Block 3

HUMAN CYTOGENETICS

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Introduction

Cytogenetics – the study of chromosomes as hereditary units has been an active field of research for over a century. At the turn of the last century, the merger of the two fields – cytology describing the cell structure, function and division and genetics that governs the inheritance of traits through generations emerged as a new field called “Cytogenetics”. In the following years tremendous progress was witnessed providing clear understanding of the process of cell cycle, phases of cell cycle (S, G1, G2 and G0 phases and cell division) acting in time bound manner, check points controlling events of cell cycle, governance of apoptosis (cell death) apart from knowing the structure of chromosome, nucleosomes, packing of DNA into the chromosomes, condensation of chromosomes during cell divisions etc.

The first foundation to the field of “Human Cytogenetics” was laid in the year 1956 when Tjio and Levan established the chromosome number in humans as 46. The preparation of human karyotype for the first time in 1959 led to the identification of numerical aberrations/abnormalities associated with Down, Turner and Klinefelter syndromes which implied the need for routine screening for chromosomal anomalies in certain clinical conditions. Later other numerical changes like XXX syndrome, XXXXY, XYY syndromes were recorded. Later detection of structural defect like deletion of chromosome 21 referred as Philadelphia chromosome causing chronic myelogenous leukemia opened up screening for chromosomal variations such as deletions, duplications and translocations in patients with different types of tumors. Further, development of chromosomal banding techniques and study of prometaphase chromosomes facilitated better identification of these variations with high resolution.

Culturing of free amniocytes was another breakthrough that allowed the identification of chromosomal abnormalities associated with birth defects and malformations. Later with the adoption of molecular biology techniques especially the hybridisation technique, the field of cytogenetics transformed itself into the field of “molecular cytogenetics”. Discovery of DNA probes and their tagging with fluorescent dyes evolved a new technique called “Fluorescent labeled in situ Hybridisation” (FISH) that enabled chromosomal analysis at various levels i.e. direct allocation of DNA regions to specific chromosomal sites, detection of microdeletions, duplications and several structural variations that cannot be traced by the application of age old classical techniques. FISH also helps to study interphase nuclei, cultured specimens etc. Later the detection of heterogeneous chromosomal changes found in cancer cells necessitated the determination of overall genomic changes. As a consequence “Comparative Genomic Hybridisation” (CGH) technique has emerged which enables the identification of “Copy number variations” (CNV) in the genome with cells showing abnormal number of copies of DNA sections due to either deletions or duplications. These CNVs may show association with certain clinical conditions and help in predicting extent of risk and also diagnosis.

In this block three units are covered which deal with chromosomes, their morphology, structure, cell cycle, cell divisions, karyotyping, basics of leucocyte
culturing, chromosome aberrations and the associated clinical conditions, nomenclature of normal and abnormal chromosomes, mechanisms causing abnormalities, recent trends in the analysis of human chromosomes by various advanced techniques including fluorescence in situ hybridization (FISH), sister chromatid exchanges (SCEs), comparative genomic hybridization (CGH) etc. Evaluation of several clinical conditions and their therapeutic interventions are now based on detailed cytogenetic analysis carried out at micro level using advanced technologies that proved to be of great help in establishing the reasons/etiology for clinical conditions, syndromes, cancers, their inheritance, risk for the progeny when the parents are carriers etc. These applications made the cytogenetic screening as an important diagnostic tools offered by majority of the clinical laboratories around the world.
UNIT 1  HUMAN CHROMOSOME

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Learning Objectives

After reading this unit, you would be able to:

- discuss the basics of Cytogenetics;
- understand difference in cell division;
- explain the basis of classification of human chromosomes;
- understand how the chromosome preparations are made;
- discuss different techniques available to stain the chromosome; and
- understand the methods used in chromosomal analysis and its importance.

1.1 INTRODUCTION

In this block you will learn about an important branch of human genetics known as Human cytogenetics. But before that let me begin with the important elements that will enable you to understand this block’s theme.
1.1.1 Definition of Genetics

Essentially, Genetics is the science of heredity and the study of genes. You might be aware from the newspapers and other media about the rapid expansion of the knowledge in the recent past in the field of genetics. You as an Anthropologist need to understand that genetics will play a central role not only in the new model of medical practice; but also in understanding the variations both normal and abnormal among the diverse ethnic populations spread across World.

1.1.2 What are Genes?

While heredity is understood as the biological similarity of offspring and parents, it is the gene that bears this heredity. Gene is the fundamental, physical and functional unit of the heredity, which carries information from one generation to the next.

The next question is, “Where are these genes?” They are in you. Yes! They are in each of your cell trying to maintain you in all ways, be it your growth, development or disease resistance. They reside tightly packed in what is called as chromosome.

So, chromosomes are thread like structures where a linear end to end arrangement of these genes exists.

1.2 WHAT IS CYTOGENETICS?

Now having examined some elementary terms, let us understand what cytogenetics is. This is the branch of genetics concerned principally with the study of chromosomes. It is the cytological approach to genetics, mainly consisting of microscopic studies of chromosomes. To put in another way the study of chromosomes and cell divisions, is also referred to as cytogenetics.

Fig. 1.1: German cytologist Walther Flemming
(Source: www.nndb.com)

Flemming developed a new staining technique in 1879, using synthesized aniline dyes to identify chromosomes, the structures of the cell nucleus. This allowed observation of mitosis, a term first used by Flemming for cell division, in far greater detail than ever before. He also coined the term chromatin, from the Greek word for color, after noting that his red dye was thoroughly absorbed by structures in the nucleus. He is usually credited as the father of cytogenetics (analysis of human chromosomes for the detection of inheritable diseases).
1.2.1 The Human Genome

The entire complement of genetic material in a chromosome set of a species is known as genome. A unifying theory (known as chromosome theory of inheritance) states that inheritance patterns may be generally explained by assuming that the genes are located in specific sites of chromosomes.

Fig. 1.2: Electron micrograph of the human genome
Source: http://www.ocf.berkeley.edu/~edy/genome/

1.2.2 Cell Theory

With the invention of compound microscope around 1600, a whole new world of science to marvel opened up for us. It was between 1655 and 1879 when researchers described the many kinds of cells seen in very thin slices of plant and animal tissues. The darkly staining long threads (chromosomes) were present in every cell. They had a remarkable dance like behaviour during the cell division. It is during this time, that the cell theory was put forth. Cell theory states that the cell is the underlying unit of structure in all the living organisms. They also proposed that new cells come only from pre-existing cells. With this idea, now just imagine a continuous unbroken series of cell divisions, from the point called “you” backward and backward, continuously to your ancestors, their forefathers and so on. So you would now agree with me, but you are here because of this marvellous unbroken chain of cell divisions running to the remote past. Yes in 4000 million years of earth history, you are lucky enough to be alive because of this continuum!

1.2.3 The Cell and its Types

The dark staining threads as I mentioned, in each cell are called as chromosomes (Greek chroma, “colour”, soma “body”) located in a nucleus. The nucleus is separated from the remaining cell contents, known as cytoplasm by a double membrane wall. Organism with this kind of cell structure is called eukaryotes.
Single celled prokaryotes (bacteria and archaebacteria) lack nuclei and have simpler chromosome. The hereditary material that is faithfully passed on from your ancestor to you resides in the chromosome.

Your body contains about 10 trillion cells. All of them came from just one cell, the fertilised egg. Yet the 10 trillion cells you are made up of are now no longer identical to each other. The skin, bones, muscles, brain, internal organs all are made up of different somatic cells. Yet they are a product of two germ cells that are also called as reproductive cells- the egg in your mother and the sperm in your father that united sexually to produce the new individual called you. Around 200 different types of somatic cells exist; each specialised to perform one or more unique functions.

Inspite of differences in the structure and function of the different cell types there are certain basic structure and functions common to virtually all cells. (Fig.1.3). But remember, that cells have complex organisations and multiple roles. How they develop and carry on their work is taken care by the information packed in their nuclei. The nucleus forms the large central compartment. As I told you earlier the nuclear envelope consists of two membranes that contain many pores, through which materials are exchanged with the surrounding cytoplasm; within the nucleus, are at least one dark staining nucleolus and many finely dispersed chromosomes.

### 1.2.4 Cell Cycle

Many cell divisions occur before a fertilized egg transforms itself into an individual with trillion cells. Events occurring during cell cycle (Fig 1.4) indicates whether a cell is dividing or not. The rate of cell cycle differs with the tissue
type. For e.g. cells lining the small intestine divide through out the life while nerve cells may not divide at all during the life time. You may define the “Cell Cycle” as a continuous process with two major stages: 1) Interphase (stage with no division of cell), 2) Mitosis (stage with cell division).

Interphase is the time in the cell cycle with great activity like carrying out several biochemical functions concerned with life processes, DNA replication and subcellular structures that are distributed to daughter cells after cell division. Interphase is divided into two gap phase called G₁ phase and G₂ phase. A cell exists from G₁ phase to enter G₀ phase also called as “醌cent phase”. A cell in G₀ phase maintains its characteristic and DNA replication or division does not occur at this stage. From here the cell may proceed for division or may lead to “apoptosis” or cell death. In G₁ phase, synthesis of proteins, carbohydrates and lipids occur which are required for the daughter cells. The time duration of G₁ phase varies from cell to cell.

Fig. 1.4: The Cell Cycle
During the next phase called S phase, cell replicates its entire genome, each chromosome replicates longitudinally held together by the centromere. This phase lasts for 8-10 hours and synthesize protein required for the formation of spindle-the structure that pull the chromosomes to the poles of the cells during anaphase stage of cell division.

G2 phase occurs after the replication of DNA and before the cell enters the mitotic division. In this phase cell synthesizes more of protein, membranes are formed from the proteins produced in G1 phase and stored in small vesicles that will enclose the daughter cells at later stage.

There are certain check points (group of interacting proteins) that ensure that the events happen in proper sequence. These points are a) DNA damage check point- that acts during S phase. It inhibits cell cycle from proceeding further till the damage is rectified, b) Apoptosis Check Point- which acts as the mitosis begins. In apoptosis check point, proteins called “survivins” override the signals causing cell death. Thus cells are kept at mitosis., c) Spindle assembly check points- take care of spindle formation to which chromosomes are attached helping their movement towards the poles during cell division.

1.2.5 Types of Cell Divisions

Before going further, let us recollect from our previous biology lessons that cell divisions are of two types. They are Mitosis and Meiosis.

Mitosis

Mitosis is the normal form of cell division. As a person develops from an embryo through fetus and infant to an adult, cell divisions are needed to generate the large number of cells required. Remember that many cells have a limited life span, so there is a continuous requirement to generate new cells in the adult. All these cell divisions occur by Mitosis. Mitosis is the normal process or cell division, from cleavage of the zygote to death of the person. It is estimated that in the life time of a human, there may be something like $10^{17}$ mitotic divisions.

![Mitosis (M) phase: The division of genetic material and cellular contents](source:sparkcharts.sparknotes.com)
Meiosis

Meiosis is a specialized form of cell division giving rise to the sperm and egg cells. Primordial germ cells migrate into the embryonic gonad and engage in repeated rounds of mitosis to form oogonia in females and spermatogonia in males. Further growth and differentiation produces primary oocytes in the ovary and produces primary spermatocytes in the testis. These specialized diploid cells can undergo meiosis.

An important point to remember is, that meiosis involves two successive cell divisions first, reduction stage during which chromosome number is halved (i.e it becomes haploid or n) and second, the multiplication stage (also known as equational stage) with mitotic division maintaining the haploid number of chromosomes. Only one round of DNA replication occurs so the products are haploid.

![Fig. 1. 6: The different phases of meiosis (Source: diffen.com)](image)

**1.3 HUMAN CHROMOSOME COMPLEMENT**

Prior to the 1950s, it was believed that each human cell contained 48 chromosomes and that human sex was determined by the number of X chromosomes present at conception. With the help of realise methods for chromosome preparation it was realized that the correct chromosome number in humans is 46 and that maleness is determined by the presence of a Y chromosomes irrespective of the number of X chromosome present in each cell. Further it was also realized that abnormalities of chromosome number and structure could seriously disrupt normal growth and development.

**1.3.1 Morphology of Human Chromosome**

At the sub-microscopic level chromosomes consist of an extremely elaborate complex, made of supercoils of DNA (Fig. 1.7). So each chromosome contains one molecule of DNA. The DNA molecule is incredibily long and has to fold itself in compact way so as to fit inside a cell which is only one millionth of an inch. Various proteins help in the compression of DNA molecule with out damaging it. A frame work of scaffold proteins guide the DNA molecule to
Human Cytogenetics

Compress. The DNA coils around the proteins known as “Histones” which give beaded appearance. The beaded structures are called “Nucleosomes” which get packed tightly during cell division so that the chromosomes become condensed and are visible as the cell division progresses specially at metaphase and telophase. A chromosome consists about 1/3 of DNA, 1/3 of histone protein, 1/3 of other DNA binding proteins and a small amount of RNA. The chromosome material is referred as “Chromatin” since it takes the color of the dye used while staining.

In a diploid nucleus, the two members of a chromosome pair are called homologous chromosomes or just homologs. Thus in diploids, each gene is present as a gene pair. Although the nucleus in a human cell contains pairs of chromosomes, they are not physically paired, in the sense of being next to each other.

Most of our knowledge of chromosome structure has been gained using microscope.

Special stains selectively taken up by the DNA have enabled each individual chromosome to be identified. These are best seen during cell division when the chromosomes are maximally contracted. During this contracted phase the constituent genes can no longer be transcribed. At this point of time, you can see that each chromosome consists of two identical strands known as chromatids, or sister chromatids, which are the result of DNA replication having taken place.
during the S (synthesis) phase of the cell cycle. The point or the primary constriction at which the two sister chromatids are joined is called as the centromere. This is the spot that is responsible for the movement of chromosomes at cell division. Each centromere divides the chromosome into short or petite arm (designated as p) and long arm (designated as q). The tip of each chromosome arm is known as the telomere. Telomeres seal the chromosome tips and their DNA caps have a unique chemical structure that keeps chromosomes from shortening during replication.

But with aging and with certain types of cancer there is a gradual accumulation of changes (mutations) in telomeres. Please remember that for unknown reasons the regions next to telomeres contain a high concentration of genes. Telomeres are known to be highly conserved throughout evolution and in humans they contain many tandem repeats of a sequence TTAGGG Sequence.

Morphologically chromosomes are classified according to the position of the centromere.

If this is located centrally, the chromosome is metacentric, if terminal it is acrocentric, and in case the centromere is located in an intermediate position, the chromosome is sub-metacentric.

Fig. 1.9: The telomeres are the ends of the chromosome
(source:wikispaces.psu.edu)

Fig. 1.10: Telomeres are similar to shoe lace tips
(source: lucashbrouwers.nl)

Fig. 1.11: Morphologically chromosomes are described as metacentric, submetacentric or acrocentric depending on the position of the centromere
(source: http://learn.genetics.utah.edu/content/begin/traits/scientists/).
Besides centromeres, some chromosomes have additional pinched-in sites called secondary constrictions. Acrocentric chromosomes sometimes have stalk like appendages called satellites that form the nucleolus of the resting interface cell and contain multiple repeat copies of genes for ribosomal RNA.

### 1.3.2 Classification of Chromosomes

Another parameter that varies among the chromosomes is their overall length. So three parameters form the basis for classification, length, position of the centromere and the presence or absence of satellites. The early researchers in cytogenetics recognized the 23 chromosomes and divided them into groups.

### 1.3.3 The Groups of Chromosomes

The groups span from A to G (seven classes) in the alphabetical order based on the overall morphology. In many standard text books you will see that the human mitotic chromosomes* of humans are generally grouped according to the following cytological criteria:

**Group A** (chromosomes 1-3) Large chromosomes with approximately median centromeres.

**Group B** (chromosomes 4-5) Large chromosomes with sub median centromeres.

**Group C** (chromosomes 6-12 and the X chromosome) Medium sized chromosomes with sub median centromeres.

**Group D** (chromosomes 13-15) Medium sized acrocentric chromosomes. Chromosome 13 has a prominent satellite on the short arm. Chromosome 14 has a small satellite on the short arm.

**Group E** (chromosomes 16-18) Rather short chromosomes with approximately median (in chromosome 16) or sub median centromeres.

**Group F** (chromosomes 19 and 20) Short chromosomes with approximately median centromeres.

**Group G** (chromosomes 21, 22 and the Y chromosome) Very short acrocentric chromosomes.

* J.H.Tjio and A. Levan in 1956 demonstrated that the diploid chromosome number for humans is 46.

* M.L.O’Riordin and three colleagues in 1971, reported that all 22 pairs of human autosomes can be identified visually after staining with quinacrine hydrochloride. They demonstrated that the Philadelphia chromosome is an aberrant chromosome 22.

(Philadelphia chromosome was first observed by researchers at the university of Pennsylvania and named after the city where the discovery was made. The chromosome is often found in myeloid leukemia, a disease in which several bone marrow derived cell lineages proliferate uncontrollably. Philadelphia chromosome ($\text{Ph}^1$) is generally a reciprocal translocation between human chromosomes 9 and 22 involving break points at 9q34 and 22q11. The translocation generates a fusion gene made up of elements from a gene on chromosome 22 and the majority of a proto-oncogene from chromosome 9).
The regions in the chromosomes where genes are actively expressed stains lightly and these are called as euchromatin regions. On the contrary heterochromatin regions stain darkly and is made up of largely inactive, unexpressed, repetitive DNA.

Sex chromosomes have a critical role in the determination of one’s gender. In humans both males and females have two sex chromosomes. XX in females and XY in males. The most important gene on the Y chromosome is the testis determining factor known as SRY. Other genes on the Y chromosome are important in spermatogenesis. Each of the ovum in the female carries one copy of X chromosome while in male each sperm carries either an X or a Y chromosome.

1.4 METHODS USED IN CHROMOSOME ANALYSIS

Tijo and Levin were the first group of scientists who in 1956 demonstrated that each human cell contains 46 chromosomes and broke then prevailing belief that it is 48 chromosomes.

1.4.1 Chromosome Preparation

Having recollected the features of the two types of cell divisions, let us move to the methods of chromosome analysis. These methods are commonly employed in cytogenetics laboratories to analyze the chromosome constitution of an individual, which is known as karyotype. The word karyotype is also used to describe photomicrograph of an individual’s chromosome arranged in a standard manner.

The entire chromosome complement of an individual organism or cell as seen during mitotic metaphase is used to arrange the karyotype.

Any tissue with living cells having a nucleus that can undergo cell division is suitable for studying human chromosomes. The commonest method is to use circulating lymphocytes from peripheral blood.

Some steps in the chromosome preparation are given below:

1) The venous blood sample is added to a small volume of nutrient medium containing phytohemagglutinin, which stimulates T lymphocytes to divide.

2) The cells are cultured under sterile conditions in an incubator at 37°C for about for 72 hours, at the end of which the culture is terminated.

3) At 70 hours i.e. 2 hours prior to the termination of the culture, colchicine is added to each culture which now stops the cell division at metaphase. Colchicine is a chemical that has a special property of preventing formation of the spindle. Once the spindle is not formed, the cell division gets arrested at metaphase. Metaphase is the time when the chromosomes are condensed to a maximum extent and because of this condensation are very clearly visible.

4) Hypotonic saline is then added to the cultures, which causes the cells to swell which helps in the lysing or breaking of the cells with ease.
5) Then the cell suspensions are dropped on the pre chilled glass slides by holding the pipettes at a distance so that good metaphase spreads are obtained.

6) The cells are then fixed and mounted on a slide.

7) The slides are further processed for staining.

**Fig. 1.12: Preparation of a karyotype**


### 1.4.2 Chromosome Banding Techniques

Now there is an important step which is called as the staining step. Many different stains and methods are used to identify individual chromosomes, using:

i) **G (Giemsa) banding** is the most common method used. The chromosomes are treated with Trypsin. Trypsin denatures their protein content; following this, the cells are stained with a DNA-binding dye known as Geimsa. On staining with Geimsa each chromosome takes up a characteristic pattern of light and dark bands. These light and dark bands can be reproduced in the same pattern for each chromosome. In other words the banding pattern is repeatable.

ii) **Q (quinacrine) banding**: This gives a banding pattern which is similar to the bands, obtained in Giemsa staining. However a ultraviolet fluorescent microscope is required to view these chromosomes.

iii) **R (reverse) banding**: In this method the chromosomes are denatured by heating and then stained with Giemsa. This gives light and dark bands which are the reverse of those obtained using conventional G banding.
Meaning- there will be a dark band in the region where the Giemsa stain shows up as a light band. Similarly it will be a light band in the corresponding region where the G staining gives a dark band.

iv) C (centromeric heterochromatin) banding: Here the chromosomes are pretreated with acid followed by alkali before G banding; the Centromeres and other heterochromatin regions containing highly repetitive DNA sequences are stained preferentially.

1.4.3 High Resolution Banding

G banding gives approximately 400 to 500 bands per haploid set, enabling us to do a high-quality chromosome analysis. Each band approximately corresponds on an average to 6000 to 8000 kilobases (kb) or 6 – 8 megabases (mb) of DNA (deoxy ribo nuclei acid, which is the ultimate molecule of life - the polymer of which genes are composed). One thousand base pairs in a DNA sequence is equal to one kilobase.

If you want a more detailed banding pattern, which is known as high resolution banding, say with 800 bands can also be obtained. The only condition is that it is technically more skill based and laborious.

Here the cells have to be arrested precisely at even an earlier stage of mitosis called as prophase or prometaphase that results in greater sensitivity to give upto 800 bands per haploid set. The agent used to inhibit cell division is methotrexate or thymidine.

Folic acid or deoxycytidine is added to the culture medium, releasing the cells into mitosis. Colchicine is then added for a specific time interval, leading to a high proportion of cells to remain in prometaphase. They will also be in fully contracted state, giving a more detailed banding pattern.

1.5 KARYOTYPE ANALYSIS

1.5.1 Counting the Number

The most important step in chromosome analysis involves first counting the number of chromosomes present in a particular number of cells. The cells that are counted to know the chromosome number in them are called as the metaphase spreads. One has to first count each metaphase spread and find out how many chromosomes are there in each of the spread.
Generally the total chromosome count is determined in 10 to 15 cells.

But if there is a state of mosaicism (a condition in which the same individual has two or more types of cells with different number of chromosome in one metaphase spread, and another chromosome number in the second spread) then 30 or more metaphase spreads are counted.

Once counting the number of chromosomes in each spread is completed, a little more detailed analysis is done.

Fig. 1.14: Metaphase spread of unbanded chromosomes from a normal male (XY)  
(source: physics.uwo.ca)

1.5.2 Analysis of the Banding Pattern

This is followed by a careful analysis of the banding pattern of each individual chromosome in selected cells. This will be observed on both members of each pair of homologs say the “1st chromosome pair”, “2nd chromosome pair” and so on. Three to five metaphase spreads, which show high-quality banding, are chosen for this purpose of detailed banding analysis.

1.5.3 Idiogram

The banding pattern of each chromosome is specific and can be shown in a particular style known as idiogram. The chromosome pairs are conventionally presented in a karyotype also called as karyogram with each pair of chromosomes arranged in descending order of their size.
Fig. 1.15: Idiogram of G banded chromosome (source: pathology.washington.edu)

So, having introduced the important steps to you, I will now sum up that, karyotype is the chromosomal complement of a cell, individual, or species. It describes the light microscopic morphology of the component chromosomes, so that their relative lengths, centromere positions and secondary constrictions can be identified. Attention should be paid to heteromorphic sex chromosomes (homologous chromosomes that differ morphologically).

The karyotype is often illustrated with a figure showing the chromosomes placed in order from largest to smallest as I mentioned earlier. This illustration is called as idiogram, which can be constructed by aligning photomicrographs of individual chromosomes, or it may be an inked drawing, summarizing the data from a series of analyses of metaphase chromosome spreads.

**Importance of chromosome analysis**

Chromosome analysis is important to detect the structural and numerical abnormalities. An extra chromosome can result in a trisomy of that particular chromosome. Sometimes the whole haploid complement can be present resulting in polyploidy instead of the usual diploid complement. Advanced chromosomal techniques like molecular cytogenetics helps to quickly detect the structural abnormalities like deletion of the segment of the chromosome, insertion of an
extra bit of chromosome or an inversion of a segment of the chromosome. Sometimes a ring chromosome is found when a break occurs on each arm of a chromosome leaving two ‘sticky’ ends on the central portion that reunite to form a ring.

1.6 SUMMARY

The normal human karyotype is made up of 46 chromosomes consisting of 22 pairs of autosomes and a pair of sex chromosomes XX in the female and XY in the male. Specific banding patterns help to identify the different chromosomes after using special procedures to culture the cells. Mitosis and meiosis are the two types of cell divisions. Mitosis takes place in the somatic cells while meiosis occurs during the final stage of gametogenesis. Homologous chromosomes at this stage exchange segments and then segregate independently to the matured daughter cells. DNA, the genetic material consists of two nucleotide chains wound in a helix. In the backbone of each chain the sugar deoxyribose alternates with phosphate groups. Attached to the sugars of both strands are the paired basis: A opposite T and G opposite C. Chromosomes are best seen during cell division especially at metaphase when they are tightly coiled. Each replicated chromosome at first consists of two identical chromatids joined by an undivided centromere. Each chromosome can be identified in metaphase are late prophase by its size, shape and banding pattern. Chromosome analysis is important to detect the structural and numerical abnormalities, which lead to the development of clinical conditions or syndromes.

Further Reading


Sample Questions

1) Write in brief about the human chromosome complement.
2) What is the basis for the classification of the chromosomes?
3) Highlight the seven classes of human chromosomes described in a karyotype.
4) What is a telomere?
5) What is the importance of chromosome analysis?
6) Define Idiogram.
7) Write on the various chromosome banding techniques available.
8) Differentiate between mitosis and meiosis.
9) Write briefly on the morphology of human chromosomes.
10) What is cell theory?
UNIT 2  CHROMOSOMAL ABERRATIONS

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Suggested Reading
Sample Questions

Learning Objectives

After reading this unit, you would be able to:

- explain different types of chromosomal aberrations;
- discuss the role of chromosomal aberrations in human disorders; and
- evaluate the causes and consequences of chromosomal aberrations.

2.1  INTRODUCTION

In this unit we shall discuss genetic changes at the level of the chromosomes and their effects in humans. Almost all individuals of a species contain the same number of chromosomes specific for that species. For example, you and I contain, within each of our cells, a total of 46 chromosomes which is specific for Homo sapiens. However, there are individuals who show variations from this normal complement. These variations could be changes in number of chromosomes or structural changes within and among chromosomes – together such changes are called chromosomal aberrations.

The genetic component of an organism regulates its development and interaction with the environment. Thus, any change in this genetic component leads to
variation in phenotypic characters. Depending on the extent of the aberration, these effects can range from being lethal to being harmless variations. We shall discuss the different types of aberrations, their phenotypic effects, the causes of such aberrations, and their roles in human disorders.

2.2 TYPES OF CHROMOSOMAL ABERRATIONS

Chromosomal aberrations are broadly classified as numerical or structural aberrations. They are further classified as shown in Figure 2.1

![Fig. 2.1: Classification of chromosomal aberrations](image)

2.2.1 Numerical Aberrations

Numerical aberrations are those that cause a change (addition or deletion) in the number of chromosomes. They are further classified as euploidy changes or aneuploidy changes.

- Euploidy is the condition when an organism gains or losses one or more complete set of chromosomes, thus causing change in the ploidy number. For example, triploid (3n), tetraploid (4n) etc. (Table 2.1).

- Aneuploidy is the condition when an organism gains or losses one or more chromosomes and not the entire set. For example, trisomy (2n + 1), monosomy (2n – 1) (Table 2.1).

In humans, euploidy conditions do not exist because the extent of abnormality is too large to sustain life. Aneuploidy conditions, however, are more common and are manifested in disorders such as Down syndrome, Klinefelter syndrome and Turner syndrome. We shall discuss these changes in detail later in the unit.

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<th>Representation</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Aneuploid</td>
<td>2n ± x</td>
<td>Gain or loss of one or more chromosomes</td>
</tr>
<tr>
<td>Monosomy</td>
<td>2n – 1</td>
<td>Deletion of one copy of any one chromosome</td>
</tr>
<tr>
<td>Nullisomy</td>
<td>2n – 2</td>
<td>Deletion of both copies of any one chromosome</td>
</tr>
<tr>
<td>Trisomy</td>
<td>2n + 1</td>
<td>Addition of one extra copy of any one chromosome</td>
</tr>
<tr>
<td>Tetrasomy</td>
<td>2n + 2</td>
<td>Addition of two copies of any one chromosome</td>
</tr>
<tr>
<td>Euploid</td>
<td></td>
<td>Gain or loss of entire sets of chromosomes</td>
</tr>
<tr>
<td>Triploid</td>
<td>3n</td>
<td>Addition of one entire set to 2n</td>
</tr>
<tr>
<td>Tetraploid</td>
<td>4n</td>
<td>Addition two entire sets to 2n</td>
</tr>
<tr>
<td>Polyploid</td>
<td>5n, 6n,7n,…..</td>
<td>Addition of more than two entire sets to 2n</td>
</tr>
</tbody>
</table>
2.2.2 Structural Aberrations

Structural aberrations are those that involve a change in the chromosome structure. These include deletions, duplications and rearrangements (inversions and translocations). Structural changes occur when chromosomes break and later rejoin in combinations that are different from the original. When there is a net loss or gain or chromosomal segments, the change is called an unbalanced structural change. When there is no net loss or gain of chromosomal segments, instead there is only a rearrangement; it is called a balanced structural change (Figure 2.2). Thus, balanced changes usually do not show any abnormal phenotypes, which unbalanced changes do. You should keep in mind that these changes are not mutations in genes; they only cause the number and order of genes to be changed.

As with aneuploidy changes, structural changes are also seen in humans, and manifest in disorders such as Cri-du-chat syndrome, Wolf-Hirschhorn syndrome, Prader-Willi syndrome and Angelman syndrome. We shall discuss each of these changes and their effects later in the unit.

![Fig. 2.2: Balanced and unbalanced structural changes in chromosomes](image-url)
2.3 ANEUPLOIDY CHANGES IN HUMANS

As was discussed previously, aneuploidy conditions are non-lethal and result in abnormal phenotypes described as syndromes. The effects of aneuploidy changes differ significantly depending on the type of chromosome involved. For example, changes in chromosomes involved in sex determination (allosomes) results in changes in the primary and secondary sexual characters of that individual, whereas changes in other chromosomes (autosomes) do not. As you can see, there are various factors that affect the phenotypic manifestations of chromosomal disorders. We shall now look at the different aneuploidy conditions in humans and their clinical manifestations.

2.3.1 Autosomal Trisomies

Trisomy is the condition where there is an additional copy of one chromosome. It is represented as 2n+1. Individuals, who are trisomics, thus show three copies of the chromosome rather than the normal two. It is usually observed that trisomies of the smaller chromosomes are more tolerated than trisomies of the larger chromosomes. This is expected, because additional copies of larger chromosomes contribute to larger genetic imbalance than additional small chromosomes. You will find it no surprise that the most common trisomy is of the shortest chromosome in human – chromosome 21. The other trisomies that have been reported include trisomy 13 (Patau syndrome) and trisomy 18 (Edward syndrome).

Down Syndrome

Down syndrome was one of the first reported chromosomal abnormalities in humans. It was described as Mongolian Idiocy by John Langdon Down in 1866. It wasn’t until 1959 that it was shown to be caused by the presence of an extra chromosome 21, resulting in an increase of number of chromosomes to 47 (karyotype 47, XX/XY, +21). Thus, this disorder is also known as trisomy 21 or Down syndrome. Figure 2.3 shows the karyotype of an individual with 3 copies of chromosome 21, or trisomy 21. With an incidence of 1 in 800 live births, this is one of the common trisomies seen in humans. This incidence increases to 1 in 350 when the woman conceives beyond 35 years of age and to 1 in 25 when she conceives beyond 45 years. Down syndrome is caused by trisomy 21 in almost 90% of the cases. 6% of the cases are also shown to be caused by a translocation rather than a numerical change (see Section 2.5.2) and the other 4% are known to be caused by mosaicism (see Section 2.3.4).

There are many phenotypic manifestations that are typical in patients of this syndrome. However, as in other syndromes, not all affected individuals show all the symptoms. Any single individual usually expresses only a subset of the manifestations. Some of the most common are:

- Flat face, round head, and typical epicanthic fold of the eyes
- Short, broad hands
- Mental retardation
- Hypotonia – poor muscle tone
- Short stature
- Protruding furrowed tongue
- Mild to moderate developmental disabilities
- Typical dermatoglyphic patterns (palm and fingerprint patterns)

Fig. 2.3: Karyotyping of the Down syndrome and the image of an affected baby (http://www.hhmi.org/news/media/981432.gif)

The most common cause of this trisomy is the non-disjunction (see Section 2.5.1) or the failure of separation of the chromosomes during meiotic division. Due to this one of the gametes undergoing fertilization contains two copies of chromosome 21 instead of the normal one copy (gametes are haploid containing one copy of each chromosome). This non-disjunction can occur at either meiosis I or II. Chromosomal analysis has shown that 75% of the cases are due to non-disjunction occurring at meiosis I. When such a gamete is fertilized by a normal gamete, it results in trisomy 21.

2.3.2 Autosomal Monosomies

Autosomal monosomies have not been reported beyond birth in humans. Even the loss of the smallest chromosome is not compatible with life. Most known cases, therefore, are stillbirths and spontaneously aborted fetuses. It seems that loss of whole chromosomes cause too much genetic imbalance, which cannot support life. However, partial monosomies have been reported and well-documented. Partial monosomy refers to the loss of a part of a chromosome while the rest of the chromosome is retained. Since such a partial monosomy is a chromosomal deletion, strictly speaking, we shall discuss it under Section 2.4.1.

2.3.3 Allosomal Aberrations

Changes in number of allosomes (X and Y chromosomes in humans) are termed allosomal aberrations. The gain or loss of these chromosomes alters the phenotype leading to syndromes. For example, the loss of one X chromosome in females leads to turner syndrome (XO) and the excess of one X chromosome in males leads to Klinefelter syndrome (XXY). As mentioned earlier changes in allosomes cause changes in the primary and secondary sexual characters along with other manifestations. We shall look at the consequences of two such changes and the contrasting variation they produce.
**Turner Syndrome**

This syndrome is characterized by the partial or complete absence of one of the X chromosomes in females (Fig. 2.4). This results in a reduction of the total number of chromosomes to 45 (karyotype – 45, X). Thus, this syndrome is also called Monosomy X. Its first description as a syndrome was by Henry Turner in 1938. Later, in 1954, the absence of barr body (inactivated X-chromosome seen in buccal cells) and presence of only one X chromosome was noted. As we saw in Down syndrome, monosomy of X is not the only cause of this syndrome. Mosaicism, deletions and isochromosome (see Sections 2.3.4 and 2.4.6) have also been shown to cause this condition.

[Fig. 2.4: Turner syndrome. A: Karyotype showing the absence of one sex (X) chromosome](http://www.geneticsofpregnancy.com/images/Turner_Syndrome.jpg); B: Classical Features](http://img.tfd.com/mk/T/X2604-T-53.png).

It is well known that, of the two X chromosomes in females, one is inactivated throughout her lifetime. If normal females have only one active X chromosome, then why should the loss of one X chromosome cause abnormal phenotype? The answer lies in the fact that although we speak of inactivated X chromosome, not all genes on that chromosome are being inactivated. There is a small subset of genes on the X chromosomes that are required to be expressed by both chromosomes for normal female development. Thus, individuals who lack one X chromosome fail to develop normal female character.

Some of the commonly seen manifestations of Turner syndrome are:

- Primary hypogonadism – poor ovary development
- Short stature
- Minimal breast development
- Broad shield-like chest with widely spaced nipples
- Absence of menstrual periods
Chromosomal Aberrations

- Absence of secondary sexual characteristics
- Horseshoe-shaped kidney
- Inability to produce gametes - sterility

**Klinefelter Syndrome**

The presence of an additional X chromosome in males causes abnormal sexual development and is described as Klinefelter syndrome. This set of characteristics was first described by Harry Klinefelter in 1942. In 1959 it was shown to be due to the presence of an additional X chromosome in males by the presence of barr bodies in these males (normal males do not show barr body). The additional X chromosome results in an increase in the total number of chromosomes to 47 (karyotype 47, XXY). It has an overall incidence of 1 in 1000 live male births. While most patients show the XXY condition, individuals showing variations like XXXY or XXYY have also been reported (Fig. 2.5).

![Fig. 2.5A: Karyotype of individual with Klinefelter Syndrome. Note the presence of two X chromosomes along with a Y chromosome (arrow) (source: http://www.geneticsofpregnancy.com/images/Klinefelter_Syndrome.jpg); B: Symptoms of Klinefelter Syndrome (Source: http://img.tfd.com/mk/K/X2604-K-07.png)](http://www.geneticsofpregnancy.com/images/Klinefelter_Syndrome.jpg)

The additional X chromosome arises due to non-disjunction (see Section 2.5.1) during meiosis. Due to this, the gamete contains two X chromosomes rather than one. When such an egg containing XX is fertilized by sperm containing Y, an XXY zygote is formed that develops into a Klinefelter male. The extra X chromosome may be either of maternal or paternal origin, but it is more often to be of maternal origin.

Individuals with this syndrome show hypogonadism and reduced fertility. These males do no develop masculine secondary sexual characteristics and show female-type characteristics. Some of the clinical manifestations include:
• Primary male hypogonadism
• Reduced facial, body and pubic hair
• Small and soft testes
• Slight learning difficulties
• Increased breast tissue – gynacomastia
• Long limb bones and lanky body
• Azoospermia – absence of sperm production leading to infertility

2.3.4 Mosaicism and Chimerism

So far we have seen how changes in chromosome number can affect an individual. We also saw that the extent of these effects vary between individuals. Some individuals show very mild conditions. These individuals provided examples of a phenomenon known as mosaicism. This is defined as the presence of more than one cell line in an individual derived from a single zygote. These cell lines can differ in their chromosome constitution, with a percentage of the individual’s cells showing numerical changes. Karyotype analysis from these individuals show that some cells have the normal number of chromosome while other have either losses or gains.

A classic example of this mosaicism is Down syndrome. These individuals show milder symptoms of the syndrome because only a portion of their body cells have the associated abnormality. Other numerical abnormalities also include mosaic individuals such as for Turner syndrome and Klinefelter syndrome. The severity of symptoms in these individuals is dependent on two things: what percentage of their cells shows the abnormality and which of their cells show the abnormality. For example, because the ovaries are the most affected organs, a Turner syndrome female whose ovary cells are from the normal cell line would show much milder symptoms than a Turner syndrome female whose ovary cells have the monosomy.

Like aneuploidy, mosaicism too can result from non-disjunction of chromosomes. But here the non-disjunction occurs in one of the early mitotic divisions of the zygote. This gives rise to three cell lines – normal, trisomic and monosomic (fig 2.6). The monosomic line usually does not survive; the normal and trisomic lines do. Thus, the embryo is formed of normal and abnormal cells.

Chimerism is similar to mosaicism in that the individual possesses cell lines of more than one type. The difference is that, while in mosaicism the cell lines are derived from the same zygote, in chimerism the cell lines are derived from different zygotes. A temporary chimeric condition is developed when a person undergoes blood transfusion. For a few days after the transfusion, the individual will have his own cells and the donor’s cells circulating in his body. Since the donor cells in his body are not of his own origin he is said to be chimeric.

A more permanent form of chimerism can develop if cells from a different zygote get incorporated into the developing embryo. There is an increased risk of this during in-vitro fertilization methods. Chimeric individuals often do not show any abnormal phenotypes, but their fertility and type of offspring would depend on which cell line gave rise to the reproductive organs. Especially, ambiguous genitalia (genitalia that look neither completely like male nor female),
hermaphroditism, and intersexuality can result if one cell line is genetically female (XX) and the other is genetically male (XY).

![Diagram of cell lines](image)

**Fig. 2.6: Formation of multiple cell lines in mosaic individual due to non-disjunction during mitosis**

### 2.4 STRUCTURAL CHROMOSOMAL ABERRATIONS

So far, we have described the types and effects of numerical chromosomal changes. We also saw that these syndromes can also be caused by certain types of structural changes. In this section you will learn the different structural changes and their consequences.

#### 2.4.1 Deletions

A deletion refers to the loss of a segment of a chromosome. This leads to the loss of the genes present in the missing region. A single break in the chromosome leads to the loss of the terminal segment and is called terminal deletion (see Figure 2.2). Intercalary deletion, however, involves two breaks in the chromosome, loss of the segment, and rejoining of the two chromosomal parts. Very large deletions are usually lethal because the monosomic condition of the large number of genes of the missing fragment reaches the level of genetic imbalance that cannot sustain life. Usually any deletion resulting in loss of more than 2% of the genome has a lethal outcome. Microdeletions, however, are reported and documented for specific disorders.

**Cri-du-chat Syndrome**

This syndrome results from a deletion on the short arm of chromosome 5. It is also known by other names such as 5p deletion syndrome and Lejeune’s syndrome. The disorder gets its name from the characteristic cat-like cry of affected infants.
Described first by Jérôme Lejeune in 1963, this disorder has an incidence of 1 in 25,000 live births. This disorder, being autosomal, should affect males and females in equal frequencies; but incidence is seen to be more in females by a ratio of 4:3 of females: males affected.

The deletion occurring on the short (p arm) arm of chromosome 5 varies in different affected individuals. The phenotypic effects are also shown to vary between individuals. Most cases show deletion of 30 to 60% of the terminal region of the short arm. Studies show that larger deletions tend to result in more severe intellectual disability and developmental delay than smaller deletions. Figure 2.7 shows the chromosome 5 pair from a karyotype of an individual with this syndrome. You can see that one of the chromosomes (left) has the normal length of the short arm while the other (right) has significantly reduced short arm. More specifically, a dark band is prominently seen in the normal chromosome, which is missing in the deletion chromosome.

Fig. 2.7: Chromosome 5 pair from karyotype of individual with Cri-du-chat syndrome. Note the deletion (arrow)

Affected individuals characteristically show a distinctive, high-pitched, catlike cry in infancy with growth failure, microcephaly, facial abnormalities, and mental retardation throughout life. Some common clinical manifestations are:

- Cry that is high-pitched and sounds like a cat
- Downward slant to the eyes
- Low birth weight and slow growth
- Low-set or abnormally shaped ears
- Mental retardation (intellectual disability)
- Partial webbing or fusing of fingers or toes
- Slow or incomplete development of motor skills
- Small head (microcephaly)
- Small jaw (micrognathia)
- Wide-set eyes

2.4.2 Duplications

Duplications, like deletions, can cause abnormal phenotypic effects. They usually arise by errors in homologous recombination (unequal crossing-over). Duplications have their importance not only in medical genetics, but also in evolutionary genetics. The presence of an extra copy of the gene virtually makes it free of selection pressure. Thus, it contributes to diversification of protein functions resulting in families of proteins. Proteins of such families have related functions differing in the task they are specialized for. A classic example is that of the globin genes. Different globin proteins express during different times of development, each of which is specialized to transport oxygen under those...
Chromosomal Aberrations

conditions. These differences arose by gene duplications. Without digressing too much we shall now look at the clinical significance of duplications exemplified by Charcot–Marie–Tooth disorder.

**Charcot–Marie–Tooth (CMT) Disorder**

This disorder results from duplication in the short arm of chromosome 17 in the region 17p12. It is a hereditary motor and sensory neuropathy that affects the nerve cells of the individual. Affected individuals typically show loss of touch sensation and muscle tissue. The chromosomal basis of this disorder is varied, with 17p12 duplication being one of the causes. The severity and symptoms shown depend on the region affected, and the presence of other chromosomal abnormalities associated with the duplication. In CMT type 1A, the duplication causes more of the protein to be produced from the genes in that region. This causes the structure and function of the myelin sheath around nerve fibres to be abnormal, causing various clinical manifestations such as:

- Weak feet and lower leg muscles
- Foot deformities (e.g., high arch)
- Difficulty with fine motor skills due to muscle atrophy
- Mild to severe pain as age progresses
- May lead to respiratory muscle weakness

**2.4.3 Robertsonian Translocation**

Translocations generally do not result in loss of genetic material. Robertsonian translocations, however, result in the loss of small parts of the chromosomes involved. The fusion of two acrocentric chromosomes with the subsequent loss of the two short arms is termed Robertsonian translocation or centric fusion (Figure 2.8). Although this translocation causes loss of the short arms, it is maintained as a balanced translocation. This is explained by the fact that the genes on the short arms are most rRNA genes that are present in many copies on other chromosomes; thus deletion of these copies doesn’t have much phenotypic manifestation as you might expect.

![Fig. 2.8: Robertsonian translocation between two acrocentric chromosomes–14 and 21](image)

One of the commonly seen such translocation is between chromosome 14 and 21, that gives rise to individuals showing characteristics of Down syndrome. Since this translocation is functionally a balanced translocation, individuals with
this aberration usually do not show any abnormal phenotype. Their effects are only seen in the next generation due to production of abnormal gametes. Balanced changes cause disturbances during the meiotic segregation. Due to this, the resulting gametes end up with loss of chromosome, or gain chromosome. How they cause such aberrations will be discussed in Section 2.5.2.

2.4.4 Reciprocal Translocation

Reciprocal translocation involves breaks in two chromosomes and the subsequent exchange of segments between the two chromosomes (Figure 2.9). Reciprocal translocation do not change the number of chromosomes. However, they may change the size and type of chromosome if the segments being exchanges are differing in size (Figure 2.9-b). For reasons not yet clear, reciprocal translocations involving chromosomes 11 and 22 are fairly common in the population.

![Fig. 2.9: Reciprocal translocation between two chromosomes](image)

![Fig. 2.10: Fluorescence photomicrograph of translocation between chromosomes 5 and 14. A segment from the short arm of chromosome 5 (yellow) has been mutually exchanged with a segment from the long arm of chromosome 14 (red). Courtesy: Science Photo Library](image)
Reciprocal translocations can give rise to deletion-duplication conditions and can cause disorders associated with such conditions. However, as in the case of Robertsonian translocation, an individual possessing the translocation himself would not show any abnormal phenotype. Due to disturbances during meiotic segregation of these translocated products, individual of the next generation have a possibility of showing abnormal phenotype. This is discussed in Section 2.5.3.

### 2.4.5 Inversions

An inversion is a condition wherein a segment of a chromosome is inverted. This is caused by two breaks in the chromosome and the subsequent rejoining in a reverse manner. This changes the order of genes on that chromosome and does not cause any changes in the chromosome number (Figure 2.11). Depending on the involvement of the centromere, inversion are of two types – pericentric and paracentric.

- **Pericentric inversions** occur when the inverted segment that includes the centromere. The product after inversion can differ significantly in the arm length and thus change the type of chromosome (eg: sub-metacentric to metacentric as shown in Figure 2.11-a).

- **Paracentric inversions** occur when the inverted segment does not include the centromere. The product after inversion remains the same type as the original except for a change in the order of genes (Figure 2.11-b)

![Fig. 2.11: Pericentric and paracentric inversions](image)

Pericentric and paracentric inversion are both balanced rearrangements because they do not cause any net loss or gain of genes. Except in the very rare cases that the break points in the chromosome is within a gene (which gets disrupted), individuals with inversions do not show any abnormal phenotype. As you may expect, their effects are seen as deletion-duplication only in the next generation. Hence we shall discuss it under the causes of aberrations in Section 2.5.4.

### 2.4.6 Isochromosomes and Ring chromosomes

**Isochromosomes**

An isochromosome is an abnormal chromosome with two identical arms – either having two short (p) arms or two long (q) arms. An isochromosome, thus, has an entire arm deleted along with the duplication of the other arm. This type of aberration is caused due to the transverse separation of the centromere during cell division instead of the normal lateral separation (Figure 2.12).
Isochromosomes, due to their deletion-duplication nature, cause abnormal phenotypes in individuals possessing them. The most common isochromosome is that of the X chromosome. This condition leads to the individual showing phenotypic characteristics of Turner syndrome. The missing genes on the missing arm contributes to the development of Turner syndrome in these females. Such X-isochromosomes account for as much as 15% of Turner syndrome cases.

**Ring chromosomes**

Ring chromosomes are formed when a chromosome losses its telomere regions and joins back on itself end-to-end. Breaks at the terminal regions cause the chromosome to have “sticky ends” because of loss of telomere region. These end, thus, join with each other causing the chromosome to become circular or ‘ring-like’ (Figure 2.13). Since the two terminal fragments are lost, loss of genes in those regions can have an effect on the phenotype. If these regions have important genes, their consequences can be serious abnormality in the phenotype.
Fig. 2.14: Fluorescence photomicrograph showing normal chromosome 2 and ring chromosome 2. Note the presence of telomere (green) in the normal chromosome and its absence in the ring chromosome. Courtesy: Journal of Medical Genetics

Disorders caused by ring chromosomes are not due to the ring formation itself, but due to the deletion of the genes in terminal regions. Also, ring chromosome are unstable during mitosis, hence the daughter cells may have lost the chromosome altogether. This results essentially in a monosomy. As much as 5% of Turner syndrome cases are shown to be due to ring chromosome-X. Some of the other disorders include ring chromosome 20 syndrome where a ring formed by one copy of chromosome 20 is associated with epilepsy; ring chromosome 14 and ring chromosome 13 syndromes are associated with mental retardation and dysmorphic (malformation) facial features; ring chromosome 15 is associated with mental retardation, dwarfism and microcephaly (small head).

2.5 CAUSES OF CHROMOSOMAL ABERRATIONS

In the preceding sections we looked at the different types of numerical and structural aberrations, and their effects on phenotypes. We briefed through the causes and origins of these aberrations. In this section we will discuss them in detail.

Table 2.2 gives an overview of the types of aberrations and the origin of their causes. It is evident that the abnormality can occur not only during gamete formation, but also in the previous generation as well as after fertilization. Since the time of occurrence would lead to different consequences, it is important to analyze existing aberrations and offer effective methods for those who are at risk. Some of these methods are explained in the following unit. If an abnormal child is born into a family, it is strongly advised that the family should undergo genetic counseling. Finding the cause of the abnormality and taking steps to reduce future abnormalities is just as important as learning to deal with an affected child.
**Table 2.2: Causes of different chromosomal aberrations**

<table>
<thead>
<tr>
<th>Type of aberration</th>
<th>Possible causes</th>
<th>Time of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerical (aneuploidy)</td>
<td>Non-disjunction</td>
<td>Parental meiosis, early zygote mitosis</td>
</tr>
<tr>
<td>Large deletion/duplication (whole chromosome arms)</td>
<td>Robertsonian translocation</td>
<td>Parental generation, subsequent segregation at meiosis</td>
</tr>
<tr>
<td></td>
<td>Isochromosome</td>
<td>Parental meiosis</td>
</tr>
<tr>
<td>Small deletion/duplication (part of chromosome arms)</td>
<td>Reciprocal translocation</td>
<td>Parental generation, subsequent segregation at meiosis</td>
</tr>
<tr>
<td></td>
<td>Pericentric inversion</td>
<td>Parental generation, crossover within inversion during meiosis</td>
</tr>
<tr>
<td>Micro deletion/duplication (one to 5 genes involved)</td>
<td>Unequal crossing over</td>
<td>Parental meiosis</td>
</tr>
<tr>
<td></td>
<td>Break without joining (terminal deletion)</td>
<td>Parental germline cells, early zygotic cells</td>
</tr>
<tr>
<td>Ring chromosome</td>
<td>Terminal breaks producing sticky ends</td>
<td>Parental germline</td>
</tr>
</tbody>
</table>

### 2.5.1 Non-disjunction

Non-disjunction is the failure of separation of the chromosomes during mitosis or meiosis. Normal division involves the separation of the two arms (mitosis and meiosis-II) of the chromosomes or separation of the two chromosomes (meiosis-I) during the anaphase stage. This ensure that one copy of each is moved to each pole and consequently each daughter cell receives one copy. When this separation fails, both copies will move to one pole. Hence, one of the daughter cells will now have two copies while the other has no copies of that chromosome. Simply put, this is the basis of aneuploidy changes where there is one extra copy present or one copy missing in the cells. Figure 2.15 shows the normal meiotic and mitotic division and the consequences of non-disjunction at meiosis-I, meiosis-II and mitosis anaphase stages.

The occurrence of non-disjunction is itself dependent on many factors. Some of these factors are:

- Advanced maternal age has been well correlated with an increase in the chances of non-disjunction. This is well illustrated in the fact that incidence of Down syndrome increases drastically as the maternal age increases. Figure 2.16 shows a graph correlating maternal age and incidence of Down syndrome (here Down syndrome is indicative of non-disjunction). This increase is attributed to the aging of the primary oocyte as age progresses and a reduction of the maternal competence to identify and abort abnormal fetuses.
Fig. 2.15: (A) Normal segregation during meiosis-I and II. (B) Non-disjunction occurring at meiosis-I producing gametes that can cause trisomy. (C) Non-disjunction at meiosis-II producing gametes that can cause monosomy and trisomy. (D) Normal mitotic division and Non-disjunction during early zygotic mitosis that can give rise to different cell lines leading to mosaicism.

Fig. 2.16: Incidence of Down syndrome in relation to maternal age
Increase in the time between ovulation and fertilization is well documented in animals to increase the rate of non-disjunction. As the frequency of copulations reduces there is an increased chance that there is a delay between ovulation and fertilization.

Exposure to mutagens in general increases the chances of non-disjunction. Especially those who are constantly exposed to radiations have a high risk of non-disjunction.

Genetic control of non-disjunction has been shown in a few species of Drosophila (fruit fly). These findings accounts for those few families that have shown to be prone to recurrent non-disjunction.

2.5.2 Robertsonian Translocation

As discussed before, Robertsonian translocation or centric fusion, causes a balanced rearrangement in the individual without any phenotypic abnormalities; however, due to improper meiotic segregation they give rise to trisomy-like and monosomy-like conditions in the offspring of such individuals.

A normal chromosomal complement in humans consists of two copies each of the 22 chromosomes and XY (for males) or XX (for females) – total of 46. Let us consider an individual who has a Robertsonian translocation between chromosomes 14 and 22. This person has a total of only 45 chromosomes. He has two copies of all the other chromosomes except 14 and 21. For this pair of chromosomes he has one chromosome 14, one chromosome 21 and one translocation 14/21 chromosome (Figure 2.17).

Fig. 2.17: Chromosome complement of 14 and 21 in a balanced translocation carrier

In a normal individual, during meiosis, one copy of each of these chromosome moves to each pole. This results in daughter cells each containing one copy of 14 and one of 21. In a translocation individual, however, because there are three chromosomes instead of four two of them move to one pole and one moves to another pole. This causes abnormal chromosomal constituents in the daughter cells (gametes). There are different possible ways of these three chromosomes segregating as shown in Figure 2.18. It is clear that only a small portion of gametes produced by individuals with such balanced translocations can produce normal offspring. Thus, a Robertsonian translocation can give rise to monosomies and trisomies of different chromosomes and their associated syndromes.
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Fig. 2.18: Segregation products of a balanced translocation carrier. The last three possibilities (monosomy 21, trisomy 14, and monosomy 14) are lethal conditions. Only trisomy 21 is compatible with survival.

You should note that although these abnormalities do not cause true trisomies or monosomies, they give rise to conditions that are akin to true trisomies and monosomies. This is because, as stated before, the long arms of these chromosomes contain the bulk of the genes for that chromosome; presence of extra copies of the long arm has the same effect as having an extra copy of the entire chromosome.

2.5.3 Reciprocal Translocation

Translocations not only cause trisomy-like and monosomy-like conditions, they also produce deletion-duplication conditions. A deletion-duplication is a condition where one segment of the chromosome is missing (deletion) and another is present in an extra copy (duplication). Figure 2.19 shows an example of a deletion-duplication condition.

![Deletion-Duplication Diagram](image)

Fig. 2.19: Deletion of genes FGHI present in only one copy) and duplication of genes QR (present in three copies). All other genes are present in two copies which is the normal complement.
Reciprocal translocations, wherein there is a mutual exchange of segments between two chromosomes, cause abnormal meiotic segregation. This abnormality is due to the formation of a quadrivalent of the four chromosomes during pairing (Figure 2.20). This structure is formed because the chromosomal segments always pair with their homologous regions. When such a complex structure is formed, separation of the chromosomes can happen in different ways depending on their orientation in the spindle. Figure 2.20 shows the different possibilities of segregation of a quadrivalent formed from reciprocally translocated chromosomes.

By analyzing the segregation products you should be able to predict the condition of the offspring from such a gamete. The first two segregation patterns produced phenotypically normal offspring. The next two segregation patterns may produce surviving offspring, but they will show abnormal phenotype due to the deletion-duplication condition. Depending on the size of the del-dup segment the severity may vary. The last two segregation patterns are usually lethal. This is due to the del-dup segments being very large in these cases. If you recall, deletions of over 2% of the genome is incompatible with survival.

Hence, translocations by themselves do not cause deletions or duplications; it is only in the next generation that their effects are seen. It cannot be emphasized enough that a balanced translocation carrier will most probably have normal phenotype, unless the breakpoint disrupts some gene. As with all balanced rearrangements, we shall see that inversions, too, cause deletions and duplications because of abnormalities in meiotic division.
2.5.4 Inversions

Inversions are balanced genetic rearrangements that invert segments within the chromosome. Depending on the involvement of the centromere they are either paracentric or pericentric (see Section 2.4.5). It is important to distinguish between these two types because the crossover products after meiosis is different for each.

In Section 2.5.3 you saw how a reciprocal translocation gives rise to an abnormal complex during meiotic pairing. By the same logic, inversions too cause the formation of “inversion loops” during meiotic pairing. Because one of the two homologous chromosomes contains the inversion, it folds back into a loop to allow for maximum homologous pairing (Figures 2.21 and 2.22). Crossing over is a unique event in meiosis that causes recombination between the homologous pair of chromosomes. When crossing over occurs in a region within an inversion loop, it gives rise to recombinant products that contain deletion and duplication.

Look at Figures 2.21 and 2.22. You can see that the formation of the inversion loop produces maximum homologous regions to be paired up. In pericentric inversions the inversion loop contains the centromere and in paracentric inversion the centromere is outside the inversion loop. Crossing over outside the inversion loop will give rise to normal chromosomes and inversion chromosomes. A crossover within the inversion loop, however, produces two non-recombinants (one normal and one inverted) and two recombinants (that contain deletion and duplication). These recombinants will contain duplication of certain genes along with deletion of other genes.

![Fig. 2.21: Pericentric inversion and its products due to crossing over within the inversion loop. NCO-Non cross-over; SCO-Single cross-over.](image-url)
In pericentric inversions the deleted and duplicated segments do not involve the centromere; hence four types of gametes will be produced. Two of these will contain the aberrations; depending on the extent of the aberration it may or may not be compatible with survival. In paracentric inversions the deleted and duplicated segments involve the centromere, hence we get one dicentric (containing two centromeres) and one acentric (containing no centromere) chromosome as recombinants. The dicentric chromosome forms a dicentric bridge during anaphase and thus arrests cell division (does not produce a gamete). The acentric chromosome is lost during division and thus doesn’t produce any viable gamete. Hence, only two types of gametes are produced from such individuals—one normal and one containing the inversion. These offspring will have normal phenotype because the inversion itself is a balanced rearrangement. Hence, the inversion itself will tend to persist in the population.

2.6 SUMMARY

Chromosomal aberrations are, broadly speaking, any kind of changes in the number and structure of chromosomes. Changes in number are classified as numerical changes; and changes in structure and size are classified as structural changes. Changes in ploidy level (euploidy changes) are seen only in plants and lower organisms. Aneuploidy changes are, however, seen commonly in animals including humans. Trisomy disorders in humans are a commonly occurring chromosomal aberration. They cause conditions such as Down syndrome (trisomy 21), Edward syndrome (trisomy 18), and Patau syndrome (trisomy 13). Changes in the sex chromosome constitution causes conditions such as Turner syndrome (monosomy X – XO) and Klinefelter syndrome (XXY).
Numerical aberrations needn’t necessarily be present in all of the affected individuals. Cases of milder symptoms have been shown to be due to mosaicism whereby only a subset of the individual’s cells contains the aberration. The presence of normal cells in the individuals lessens the severity of the symptoms. The variability of the symptoms also depends on which organ’s cells contain the aberration. If the aberrant chromosomal genes are not normally expressed in the organ containing cells with the aberration, no symptoms will develop. Chimerism is similar to mosaicism differing only in the origin of the different cell lines being from different zygotes.

Structural aberrations include deletions, duplications, translocations and inversions. They also include isochromosome and ring chromosome that are the cause for a small percentage of syndromes like Turner syndrome. Among the structural aberrations, it is only the deletions and duplications (unbalanced changes) that majorly cause abnormal phenotypes such as Cri-du-chat syndrome and Charcot-Marie-Tooth disorder. Balanced rearrangements (translocations and inversions) by themselves do not cause abnormal phenotype because there is no actual loss or gain of genes – only their rearrangement. They, however, cause deletions and duplications in the next generation.

Numerical aberrations usually arise due to non-disjunction (failure of chromosome separation during division). Non-disjunction can occur during meiosis-I, meiosis-II or mitosis. The products of non-disjunction usually causes either monosomy or trisomy. Monosomy of any chromosome is usually a lethal condition, except for X chromosome (Turner syndrome). Trisomy for the larger chromosomes is usually lethal. Trisomy of small acrocentric chromosomes is most commonly seen (chromosomes 21, 13, and 18). Trisomy-like conditions can also be produced by abnormal segregation of centric fusions (Robertsonian translocations) as is seen in a percentage of cases of Down syndrome.

Coupled deletions and duplications (Del-Dup) can arise from reciprocal translocations and inversions. These aberrations are balanced and do not cause any abnormal phenotype themselves (except if the breakpoint disrupts a gene). However, these rearrangements cause abnormal pairing up and segregation during meiosis giving rise to the deletions and duplications.

Chromosomal aberrations not only have their significance in medical genetics, but also in evolutionary biology. Certain aberrations, such as inversions, occur naturally and become stable in populations. They alter the structure of the genome and contribute to the evolutionary course for that species. Other aberrations, such as deletions, cause genome instability to the extent that they are lethal. Still others, such as centric fusion, play a role in speciation. For example, centric fusion of two ape chromosomes (acrocentric) gave rise to the human chromosome 2 (metacentric). The study of chromosomal aberrations thus has its importance in multiple fields of life sciences.

**Suggested Reading**


Sample Questions

1) What are the structural chromosomal aberrations, give a note with examples?

2) Give an account of common genetic syndromes caused by aneuploidy.

3) Describe the phenomenon of Genetic Imprinting.

4) Write short notes on Non-disjunction and translocations?
UNIT 3  RECENT TRENDS IN HUMAN CYTOGENETICS

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   3.6.4 Array CGH (aCGH)
3.7 Flow Karyotyping
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Learning Objectives

After reading this unit, you would be able to:

- define the role of cytogenetics and understand the Human Karyotype;
- explain the efficacy and drawbacks in conventional karyotyping;
- elucidate the role played by molecular biology in the evolution of Molecular Cytogenetics;
- discuss in detail the principle and process of Fluorescent In Situ Hybridization; and
- describe the newer methods of FISH based cytogenetic analysis and their applications and drawbacks.

3.1 INTRODUCTION

The branch of genetics which deals with the study of the structure and function of the cell, especially the chromosomes is known as Cytogenetics. Cytogenetic analysis can be carried out virtually for any cell in the body. However analysis of certain cells such as lymphocytes yield the best quality of chromosomes for study. Generally to understand the complete chromosomal complement, Karyotyping is done. A Karyotype is defined as the identification of chromosomes based on their size, centromere location and banding pattern. When the chromosomal image is organized based on the same criteria then we get an Ideogram.
Human Cytogenetics

The human karyotype is written as 46, XX for females and 46, XY for Males. The nomenclature and correct method of denoting the karyotype, normal chromosomes and aberrations (which have been described in Unit 2) are done in accordance to the International Society for Chromosome Nomenclature (ISCN) guidelines.

3.2 CELL CULTURE MEDIUM: PERIPHERAL BLOOD LYMPHOCYTES FOR CHROMOSOME STUDIES IN HUMANS

As the culture is essentially set up outside a living system in a laboratory and under artificial conditions, it is critical that the cells be made to ‘feel at home’. Hence macro-environmental conditions such as pH, Temperature, O₂/ CO₂ concentrations, humidity and sterility have to be carefully maintained while setting up the cell cultures. In addition to this, other micro environmental conditions like nutrients, growth factors, signal molecules etc., should also be carefully regulated.

Peripheral blood forms an ideal source for studying Human Chromosomes. This is because it contains lymphocytes and is easily collected without much discomfort to the subject. These types of cultures are of the primary type as whole blood is used, the culture is finite i.e the cells are only viable for a maximum period of 72 hours after it is initiated. These cells have large nuclei and yield high quality Metaphases for analysis. Also genetic damages if present can be easily observed on analysis. However the drawback of using these cells is that they are mature cells. In in vitro cultures often a mitogen be used. The function of mitogen is to stimulate division of the lymphocytes that are under culture. One commonly and sucessfully used mitogen is Phytohemaglutinin (PHA) an extract from the plant Phaseolus vulgaris. During culture in order to arrest the cells at metaphase certain mitotic spindle inhibitors like Colchicine or Methotrexate are added at two to four hours before the termination of the culture.

RPMI 1640 has traditionally been used for the serum-free expansion of human lymphoid cells. (RPMI- Roswell Park Memorial Institute). It has traditionally been used for growth of Human lymphoid cells. This medium contains a great deal of phosphate and is formulated for use in a 5% carbon dioxide atmosphere. Generally a bicarbonate buffer is required. However several modifications are available. pH indicator is Phenol Red.

3.3 CHROMOSOME BANDING AND THE HUMAN KARYOTYPE

Maximo Drets and Margery Shaw established methods to stain metaphase chromosomes using a dye called Giemsa, which produces a signature banding pattern, called G-bands, for each of the 24 different human chromosomes. G-banding patterns can be used to detect chromosomal translocations, deletions, and insertions, and localising the genes to specific regions of the chromosomes.

Chromosomal Banding Patterns: Chromosomes are composed of chromatin. Chromatin is nothing but the DNA Polynucleotide encased within several different proteins including Histones. Chromatin itself is of different types; the more active
regions are known as Euchromatin where as the less functional and more structural regions are known as heterochromatin. On staining ‘Banding’ pattern that is specific to each chromosome is observed that helps in identifying the chromosomes and arranging them in the form of karyotypes. The differential pattern of staining of chromosomes occurs with the additional treatment of the cultures with proteolytic enzymes such a Trypsin, Alkali or even Heat.

Banding techniques are often referred to by a single alphabet (such as G, R, C, Q, NOR) that denotes the type of banding and more precisely by three alphabets that denote: 1) The type of banding observed, 2) The treatment being used and 3) by the stain being used.

For example: G Banding is also known as GTG banding indicating

G for G Banding
T for Trypsin when used in culture
G for Geimsa stain when used in culture

a) **G-banding:** This technique does not involve a fluorochrome-based pretreatment. During mitosis, the 23 pairs of human chromosomes condense and are visible under a light microscope. A karyotype analysis usually involves blocking cells in mitosis and staining the condensed chromosomes with Giemsa dye at metaphase stage. The dye stains regions of chromosomes that are AT rich (i.e. rich in the DNA base pairs Adenine and Thymine) producing a dark band. The G-light bands are thought to be relatively GC-rich (rich in the DNA base pairs guanine and cytosine). Furthermore, the light bands represent the regions which are relatively open and which contain most of the genes, including house keeping genes (genes active in every cell type). On the other hand, the G-dark bands represent regions which are relatively compact and contain few genes. The genes in the dark regions are mainly tissue-specific (Fig. 3.1).

b) **R-banding:** This method involves a moderate use of alkaline with or without heating to obtain a banding pattern that is reverse to G Banding. Here the AT rich region with more heterochromatin is lightly stained and the more active GC regions form the dark bands. In this method centromeric regions do not take up staining.

c) **C-banding:** In this method, method of staining only the constitutive heterochromatin regions of chromosomes is stained. That is only the centromeres and the satellite region on chromosomes gets darkly stained. Hot alkali is used to accomplish this effect; treatment with same would bring about depurination of DNA at regions that are vulnerable. As constitutive heterochromatin represents the most resistant region on the chromosome to such treatment, it remains intact and is stained.

d) **Q-banding:** This banding pattern is obtained by treating with a fluorochrome or the fluorescent dye quinacrin. They can be identified by a yellow fluorescence of different intensity. Most parts of the stained DNA are heterochromatin. Quinacrin binds those regions which are rich in AT and G-C, but fluorescences only A-T-quinacrine regions. A-T regions are seen more in heterochromatin than in euchromatin. Therefore, by this banding method heterochromatin regions are labeled preferentially. The characters of the
banding regions and the specificity of the fluorochrome are not exclusively
dependent on their affinity to regions rich in A-T, but it depends on the
distribution of A-T and its association with other molecules such as histone
proteins.

e) **NOR Banding:** Human chromosomes belonging to the D (13, 14 and 15) as
well as the G (21 and 22) group of chromosomes contain secondary
constrictions called satellites. These regions are in fact the Nucleolar
Organizing Regions, which come together to form the Nucleolus. This is the
site for rRNA synthesis- which is the most abundant type of RNA found in
mammalian cells. Here in addition to alkali treatment, Sivler Nitrate staining
is used which gets preferentially deposited in these regions, making them
darkly stained.

![GTG Banded Male Chromosomes](http://carolguze.com/images/chromosomes/ideochrn.gif)

**Fig. 3.1: GTG Banded Male Chromosomes**

**Source:** http://carolguze.com/images/chromosomes/ideochrn.gif

### 3.4 CYTOGENETIC APPROACHES TO MAP GENES

The very nature of cytogenetic analysis lends to identifying the location of gene
i.e. Gene Mapping. This is because of the fact that most disorders are caused by
mutations in genes. If the mutation involves a chromosomal aberration, then it
follows that by identifying the defect we can trace the location of the gene on to
a specific chromosome. Cytogenetic approaches in addition to providing
associations, between chromosomal abnormalities and disease, have also allowed
researchers to map genes to particular chromosomes. However in conventional
chromosomal analysis some of the following limitations are:

1) **Low resolution limit:** Unable to identify subtle chromosomal aberrations
such as microdeletions and cryptic translocations, both of which involve
very small chromosomal segments but are the genetic cause for disease.
3.5 FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Overview

It essentially involves the hybridization of a genetic ‘probe’ sequence to its complimentary region in the human genome. In order to accomplish this, the target chromosomes are first denatured. The probe of course is composed of DNA/RNA or cDNA from the gene of interest. The probe is essentially a stretch of labeled oligonucleotide that is used to identify the location of a gene on a chromosome. The principle of this method lies in choosing probes that have a greater specificity.

In early work, probes were labeled with radioactive isotopes and target sequences were identified by autoradiography. This method of labeling and detection limits both the sensitivity of the technique and its resolution. In particular, the original protocol only allowed the detection of tandemly repeated sequences such as the ribosomal genes and satellite DNA. By 1981, however, investigators had optimized the in situ protocol for use in mapping single copy mammalian sequences, and in 1984 an improved method was developed for better resolution of chromosome banding patterns.

Nevertheless, the technique is still not ideal because with single-copy radioactive probes, localization of genes can not be determined within the chromosomes of a single cell; instead, it is necessary to perform a statistical analysis of silver grain distributions in 50-100 sets of metaphase chromosomes.

Two critical changes in the protocol now allow the detection of single-copy sequences and their high-resolution mapping through the direct observation of single chromosomes. The first change made is in the nature of the label with the substitution of fluorescent tags for radioactive ones and dramatic improvement of the physical resolution of the hybridization site. The modified in situ protocol that utilizes fluorescent tags is referred to as FISH (fluorescent in situ hybridization).

The second change was in the nature of the hybridization cocktail. With the inclusion of a large excess of unlabeled total genomic DNA, it is possible to block dispersed repetitive sequences — present in essentially every genomic region larger than a few kilobases in length — from hybridization to their targets throughout the genome.

FISH (fluorescence in situ hybridization) is a cytogenetic technique developed by biomedical researchers in the early 1980s that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification. FISH can also be used to detect and localize specific
mRNAs within tissue samples. In this context, it can also help define the spatial-temporal patterns of gene expression within cells and tissues.

Fig. 3.2: Overview of the FISH Method

Source: http://www.bio.davidson.edu/courses/molbio/molstudents/spring2003/baxter/FISH.gif

The FISH method involves four steps: fixation, hybridization, washing, and detection.

Fig. 3.3: Overview of the Steps Involved FISH Technique
Types of Probes

A probe is a stretch of DNA sequence that hybridizes with DNA/RNA based on the complementary base pairing property. When probes developed from known sequence of a gene hybridize with test DNA sample, it indicates the presence of the complementary sequence or the gene in the sample tested. The different types of probes are:

a) **Locus specific probes:** These probes bind to a particular region of a chromosome. This type of probe is useful when scientists have isolated a small portion of a gene and want to determine on which chromosome the gene is located.

b) **Repeat binding probes:** These probes bind to part of the human genome that contains certain types of repeats. Some such elements include Centromeric, Telomeric and Alu repeat (a type of transposon) probes. These probes are used to detect the presence of the repeats and detect centromere related aberrations.

c) **Whole chromosome probes:** They actually are collections of smaller probes, each of which binds to a different sequence along the length of a given chromosome. Using multiple probes labeled with a mixture of different fluorescent dyes, scientists are able to label each chromosome in its own unique color. The resulting is a full-color map of the chromosome. Whole chromosome probes are particularly useful for examining chromosomal abnormalities by screening the whole genome-for example, when a piece of one chromosome is attached to the end of another chromosome.

d) **Arm specific probes:** These probes hybridize to unique sequences either p or q arms of all chromosomes (except p-arm of acrocentric chromosome). Their use in molecular cytogenetic examination of patients include analysis of chromosome patterns involved in translocations, to study mutagenesis in human chromosomes, analysis of complex chromosomal rearrangements in

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**Fig. 3.4: Types of Probes Used in FISH Technique**

neoplastic cells. Arm specific probes are available in two colors: green and red.

![FISH Image Showing Chromosome with Telomeric Probes](http://childrenshospital.org/az/Site1198/Images/Karyotype_Fluorescent_in_situ_hybridization.jpg)

**Fig. 3.5: FISH Image Showing Chromosome with Telomeric Probes**

The chromosomes are counter stained with DAPI

**Source:** [http://childrenshospital.org/az/Site1198/Images/Karyotype_Fluorescent_in_situ_hybridization.jpg](http://childrenshospital.org/az/Site1198/Images/Karyotype_Fluorescent_in_situ_hybridization.jpg)

e) **Band specific probes:** Band specific probes are capable of detecting small chromosomal segments those involved in subtle translocations with break points. These particular probes increase the resolution typically obtained with whole chromosome probes when identifying chromosomal abnormalities.

The **NOR probe** is specific for p-arm of acrocentric chromosome, whereas the **Alu probe** will detect Alu repeat sequences found in primate chromosomes.

### 3.6 ADVANCES IN MOLECULAR CYTOGENETIC ANALYSIS

After the development of FISH several advances in FISH based methodology were developed, some of these are described below.

#### 3.6.1 Whole Chromosome Painting and M-FISH

In some cases it is advantageous to label larger segments of chromosome such as part of an arm, one arm or the entire chromosome itself. All these can then be used to track chromosomal aberrations including translocations (as illustrated in Fig. 3.6). However it is not possible to span the entire chromosome using a single probe. Hence chromosome paint probes are used. These are probes that contain overlapping ends such that they will hybridize at different points along the desired target chromosome giving a fluorescent label to the entire region. By using several combinations of probes each chromosome in the human genome can be given a separate color. Such a combinatorial approach is known as M-FISH or Multicolor FISH. Spectral Karyotyping (SKY) and Comparative Genomic Hybridization (CGH) are types of M-FISH.
The figure shows a translocation of \textit{SRY} material to the q-arm of the del(X). The X chromosome is painted in green and the Y chromosome in red/orange. Normal cross hybridization of the Y painting probe is seen in proximal Xq of both the normal X chromosome and the del(X), whereas normal cross hybridization to Xp is only seen in the normal X chromosome as these sequences are missing from del(X). The del(X) also shows a signal at distal Xq, corresponding to translocated Y sequences. Inset (A): \textit{SRY} material (red/orange) is located at distal Xq while X centromere is in green. Inset (B): The G- banding of del (X) and normal X.

\textbf{Advantages}

1) Easy to detect structural and numerical aberrations.

2) Each chromosome can be given a different label allowing screening of the entire genome.

\textbf{Disadvantage}

1) Costly to label the entire genome using probe combinations.

2) Cannot detect paracentric inversions.

\textbf{3.6.2 Spectral Karyotyping (SKY)}

Spectral karyotyping is a molecular cytogenetic technique used to simultaneously visualize all the pairs of chromosomes in an organism in different colors. Fluorescently labeled probes for each chromosome are made by labeling chromosome-specific DNA with different fluorophores. Because there are a limited number of spectrally-distinct fluorophores, a combinatorial labeling method is used to generate many different colors. Spectral differences generated by combinatorial labeling are captured and analyzed by using an interferometer attached to a fluorescence microscope; this device is used to distinguish minor changes in the fluorescent signal that cannot be observed by the human eye.
Then the image processing software assigns pseudocolors to each spectrally different combinations, allowing the visualization of the individually colored chromosomes. Hence this method can be used as a screening method for analyzing the entire chromosomal compliment at once.

**Advantages**

1) A sensitive method, can detect complex translocation involving two or more chromosomes.

2) Provides a critical screening method that can analyze all the chromosomes at once.

**Disadvantages**

1) Cannot detect inversions especially paracentric inversions.

2) Due to the nature of the probes they are very expensive.

Fig. 3.7: Schematic Representation of SKY Hybridization Experimental Set Up

Source: http://atlasgeneticsoncology.org/Deep/Images/ComparCancCytogFig2.jpg
3.6.3 Comparative Genomic Hybridization (CGH)

This method is based on ratiometric analysis. This method uses the following approach:

1) DNA is extracted from the sample that needs to be tested. It is labeled using a fluorescent dye (such as TRITC- Tetramethyl Rhodamine Isothiocyanate) by the Nick Translation method.

2) A suitable control is also taken; this too is labeled but with a distinctly different colored fluorescent dye (for. Ex. FITC- Fluoresce in Isothiocyanate which gives a green color).

3) Both these labeled DNAs will then be hybridized on to a slide containing Normal Human Metaphase Chromosomes.

4) Once the hybridization is completed the analysis is done.

Here the analysis is done by comparing the test or sample DNA as compared to the control DNA.

Three outcomes are possible while applying this procedure. They are:

1) No loss or gain of genetic material in a particular region. This results in a balanced color being formed and balance between both the dyes (in this case it would appear orange= Red labeled DNA = Green labeled DNA).

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Fig. 3.8: Schematic Representation of the CGH Process

Source: http://atlasgeneticsoncology.org/Deep/Images/ComparCancCytogFig3.jpg
2) Regions which have been deleted in the test sample will be showing a greater signal of the normal DNA. In this case it would result in a red signal. (Green label DNA< Red Labeled DNA).

3) Region where there has been a gain of genetic material in the sample DNA as compared to the normal DNA. In this case it would show an excess of the sample’s fluorescent color (Green label DNA>Red Labeled DNA).

This method is greatly used in the analysis of copy number variants (CNVs) in genes. CGH is widely used in Cancer diagnosis and research as well as to study evolutionary changes in the human genome by carrying out CGH using ancestral DNA (Ex. Chimpanzee) with Human DNA.

![Microphotograph of a CHG Metaphase and a Schematic Representation of the Ratiometric Analysis](http://www.aist.go.jp/aist_e/aist_today/2003_07/p20_1.jpg)

**Advantages**

1) Highly sensitive method can detect minor changes in copy number variations (CNVs.)

2) A screening method that can screen the entire genome for CNVs.

**Disadvantages**

1) Whole genome labeling is expensive.

2) Can detect only gain/loss in the sample’s DNA but can not detect any other type of aberration.
3.6.4 Array CGH (aCGH)

DNA microarrays allow for simultaneous analysis of genes products or DNA copy numbers for thousands of loci. These microarrays contain defined human chromosomal segments (isolated from a chromosomal library), that have been ‘spotted’ or fixed onto a glass slide. The spotting is done in a very precise manner such that the location of each spot as well its content is clearly known. The microarray will contain several hundreds of such ‘spots’. Once this is complete, then standard CGH protocol will be followed to determine gain or loss of genetic material for all the spots on the microarray. The microarray is then incubated with the labeled test and control DNA. The array is then washed to remove DNA that is not bound, and the positions in the microarray with labeled DNA fragments.

The resolution that can be achieved with this type of analysis is greater than what can be done with conventional CGH. This is because the spots on the microarray can have a defined size. In addition to this the analysis can be done for Single Nucleotide Polymorphism which can be mapped using aCGH. However, the resolution of SNP arrays is currently limited to about 10,000 SNPs per chip.

![Schematic Representation of the Array CGH Assay and Analysis of the Results](http://www.ebioservice.com/sbc_2009/images/pic_11.jpg)

**Advantages**

1) Highly sensitive, can carry out analysis for several thousands of variants simultaneously.

2) CNV as well as SNP analysis can be done.

**Disadvantages**

1) Very costly to carry out due to high cost of array production and analysis.

2) Low reproducibility of tests.

3.7 FLOW KARYOTYPING

Flow Cytometry is a method that is used to distinguish and separate particles such as cells or chromosomes. The mechanism involved uses the combinations of fluorescent labels to create unique labels for the desired chromosomes. The
chromosomes in a suitable buffer are then passed through a narrow nozzle of the
flowcytometer. The nozzle is so narrow that each droplet will contain only a few
chromosomes. The drops are then scanned with a laser beam that excites the
flours present. The fluorescent signals are then picked up by a detection system;
the signal is then amplified and based in the presence or absence of a particular
signal the drop is charged using a charging ring. This charged droplet is deflected
using charged deflection plates into suitable collection vials. In this method the sorting
can be done based on the type of signal being observed as well as the strength of the
signal. The overview of the method has been represented in Fig. 3.11.

One of the most common methods is the use of two different dyes. One is, called
Hoechst 33269, binds to A-T base pairs of DNA the other is Chromomycin A
which binds to the G-C rich regions. This combination can be used to sort all the
chromosomes based on the principle that larger the chromosome, greater will be
the AT and GC content, the greater this content the greater will be the resultant
signal strength. Using the strength of the signal the chromosomes can be sorted.

As illustrated in Figure 3.11, the smallest chromosome i.e. 21 shows the lowest
Hoechst and chromomycin staining intensity. Chromosomes 1 and 2, which are
the largest, show the highest Hoechst and chromomycin staining.

Advantages

1) Highly sensitive, capable of carrying out analysis in real time.
2) High throughput, large number of sample can be analyzed.

Disadvantages

1) Trained personnel required to handle instrument.
2) Currently can only detect numerical aberration accurately, used most
commonly to determine ploidy in samples.

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Fig. 3.11: Schematic representation of the discrimination and sorting of human chromosomes
FACS (Source: http://www.nature.com/scitable/nated/content/6044/10.1038_nrg
905-f3_thumb_0.gif)
Chromosomes that are released from mitotic cells are stained with two DNA-binding dyes with different base-pair specificities, and the fluorescence intensities of each of several thousand chromosomes are measured in a two-laser flow cytometer. In the example shown, the two dyes are Hoechst 33258, which binds preferentially to A-T base pairs, and chromomycin A3, which binds to C-G base pairs. The resulting bivariate “flow karyotype” (bottom right panel) resolves all chromosomes except for the 9–12 group. In this example, maternal and paternal homologous of both chromosomes 21 and 19 are resolved into separate peaks owing to differences in their DNA content. After measurement, droplets that contain desired chromosomes, such as chromosome 3 in this example can be deflected into tubes for molecular analyses.

3.8 SUMMARY

Traditional banding methods like GTG banding offer cost-effective methods for screening chromosomes for structural changes. Development of ‘Molecular Cytogenetics” techniques have revolutionised the analysis of Human Chromosomes. It involves the use of modern molecular tools to study different aspects of chromosomal alterations. These newer techniques are more sensitive and can detect smaller changes. The discovery of Fluorescent probes have enabled wide spread use of molecular cytogenetics in a variety of fields. Techniques such as SKY and CGH are being used to provide valuable insight into complex chromosomal rearrangements as well as change in the copy number of the genes (CNVs). Such cytogenetic analysis is particularly useful in the field of prenatal genetic diagnosis and genetic counseling. Although each technique has several advantages the disadvantages have limited their applications. Hence a more common practice would be to combine more than one of the methods described above. The type of analysis show that the focus is now shifting to cost effective high throughput chromosomal analysis that is required for genetic diagnosis and adoption of prophylactic/preventive measures.

Further Reading and References


Sample Questions

1) What is a Karyotype?

2) Describe the medium most commonly used for Lymphocyte Culture.
3) What are the drawbacks in conventional karyotyping?
4) Describe different Chromosomal banding techniques.
5) What is FISH? What are the different types of Probes used in FISH analysis?
6) What is SKY? Describe its applications, advantages and disadvantages.
7) Describe in detail the methods of CGH and aCGH.
8) What is Flow Karyotyping?