present in a person.

Type I WS—It is represented by persons having wide-set eyes (hypertelorism) due to a prominent, broad nasal root (dystopia canthorum). Hearing impairments occur in about 20% of individuals with this type of Waardenburg syndrome.

Type II WS—Persons who do not have a wide set eyes but who have many other WS characteristics are described as having Type II WS. About 50% of persons with WS Type II have a hearing impairment or are deaf. Type II WS can be further classified into 4 different sub types.

Genetics

Inheritance: This condition is usually inherited in an autosomal dominant (AD) pattern. A small percentage of cases result from new mutations in the gene. Some cases of type II Waardenburg syndrome appear to have an autosomal recessive pattern of inheritance. Most often the parents of a child with an autosomal recessive disorder are not affected but are carriers of the disease.

The genes that cause Waardenburg syndrome are involved in the formation and development of several types of cell including pigment-producing cells called melanocytes. Melanocytes make a pigment called melanin that contributes to the coloration of skin, hair, and eye and also plays an essential role in the normal function of the inner ear. Mutations in any of these genes disrupt the normal development of melanocytes leading to abnormal pigmentation of the skin, hair, and eyes and problems with hearing. In addition to melanocyte development these genes are important for the development of nerve cells in the large intestine. Mutations in any of these genes result in hearing loss, changes in pigmentation, and intestinal problems related to Hirschsprung disease.

Type I Waardenburg syndrome is caused by mutations in the PAX3 gene. Mutations in the MITF and SNAI2 genes are responsible for type II Waardenburg syndrome.

Paired box 3 (PAX3) gene is active in cells called neural crest cells. These cells migrate from the developing spinal cord to specific regions in the embryo. The protein made by the PAX3 gene directs the activity of other genes (such as MITF) that signal neural crest cells to form specialized tissues or cell types such as limb muscles, bones in the face and skull (craniofacial bones), some nerve tissue and pigment-producing cells called melanocytes. Melanocytes produce the pigment melanin, which contributes to hair, eye, and skin color. Melanocytes are also found in certain regions of the brain and inner ear.

Microphthalmia-associated transcription factor (MITF) gene helps to control the development and function of pigment-producing cells called melanocytes. The official name of this gene is "microphthalmia-associated transcription factor" and 'MITF' is the gene's official symbol. The MITF gene is also known by a few other names listed below. The Snail 2 (SNAI2) gene probably plays a role in the formation and survival of melanocytes.

The mapping analysis by Farrer, et al (1994) indicated that WS type I is linked to PAX gene situated on chromosome number 2; WS IIA is linked to mutation in gene MITF located on short arm of chromosome 3 (3p): WS IIB is due to mutation in gene located on short arm of chromosome 1 (1p) and WS IIC is due to mutation in gene situated on short arm of chromosome number 8 (8p).

Treatment

No effective treatment is available for persons with Waardenburg syndrome.

OSTEOGENESIS IMPERFECTA

The osteogenesis imperfecta (OI) is a serious inherited disorder. The bones formed are defective, i.e. they are brittle and easily fractured. This condition results due to an abnormality in the formation of type I collagen. It is formed either in less quantity or poor quality. 90% of body collagen is type I. This type of collagen fibers is found in bones, capsule of organs, cornea, dentin, sclera, tendon, dermis, fascia, the dura and ligaments.

Incidence: the disease is observed in 7 per 100,000 persons. Type II and IV are more common than the rest.

The disorder is classified in various types, i.e. OI type I to OI type VIII. The genetic cause of OI type V and VI are not known. The types I to IV are inherited as autosomal dominant and type VII and VIII as autosomal recessive traits. Clinical features of only first four types of OI are described in brief:

OI, type I—It is the mildest form and may remain undetected till the occurrence of the first fracture in a child. The collagen formed in this type of OI is of normal quality but produced in insufficient quantity resulting in the bones getting fractured easily and the spine showing slight curvature defects. Joints are loose or sub-laxed.

The sclera looks blue (sometimes purple or gray) instead of white as the sclera is very thin and the choroidal veins present beneath the sclera gives a blue look to the sclera. Sometimes Type I OI is associated with DGI characterized by opalescent teeth.

OI, type II—This is also known as congenital OI. It is the most severe form of OI and it is lethal at birth or shortly thereafter. As most cases die within first few years, this type of OI may not be encountered in dental practice. The collagen produced in neither of good quality nor of sufficient quantity. This leads to severe bone deformity and small stature in persons who survive the disease.

OI, type III – This type shows progressive deformities with increasing age, i.e. neonates present with mild symptoms that bloom into severe symptoms with age. The collagen produced is of sufficient quantity but of poor quality. Bone deformities are severe and bone gets fractured easily. Joints are loose. Sclera shows discoloration.

OI, type IV—Collagen produced is of sufficient quantity but poor quality. Affected person is of short stature with deformed and fractured bones and spinal curve disorders. There may be early loss of hearing.

Genetics of OI

The formation of collagen begins as procollagen molecules. Each rope-like procollagen molecule is made up of three chains, the two *proalpha 1* chains and one *proalpha 2* chain.

The *proalpha 1* chain is produced by gene **COL1A1 (*Collagen type 1 alpha 1*)** and *proalpha 2* chain is produced by gene **COL1A2** (***collagen type 1 alpha 2***) (Byers, et al 1991 and Byers, 1993). Gene COL1A1 is located on the long arm of chromosome number17 between 21.3 and 22.1. Gene COL1A2 is located at 7q22.1.

Two other genes are also involved in the synthesis of collagen type I, i.e. **CRTAP** (***Cartilage associated protein)*** and **LEPRE 1** (***Leucine proline-enriched proteoglycan 1***). The CRTAP gene codes instruction for making a protein called *cartilage associated protein.* This protein is critical for normal folding and assembly of collagen. It plays an important role in bone development. This gene is located on short arm of chromosome number 3 at position 22.3. The LEPRE1 gene is also known as P3H1. It provides the code for making an enzyme called *prolyl-3 hydroxylase 1.* This enzyme modifies an amino acid called proline in collagen molecules. Proline is necessary for proper folding and assembly of collagen. This gene is located on the short arm of chromosome number 1 at position 4.1.

Mutation of COL1A1 Gene

The mild mutation of this gene leads to OI type I. There is a reduction in the production of proalpha 1 chain thus reducing the amount of collagen I produced. A severe mutation of this gene leads to production of type II to type IV varieties of OI. Deletion of segments of DNA from COL1A1 gene results in nonfunctional *proalpha1* chain. Some mutations may replace the amino acid glycine with some other amino acid. Sometimes mutation may also interfere with the assembly of collagen molecules. Thus the mutation on gene COL1A1 leads to the formation of abnormal types I collagen.

Mutation of COL1A2 Gene

This gene codes for proalpha 2 chain of collagen molecule. The mutation of COL1A2 gene produces abnormal collage type I. The mutation of this gene leads to severe types of OI (type II, type III and type IV).

Mutation of CRTAP Gene

Mutation of this gene leads to type VII variant of OI. *Cartilage associate protein* is not formed due to the underlying mutation. This results in the production of abnormal cartilage.

Mutation of LEPRE 1 Gene

The mutation leads to abnormal production of enzyme prolyl-3 hydroxylase 1 which ultimately leads to incorrect folding and assembly of collagen molecules. The mutation of this gene is associated with OI type VIII.

Modes of inheritance—Type I to type IV OI are inherited as autosomal dominant entities while type VII and VIII are inherited as autosomal recessive ones.

Treatment

No genetic cure is available at present. Putting metal rods in these bones can prevent fractures of long bones.

DOWN'S SYNDROME

Refer Chapter 5.

ACHONDROPLASIA

Refer Chapter 6

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Chapter

12 Genetics of Cleft Lip and Cleft Palate

- Molecular regulation of the development of the palate
- Etiology of cleft lip and cleft palate
- Nongenetic (Environmental) risk factors
- Syndromic form of CL/P and CP
- Nonsyndromic clefting

MOLECULAR REGULATION OF THE DEVELOPMENT OF THE PALATE

The palate is formed by the fusion of one median palatine process (*primary palate*) and two lateral palatine processes. The median palatine process is formed by the fusion of right and left medial nasal processes. The fused medial nasal processes form median part of upper lip, the part of upper jaw (which carries four incisors) and the primary palate.

The two lateral palatine processes are formed by shelf-like outgrowths from the maxillary processes in the 6th week of development (Fig. 12.1). The growth of shelf-like process depends upon the interaction between ectoderm and mesenchyme. The following important genes play important role in the development of the palate. The sonic *hedgehog* **(SHH),** *bone morphogenetic proteins* **(BMP)**, *fibroblast growth factors* **(FGF)** and members **of** the *transforming growth factor* β (**TGF** β) gene superfamily determine the formation of the palate (Carlson, 2004).

- The mesenchyme of the palatal shelf expresses **MSX-1** that stimulates BMP-4 signaling in the mesenchyme.
- This leads to expression of SHH signaling in the apical ectoderm.
- SHH further induces BMP-2 signaling in the underlying mesenchyme.
- Both BMP-2 and BMP-4 stimulate mesenchyme proliferation leading to the growth of the shelf like palate.
- *Epidermal growth factor* (**EGF**) stimulates glycosaminoglycan production within the palatal shelves.

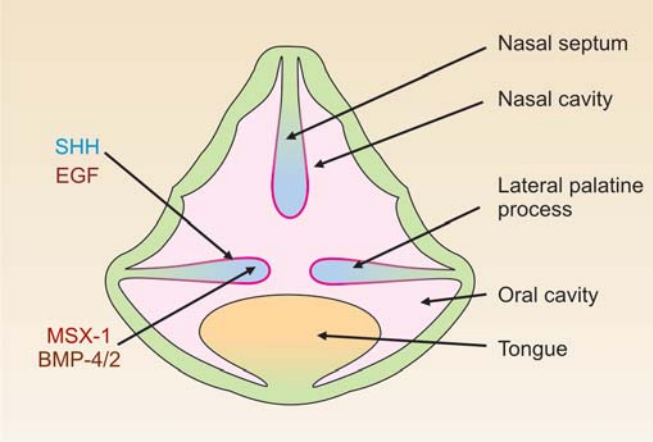

FIGURE 12.1: Coronal section passing through developing nasal and oral cavities. MSX-1 acts in the mesenchyme of palatal shelf and stimulate BMP-4, which leads to SHH signal in apical ectoderm. The SHH then induces as BMP-2 signal in mesenchyme. Both BMP-4 and BMP-2 stimulate the growth of palate

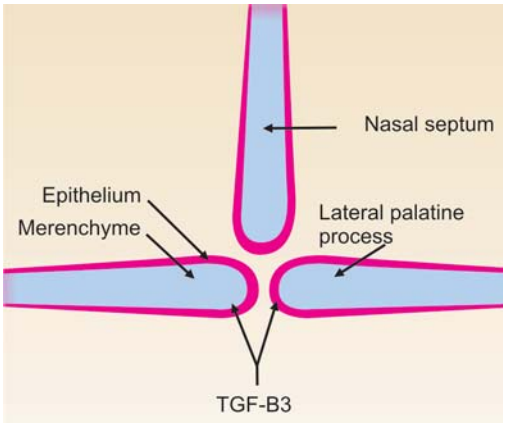

FIGURE 12.2: At the site of fusion between two lateral palatine processes the epithelium undergoes transformation to mesenchyme by the action of TGF-β3

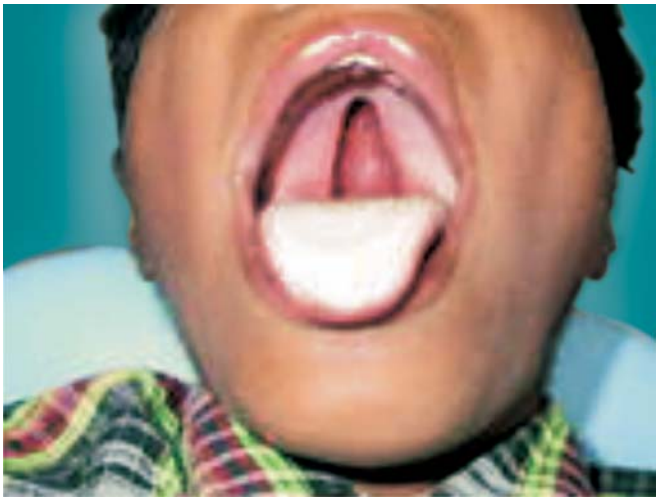

FIGURE 12.3: Patient showing cleft palate. (*Courtesy* of Deptt. of Oral Pathology, Modern Dental College and RC, Indore, India)

As the right and the left palatal shelves start fusing with each other in the midline, they are covered by epithelium. Some of these fused midline epithelial cells soon disappear by the process of apoptosis, while some other cells transform themselves from epithelial to mesenchymal cells. This transformation of cells is mediated due to the release of transforming growth factor-β3 (***TGF-β3***) (Fig. 12.2). It is well known that TGF-β3 is expressed in the epithelium just before fusion. Mutation of TGF-β3 gene leads to formation of isolated ***cleft palate*** (Fig. 12.3).

(The TGF-β3 knock out mouse exhibits an isolated cleft palate due to failure of palatal shelf fusion. Although the palatal shelves otherwise develop normally, they show a marked reduction in the filopodia present on the midline epithelial cell surface and show a down-regulation of chondroitin sulphate proteoglycan on the apical surface of the epithelia. Both of these are required for efficient midline epithelial adhesion).

Thus, it is clear that a tight control of a cascade of genes is required to complete normal palatogenesis. Anteriorly each palatal shelf joins with the primary palate. The fusion between two palatal shelves occurs anteroposteriorly and the process is completed by the 11th or 12th week.

ETIOLOGY OF CLEFT LIP AND CLEFT PALATE

Cleft lip (Fig.12.4) with or without cleft palate (CL/P) and cleft palate (CP) are common birth defects that represent a major public health burden both in the social and medical contexts. The prevalence of CL/P varies from 0.3 to 2.5 per 1000 livebirths worldwide. Cleft lip and palate (CLP) and cleft lip (CL) comprise 45 and 25% of all children respectively born with an oral cleft. The population-based studies have shown that 50 to 70% of CL/P births had no other major malformation while upto 30% are associated with other anomalies. 10 to 15% of all CL/P cases report a positive family history. The median cleft lip and lateral facial cleft are rare conditions (Figs 12.5 and 12.6).

The etiology of CL/P is complex and thought to involve genetic influences with variable interactions

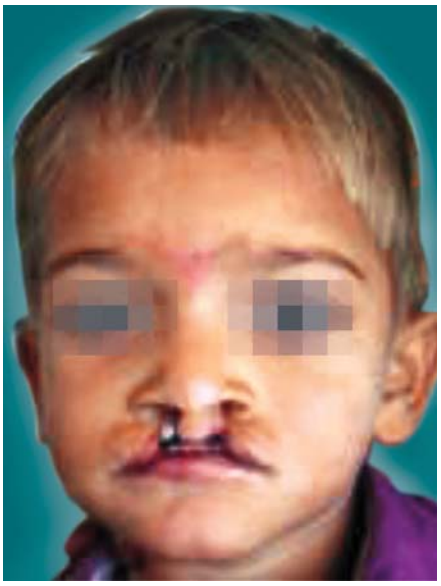

FIGURE 12.4: Patient showing bilateral cleft-lip. (*Courtesy:* Deptt. of Oral Pathology, Modern Dental College and RC, Indore, India)

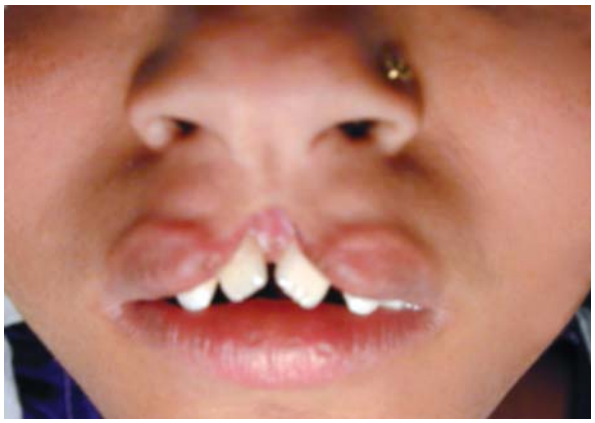

Figure 12.5: The median cleft lip. This condition results due to defective development of the lower most part of frontonasal process. (*Courtesy:* Dr Prakash Chhajlani, Plastic Surgeon, Indore, India)

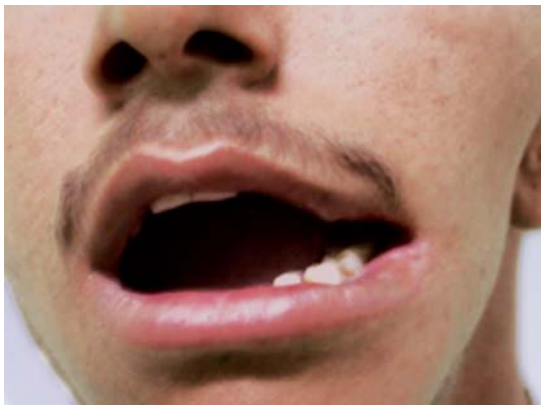

Figure 12.6: Unilateral lateral facial cleft. Not the abnormal wide mouth (macrostomia). This condition results due to inadequate fusion of mandibular and maxillary process with each other. (*Courtesy:* Dr Prakash Chhajlani, Plastic Surgeon, Indore, India)

from environmental factors. The etiological factors of cleft lip and cleft palate can be grouped as under:

A. Nongenetic—This includes various environmental (teratogenic) risk factors which may cause CL/P.

B. Genetic—Genetic causes include:

- *Syndromic:* Here the cleft is associated with other malformations. Usually, it is due to a single gene (monogenic or Mendelian) disorder. The clefting may also occur due to other chromosomal abnormalities due to multiple gene involvement.
- *Nonsyndromic:* Here the cleft is mostly an isolated feature and occurs in the vast majority of individuals having a cleft-lip or palate (up to 70% cases). In this form of cleft neither a recognized pattern of malformation nor a known cause for the disorder can be identified.

Overall, environmental factors are considered much less important agents than genetic factors in the etiology of oral clefts.

NONGENETIC (ENVIRONMENTAL) RISK FACTORS

Scientists have investigated many nongenetic environmental factors (teratogens) that increase the possibility of clefting. The lack of total concordance found in monozygotic twins (Spritz, 2001) indicated environmental etiology. Following is a list of these teratogens:

1. *Drugs*

Vasoactive drugs—Pseudoephedrine, aspirin, ibuprofen, amphetamine, cocaine.

Anticonvulsant drugs— Phenobarbitol, trimethadione, valproate, and dilantin.

Anticancer drug—Aminopterin.

Antinausea or vomiting drug—Diphenhydrinate.

Others—Corticosteroids, isotretinoin (Accutane), sulfasalazine, naproxen.

2. *Maternal smoking*—Leads to embryonic hypoxia (Wyazynski et al, 1997).
3. *Alcohol intake*—May also lead to fetal alcohol syndrome (Jones et al, 1973; Goslin et al, 2001).
4. Maternal fever
5. *Exposure to chemicals*—Xylene, toluene, acetone, benzene and pesticides.
6. *Nutrition*—Folic acid and vitamin A deficiency during pregnancy (Hartridge et al, 1999).
7. *Altitude Hypoxia* (Castilla et al, 1999).
8. *Traumatic stress* by releasing hydrocortisone.

SYNDROMIC FORM OF CL/P AND CP

It is monogenic (Mendelian) form of clefting. This form of cleft is associated with syndrome (linked to varieties of other congenital anomalies). Since these syndromes are determined by the mutation in single gene they are of a high-risk type. Over 300 syndromes are known to be associated with cleft lip or palate as an associated feature. The syndromic CL/P can be subdivided into following categories (Table 12.1):

a. CL/P associated with syndromes caused by mutation in single gene.
b. CL/P associated with syndromes caused by chromosomal abnormalities.
c. CL/P associated with syndromes caused due to known teratogens.
d. CL/P associated with syndromes whose cause is not known.

About 300 syndromes are known to have clefting of the lip or palate as an associated feature. The following table describes in brief a few well-known syndromic forms of CL/P.

TABLE 12.1: Syndromic forms of CL/P and CP

Syndrome	*Gene name (symbol)*	*Location on chromosome*	*Inheritance*
Stickler syndrome, type II	Collagen type XI, alpha-2 chain (COL11A2)	6p21.3	AD
Simpson dysmorphia syndrome	Glypican-3 (GPC3)	Xq26	X-linked
Phenylketonuria	Phenylalanine hydroxylase (PAH)	12q24.1	AR
Holoprosencephaly, type 3	Sonic hedgehog (SHH)	7q36	AD
Retinoblastoma	Retinoblastoma (RB1)	13q14.1-q14.2	AD
Crouzon craniofacial dysostosis (including Apert and Pfeiffer syndromes)	Fibroblast growth factor receptor-2 (FGFR2)	10q26	AD
Zollinger syndrome-3	Peroxisomal membrane protein-3 (PXMP3)	8q21.1	AD
Diastrophic dysplasia	Diastrophic dysplasia sulfate transporter (DTDST)	5q32-q33.1	AR
Gorlin syndrome (Basal cell nevus syndrome)	Patched (PTCH)	9q22.3	AD
Waardenburg syndrome, type I	Paired box homeotic gene-3 (PAX3)	2q35	AD
Waardenburg syndrome, type IIA	Microphthalmia-associated transcription factor(MITF)	3p14.1-12.3	AD
DiGeorge syndrome	DiGeorge syndrome chromosome region (CATCH22)	22q11	AD
Treacher-Collins mandibulofacial dysostosis	Treacle (TCOF1)	5q32 -q33.1	AD
Van der Woude syndrome	Interferon regulatory factor 6 (IFRF 6)	1q32-q41	AD
CLP-ectodermal dysplasia synderome	Poliovirus receptor related –1 (PVRL1)	11q23.3	AR
Ectrodactyly, ectodermal dysplasia orofacial cleft syndrome (EEC)	p63	3q27	AD

Besides above-mentioned syndromes, X-linked Optiz syndrome (due to mutation in MIDI gene on chromosome Xp22) and mutation in the MSX1 gene and mutation in TBX22 genes are also known to be responsible for syndromic form of clefting.

Genetic Mechanism for Syndromic Form of Clefting

We have started, though recently, to understand genetic mechanisms implicated in some of the syndromic forms of CL/P.

DiGeorge syndrome (arising due to a deletion in chromosome 22q11) results from the failure of migration of neural crest cells in third and fourth branchial arches. These individuals also have associated CP (Seambler, 2000). We now know that the mutation in TBX22 gene encompasses almost all the common features of DiGeorge syndrome (Lindsay et al, 2001).

- The geneTCOF1 in Treacher-Collins syndrome encodes the protein called ***treacle***. The exact function of treacle is unknown at present. It is speculated that treacle is involved in shuttling proteins between nucleolus and cytoplasm of the cell (Marsh et al, 1998). At present we do not know how this function can cause various malformations observed in Treacher-Collins syndrome.
- The mutation of SHH (Sonic hedgehog) gene leads to holoprosencephaly (Belloni et al, 1996). Holoprosencephaly is associated with midline defects like incomplete or deformed forebrain

division, cyclopia, etc. The SHH signaling peptides play the role in midline patterning of human embryo.

- The genetic mechanism of CP in Stickler syndrome can be explained on the basis of defective collagen biosynthesis. Stickler syndrome (type I) occurs due to mutation in COL2A1 gene which encodes for type II collagen (Snead et al., 1999). Stickler syndrome (type II) has also been noted to occur due to mutation in the COL11A1 gene encoding type XI procollagen.

NONSYNDROMIC CLEFTING

Nonsyndromic cleft lip and/or palate (NSCLP) is a malformation characterized by an incomplete septation between the nasal and oral cavities without any associated anomalies. The distinction between syndromic and nonsyndromic clefting is very easy. In case of syndromic clefting there is always the presence of other associated anomalies in addition to CL/P and CP. In cases of NSCLP the clefting is not associated with any other anomaly. Nonsyndromic orofacial clefts (OFC) are one of the most common malformations among livebirths and are composed of two separate entities: cleft lip with or without cleft palate (CL/P) and isolated cleft palate (CP).

The nonsyndromic clefting is said to be polygenic in nature. It is produced out of interaction between a number of genes; each producing a small effect that add up together to create the clefting. In other words, a cleft occurs when the total genetic liability of an individual reaches a certain minimal level termed the threshold. It should be noted that every individual carries some genes that predisposes cleft formation but if the liability due to these genes is less than the threshold or the critical levels, no cleft results.

NSCLP is also said to be a complex multifactorial trait with interactions between genetic and environmental factors playing an important role in its causation. As the etiology of NSCLP is complex, many reports in the literature are contradictory (Murray, 1995).

The genetics of nonsyndromic clefting has been investigated by following methods:

1. **Familial and Segregational Analysis:** Many studies have shown an increased risk of clefting in siblings. The risk was found to be 30% higher in siblings as compared to normal population prevalence. This clearly shows that NSCLP is genetically determined. The mode of inheritance of nonsyndromic cleft lip with or without cleft palate (NSCLP) is still a matter of dispute. Many studies have indicated towards a multifactorial mode of inheritance while others are of opinion that the inheritance has a mixture of monogenic and multifactorial patterns.
2. ***Twin Studies:*** NSCLP has shown higher concordance rates in monozygotic twins. The concordance rate in monozygotic twins is approximately 25 to 45% as opposed to 3-6% in dizygotic twins. On the other hand, lack of complete concordance was also found similar to any other multifactorial trait. This suggested involvement not only of genetic but also of environmental factors in the causation of nonsyndromic clefting.
3. ***Linkage Analysis:*** Linkage analysis is used to map a disease (mutant) gene to specific region on a chromosome. Today we know that mutations in many candidate genes or loci and their chromosomal locations are associated with nonsyndromic clefting (Refer Table 12.2). (*A candidate gene is a gene known to be located in a region of interest in the genome. The product(s) of a candidate gene has/have biochemical or other properties suggesting that it may be the gene being related to a condition).*
4. ***Association studies:*** Both kinds of the approaches, i.e. *candidate gene approach* and *genome wide association studies* are reported for nonsyndromic clefting. Association studies have identified many genes for the clefting.

The following candidate genes have been identified as etiological factors of nonsyndromic CL/P (Table 12.2).

It has been observed that some of the genes that are responsible for causing syndromic forms of clefting are also responsible for causing nonsyndromic form of clefting. These overlapping genes are MSX-1, IRF 6, PVRL1, CATCH22, TGFβ3 and TBX22. It is now becoming apparent that the same genes contribute to the population of nonsyndromic clefts perhaps through variable penetrance or through the action of different modifiers.

In conclusion, one may say that genetic analysis of CL/P is quite confusing as mutation screening of

TABLE 12.2: Possible genes whose mutation may result in the nonsyndromic clefting

Name of the candidate gene	*Symbol*	*Ch. Location*	*Reference*
Blood clotting factor XIII gene	F13A	6p24-25	Eiberg et al, 1987
Endothelin-1 gene	ET1	6p24	Carinci, 1995
Proto-oncogene BCL3	BCL3	19q13.2	Stein et al, 1995
Retinoic acid receptor alpha gene	RARA	17 (t15/17)	Shaw et al, 1993
Transforming growth factor-alpha	(TGFA)	2p13	Murray, 1995
Transforming growth factor-β3	(TGFβ3)	14q24	Proetzel, 1995
MSX-1	(MSX-1)	4q25	Lidral, 1998
Methylene tetra-hydrofolate reductase	MTHFR	1p36.3	—

specific candidate genes, association studies and even genome-wide scans have largely failed to reveal the exact molecular basis of human clefting. There is also overlapping of genes determining syndromic and non-syndromic forms of CL/P. Greater efforts are necessary in order to have a complete picture of the main factors involved in lip and palate malformation. These elements will permit us to better understand and better treat patients affected by clefting.

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Chapter

13

Genetics of Dental Caries

- Twin studies
- Risk factors in dental caries
- Microorganisms of the oral cavity and host immune response
- Cariogenic diet
- Role of saliva in protection against caries
- Morphology of tooth and composition of enamel matrix
- The search of candidate gene(s) for dental caries

Dental caries is the medical term for tooth decay or cavities. Tooth decay is one of the most common of all disorders of teeth.

Bacteria, that are normally present in the mouth, convert all foods especially sugar and starch into acids. Bacteria, acid, food debris and saliva combine in the mouth to form a sticky substance called plaque that adheres to the teeth. Tooth decay begins if this plaque is not removed thoroughly and routinely. As stated earlier some of the plaque forming bacteria converts sugar and carbohydrates (starches) in the foods we eat into acids. These acids dissolve minerals on the surface of the tooth. This erodes the enamel or creates pits on the enamel that are too small to see at first but they get larger over time. Cavities are usually painless until they grow very large and affect nerves or cause a tooth fracture. A tooth abscess can develop. Untreated tooth decay also destroys the internal structure of the tooth (pulp) and ultimately causes loss of the tooth (Fig. 13.1).

The etiology of dental caries has been studied for many years. Multiple factors contribute to a person's risk for caries.

(1) *Environmental factors*: Diet, oral hygiene, fluoride exposure and the cariogenic bacteria.

(2) *Host factors*: Salivary flow, salivary buffering capacity, position of teeth relative to each other, composition of tooth enamel and host immune response.

In spite of all that is known about this disease there are individuals who still appear to be more susceptible to caries and those who are extremely resistant to dental caries regardless of the environmental risk factors to which they are exposed.

Similar to periodontitis, malocclusion and cleft lip or cleft palate, dental caries is also said to be a multifactorial (complex) disease. Though dental caries are seemingly caused out of interaction between environmental and genetic factors, yet the disease is to a great extent influenced by environmental factors. Most scientists agree that the genetic component of dental caries has a minor one in comparison to the overall effect of the environment.

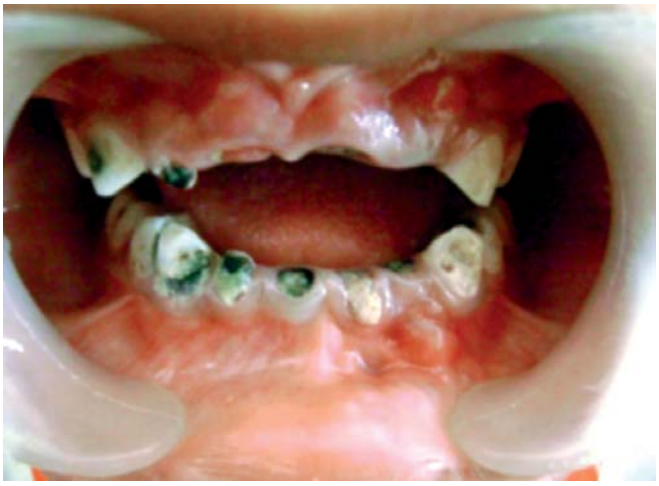

FIGURE 13.1: Full mouth case of early childhood caries. (*Courtesy:* Dr Priyanka Airen, Department of Pediatric Dentistry, Modern Dental College and RC, Indore, India)

TWIN STUDIES

How We Come to Know that Genetics Plays a Role in the Etiology of Caries

Twins studies were carried out to investigate the role of genetics in the etiology of caries. In case of multifactorial diseases where genetic and environmental factors play important role in the causation of the disease, twin studies can be used as useful tools to evaluate the roles of the genetic component of the disease. Presence or absence of the trait or disease in a large number of the two types of twins (mono and dizygotic varieties) is calculated in percentage. Diseases in which the percentage of twin pairs, where both the twins of the pair are affected, is greater in the monozygotic group as compared to the dizygotic group, the diseases can be confirmed to have a definite genetic etiology. The genetic basis of a disease is tested in monozygotic twins who are reared together in the same environment or separated after birth and reared in two different environments. All the similarities in these twins would expectedly be due to common genes and all the dissimilarity will be due to environmental factors.

The early twin studies carried out at the beginning of the 20th century provided some indications that inheritance played a role in caries but the evidence were not conclusive. They only pointed that inheritance was only a contributor to the process (Bachrach et al, 1927; Horowitz et al, 1958; Mansbridge et al, 1958 and Goodman et al, 1959). Twin studies conducted later in the century compared the incidence of caries in monozygotic twins and dizygotic twin groups. Many studies detected a statistically significant genetic component in the susceptibility to caries and demonstrated that caries experience in monozygotic twins had a greater concordance (Bordoni et al, 1973, Hassell et al, 1995; Townsend et al, 1998). These studies concluded that not only environmental factors clearly have a greater influence but that genetic factors also contribute to the causation of dental caries (Niswander et al, 1975).

A major advance in the understanding of the role of inheritance and the incidence of dental caries was achieved by analyzing twins reared apart. These patients had an average age greater than forty and did not share similar environments from shortly after birth until the time of analysis. The analysis of twins raised apart provides the strongest evidence of a genetic contribution to the incidence of dental caries (Borass et al, 1988 and Conry et al, 1993).

The analyses of dental caries incidence in monozygotic and dizygotic twins also indicate that a large number of different genes contribute to the observed outcomes. Although the twin studies provided some strong evidence of genetic contribution to the risk of dental caries, none provided any evidence of linkage to specific genes (Shuler, 2001).

RISK FACTORS IN DENTAL CARIES

The most important components contributing to the risk of dental caries are as under:

- Microorganisms present in the oral cavity and the host immune response
- Cariogenic diet
- Role of saliva in protection against caries
- Morphology of tooth and composition of enamel matrix
- Gene(s) for dental caries.

Many studies have indicated that even if all the environmental factors are identical and monitored to be under controlled and standard conditions, variability in the susceptibility to dental caries still exists and differs within the tested individuals.This indicates that certain environmental factors are more cariogenic for some individuals as compared to others. This may be explained by the existence of genetic variability among individuals. The genetic influences modify the expression of disease in the individual.

MICROORGANISMS OF THE ORAL CAVITY AND HOST IMMUNE RESPONSE

Dental caries will not occur if the oral cavity is free of bacteria. These bacteria are organized into dental plaque. Of the many types of bacteria in the mouth the most caries active appear to be *Streptococcus mutans, Lactobacillus spp., Veillonella spp. and Actinomyces spp.* These bacteria can be transferred from the mother to child and are present at varying levels in all human mouths. A variety of carbohydrates provide substrates for these organisms to grow on.

Most research on the bacteriology of dental caries has focused on the ubiquitous *S. mutans* and its ability to ferment sucrose (Loesche, 1986). This organism

preferentially ferments sucrose to produce significant amounts of acid and extracellular polysaccharide (plaque). However, most researchers now agree that other organisms present in the mouth are capable of plaque formation and acid production from a variety of fermentable carbohydrate substrates besides sucrose which is present in the normal mixed diet.

The individual's genotype may influence the likelihood of intraoral colonization of cariogenic bacteria. Scientists have shown that persons may be caries-resistant or caries-susceptible for a particular strain of bacteria. Even if cariogenic bacteria are present in the oral cavity the caries-resistant person usually doesn't develop caries. This confirms the presence of important genetic elements influencing susceptibility to dental caries.

Streptococcus mutans was first isolated from human carious lesions in 1924 by Clark. This indicated that dental caries had a bacterial etiology and were transmissible infections. *S. mutans* isolates have been divided into eight serotypes. Human isolates represent serotype *c, e* and *f.* The *S. mutans* serotype *c* being the most prevalent streptococci isolated from human dental plaque.

Salivary immunoglobulin A (sIgA) is the major antibody present in the saliva. It is the host's first line of immune defense against *Streptococcus mutans.* Salivary IgA is synthesized and secreted by plasma cells located in salivary glands. The host's immune system protects individual from caries by producing various types of antibodies. In humans majority of these antibodies are of the IgA type but types IgG and IgM are also present in the saliva. Salivary IgA acts in the following ways to restrict infection:

- It neutralizes bacterial exotoxins.
- It neutralizes enzymes contributing to the disease processes such as **glucosyltransferases**. *(Caries only form when this enzyme acts in presence of sucrose. Glucosyltransferases breakdown sucrose into its components like simple sugars known as fructose and glucose. Mutans streptococci use these sugars to form plaque. The enzyme glucosyltranferase could be used in making effective vaccines for the reduction in dental caries. Certain components of these enzymes when injected into an individual produce antibodies against the enzymes and inactivate bacterial activity to reduce plaque formation. In the past this enzyme was used as an antigen and was injected in the salivary glands of rats. The immunized rats produced the antibody IgA in their saliva and had fewer dental caries. Thus in future this enzyme may be used as vaccine in humans against caries).*
- Inhibits the attachment of bacteria on epithelial or tooth surface.

Because of the above function of the salivary IgA most of the caries vaccines induce salivary IgA antibody response to *S. mutans* antigens (Han, 2007).

Human studies in the past have measured the levels of antibodies present in blood serum and saliva of caries patients. Some of these studies found a negative association between IgA and caries activities while other reports have shown a positive association. Few reports found no correlation between the two. Thus the association between the levels of IgA and the development of dental caries has been studied with conflicting results. However, the number of papers is growing that report increases in immunoglobulin IgA contents in the saliva in cases with high caries experience (Weyna et al, 1979 and Dens et al, 1995). One group of research workers studied pair-matched patients between a group of *IgA deficient* patients and a section of immunocompetent normal subjects by age and plaque index. During a two years period they observed less caries experience in *immunodeficient* patients than found in normal controls (Robertson et al, 1980). Another study observed that IgA-deficient children showed caries scores lower than those of healthy children (Fernandes et al, 1995).

Major Histocompatibility Complex (MHC) Antigens or Human Leukocytes Associated (HLA) Antigens

The plasma membrane surface of almost all the body cells (except RBCs) presents "self antigens" called the major histocompatibility complex (MHC) antigens. These are also called HLA (human leukocyte associated) antigens because they were first detected on the white blood cells. These self-antigens are integrated membrane glycoproteins. Thousands to several hundred thousands of MCH antigens are present on all body cells.

The success of tissue transplantation depends on histocompatibility matching between the donor and the recipient. The chances of rejection of a graft are less if a large number of MHC antigens are similar in the donor and recipient. Graft rejection doesn't occur

between identical twins as they have exactly the same genetic constitution. The other function of MHC (HLA) antigens is to help T cells of the immune system to recognize the 'foreignness' of an antigen (nonself) to mount an immune response.

There are three classes of HLA antigens:

- Class I MHC (MHC-I) antigen molecules are present on the plasma membrane of all the cells of the body (except RBCs). They are involved in signaling to cytotoxic T cells.
- Class II MHC (MHC-II) antigens are present only on the antigen presenting cells (macrophages, B cells and dendritic cells), B cells and d. They are involved in signaling helper T cells, B cells and macrophages.
- Class III MHC (MHC-III) include a number of proteins with a variety of immunological functions, e.g. tumor necrosis factor (TNF), heat shock proteins and various types of "complement proteins".

The MHC antigens play a major role in immune response of an individual. The genes coding for class I MHC (A, B, C, E, F and G), class II MHC (DR, DQ and DP) and for class III molecules are located on chromosome number 6.

The HLA system is highly polymorphic, i.e. many different combinations of gene alleles are possible at a given HLA loci. Two unrelated individuals are therefore unlikely to have identical HLA antigens. Only siblings may match for similar HLA antigens. Therefore brother and sister are usually selected as donors of organs or tissues for grafting to the other.

Why Some Diseases are Associated with Certain HLA Type?

It is well known that certain diseases are associated with certain HLA types (Turnpenny and Ellard, 2005). The celiac disease and rheumatoid arthritis are associated with DR4 HLA type; ankylosing spondylitis with B27 HLA type; myasthenia gravis with B8 and so on. It should be noted that possession of certain HLA type antigen does not mean that the associated disease will affect the person. The person is, however, at a greater risk to be affected by that particular associated disease as compared to persons not having that particular HLA type. Although the mechanism involved in the association between certain disease and HLA type is not fully understood, it may result from the close proximity of a susceptibility gene and the HLA complex on the same chromosome. An example of close linkage is congenital adrenal hyperplasia (CAH) linked with HLA A3 / Bw47 / DR7. This is because the mutant gene CYP21 (responsible for CAH) is located within the HLA major histocompatibility locus on chromosome 6p21.3. Thus the disease CAH is associated with HLA A3 / Bw47 / DR7.

Association between HLA Antigens and Susceptibility to Dental Caries

Current evidences support the relationship between immune complex genes (HLA) and caries and the association of different levels of cariogenic bacteria and enamel defects. Many studies are now available which show the association between increased risk for caries and immune complex (HLA) genes. One study reported the strong association between HLA DRw6 loci and DMFS index (Lehner et al, 1981). The same immune complex locus showed a low dose response to *Streptococcus mutans* antigens. However, few studies could not detect a relationship between HLA DR type and dental caries (De Vries et al, 1985 and Acton et al, 1999).

Celiac disease is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages. Symptoms include chronic diarrhea, weight loss and fatigue. The vast majority of celiac patients have one of the two types of HLA DQ. This gene is a part of the MHC class II antigen-presenting receptor. The gene is located on the short arm of the sixth chromosome and as a result of this linkage the locus has been labeled *CELIAC1*. Celiac disease patients exhibit an increased incidence of dental caries. This might be due to the fact that these patients have defective enamel that predisposes the tooth to dental caries (Aine et al, 1990; Aine, 1996 and Aguirre et al, 1997). However, the cause of this defective enamel in celiac disease patients has not been well-understood. These patients show significant positive correlation between their HLA type and presence of the enamel defect. The HLA –DQ 2 and DQ 8 is strongly associated with enamel defects and dental caries in celiac patients.

The association between HLA complex and caries has indicated that a few genes in the HLA complex are responsible for dental caries resulting from altered

enamel development and also due to low dose response to cariogenic bacteria (Lehner et al, 1981), i.e. less aggressive immune response to bacterial invasion. We still do not know whether specific genes dedicated to the development of enamel or responsible immune response to cariogenic bacteria are located close enough (linked) to certain HLA complex. It is expected that the existence of any such association would be determined in the near future.

It is well known that individuals with immune deficiency diseases are susceptible to dental caries and have a greater frequency of harboring *S. mutans* than do normal persons, e.g. as seen in HIV infection.

CARIOGENIC DIET

The dietary components that contribute most to the process of caries formation are fermentable carbohydrates. These need to be retained in the mouth long enough to be metabolized by oral bacteria (principally *Streptococcus mutans*) to produce acid. The acid attacks the tooth enamel and gradually dissolves it (demineralization). A repair process known as remineralization offsets this demineralization process. The balance between remineralization and demineralization determines the occurrence of caries.

The presence of fat in experimental diets has been shown to affect cariogenicity of sugars. The effects have been ascribed to enhance clearance of sugars from the mouth. It is also conceivable that several fatty acids express a potent antibacterial effect. The presence of calcium and phosphorus has been shown to influence the cariogenicity of foods; the effect, however, is restricted to the food containing the minerals. Evidence suggests that pyridoxine (vitamin B6) may exert a cariostatic (stopping caries) effect by enhancing decarboxylation activity in dental plaque.

Studies have indicated that the persons suffering from *hereditary fructose intolerance* are free from caries (Newburn et al, 1980 and Saxen et al, 1989). They are free from caries because there is absence of sugar in their diet and not because a hereditary fructose intolerance provides some kind of resistance to the production of caries.

Very few studies are available that has investigated the heritability of caries in relation to sucrose. A twin study in 2003 was aimed to determine heritability estimates for dental caries traits and sucrose sweetness preference. Results indicated that variations in dental caries traits and sucrose sweetness preferences have a significant genetic contribution mediated independently (Bertz et al, 2003).

Many studies have revealed that higher and more frequent sugar intake may increase the risk of caries formation in children. The high sugar intake reflects a preference for sweet substances. Inherited behavior and taste thresholds may play an important role in the frequency of carbohydrate intake. Genetic sensitivity to taste may be associated with a preference for or rejection of some food by children. Many studies indicate that children belonging to the group "*non-tasters*" (with high threshold for taste) were sweet likers and prefer strong tasting food (Verma et al, 2006) while children belonging to group "*tasters*" are sweet dislikers and preferred weak tastes. The incidence of dental caries was significantly higher in nontasters as compared to tasters.

ROLE OF SALIVA IN PROTECTION AGAINST CARIES

Saliva is body's natural protective mechanism against decay. It contains salivary proteins that adsorb strongly onto the teeth, protecting enamel against acid dissolution. This adsorbed protective layer is referred to as the pellicle. Salivary proteins also act as antibacterial agents. Saliva is the primary resource of calcium, phosphate and fluoride; materials used to remineralize the enamel. Saliva also acts quickly to clear away food debris from the mouth and to buffer the organic acids that are produced by the bacteria. Saliva is therefore a very vital and complex material in the prevention of dental caries. Salivary dysfunction can lead to rapid deterioration of dental enamel. Salivary dysfunction may occur as a result of certain medications or as side effects of medical treatments such as radiotherapy.

There is a strong correlation between the composition of saliva and the production of caries. The formation of dental plaque is the result of interactions between environmental and genetic factors. The caries-susceptible plaque is formed due to presence of certain chemicals in saliva. A group of saliva proteins known as proline-rich proteins (PRPs) are responsible for early plaque and pellicle formation (Mayhall, 1970 and Bennick et al, 1983). At present eight different kinds

of PRPs are known that are thought to be produced by a cluster of genes located on the short arm of chromosome number 12 (Goodman et al, 1985 and Mamula et al, 1985). People show variations in the type of PRPs produced in their saliva due to variations in their genotype at these regions. Some people with certain protein genotypes (especially Pa+ and Pr22) are more susceptible to dental caries (Yu et al, 1986). On other hand individuals with genotypes Pa– and Pr11 are resistant to the dental caries. Similarly low levels of salivary calcium and phosphate have been shown to be associated with the increased risk of caries.

Different individuals respond in a different way to specific biochemical differences in oral environment depending on their genetic constitutions. For example there is significant difference between monozygotic and dizygotic twins in terms of salivary flow, pH, and salivary amylase activity when compared between the two groups. On the contrary, both the monozygotic twins of a pair will respond similarly (because of same genetic constitution) to these factors whereas such similarity may be lacking between the two individuals of a dizygotic pair (because of difference in their genetic constitutions).

Literature is nearly equally divided both in favor of and against the anticaries role of salivary immunoglobulins, especially **sIgA**. Many studies have indicated inverse relations between sIgA and caries (Camling et al, 1987 and Rose et al, 1994). Few studies have also reported increased levels of sIgA (Prakash et al, 1994). However, some studies have indicated no correlation at all (Kristila et al, 1994).

Xerostomia or decreased secretion of saliva (due to pathological dysfunction of salivary glands) has been demonstrated to be responsible for increased rate of caries. Studies indicate that a low salivary flow rate (less than 1 ml/min after salivary stimulation) is associated with an increased risk of caries. **Sjögren's syndrome** is an autoimmune disorder in which abnormally activated immune cells attack and destroy exocrine glands that produce tears and saliva. The primary and secondary variants of Sjögren's syndrome are found to be associated with increased caries risk (Ravald et al, 1998). This is due to the fact that Sjögren's patients have a decreased flow rate of saliva. Similarly, the condition of scleroderma related xerostomia is also associated with caries tooth (Wood and Lee, 1988). Several medical conditions including therapeutic radiations administered to the head and neck regions (Nasman et al, 1994) and pharmacological agents with xerostomic side effects (Ryberg et al, 1990) lower salivary flow rate dramatically to pathological levels and elevate the patient's risk of caries. The evidence therefore indicates that normal salivary flow rate is strongly protective against caries and clinicians should identify individuals with reduced salivary output to modify their treatment.

Dental caries has a higher prevalence rate in females as compared to males. A recent study indicated that biochemical composition of saliva and salivary flow rates are modified in women due to hormonal fluctuations during events such as puberty, menstruation and pregnancy. This makes the oral environment significantly more cariogenic for women when compared to men.

Saliva is a major carrier of topical fluoride. The concentration of fluoride in the ductal saliva, as it is secreted from salivary glands, is low. This concentration of fluoride is not likely to resist cariogenic activity. Drinking fluoridated water, brushing with fluoride toothpaste or using other fluoride dental products, on the other hand, can raise the concentration of fluoride in the saliva present in the mouth to about 100- to 1,000-folds. Saliva and the extraneous sources thus serve as important sources of fluoride that gets concentration in the plaque and aid tooth remineralization. Fluoride concentrated in plaque and the saliva inhibits demineralization of the sound enamel and enhances the remineralization or recovery of demineralized enamel (Featherstone, 1999). Fluoride also inhibits dental caries by impairing cellular mechanisms of cariogenic bacteria.

High caries prevalence has been reported for individuals suffering from the deletion at 22q11; the 22q11 Deletion Syndrome (22q11 DS). It was observed that patients with 22q11 DS had impaired salivary secretion rates, higher numbers of cariogenic bacteria, increased salivary protein concentrations and reduced output of electrolytes in the saliva compared to the controls. This indicated that the salivary function is affected in 22q11 DS explaining increased caries risk seen in these subjects (Klineberg et al, 2007).

MORPHOLOGY OF TOOTH AND COMPOSITION OF ENAMEL MATRIX

The morphology of teeth related to their shapes, sizes, pit and fissure morphology, enamel structure and composition, arch forms, dental spacing and order of the teeth are some of the important factors that regulate the "washing" effects of saliva and thereby may profoundly influence the production of caries. These factors are in fact largely determined by hereditary factors. Both kinds of twin studies ("twins reared together" and "reared apart") have indicated that the morphologies of teeth, arch forms, dental spacing, malocclusion, etc. have strong genetic contributions.

Teeth are composed of a thin layer (1-2 mm) of dental enamel, which forms the hard protective coating over the tooth. This layer mainly consists of calcium, phosphate and other ions in a structure known as "hydroxyapatite". Dental enamel is porous and is susceptible to acid dissolution during the process of demineralization. Many genes are known which are active in the formation of enamel (AMELX, ENAM, KLK-4 and MMP20 which encode various proteins like *ameloblastin*, *amelogenin*, *enamelin*, *tuftelin-1*, and *tuftelin interacting protein 11).* Certain variations in some of these enamel matrix genes may be associated with enhanced caries susceptibility. Some of these genes are linked to specific syndromes where the process of tooth development itself is altered. This is due to an alteration in the proteins responsible for biomineralization of the enamel matrix. Patients with alterations in the morphology of teeth and formation of the enamel are susceptible to caries. Syndromes, which are associated with caries and well-defined altered craniofacial phenotypes, are usually determined by mutation in a single gene. These syndromes are Turner's syndrome (Takala et al, 1985), the fragile X syndrome (Shellhart et al, 1986), ectodermal dysplasia, cleft lip and/or cleft palate (Dahllot et al, 1989), diastrophic dysplasia, etc. We do not know much about specific genes responsible for these disorders yet.

Nonsyndromic forms of Amelogenesis Imperfecta (AI) present with abnormal formation of the enamel. The enamel is composed mostly of minerals that are modified and regulated by the activities of the proteins in it. AI is caused due to malfunction of proteins in the enamel: Ameloblastin, enamelin, tuftelin and amelogenin (Refer Chapter 10).

People afflicted with amelogenesis imperfecta have teeth with an abnormal color. The teeth have a higher risk for dental cavities. The AMELX, ENAM, KLK-4 and MMP20 genes provide instructions for making proteins that are essential for normal development of tooth. Mutations in the AMELX, ENAM, MMP20, and KLK-4 genes have been found causative for amelogenesis imperfecta (nonsyndromic form). Mutations in any of these genes alter the final structure of these proteins or completely prevent synthesis of any protein at all (Refer Chapter 10). As a result tooth enamel is abnormally thin or soft and has high a risk of developing dental caries.

Epidermolysis Bullosa (EB)

The syndrome epidermolysis bullosa (EB) has been shown to be involved with an alteration in the enamel and as well as with an increased incidence of caries (Wright et al, 1994 and Kirkham et al, 2000). Epidermolysis bullosa is a group of inherited disorders presenting with skin blisters that develop in response to minor injuries such as rubbing or scratching. Blisters may also occur in the oral mucosa.

There are three main types and various subtypes of epidermolysis bullosa:

- Epidermolysis bullosa simplex
- Junctional epidermolysis bullosa
- Dystrophic epidermolysis bullosa.

EB simplex is the most common form. It is due to a mutation in the gene involved with the production of ***keratin***. As the name of the disease suggests, the condition is associated with spontaneous splits in the basal epidermis that cause formation of blisters within the epidermal layers.

Junctional EB is caused due to mutations in genes responsible for formation of ***hemidesmosomes*** (microscopic structures that attach the epidermis to the basement membrane). Blistering takes place in the basement membrane zone (lamina lucida of basement membrane).

Dystrophic EB is affected due to mutations in genes involved in the production of a type of collagen that attaches the epidermis to dermis. In this form of EB blistering appears between the epidermis and the dermis.

Genetics of EB

EB Simplex

Inheritance: EB simplex is inherited as an autosomal dominant (AD) trait. But it may also be inherited in an AR pattern also.

Incidence: 1 in about 35,000 to 50,000 individuals.

It is caused due to mutations in the KRT5 and KRT14 genes. These genes code for the proteins *Keratin* 5 and 14. Both of these proteins combine to form intermediate filaments in the basal keratinocytes.

The KRT5 gene is located on long arm of chromosome 12 at the locus 13 (12q13) while the KRT14 gene is located on the long arm of chromosome number 17 (17q12-q21).

Junctional EB

Inheritance: This syndrome is inherited as an autosomal recessive (AR) condition.

Incidence: It is rare in occurrence.

The disease is linked to mutations in the LAMB3, LAMA3, and LAMC2 genes. The LAMB3 gene locus is 1q32 and the LAMA3 gene is located at 18q11.2. The LAMC2 is situated on 1q25-q31. These genes encode the protein *leminin5* produced by basal keratinocytes. Some of the subtypes of this variety of EB may also be caused due to mutations in the ITGB6 and ITGB4 genes encoding the protein *integrin*. ITGB4 is situated at the locus17q11 and the ITGB- is located on chromosome 2. Another form of JEB may occur due to mutations in the BPAG2 gene that code for *collagen type XVII.* BPAG2 (COL17A1) is located on 10q24.3.

Dystrophic EB

Inheritance: It is mostly inherited as an AR trait but sometimes it may also be inherited as an AD entity. *Incidence*: 6.5 per million newborn.

Dystrophic form of EB results from mutations in the COL7A1 gene involved in the assembly of type VII collagen. Collagen VII forms anchoring fibers that anchor the epidermis with the dermis. Two layers of the skin get separated from each other in absence of anchoring fibers (due to mutation of COL7A1 gene) with formation of blisters.

The COL7A1 gene is located on long arm of chromosome number 3 at position 21 (3q21).

Risk of Dental Caries in EB

There is marked dental involvement with certain types of EB. Genetic defects related to the syndrome directly alter the hard tissue of the tooth and renders the tooth more susceptible to dental caries. EB-junctionalis and EB dystrophic syndromes are associated with increased incidences of dental caries. Various studies have shown that the DMFS (decayed, missing, filled surface) scores were always high in junctional EBs and dystrophic EB. Junctional EB is always associated with a generalized enamel hypoplasia. Thus the primary defect lies in the enamel that further predisposes it to risk of dental caries. In EB-junctionalis the enamel suffers greater porosity and thus it is imparted with an increased surface area for acids to work upon. Acid is generated by the cariogenic bacteria. The enamel also contains a large amount of serum albumin. Albumin inhibits crystal formation and remineralization of altered sites and felicitates tooth decay.

Though generalized enamel hypoplasia appears to be a feature limited to junctional EB, dystrophic forms of recessive EB have also been associated with dental caries.

Why is EB associated with enamel hypoplasia? Though the exact mechanism is not yet known to us, it appears that there is an altered relationship between the ameloblasts and the extracellular matrix of the developing enamel leading to a primary defect in the enamel hard tissue.

Treatment: At present there is no cure of EB. Care of pain, infection and other complications are the only lines of treatment available. Gene therapy seems to be a promising alternative.

THE SEARCH OF CANDIDATE GENE(S) FOR DENTAL CARIES

Very recently (postgenomic) quite a few studies have tried to locate the candidate gene for caries but with little success. A candidate gene is a gene known to be located in a region of interest in the genome. Product/s of the candidate gene has/have biochemical or other properties the presence or absence of which can be directly related to a disease. Findings form the following significant studies are as under:

- A study on mice indicated that major gene(s) responsible for the regulation of susceptibility to dental caries or resistance are located on chromosomes number 1, 2, 7 and 8 (Nariyama et al, 2004).
- Genome wide genotype data and DMFT scores in a large number of families were evaluated (Vieira et al, 2008). Low caries susceptibility loci were found on chromosomes number 5 (5q13.3), 14 (14q11.2) and X (Xq27.1). The high caries susceptibility genes were identified on chromosome number 13 (13q31.1) and 14 (14q24.3). The presence of genes for caries on the X chromosome may account for the sex differences observed in the incidence of caries. This study was the first of genome wide scans introduced for dental caries.
- In a recently concluded study single nucleotide polymorphism (SNP) assays were performed for 6 candidate genes. The candidate genes selected for this study were the amelogenin (AMELX), ameloblastin (AMBN), tuftelin (TUFT1), enamelin (ENAM), tuftelin-interacting protein (TFIP11) and kallikrein 4 (KLK4) genes. There were no significant associations concluded between single candidate genes and caries susceptibility. A significant interaction between tuftelin and *S. mutans* was however observed (Slayton et al, 2005).
- The Osteopontin (OPN) gene plays an important role in mineralization. In a recently conducted study OPN was chosen as candidate gene with respect to caries susceptibility as OPN gene was found to be associated with incidences of enamel hypoplasia in primary dentition. Results indicated an association between the OPN gene and caries in the primary dentition (Willing et al, 2006).
- A new study applied scanning of single-nucleotide polymorphism (SNP) markers with relation to selected candidate genes (*ameloblastin*, *amelogenin*, *enamelin*, *tuftelin-1* and *tuftelin interacting protein 11)* that influence enamel formation. One copy of a rare *amelogenin* allele was found to be associated with caries experience. This result suggested that variations in *amelogenin* may contribute to caries susceptibility (Deeley et al, 2008).

In conclusion one may say that the search for genetic variables in the etiology of dental caries has just begun.

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Chapter

14 Genetics of Periodontitis

- Familial aggregation of periodontal diseases
- Segregation analysis
- Twin studies
- Linkage studies for periodontitis
- Association studies for periodontitis
  - Association of HLA (human leukocyte antigens) with periodontitis
  - Association between periodontitis and interleukin-1 (IL-1) gene polymorphism
  - Tumor necrosis factor (TNF-α) and periodontitis
  - Association between neutrophil IgG receptor (FcγR) polymorphism and periodontitis
  - Association between IgG2 production and periodontitis
  - Association between polymorphism in matrix metalloproteinase, cathepsin C and vit D receptor with periodontitis
- Syndromic form of periodontitis
  - Neutrophil functional disorders
  - Deficiency in neutrophil number (neutropenias)
  - Genetic defects of structural components

Periodontitis is a dental disorder involving inflammation and infection of the ligaments and bones that support the teeth and results from progressive and uncontrolled gingivitis.

Classification of periodontitis is based on the rate of disease progression. Periodontitis can be divided into two major types, e.g. ***aggressive (Fig. 14.1)*** (localized aggressive and generalized aggressive) and ***chronic (Fig. 14.2)***.

Until recently, periodontitis was thought to be strictly determined by environmental factors like poor oral hygiene, smoking, low socioeconomic condition, bacterial infections of oral cavity, etc. alone. It is a common observation that given the same status of poor oral hygiene and sharing the same environmental factors, some people show severe periodontal disease while some of the same people suffer from mild or no disease. This indicates the existence of individual differences in susceptibility to the disease and perhaps towards a genetic basis for the susceptibility. Scientists are trying to find out the genes responsible for the disease. It is now well known that periodontal disease is multifactorial (complex) and its susceptibility is influenced both by genetic and environmental factors. Genes responsible for multifactorial disease are also known as susceptibility genes. However, these genes alone do not produce the disease unless they are exposed to the necessary environmental factors.

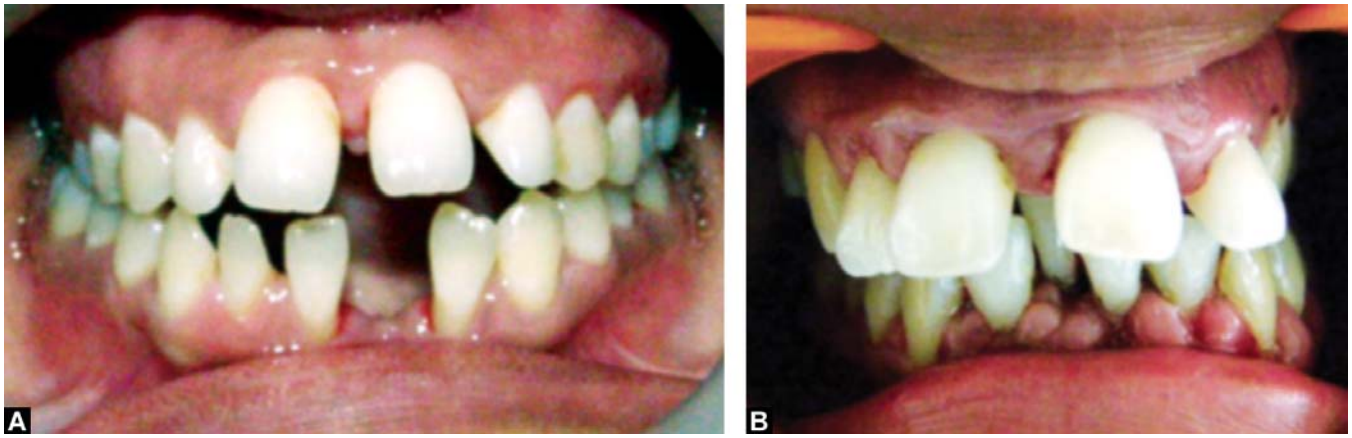
Periodontitis is also associated with many types of syndromes and genetic disorders. Periodontitis presents itself as one of the clinical manifestation of the syndrome. This establishes the genetic basis of periodontitis as these syndromes result due to mutation in a single gene/genes.

Verification of the Genetic Basis of Periodontitis

Following genetic analytical methods have been used to evaluate the genetic basis of periodontitis.

FAMILIAL AGGREGATION OF PERIODONTAL DISEASES

Several members of the same family suffering from periodontitis is indicative of a genetic etiology. Many scientific studies have indicated familial aggregation



FIGURES 14.1A and B: Patient showing localized aggressive (A) and generalized aggressive (B) periodontitis. (*Courtesy:* Dr Arvind Sahu, Dept of Periodontology, Modern Dental College and RC, Indore, India)

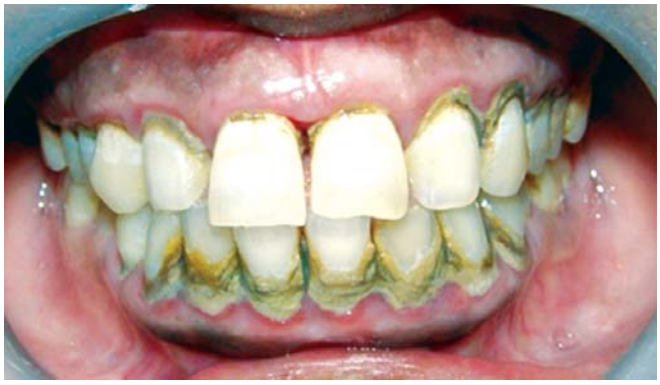


FIGURE 14.2: Patient showing chronic generalized periodontitis. (*Courtesy:* Dr Arvind Sahu, Dept of Periodontology, Modern Dental College and RC, Indore, India)

for both aggressive (Boughman et al., 1988; Long et al, 1987; Marazita et al, 1994 and Hart and Korman, 1997) and chronic periodontitis (Hassell and Harris, 1995). Reports of the familial nature of chronic forms of periodontitis are less frequent. This aggregation within families strongly suggests of a genetic predisposition to the disease. However, the familial aggregation of the disease may also reflect on the common environmental factors prevalent in the family, i.e. levels of oral hygiene, shared transmission of bacteria, same socioeconomic condition, sanitation, etc. Therefore familial aggregation is only suggestive of a genetic basis but does not prove a definite genetic etiology. To substantiate evidence in favor of a genetic basis of familial aggregation, application of more specific tools such as segregation and twin studies are imperative.

SEGREGATION ANALYSIS

Segregation analysis is conducted in affected families to determine the mode of inheritance of a disease whether it follows an autosomal-dominant, autosomal-recessive, X-linked dominant, X-linked recessive or multifactorial mode of inheritance.

Many studies have been conducted to determine the mode of inheritance of diseases. These studies have indicated many patterns of inheritance in periodontitis. The most definitive segregation analyses in North American families were performed by Marazita and coworkers (1994) who studied more than 100 families segregating aggressive forms of periodontitis and found support for an autosomal-dominant transmission. They concluded that autosomal-dominant inheritance with approximately 70% penetrance occurred for both Blacks and nonBlacks. It is believed that various forms of aggressive periodontitis are due to single gene defects that are inherited as autosomal-dominant disorders with incomplete penetrance. Some studies have indicated an autosomal recessive mode of inheritance (Saxen, 1980; Saxen and Nevanlinna, 1984). Few workers have also reported X-linked inheritance (Melnick et al, 1976) in the same context.

Why were these studies unable to point towards a single type of mode of inheritance? It might be because of the multifactorial or polygenic nature of the disease or probably due to the fact that the origin of periodontitis is heterogeneous in nature. There is a common precedent in genetics, with relation to heritable pathologic conditions, to show different modes of inheritance in different families. These findings reflect that different genetic loci are capable of causing the same disease in both dominant and recessive manners.

Even though segregation studies have shown different modes of inheritance, they all support the involvement of genes in the causation of the disease.

TWIN STUDIES

Many human traits are multifactorial (polygenic). The modes of inheritance in which can be manipulated with environmental modifications. Twin studies allow evaluation of the importance of genetic as well as environmental factors in causation of a disease. As early as in 1940, Noack recognized similarities of features in periodontal conditions seen in identical twins. Many twin studies in the past were carried out on monozygotic adult twin groups. These twins were grouped as the reared-together and reared-apart sections. Following conclusions were drawn that:

- The periodontal conditions in identical twins were similar.
- A significant genetic influence on various parameters of periodontal diseases was found.
- Findings in the reared-together and reared-apart groups were similar which strongly suggested a genetic basis of the disease. This finding also indicated that environmental factors had no significant influence on development of the disease.

A study by Corey et al (1993) on monozygotic and dizygotic twins indicated a higher concordance rate for monozygotic twins (23% for monozygotic twins as compared to 8% for dizygotic twins). Concordance implied that both the twins of a pair were suffering from periodontitis. This strongly suggested the involvement of a genetic component in the etiology of periodontitis as we know that monozygotic twins possessed identical genes. Michalowicz et al (1991) investigated alveolar bone height and probing depth in twins from the Minnesota study and showed significant variations in them, according to the differences in their genotype. The twin groups had similar smoking histories and oral hygiene practices. It was concluded that genetics plays a role in moulding the susceptibility to periodontal disease. In another study Michalowicz (1994) found that monozygotic twins reared either together or apart have been found to bear a more similar type of periodontal disease experience than found between dizygotic twins. These findings indicate that genetic factors may influence the manifestations of periodontal disease.

In a more recent study (Michalowicz et al, 2000) of another large human twin cohort, hereditary factors accounted for approximately 50% of "adult periodontitis". The heritable component for periodontitis was not associated or influenced by behaviors such as smoking, utilization of dental care, and oral hygiene habits. This indicated that development of periodontitis was influenced by genes that mediated biological mechanisms.

Twin studies were also used to know about the effect of host genes on the composition of microbacteria in the oral cavity. The long-term colonization of bacteria in the oral cavity may be due to the genetic constitution of the host. Studies have revealed that adolescent twins were more similar in their oral microbiota than pairs of unrelated individuals (Moore et al, 1993). However, the study of Michalowicz et al (1999) in adult twins indicated that neither the host genes nor the environment played a significant influence on the presence of bacteria in subgingival plaque. These two studies suggested that although the host genes influence initial bacterial colonization but this influences do not persist till adulthood.

LINKAGE STUDIES FOR PERIODONTITIS

Linkage studies are conducted to localize the disease causing gene on a specific chromosome. Till date only very few linkage studies analyzing aggressive periodontitis are reported.

- Saxen and Koskimies carried out the first linkage study in 1984 on Finnish families to test the claim for association of aggressive periodontitis with HLA antigens. This study concluded that the gene responsible for aggressive periodontitis was not linked with HLA antigen in these families.

- Boughman et al reported the second linkage study in 1986 for a Maryland population. According to this study the gene responsible for aggressive periodontitis was situated on the long arm of chromosome number 4 (4q 11-13). The inheritance pattern of this gene was determined as autosomal-dominant. The gene was found to cosegregate with the gene responsible for *dentinogenesis imperfecta*. Both the genes were linked, i.e. present close to each other on the long arm of chromosome number 4.
- However, another study in 1993 (Hart et al.) on a different population of USA failed to locate the gene for aggressive periodontitis on chromosome number 4. This finding indicated that aggressive periodontitis was heterogeneous in nature, i.e. different genes may be responsible for different forms of aggressive periodontitits.
- Prepubertal periodontitis (PPP) is a rare and rapidly progressive disease of young children that results in destruction of the periodontal support of the primary dentition. The condition may occur as part of a recognized syndrome or may occur as an isolated finding. Both autosomal dominant and recessive forms of Mendelian transmission have been reported for PPP. Hart et al. (2000) have localized a *gene of major effect* for PPP on chromosome 11q14. This PPP candidate interval overlaps the region of chromosome 11q14 that contains the cathepsin C gene responsible for Papillon-Lefèvre and Haim-Munk syndromes.
- Recently in 2004, a linkage study by Li and coworkers mapped the gene responsible for localized aggressive periodontitis on the long arm of chromosome number 1 (1q25).

ASSOCIATION STUDIES FOR PERIODONTITIS

The susceptibility to a disease depends upon the genetic background of a person. Since periodontitis is an inflammatory disease, it is believed to be affected by genes that are involved in the regulation of inflammatory cell functions. Associations between periodontitis and genes responsible for control of inflammation response are of great value in understanding the genetics of periodontitis. The immune system controls inflammatory responses against a disease. However, the immune response to a disease varies from person to person and the pattern of immune response in an individual is determined by the genetic make-up unique to the person. The clinical presentation of a disease, therefore, varies in different persons reflecting the diversity of immune response shown by each individual. The same concept is also applicable to developing specific and targeted medical treatment. Thus if we are able to identify the variations in the genes involved in the control of inflammatory process and ultimately the disease, we can successfully identify the degree of susceptibility, severity and prognosis of the disease and perhaps design medical interventions custom made according to a particular etiology. In recent past many studies have identified the association between periodontitis and variation in the genes (single nucleotide polymorphism) responsible for regulation of immune and inflammatory response.

Association of HLA (Human Leukocyte Antigens) with Periodontitis

The human leukocyte antigen (HLA) complex plays an important role in immune response. Many autoimmune diseases are found to be associated with various HLA antigens. Genes for class I and II antigens are located on chromosome number 6. At present more than 150 HLA antigens are known. The HLA molecules are involved in antigen recognition of periodontal pathogens, interaction between T and B-lymphocytes and in production of IgG. Following positive association between HLA and periodontitis was found:

- A positive association was reported between aggressive periodontitis and HLA—A9 and B15 antigens (Sofaer, 1990). Persons having these two antigens are at 3.5 times higher risk of developing the disease as compared to those who are negative for these antigens. Thus HLA -A9 and B15 seem to represent susceptibility factors for aggressive periodontitis.
- Class II DR4 antigen is in association with type I diabetes mellitus (Rotter et al, (1992). Since periodontitis is a diabetes related complication, DR4 antigen and periodontitis are said to be in association with each other. The DR4 antigens were found to be more prevalent in patients with aggressive periodontitis than in controls (Katz,

Goultschin, Benoliel et al., 1987). However, a few studies have been unable to find any association between the two.

- HLA-A2 and HLA-B5 antigens appear to be less prevalent in patients of aggressive periodontitis as compared to controls (Kaslick, West, Chasens et al, 1975). This indicates that these HLA antigens are protective in nature.

Though above studies have indicated a positive association, many other studies have also pointed towards a negative association between HLA and periodontitis. The cause of this discrepancy appears to be the involvement of environmental factors and racial differences acting as variables.

Association between Periodontitis and Interleukin-1 (IL-1) Gene Polymorphism

Interleukin-1 plays an important role in the initiation and progression of periodontal disease. Il-1 is found in two different forms; IL-1a and IL-1b. Genes for IL-1 are present on the long arm of chromosome number 2 (2q13). IL-1 is mainly produced by activated monocytes. There is an increased level of IL-1 in periodontal tissue which stimulates bone reabsorption, inhibits collagen synthesis, up regulates matrix metalloproteinase activity and synthesis of prostaglandin.

- An initial study (Korhman et al, 1997) reported a positive association between polymorphism in the genes encoding for IL-1 and IL-1B (composite genotype) and increased severity of periodontitis. This association was seen in nonsmokers. Genotype positive nonsmokers were 6.8 times more likely to have severe periodontal disease.
- Another study (Mc Guire and Nunn, 1999) reported that the IL-1 genotype increased the risk of tooth loss by 2.7 times and heavy smoking increased it by 2.9 times. The combined effect of heavy smoking and IL-1 genotype positive increased the risk of tooth loss for 7.7 times.
- A study carried out in 2001 by Axelsson indicated that genetic polymorphism of IL-1 and smoking seems to have synergistic risk factors. These factors when combined leads not only to tooth loss but also for alveolar bone loss. Nonsmokers are at low risk of tooth loss.

(*Thus there are contrasting findings regarding interactions between genetic polymorphism and smoking to affect the risk of periodontitis. Though the IL-1 genotype and smoking habit seem to interact with each other, the nature and direction of this interaction is poorly understood. More studies with large sample size are needed to evaluate the exact correlation between smoking and IL-1 interactions).*

- A variant of the IL-1B coding region with a single nucleotide base pair substitution is associated with a four-fold increase in IL-1B production.
- Generalized aggressive periodontitis is in linkage disequilibrium with an allele at IL-1B. This suggests that the gene for periodontitis is located close to the gene for IL-1B (Diehl et al, 1999).
- The IL-1B allele is found to be more prevalent in patients suffering from advance chronic periodontitis.
- Though the association between periodontitis and polymorphism of IL-1 gene cluster was observed in about 30% of European population, it was observed so only in 2% of Chinese population (Armitage, 2000).
- Many studies have indicated the lack of association between interlukin-1 polymorphism and aggressive periodontitis.

Where do we stand now? Studies in both chronic and aggressive periodontitis have yielded mixed results. It is evident that to get a clear picture of the association between IL-1 polymorphism and various types of periodontitis we need more comprehensive studies.

Tumor Necrosis Factor (TNF-α) and Periodontitis

The TNF-α is the proinflammatory cytokinase which is involved in pathogenesis of periodontitis. The TNF-α gene is located on chromosome number 6. Two different polymorphisms have been reported in the promoter region of the gene (Fassmann et al, 2003). This results in increased production of TNF-α. The level of TNF-α molecules is high at the sites of active tissue destruction and low at healthy sites.

Individuals expressing the TNF-α poly-morphisms manifest greater susceptibility to certain infections. Although till date TNF-α poly-morphism has not directly been linked to susceptibility to periodontitis, there is a strong possibility that such a link exists (Craandijk et al, 2004).

Association between Neutrophil IgG Receptor (FcγR) Polymorphism and Periodontitis

FcγRs are the group of receptors that are expressed on the cell surface of leukocytes binding to IgG antibodies. The interactions between FcγRs and IgG trigger a variety of immune responses like phagocytosis, endocytosis, antibody dependant cellular cytotoxicity and enhancement of antigen presentation.

Polymorphisms in the genes coding FcγRs (especially in FcγRII) receptors (Osborne et al, 1994) are common. The FcγRII receptor is the only receptor that recognizes bacteria opsonized with IgG2. These polymorphisms result in the expression of receptors of high, low and intermediate affinities. The low affinity receptors lead to a decreased immune function and subsequently to increased susceptibility to periodontal pathogen and to severe periodontitis even in presence of high level of antibodies.

Kobayashi et al (2000) found functional polymorphisms of IgG Fc receptors (FcγR) in Japanese patients with AgP. They found that FcγRIIIb (NA2 allele) was linked with the aggressive form of periodontitis and possibly also linked to an FcγRIIIa allele. Kobayashi et al (2001) reported that 2 FcγRIII alleles may be associated with the degree of severity in chronic periodontitis in a Japanese population. Meisel et al (2001) analyzed the association between FcγRIIIa (high-affinity receptor) and FcγRIIIb (low-affinity receptor) and chronic periodontitis (CP). Thus a large number of studies have recognized causal association between polymorphisms of FcγR genes and both aggressive and chronic periodontitic conditions. The alleles of FcγR gene are linked with genes causing periodontitis. The genetic poly-morphism of FcγRII receptor is a promising marker for the susceptibility of periodontitis.

Association between IgG2 Production and Periodontitis

Immunoglobulin G2 (IgG2) is produced in response to periodontal infections. The production of IgG2 is under the control of specific genes. The levels of production of IgG2 vary from person to person. The reduction in the production of IgG2 during the course of periodontal infection may lead to an increased susceptibility to disease. On the other hand an increase in the production of IgG2 provides sufficient protection against the disease. Marazita et al, (1994, 1996) studied families with aggressive periodontitis and found a major locus accounting for approximately 62% of the variance of IgG2 production.

Patients with periodontitis and normal subjects vary greatly in their capacity to produce IgG2. Patients with high titers of IgG2 antibodies have significantly less attachment loss than do patients with low titers. It is observed that patients with localized aggressive periodontitis have high titers of IgG2 compared to patients with generalized periodontitis. This indicates that IgG2 provides sufficient protection against the spread of disease and tries to limit the same.

Associations between Polymorphism in Matrix Metalloproteinase, Cathepsin C and Vit D Receptor with Periodontitis

The matrix metalloproteinase (MMP) are enzymes involved in connective tissue destruction. Single nucleotide polymorphisms are observed in MMP genes and are designated MMP-1, MMP-2, MMP-3 polymorphisms. Very few studies have evaluated the correlation between MMP polymorphisms and periodontitis (Holla et al 2005; Itagaki et al, 2004).

A recent study has indicated a decreased level of Cathepsin C activity in chronic periodontitis. Cathepsin C is a lysosomal enzyme that plays an essential role in immune and inflammatory processes and hence may play an important role in periodontitis (Hewitt et al, 2004). A mutation of the cathepsin C gene leads to ***Papillon-Lefvre syndrome*** (see later).

Polymorphism in Vit D receptor has been associated with aggressive periodontitis. A very few studies, though, have verified their association.

SYNDROMIC FORM OF PERIODONTITIS

It has been observed that there is a clear association between some type of genetically determined syndromes/disorders and periodontitis. These syndromes increase the susceptibility of the individual to periodontitis by interfering with the structural integrity of periodontal tissue or periodontitis may be a concomitant feature of the syndrome. These syndromes are due to mutations in a single gene (monogenic or Mendelian syndromes). Transmission of the disease from one generation to the next follows

Mendelian patterns of inheritance as autosomal-dominant (AD), autosomal-recessive (AR) or X-linked dominant or recessive (XLD or XLR) traits.

As stated above, periodontitis presents itself as a part of a wider spectrum of clinical manifestations of a number of syndromes that occur as a result of mutations in genes. This itself points towards the clearest and most direct clinical evidence that periodontitis has strong genetic etiology. Following is a brief description of some of the genetic disorders where periodontitis is observed as a clinical manifestation.

1. Neutrophil Functional Disorders

(a) Leukocyte Adhesion Deficiency Syndromes (LAD Syndromes)

Polymorphonuclear leukocytes play an important role in the restriction of the bacterial infection. There are several adhesion receptors on the surface of polymorphonuclear leukocytes. Adhesion receptors are necessary for the proper functioning of leukocytes (phagocytosis and chemotaxis). If the circulating leukocytes have defective or reduced adhesion surface receptors they will not adhere to vascular endothelium. As a result leukocytes would not be accumulated at the site of inflammation where they are needed to combat the infection. This leads to an increased susceptibility to an infectious disease as exemplified by the *leukocyte adhesion deficiency syndrome* (LADS). Recurrent bacterial infections, impaired pus formation and impaired wound healing clinically characterize the syndrome. A number of these infections are associated with increased susceptibility to periodontitis. LAD occurs in two forms, LAD syndrome type I and LAD syndrome type II.

Inheritance: Both types of LAD syndromes are inherited as AR.

Mutation in LAD type I: The mutation occurs in the B2 integrin chain (ITGB2) gene –21q22.3 (Arnaout et al, 1990). At present more than 20 mutations of the integrin B2 gene are known. The gene mutation causes defects in the integrin receptors of leukocytes. This mutation leads to defects in cell adhesion and chemotaxis ultimately resulting in increased susceptibility to severe infections including prepubertal aggressive periodontitis.

Mutation in LAD type II: This is a rare variety of the LAD syndrome and is usually associated with psychomotor retardation (mental retardation, short height and recurrent infection). The syndrome is caused due to a mutation in the *guanosine 5′-diphosphate- fucose transporter 1* (SLC 35C1) gene (Lowe et al, 1990; Ishikawa et al, 2005). The infectious episodes and severity of disease is much milder as compared to LAD type I. This syndrome results in chronic periodontitis.

(b) Chédiak-Higashi Syndrome

The syndrome is clinically characterized by a decreased pigmentation of eyes and hair, photophobia, nystagmus and susceptibility to infection.

Inheritance: This syndrome is inherited as an autosomal recessive (AR) trait.

Mutation: The mutation occurs in lysosomal trafficking regulator (LYST) gene –1q42.1-q42.2 (Nagle et al, 1996). This leads to an abnormal transport of vesicles to and from neutrophil lysosomes or to a defect in the ability of cells to produce lysosomes (Charette et al, 2007). Chédiak–Higashi syndrome is associated with severe periodontitis unresponsive to conventional periodontal treatment. However, periodontitis is only seen in its severe form with the syndrome.

2. Deficiency in Neutrophil Number (Neutropenias)

Neutropenia is defined as an abnormally low number of circulating neutrophils. The condition is associated with increased susceptibility to infections such as aggressive periodontitis. Table 14.1- includes various types of neutropenias and associated gene mutations in brief.

3. Genetic Defects of Structural Components

(a) Papillion-Lefèvre Syndrome (PLS)

This syndrome is characterized by severe early onset periodontitis, which affects both primary and permanent dentitions and hyperkeratosis of palmar and plantar surfaces.

Inheritance: Autosomal recessive inheritance (AR).

Mutation: It is due to mutations in the cathepsin C gene which is located on chromosome 11 (11q14-q21). At present more than 50 mutations have been identified in cathepsin C gene. Cathepsin C is a cysteine protease that plays a role in degrading proteins and activating proenzymes in immune and inflammatory cells. Inactivation of cathepsin C results in failure to cleave and activate the neutrophil serine proteases cathepsin G, neutrophil elastase and proteinase 3 (Pham et al, 2004).

It was observed that in some of the PLS patients aggressive periodontitis were associated with a kind of virulent microorganism. Elimination of this microorganism prevented the periodontal destruction. This suggested that the periodontitis was not a direct effect of mutation of the gene but the gene mutation increased the susceptibility of a person to infection by the specific microorganism instead.

(b) Ehlers-Danlos Syndrome (EDS)

This syndrome is associated with connective tissue disorders. EDS is characterized by defective formation of collagen fibers. The abnormal collagen in the patient leads to fragility and hyperextensibility of the skin, hypermobility of joints, easy bruising and is also associated with early onset of periodontitis. Though at least 17 different types of EDS have been reported but early onset periodontitis has been associated only with two of its subtypes: EDS type IV and EDS type VIII.

Inheritance: Both the types IV and type VIII EDS are inherited as autosomal-dominant (AD) conditions.

Mutation: The mutation leading to the type IV EDS was found to be linked to the gene responsible for the synthesis of type III (COL 3A1) collagen (Superti-Furga et al, 1988). This leads to the defect in synthesis of collagen III resulting in decreased levels of collagen III.

TABLE 14.1: Summary of Genetic disorders/syndromes associated with periodontitis

Disorders/Syndromes	*Inheritance*	*Gene*	*Functions of normal gene*
1. Severe congenital neutropenia type I	AD	Neutrophil elastase gene(ELA2) - 19p13.3	The products of elastase gene (elastase) degrade membrane protein A of bacterial cell wall.
2. Severe congenital neutropenia Type II	AD	Growth factor independent gene (GFI 1) - 1p22	GFI 1 gene function to replaceELA2
3. Severe congenital neutropenia type III	AR	HCLS1 associated protein X1 (HAX1) -1q21.3	Controls the development of neutrophils.
4. Severe congenital neutropenia	AD	Granulocyte colony stimulating factor gene (G-CSF) - 17q11.2-q12	Stimulation of granulocyte colony
5. Leukocyte adhesion deficiency (LAD) syndrome type I	AR	Integrin B2 (ITGB2) –21q22.3	Adhesion and chemotaxis
6. LAD syndrome type II	AR	Solute carrier family 35 member C1 (SLC35C1) 11p11.2	Secrete guanosine 5'-diphosphate fucose transporter-1
7. Chediak-Higashi syndrome (CHS)	AR	Lysosomal trafficking regulator gene (LYST)-1q42.1 – q42.2	Transport of vesicles to and from neutrophil lysosome.
8. Papillion-Lefevere syndrome	AR	Cathepsin C gene -11q14-q21	Degrading proteins and activation pro-enzymes in immune cells.
9. Ehlers-Danlos syndrome (EDS) type IV	AD	Type III collagen gene (COL 3A1)- 2q31	Synthesis of type III collagen
10. EDS type VIII	AD	Ehlers-Danlos syndrome8 (EDS 8) 12p13	Unknown. Defect in collagen 1?
11. Phosphatasia	AD/AR	Alkaline phosphate liver/bone/kidney (ALPL) -1p36.1-p34	Maintains normal level of alkaline phosphate
12. Kindler syndrome	AR	Kindlin 1 (KIND 1)- 20p13	Cell to cell contact

Mutations causing type VIII EDS, though, have not yet been identified. This disease has been, nevertheless, linked to chromosome number 12 (12p13). The association of early onset periodontitis with type VIII EDS is more common as compared to that of type III.

(c) Hypophosphatasia

These patients have decreased levels of alkaline phosphatase in the serum. The condition is associated with abnormal bone mineralization, skeletal abnormalities and cementum hypoplasia. These patients present a severe loss of alveolar bone and premature loss of primary teeth. Pulp chambers may also get enlarged. Lack of connective tissue attachment to bone is responsible for early exfoliation of the primary teeth. Hypophosphatasia may be considered as an etiology of aggressive periodontitis. The condition leads to premature loss of primary teeth and occasionally permanent teeth**.**

(Mode of) inheritance: Autosomal dominant or recessive.

Mutation: Mutation occurs in the ALPL (alkaline phosphatase, liver/kidney/bone) gene. The gene is localized on the short arm of chromosome 1 (1p36.1-p34). Mutations result in abnormal production of alkaline phosphatase leading to poor mineralization of tissues.

(d) Kindler Syndrome

It is caused due to a mutation in the Kinderlin gene (KIND1) (Jobard et al, 2003). The gene is expressed in many tissues including epidermal keratinocytes. In skin it plays a role in cell adhesion process. Patients of Kindler syndrome show multiple dermatological findings (hyperkeratosis, pigmentation, eczema, skin fragility, etc.), the syndrome is associated with severe aggressive periodontitis both in the primary and secondary dentition.

(Mode of) inheritance: Autosomal recessive (AR) in nature.

Mutation: Mutation occurs in the Kindlin gene (KIND1) situated on the short arm of chromosome number 20 (20p13).

From the above description one can conclude that isolated periodontitits is clearly behaves as a multifactorial disease. Researchers have shown associations between complex form of periodontitis and abnormalities in genes (single nucleotide polymorphism) responsible for regulation of immune and inflammatory responses. Further research is needed to examine the role of both environmental and genetic factors in the causation of periodontitis. We have also seen that monogenic (single gene) inheritance of periodontitis is possible but these incidences are associated with certain syndromes. We have successfully located many mutant genes (refer table) and understood to some extent the mechanisms of these mutations that disrupt normal homeostasis in the periodontum.

Though a large number of studies are being conducted to identify the genetic basis of periodontitis, we are still far away from determining the risk factors, prevention and treatment of aggressive and chronic periodontitis, genetically.

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Chapter

15

Genetics of Malocclusion

- Family and twin studies
- Mode of inheritance of class III malocclusion multifactorial or autosomal-dominant?
- Malocclusion associated with syndromes
- Malocclusion and dental anthropology
- Human evolution and malocclusion
- Linkage studies

Occlusion means alignment of the upper and lower teeth together. Normally all upper teeth fit slightly over the lower teeth. The upper teeth keep the cheeks and lips from being bitten and the lower teeth protect the tongue. Malocclusion of teeth denotes improper or misalignment of teeth. The maxillary and mandibular teeth are incorrectly positioned with relation to each other. Malocclusion also occurs because of altered relation between the upper and lower jaws.

Classification of Malocclusion

- ***Class I malocclusion*** is the most common variety of malocclusion. The bite is normal as per the permanent Ist molar relationship but the teeth are crowded or not positioned correctly. The upper teeth slightly overlap the lower teeth (Fig. 15.1).
- ***Class II malocclusion*** is also called as retrognathism. It occurs when the upper jaw/upper teeth are forwardly placed (lower teeth/lower jaw are placed distally) (Fig. 15.2).
- ***Class III malocclusion*** is also called prognathism. It occurs when the lower jaw protrudes or just forward causing the lower jaw and teeth to overlap the upper jaw and teeth from beneath the upper jaw (Fig. 15.3).

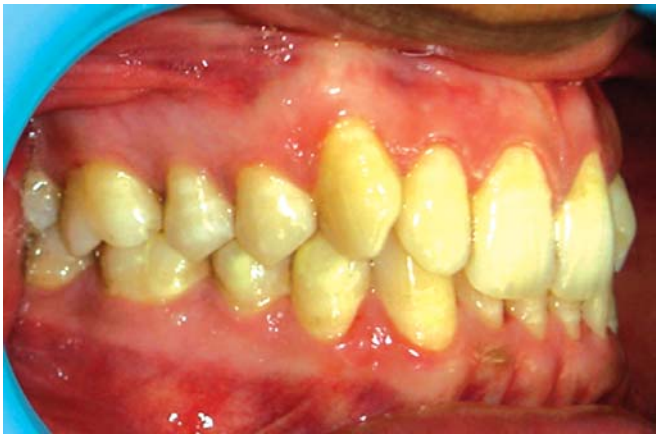


FIGURE 15.1: Class I malocclusion. *Courtesy* Prof PG Makhija, Dept of Orthodontics, Modern Dental College and RC, Indore, India

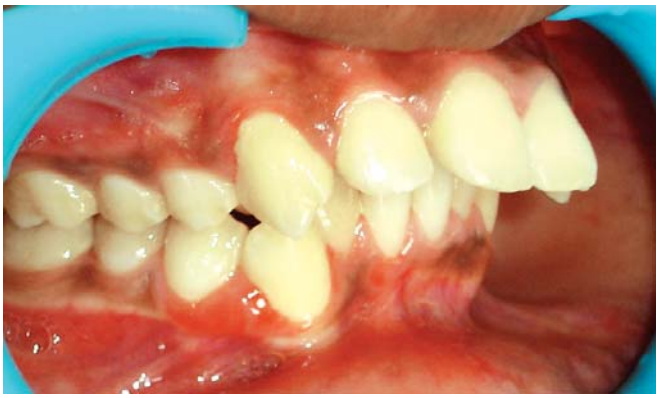


FIGURE 15.2: Patient showing Class II malocclusion. *Courtesy* Prof PG Makhija, Dept of Orthodontics, Modern Dental College and RC, Indore, India

Causes

Malocclusion is caused due to:

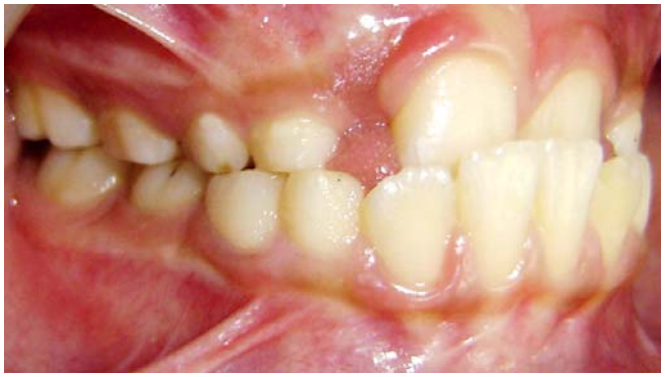


FIGURE 15.3: Patient showing Class III malocclusion. *Courtesy* Prof PG Makhija, Dept of Orthodontics, Modern Dental College and RC, Indore, India

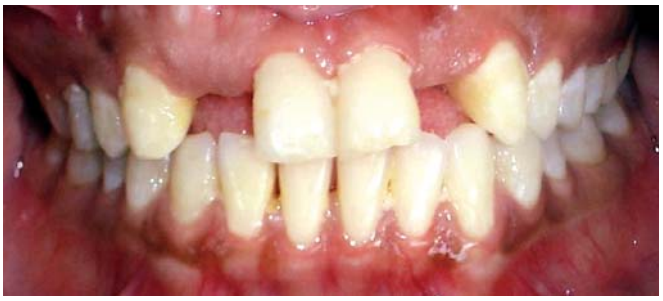


FIGURE 15.4: Malocclusion may also be associated with partial anodontia. Note the absence of maxillary lateral incisors associated with Class I malocclusion. *Courtesy* Prof PG Makhija, Dept of Orthodontics, Modern Dental College and RC, Indore, India

Acquired Factors

- Alteration in the shape or size of jaws; if teeth in a small jaw jostle for space and grow crowded in small area pushing each other.
- Alteration in the shape or size of teeth.
- Tooth loss.
- Thumb or finger sucking, use of pacifier and mouth breathing (due to enlargement of tonsils) and tongue thrusting.

Genetic Factors

Inherited conditions include:

- Inheritance of too many or too few teeth.
- Inheritance of too much or too little space between teeth.
- Inheritance of irregular mouth and jaw size and shape.
- Abnormal formations of the jaws and face, e.g. cleft palate.

The etiology of malocclusion is a complex subject and not fully understood. The above brief description of etiology indicates that the bony factors (size and shape of maxillary and mandibular arches) and dental factors (size and shape of teeth, failure of eruption (Fig. 15.4), supernumerary teeth and early loss of teeth) can be determined by both environmental and genetic factors. For example, the failure of eruption of upper lateral incisor may be due to acquired as well as genetic causes. The presence of supernumerary teeth may lead to the failure of eruption of incisor. Supernumerary tooth again may feature due to an inheritance (as supernumerary tooth may also be present in a parent of the patient). On the other hand failure of eruption of central incisor may be also due to early loss of many deciduous teeth (due to caries) that leads to forward drift of first permanent molar teeth resulting in the crowding of teeth. Thus it sometimes becomes difficult to assess the clear-cut role of acquired and genetic factors in the causation of malocclusion as there is a complicated interplay between various factors.

Malocclusion may result due to inheritance of disproportionate size of the teeth and jaw, resulting in crowding or spacing of teeth with a small jaw leading to crowding and large jaw giving abnormal spacing between teeth. The other important factor leading to malocclusion is the disproportion between the size and shape of upper and lower jaws. These skeletal variables are primarily genetic in nature. It should be noted that genetic influence on the shape and size of jaws is not due to a single gene defect but is mostly determined by the additive effects of many genes (i.e. polygenic in nature). The environmental factors play an influential role on shaping of the genetic factors. Thus, the etiology of malocclusion is mostly multifactorial in nature (due to interaction between genetic and environmental factors). Though it is simple to analyze the genetics of a single gene (Mendelian trait), analysis of multifactorial traits (cleft palate, cleft lip, caries, periodontitis and malocclusion) are difficult to analyze. For the analysis of the multifactorial inheritance one has to evaluate the role of genetic as well as environmental factors and the interaction between the two. The time-tested methods to analyze the role of genetic and environmental factors for a particular multifactorial disease in humans are familial and twin studies.

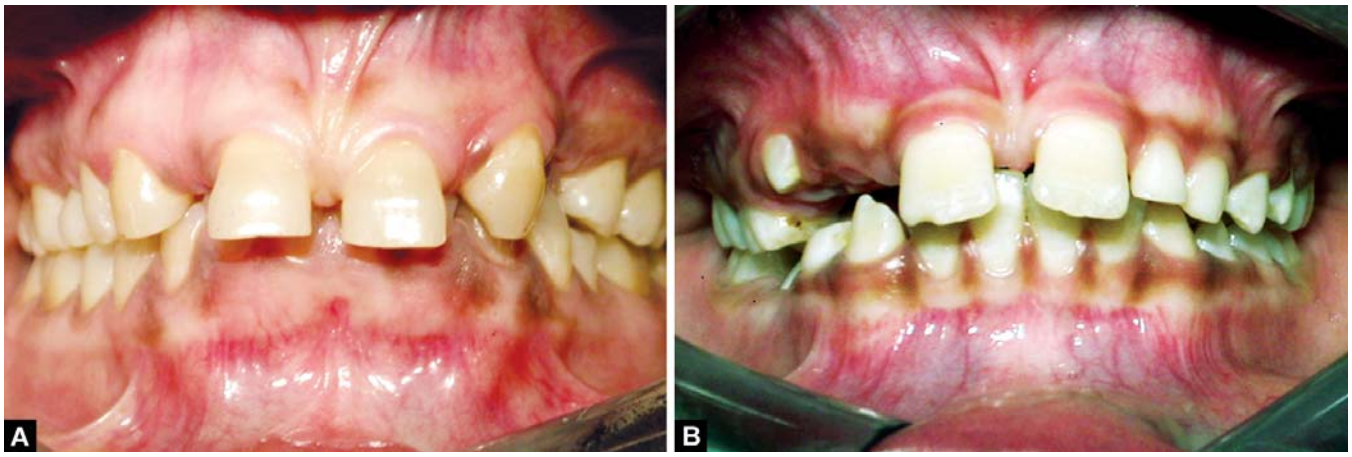
FAMILY AND TWIN STUDIES

In familial studies one has to observe the similarities as well as differences between the mother and the child (Figs 15.5A and B), father and the child and between sibling pairs. Correlation coefficients of the trait are obtained between parents and offspring or between sibling pairs and half sibs. For most measurements of facial skeletal dimensions correlation coefficients for parent-child pairs are about 0.5 which is the upper limit of correlation between first-degree relatives. The correlations for parent-child in relation the dental characteristics range from 0.5 to 0.15. This is also reflected in the Harris and Johnson (1991) study which revealed high heritability of craniofacial (skeletal) characteristics and low heritability of dental characteristics. This indicated that malocclusion resulted mainly from facial skeleton deformities that could be inherited while pure dental variations were due to environmental factors. Many other family studies have indicated the role of heredity in determination of the craniofacial and dental morphology (Korkhaus, 1930; Rubbrecht, 1930; Trauner, 1968 and Peck et al, 1998).

As stated earlier twin studies are useful in identifying the genetic and environmental factors determining multifactorial traits. Differences in features between monozygotic twin pairs implicate environmental factors while similarities points towards genetic influences as the primary causal factors of the disease. The similarities in disease features between dizygotic twin pairs are thought to result from environmental influences and genetic factors. A comparison of the differences observed within twin pairs in the two categories should provide a measure of the degree to which monozygotic twins are more alike to each other than dizygotic twins between themselves in a pair. Studies with twins reared apart are more useful as compared to twins reared together in a common environment as these studies overcome the problems of twins displaying similarities because of being reared in a common environment.

Following is a brief review of family and twin studies in relation to the heritability of craniofacial and dental characteristics. Most of the familial and twin studies indicated that heredity plays a significant role in the etiology of malocclusion.

Lundstrom (1948) reported that characteristics like width and length of dental arch, height of palate, spacing and crowding of teeth, tooth size and degree of overbite are genetically determined. A study on triplets by Kraus et al (1959) investigated the cephalometric parameters and concluded that the morphology of craniofacial bones are under strict genetic control. Nevertheless, environment plays a major role in determining how these bony elements combine with each other to produce normal occlusion or malocclusion. This observation explains why sometimes differences are observed between a pair of monozygotic twins in spite of having the same genetic constitution. Markovic (1992) conducted a cephalometric twin study and concluded that 100% of monozygotic twin pairs demonstrated concordance for malocclusion while 90% of dizygotic twin pairs were



FIGURES 15.5A and B: See the similarity of malocclusion (Deep bite) between mother (A) and teenage daughter (B). *Courtesy* Prof PG Makhija, Dept of Orthodontics, Modern Dental College and R C, Indore, India

discordant. This is strong evidence in support of genetic etiology of malocclusion.

Schulze and Weise (1965) reported the polygenic nature of mandibular prognathism (class III malocclusion). They found that the concordance in monozgotic twin pairs was very high as compared to dizygotic twins. Litton et al (1970) also investigated the polygenic nature of class III malocclusion (mandibular prognathism). The Harris (1975) study indicated that craniofacial skeletal patterns of children with class II malocclusion were heritable. In a large familial group Nakasima et al (1982) studied the inheritance of class II and class III malocclusion. High correlation coefficient values were seen between parents and their offspring in the class II and class III groups. Thus, there appears to be a strong familial tendency in the development of class II and class III malocclusions. They concluded that the hereditary pattern must be taken into consideration in the diagnosis and treatment of patients with these classes of malocclusion.

Environmental factors have also been suggested as contributory to the development of mandibular prognathism. Among these are enlarged tonsils (Angle, 1907), nasal blockage (Davidov et al, 1961), congenital anatomic defects (Monteleone and Davigneaud, 1963), hormonal disturbances (Pascoe et al, 1960), endocrine imbalances (Downs, 1928), posture (Gold, 1949) and trauma/disease including premature loss of the first permanent molars (Gold, 1949).

Development of both the maxillary and mandibular arch shapes in an Australian twin study was found to be under genetic influence. However, authors (Richards et al, 1990) also have reported the existence of some independent variables that determine the shape of mandibular and maxillary archs. Hughes et al (2001) quantified the extent of variations in different occlusal features in Australian children of European descent with complete primary dentitions but no permanent teeth present in the mouth. Occlusal traits including interdental spacing, incisoral overbite and overjet, arch breadth and arch depth were studied. Estimates for overbite and overjet were 0.53 and 0.28 respectively and estimates for arch dimensions ranged from 0.69 to 0.89. These results indicated a moderate to relatively high genetic contribution to the observed variations. The aim of a study by Eguchi et al (2004) was to quantify the relative contributions of genetic and environmental factors to variations in dental arch breadth, length and palatal height in a sample of Australian twins. The study brought forth information that the heritability load for dental arch breadth ranged from 0.49 to 0.92, those for the arch length from 0.86 to 0.94 and those for palatal height were 0.80 and 0.81 respectively. These results indicate a high genetic contribution to the variation in dental arch dimensions in mainly teenage twins.

A twin study by Corruccini et al (1980) indicated the importance of environmental factors in the etiology of malocclusion. The researchers showed that heritability of overjet is almost zero. The study on Australian twins conducted by Townsend et al (1988) for occlusal variations has shown the importance of environmental influences in shaping overjet. The cross bite was found to be determined by environmental factors. They also indicated that genetics may perhaps play an important role for overjet, a lesser role in overbite and least involved in determining molar relationship.

The masseter muscle electrical activity and its morphology were analyzed in twin studies and it was found that both its activity and morphology were genetically influenced. These twin studies revealed for the first time that soft tissue function and morphology could also be inherited (Lauweryns et al, 1992 and 1995).

A study by Dempsey et al (1996) on tooth size in Australian twins indicated a relatively strong genetic and environmental influence on the development of the canines and first premolars. The findings of the canine and first premolar mesiodistal dimensions support the evolutionary theory that indicates the presence of dominance variation in morphological features that have been subjected to strong selective pressure in the past.

Twin studies have shown that tooth crown dimensions are strongly determined by heredity (Osborne et al, 1958). The molecular genetics of tooth morphogenesis with the homeostatic MSX1 and MSX2 genes have been linked to stability in dental patterning. It has been reported that inheritance of tooth size fits the polygenic multifactorial threshold model.

Markovic (1982) found a high rate of concordance for hypodontia in monozygous twin pairs while he observed discordance patterns in dizygous twin pairs. This and other previous studies concluded that a single autosomal dominant gene with incomplete penetrance could explain such a mode of transmission.

Niswander and Sugaku (1963) analyzed data from family studies and have suggested that the development of *supernumerary* teeth, most frequently seen in the premaxillary region, appears to be genetically determined. The etiology of ectopic canines is controversial with divergent opinions regarding its genetic or environmental mechanism of occurrence. Mossey et al, 1994 indicated existence of an association in inheritance between ectopic maxillary canines and class II malocclusion demonstrating the involvement of genetic factors in the inheritance of the trait. The study of Camilleri et al (2008) addressed the hypothesis that genetic factors play a role in the etiology of ectopic maxillary canines. Sixty-three probands were identified and information on the dental status of 395 of their relatives was determined. Only two of seven pairs of monozygotic twins were concordant for ectopic canines. This is consistent with environmental or epigenetic variables affecting the phenotype. The low concordance rate is consistent with the low penetrance determined by segregation analysis studies further supporting the existence of environmental etiological factors.

Environmental influences during the growth and development of face, jaws and teeth are mainly exerted through pressures and forces acting on these structures. Mossey (1999) states, "*it is difficult to determine the precise contribution from hereditary and environmental factors in a particular case. For example, the simultaneous appearance of proclined maxillary incisors and digit sucking may lead to the assumption that the digit was the sole causative factor, but the effect of the digit may very well be either potentiated or mitigated by other morphological or behavioral features in that particular individual. A similar argument may apply in cases of mouth breathing where the influence of the habit and associated posture is very much dependent on the genetically determined craniofacial morphology on which it is superimposed, and the reason for the habit developing may well be dependent on the morphology in the first place. These scenarios are classical examples of the interaction of genotype and environment, and ultimately success of treatment will depend on the ability to ascertain the relative contribution of each.*"

MODE OF INHERITANCE OF CLASS III MALOCCLUSION – MULTIFACTORIAL OR AUTOSOMAL-DOMINANT?

Mandibular prognathism typically shows familial aggregation. Various genetic models have been described in relation to its inheritance and it is assumed to be a multifactorial and polygenic trait exhibiting a threshold for expression.

Many familial studies carried out in the first half of the twentieth century (Castro, 1928; Keeler, 1935; Moore and Hughes 1942; Gottlieb and Gottlieb, 1954) suggested that class III malocclusion is inherited in an autosomal-dominant or autosomal-recessive pattern with variable expressivity and penetrance. Strohmayer (1937) studied several generations of Hungarian and Austrian royal families for Hapsburg jaw. He concluded that mandibular prognathism was transmitted as an autosomal trait. However, more recent studies revealed the mode of its inheritance as multifactorial. The Hapsburg jaw, as seen in many generations, may have been caused by small number of segregating major genes.

Litton et al (1970) carried out a familial study and reviewed the literature to analyze the mode of inheritance of the disorder. They ruled out the possibility of an autosomal-dominant or autosomal-recessive inheritance. The study also indicated that the inheritance was not sex-linked as males and females both had malocclusions in almost equal proportions. They proposed a multifactorial polygenic model of inheritance for class III malocclusion. However, two recently concluded studies reported that class III malocclusion is inherited as an autosomal dominant trait. A study by El-Gheriani et al (2003) performed segregation analysis on 37 families of patients of mandibular prognathism. Segregation analysis of a prognathic mandible in the entire dataset supported a transmissible Mendelian major effect with a dominant mode of inheritance. Similarly, a genome wide scan of familial class III dentofacial deformity by Frazier-Bowers et al (2007) revealed that the broad class III phenotype is inherited as an autosomal dominant trait. Another recent study conducted by Richard M Cruz and colleagues (2007) indicated the mode of inheritance as autosomal-dominant with incomplete penetrance. They conclude "there is a major gene that influences the expression of mandibular prognathism with clear signs of Mendelian inheritance and a multifactorial component".

Thus, even after a century, many familial segregational studies conducted to determine the inheritance of class III malocclusion could not resolve the controversy yet.

MALOCCLUSION ASSOCIATED WITH SYNDROMES

1. ***Associated with Facial Asymmetry***
   Hemifacial microstomia
   Neurofibromatosis
2. ***Associated with Mandibular Prognathism***
   Gorlin's syndrome (Basal cell nevus syndrome)
   Klinefelter syndrome
   Marfan syndrome
   Osteogenesis imperfecta
   Warden burg syndrome
   Crouzon syndrome (Fig. 15.6)

FIGURE 15.6: An eleven years old boy with Crouzon syndrome. Note the mandibular prognathism. *Courtesy* of Dr S Dilliwal Dept of Orthodontics, Modern Dental College and R C, Indore, India

3. ***Associated with Mandibular Deficiency***
   Hemifacial Microstomia (Goldenhar Syndrome)
   Treacher Collins syndrome
   Pierre Robin anomalad

MALOCCLUSION AND DENTAL ANTHROPOLOGY

In the past anthropological observations have suggested that endogamous populations (isolated populations/primitive populations) have homogenous genetic constitution and this kind of population show normal occlusion. If everyone in the population carried the same genes for tooth size and jaw size, there would be no possibility of a child inheriting malocclusion. This is supported by the fact that the pure racial populations (like Melanesians) do not show malocclusion. However, it has been debated whether this is due to the lack of admixture with other population or as a result of a less refined diet often eaten by the isolated groups. The primitive human populations use tough diet that produces good masticatory function.

Anthropologists also observed that racial admixture increases the occurrence of malocclusion. The increase in the incidence of malocclusion in the modern human populations is claimed to be due to increase in out-breeding associated with acquired mobility of various populations in recent centuries. This notion was supported by the work of Stockard and Johnson (1941) in which deformities in the jaws of dogs were produced by cross breeding different inbred strains. These experiments seemed to confirm that independent inheritance of facial characteristics could be a major cause of malocclusion and that rapid increase in cases of malocclusion accompanying urbanization was probably the result of increased out-breeding. Stockard was severely criticized later by Proffit (1986) for his interpretation of the experimental results. He noted that the Stockard's results reflected segregation of the genes for achondroplasia which is present in small dogs but rare in humans. Animals or humans affected by this condition have deficient growth of cartilage. As a consequence individuals have short height and underdeveloped midface. Thus malocclusion produced in the dogs was not due to inherited jaw size but because of the expression of achondroplasia. Hence, extrapolation of the Stockard results on human population was thought to be invalid. The study of Chung and colleagues (1971) tested the hypothesis of admixture in a Hawaiian population group and showed that there was no increased risk associated due to an admixture in human populations. Before the 18th century, the Hawaii islands had a homogenous Polynesian population. Later the Chinese, Japanese and European groups migrated to the island that resulted in a heterogeneous mixture of modern population. It was observed that the out-breeding in this modern

population has not shown any significant rise in malocclusion or dramatic facial deformities like those seen in crossbred dogs. Chung's study concludes that if malocclusion is inherited, the mechanism is not just an independent mode of inheritance of malocclusion as a trait but it is inherited as a group of discrete morphological characteristics like tooth and jaw sizes.

HUMAN EVOLUTION AND MALOCCLUSION

Crowding and malalignment of teeth have been also recorded in specimens from the prehistoric period. However, since then there has always been an increase in the incidence of malocclusion. The frequency of malocclusion is definitely much higher in modern population as compared to the distant past. The reasons (genetic or nongenetic) for this are not fully understood. One explanation given for this is the adoption of modern industrialized life styles. The change from a more tough diet to soft cooked diet is an environmental factor responsible for increasing incidence of malocclusion in the modern day populations. Corruccini and Townsend (1990) reported that occlusal variation increased significantly in the Yuendumu people within one generation after adopting of a more westernized diet. It was observed that the vigorous masticatory function in aboriginals leads to alveolar bone development and tooth migration thus providing space for easy eruption and alignment of permanent teeth. As these vigorous masticatory functions are lacking in modern population individuals, their dental arches are crowded. The above discussion indicates that the heritability of maxillary arch size variability is low in the aboriginals. The arch size variability is mainly a function of environmental factors.

The following statement is based on Butler's field theory -*"As dietary habits in humans adapt from a hunter/gatherer to a defined food culture, evolutionary selection pressures are tending to reduce tooth volume, which is manifest in the third molar, second premolar and lateral incisor."*

LINKAGE STUDIES

Linkage studies identifying chromosomes or gene(s) responsible for craniofacial and dental variables of malocclusion in humans are very limited.

- The role of X and Y-chromosomes on craniofacial morphology has been investigated by Gorlin et al (1965). X and Y-chromosomes exert growth-promoting effects on human tooth crown size. The X-chromosome appears to regulate mainly enamel thickness while Y-chromosome affects both enamel and the dentine. Amelogenin is a matrix protein secreted by ameloblasts and it is thought to direct the growth of hydroxyapatite crystals. The gene for amelogenin is located on X and Y-chromosomes (Lau et al, 1989). The amelogenin gene is located on the distal portion of short arm of X-chromosome and to the pericentromeric region of Y-chromosome. The gene on the X-chromosome is the predominantly functional one. Its mutation leads to amelogenesis imperfecta. This dental deformity (amelogenesis imperfecta) is associated with malocclusion (vide infra).
- Mutation in the novel enamelin gene (ENAM) is responsible for autosomal recessive amelogenesis imperfecta and localized enamel defects. It was discovered by Hart et al (2003). In this study 20 consanguineous families with amelogenesis imperfecta were identified with probands suggesting an autosomal recessive transmission. Linkage studies indicated the presence of amelogenesis imperfecta gene on chromosome 4q region. The mutation of this gene resulted in generalized hypoplastic amelogenesis imperfecta phenotype and a class II open bite malocclusion. A strong association was thus established between amelogenesis imperfecta and malocclusion due to homozygous mutation in ENAM gene.
- Ravassipour et al (2005) conducted an investigation to evaluate the association of the AI enamel defect with craniofacial features characteristic of an open bite malocclusion. Open bite malocclusion was found to have occurred in individuals with AI caused by mutations in the AMELX and ENAM genes even though these genes are considered to be predominantly or exclusively expressed in the teeth. The purpose of this investigation was to evaluate the association of the AI enamel defect with craniofacial features characteristic of an open bite malocclusion. Affected AI individuals with cephalometric values meeting

criteria of skeletal open bite malocclusion were observed in all major AI types. The pathophysiological relationship between AI associated enamel defects and open bite malocclusion remains unknown.

- The study by Frazier-Bowers et al (2007) investigated whether the class III trait was present in several of the pedigrees affected with AI. Their results suggested that the class III trait factor co-segregated with AI in the experiment population.
- The Klinefelter males (47, XXY) show pronounced mandibular prognathism and reduction of cranial base angle (Brown et al, 1993). Similarly, 45,X females show imbalance of growth in the craniofacial skeleton (Peltomaki et al, 1989). They show retrognathic face with short mandible and flattened cranial base angle. There is a tendency to have a large maxillary overjet and crossbite. The X-chromosome seems to be responsible for altering the growth of cranial base by acting on cartilaginous joint at the base of the skull. This has a direct effect on the shape of the mandible.
- A linkage study for the growth of maxilla in mouse was conducted by Oh et al (2007). They found that the gene(s) regulating the shape of maxillary complex was situated on the mouse chromosome 12 at 44cM.
- A recent study by Fraziers-Bowers et al (2007) identified the chromosomal locus responsible for the class III trait. DNA samples were processed and subjected to a genome-wide scan and linkage analysis. The linkage analysis of these families revealed that a region on chromosome 1 was suggestive of linkage with the class III trait.
- The growth hormone receptor (GHR) gene was found to be associated with mandibular height in a Chinese population. Zhou et al (2005) evaluated the relationship between craniofacial morphology and single-nucleotide polymorphisms (SNPs) in GHR in a healthy Chinese population. Their results indicate that the GHR gene polymorphism I526L is associated with mandibular height in the Chinese population.

As far as genetics of malocclusion is concerned, much progress is still awaited. We do not know the exact mechanisms of genetic or environmental interaction that combine to produce malocclusion. Similarly, interactions between genetic and environmental factors are least understood. A clear understanding of mechanisms involving environmental factors would help in designing therapies along with the manipulation of environmental factors for orthodontic treatment. Multifactorial traits determined by the additive effects of many genes need to be investigated better to find out the exact number and specific locations of genes involved in their etiology.

More precise research tools and methods should be applied to understand aspects of genetics associated with malocclusion. It is hoped that sequencing of human genome and the use of single nucleotide polymorphisms (SNPs) will help to attain the desired levels of understanding.

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Chapter

16 Genetics of Cancer

- Characteristics of cancer cells
- Signal transduction in cell proliferation
- Signal transduction (genes and cancer)
- Oncogenes
- Tumor suppressor genes (TSG)
- Cell cycle control
- Cell cycle control genes and cancer
- Viruses and cancer genes
- Apoptosis

The term "cancer" is derived from the Greek word for crab. Hippocrates likened the spreading of cancerous tumor to the shape of a crab's claws. Cancer is a disease characterized by uncontrollable and unwanted growth of body cells due to the loss of their normal regulatory controls. Majority of cancers manifest in the form of solid tumors. A cancerous tumor is often a collection of many abnormal cells, most of which divide wildly. Cancerous tumors infiltrate neighboring tissues by forcing their way between normal cells and may spread to distant places in the body through blood or lymph vessels.

CHARACTERISTICS OF CANCER CELLS

Cancer cells are different from the normal cells. Cancer is a disorder involving dynamic changes in the structure as well as the function of the cellular genome in the cancer cells. Following changes are observed in the cancer cells:

1. *Unrestricted cellular proliferation*—Cells affected with cancerous changes lose their usual control over growth and division. The unrestrained growth and division of the cancer cells hamper the normal functioning of the body as a whole by disrupting metabolic activity of the organism and also by causing local effects by the growing mass.
2. *Transformation*—Cancerous cells are transformed cells. These abnormal cells are transformed and become independent of factors usually needed for normal cell growth and proliferation.
3. *Ability to invade*—One of the potent properties of cancer cells is their ability to invade from their site of origin into the surrounding healthy tissue.
4. *Metastasis*—Cancer cells characteristically scatter away from their origin and disseminate to distant parts of the body where they seed and proliferate.
5. *Suppression of apoptosis*—The program for normal cell death (apoptosis), that usually operates in a healthy cell is altered and suppressed in cancer cells.
6. *Angiogenesis*—Cancer cells have the ability to induce new vessel formation or neovascularization in the tumor mass to facilitate the availability of oxygen and nutrients.

Factors causing or aiding cancer can be grouped as environmental and genetic. Certain environmental components account for the occurrence of approximately 80% of all human cancers and are hence preventable.

Factors Responsible for the Causation of Cancer

Cancer may develop either due to environmental as well as genetic factors.

Environmental Factors of Cancer

Chemicals: Many chemicals like polycyclic aromatic hydrocarbons (3, 4 benzpyrene), aromatic amines (B-naphthylamine), vinyl chloride and arsenical

compounds are known carcinogens and may cause cancers of lung, skin, bladder and liver. Substances like tobacco and alcohol are associated with cancers (squamous cell carcinoma) of the oral cavity.

Radiations: Similarly ultraviolet light (exposure to sunlight) is proven to be carcinogenic for skin (malignant melanoma and basal cell carcinoma) in fair skin people. High dose of ionizing radiation is well known carcinogen especially in people working with radioactive materials. Ionizing radiation is responsible for leukemia and cancers of skin, thyroid, bone and breast. Melanomas (Fig.16.1) are common forms of cancer that involve oral mucous membrane in addition to their usual site of occurrence, the skin.

Viral infection: Many viruses are considered strong carcinogenic agents. About 15% of all human cancers are due to viruses. Several human tumors have known viral etiology, e.g. infection with the human papilloma virus (HPV) causes carcinoma of cervix. HPV infection is incriminated in development of squamous cell carcinoma (Fig. 16.2) that constitutes about 95% of all oral cancers. Epstein-Barr virus is responsible for formation of Burkitt's lymphoma and nasopharyngeal carcinoma. This virus is associated with oral lesions called hairy leukoplakia, especially in patients suffering from AIDS. Certain leukoplakias of the oral cavity may be precancerous conditions. Hepatitis C and B virus produce liver cancer and RNA retrovirus leads to T-cell leukemia and lymphoma. (*The association of virus and cancer is described in detail later in this chapter).*

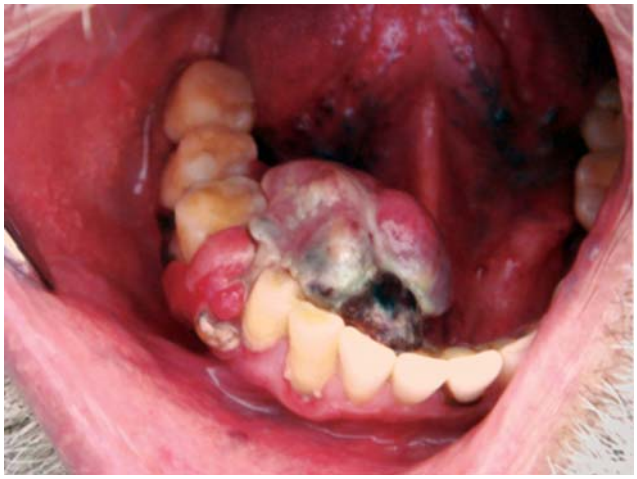

FIGURE 16.1: Patient showing a malignant melanoma lesion. *Courtesy* of Dept. of Oral Pathology, Modern Dental College and RC, Indore, India

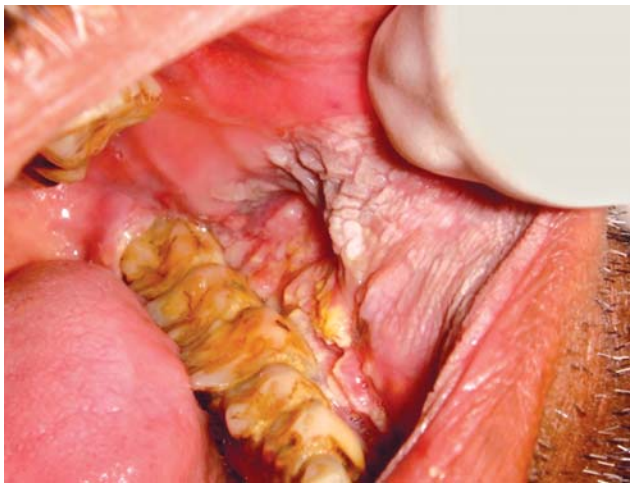

FIGURE 16.2: Patient showing a squamous cell carcinoma lesion. *Courtesy* of Dept. of Oral Pathology, Modern Dental College and RC, Indore, India

Bacteria and other microorganisms: Cancer may also result from bacterial infection (*H. pylori* can produce lymphoma and gastric carcinoma), toxins of fungi (aflatoxins can cause cancer of liver) and parasites like schistosoma can cause bladder cancer.

Production of Cancer by Carcinogens

The Production of Cancer by a Carcinogen Involves a Multistep Process

In the first step the presence of a carcinogen causes a lesion in the cell's genome (in the DNA of the target cell) that leads to the transformation in the cell. In the second step this transformed cell divides repeatedly (clonal proliferation). This uncontrolled cellular proliferation is the main event leading to formation of carcinoma. In the third step the clonal proliferation of tumor cells acquire autonomous growth, i.e. they no longer require the stimulation by carcinogen or other intrinsic factors and rapidly proliferate themselves. In still later stages tumor cells acquire the ability to invade the surrounding tissue, metastasize to distance places in the body and induce vascularization of the tumor.

What is Cell Proliferation and How is it Controlled?

Unrestricted cell proliferation is the main characteristic of cancer. Carcinomatous changes in a cell are brought about by disruption in the normal mechanisms that control cellular proliferation and differentiation. Thus

in order to understand the dynamics of cancer we need to understand what is cell proliferation and how it is regulated. Normal proliferation, differentiation and growth in cells are sequentially controlled by the following events:

1. Binding of growth factors to specific receptors on the cell membrane.
2. Activation of growth factor receptors that further activates signal transducing proteins on the inner surface of the plasma membrane.
3. An appropriate signal is then transmitted to the nucleus through certain messenger proteins across the cytoplasm.
4. DNA transcription is initiated by the activation of transcription factors that bind at specific regions on the genome to activate transcription.
5. Cell enters into mitosis after passing through the checkpoints and eventually undergoes nuclear and cytoplasmic division.

The events mentioned above operate under strict genetic control. Abnormal proliferation of cells may result from mutations that alter the functions of genes governing cellular proliferation. Uncontrolled cellular proliferation can be studied vis-à-vis the mechanisms of normal cellular life cycle.

SIGNAL TRANSDUCTION IN CELL PROLIFERATION

Several of growth factors (GFs) and growth factor receptors (GFRs) exhibit significant role in normal cellular growth and differentiation. Various types of these factors initiate specific course of action. It has been observed that factors like the epidermal growth factor (EGF) stimulates epidermal cells, fibroblast growth factor (FGF) stimulates fibroblasts, platelet derived growth factors (PDGF) stimulates proliferation of connective tissue, etc. These factors bind to the receptors on the cell membrane in order to exert their action. The sequential activation of successive events through cascading pathways resulting in cellular activity, growth, differentiation or proliferation is termed as **signal transduction**. Thus extracellular growth factors trigger cellular events through complex pathways. Each of the steps in the pathway is controlled through specific genes and their activity.

Binding of a growth factor to its specific receptor leads to the activation of the receptor.

- A series of cytoplasmic proteins get activated by the receptor in a cascade of reaction. These proteins are called **signal transducing proteins**. Many of such proteins are present on the inner surface of plasma membranes.
- Two important signaling proteins are produced by the **ras** and **abl** genes.
- During the resting state of the cell the **ras** families of proteins bind to GDP (guanosine diphosphate) constitutively.
- On stimulation by growth factor receptors, inactive **ras** becomes active by releasing the attached GDP and binding to a new GTP (guanosine triphosphate) molecule.
- The activated **ras** further turns on cytoplasmic kinases that pass signals to the nucleus for cellular proliferation.

  [The life of activated **ras** proteins is very short. The enzyme guanosine triphosphatase (GTPase) hydrolyzes GTP to GDP and inactivates **ras** proteins thereby downregulating cytoplasmic kinases. As a result the cell no longer responds to a signal till further activation. The **abl** gene induces different signal transducer proteins. This gene is located on chromosome 9.
- Cytoplasmic kinases enter the nucleus and activate a large number of genes immediately and very early (**myc, myb, jun, fos** and **rel gene**). The activity in these genes further regulate transcription of specific DNA segments.
- The **myc** protein is the one to get frequently bound to specific DNA sites after a cell receives a signal for proliferation (Fig. 16.3).
- The activity of **myc** proteins induces transcriptional activation of several growth related genes including **cyclin D** (see later in cell cycle). The quantity of myc protein reduces back to basal levels once the cell enters the cell cycle.

SIGNAL TRANSDUCTION (GENES AND CANCER)

Defective signaling in growth regulating pathways can lead to abnormal growth. Overexpression of growth factors can result in nonneoplastic disorders like **psoriasis**. Abnormalities at the level of growth factor receptors can lead to conditions like **insulin-resistant diabetes** (insulin receptor) and **dwarfism** (fibroblast growth factor receptor). Development of carcinomas,

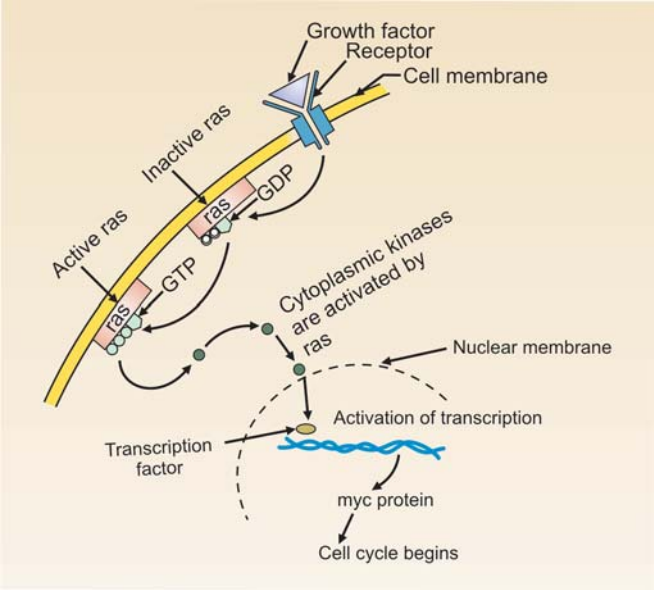

FIGURE 16.3: Schematic diagram showing signal transduction for cell cycle. When growth factor binds to its receptor the inactive ras gets activated and sends signals to the nucleus through cytoplasmic kinases. The expression of myc proteins begins the cell cycle

however, involves multiple steps and show other features such as invasion and metastasis. These multiple steps include unregulated expression of growth factors, receptors or components of other signaling pathways. As discussed in earlier chapters, abnormal expression of components regulating signaling pathways is caused by mutations in the responsible genes. These mutant genes are called **oncogenes**.

ONCOGENES

Oncogenes are responsible for the production of cancer. Oncogenes are generated from several normal genes that are constitutively active and involved in coding for proteins, which act as growth factors, growth factor receptors, signal transducing proteins or transcription factors. These genes are called "**proto-oncogenes**". Following are the well known proto-oncogenes: *Cyclin D, Cdk4, EFGR (epidermal growth factor receptor), FGFR (fibroblast growth factor receptor), Ras, Bcl2 and Mdm2.*

A nonactivated proto-oncogene can be transformed into an activated cancer producing oncogene by a ***point mutation, chromosomal translocation*** or ***viral infection***. The activated oncogenes induce cellular proliferation and therefore lead to tumor development. Based on the functions of proteins produced by oncogenes these can be divided into five groups:

1. **Growth factor oncogenes**, e.g. ***sis*** gene which codes for platelet derived growth factor.
2. **Growth factor receptor oncogenes,** e.g. ***erb B*** gene which codes for epidermal growth factor receptor.
3. **Cyclic nucleotide binding oncogenes,** e.g. ***ras*** and ***GTP***.
4. **Tyrosine kinase activity oncogenes**, e.g. ***src***

TUMOR SUPPRESSOR GENES (TSG)

It has been stated earlier that a normal cell contains proto-oncogenes that promote cell growth. On the other hand a normal cell also contains genes which are called tumor suppressor genes. The function of these genes is to apply brakes to cell growth thus they possess tumor suppressor activity. The products of these genes check undue cell proliferation and also induce the repair of damaged DNA when detected. These kinds of TSG are thus called "***caretaker genes***" (BRCA 1, BRCA 2, and MLH 1). In cases of damaged DNA that are beyond repair, these genes induce cell death (apoptosis) in them. These kinds of TSGs are called "***gatekeeper genes***" (p53, p21, Rb 1, Bax, APC). Thus a loss or inactivation of these genes takes off the regulation and constraints imposed by these genes on cell growth and proliferation resulting in carcinogenesis. Consequently, the activation of proto-oncogenes or inactivation of tumor suppressor genes both lead to the progression of many tumor to full malignancy. The tumor suppressor genes are a class of cellular genes whose normal function is to suppress inappropriate cell proliferation. These genes are intimately involved in the control of the cell cycle. The development of cancer due to loss of function or mutation in these (TSG) genes requires the inactivation or mutation to be present in both its alleles on both chromosomes of the pair. Thus, tumor suppressor genes function in the recessive mode.

Over 100 proto-oncogenes and about 30 TSGs have been known yet. Some of these genes are listed in Table 11.1 and 11.2. Some of well known tumor suppressor genes are **BRCA-1**, **WT-1**, **Rb** and **p53**. The descriptions of few TSGs and mechanisms by which the loss of their functions lead to cancer transformation is described in next section (cell cycle and cancer) of this chapter.

The analysis of functions of various known oncogenes and TSGs has shown that they code and control growth factors, growth factor receptors, adapter molecules, protein kinases, nuclear transcription factors, cell cycle genes, various checkpoints of cell cycle and apoptosis. Any impairment in the functioning of the above components of signal transduction cascade leads to the over proliferation of cells which ultimately results in tumor formation.

Growth Factors and Cancer

Genes coding for growth factors may acquire oncogenic properties after mutation. For example, the gene coding for PDGF after mutation over expresses the growth factor which give rise to cancers like **osteosarcoma** and **astrocytoma**.

Growth Factor Receptors and Cancer

Genes coding for growth factor receptors have been found to be mutated in several carcinomatic conditions. Such mutations are believed to induce continuous signals for cell growth and proliferation, even in the absence of growth factors.

Signal-transducing Proteins and Cancer

The **ras** genes that produce signal transducing protein are susceptible to mutations. Such mutations are responsible for almost 30% of all human tumors. As a consequence of mutation the enzyme GTPase is unable to hydrolyze the active GTP back to inactive GDP. Thus the ras protein remains constantly active and the cell continues to proliferate. Similarly a mutation in the GTPase protein itself leads to a defective enzymatic action that fails to restrain the activated ras protein. This eventually results in cancer.

Transcription Factor and Cancer

Several early and immediate gene products link the activities of growth factors to other factors that results in transcription of the DNA. The **myc** gene binds to DNA and activates many transcription factor elaborating genes involved in growth. Mutations in transcription factors with overexpression contribute to sustained proliferation.

CELL CYCLE CONTROL

The Cell Cycle

The cell cycle includes all events of cell growth, cell activity, replication of the DNA content and cell division that gives forth the daughter cells. This process is divided into four sequential phases (Fig. 16.4).

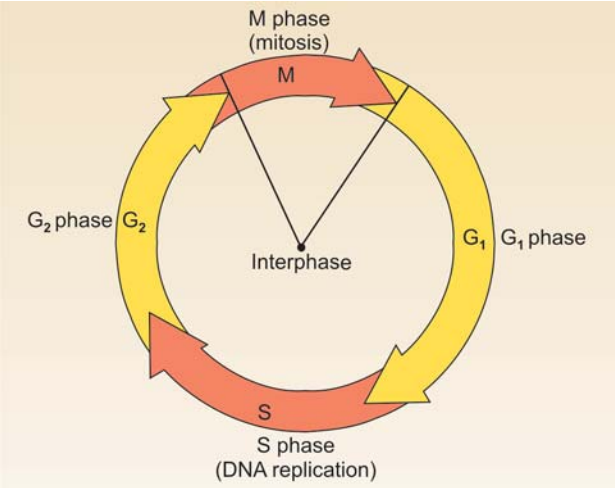

FIGURE 16.4: The cell cycle. It consists of two subdivisions, i.e. interphase and mitosis. The interphase consists of G1, S and G2 phases. M phase is the phase in which cell divides to give rise to two daughter cells

- *G1 phase* (Gap phase or presynthetic phase) – This phase starts immediately after completion of cell division. The chromosomes gradually become thinned and extended. Cells are responsive to growth signals. They may or may not enter the next cell division depending whether signals are positive or negative with respect to cell division. Cells like neurons that are highly differentiated and lose their capability to divide further are shifted to **G0** phase. Cells in the G0 phase usually subserve their functions and perish after their life is over. These cells may reenter into mainstream G1 phase for replication under special circumstances.
- *S phase* (Synthetic phase) – It is called S phase as DNA replication or synthesis occurs in this phase.
- *G2 phase* (premitotic phase) – G2 phase is short where chromosome begins to get condensed in

preparation for the cell division phase that comes next. All the above three phases described above constitute the interphase of the cell cycle. Cells usually spend the bulk of their functional lives in the interphase.

- *M phase* (Mitotic phase) – The M phase results in complete nuclear and cytoplasmic division of a cell into the daughter cells.

Cell Cycle Checkpoints

As cells transit from one phase to the next in the cell cycle, all the events during such transitions are scrutinized and regulated at a number of specific and regular points within the cell cycle. These locations are known as checkpoints (Fig. 16.5). These checkpoints verify the structural integrity of the genome, ensure that the DNA is free of any breaks and monitor the cellular environment as a pre-requisite for a given phase in the cell cycle. The check points of the cell cycle are shown below.

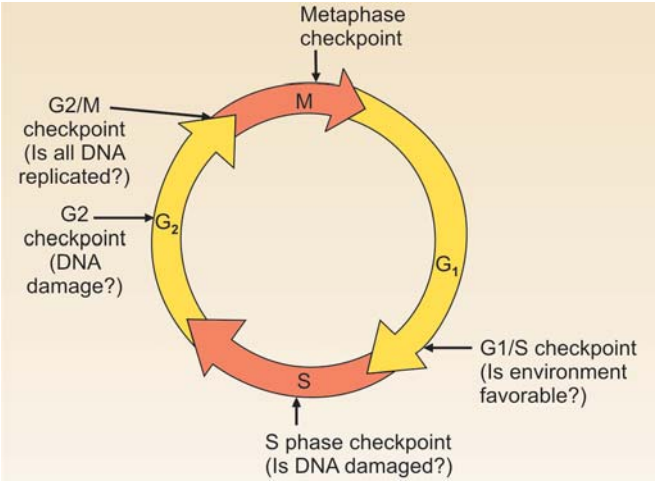

FIGURE 16.5: Checkpoint of cell cycle

- *Restriction point*—This restriction point (R) occurs late in the G1 phase between the middle and the termination of the G1 phase. It is the time when a cell verifies whether it has appropriately been instructed by the growth signals to proceed to the S phase for DNA replication. Growth signals sufficient to trigger cells to go into S phase would induce replication of DNA or else the cells would shift to the G0 phase.
- *G1/S DNA damage checkpoint* – The G1/S phase transition forms a major checkpoint for detection of any damage in the DNA molecule entering the synthetic phase.
- *S phase DNA damage checkpoint* – This checkpoint is strategically located at the later part of the S phase. Defective synthesis of DNA is detected at this stage and appropriately dealt with.
- *G2/M checkpoint* – Acts as a DNA damage check point.
- *Centrosome duplication checkpoint*- A defect in duplication of the centrosome or chromatid segregation arrests cells at the G2/M transition.
- *Mitotic checkpoint* – The M phase checkpoints observes the formation of normal mitotic spindles. The detection of any chromosome that is not attached at a spindle blocks the onset of anaphase.

How is Cell Division (Cycle) Controlled?

The steps in cell division are usually controlled by proteins called **cdk-cyclin complexes**. The cdks (cyclin dependent kinases) belong to a family of **kinases**. The kinases act as catalytic subunits and are named *cyclin dependent kinases* (cdks) as their activity is dependent on certain **cyclins**. Cyclins are types of regulatory subunits. The catalytic and regulatory subunits always occur as associated pairs. Thus a specific cdk is fully activated only when its cyclin partner is expressed in association. As exemplified at specific cell cycle stages, the G1 phase cdk4 and cdk6 act in association with cyclin subunits D1, D2 and D3, while the cdc2/cyclin B complex (cdc = cell division cycle) is expressed in G2/M phase of cell cycle (Fig. 16.6).

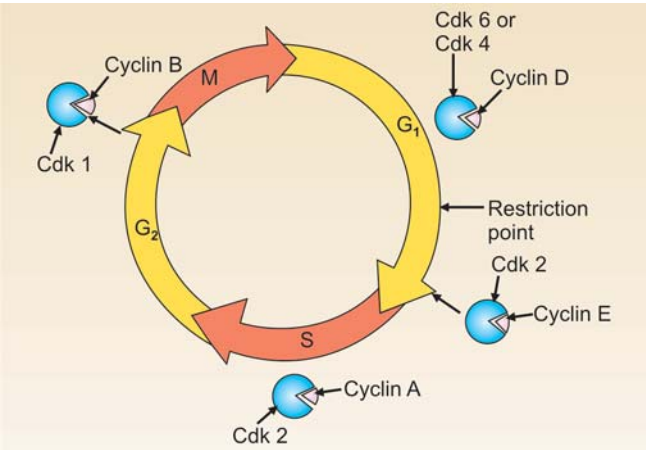

FIGURE 16.6: Some major cyclins and cdks. The cdk-cyclin complexes are required for different phases of cell cycle

Multiple proteins actively regulate different stages of cell division. The cdks control the phosphorylation of regulatory proteins at different stages in the cell cycle progression, e.g. the retinoblastoma (RB) tumor suppressor gene product (pRb) is a key regulatory protein of the G1 phase that is phosphorylated by a cdk/cyclin complex.

- In the first part of the G1 phase (very early interphase) pRb is bound to E2F. The transcription factor E2F needs to be in an unbound form for the cells to transit form the G1 to the S phases (Fig. 16.7).
- The pRb with E2F forms a complex that inhibits expression of other transcription factors needed for the initiation of S phase.
- Cells remain in the G1 phase or G0 phase in presence of the complex.
- The synthesis of D cyclin is activated subsequent to the action of growth factors. This event stimulates cells to reenter the cycle from G0 or G1 phases.
- Cdks and cyclins couple to form active complexes regulating further steps in the cell cycle. The cdk 4/cyclin D and cdk6/cyclin D become active in the early phase of G1 and cdk2/cyclin E complex function in late phase of G1.

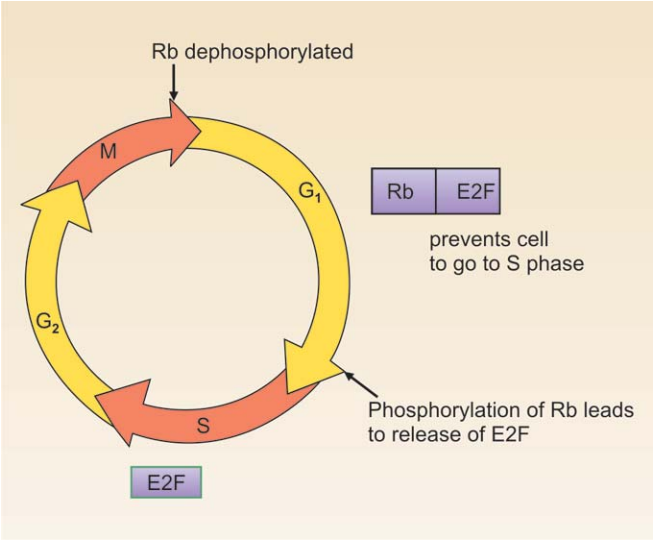

FIGURE 16.7: In the resting cell Rb binds to E2F which prevents the initiation of S phase. As Rb of Rb-E2F complex is phosphorylated by Cdk/cyclin complex, E2F is released. E2F is required for the beginning of S phase

- As the cdk/cyclin complexes phosphorylate the protein pRb in the pRb and E2F complex, the trapped E2F is released (Fig. 16.7).
- The free E2F subsequently activates transcription of genes that are essential for initiating the S phase.
- The inactivation of certain other genes caused by pRb-E2F complex is also disinhibited.
- Thus with the inactivation of pRb the cell now enters in S phase.

Alternatively the cdk-cyclin kinases also can be inactivated to retain a cell in the G0 or G1 phases. Such inactivation of cdk/cyclin complex can be achieved with the binding of certain inhibitory proteins to the cdk/cyclin complexes. The inactivated complexes are now unable to phosphorylate the pRb and the cells fail to transit into the S phase. There are two families of the cdk/cyclin inhibitor proteins.

- *INK4 family* (Inhibitors of cdk4 family) – INK4 family of proteins specifically bind and inactivate cdk4 and cdk6. The p16, p15, p18 and p19 are the four members of the family.
- *Cip or Kip family* (cdk interacting protein or kinase inhibitory protein). The p21, p27 and p57 are the three members of the family. p21 binds to all complexes of cdk2, cdk4 and cdk6 and forms the universal cdk inhibitor that can block all stages of G1 and S phase.

CELL CYCLE CONTROL GENES AND CANCER

G1 Phase

The cyclin D-dependent kinases integrate the extracellular signals towards progression of the cell cycle. Alterations in pRb and cyclin D-dependent kinases may lead to inappropriate and unbalanced phosphorylation of pRb. This may result in uncontrolled signaling and proliferation of cells.

Deletion or mutation of the suppressor gene CDKN2 has been implicated in multiple cancer states. The RB (retinoblastoma) gene that acts as a tumor suppressor gene has been found to be associated with cancer. Retinoblastoma is a tumor of the retina seen in children. The retinoblastoma (RB) gene is located on the q arm of chromosome 13. Retinoblastoma arises when both the copies of the RB genes are deleted or

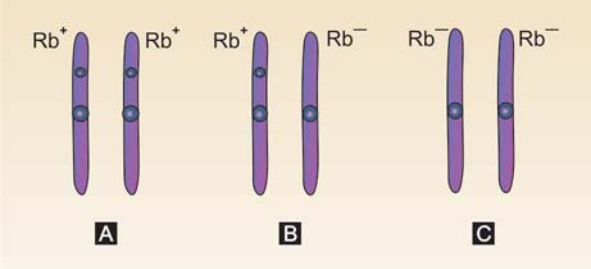

FIGURE 16.8: Presence of Rb genes on a homologous pair of chromosome (A); Heterozygous loss of one allele (B); and loss of both alleles (C); Retinoblastoma arises when both copies of genes are absent (as in case of C). Absence of one gene (as in case of b) has no effect as the other gene compensates for the loss

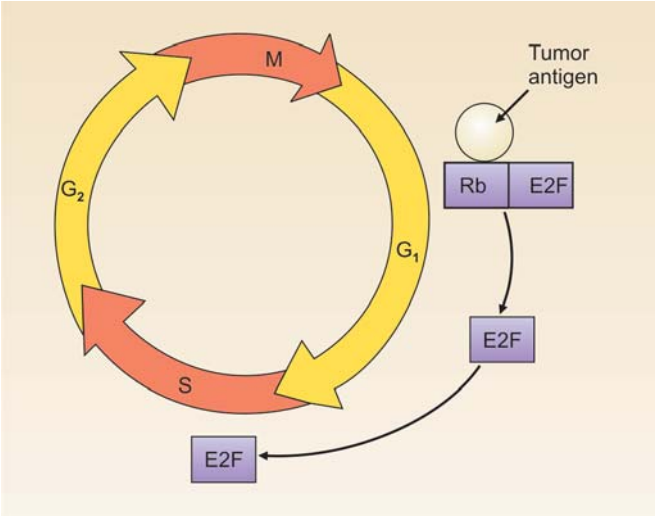

FIGURE 16.9: As a result of binding of tumor antigen to Rb, E2F remains free throughout the cell cycle. This results in unrestricted cell proliferation

inactivated. Usually the child inherits one defective allele and acquires a fresh mutation in the normal allele in childhood. Thus these tumors are generally sporadic in occurrence resulting from new mutation; homozygous mutations can also be inherited giving rise to the condition (Fig. 16.8).

A Condition like retinoblastoma arises due to the loss of protein pRb (product of gene RB) that leads to unrestrained cellular proliferation. Absence of pRb has been linked to osteosarcoma and lung cancer.

RB gene yields a nuclear phosphoprotein (pRb) that influences crucial activities in the cell cycle. The protein pRb is usually kept bound to the E2F group forming an inactive pRb/E2F complex in cells that don't proceed towards cell division. Once the cdk/cyclin complex phosphorylates the pRb faction of the pRb/E2F complex, it sets the E2F component free to bind and activate the next set of transcription factors.

Protein pRb can also bind certain viral tumor antigens like SV40T and E1A. In this situation pRb doesn't bind E2F and remains as a pRb-tumor antigen complex. The free E2F helps the cell to pass from G1 to S phase as the viral antigen induces unrestrained growth by blocking normal activity of pRb. (Fig. 16.9).

Cell proliferation can be arrested if—

- pRb is not phosphorylated (remains coupled to E2F).
- D-cyclin is absent (disabled cdk complex).
- p16, p21 and p27 inactivates cdk-cyclin complex.

On the contrary the loss of the above functions may lead to unrestrained growth or tumor formation.

G1/S Checkpoint

Damage caused by double strand breaks (DSBs) in the DNA activates specific events at this check-point. Ionizing radiation or genotoxic chemicals usually cause such damage in the DNA strands. Escape of undetected damaged DNA on to daughter cells in somatic and germ cells may have devastating consequences.

Operation of the G1/S cell cycle checkpoint is governed by the tumor suppressor gene TP53. This gene gives rise to the protein p53 that acts as receiver of stress signals including DNA damage. Any damage to DNA leads to the activation of p53 which then acts as a transcription factor inducing cell destruction (Fig. 16.10).

The levels of p53 are generally low in normal cells. To function as transcription factor p53 protein must be activated by phosphorylation and acetylation. The factor Mdm2 prevents phosphorylation and acetylation of p53 and removes p53 from the nucleus. This leads to degradation of p53 by proteosomes in the cytoplasm.

The level of p53 thus is kept low by continuous export of p53 by Mdm2 from nucleus to cytoplasm followed by degradation. On the other hand DNA damage itself results in phosphorylation and acetylation of p53. Once p53 is phosphorylated, Mdm2

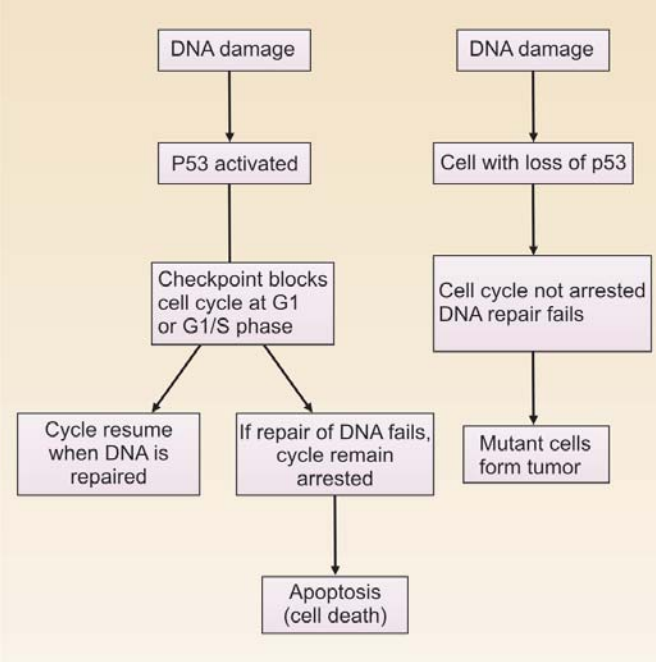

FIGURE 16.10: In case of DNA damage p53 gene gets activated and helps to repair DNA. In case of loss of p53 gene DNA damage cannot be repaired. Damaged DNA leads to tumor formation

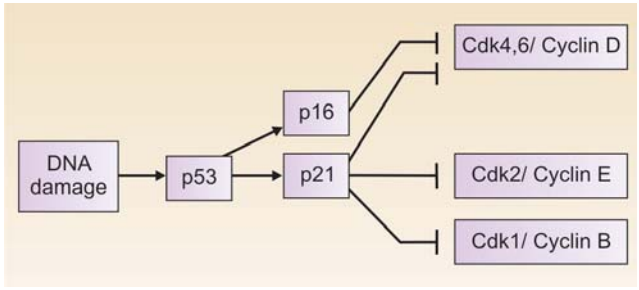

FIGURE 16.11: Genes p16 and p21 when induced by gene p53 binds to various cdk/cyclin complexes and leads to arrest of different checkpoints. Thus genes (p53, p21 and p16) act as tumor suppressors. (→ = activation, —| = inhibition)

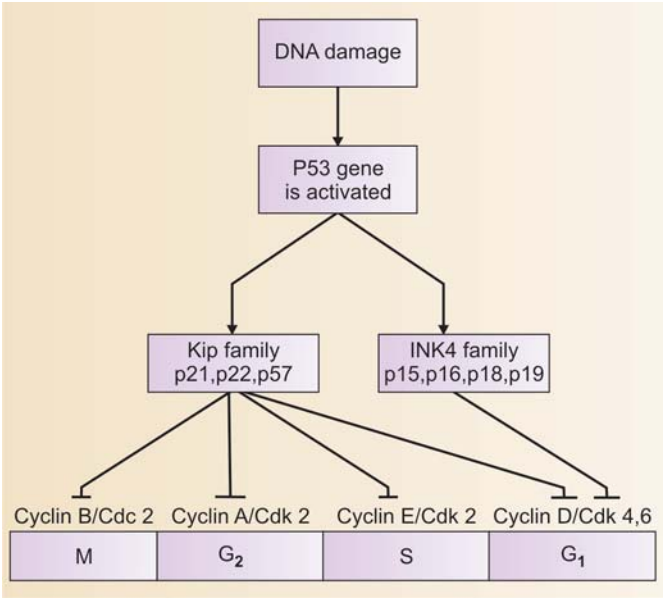

FIGURE 16.12: Note that the inhibitors of INK4 family bind specifically to cdk4 and cdk6. While kip family (p21) is a universal inhibitor. All these inhibitors block the cell cycle and thus prevent tumor formation

cannot bind to the modified (activated) p53. The activated p53 remains in the nucleus.

Transcription of a number of genes is brought about by the p53 protein to trigger cell cycle arrest and apoptosis p53 induced gene p21C1P1 binds to cdk2/cyclin E resulting in the arrest of cell cycle at G1/S checkpoint (Figs. 16.11 and 16.12)

Activities of p53 have earned it the name "**Guardian of Genome**". *Mutations in TP53 gene are responsible for about 50% cancers in humans*. p53 is located on chromosome 17p13.1. Virtually all types of cancers are associated with defects in the p53 gene. Such defects or mutations generally exist in a homozygous pattern.

Mutant p53 alleles are inheritable. Individuals having such mutations are susceptible to develop malignant tumors. Carriers of heterozygous defects are said to have the **Li-Fraumeni syndrome** and may develop a varied type of tumors (carcinomas, lymphomas, brain tumors, sarcomas, etc).

p53 protein has a vital function of arresting the cell cycle on detection of breaks and damages in the DNA content of a cell. The protein gets accumulated in the nuclei and inhibits the cell from crossing over to the S phase.

As mentioned above, p53 induces the action of p21 to complete the arrest. p53 also helps in the repair of broken DNA molecules by activating certain other transcription factors and DNA repair enzymes. In the event of a complete repair, the cell is allowed to advance to the next step in the cycle.

In case of an incomplete or failed repair, p53 stops the cell division and induces apoptotic mechanisms in the cell.

S Phase

The S phase checkpoints are also invoked by structural changes in the DNA. Cell cycle is stopped with the action of the dephosphorylated pRb. Arrest of progression of cell cycle may also be brought about by p21 that blocks cdk.

G2/M Checkpoint

This checkpoint is located at the junction between the G2 and the M phase and it is triggered by DNA breaks in the genome. Cell cycle is inhibited at this stage by inactivation of the Cdc2/cyclin B complex. The inhibition of the complex stops the transit of a cell from G2 to mitosis. The action of the checkpoint is to maintain the Cdc2/cyclin B1 complex in an inactive state with the help of p53 protein.

The induction of p21 and its binding to Cdc2/cyclin B in the nucleus causes cell cycle arrest at G2/M checkpoint.

VIRUSES AND CANCER GENES

The study of viral carcinogenesis has shed light on the genetic mechanisms involved in cancer formation. Retroviruses (RNA viruses) are the ones that cause most of cancers in animals and a very few in humans. Some of the DNA viruses also cause a few cancers in man. The understanding of replication in the retrovirus helps us analyze events occurring in cancer.

Retroviruses

The retroviral genome consists of a diploid, double stranded RNA molecule. These viruses are incapable of replication till they infect a cell and use the cellular machinery of the host to replicate its genome and assemble other constituents of its structure. The presence of the key **reverse transcriptase** enzyme forms a double stranded DNA copy from the viral RNA. The transcribed DNA strands easily incorporate into the host DNA and are called "**provirus**". A provirus constitutes three genes that are adequate for complete viral replication.

- **gag –** the gene codes for structural protein of the virus.
- **pol –** this segment codes for the enzyme reverse transcriptase.
- **env –** the sequence codes for protein of the outer envelope.

The promoter and enhancer elements are integrated at each end of the genome. These elements are constitutively associated with the provirus and are termed **long terminal repeats** (LTRs) (Fig. 16.13).

The normal replication of the host DNA also results in the replication of the viral genome by default. Transcription of the integrated viral genome, on the other hand, gives rise to different cellular components of the virus. All viral components assemble in the cell and come out as viral progeny in multiplied numbers after lysing or destroying the host cell.

Once a provirus is integrated in the host genome, it stays in the cell for its life; even passing to the daughter cells of the infected host cell. Proviruses have also been found integrated into DNA of gametes (eggs and sperms) where they reach after infecting the germ cells in an individual.

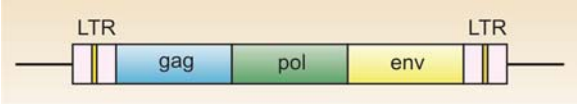

FIGURE 16.13: Schematic diagram showing genome of a retrovirus

In addition to the **gag**, **pol** and **env** genes found in common retroviruses a potent fourth gene exists in certain viruses (as identified during the study of Rous sarcoma virus). This gene is capable of carcinomatous transformation in a host cell. It is called the **src** gene (Fig. 16.14) with its action of coding for a protein kinase and inducing cancerous transformation of host cell well-documented. A viral gene that can transform the infected host cell is termed **oncogene**.

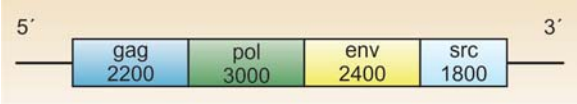

FIGURE 16.14: Genetic map of sarcoma virus

Oncogenes carried on viruses are called viral oncogenes or **V-onc** whereas oncogenes located in host cell genome are called cellular oncogenes or **C-onc** genes.

Some DNA sequences in the host cell are homologous or identical to the viral oncogenes and

thus are called **proto-oncogenes**. As discussed earlier proto-oncogenes regulate normal cell growth and do not cause cancer in normal circumstances. The cellular proto-oncogenes are potentially carcinogenic and can be transformed to act as oncogenes by point mutation, amplification and chromosomal translocation.

How Viral Oncogenes are Formed

It is interesting to note that retroviral oncogenes originates from cellular genes in the host. Any error in the replication of retroviral genome, after their integration in host genome, gives rise to retroviral oncogene. This viral oncogene is structurally similar to its cellular counterpart (the viral oncogene **sis** is almost similar to the gene for platelet-dependent growth factor [PDGF]) but different in its function.

Conversion of Proto-oncogenes to Cellular Oncogenes (c-onc)

Proto-oncogenes can be converted into cellular oncogenes in the following ways:

1. *By increase in the amount of proto-oncogene product:* This can be achieved in two ways.
   a. Integration of the viral oncogene to the host DNA close to a proto-oncogene may induce uncontrolled expression of proto-oncogene through the action of the LTRs of the v-onc. (Fig. 16.15). The LTR of the Epstein - Barr viruses have been observed to overexpress myc gene in infected human cells leading to **Burkitt's lymphoma**.
   b. Multiple copies of the proto-oncogenes can be formed in a cell through gene amplification. Activity of several copies of the gene yields a large amount of the transcriptional product that leads to transformation of proto-oncogenes to cellular oncogenes.

      The **N-myc** gene is amplified manifolds in **neurofibromatosis**. **C-myc**, **N-myc** and **L-myc** amplifications are associated with lung carcinomas and **c-neu** or **erb**-B2 genes with types of breast carcinomas.
2. *Mutation in coding sequence:* Oncogenes are also formed by mutations in the proto-oncogenes. As discussed, these proto-oncogenes are potential oncogenes and are activated through mutations to produce cancer. Mutations in the ras gene account for approximately one-third of all human cancers.
3. *Chromosomal translocation:* A good percentage of human cancers are caused due to translocation of chromosomes with rearrangement of the genome. **Chronic myeloid leukemia** and **Burkitt's lymphoma** are two common examples of the group. The **Philadelphia chromosome** seen in the white blood cells of the CML patients is formed by rearrangement of chromosomal segments as a result of translocation

FIGURE 16.15: Schematic diagram to show the integration of provirus in the host genome adjacent to the proto-oncogene. This integration causes the activation of oncogene

A reciprocal translocation between the long-arm of chromosome number 22 and the 9th chromosomes transfers cellular **abl** oncogene from chromosome 9 that fuses with **bcr** (break-point cluster region) gene of chromosome 22. This fused gene (chimeric gene) manufactures protein that contains about 900 amino acids of **bcr** region and 1100 amino acids of **c-abl** region. The resultant condition gives rise to abnormal proliferation of the cells.

Similar translocation between the long arm of **chromosome** 8 containing the **c-myc** gene and the chromosome 14 at the locus carrying the gene for immunoglobulin heavy chains is implicated in the formation of **Burkitt's lymphoma**. This rearrangement brings the translocated **c-myc** gene under the regulatory influence of the immunoglobulin gene resulting in about 20 fold increase in the levels of **c-myc** transcription.

APOPTOSIS

Apoptosis or cell death is a programmed event to maintain a balance between the generation of new cells and the death of senescent or defective cells.

Apoptosis occurs due to several events that causes damage to the growth regulating genes, loss of check-point genes and also when the telomerase enzyme is unable to protect the integrity of the terminal part of the chromosomes after each cell division. (With each

cell division, the tails of each chromosome gets shortened due to progressive loss of nucleotides to reach a certain limit where the cells automatically undergo self-destruction. This threshold is referred as the ***Hayflick's*** limit. This phenomenon occurs due to gradual dwindling in the levels as well as activity of the telomerase enzyme that is needed to repair damaged ends of the chromosome).

Cells usually die when they grow old and are unable to furnish proteins like the telomerase enzyme that are needed to cross the checkpoints in the cell cycle. Old cells also suffer breaks in the DNA and are directed towards self-destruction. Cells also die if they are subject to sustained injuries such as heat, oxidative stress, UV irradiation damage or get killed when they become vulnerable and infected with a virus or other intracellular pathogen that destroys the cell.

Thus apoptosis is a form of programmed cell death initiated by extracellular or intracellular signals in which enzymes are activated that break down the cytoplasmic and nuclear skeleton, degrade the chromosomes, disintegrate the DNA and shrink the cells. Initiation of the process of apoptosis begins either with extracellular or intracellular signals.

Execution of cell death is effected with the release of ***caspases*** (cysteine containing aspartase specific protease). Caspases are the ultimate destroyers of cell. These are a family of proenzymes that are activated in a cascade. The targets of these proteases are the DNA, several cytoskeletal proteins, DNA repair enzymes, etc. The caspase family includes at least 13 proteins and is divided into 3 groups.

Morphological changes occur in the dying cells. Surrounding cells like macrophages remove the dead cells.

SUMMARY

(a) Cancer cells are characterized by:
   i. Uncontrolled proliferation.
   ii. Transformation to abnormal cells.
   iii. Capacity to invade surrounding tissues.
   iv. Property to metastasize to distant places.
   v. Suppression of apoptosis.
   vi. Induction of angiogenesis.

(b) Cancer is due to the loss of the normal mechanisms which control cellular proliferation and differentiation.

(c) Cellular proliferation is under the control of genes.

(d) Genes responsible for causing cancer are known as **oncogenes**.

(e) Tumor suppressor genes (TSG) apply brakes to unrestrained cell growth as they induce tumor suppressor activity. Thus cancer may develop due to loss of function (mutation) of TSGs.

(f) About 100 oncogenes and about 30 TSGs are now known.

(g) **Signal transduction** is a process by which extracellular growth factor regulate cell growth and differentiation by a complex pathway. [Growth factor – activation of receptor – cytoplasmic proteins (**ras** and **abl**) are activated – ras binds to GTP – cytoplasmic kinases are activated – Kinases enter the nucleus – activate **myc** which regulate transcription of DNA – cell cycle begins].

(h) Genes that code for growth factors and growth factor receptors, after mutation may acquire oncogenic properties.

(i) The mutation of ras gene (which codes for signal transducing protein) and myc gene coding for transcription factor may cause cancer.

(j) The cell division cycle is divided into four sequential phases, i.e. G1, S, G2 and M phase.

(k) The transition from one phase to the next is regulated by **checkpoints**.

(l) Cell cycle is controlled by cdk/cyclin complexes.

(m) Various proteins act as key regulatory proteins during cell cycle. For example the product of **retinoblastoma (RB)** tumor suppressor gene **(pRb)** is a regulator protein of G1 phase that is phosphorylated by cdk/cyclin complex.

(n) Cell cycle checkpoints are under genetic control and surviallence. In case DNA (gene) is damaged, cell cycle progression is checked till the damaged DNA (gene) is repaired.

(o) Any alteration (mutation) in these genes will lead to the formation of tumor due to uncontrolled cell proliferation.

(p) The maintenance of the G1/S cell cycle checkpoint is dependent on the tumor suppressor gene (TP53). The mutation of TP53 gene is responsible for about 50% cancers in human.

(q) The gene p21 when induced by p53 binds to cdk2/cyclin E which leads to cell cycle arrest at G1/S

checkpoint. Thus mutation of p53 and p21 may lead to nonfunctioning of G1/S, S and G2/M checkpoints leading to formation of tumor.

(r) Retroviruses (RNA viruses) and DNA viruses are responsible for causing cancers in man.

(s) The oncogenes present in viruses are called **viral oncogenes (V-onc)** those in host cells are called **cellular oncogenes (C-onc)**. In host cells there are DNA sequences homologous to the viral oncogenes and are called **proto-oncogenes**. *Proto-oncogenes are responsible for promotion of* normal cell growth.

(t) Apoptosis is triggered by DNA damage (programmed cell death). A cascade of proteolysis is initiated with intracellular or extracellular activation of apoptotic pathway. The proteases involved are called **caspases**. Cell death is brought about by the action of caspases.

Section

Advancements in Genetics

Chapter

17

Gene Therapy in Dentistry

- Common strategies to treat genetic diseases
- Concept of gene therapy and its applications
- Applications of gene therapy in dentistry

We have seen already in the preceding sections of this book that diseases are caused by defective gene structure and function. Several strategies have been adopted to treat genetic diseases caused by such defects. These strategies are either direct or indirect interventions directed to correct those defects. Indirect methods apparently try to treat the 'results' of the disease whereas direct interventions try to mend the 'cause' (genetic defects) of these diseases. Attempts have been made to cure genetic diseases with the correction or replacement of defective genes by molecular tools of genetic engineering. Several methods that have been adopted to treat genetic diseases at different levels have been discussed in the subsequent sections of this chapter.

COMMON STRATEGIES TO TREAT GENETIC DISEASES

Prenatal Treatment

The scope of treating genetic diseases in fetuses inside the uterus is absolutely negligible till date. Positive prenatal detection of any genetic disease mostly results in its spontaneous or voluntary termination due to the absence of the option of a permanent cure at the genetic level. There is of course some hope that prenatal treatment for few diseases may evolve in the near future.

A few disorders like **congenital adrenal hyperplasia** (CAH) and **severe combined immunodeficiency** can be treated inside the uterus (in-utero) to some extent. Low doses of dexamethasone are given throughout the pregnancy on detection of **congenital adrenal hyperplasia** (CAH). The later disorder may be corrected by transfusion of stem cells that give rise to immune precursor cells. In both the cases the intervention or the therapeutic correction is aimed at the level of the gene products and not at the level of the genes.

It is hoped that gene therapy may become a possibility in near future to treat a genetic disease at the level of the genes. This treatment can be extended not only to living patients but to fetuses detected with the disease *in-utero*. It is hoped that stem cell transplantations *in-utero* may treat many serious early onset genetic diseases. *In-utero* gene therapy has successfully treated cystic fibrosis in mouse. Treatments at the molecular level include transplantation of stem cells (containing normal genes) at the site of defects or comprise replacement of the defective genes from affected cells within the body. Somatic cells can also be taken out of the body, injected with the normal replacement of genes and then put back inside the body.

Postnatal Treatment

Cure for most of the genetic disorders is not available due to our incomplete understanding of links between defective genes and their products. The therapies directed towards genetic disorders today mainly depend upon supplementation of deficient gene products (enzyme, protein, etc.) from extraneous

sources. Compounding our limited knowledge of the dynamics of genetic disorders is the difficulty to deliver gene products into the cell for intracellular metabolism. Replenishment of secretory products of cells into the extracellular milieu is comparatively a better strategy to combat a genetically mediated deficiency. On the other hand corrections have been attempted in the abnormal genes themselves. Genes in the germ-line as well as somatic cells in affected tissues have been manipulated in order to integrate normally expressing elements in them. Though no single and foolproof therapy exists in treating genetic diseases, strategies as described below have evolved to minimize the disabilities rising thereof.

The following section discusses the strategies that have been conceived and are being worked upon for developing therapies for treating genetic disorders including dental diseases.

Supplementing a Gene Product

Genetic disorders resulting in the deficiency or reduced effectivity of a gene product (enzyme or protein) can be treated with supplementation of the product from outside. Recombinant DNA technology has proven to be a boon in this regard as it can yield large amounts of polypeptides that can be introduced in affected individuals.

Treating with Drugs

Drugs with varied pharmacotherapeutic effects intervene and allay symptoms in a few of the genetic disorders of metabolism. **Cholestyramine** helps to reduce cholesterol levels in familial hyper-cholesterolemia as does the chelating agent penicillamine in Wilson's disease (in defective copper metabolism).

Transplantation or Removal of Tissue

Several approaches of reconstructive surgeries (autologous or allograft bone replacement) are usually tried in the patients who suffer from a major loss of alveolar bone loss. It is a priority to preserve the affected tooth and/or restoration of the diseased tooth is favored instead of sacrificing the tooth. The results of conventional therapeutic modalities in treating genetic diseases are mostly unpredictable at best. Activation of body's own reconstructive mechanisms can be targeted with gene therapy in such cases to hasten recovery. Manufacturing of tissues like bone has been attempted from within the body rather than from without.

Stem Cell Transplantation

Stem cell transplantation seems to be a viable option in the near future as a strategy directed for treating genetic diseases. Patients suffering from certain genetic disorders involving the blood cells can be injected with precursor stem cells that differentiate into the required population of matured blood cells. Compatibility matching between the donor and the recipient is mandatory except for administration of stem cells derived from fetal umbilical cord or bone marrow derived mesenchymal stem cells.

CONCEPT OF GENE THERAPY AND ITS APPLICATIONS

Gene therapy is based on intricate principles of genetic engineering that involves correction of defective genes or their replacement with normally functioning genes in cells. Gene therapy may be of two kinds, **Germ line gene therapy** and **Somatic cell gene therapy**. Germ line gene therapy involves genetic manipulation of the defective gamete producing cells so that a normal gamete is produced and a corrected haploid complement of chromosome is transferred to the future generations. This kind of gene therapy is of course associated with its own moral and ethical issues. Somatic cell gene therapy on the other hand targets only particular tissues or organs resulting in the change of a given somatic environment of an individual. This kind of therapy is universally accepted.

Treatments with protein delivery systems have been tried for sometime now. Supplementation with growth factor enhancement can be useful in replenishing bone loses in the alveoli of the mandible. These factors increase the turnover of bone production. The effect of externally introduced growth factors is extremely short-lived with the factors getting dissolved or being broken down by proteolysis. Gene therapy can be adopted as an alternative option for sustaining the delivery of such factors for a prolonged period.

The transfer of genes can be achieved in two ways. In one procedure the desired gene and the vector (within which resides the injected gene) is introduced directly into the area of interest or indirectly through the intravenous route. The vectors are taken up by the target cells. This direct application of target gene is called the *in-vivo* process. On the other hand genes may be introduced into cells after being taken out of the body (biopsy) in the laboratory, with the help of inoculating vector viruses. These cells are further cultured (multiplied) and then put back into the host. This process is termed as the *ex-vivo* method of gene transfer.

Gene Therapy Involves the Following Steps

Identification of the Defective Gene

Several molecular techniques are used to detect defective genes (structural genes, promoter genes, etc.) that are responsible for causing disease. Identification of such genes can be done both in somatic as well as in the germ line cells.

Cloning of Normal Healthy Gene

Cloning or duplication of DNA sequences involves copying of structural genes, promoter regions and other segments of DNA that regulate the expression of that gene. The desired gene is generally cloned or copied inside a vector. The vector is capable of penetrating and depositing the foreign or corrected gene into a target cell. Once inside the cell, a structural gene may take the help of promoters that are already present in the cell for its activation.

Identification of Target Cell/Tissue/Organ

Target cells or tissues are the ones that are affected by the functioning of the abnormal gene or genes, e.g. alveoli of the mandible that suffer from bone loss. Cells are taken out from the organ/tissue, genetically manipulated and then they are introduced back into the blood stream. The engineered cells 'home' at the target regions to resume normal function. Corrected genes may also be introduced directly at a desired location inside the organism.

The Method of Insertion of a Normal Functional Gene in the Host DNA

A ***physical and chemical method*** of gene transfer includes microinjection of DNA into the cells by electroporation (permeability of the cell membrane is increased by application of electric current), Calcium-phosphate precipitation where endocytosis of the DNA element is facilitated by precipitating it with calcium-phosphate. Cationic liposome mediated gene transfer is another technique in which synthetic cationic lipid vesicles encapsulating DNA particles fuse with specific cell membranes and release DNA into the cell. The methods of physical gene transfer techniques have evolved with time and graduated from the most basic direct injection of the DNA (micro-seeding technique), usage of electrically charged aqueous liposomes (bags of lipid associated DNA) that pass through the cell wall, to the more sophisticated processes of gene delivery by the macromolecular conjugate method where a negatively charged target DNA molecule is attached to an oppositely charged chemical substance or antibodies that bind to certain receptors on the cell wall with subsequent endocytosis of the DNA construct to the interior of the cell. More advanced physical methods include the transfer of genes with the gene activated matrices (GAMS) where naked DNA fragments are carried on polymer matrix sponges. Non-viral or physical methods are attractive tools for gene delivery as they are safer than the viral methods. DNA of relatively large size also can be delivered with the physical technique. The drawback of the physical method is that it is not as efficient as the viral methods because of its complicated designing and application. Though repeated application of gene transfer is possible with the physical technique but the effect of transfer is short-lived.

The most common method used in gene therapy is the ***viral vector method for gene transfer***. Adeno-viruses and retroviruses are the most used vectors. Adenoviruses are DNA viruses and do not integrate their DNA into the host genome. The disadvantage with this vector is that the introduced gene may be unstable. The inserted gene is activated outside the host genome. Retroviruses are RNA viruses that integrate into the host DNA. The inserted stretch of viral RNA uses the cellular machinery of the host to synthesize selected proteins from the viral genome but multiplication of the entire virus particle is not allowed by deliberately silencing certain regions of the incorporated viral genome. This prevents propagation of the virus themselves. Though their uses have not been widely reported, lentivirus and herpes simplex

virus are some of the other example of viruses used for this purpose. Adeno-associated viruses are also gaining acceptance for their selective benefits for this technique. All said and done, the transferred gene would only function normally when the coding regions for the gene as well as their regulatory elements are present in the host and more so, when they are correctly aligned. The designing of a perfect genomic architecture is the biggest challenge for the scientists. The selection of types of promoters that influence the expression of introduced genes is an important aspect of gene transfer. The promoters are responsible for persistent, stable and elevated levels of gene expression. Erroneous selection of viral promoters has shown unregulated expression of undesired mammalian host genes. Promoters are varied in function and as such are put to trials before tagging with specific transferrable genes. The application of tissue-specific promoters is gaining popularity as they allow the genes to be expressed only in specified tissues.

Characteristics of Different Viruses used in Gene Transfer

Viral methods are actually nature's own mode of gene transfer. Scientists have adopted this technique for delivery of genes to the target cells. Though an efficient technique, viral transfers of genes have their own safety concerns. The criteria for selection of a definite type of viral vector depend upon the tissue target, the duration for which the expression of the transferred gene is desired and the size of the concerned gene to be transferred. Viruses have different characteristics in terms of their replication. Retroviruses infect only dividing cells whereas adenoviruses and adeno-associated viruses infect both dividing and non-dividing cells. Retroviruses can attach into desired region of the host cell DNA leading to a prolonged and stable expression of the gene. The disadvantage with retroviruses is that its application may cause mutations in the host genome by integrating the gene at 'risky' regions in the genome.

Adenoviruses on the other hand introduce DNA into the host cell where these DNA remain independent (called Episomes) and do not integrate into the host genome. Thus with each cell division the number of cells that contain the introduced DNA is reduced. This results in the period of expression of the introduced gene being reduced. However, adenoviruses can be generated in huge numbers and as such the viruses can be introduced in large numbers directly to the desired tissue (*in-vivo*). Adeno-associated viruses integrate desired genes to sites in the genome that are not 'risky' in terms of mutagenesis.

Retroviruses are preferred and used for an *ex-vivo* type of gene transfer where cells like the blood or bone marrow cells are briefly taken out of the body and infected with the virus. These cells are then reintroduced into the body. The size of the introduced gene is a limiting factor in developing a fully functional vector. Adenoviruses are the tiniest of viruses and can accommodate a foreign DNA that is only a fraction of the size of its own DNA.

An important step before introduction of the vector virus into the host cell is to render it completely harmless and incapable of self replication within the host cell to cause damage or disease. Viruses are rendered deficient in replication by means of deleting certain elements from their genomes that are involved in replication. These viruses can be manipulated to grow only in laboratory settings and not in any settings outside the laboratory.

Ribozymes are certain types of RNA molecules that can act like an enzyme to cleave and destroy mRNA transcripts of cancer producing genes. Experimentally designed ribozymes directed against transcripts of the E6 and E7 genes of the oral cancer producing Human Papilloma Viruses (type 16 and 18) have been shown to cut and destroy the mRNA of those E6 and E7 proteins that cause defects in the cell growth regulation and produce tumors, especially oral cancers. The DNA encoding such ribozymes can be introduced inside replication–deficient viral vectors and then these vectors could be used to transfer the gene into the oral mucosal cells to stop E6 and E7 translation and prevent unregulated cellular proliferation.

As discussed in the previous chapter as well as in appropriate segments in the book, the advancement in molecular biology has enabled us to understand the nuances of the development of human structure and the importance of several molecules that work in tandem and with immaculate precision to bring forth flawless and wonderful functional structures. The concept of molecular dentistry is fast gaining its due acceptance as research is progressing toward a detailed

understanding of dental diseases and their management. The human genome project, transcriptomics, proteomics and related developments have revolutionized the discipline of basic sciences. Clinical research is facilitating the application of the ideas of basic science to the benefit of the patients. The oral health professional community, of late, has emphasized their commitment to the need of improving standards of oral health care, education and training about research innovations, discoveries and their clinical applications like never before.

The capacity to design and fabricate tissues and organs has been achieved with interdisciplinary research involving material scientists and biologists, and is no longer a distant dream. Revelation of the regulations of molecular biology has enabled scientists to design models that simulate or mimic biological system. 'Biomimetics' is a new concept that uses genetics and stem cell biology methods to engineer biomimetic cartilage, bone, muscle and nerve tissues that have been applied to tackle clinical problems. Such an approach can be applied through molecular dentistry to improve soft and hard tissue engineering and towards regeneration of tooth and salivary glands.

It is to the credit of scientific advancement that it has also transformed imaging procedures. Starting from the application of simple dental X-rays, to the use of magnetic resonance imaging (MRI), 360 degrees craniofacial-oral-dental imaging, computer-assisted tomography, ultrasound imagining, digital radiography and innovations such as biomarker reporter molecule detection, usage of these modalities have changed dimensions of medical intervention. Recent advancement in molecular genetics has not only aided to the diagnostic confirmation of a disease but also have pinpointed to the etiology of a disorder. Molecular techniques have identified the disease causing events or molecules to ultimate perfection and these tools have also given precise insights to the genetic maps and mechanisms dynamically involved in producing the disease.

Genetic as well as environmental factors affect tooth agenesis. Hypodontic individuals may show the characteristic in isolation or as a feature along with other traits of a syndrome. In both the cases though, hypodontia is determined genetically. Non-syndromic hypodontia involve the Msx1, Pax9, and Axin2 genes. A few important genes associated with early embryonic development like the Shh, Pitx2, Irf6, and p63 have been implicated in several syndromes that induce dental agenesis. Molecular therapies and bioengineering methods can be used along with dental implants and other conventional treatment modalities for treating tooth agenesis.

The interaction between the genetic and environmental factors is complex and thus it is more difficult to implicate a single factor in the development of a dental disorder. Yet we can simplify to understand that there are conditions that are simple and result from single gene defects. The more complex conditions result from the interaction between a set of defective genes and environmental influences.

It is also imperative that one understands the mechanisms and events that shape the development of the craniofacial complex. These events are guided under strict molecular control. Details of each of the genetically defined dental anomalies are available in the book. In the subsequent section of the chapter we would preview the potential application of molecular treatment in dental disorders.

Genetic conditions may be simple (single gene regulated) and complex (multiple gene and environmentally regulated) situations. Genetics and molecular events related to single and multiple gene disorders are discussed in the appropriate sections of the book.

Gene therapy was tried for the first time on a child suffering from ADA deficiency. Absence of the adenosine deaminase (ADA) enzyme results in inactivation of the white blood cells leading to incapacitation of the immune system. WBC's culled from the boy were allowed to mix with viral vectors containing normal ADA genes. The normal genes got transferred into the white blood cells through the vectors. These WBC's were cultured further and transfused back in large numbers into the patient. Though the patient required repeated transfusions of the same kind at repeated increasing intervals, this effort paved the way for others improved techniques to follow.

APPLICATIONS OF GENE THERAPY IN DENTISTRY

Use in Bone Repair

In-vivo gene transfer technology is utilized with adenovirus acting as vectors to carry the BMP genes

to the diseased area. This recombinant adenovirus (Ad-BMP) population is directly injected to the site of the bony lesion. Lesions of periodontal diseases or surgical wounds can be healed and osseous defects can be treated with new bone replacement. After being delivered, the genes encoding for bone morphogenetic protein-7 (BMP-7) in the virus tend to upregulate the bone forming mechanisms in the local diseased area and heal large wounds around dental implants in the supporting bones. BMP-7 belongs to the family of cartilage and bone producing gene family.

A mixture of the BMP gene and Adeniovirus has been successfully introduced into target cells at the defect. When inside the host cell, BMP-7 genes are seen to be guided near the host genome by the virus to precise locations where they are required to be present. The host cell stimulates the expression of the BMP that peaks in about ten days. The expression gradually tapers with time as the target gene does not get integrated into the target cell genome and do not get multiplied or replicated at the time of cell division. Thus the effect of the gene is temporary and to the advantage of the treatment.

Ex-vivo methods are also used to transfer BMP 2 and BMP 7 to the target cells. These genes are introduced into cultured keratinocytes outside the body and then introduced to the desired affected areas. The genes help to repair bones, ligaments and the cementum. New bone and blood vessels can also be formed from stem cells that are induced to express bone morphogenetic proteins.

Use in Salivary Glands

Gene transfer has successfully been tried in the salivary glands both with the *in-vivo* and *ex-vivo* models. The salivary glands are vulnerable to radiations applied to treat cancers of the head and neck regions. These structures also commonly get affected irreversibly with several *autoimmune* diseases (Sjögren's syndrome, etc.). Repair in salivary glands has been achieved by inserting the gene that encodes the water channel protein aquaporin – 1 or AQP-1 into the ductal cells of the gland. This results in the nonsecretory cells of the ducts of the glands being converted into secretory cells thereby restoring the function of the gland. In another example of gene therapy, a definite gene for example, the one responsible for synthesis of the polypeptide histatin is delivered into the cells in the gland resulting in increased levels of its production in the saliva. Histatin being a natural anticandidal polypeptide is postulated to be effective in preventing or treating resistant oral candidal infection. Oral candidiasis is common in AIDS and also occurs secondary to dental implants.

Though the salivary glands are exocrine glands, they can be manipulated to act as an endocrine gland by gene transfer. Genes encoding hormones like the growth hormone can be introduced into the salivary gland. The new endocrine secretions from these glands are carried from the acini directly into the blood and serum.

Application of immunomodulatory properties of stem cells can be utilized to combat autoimmune diseases. Specific and local activation of certain genes also can act as mediators of immunomodulation and can prove to be good methods for restricting autoimmune diseases related to salivary glands found commonly in dental practice.

Use in Pain Management

The management of pain involves the participation of maximal resources in dental as well as medical practice. As it is well-established that the intrinsic mechanisms in the body to combat pain depends upon the expression of the endogenous opioids and their receptors, gene therapy has emerged as a promising tool for the management of pain at different levels. Managing or eliminating pain is a major part of dental practice. The use of viral vector mediated gene transfer is being experimented as the technique to achieve expression of specific genes in the host cell. The genes enhance the expression of endorphins and enkephalins and simultaneously upregulating the expression of the μ, delta and kappa receptors. This activation of opioid systems at the levels of the peripheral and the central nervous system causes delayed conduction of nociception with induction of analgesia.

Use in Periodontal Diseases

The introduction of the *Porphyromonas gingivalis (P. gingivalis)* fimbrial gene into the salivary glands through plasmids has been tried successfully with adenovirus recombination. This experiment has resulted in two outcomes. The DNA delivered directly

into the salivary glands of the mice has lead to the production of immunoglobulins like IgA, IgG in the saliva as well as antibodies IgG in the serum. The salivary antibodies are able to reduce plaque formation by neutralizing the plaque forming organism *P.gingivalis*. Researchers have also identified and isolated the fimbrillin gene. The fimbrillin protein is one of the surface proteins of the organism *P.gingivalis*. Recombination and transfer of the fimbrillin gene through adenovirus vectors into salivary glands is expected to secrete the protein fimbrillin locally around the gland and in the saliva. The availability of fimbrillin in the saliva would attach to the pellicle elements and thus prevent the harmful *P. gingivalis* to attach to the pellicle and form plaque.

Periodontal diseases can be controlled by preventing the process of tight microbial attachment to the infecting surfaces. The degree of virulence of a pathogen depends to a great extent to the levels of attachment of the pathogen to a surface. Adherence is brought about by the expression of "tight adherence genes" as found in a certain strain of Actinobacillus. Localized and destructive periodontitis results from Actinobacillus. The strength of adherence adds to the degree of pathogenicity of organisms. A strategy has been evolved that uses application of artificially mutated strains of the organism deficient in the 'Tight adherence' gene. These strains when introduced with the virulent strains of organism colonize with them. This cocolonization of the mutated with the virulent strain causes limitation in the extent of pathogenic colonization of the organism. The spread of periodontitis can be prevented with the help of application of this model of genetic engineering.

Similar to the strategy applied to expedite bone growth, osseous defects in the periodontal region can be addressed by the application of *in-vivo* or *ex-vitro* gene transfer of BMP 7 and BMP 9 genes with the help adenovirus vectors into affected regions in the oral cavity. BMP 2 can expedite the formation of blood vessels.

Stem cells with specifically activated genes may also differentiate into osseous tissue on application into the defects.

Hard and soft tissue regeneration is distinctly related to the growth factor called the Platelet Derived Growth Factor (PDGF). This factor is a potent substance and has profound action on cellular proliferation. In situations of tissue injury the interactions between the receptors for this molecule and the PDGF is disrupted that limits the activity of the growth factor. Investigators have tried to transfer the PDGF gene through an adenovirus to the injured areas in order to enhance cell signaling and proliferation.

Use in Keratinocytes

Keratinocytes are preferentially used as targets for the study and therapeutic application of gene therapy. This is due to the fact that keratinocytes being epidermal cells are easily accessible. Culture models for keratinocytes are well-founded techniques. The technique of gene transfer as well as their subsequent therapeutic application and monitoring are simpler in keratinocytes.

Researchers have used the *ex-vivo* method to transfer genes into cultured keratinocytes with retroviral vectors. These viruses insert the foreign gene permanently into the keratinocyte genome. The keratinocytes are then cultured easily in sheets and are applied for treatment in specified areas. This technique can be used as gene product delivery systems in the oral mucosa and elsewhere. As keratinocytes are well-designed to deliver proteins, epithelial sheets have already been experimentally made that deliver proteins like *apolipoprotein E.*

Use in Cancers of the Head and Neck

As mentioned elsewhere in this book the role played by the p53 molecule in detecting structural DNA damage is of immense importance. This system of surveillance identifies defective DNA and stops the progress of the cell cycle and instructs either a DNA repair or cellular apoptosis. Efforts are being made to develop adenoviruses that when introduced into the system replicate and destroy only those abnormal cells that contain a mutated p53 gene. Normal cells remain unaffected and re-populate the tissue. Such a therapy can boost the outcome of treatment in cancers if they are tried along with the conventional modes of cancer therapy. The genomes of these viruses are manipulated in such a way that their propagating machinery is activated only in conditions where it detects an abnormality in the host p53 molecule.

As discussed earlier, the application of the ribozymes to inactivate the Human Papilloma Virus (HPV 16 and HPV 18) proteins E6/E7 that mediate

cancerous growth in the oral cavity has led scientists to create recombinants using the DNA coding for those ribozymes from the protein mRNA. This strategy is under development and investigators hope that its application would not only halt progression of a primary tumor but also help to scavenge dysplastic cells not yet turned malignant.

Use in Growing New Teeth

Though quite futuristic in outlook, the idea of growing teeth in the laboratory and transplantation to edentulous patients has been worked upon for some time now. This feat of bioengineering would create teeth almost with the composition similar to normal teeth but without nerves or blood vessels. This effort would involve the identification and activation of several genes that are associated with synthesis of over more than 25 proteins constituting dental tissues. The discovery of the role of the master gene PAX 9 will help to understand the sequence of gene activation critical for fabrication of tooth in time to come.

Dental tissues or dissociated dental cells have been used for at least sometime now for tooth engineering purposes as a part of recombination experiment. Recently, of course, certain type of stem cells and types of non-dental cells have been applied in tooth bioengineering. These cells range from mesenchymal stem cells, bone marrow stromal cells to dental pulp stem cells. In 2009, researchers at the Akita University in Japan have reported a novel epithelial-mesenchymal interaction experiment. The report explains an attempt of tooth regeneration by recombination of intact dental epithelium with a transformed, continuous dental mesenchymal cell line (see Suggested Readings) called the odontoblast-lineage cells (OLC). Interestingly, these cell lines were grown on three dimensional, *in-vitro* organ culture constructs and also transplanted beneath the renal capsule in mice as an *in-vivo* experiment. The OLC seem to have shown induction of dental development in both the *in-vivo* and the *in-vitro* models.

Other Modalities of Bone Repair with Gene Therapy

The introduction of *Bone sialoprotein (BSP)* in areas deficient in osseous tissue can trigger alveolar and periodontal bone proliferation. BSP is expressed in the event of bone repair and regeneration. This gene controls cell differentiation. It has also been found that the BSP is under the control of the mastergene Cbfa. Bone sialoprotein is non-collagenous in nature and one of the chief constituents of bone.

The application of the new NTF-hydrogel technology is based upon the delivery of a nonviral gene mixed with a hyaluronic acid-derived, non-immunogenic gel at the site of an osseous defect. This technique can be used as an adjuvant to conventional therapies. This method does not invoke any immune reaction and helps in bone regeneration by inducing the resident cells at the neighboring sites of the wound to add new bone to fill the defect.

Vascular endothelial growth factor (VEGF) delivery into rat mandibular condyles involving *in-vivo* technique have proven to be of help in cases of craniofacial deformities. This growth factor when delivered using *adeno-associated virus* (rAAV), have shown subsequent increases in certain osteogenetic and chondrogenetic markers accompanied by increase in the size of the mandibular condyle (See Suggested Readings).

Delivery of antiapoptotic genes like the Bcl2 gene to the site of tissue injury could be effective in recovery. This process involves much more localized delivery of the gene. The gene is actually processed with the *gene activated matrix (GAM)* technology (as done with NTF-Hydrogen) prior to its application. These "*DNA devices*," are the latest concepts in fabrication of special dental implants. Implantable products are made biocompatible by coating them with polymers capable of incorporating intact DNA molecules. The delivery of specific genes at the required sites creates implants with site-specific gene delivery.

Use in Periodontal Vaccination

The immunization of the salivary glands with non-virulent DNA encoding *P. gingivalis* and its fimbrial protein using plasmids and adenovirus has been discussed in the preceding paragraphs. Vectors like the *Streptococcus gordonii* have successfully been tested in animal models against the organisms like the *P. gingivalis* that cause periodontitis. It has been observed that inoculation of hemagglutinin in a certain variety of rats increases the levels of IgG antibodies as well as enhances the production of interleukins as an immune response. The availability of these immune

mediating factors induces protection against attack of *P. gingivalis*. Since hemagglutinin has been identified as one of the virulence factors of *P. gingivalis*, the production of antibodies against hemagglutinin provides such a protection.

Genetic Approach to Biofilm Antibiotic Resistance

It is interesting to note that certain microorganisms become resistant manifolds to antibiotics as they start living in microbial colonies attached to surfaces. This phenomenon is called biofilm formation. The reasons for the development of such resistance are not well understood and may be attributed to the activation of definite genes like the ndvB, which is related to the synthesis of the enzyme glycosyl transferase. Glycosyl transferase is further linked to the production of periplasmic glucans that impart them resistance against disinfectants and antibiotics. Scientists have been able to identify, isolate and replicate a mutated version of the ndvB gene. This gene when introduced into some of the replicating cells in a pseudomonas biofilm, rendered the other members of the biofilm vulnerable to common antibiotics. Such an approach can be adapted for application in dentistry to destroy resistant bacteria in a biofilm.

Use in Alveolar Remodeling

Alveolar remodeling is a natural phenomenon that occurs due to stress, injuries and inflammation of the periodontal tissue. The alveolar structures including the bone undergo active remodeling as a reaction to mechanical stimulation. The process of remodeling can be expedited by enhancing the expression of several factors that induce and maintain alveolar remodeling. This can be achieved by the transfer of the LacZ gene into the periodontal tissue directly with the help of a plasmid. The integrated gene within the plasmid can be introduced into cells with the application of an electric impulse (electroporation).

Used in Antimicrobial Control Disease Progression

Host defense mechanisms can be boosted with the introduction of genes that contribute to host cell defense against pathogens. This boosting may be done with supplementation of genes encoding certain antimicrobial agents. These factors or genes can be introduced into the host cell through retroviral mediated *in-vivo* techniques into the host genome at areas susceptible to infections. Some proprietary products are available that applies the defensin-2 gene for this kind of an effect. The above discussion on the application of gene therapy can be reviewed in terms of the basic designs of gene transfer into the cell. The approach adopted for gene delivery may be an *in-vivo* technique involving gene constructs trapped in physical or viral agents and delivered into the cell. The *ex-vivo* method transfects cells in culture *in-vitro* and then introduces them into the target cells in the body. The protein-based methods apply the gene products to the required regions and the cell-based approach uses mesenchymal stem cells for activation of tissue repair.

Constraints and Limitations of Genetic Therapy

Though a lot has been written both in favor and against the application of gene therapy, the message is clearly home that a foolproof therapeutic package involving gene therapy still needs some more ground-work to become a practicable reality. The regulatory authorities have been rightfully alarmed by outcomes of certain trials and are skeptical about the safety as well as the feasibility of such therapies. Planners have reiterated the need of extensive preclinical trials of novel therapies before they become standard modes of treatment. Other issues related to the confidentiality of genetic information, disclosure of susceptibility concerns and the risk statuses of individuals are a few of the ethical aspects that need to be addressed in context of gene therapy.

There are several systemic disorders associated with specific types of periodontal diseases. Treatments in such cases are basically framed on the logic of treating not only the defect within terms of the parameters of dentistry but treating the symptoms of the disorder as a whole. Chronic and early-onset periodontitis need chemical and mechanical control of bacterial plaque. Severe congenital neutropenia or depletion in IgA levels my cause premature loss of teeth and need antibiotic prophylaxis along with chemical and mechanical control of bacterial plaque.

Conditions associated with hormonal changes and arising due to unresponsive bacteria call for extensive and rigorous bacterial control.

Diseases and traits that are genetically transmitted have been studied extensively and analyzed for their causative molecular defects and the modes of their inheritance. The frequency of occurrences of the coding elements of the genome has been studied along with that of noncoding sequences in the DNA. Certain disease causing genes have always been found to occur along with certain noncoding sequences. They have been identified always to occur together and the details of this occurrence is analyzed in linkage studies. This phenomenon occurs perhaps due to the close proximity of these two segments in the genome that always segregate together in the gamete. As stated earlier, molecular research has revealed that specific regions of the non-coding regions are intimately associated with the inheritance of a particular gene. HLA associations of the disease producing genes have also been discussed earlier. Several thousands of similar genes have also been found existing across different organisms. The sequences of these stretches of DNA have not been defiled or disturbed by time and evolution in the organisms. The genes and allied segments in the genome are said to be highly conserved in terms of structure and function. The origin of these genes and their subsequent distribution in the nature can be studied by analyzing their inheritance and linkage patterns.

The virulence of certain microorganism as well as susceptibility to diseases in an individual is determined by the genetic make-up of the microorganism as well as the individual. Craniofacial birth defects, orthognathic disorders, abnormal tooth size and shape, cancers, temporomandibular joint diseases and several others are linked to outcomes of gene-gene, gene-environment interactions.

Though gene therapy seems to be the panacea for all genetic disorders, it has its own share of limitation and pitfalls. The technique of gene delivery is tedious and difficult. Even if the gene causing a disorder is identified and mutations are well-defined, an attempt to introduce the corrected version of the gene in a cell may not be successful. The limitations range from difficulty to pinpoint the exact gene responsible for a disease (except for a single gene disorder), developing an ideal vector for a gene, identification of the site of delivery, compatibility of the environment in the host tissues and eventually the normal and desired expression of the inoculated gene in the system. The success of gene transfer cannot be predicted successfully specially in cancers as there are multiple affected sites in the system, which makes the decision of selection of the target region difficult. Some of these problems can be circumvented with the understanding of the mechanisms of viral replication and gene regulatory pathways.

Issues related to the durability and integration of the transferred gene is of immense significance as the desired period for sustenance of therapeutic benefits from a gene transfer varies with the type of the disease being addressed. Genetic integration of the transferred material into the host genome provides a long-term replication as well as expression of the gene. Such functional durability comes at the cost of certain risks of undesirable effects. Unwarranted and unexpected integration of genes at different locations may trigger and disastrous consequences. Multiple introduction of gene therapy is possible with physical agencies but frequent repetition of gene transfer using viral vectors is not recommended.

Precise introduction of genes is the prime requisite for delivering nonspecific apoptotic genes that kill cancer cells. These genes don't need to integrate into the genome but become active anywhere inside the cell. Applications of these 'suicide genes' produce more immediate effects irrespective of the site of application.

The success of gene therapy also depends upon the degree of immune responses elicited by the host especially against viral vectors. Viral vectors elicit immune responses in the host against themselves if the host cell recognizes the vector as "foreign". In fact development of such immune responses is desired in the host immune system if the therapy is directed against cancer cells in treating carcinomas. Undesired immune responses reduce the efficacy of the therapy. Repeated applications of viral vector mediated gene delivery may cause increased immune mediated destruction of the viral vectors or may result in serious side effects. Usage of viral vectors may be a potential cause of toxicity, immune and inflammatory responses with the very first instance of its application.

Other than perhaps single gene disorders, more commonly occurring diseases like hypertension and diabetes are dependent on more than a single factor and hence the applicability of gene therapy in such situations is debatable. The other more contentious issues with gene therapy are related to ethical considerations like questions regarding the criteria that decide what is 'normal' and what defines 'abnormality'. 'Whether a disability can be viewed as a disease' and 'whether a somatic gene therapy is more ethical than germ line therapy' are some of the probing questions that remain to be answered. The issues of the feasibility of developing such expensive treatment modalities and the affordability of these regimes by less affluent population are also unanswered. Majority of diseases in dentistry are difficult to treat with single gene transfers.

New interventions that combine gene therapy with other approaches such as stem cell therapy are fast emerging. Gene therapy has the potential to treat diseases such as cystic fibrosis, cancers, heart diseases and human immunodeficiency virus infection. However, no clinical trial of gene therapy has resulted in the development of a commercially available treatment till date. Unsettled issues in gene therapy also include the effectiveness of delivery, longevity of the therapy and safety of the procedures. While patient groups are largely satisfied with the current disease-based approach to gene therapy research, scientists have called for more studies on vector safety, delivery techniques, identification the molecular causes of diseases and finding the reasons for uncertainty of outcomes of current applications.

SUMMARY

(a) Control of genetic diseases have been tried with several strategies applied both as prenatal as well as postnatal treatment modalities.
(b) Common strategies to treat genetic diseases include supplementing a gene product, treating with drugs, transplantation or removal of diseased tissue and stem cell therapy.
(c) Therapy at the level of genes is called gene therapy. Gene therapy may be applied to the germ line cells or directed towards somatic cell lines.
(d) Gene therapy involves the steps of identification of the defective gene, cloning of normal healthy gene, identification of target cell (tissue or organ) and insertion of a normal functional gene into the host DNA.
(e) Physical and chemical methods as well as viral vectors are used for gene transfer.
(f) Genes can be transferred directly into affected tissues (*in-vivo* process) or may be introduced into cells taken out of the body (biopsy) and then put back into the host (*ex-vivo* process).
(g) Ribozymes are certain types of RNA molecules that can act like an enzyme to cleave and destroy harmful mRNA transcripts.
(h) Gene therapy can be used in bone repair, in treating diseased salivary glands, for pain management and in conditions of periodontal diseases. Applications are also being tried to treat cancers of the head and neck region and for active alveolar remodeling.

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Chapter

18 Techniques Used in Genetics

- Recombinant DNA technology
- Polymerase chain reaction
- Nucleic acid probes
- DNA sequencing of gene or a DNA segment
- DNA fingerprinting
- Human genome project
- Stem cell research
- Prenatal diagnosis, techniques and genetic counselling
- Diagnosis of genetic disease
- Management of genetic disease

The understanding of the science of genetics has evolved along with the development of several molecular techniques. These techniques are based on basic principles of genetics and are applied to use genetic mechanisms for the benefit of humanity. Overviews of certain techniques are elicited in this chapter. Details of the techniques can be found in standard biotechnology textbooks.

RECOMBINANT DNA TECHNOLOGY

In very basic terms recombination of DNA implies the insertion of fragments or more specifically, insertion of desired genes into certain host cells to utilize the inherent replication mechanisms of the host to produce multiple copies of the gene. This is nothing but cloning of the specific sequences of DNA and thus the process is also termed 'genetic engineering'.

The specific gene to be cloned may be derived from sources such as another genome of an organism or artificially synthesized in the laboratory. Before the desired fragment of DNA is inserted into a suitable host cell, it is processed by separation from its source. This processed segment has two cut ends that integrate into the host genome and is called the recombinant DNA.

Idea of Recombination from the Nature

Interesting observations on the genetic behavior of bacteria and viruses have inspired the application of those mechanisms to evolve genetic techniques.

Bacteria and virus are called **prokaryotes** as they do not have cell nuclei. **Eukaryotes** are all the other organisms that possess a well-defined nuclear membrane. Bacterial DNA exists in the form of a looped thread-like chromosome or in the form of several smaller ring-shaped genetic material called **plasmids** (Fig. 18.1). The enormously rapid replication rates of bacteria and virus make them favorites for becoming the host cells in recombination techniques.

Plasmids have the unique property of easily entering a cell and promptly using the cellular mechanism for its replication. Scientists target plasmids for attaching the desired gene to transport them into the host cell. Thus the plasmids act as vectors of DNA.

Though the virus contain nuclear material inside their protein coat, it is mandatory for them to take the help of any other host cell for replication as they lack replicatory enzyme mechanisms of their own. The

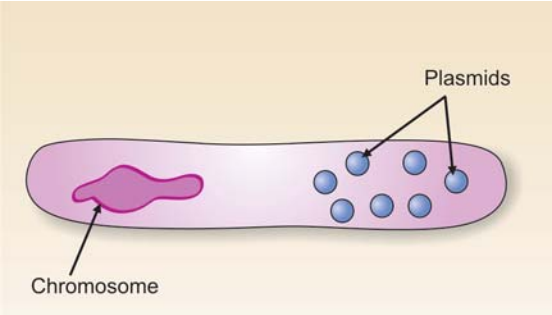


FIGURE 18.1: Schematic diagram of bacteria: The DNA of bacteria is found in the form of closed loop chromosome and plasmids

viruses usually infect bacteria as the host cell, replicate its components inside them, assemble and eventually rupture the bacteria to come out. Recombinant DNA is integrated into viral (bacteriophage) genome and then the virus acts as a vector for the integrated genome.

On the other hand certain enzymes evolve in the bacteria to fight such an invasion by viruses. One of them, the **restriction enzymes** is used as important tool by scientists in genetic engineering. Viral DNA segments can be fragment at desired sites with the enzyme. This enzyme is used extensively in cleaving required DNA segments from its source. Other enzymes like the ligases are used appropriately to anneal or join ends of DNA fragments.

Process of Recombinant DNA Technique

The sequential procedure of obtaining the desired fragment of DNA (gene) which is to be cloned, multiplication of the obtained gene in a suitable vector, combining the DNA fragment with that of the DNA of vector and transferring of the recombinant vector to the host organism comprise the process of the technique.

Production of the DNA Fragment

The cloning begins by cutting off the DNA at specific sites with enzymes like restriction endonucleases that recognize a set of short DNA sequences (4 to 8 base pair long). The enzymes are named as per their sources, e.g. Eco RI is from the organism *E. coli* and Hind III from *Hemophilus influenzae,* and number at about 300.

The endonucleases cleave both the strands of DNA but between specific pairs of bases.This cleaving produces either *staggered ends or blunt ends* at the interface of the cut ends of the DNA. These ends are called 'sticky' ends as these cut ends can unite with complementary sequences at any other cut end produced by the same enzyme on a DNA molecule (Fig.18.2).

Processing of the Vector

Vectors can be obtained from natural sources that can incorporate the desired DNA segment easily into its own genetic environment and transfer the integrated molecule into the host cell for independent and rapid replication. Plasmids, bacteriophages and cosmids are some of the common examples of vectors.

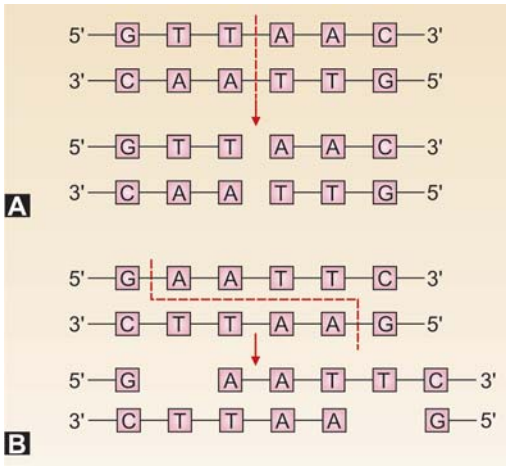


FIGURE 18.2: Double stranded DNA can be cleaved by endonuclease enzymes. A = Blunt end and B = staggered ends

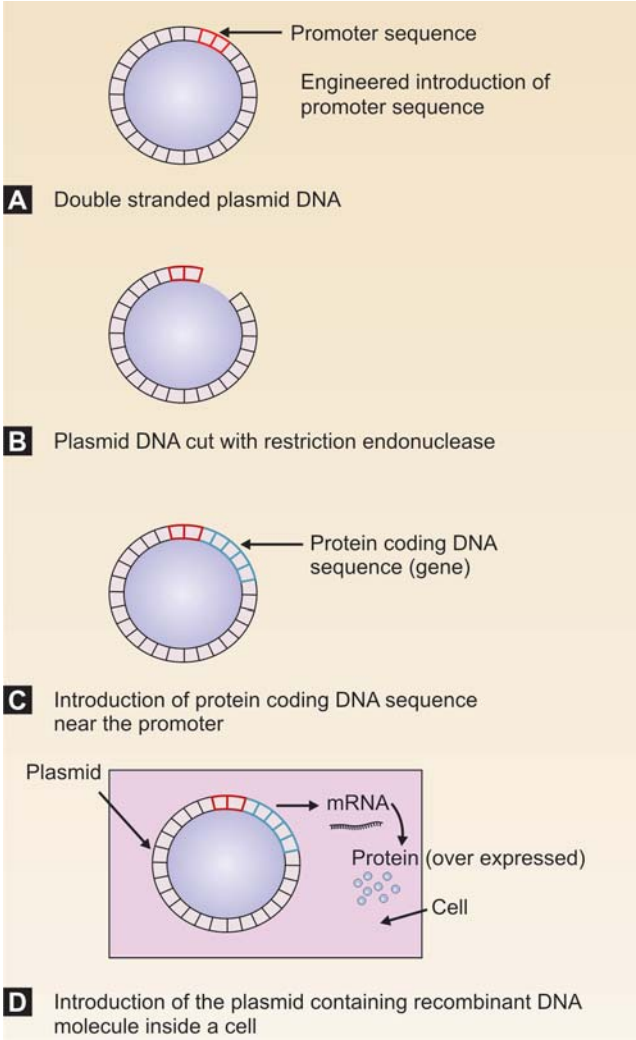
Plasmids, as already mentioned, consist of circular duplex of DNA and occur naturally in bacteria. Plasmids are obtained after disruption of bacteria and then cleaved by restriction enzymes. Restriction enzymes only act at specific sites on the plasmid. Along with the desired DNA the plasmids also may contain genes expressing factors for antibiotic resistance and thus a particular strain of plasmid can be identified by detecting its resistance against a specific antibiotic.

Integration or Recombination of Desired DNA Fragment with the DNA of Vector

Same restriction enzymes are used to cleave out the desired DNA fragment from its source as well as to break the plasmid between specific bases (Fig.18.2). This type of breaks in the DNA fragment and in the plasmid's DNA produces reciprocally complementary ends in both the 'foreign' DNA and the cleaved plasmid that combine easily. As mentioned earlier, the ends of the cleaved DNA are termed 'sticky' as they easily combine with cut ends of the plasmid. The enzyme **DNA ligase** seals and secures the attached ends. The united DNA and the plasmid molecule are then called the recombinant DNA molecule.

Transfer of Recombinant Vector to Host Organism

After the recombination of the DNA fragment and the plasmid, this particle is introduced into the host cell by increasing the porosity of the cell membrane with the application of certain chemicals or high



FIGURES 18.3A to D: Basic mechanism of recombinant DNA technique

electric voltage across its membrane (Fig. 18.3). The recombinant molecule starts replication within the cell along with the nuclear material of the cell at each cycle of cell division. Eventually hundreds and thousands of copies of the desired DNA are produced with the help of the host cell machinery.

Screening of Recombinant Vectors

The host cell or bacteria does not accept all plasmids. This selectivity of acceptance creates two kinds of bacteria in the culture media—one type containing the DNA fragment whereas the other not containing it.

The plasmids contain certain genes along their genome that impart them resistance against certain antibiotics. In case the foreign DNA is inserted into the plasmid by cleaving a particular gene responsible for developing resistance for a particular antibiotic, such resistance would be lost in this plasmid though the same plasmid would maintain resistance against some other antibiotics, the genes for which remain intact.

This property of loss of resistance due to recombination is utilized for the detection of bacteria that have accepted the DNA fragments. Separate colonies of bacteria are exposed to the recombination. Bacteria from these colonies are cultured separately and representative bacteria from individual colonies are tested for susceptibility for different antibiotic. The colony in the subculture showing susceptibility to a particular antibiotic specifies the cleavage in the plasmid and integration of the DNA fragment. These colonies from the master (primary) plate are picked and cultured separately and will contain only bacteria (host cells) with recombinant vectors (plasmids).

Screening of Clones with Specific DNA Sequence

The detection of the recombinant DNA integrated bacterium can be pursued with more refined techniques including **nucleic acid hybridization.** This method entails direct hybridization of labeled probes on to specific sequences on the recombinant molecule. The identified bacteria are isolated and culture to obtain the desired DNA segment.

The recombinant DNA molecules are thus generated and collected and this collection constitutes the **DNA library**.

Some Important Applications of Recombinant DNA Technology

Application of the recombinant DNA technology is widely accepted now as an important tool for several useful purposes such as:

- Preparation of chromosome maps and analysis of DNA sequences.
- Production of drugs like insulin, somatostatin, blood clotting factors, growth hormones, synthetic vaccines like antirabies, antimalarial, antihepatitis and cholera vaccines, interferon from genetically engineered *E. coli* to combat viral infections and monoclonal antibodies against certain organisms.
- Using in the diagnosis of genetic diseases and gene therapy.

POLYMERASE CHAIN REACTION

The amplification of DNA sequences (genes) described in the above sections is based on utilization of the host cellular mechanisms and thus is known as *"in vivo"* **cell-based cloning**. Genes can be cloned by non cellular *"in vitro"* techniques such as the **polymerase chain reaction (PCR)** that is done in machines. Copies of DNA sequences can be produced in large amounts with PCR. The essential prerequisite for this technique is that we must know the sequences of the DNA of the either sides (flanking regions) of the desired segment to be cloned. This knowledge is mandatory for the formulation of the 'primers' (discussed later). Only a very small amount of DNA (even of a single cell) is needed to produce millions of copies of the DNA fragment.

Concept of PCR Technique

As shown in the figure below, (Fig. 18.4) DNA replication needs at least a single molecule of a double stranded DNA to begin with. The two strands are separated (denatured) with regulation of temperature. Enzymes, nucleotides and primers are then added to make-up a mixture in the PCR machine (thermo-cyclers). Once added, the nucleotides get arranged on each of the denatured DNA single strand. Thus the new complementary strand along with the old strand together forms the double helix. The nucleo-tides are attached one by one to the primer to their 3'ends. The primers, as seen in the figure below, are attached one on each of the denatured starting molecule of the DNA at opposite ends. In the PCR technique, the primers (short nucleotide stretches) are added in the machine along with enzymes and the nucleotides. The primers are actually very short DNA sequences called **deoxyoligonucleotides**. DNA polymerase as well as the four nucleotides are added to the cloning mixture. It is therefore essential to have an idea of the flanking sequences of the desired gene to be cloned in order to produce the primers. These primers essentially limit the stretch of the big DNA molecule to be replicated. The DNA molecule confined between the primers is acted upon by the artificial cloning machinery to replicate the trapped segment of the DNA.

The DNA fragments, the primers, the oligonucleotides and DNA polymerase enzyme (the heat stable "**Taq polymerase**" derived from the *Thermus aquaticus*) are all incubated in the machine and the required temperature for amplification is regulated externally.

Each cycle of replication is repeated with fresh denaturation of the double helix and annealing of added nucleotides to the annealed primers. This results in the replication of a DNA segment in an exponential proportion. PCR thermal cyclers are automatic and need not be set again after each round of amplification. Modification of techniques can also produce mutations in DNA fragment, as desired.

PCR is a valuable technique used for detecting infectious agents like viruses, for prenatal investigations, tissue typing for transplantation, studying polymorphisms, evolution and several other applications.

NUCLEIC ACID PROBES

Radiolabeled probes help to recognize complementary sequences in DNA or RNA molecule. This helps to identify and isolate the specific DNA sequences from an organism. Nucleic acid probes are small stretches of DNA that can be derived from various sources. These probes anneal to

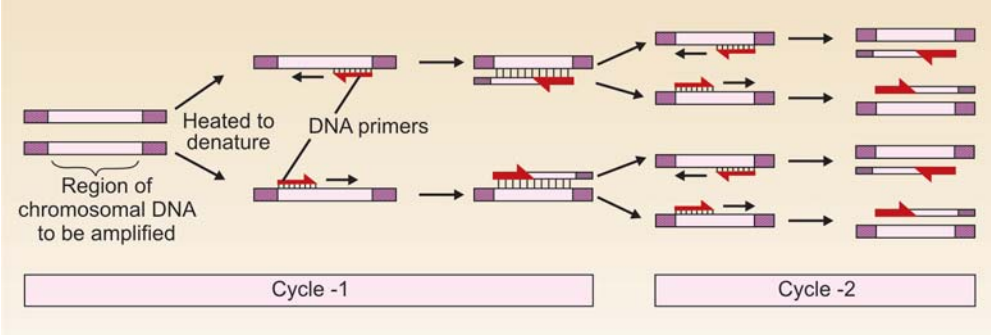


FIGURE 18.4: Schematic diagram showing polymerase chain reaction (PCR). In this diagram only first two cycles are shown

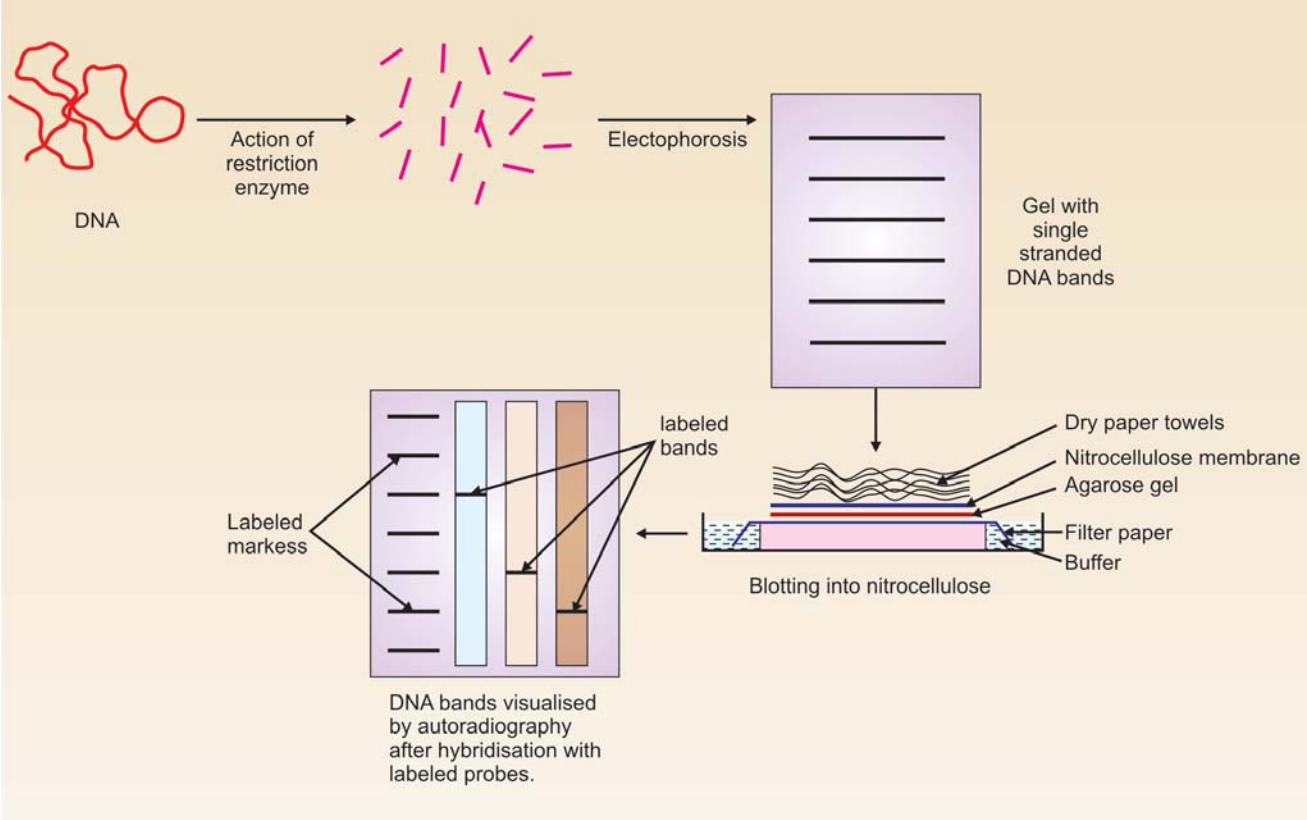


FIGURE 18.5: Southern blotting for analysis of DNA segments

complementary sequences, if these complementary target sequences are present in the sample DNA.

These probes also help in the diagnosis of infectious diseases and identification of specific causal organisms. Forensic tests (DNA fingerprinting) are based on the same principle.

Detection of DNA Segments with Nucleic Acid Hybridization

The following steps are followed sequentially to identify DNA segments of interest from a given genomic population

- After the DNA molecules are extracted, they are digested with application of restriction enzyme so that they are cleaved into multiple segments of different sizes.
- The DNA sample is run in electrophoresis where the fragments are arranged according to their sizes along the gel.
- **Bands** appear on the gel at specific intervals depending on the molecular weights of the fragments.
- These bands are stained and visualized directly in the gel.
- These bands can be isolated for analyzing their DNA sequences. A particular gene (DNA segment) can be identified within those bands with the help of radio tagged molecular probes that bind to definite denatured strand.

A particular segment in a band in the gel can be identified by hybridization with molecular probes. This process requires transferring of the band from the gel to a nitrocellulose paper. This transferring technique is called **'blotting'**. Several types of blotting are enumerated according to the involvement of different molecules.

Blotting of DNA bands on nitrocellulose paper—*Southern blotting* (Fig. 18.5).

Blotting of mRNA bands on nitrocellulose—*Northern blotting*.

Blotting of protein on nitrocellulose membrane—*Western blotting*.

Southern Blotting

Southern blotting is one of the most used techniques in genetics. DNA is extracted from a source and then digested using specific restriction enzymes that cut the DNA strand into several smaller segments at sites specific to the enzymes. This sample is then run in a gel electrophoresis equipment. The bands thus formed on the gel are then denatured with alkali (Fig.18.5). This gel is now placed between a buffer saturated paper and a sheet of nitrocellulose membrane. The movement of the buffer from the paper to the nitrocellulose membrane passes through the gel and carries the denatured single strands of DNA from the gel to the membrane (blotting). The transferred DNA is now fixed on the membrane by heating it at 80°C for 2 to 3 hours.

The membrane can now be subjected to exposure to radio-labeled probes that hybridize to specific sequences in the DNA. After proper washing of the membrane, an autoradiograph of hybridized DNA may be taken on an X-ray film for the presence of the desired strand of molecule in any given band on the membrane.

The appearance of a band at a particular level will happen only when a DNA segment of the particular length is present in the sample after the application of restriction enzyme. Similarly, attachment of specific probes will depend upon the presence of the particular DNA segment complementary to the probe. Bands at a particular level denote the presence of similar fragments that can be compared with a given reference (Fig. 18.5). Bands may be of different thickness at the same levels of reference and across the length of the gel.

DNA SEQUENCING OF GENE OR A DNA SEGMENT

As an extension to the step of DNA isolation and segment identification, one may proceed to determine the sequencing of nucleotides on the DNA molecule (DNA sequencing). Several methods are utilized as tools for sequencing and are designed on the basis of different principles of genetics.

The most commonly used technique has been the dideoxy chain termination method. The more recent automatic sequencers apply a variant of this method.

Dideoxy Chain Termination Method (enzymatic)

This is one of the earliest processes employed in the late 70's to determine DNA sequences. Denatured single stranded DNA fragments that are to be sequenced are taken in four different reaction tubes as the first step. All the tubes contain several identical copies of fragments of DNA molecules to be sequenced.

Radioactively labeled four different deoxynucleotides, enzyme DNA polymerase I and oligonucleotide primers all are added to each of the four tubes. The deoxynucleotides are molecules that anneal against complementary nucleotides on the denatured DNA strand and maintain the elongation of the new strand that is being synthesized.

Each tube also receives one of the four dideoxynucleotides and as such each tube has a different dideoxynucleotide. The dideoxynucleotide molecules are different from the deoxynucleotide molecules as the former lack in a hydroxyl group in one of their carbon atoms. The attachment of a dideoxynucleotide to the growing strand immediately stops the chain elongation. This termination of chain elongation is randomly affected. This means that the termination of chain elongation depends on the 'chance' of attachment of the particular type of a dideoxynucleotide to its complementary nucleotide in the template strand. Therefore, in a given tube we can find millions of chains of different lengths terminated randomly on the event of attachment with the specific dideoxynucleotide in the tube (Fig.18.6).

Once (in a given tube) the particular dideoxynucleotides are incorporated in the chain with stopping of chain elongation, the fragments are taken out from the tube and run in electrophoresis. Terminated chains in all the four tubes are run in electrophoresis equipment on four adjoining lanes, each lane denoting chain terminations due to attachment of four different dideoxynucleotides. These attachments are random, giving rise to chains of different lengths that arrange according to their lengths in the gel. From this information we can identify the particular terminal dideoxynucleotide that stops chain elongation and hence identify the complementary terminal nucleotide in that chain. In this way all the terminal nucleotides in all the chain fragments in all the four lanes can be computed in a

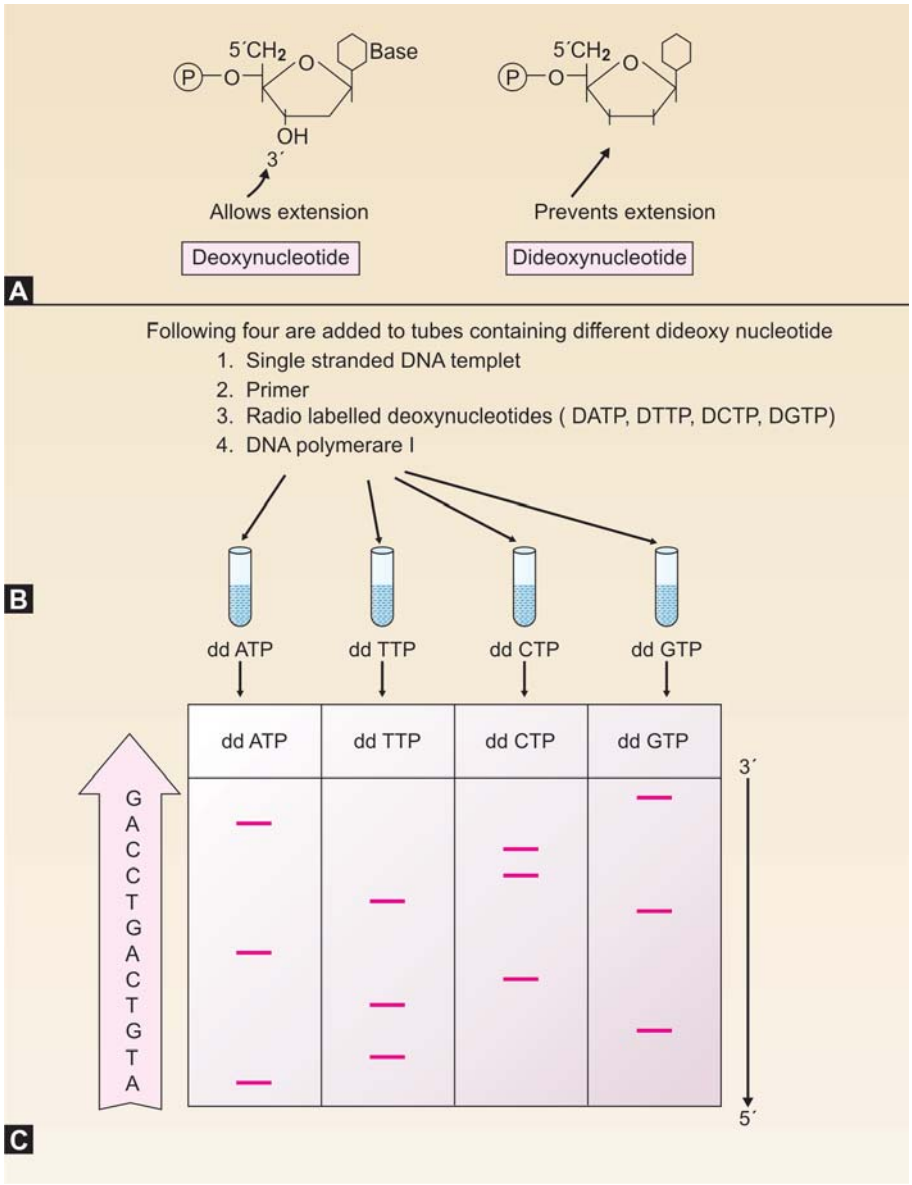


FIGURE 18.6: DNA sequencing by dideoxy chain termination method. A = showing the structural formula of deoxynucleotide and dideoxynucleotide. The absence of OH group at 3' position prevents the extension of sequence (its bond with next nucleotide). B = Synthesis and termination of segments in reaction tubes, C = Electrophoresis (Sequence of DNA given inside arrow)

sequence yielding precise sequencing of a DNA molecule. Autoradiographic methods are applied in detection of the radiolabeled dideoxynucleotides in the electrophoretic bands.

Automatic Sequencers

Modern day automatic sequencing machines are computerized and highly accurate and rapid. Different fluorescent dyes are attached to the oligonucleotide primer in each of the four reaction tubes. The resultant gel mixture is then electrophoresed in a single gel tube instead of four. A fluorescence detector measures the color from the gel tube and automatically records sequences. This method is of course a further modification of the dideoxy process. The credit for rapidity with which the human genome project was conducted goes to these automatic sequencers.

DNA FINGERPRINTING

In all humans the genome comprises of the coding as well as noncoding regions. In the noncoding DNA regions the sequences are very repetitive and are called tandemly repeated DNA sequences. The collection of these repetitive sequences imparts unique identities to individuals. The pattern of occurrence, length and number of these repeats are unique and specific for each individual. The concept of DNA fingerprinting is based on the above principle.

DNA fingerprint is an important tool for identification of individuals, settlement of disputed paternity, criminal investigations and related purposes. DNA fingerprints in identical (monozygotic) twins are exactly the same.

The DNA Fingerprinting Technique

The technique begins with obtaining DNA from a source that may be as varied as the body fluids, cells or sequestrated dead tissues often several years old (DNA is usually a very stable molecule). Obtained DNA is cleaved into smaller fragments with the help of endonuclease enzymes. The action of the endonuclease enzyme differs in individuals as the enzyme cuts individual genomes at different places due to the presence different patterns of the tandem repeats sequences in different individuals. These ununiform cuts in the genome give rise to DNA fragments of different lengths in individuals. The fragments of DNA are subsequently separated by agarose gel electrophoresis. Southern blotting is then applied to transfer the bands on to nitrocellulose (Fig.18.7)

As mentioned earlier, variability in individual genomes occurs specially in the noncoding regions in the forms of several polymorphisms. These polymorphisms exist as short (2-3 bp), inherited tandem sequence repeats like CACACA……that run repeatedly at several locations (loci) in the genome. The sequences are called microsatellites which occur throughout the genome at microsatellite loci. The numbers of such tandem sequences running at each locus may vary from 4 to 40 in different individuals. This variation in the number of nucleotide runs at these loci is called Variable Number of Tandem Repeats (VNTR) or a hypervariable satellite sequence. The numbers of these variations are different in individuals and when several of these loci are taken into account, the diversity between individuals is enormous and the chance of two random individuals sharing the same genetic pattern is one in a billion. Primers that bracket important loci with VNTRs have been developed. These bracketed variable sequences can be amplified with the help of PCR. A set of 5 to 10 such VNTR loci are chosen and amplified for the complete comparison between individuals. The band patterns of these variable sequences produced after electrophoresis are akin to the molecular fingerprint of an individual.

As discussed earlier, the presence of these variable sequences also alter the sites of action of restriction enzymes. Action of these enzymes on different individual genomes would cut the DNA at different places in different individuals. This polymorphism is called the Restriction Fragment Length Polymorphism (RFLP). RFLP in the DNA produce bands at different levels (according to the different lengths of DNA segments) in the electrophoretic gel. This property of variance is used to detect the molecular fingerprinting of a person or used to detect associations of a type of RLFP with a disease.

In the given example, a sample from the site of crime (e.g. a strand of hair from the victim's nails) is amplified by PCR. A set of 3 variable loci are selected and amplified from the sample. Since each of the loci is represented in two homologous chromosomes, 6 bands are obtained after the amplification. Samples are collected from the suspects and the same loci are amplified and banded.

Identification of the culprit (individual 2 in the example) is accurate using this technique when the overall pattern of its bands matches significantly with that with the sample (Fig. 18.7). This approach can be used in the testing of paternity. In cases of paternity disputes matching of at least half of the bands usually settles the issue.

HUMAN GENOME PROJECT

A monumental project was started in 1991 in the USA involving 16 laboratories, 1,100 biologists, computer scientists and technicians from countries like USA, UK, France, Japan and others. The design behind the **human genome project (HGP)** was to sequence the entire human genome with a view to provideing an extensive understanding of DNA sequences, the

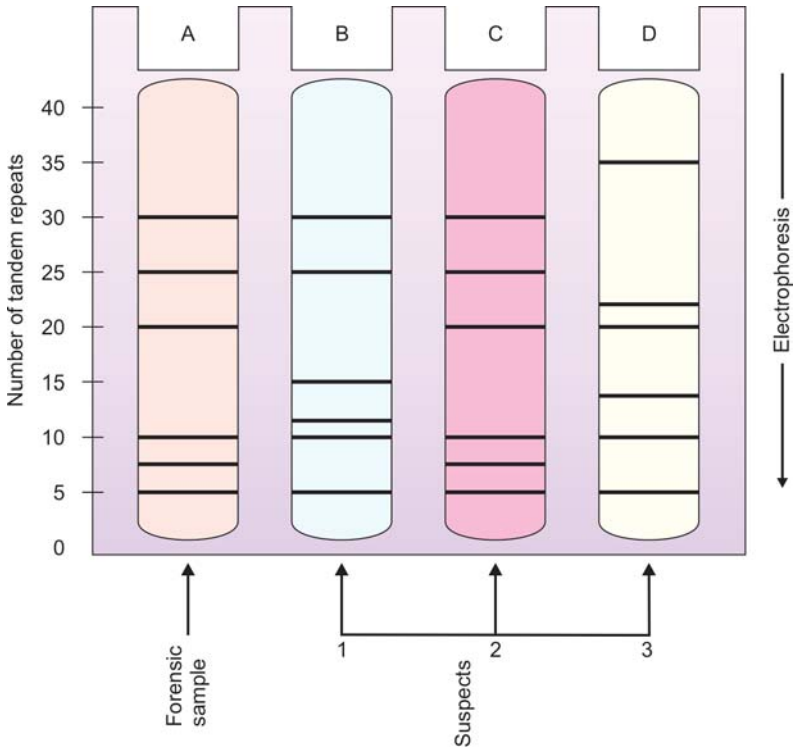


FIGURE 18.7: DNA fingerprinting.
DNA fingerprints of 3 suspects compared with that of the forensic sample (in well A). DNA bands are matched after electrophoresis. Comparison reveals that DNA bands of suspect 2 mathces closest to the sample establishing the identity of the criminal

organization, function and evolution of the human genome , to map both normal and anomalous disease-specific genes, give boost to functional and comparative genomics and bioinformatics.

Constant upgradation of techniques helped to complete this public funded project in February 2001; well before the stipulated time of 15 years. Two research facilities **the Celera Genomic Corporation** and the **HGP** working on the project released their data simultaneously to the world.

The project yielded several important observations about the human genome. About **3.2 billion** base pairs constitute the human genome with approximately **30,000** genes in contrast to the earlier concept of presence of greater number of genes in the genome. This finding, supplemented with further studies in proteomics, has forced us to reconsider the validity of the "**one gene-one function (enzyme)**" concept in favor of a better explanation for protein synthesis. The genes comprise only about 5% of the genomic bulk. Rest of the genome is made up of noncoding DNA (junk DNA) containing segments of repetitive DNA.

Human DNA can be dated (**DNA dating**) with the analysis of such repeats and family trees can be assembled that explain the source, the point of first occurrence and subsequent evolution and dispersion of any segment of the genome in an individual, family, clan or an entire race.

It is interesting to observe that several bacterial genes have been introduced by nature directly into the human genome without undergoing the grind of evolution.

Though smeared with its share of controversies, the HGP was a historical and colossal endeavor in science that has brought forth vast data and understanding that would eventually benefit the mankind, with its proper utilization.

STEM CELL RESEARCH

Stem cells are those cells that are capable of self-renewal (can divide to produce cells with same properties) and also are able to differentiate into specific types (lineages) of cells. Thus stem cells are basically pluripotent and multipotent (if not

totipotent) progenitor cells that not only divide to produce cells of the same nature but given the appropriate environment, they differentiate into other tissues as well.

It is evident that potency of cells diminishes as an individual grows from a zygote towards an adult. Therefore, it is understood that early embryos are the best sources of stem cells (embryonic stem cells). Stem cells can also be harvested from the umbilical cord at a later stage of life (cord blood stem cells).

In adults the bone marrow is a good source of stem cells (mesenchymal stem cells). It is to be noted that the potency of the stem cells depends on its source. After collection of the stem cell rich tissue, the stem cells are separated by various cell sorting techniques using sophisticated equipment as per the requirements. The stem cells bear several cell markers on their cell walls that give them specific identities. Cells are preserved at very low temperatures (cryopreservation). When desired the cells can be thawed back to normal conditions. Cells can be then cultured for multiplication or put in an environment that leads the cells to differentiate into required progenitor cells. These cells are then injected into damaged tissues where they are thought to replicate and repair the damaged organs. Embryonic stem cells are good models to study the effects of bacterial toxins, drugs, etc. One can preserve his/her own cord blood stem cells (banking) in case of a need to him (autologous transplantation) or others (allogenic transplantation) in the future. An important aspect of stem cell therapy is that it does not need stringent compatibility match for allogenic transplantations.

PRENATAL DIAGNOSIS, TECHNIQUES AND GENETIC COUNSELLING

The term "Prenatal diagnosis" is used to define the process of detecting a disease or the risk of occurrence of a disease in humans. These tests are done well before the birth of the fetus. This approach of detecting severe debilitating or fatal diseases proves to be very helpful in pregnancies with a high risk for any such genetic disease. This gives an opportunity to the couple to decide the fate of the affected pregnancy before it reaches to a very advanced stage.

Prenatal detection of dental diseases is not practiced. Prenatal diagnosis of congenital and other genetic disorders is common in cases of diseases that are incompatible to life or diseases that cause severe physical and mental disability. Detection of dental disorders may be a matter of correlation or coincidence occurring as a part of these systemic diseases.

Prenatal detection of diseases is advised in cases of high-risk pregnancies with the history of previous incidence of the disease in the family.

Indications for Prenatal Diagnosis and Techniques

Couples having a history of any genetic disorder or undiagnosed physical abnormality in the family are ideal candidates for prenatal diagnosis of the fetus. History of a neural tube defect in the family or any genetic syndrome in a previous child, pregnant women of 35 years or above calls for a prenatal diagnostic test in the unborn child. In case of suspicion of an X-linked recessive disorder, mothers should be screened for their carrier status.

Few of the prenatal diagnostic techniques are summarized below:

Amniocentesis

Amniocentesis entails the process of collection of fetal cells for chromosomal analysis. The technique is preferably applied between 14-16 weeks of gestation. 10 to 20 ml of amniotic fluid is tapped through the abdominal wall of the mother and the cells that are collected are used for karyotyping (Refer Chapter 3).

Neural tube defects can be diagnosed in the prenatal state by estimation of α-**fetoprotein** levels in the amniotic fluid. Its level is raised in the amniotic fluid as a result of leakage from the open neural tube defects. The procedure of amniocentesis carries only 1% risk of abortion.

Chorionic Villus Sampling

Chorionic villi samples are aspirated with a catheter introduced through the cervix under strict asepsis and ultrasound guidance. Analysis of the cells is possible even without culturing them as the cells in these tissues grow rapidly. The biopsy of the tissue is used for biochemical assay or DNA analysis to detect any genetic disorder.

Chorionic villus sampling is preferably done during 10 to 11 weeks of pregnancy, i.e. a few weeks

earlier than the designated period for an amniocentesis (14-16 weeks). The risk of abortion is enhanced due to the small gestation size and manipulation of the vital villus structures. It is rated at 2 to 3% and is higher than that with amniocentesis. The procedure may be associated with limb anomalies if carried out prior to 9 weeks of gestation.

Ultrasonography

It is a safe method of prenatal diagnosis both for the fetus and the mother. It is routinely performed at 12 weeks of pregnancy for detection of multiple pregnancies, fetal malformations, fetal age determination and classification of the type of placenta. Nuchal translucency (NT), exomphalos, rocker-bottom foot, etc. are some of the features related to certain chromosomal abnormalities detected with ultrasonography.

Serum Screening in the Mother and Blood Sampling in the Fetus

Detection of certain elements in maternal blood sample may act as clues for a probable disease in the offspring. The presence of "**alpha-feto-protein**" (AFP) in maternal serum at 16 weeks gestation indicates towards a neural tube defect (anencephaly or spina bifida).

Fetal blood is drawn under ultrasound guidance from one of the umbilical vessels by putting a transabdominal percutaneous needle into the mother's abdomen. The procedure is often referred to as "cordocentesis". The indication of this procedure is to use the collected fetal blood to arrive at a prenatal diagnosis of blood disorders and for chromosomal analysis. A high risk of abortion (about 10%) is associated with the procedure.

Genetic Counselling

At the existing levels of medical understanding and therapeutics the scope of curing genetically incurred diseases is quite remote. Interventions in terms of replacement of defective genes or their products by genetic engineering are not commonly practiced. Therefore, the knowledge of the principles of genetics can be used to detect the risk of incidence of some of the genetic diseases. This information, then, can be used to prevent their occurrences or reduce the severity of their outcome.

The services of a counselor should be sought by patients distressed with genetic disorders to help them attenuate their sufferings or couples, who are at risk of having a genetically abnormal child, to consider specific detection and treatment measures. The counselor should provide realistic and appropriate suggestions about the disease.

DIAGNOSIS OF GENETIC DISEASE

It is vital that the patient and his relatives should be informed about the correct diagnosis of the disease as well as the mode of its inheritance. The risks for occurrence of the disease are calculated on the basis of the understanding of laws of Mendelian inheritance. The prognosis and availability of treatment, if any, should also be positively discussed. Arriving at a diagnosis may involve 3 pertinent steps.

- *Family history*
  One should make a detailed pedigree chart to analyze the mode of inheritance of the trait or disease.
- *Examination of patient*
  A careful clinical examination of patient will help to reach a correct diagnosis.
- *Laboratory investigations*
  This may include biochemical investigations, chromosomal analysis and molecular studies. Antenatal diagnostic interventions may be sought in high-risk pregnancies, e.g. amniocentesis, chorionic villi sampling, imaging, etc. that would aid correct diagnosis.

MANAGEMENT OF GENETIC DISEASE

Parents as well as individuals should be clearly told about the diagnosis and the risk of occurrence or recurrence (as applicable) involved in case they decide to continue an affected pregnancy or to have children in future. A counselor should also tell them about all the options available for the management of any genetic disease. As most of the genetic disorders are incurable, one should try to prevent or limit the disabilities of the disorder. Couples left with no other alternatives of having a normal child, should be recommend to think for an adoption. Subsequent to a positive prenatal detection and all explanations given by the counselor, the decision regarding the fate of the pregnancy has to be made only by the couple involved.

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