Genetics for Clinicians

Genetics for Clinicians

SHUBHA R. PHADKE
Additional Professor
Department of Medical Genetics
Sanjay Gandhi Post-graduate Institute of Medical Sciences
Raebareli Road, Lucknow
India

To,

My parents

Mrs Mandakini

and

Mr E.B. Sangitrao

Contents

SECTION IV: DIAGNOSIS, TREATMENT AND PREVENTION OF GENETIC DISORDERS

Preface

The knowledge of genetics and the genome has increased rapidly over the past few decades. The application of this knowledge has become an integral part of patient care and the preventive thrust of modern medicine. The aetiology of the majority of disorders has some genetic component and, hence, understanding the basic concepts of genetics is essential for medical professionals from all subspecialties, as well as for general practitioners.

Although genetics is the fundamental science of biology and medicine, the subject has not found adequate emphasis in medical education in India. Medical professionals often tend to shy away from the genetic aspects of the disease being treated. This leaves lacunae in appropriate counselling, especially concerning the issue of recurrence of the disorder in a family. The aim of this book is to introduce genetics to clinicians and to remove the inhibitions of medical professionals towards the subject.

This book is not a comprehensive textbook of genetics. Instead, it takes a look at the basic concepts as well as interesting and clinically applicable aspects of genetics. Diagnosis and genetic counselling for chromosomal disorders, monogenic disorders and congenital malformations are discussed in depth so that the interested clinician will be able to deal with common presentations of genetic disorders. The need and utility of genetic counselling are stressed throughout the book. This should help treating physicians to identify clinical situations which require referral to a genetic centre.

A knowledge of molecular genetics is essential to understand the latest developments in medicine. An attempt has been made to outline the fundamentals of molecular genetics so that the reader gets familiar with the terminologies and techniques used in this field. This book attempts to introduce medical students to the fascinating and ever-advancing field of genetics and prepares them for the coming era of molecular medicine. The students from various nonmedical backgrounds

will also find this book useful in familarizing them with the clinically applied aspects of genetics.

I wish to express my gratitute to Professor S.S. Agarwal, former Head, Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. He introduced me to the fascinating field of medical genetics and taught me the basics of the subject. I also thank my students whose enthusiasm and inquisitiveness have always compelled me to stay updates in the ever-expanding field of genetics. I can not forget to acknowledge my patients and their families who, over the years, have given me the opportunity not only to learn about various common and rare genetic disorders but also made me experience various shades of emotions and coping strengths the circumstances demand. The journey with them taught me to deal with the complex counseling situations with a humane angle.

I hope this book generates curiosity and enthusiasm for genetics and encourages both medical professionals and students to utilize the knowledge in their clinical practice. It is for the readers to judge the success of this endeavour.

Lucknow SHUBHA R. PHADKE

APRIL 2006

Abbreviations

A	adenine (base), adenosine (nucleoside)
AAMR	American Association of Mental Retardation
ACE	angiotensin-converting enzyme
AFP	alphafetoprotein
AgNOR	silver staining of nuclear organizing regions
ALV	Abelson leukaemia virus
ARMS	amplification refractory mutation system
ADA	adenosine deaminase
ApoE	apolipoprotein E
ASO	allele-specific oligonucleotide
BACs	bacterial artificial chromosomes
bcr	breakpoint cluster region
BMD	Becker muscular dystrophy
BMT	bone marrow transplantation
bp	base pair
C	cytosine (base), cytidine (nucleoside)
CAD	coronary artery disease
CCM	chemical cleavage mismatch
cDNA	complementary deoxyribonucleic acid
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance receptor
CGH	comparative genomic hybridization
CHARGE	coloboma, heart disease, atresia choanae, retarded growth, genital and ear abnormalities
CML	chronic myeloid leukaemia
CPEO	chronic progressive external ophthalmoplegia
CPM	confined placental mosaicism
CVS	chorionic villus sampling
DAPI	4,6-diamidino-2-phenyl indole
DDAVP	desmopressin / 1-deamino-8 arginine vasopressin
DDS	Denver Development Schedule
DGGE	denaturing gradient gel electrophoresis
DMD	Duchenne muscular dystrophy

DNA	deoxyribonucleic acid
DNPH	2,4-dinitrophenylhydrazine
dNTPs	deoxynucleotide triphosphates
ddNTPs	dideoxynucleotide triphosphates
ESAC	extra structurally abnormal chromosome
ELISA	enzyme-linked immunosorbent assay
FBS	foetal blood sampling
FISH	fluorescence *in situ* hybridization
FGFR	fibroblast growth factor receptor
FAP	familial adenomatous polyposis
FITC	fluorescein isothiocyanate
G	guanine (base), guanosine (nucleoside)
G6PD	glucose-6-phosphate dehydrogenase
GDNF	glial cell line-derived neurotrophic factor
GSD	glycogen storage disorder
hCG	human chorionic gonadotrophin
HGP	Human Genome Project
HLA	human leucocyte antigen
HNPCC	hereditary non-polyposis colon cancer
ISCN	International System of Human Cytogenetic Nomenclature
IDDM	insulin-dependent diabetes mellitus
LDDB	London Dysmorphology Database
LDL	low density lipoprotein
LINEs	long interspersed nuclear elements
LHON	Leber hereditary optic neuropathy
MECP2	methyl-CpG-binding protein 2
MELAS	mitochondrial encephalopathy, lactic acidosis and stroke-like episodes
MERRF	myoclonic epilepsy, ragged red fibres [in muscles]
mRNA	messenger ribonucleic acid
MSUP	maple syrup urine disease
MsAFP	maternal serum alphafetoprotein
MoM	multiples of median
MODY	maturity-onset diabetes of the young
NIH	National Institutes of Health
NTM	normal transmitting males
NTD	neural tube defect
NIDDM	non-insulin dependent diabetes mellitus
OMIM	Online Mendelian Inheritance in Man
OTD	ornithine transcarbamylase deficiency
PAH	phenylalanine hydroxylase

PCR	polymerase chain reaction
Ph	Philadelphia chromosome
PMP22	peripheral myelin protein 22
POSSUM	Pictures of Selected Syndromes and Undiagnosed Malformations
PKU	phenylketonuria
PAPP-A	pregnancy-associated placental protein A
RDS	retinal degeneration slow [gene]
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
ROM1	retinal outer segment membrane protein 1 [gene]
rRNA	ribosomal ribonucleic acid
SCID	severe combined immunodeficiency
SHOX	short stature homeobox [gene]
SINEs	short interspersed nuclear elements
SKY	spectral karyotyping
SMA	spinal muscular atrophy
SNPs	single nucleotide polymorphisms
SSCP	single strand conformational polymorphism
STRs	short tandem repeats
T	thymine (base), thymidine (nucleoside)
tRNA	transfer ribonucleic acid
TRIC	tetramethyl rhodamine isothiocyanate
TORCH	Toxoplasma, rubella, cytomegalovirus, herpesvirus [infections]
USG	ultrasonography
UTR	untranslated region
VATER	vertebral, anal, tracheo, oesophageal and renal [abnormalities]
v-*onc*	viral oncogenes
VHL	von Hippel–Lindau [disease]
VMA	vanillylmandelic acid
WAGR	Wilms tumour, aniridia, genital abnormalities, retardation [syndrome]
YAC	yeast artificial chromosome

Index

The letters 'b', 'f' and 't' after the numbers indicate boxs, figures and tables, respectively.

Section I

Introduction to medical genetics

1 Genetics in clinical practice

INTRODUCTION

Medical genetics is the science of human biological variation relating to health and disease. An improved understanding of the genetic basis of diseases over the past four decades has led to the growth of medical genetics as a specialty. Its direct use in clinical practice is known as clinical genetics. Clinical genetics deals with the diagnosis, prevention and management of genetic disorders. This specialty came into existence in 1956 with the development of techniques to study chromosomes.

In 1959, for the first time 47 chromosomes were observed in a case of Down syndrome. Following this, molecular techniques to identify genes and disease-causing mutations were developed. The spectacular progress in this field changed the diagnosis and management of single-gene disorders and also provided a new molecular approach to diseases. Genomes carry the entire blueprint for the orderly development, differentiation and functioning of all living organisms. Therefore, the study of genes helps in the understanding of basic life processes. Research in genetics is not limited to rare single-gene (monogenic) disorders but also includes common disorders of adult life such as cardiovascular diseases, psychiatric illnesses and cancer. Genetics has a place at the forefront of medical science and is not restricted to any one specialty. All medical students and practitioners need to understand the basic principles of genetics and their applications. The twentieth century saw the establishment of the specialty of medical genetics and in the twenty-first century, the increasing importance of genetics in diagnosis, prevention and therapy is expected to revolutionize medicine.

HISTORY OF MEDICAL GENETICS

The field of medical genetics began to develop at the end of the nineteenth century and the entire human genome was sequenced in 2000. Although the growth of genetic research was tremendous during the last three decades of the twentieth century, many basics of inheritance patterns, cytogenetics, biochemical genetics and population genetics were understood long before molecular techniques became available. The important landmarks in the history of genetics are given in Tables 1.1 and 1.2.

Table 1.1 Landmarks in the history of medical genetics

1865	Gregor Mendel	Particulate inheritance
1866	Langden Hayden Down	Mongolian idiocy (Down syndrome)
1882	Walther Flemming	Human chromosomes visualized
1900	K. Landsteiner	Discovery of ABO blood groups
1902	Archibald Garrod	Concept of inborn errors of metabolism
1903	Sutton and Boveri	Chromosome theory
1908	Ottenburg and Epstein	Inheritance of blood groups
1908	G.H. Hardy and Wilhem Weinberg	Calculation of gene frequencies in a population
1910	Davenport	First US genetic clinic
1911	T.H. Morgan	Significance of linkage to gene mapping
1928	Frederick Griffith	Genetic transformation
1941	Beadle and Tatum	One gene, one enzyme hypothesis
1944	Oswald Avery, Colin McLeod and Maclyn McCarty	Nucleic acids carry hereditary information
1946	Roberts	First genetic clinic in the UK
1949	Murray Barr and Bertram	Sex chromatin
1949	Linus Pauling	Electrophoretic abnormality of sickle haemoglobin
1953	James Watson and Francis Crick	DNA structure
1956	Tjio and Levan	46 chromosomes in man
1957	Messelson and Stahl	Semi-conservative mode of DNA replication
1957	Ingram	Amino acid sequence of sickle haemoglobin
1959	Jerome Lejeune	First chromosomal abnormality—trisomy 21 as the cause of Down syndrome
1960	Moorhead	Chromosomal analysis of blood
1961	Guthrie	Biochemical screening
1961	Mary Lyon	X chromosome inactivation

(*...cont.*)

Table 1.1 (*cont.*)

1961	Nirenberg, Khorana and Holley	Genetic code deciphered
1966	Breg and Steel	First prenatal chromosomal analysis
1966	Victor McKusick	First edition of *Mendelian inheritance in man*— Catalogues of autosomal dominant, autosomal recessive and X-linked phenotypes
1968	R.P. Donahue	First gene (Duffy blood group) mapped to a specific chromosome (chromosome 1)
1970	Caspersson	Banding of chromosomes
1970	Hamilton O. Smith	Restriction enzymes
1970	Hargobind Khorana	First gene synthesized *in vitro*
1977	Shine	First human gene (chorionic somatomammotrophin) cloned
1977	Jorge Yunis	High-resolution cytogenetics
1977	Itakura	Somatostatin made by genetic engineering
1977	Sanger, Maxam and Gilbert	Methods of DNA sequencing
1978	Steptoe and Edwards	The first successful *in vitro* fertilization
1978	Y.W. Kan and A.M. Dozy	First DNA diagnosis
1985	Jeffreys	DNA fingerprinting
1985	Ward	Fluorescence *in situ* hybridization
1986	Schmickel	Concept of contiguous gene syndromes
1986	Kary Mullis	Polymerase chain reaction (PCR)
1990	Watson	Human Genome Project initiated
1991	Barton	Enzyme replacement therapy for Gaucher disease
1993	Lyon	Genetic imprinting
2000	Celera Genomics and a consortium of 13 institutions from 6 countries	Human genome sequenced

Table 1.2 Terms and scientists who coined them

• Recessive and dominant	Mendel	1865
• Chromosome	Waldeyer	1882
• Genetics	Bateson	1905
• Genome	Winkler	1920
• Dysmorphology	Smith	1960s
• Reverse genetics	Orkin	1986
• Positional cloning	Collins	1992
• Proteomics	Russel	1995
• Pharmacogenomics	Roses	2000

GENETIC DISORDERS

Genes are involved in many diseases and now it is obvious that almost all diseases have variable amounts of a genetic component in their causation. Genetic disorders are traditionally classified as chromosomal disorders, single-gene disorders and multifactorial disorders. With the identification of the role of mutations in the etiology of cancers, a fourth category of somatic genetic disorders needs to be included.

Chromosomal disorders

Many numerical and structural abnormalities of chromosomes have been described in liveborns. These include a change in the number or structure of chromosomes. The change can be duplication or deletion of a chromosome. Even a part of a chromosome—extra or missing—can be associated with severe outcomes such as mental retardation and major and/or minor structural defects. Balanced chromosomal rearrangements, such as mutual transfer of parts of two chromosomes (i.e. reciprocal translocation) or an inversion of a part of a chromosome, do not usually lead to any phenotypic abnormality as the total DNA content of the cell is not altered in these situations. In contrast, conceptuses with major chromosomal imbalances are spontaneously aborted. Disorders caused by deletions involving smaller parts of chromosomes are known as microdeletion syndromes. Such deletions, which cannot be detected with traditional cytogenetic techniques, can now be detected using advanced techniques of molecular cytogenetics such as fluorescence *in situ* hybridization (FISH). Examples include Prader–Willi syndrome, Angelman syndrome and Williams syndrome.

Single-gene disorders

Genes are the basis of life. It is not surprising that defect in a gene can lead to a disease or disorder. Disorders caused by a defect (mutation) in a single gene are known as single gene or monogenic disorders. These disorders follow the Mendelian principles of inheritance. More than 5000 monogenic disorders have been identified. The Online Mendelian Inheritance in Man: A catalogue of genetic disorders and genes (OMIM; http://www.ncbi.n/m.nih.gov/omim/) is a source of complete and up-to-date information about these mendelian disorders. These diseases are classified as recessive or dominant depending on whether a mutation in both the copies of the gene is required to produce the clinical disorder, or mutation in even one of the copies can cause the disease. These disorders are caused by various genes on autosomes, sex chromosomes

or on the mitochondrial genome. Monogenic disorders involving each system of the body have been described. Some of them predominantly involve one system of the body, e.g. thalassaemia, haemophilia, achondroplasia, while others involve many systems of the body as in metabolic disorders such as mucopolysaccharidosis, galactosaemia and mitochondrial disorders (*see* Chapter 3). Monogenic disorders are rare; their frequencies vary from 1 per 2000 to 1 per 50,000 births. Taken together, monogenic disorders affect 1%–2% of live births.

As our understanding about the pathogenesis of these diseases is improving, it is becoming obvious that no disease is truly monogenic. Many other genes and environmental factors interact with the disease-causing gene, and modify the phenotype. Similarly, the distinction between dominantly inherited and recessively inherited disorders is getting blurred (*see* Chapter 4).

Multifactorial disorders

Most diseases are the result of a combination of small variations in genes that can together produce or predispose the individual to a disease. Multifactorial disorders tend to recur in families but do not show the characteristic pedigree pattern of single-gene disorders. Multifactorial inheritance is able to explain the pattern of inheritance observed in many common conditions such as congenital malformations and adult-onset disorders such as hypertension, ischaemic heart disease, diabetes mellitus, etc. Research in the identification of specific susceptibility genes for these common disorders is in progress and it is expected that genetic testing for the identification of individuals at high risk for these diseases may become available. This will open up many preventive options such as changes in lifestyle or prophylactic pharmacotherapy.

Acquired somatic genetic diseases

Some genetic defects may not be present in the zygote but may appear during somatic cell divisions (mitosis). These mutations (in genes and chromosomes) accumulated through replication are known to play an important role in the causation of cancer. In about 1%–5% of cancers, one of the cancer-causing mutations is present in the germline, i.e. present in the zygote itself and hence, in all cells of the body since conception. When the other copy of the gene in a cell also undergoes mutation during a lifetime, the cell becomes cancerous. These cancers follow an autosomal dominant pattern of inheritance and get transmitted from one generation to the next. However, most cancers are

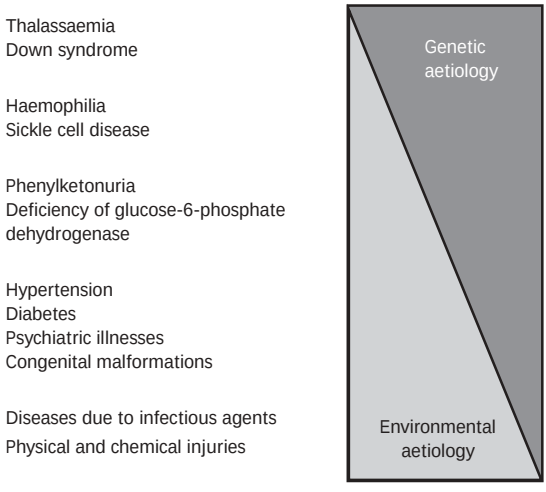


Fig. 1.1 Genetic and environmental components of the aetiology of some diseases.

seen in patients without a family history of the cancer. Such cancers are caused by mutations of cancer-related genes in somatic cells. Cancer-related genes, known as oncogenes and tumour suppressor genes, play an important role in the normal body processes of development and differentiation. It is now known that gene mutations lead to cancers but much remains unknown about the factors causing these mutations.

CLINICAL GENETICS

Almost all disorders have some genetic component in their aetiology (Fig. 1.1). A clinical geneticist should be able to evaluate these disorders from a genetic perspective (Table 1.3). The clinical geneticist provides valuable inputs for the diagnosis of chromosomal disorders, congenital malformations and multiple malformation syndromes, as well as for many rare disorders such as inborn errors of metabolism, neurodegenerative disorders, skeletal dysplasias, etc. For many other disorders, the role of the geneticist is limited, though important. This includes analysing pedigree data (family information), ordering special tests, interpreting the results of molecular and cytogenetic tests, and providing genetic counselling and prenatal diagnosis.

Pedigree drawing

Family history is an important part of the clinical information, especially for genetic disorders. A pedigree is a graphical representation of a family

Table 1.3 Approach to a disease from a genetic perspective

- Analyse data from the family history *
- Dissect the phenotypic heterogeneity *
- Dissect the genetic heterogeneity *
- Special tests: Biochemical, cytogenetic and molecular *
  —To order
  —To carry out
  —To interpret the results
- To diagnose rare disorders*
- To collect the latest information about the genetic aspects of the disease*
- Genetic counselling †
- Prenatal diagnosis †
- Coordination of multidisciplinary management

* Prerequisites for †, which is the primary responsibility of a clinical geneticist

tree using standard symbols (Figs 1.2a and b). Pedigree is the documentation of the family data in an easy-to-read form. Documentation in this form, provides more information about the disease and the concerned family. The pedigree of at least three generations is required to arrive at any fruitful conclusion. Symbols used in pedigree charts vary slightly among different genetic counselling services. Each generation is denoted by roman numerals, starting with the earliest generations. The female is shown on the right of the male in mating pairs. Offspring are depicted from left to right in descending order of age and denoted by arabic numerals.

The affected member, through whom a family with a genetic disorder is brought to attention, is the proband. The person (family member) who seeks genetic counselling is referred to as a consultand. If information about previous generations is not known to the consultand, older family members may be contacted. In a pedigree chart, members of the same generation are placed at the same horizontal level. Information about the clinical status of each member should be accurate. It should be noted whether the information is based on clinical examination, investigation reports or hearsay. In case there is doubt about the reliability of the information, it should be noted. General questions (e.g. Is everyone on the maternal side of your family alright?) should be avoided. Direct questions may need to be asked about similarly affected individuals, infertility, miscarriages, early death, consanguinity, etc.

There are two situations in which a pedigree is analysed. The first is when the mode of inheritance of the disease in the family is known. For example, take a pedigree of a family with haemophilia A. It is known that

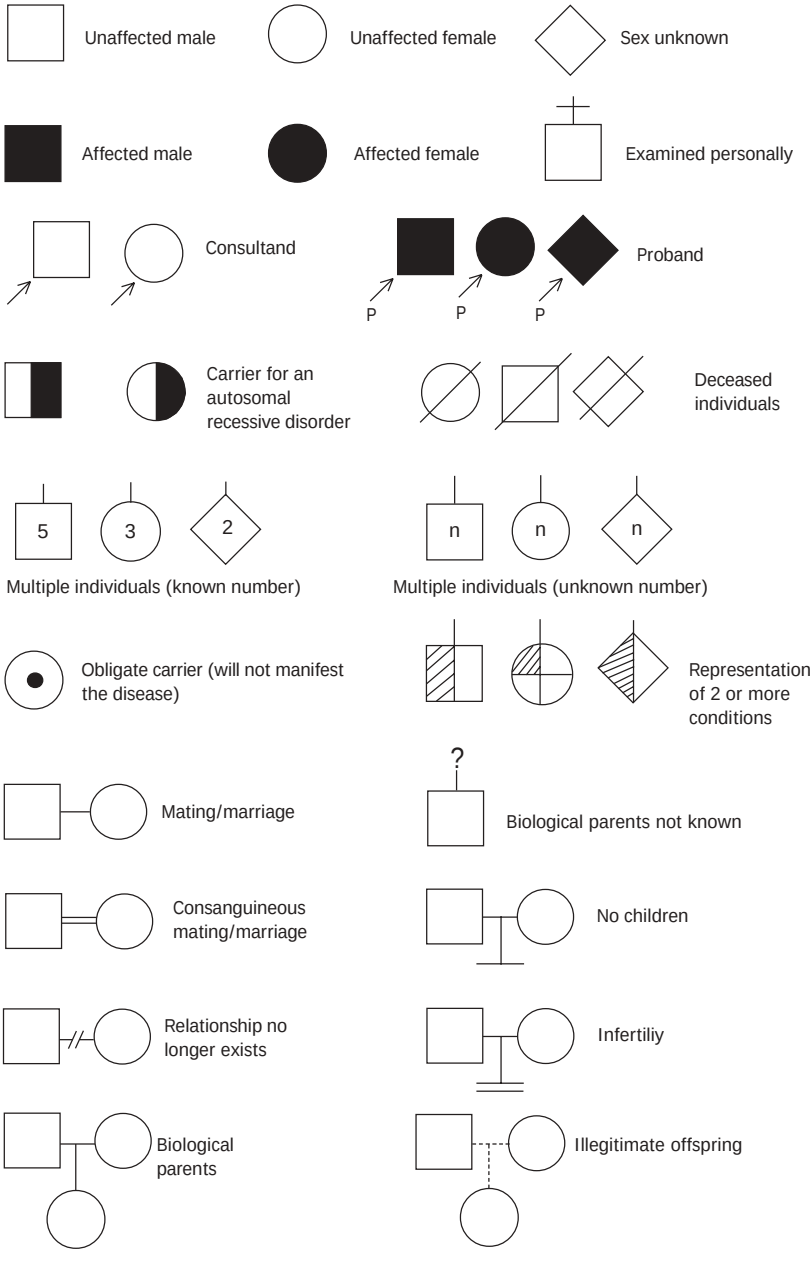


Fig. 1.2a Common symbols used in a pedigree drawing.

(*...cont.*)

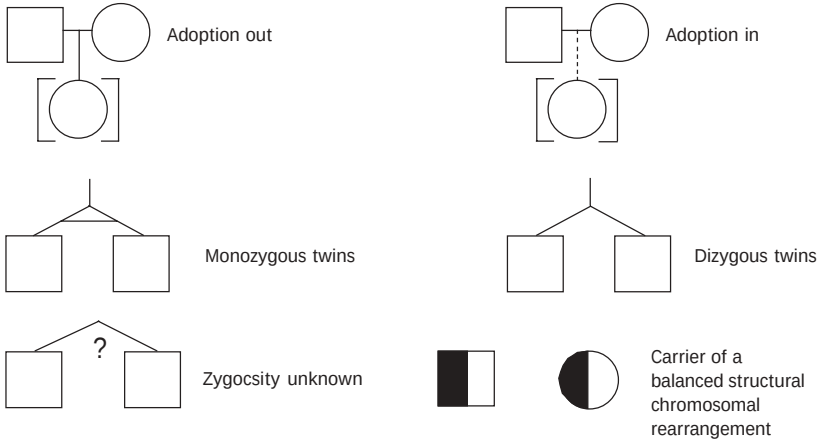


Fig. 1.2a (*cont.*) Common symbols used in a pedigree drawing.

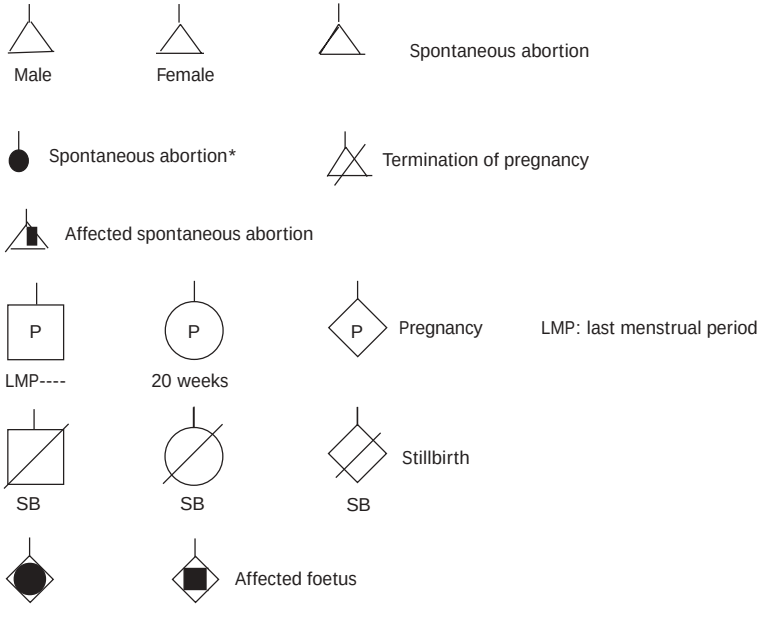


Fig. 1.2b Pedigree symbols for pregnancies not carried to term. Note that the symbols are smaller and the line is shorter. The gestational age and gender are written below if known. *- This symbol is also used for abortion.

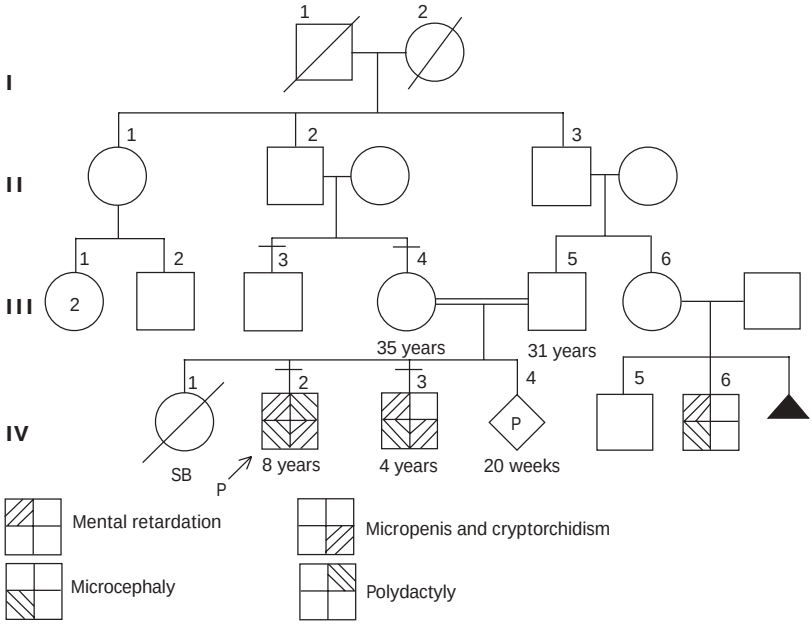


Fig. 1.3 An illustrative pedigree showing the use of various symbols and different relationships. Individual IV-6 may or may not have the same disorder as IV-2 and IV-3. In this pedigree, two similarly affected children are born to a normal consanguineous couple. This suggests a possibility of autosomal recessive mode of inheritance. However, the possibility of X-linked recessive mode of inheritance is also there as both the affected children are males.

haemophilia always follows an X-linked recessive pattern of inheritance. One then needs to refer to the pedigree to find out how many family members are affected and who are the definite carriers, possible carriers and non-carriers. If a woman has two sons with haemophilia or has an affected brother and a son, we know (even without testing) that she is definitely (obligate) a carrier of haemophilia. The second situation is when the mode of inheritance of the disease in the family is unknown; it may be autosomal dominant, autosomal recessive or X-linked. In that case, one should find out whether the pedigree shows the characteristics of any particular type of inheritance. A representative pedigree is shown in Figure 1.3.

2 Gene structure and function

A cell is the basic functional unit of life. The functions performed by a cell are influenced both by the information contained in its deoxyribonucleic acid (DNA) and the interaction with the external environment. Gene is a segment of DNA and codes for a polypeptide.

STRUCTURE OF DNA

DNA is a double-stranded polymeric macromolecule composed of several nucleotides. Each nucleotide consists of three types of units;a five carbon (pen-tose)sugar(deoxyribose), a nitrogen-containing base and a phosphate group (Fig. 2.1). The nitrogen bases found in DNA are of two types; purines (i.e. adenine [A] and guanine [G]) and pyrimidines (i.e. thymine [T] and cytosine [C]). The nucleotides are joined to each other through phosphodiester bonds between the fifth carbon atom of one deoxyribose (5') and the third carbon atom of the next deoxyribose (3'). The polynucleotide chain has a sugar-phosphate 'backbone'.The two chains of a DNA molecule wound around each other form a double helical DNA molecule with the nitrogenous bases directed towards the inside of the helix.

One end of each chain has a terminal sugar residue in which the 5th carbon atom is not linked to any sugar and is called the 5' end. Similarly, at the other end, the 3rd carbon atom of the terminal sugar molecule is free and is called the 3' end. One chain runs from the 5' to the 3' end, while the other has the opposite orientation and runs from the 3' to the 5' end. A nucleotide containing A always pairs with the one containing T, while G always pairs with C. These specific purine-

pyrimidine couples are called complementary bases and the opposite strands of a DNA molecule are known as the complementary strands. These strands are joined to each other by hydrogen bonds between the bases. There are three hydrogen bonds between C and G, and two between A and T.

Forms of DNA

DNA can adopt different types of helical structures(Fig. 2.2a). A- and B-DNA are right-handed helices (i.e. the helix spirals in a clockwise direction as it moves away from the observer). They have 11 and 10 base pairs per turn, respectively. Z-DNA is left-handed and has 12 base pairs per turn. In the majority of cells, B-DNA is present.The width of a DNA molecule (2 nm) is much less than the space left between adjacent turns, creating major and minor grooves.

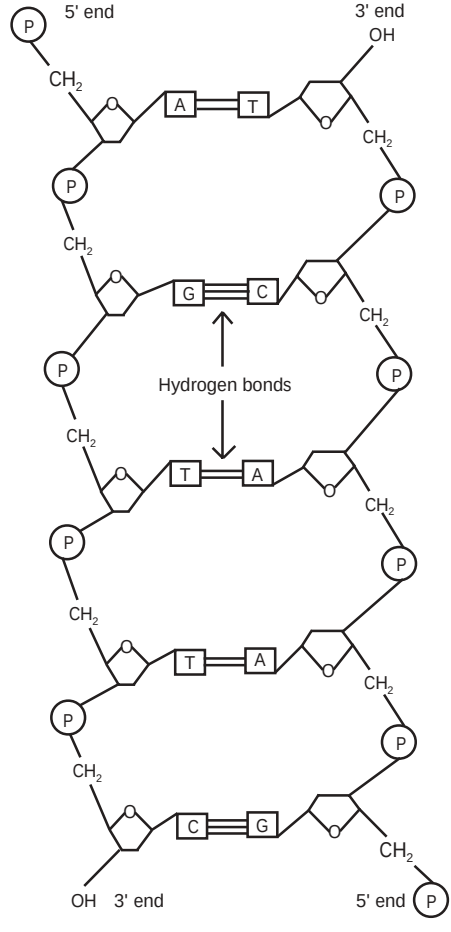


Fig. 2.1 Two complementary DNA strands oriented in the 5' to 3' and 3' to 5' direction and joined by hydrogen bonds between bases.

DNA packaging

DNA is a long molecule. The total length of DNA (6.6×10^9 base pairs [bp]) in a single human cell is 1 metre. It has to be packed in a nucleus of less than 10 µm size. Thus, it is compactly packed by different levels of coiling. At the first level, the DNA molecule is coiled around proteins called histones to form a bead-like structure known as a nucleosome(Fig.2.2b).At the tertiary level of coiling, chromatin fibres are formed. The chromatin fibres form long

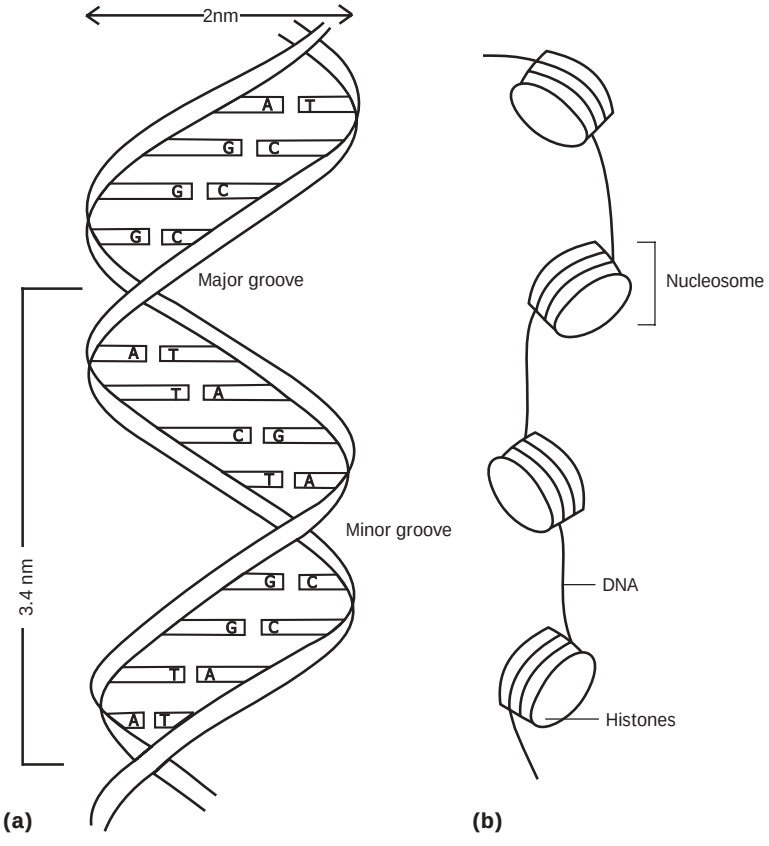


Fig. 2.2 **a** The molecular ladder of a double-stranded DNA molecule twists into a double helix. **b** First level of DNA coiling—winding around histones.

loops on a scaffold of non-histone proteins, which are further wound in a tight coil to make up the chromosome during mitosis. Such compaction of DNA allows its equal distribution to the daughter cells during mitosis. A chromosome consists of equal amounts each of DNA, histones and other non-histone proteins.

REPLICATION OF DNA

Nuclear DNA replicates before mitosis. The DNA content becomes double (4n) as compared to that of a normal diploid cell (2n). It then becomes compact in the form of visible chromosomes, which are equally distributed

to the two daughter nuclei. DNA replication is semi-conservative, both strands of the original DNA molecule are conserved as such and each daughter DNA molecule contains one original and one newly synthesized strand.

DNA replication is initiated at multiple points known as origins of replication and forms bifurcated, Y-shaped structures known as replication forks (Fig. 2.3a).It progresses in both directions from these points of origin forming bubble-shaped structures or replication bubbles (Fig.2.3b).The enzyme DNA helicase separates the two strands.Each strand then synthesizes a complementary strand through the action of DNA polymerases. The synthesis of both the strands occurs in the 5' to 3' direction. Hence, one strand of the double helix is synthesized in a continuous fashion and is known as the leading strand, while the other is synthesized in pieces called Okazaki fragments. The fragments are joined together by the enzyme DNA ligase to form a continuous strand. This strand is known as the lagging strand. DNA replication in individual replication units takes place at different times in the synthesis(S) phase of the cell cycle, with adjacent replication units fusing until the entire DNA is copied, forming two identical daughter DNA molecules.

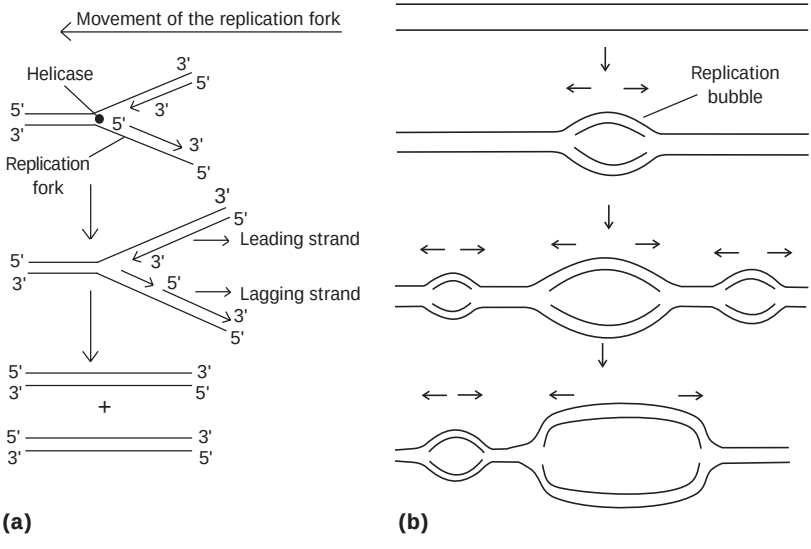


Fig. 2.3a DNA replication occurs in pieces on the 'lagging strand' and in a continuous fashion on the 'leading strand'. **b** Multiple units of DNA replication.

RIBONUCLEIC ACID (RNA)

Genetic information contained in the DNA is transferred from the nucleus to the cytoplasm by RNA molecules. The chemical structure of RNA is similar to that of DNA, except that the nucleotides in RNA have a ribose sugar instead of deoxyribose; and uracil (U) replaces thymine (T) as one of the pyrimidines. RNA is usually single-stranded whereas DNA is generally double-stranded.

Types of RNA molecules

RNA molecules are of three types:messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA).RNA carrying the coded information from the nucleus to the cytoplasm is known as mRNA. It is synthesized in the nucleus from the DNA template through a process known as transcription. The sequence of an mRNA is the same as that of the gene that transcribes it, except that U replaces T in RNA. The information in the mRNA is used for the synthesis of a polypeptide. This process, known as translation, occurs on ribosomes. Ribosomes are made up of different structural proteins in association with a specialized type of RNA, known as rRNA. Translation involves one or more types of RNA, i.e. tRNA which provides the molecular link between the coded base sequence of an mRNA and the amino acid sequence of a protein. There is a different tRNA for each amino acid.

GENE STRUCTURE

A gene can be defined as a sequence of bases in a DNA molecule along with regulatory sequences, which codes for one polypeptide (Fig. 2.4). These regulatory sequences are necessary for gene expression, i.e. the production of an mRNA and, in turn, a functional polypeptide. A gene also includes non-coding sequences (introns) that interrupt the coding segments (exons). Thus a gene is a segment of DNA molecule and is structurally continuous with the DNA on either side.

Introns alternate with exons and both are transcribed into an mRNA. Although a few genes in the human genome do not have introns, most genes contain at least one and usually several introns. The number

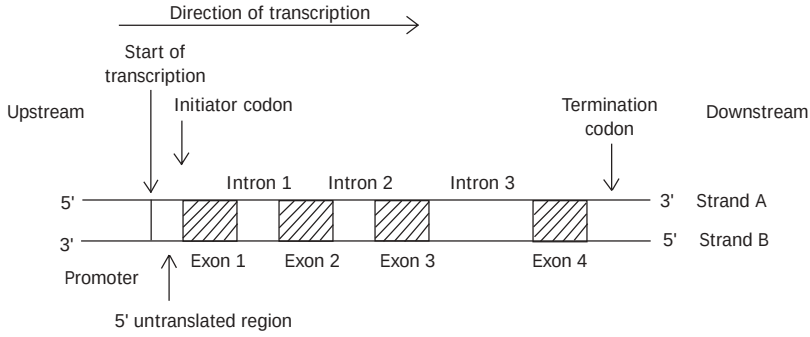


Fig. 2.4 Schematic representation of a gene.

of exons is one more than the number of introns. The size of different genes varies from ten to thousands of kilobases (kb) (1kb = 1000 base pairs or bp) of DNA. However, the size of most genes is usually hundreds of kb, e.g. the length of the antihaemophilic Factor VIII gene is 186 kb. Some genes are small, e.g. the length of the beta globin gene is 3 kb, while some are long, e.g. the dystrophin gene is 2 million bases (mb) (1 mb = 1000 kb) in length.

Theoretically, each of the two complementary DNA strands can code for an mRNA molecule. However, these complementary mRNAs would produce two entirely different proteins. In practice, however, only one of the two strands is required to code for a protein. By convention, this sequence of a gene is referred to as the sense strand or coding strand (strand A in Fig. 2.4) and the other DNA strand serves as a template for the production of an mRNA and is referred to as the antisense or non-coding strand (strand B in Fig. 2.4).

Untranscribed regions of a gene

The nucleotide sequences adjacent to the coding sequence provide the molecular 'start' and 'stop' signals for the synthesis of an mRNA. At the 5' end of the gene, immediately upstream from the transcription initiation site, lie untranscribed but important regions. One of these is the promoter region. It includes sequences responsible for the proper initiation of transcription. It is usually several hundred nucleotides long and RNA polymerase binds to it. Many promoters often contain a consensus sequence 5'-TATA-3' (TATA box), 30 to 50 bp upstream of the site at which transcription begins.

In addition to promoters, there are other regulatory elements for gene expression such as enhancers, silencers and locus-controlling regions.

Some of them may be far away from the gene or even on other chromosomes in the genome. Mutations in the promoter or other regulatory elements can also cause genetic diseases.

At the 3' end of a gene lies an important region that contains the signal for addition of adenosine residues (poly A tail) to the 3' end of the mRNA. Such closely neighbouring regulatory sequences are a part of the gene. However, at present, complete information about regulatory elements spread over the genome is not available and the limits of a gene are still ill defined.

GENE EXPRESSION

Transcription

Information in a gene is first transferred onto an mRNA. This process is known as transcription. The DNA in the region of the gene to be transcribed uncoils and the two strands separate. The antisense strand is used as a template and mRNA is synthesized. The sequence of mRNA is the same as that of the sense strand and complementary to that of the antisense strand. Transcription begins with the first exon and proceeds in the 5' to 3' direction. Thus, the initiation point corresponds to the 5' end of the final mRNA product. The initiator (first) codon of any mRNA is always AUG, which codes for methionine. Transcription continues through both the intron and exon portions of the gene,to the position on the chromosome that eventually corresponds to the 3' end of the mature mRNA.

Processing of pre-mRNA

In eukaryotes, transcription results in the production of pre-mRNA, which requires processing to generate a functional mRNA. These post-transcriptional modifications occur in the nucleus and include capping of the 5' end of the mRNA, addition of a poly A tail at the 3' end, and removal of introns and splicing of exons to produce the final mRNA. The presence of untranslated regions (UTRs) at both the 5' and 3' ends of the molecule is an additional feature of a mature mRNA molecule. The 5' untranslated region extends from the capping site to the beginning of protein-coding sequences and can be up to several hundred base pairs in length.

Capping

Shortly after the initiation of mRNA transcription, a 7-methylguanosine

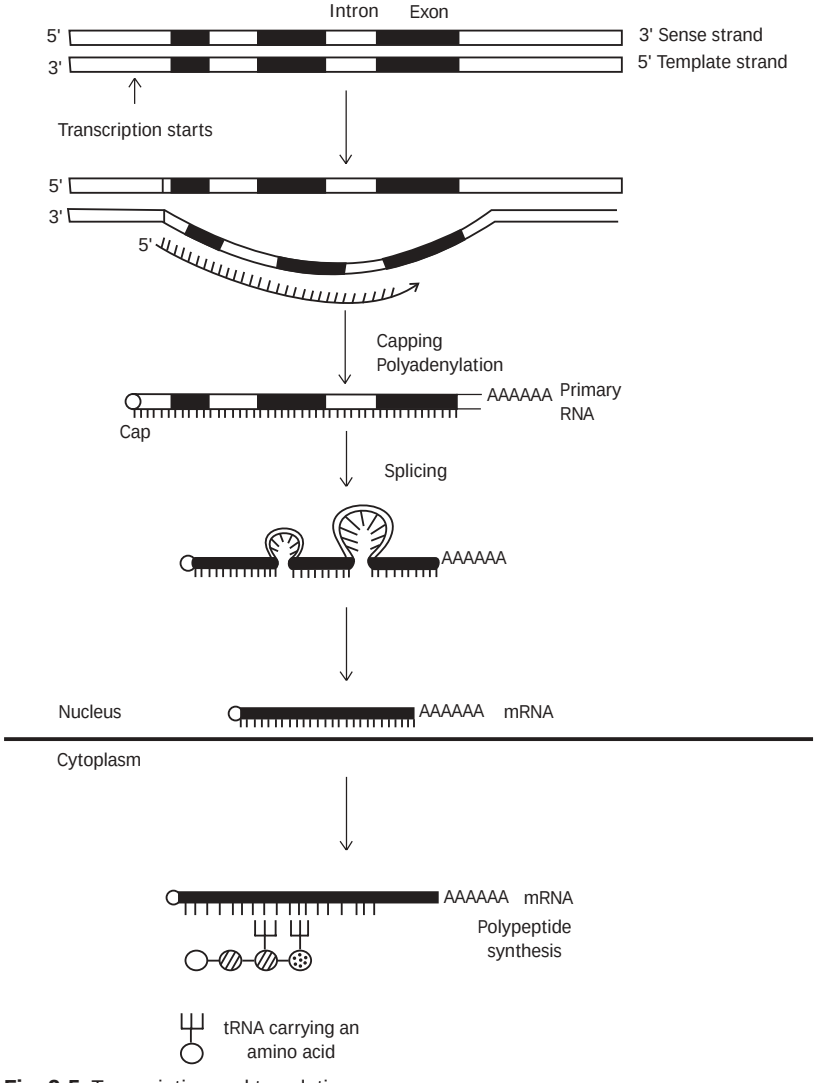


Fig. 2.5 Transcription and translation.

residue is added to the 5' end of the primary RNA transcript (Fig. 2.5). This 5' 'cap' is characteristic of every mRNA molecule.

3' cleavage and polyadenylation

Cleavage occurs at the 3' end at a specific point downstream from the end of the coding information. This cleavage is followed by the addition of a poly A tail

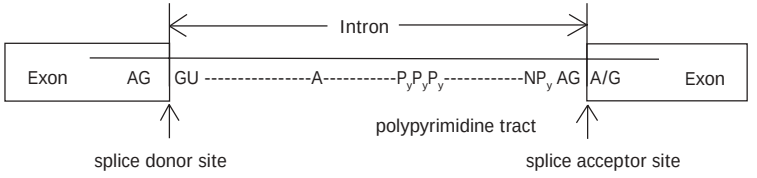


Fig. 2.6 Sequences for the donor (5' splice) and acceptor (3' splice) sites.

at the 3' end. The poly A tail appears to facilitate the transport of the mRNA molecule to the cytoplasm and increase the stability of the mRNA.

RNA splicing

Introns have to be removed before the final mRNA is formed and transported to the cytoplasm. The exon–intron junctions are marked by specific sequences. At the 5' exon–intron boundary (Fig. 2.6), the intron begins with the sequence GU and has the sequence AG at the 3' end of the boundary. These sequences, along with the sequences near the splicing site, signal the splicing. Cleavage occurs at both ends of an intron and the exonic mRNA segments are spliced.

Translation

The process of synthesis of a polypeptide from an mRNA is known as translation. After the introns are spliced, the mature mRNA is transported from the nucleus to the cytoplasm, where it is translated into a polypeptide chain. In the cytoplasm, tRNA molecules provide a bridge between the mRNA and free amino acids. There are 30 species of tRNA and each one is specific for a single amino acid. Transfer RNAs have a three-nucleotide sequence, which is complementary to the genetic code for a particular amino acid, known as the anticodon. Ribosomes are the site of protein synthesis and mRNA becomes associated with them. Messenger RNA acts as a template for polypeptide synthesis.

GENETIC CODE

The sequence of nucleotides in the mRNA decides the order of amino acids in the polypeptide. Three bases code for an amino acid and constitute a codon. The order of codons in a gene is known as the 'reading frame'. As there are four types of bases, there can be 64 different combinations of three bases each (triplet). Of these, AUG codes for methionine, which is always the starting amino acid. Three codons

Table 2.1 The genetic code

First base	Second base U	Second base C	Second base A	Second base G	Third base
U	UUU phe	UCU ser	UAU tyr	UGU cys	U
	UUC phe	UCC ser	UAC tyr	UGC cys	C
	UUA leu	UCA ser	UAA stop	UGA stop	A
	UUG leu	UCG ser	UAG stop	UGG trp	G
C	CUU leu	CCU pro	CAU his	CGU arg	U
	CUC leu	CCC pro	CAC his	CGC arg	C
	CUA leu	CCA pro	CAA gln	CGA arg	A
	CUG leu	CCG pro	CAG gln	CGG arg	G
A	AUU ile	ACU thr	AAU asn	AGU ser	U
	AUC ile	ACC thr	AAC asn	AGC ser	C
	AUA ile	ACA thr	AAA lys	AGA arg	A
	AUG met	ACG thr	AAG lys	AGG arg	G
G	GUU val	GCU ala	GAU asp	GGU gly	U
	GUC val	GCC ala	GAC asp	GGC gly	C
	GUA val	GCA ala	GAA glu	GGA gly	A
	GUG val	GCG ala	GAG glu	GGG gly	G

Abbreviations and symbols of amino acids

ala (A)	alanine	leu (L)	leucine
arg (R)	arginine	lys (K)	lysine
asn (N)	asparagine	met (M)	methionine
asp (D)	aspartic acid	phe (F)	phenylalanine
cys (C)	cysteine	pro (P)	proline
gln (Q)	glutamine	ser (S)	serine
glu (E)	glutamic acid	thr (T)	threonine
gly (G)	glycine	trp (W)	tryptophan
his (H)	histidine	tyr (Y)	tyrosine
ile (I)	isoleucine	val (V)	valine

(UAA, UAG and UGA) do not code for any amino acid and are known as 'stop' or 'termination' codons that signal the termination of translation. The remaining codons code for 20 amino acids. Each codon codes for a specific amino acid and there is no overlap in the reading frame. However, the genetic code of mitochondrial DNA for some amino acids differs from that of nuclear DNA. More than one codon can code for the same amino acid, thus, the code is said to be degenerate (Table 2.1).

HUMAN GENOME

The term 'human genome' is used to describe the total genetic information (DNA content) in human cells. It comprises two genomes:nuclear and mitochondrial. The

Box 2.1 Some single-copy and repetitive DNA sequences of the human genome

Housekeeping genes: Many proteins which serve a basic metabolic function such as energy generation or transport of nutrients are found in all cells. Genes coding for these proteins are described as housekeeping genes. These are single-copy genes.

Pseudogenes: Pseudogenes are single-copy genes which have a sequence similarity with known genes but are not expressed. These probably arise as a result of gene duplications and become non-expressing due to acquisition of mutation.

Satellites: These consist of complex, short, tandemly repeated DNA sequences 100 kb to several mb in length. These are clustered around specific regions of the chromosomes such as centromeres and telomeres. For example, the alpha satellite family of DNA is composed of tandem arrays of approximately 171 bp found at the centromere of each chromosome.

Minisatellite DNA: Hypervariable minisatellite DNA is made up of a highly polymorphic DNA sequence consisting of short tandem repeats of a common core sequence (6–64 bp). The number of repeats varies greatly in different minisatellites (0.1–20 kb). These are used in DNA fingerprinting and paternity testing. These are located close to telomeres.

Microsatellite DNA: Microsatellite DNA consists of tandem single, di-, tri- or tetranucleotide repeat sequences located throughout the genome. These can occur within or near genes. The variation in the number of repeats (usually less than 150 bp) gives rise to various possible alleles. Thus, the number of repeats on two homologous chromosomes varies from person to person. These repeats are highly polymorphic and are thus useful in gene mapping and diagnosis by linkage analysis. Trinucleotide repeats are associated with certain genetic disorders.

Short interspersed nuclear elements (SINEs): These are highly repeated interspersed DNA sequences, dispersed throughout the genome. A common example is Alu. They contain an Alu I restriction enzyme recognition site. Members of the Alu family are about 300 bp in length and their sequences are similar. There are about 750,000 copies of these repeats in the genome.

Long interspersed nuclear elements (LINEs): These are also highly repeated interspersed DNA sequences and are dispersed throughout the genome. The commonest of the LINEs is LINE-1 or L1. L1 elements are about 6000 bp in length and about 100,000 copies are present in the genome.

mitochondrial DNA accounts for 0.0005% of the total genome. Approximately 60%-70% of the human genome consists of a single- or low-copy number of DNA sequences. The rest of the DNA consists of several classes of repetitive DNA. Less than 10% of the genomic DNA is actually transcribed and these are known as functional genes (Box 2.1).

Single-copy DNA sequences

Only a small portion of single-copy DNA codes for proteins; the function of the non-coding sequences is unknown. Some genes belong to multigene families. The members of such a family have a structural and functional similarity with each other and may lie physically close to each other. Examples include the alpha- and beta globin (of haemoglobin) gene clusters on chromosomes 16 and 11, respectively. *HLA* genes on chromosome 6 and the various rRNAs that are clustered as tandem arrays on the short arms of acrocentric chromosomes are other examples.

Table 2.2 Types of repetitive DNA in the human genome

- Tandemly repeated DNA sequences
  - —Satellite: Moderately complex short tandemly repeated DNA, e.g. alpha satellite, DNA at the centromere
  - —Minisatellites: Short tandem repeats of a core common sequence
  - —Microsatellites: Single, di-, tri- or tetranucleotide repeats
- Highly repeated interspersed repetitive DNA
  - —Short interspersed nuclear elements (SINEs): About 300 bp in length, e.g. Alu
  - —Long interspersed nuclear elements (LINEs): About 6000 bp in length

Repetitive DNA

Most of the genomic DNA, other than single-copy genes, is repetitive DNA. It is not known to have any effect on the phenotype and at present its function is unknown. The various types of repetitive sequences are listed in Table 2.2.

EPIGENETICS

In each cell, only some genes are expressed at a time. The expressing genes vary from tissue to tissue. This happens because some of the genes are silenced and do not express. Silencing occurs without a change in the

nucleotide sequence of the gene. Such a type of heritable change in gene expression is known as an epigenetic phenomenon. DNA methylation is the best understood mechanism of this phenomenon.The other mechanisms responsible for the modification of gene expression are histone acetylation and chromatid structure modelling. These are interlinked with DNA methylation.

DNA methylation and CpG islands

In the human genome, C followed by G in the same strand occurs more infrequently than expected. However, in many genes, at the 5' end of the first exon, there is a high concentration of such CG sequences. These are called CpG (p for phosphate) islands. Three per cent of the cytosines in human DNA are methylated, producing 5-methylcytosine (by the addition of a methyl group at the 5' carbon atom). Most of these methylated cytosines are found in CpG islands. Methylation of CpG islands is associated with silencing of the gene.

This is an important mechanism for silencing genes in various physiological situations. The exact mechanism of how methylation stops transcription is not yet known. Abnormal methylation or demethylation of genes is an important component of the aetiopathogenesis of cancers.

In physiological situations, the expression of genes varies from tissue to tissue. Some genes express from only one chromosome depending on the parent from which it originated. The other allele inherited from the other parent will be silenced. This is known as genomic imprinting (*see* Chapter 5). In maternal imprinting, the maternally inherited copy of the gene is silenced and in paternal imprinting, the paternally inherited allele is silenced. Methylation of DNA is the mechanism responsible for genomic imprinting. DNA methylation also appears to an important mechanism of silencing the genes on lyonized X chromosome. DNA methylation is heritable and various enzymes, namely, DNA methyl transferase 2, 3a, and 3b are responsible for *de novo* methylation and maintenance of methylation.

Genetic disorders due to defects in methylation and other epigenetic phenomena are beginning to be known. Acquired abnormalities of methylation of cancer-related genes are being identified in many cancers (*see* Chapter 14). Identification of the abnormalities caused by DNA methylation in various diseases has important therapeutic implications. Drugs such as 5-azacytidine are known to prevent methylation and enhance the expression of silenced genes. These are being tried as a novel method of treatment.

3 Gene mutations

A mutation is defined as a heritable alteration or change in the genetic material, i.e. any change in the nucleotide sequence or arrangement of DNA. Mutations are of three types:

1. Genome mutation: Change in the number of chromosomes
2. Chromosomal mutation: Alteration in the structure of chromosome/s
3. Gene mutation: Change in the sequence of a gene

This chapter describes gene mutations that cause single-gene or monogenic disorders. In addition to these disease-causing mutations, there are a number of differences in the DNA sequences of two individuals and not all differences in the DNA sequence cause disease. Such diferences are known as polymorphisms. Many of these polymorphisms may be responsible for the anatomical and physiological variations between individuals, and for the variations in their susceptibility or predisposition to disease. Mutations give rise to different forms of a gene known as alleles. Mutations can be spontaneous or non-spontaneous (induced by chemical or physical agents). Errors occurring during replication and repair of DNA result in mutations.

TYPES OF GENE MUTATIONS

Mutations can occur in somatic cells during mitosis. Many of them are responsible for carcinogenesis. However, these mutations are not transmitted to the next generation. Only those mutations that are present in the germ cells (germline mutations) can be passed on to the next generation. Most germline mutations are stably (as such) transmitted to

Table 3.1 Types of stable mutations

Type of mutation	Definition	Examples
Substitution	Replacement of a nucleotide by another	Sickle cell anaemia, beta-thalassaemia
Deletion	Deletion of one or a few nucleotides, or large deletions	Duchenne muscular dystrophy, alpha-thalassaemia
Insertion	Insertion of one or a few base pairs, or large insertions	Neurofibromatosis type 1, haemophilia A
Duplication	Duplication of a gene or a few exons	Charcot–Marie–Tooth disease, Duchenne muscular dystrophy
Inversion	Inversion of a segment of a gene	Haemophilia A

the next generation and are known as stable or fixed mutations. In 1991, a new type of mutation known as dynamic mutation (*see* Chapter 10) was described.

Stable mutations

This group of mutations is further classified into subgroups depending on the type of change in the DNA sequence (Table 3.1).

Substitutions

A substitution is the replacement of a single nucleotide with another. This is the most common type of mutation. A purine can be replaced by another purine and a pyrimidine by another pyrimidine (transition) or a purine may be replaced by a pyrimidine and vice versa (transversion). Transition is more common than transversion. The commonest type of substitution is replacement of C by T.

Missense and nonsense mutations

Depending on its effect on a codon, a substitution can be classified as missense or nonsense. Missense mutations lead to a change in the codon, which in turn may or may not lead to a change in the amino acid. If the changed codon also codes for the same amino acid as the original codon, it is known as a synonymous mutation and if it codes for a new amino acid, it is a non-synonymous mutation. Synonymous mutations are silent, i.e. they do not have any phenotypic effect. In contrast, the effect of a non-synonymous mutation on the phenotype depends on the importance of the changed amino acid in the functioning of the protein.

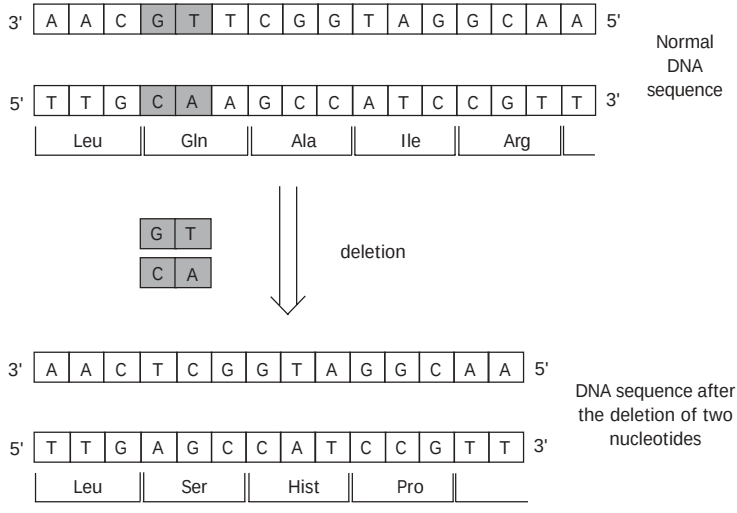


Fig. 3.1 Alteration in the reading frame due to the deletion of two nucleotide base pairs.

If a substitution changes the codon for a particular amino acid to a stop codon, then it will lead to the termination of protein synthesis at that point. Such types of mutations are called nonsense mutations.

Deletions and insertions

Deletions and insertions can be small or large. If the inserted or deleted segment involves nucleotides from the coding sequence of a gene and the number of nucleotides in that segment is not a multiple of three, then there is a shift in the reading frame of the DNA sequence downstream of the mutation (Fig. 3.1). This is known as a frameshift mutation.

Deletions of a large segment or the complete gene are commonly seen in the growth hormone and alpha globin genes. These usually occur due to a recombination of mispaired homologous sequences (Fig. 3.2). The homologous sequences can be a similar copy of the gene (e.g. in alpha-thalassaemia, each chromosome has two copies of the alpha globin gene) or repetitive sequences in the region near the gene.

Duplication

Gene duplication is an important cause of mutation in some disorders. The mechanism of duplication is the same as that of deletions (Fig. 3.2).

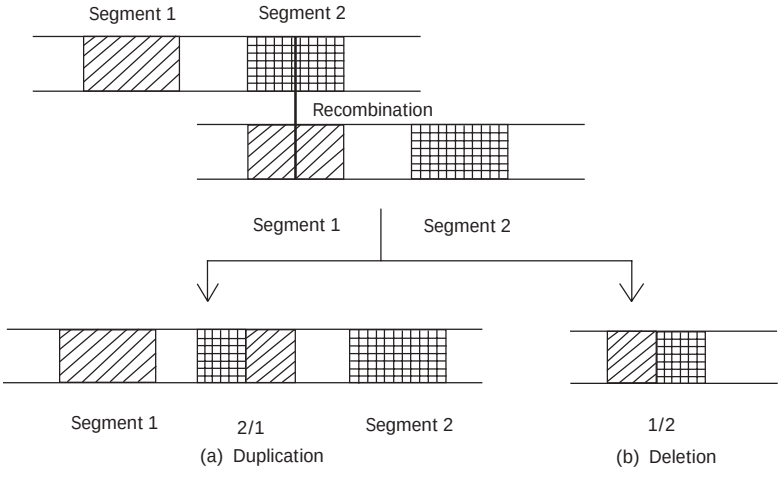


Fig. 3.2 Recombination due to mispairing of similar sequences in the region near the gene. Note that in the resultant daughter chromosomes, one has duplication (a) and the other has deletion (b).

Inversion

Inversion is a rare mutation. The most common inversion found to date is that of the Factor VIII gene, resulting in haemophilia A. In exon 22 of the Factor VIII gene, there is a 9.5 kilobase (kb) sequence (A in Fig. 3.3). This sequence is repeated twice, about 400 kb away from the first, towards the telomeric end. Recombination occurs between the sequence

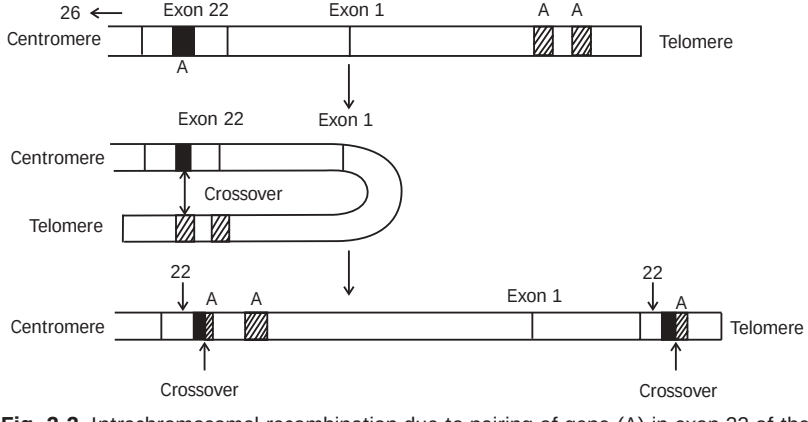


Fig. 3.3 Intrachromosomal recombination due to pairing of gene (A) in exon 22 of the Factor VIII gene and the other similar gene near the Factor VIII gene.

in exon 22 and either of these sequences on the same X chromosome. This leads to inversion of a large segment of the Factor VIII gene, making it non-functional.

Dynamic mutations

Dynamic mutations arise due to an increase in the number of triplet repeats within or near a gene. In this type of mutation, the number of triplet repeats undergoes changes while being transmitted from one generation to the next (*see* Chapter 10). These are known as dynamic mutations because of their unstable nature. These are found to be responsible for causing the fragile X syndrome. Some more disorders have been found to be caused by dynamic mutations.

Mutations of different types are commonly seen in association with one disease. Hundreds of different mutations are known to give rise to a disease, e.g. different point mutations, deletions in the beta-globin gene result in beta-thalassaemia. Different families with the same disease may have different mutations.

NOMENCLATURE FOR DESCRIBING MUTATIONS

Mutations are described according to the nomenclature laid down by the Genome Database Nomenclature Committee. The mutations can be classified in two ways; by their effects (Box 3.1) and their description.

The nomenclature for describing a mutation uses one- or three-letter codes for amino acids, e.g. R 100 H or Arg 100 His means the hundredth amino acid arginine is replaced by histidine. R stands for arginine and H for histidine. A stop codon is denoted by 'X' or 'stop'. For the codes of other amino acids, *see* Table 2.1 (p. 22).

Nucleotide substitutions can also be described in the above-mentioned way. For substitutions, the nucleotides are numbered starting

Box 3.1 Nomenclature for describing the effect of a mutant allele

Null allele or amorph: An allele that does not produce any product.

Hypomorph: An allele that produces a product in reduced amount or with reduced activity.

Hypermorph: An allele that produces a product in increased amount or with increased activity.

Neomorph: An allele that produces a product with novel activity.

Antimorph: An allele whose activity or product opposes the normal product, thus reducing the efficacy of the normal protein of the normal allele.

from +1 for the A of ATG codon (always the first codon) and –1 for the nucleotide just preceding the A of ATG. There is no zero. For example, 100 G>A means guanine at the hundredth position is replaced by adenine.

Deletions and insertions are denoted by 'del' and 'ins'. For example, F 508 del means deletion of phenylalanine at the 508th position and 602–606 del means deletion of five nucleotides starting from position six hundred and two.

MOLECULAR PATHOLOGY

Molecular pathology attempts to find out why a given genetic change should result in a particular phenotype. Thus, it tries to discover the effect of a change in the DNA sequence on the product (mRNA or protein) and how that change in product alters the structure or function of the body. For most genetic disorders, the understanding of the effect of change in the DNA sequence on the phenotype is not complete.

The functional effects of a mutation can be different (Table 3.2). Loss of function mutations are usually associated with diseases inherited in an autosomal recessive fashion. In these disorders (e.g. inborn errors of metabolism), even if only one copy of a gene is functioning normally (as in carriers of the disease), the amount of protein produced is sufficient to prevent manifestation of the disease. On the contrary, in autosomal dominant disorders, 50% of the product is not sufficient to prevent

Table 3.2 Classification of mutation depending on the functional effects

Functional effect	Description	Examples
Loss of function mutations	The gene produces little or no functional product	Thalassaemia, enzyme deficiencies of mucopoly-saccharidosis and other metabolic disorders
Gain of function mutations	The product of the gene acquires a new function	Hereditary motor and sensory neuropathy typeIa,achondro-plasia, Huntington chorea
Haploinsufficiency	The amount of protein produced by the normal allele is insufficient to prevent a clinical phenotype	Acute intermittent porphyria, familial hypercholesterolaemia
Dominant negative effect	The product of the gene interferes with the function of the protein produced by the normal allele	Osteogenesis imperfecta

manifestation of the disease. Such diseases will manifest in heterozygotes due to haploinsufficiency.

However, in some autosomal dominant disorders, the mechanism is not haploinsufficiency. In such cases, the abnormal product of the mutated copy of the gene interferes with the function of the product of the normal copy and thus causes disease. This is described as a dominant negative effect. Dominant negative mutations are particularly common in proteins that are dimers or multimers. Collagen, an important protein of connective tissue, is made up of triple helices of two types of polypeptide chains. Missense mutations in the collagen gene will lead to the formation of an abnormal chain. This chain will form complexes with the normal chains and will reduce the yield of functional collagen leading to osteogenesis imperfecta—a disease with weak bones. In such diseases, the null allele will have less severe manifestations than a mutant allele, which produces abnormal protein (antimorph). This has been confirmed in the case of osteogenesis imperfecta.

Gain of function requires much more specific change than loss of function. Thus, the mutational spectrum in gain of function conditions is limited. For example, for all types of achondroplasia, only two mutations of the fibroblast growth factor receptor 3 (*FGFR3*) are known. Other mutations in the *FGFR3* gene give rise to different disorders.

GENOTYPE–PHENOTYPE CORRELATION

Most genetic disorders have great variability in their manifestations. As the mutational spectrum of various disorders is becoming known, an attempt is being made to correlate a particular mutation with the specific disease manifestations or disease severity. This is known as genotype–phenotype correlation.

This correlation can be clinically useful to predict the phenotype during prenatal or presymptomatic diagnosis. One important way to predict the phenotype is to know the mechanism of effect of the mutation on the gene product or its activity. Unfortunately, it has become obvious that accurate prediction of the phenotype from the genotype is a rare phenomenon. Great intrafamilial variability is proof of that. Thus, even if all affected members of a family have the same mutation, there may be great variability in their phenotypes. This is partly due to the fact that the phenotype is determined not only by the type of mutation but also by the interaction among other genes (genetic background). Even in the case of so called single-gene disorders, there is significant

Table 3.3 Entries of human genes and genetic disorders in OMIM*

Autosomal	14892
X- linked	886
Y-linked	56
Mitochondrial	64
Total	15898

*Source: *Online Mendelian Inheritance in Man* (as of March 2006)*

contribution of other modifier genes and the environment in determining the phenotype.

SINGLE-GENE DISORDERS

Single-gene (monogenic) disorders or diseases arise due to a defect in a single gene. The defective gene could be present on the sex chromosomes, autosomes or mitochondrial genome (Table 3.3). The disorders are classified into dominant or recessive depending on whether it manifests in heterozygote or homozygous state respectively (Table 3.4).

Monogenic disorders are rare and their incidence varies from 1 in 2000 to 1 in 50,000 or less. Taken together, they affect 2% of the population throughout their lives. Their incidence in the paediatric population is said to be 0.36%, while among hospitalized children, 6%–8% have a single-gene disorder. All monogenic disorders and genes have been listed by Victor McKusick in the *Mendelian Inheritance in Man* (MIM) available online [OMIM]. At present there are more than 15,000 entries in OMIM and new disorders are being added. Molecular basesof more than 1600 of these disorders had been identified by the year 2006.Two single-gene disorders and their management are discussed here—beta-thalassaemia (autosomal recessive), and Duchenne muscular dystrophy (DMD) (X-linked recessive).

BETA-THALASSAEMIA

Beta-thalassaemia is a common autosomal monogenic disorder. It is caused by decreased or no synthesis of the beta globin chains. It is a heterogeneous disorder caused due to many point mutations or deletions in the beta globin gene. In the homozygous condition, the disease is characterized by hypochromic, microcytic anaemia presenting in infancy and is known as thalassaemia major. The anaemia is so severe that without blood transfusions, the child rarely survives beyond infancy. In some

Table 3.4 Examples of single-gene disorders

Autosomal dominant	Autosomal recessive	X-linked recessive	X-linked dominant
Achondroplasia	Thalassaemia major	Haemophilia A	Hypophosphataemic rickets
Huntington chorea	Sickle cell disease	Haemophilia B	Rett syndrome †
Myotonic dystrophy	Limb–girdle myopathy	Duchenne muscular dystrophy	Incontinentia pigmentosa†
Adult-onset polycystic kidney disease	Afibrinogenaemia	X-linked severe combined immuno-deficiency	Aicardi syndrome †
Crouzon syndrome	Cystic fibrosis	X-linked hydrocephalus	Fragile X mental retardation (semi-dominant)
von Willebrand disease	Phenylketonuria	Pelizaeus–Merzbacher syndrome	
Holt–Oram syndrome	Meckel syndrome	Mucopolysacch-aridosis type II	
Retinitis pigmentosa *	Short limb polydactyly syndromes	Retinitis pigmentosa *	
Spinocerebellar ataxia	Metachromatic leucodystrophy	X-linked anhidrotic ectodermal dysplasia	
Facioscapulohumeral myopathy	Retinitis pigmentosa *	Ornithine transcarbamylase deficiency	

* Indicates genetic heterogeneity † Lethal in males

homozygotes, the severity of anaemia is less, the disease manifests a little late and the patient is not dependent on blood transfusions for survival. This type of phenotype is known as thalassaemia intermedia.

Epidemiology

Beta-thalassaemia is highly prevalent in India and the Mediterranean countries such as Cyprus and Greece. Although nearly 180 mutations in the beta globin gene have been described, a relatively small number of mutations account for the majority of cases in a given population. For example, five common mutations in the beta globin gene account for about 92% of all mutations in beta-thalassaemia patients in India. These are as follows:

1. (IVS) 1–5 (G -->C)
2. IVS 1–1 (G -->T)
3. 619 bp deletion
4. +1 codon 8/9 (+G)
5. –4 codon 41/42 (–CTTT)

Aetiology

A patient with thalassaemia major may be homozygous for a mutation in the beta globin gene, i.e. both copies of the beta globin gene have the same mutation (Fig. 3.4) or, more commonly, may be a compound heterozygote, i.e. has different mutations in the two copies of the beta globin gene (Fig. 3.5). Thus, the patient has two mutations (same or different); one inherited from each parent. Homozygotes or compound heterozygotes manifest the disease while heterozygotes are usually asymptomatic. The underlying pathology of anaemia in homozygotes is either decreased production(β^+-thalassaemia) or no production (β^0-thalassaemia) of production of the beta globin chains.

Mutations causing beta-thalassaemia

Mutations known to reduce the synthesis of mRNA or proteins have been identified as a cause of beta-thalassaemia. Most mutations in the beta globin gene are point mutations. The only common deletion known to cause beta-thalassaemia is deletion of the 619 base pair at the 3' end of the gene and is seen in Indians. Beta globin gene mutations are seen in exons, introns, splice junctions, cap site, promoter and the poly A tail region.

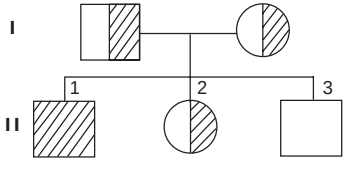


Fig. 3.4 Pedigree of a family having a child (II-1) with thalassaemia major. The child is homozygous for a mutation in the beta globin gene. The mutation inherited from the father is the same as that inherited from the mother.

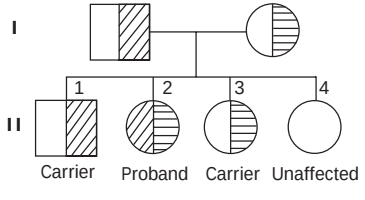


Fig. 3.5 Pedigree of a family affected with beta-thalassaemia. II-2, the proband, is a compound heterozygote for two different mutations in the beta globin gene inherited from the parents. II-1 and II-3 are carriers and have inherited mutations from the father and mother, respectively.

Exon mutations

A mutation in an exon usually changes the amino acid sequence leading to the synthesis of a variant haemoglobin. Rarely, mutation in an exon also activates a cryptic site (present in exons or introns) leading to synthesis of variant haemoglobin at a reduced rate. An example is haemoglobin E.

Intron mutations

A mutation within an intronic cryptic site may enhance its use by making it similar or identical to the normal splice site. The normal splice site being intact, the activated cryptic site has to compete with it. These mutations usually result in β^{+}-thalassaemia.

Splice junction mutations

This group of mutations includes mutations in the splice junction region or in the consensus sequences surrounding the junction. As a result, normal splicing does not take place. A cryptic site may be involved in splicing. A cryptic site, if spliced alone, will usually lead to β^{0}-thalassaemia and if it competes with the normal site, some beta globin chains will be formed, leading to β^{+}-thalassaemia.

Mutations producing non-functional mRNA

Some mutations introduce the stop codon prematurely and lead to the formation of non-functional mRNA, through substitution or deletion of one or two bases. Such frameshift mutations in the beta globin gene lead to β^{0}-thalassaemia.

Transcriptional mutations

A transcriptional mutation occurs in the promoter (non-coding) region of the gene. These are single-nucleotide substitutions in the TATA box. Such mutations usually result in β^{+}-thalassaemia and the phenotype is mild.

RNA modification defects

These defects are found at both the 5' end or cap site and the 3' end or RNA cleavage and polyadenylation signal site, which decrease the stability of mRNA, resulting in decreased production of beta globin (β^{+}-thalassaemia). In addition, mutation at the cap site may also reduce the rate of transcription.

Complex thalassaemia and deletions

In addition to the mutations discussed above, a few small deletions

have also been described. Large deletions of the beta globin gene involve the neighbouring delta gene and other genes leading to delta–beta-thalassaemia, gamma–delta–beta-thalassaemia or hereditary persistence of foetal haemoglobin. The phenotypes of these thalassaemias are clinically different from thalassaemia major.

The genotype–phenotype correlation

Depending on the site of mutation, the biochemical phenotype-β^0 or β^+ can be predicted and confirmed by measuring the ratio of the alpha and beta chains. The clinical heterogeneity of thalassaemia can be explained partly by the heterogeneity in the mutations.

Usually, heterozygotes for beta-thalassaemia mutations (thalassaemia trait or minor or carriers) are clinically asymptomatic except a rare occurrence of mild anaemia. However, they have microcytosis and hypochromia in the peripheral blood. Rarely, some thalassaemia heterozygotes present with clinically significant anaemia (less severe than thalassaemia major) and haemolysis. Mutations causing such presentations (dominant beta-thalassaemia) usually occur in the last (third) exon of the beta globin gene. At the other extreme, some mutations in the beta globin gene are mild (silent thalassaemia) and heterozygotes cannot be identified by haematological study, as they do not have microcytosis or increase in Hb A_2 or Hb F levels.

So-called homozygotes for beta-thalassaemia are usually compound heterozygotes at the molecular level as the two copies of the beta globin gene have different mutations. Homozygotes or compound heterozygotes usually present with a severe form of the disease, i.e. thalassaemia major. Patients with a phenotype of intermediate variety, namely, thalassaemia intermedia may be homozygotes for mild mutations or rarely heterozygotes (Table 3.5). In spite of having a lot of information about beta globin gene mutations, the pathophysiology of thalassaemia is still not completely understood.

Clinical features

Homozygotes and compound heterozygotes for beta-thalassaemia usually present with severe anaemia at 4 months to 1 year of age. If untreated, the child with thalassaemia major dies by the age of 1–5 years.

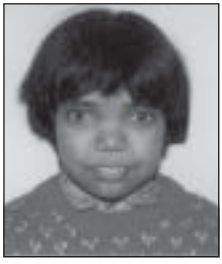
Treatment and management

The curative treatment for thalassaemia major is bone marrow transplantation. If bone marrow transplantation is not possible, then

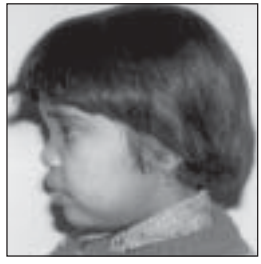
Table 3.5 Genotypes of thalassaemia intermedia and associated modifiers of the phenotype

Determinant / modifier of phenotype	Examples of genotypes associated with the thalassaemia intermedia phenotype
Mutation	• Homozygous for mild β^+-thalassaemia • Compound heterozygosity for β^0- or severe β^+- and mild β^+-thalassaemia mutation • β^0- and silent β-thalassaemia • Homozygote for silent β-thalassaemia • Heterozygote for dominant β-thalassaemia
Number of alpha genes	• Homozygote or compound heterozygote for β^0- or β^+-thalassaemia with one, two or three alpha globin gene deletions • Beta-thalassaemia heterozygote with extra copies of the alpha globin gene (alpha triplication or quadruplication)
Co-inheritance of mutations causing increased synthesis of gamma globin genes, leading to increased synthesis of foetal haemoglobin	• Homozygote or compound heterozygote for β-thalassaemia mutation with mutation for hereditary persistence of foetal haemoglobin • Homozygote or compound heterozygote with high levels of foetal haemoglobin due to unknown causes
Co-inheritance of other haemoglobin variants with β-thalassaemia	• Hb S, Hb Lepore, delta–beta-thalassaemia, Hb C, Hb E, unstable Hb S

regular three-weekly red blood cell transfusion and iron-chelation therapy provide good results (Figs 3.6 and 3.7). However, this form of hypertransfusion therapy is expensive and difficult to carry out. The disease causes a great burden on the parents and indicates a strong need for prevention by prenatal diagnosis. Beta-thalassaemia is associated with mutational heterogeneity at the genetic level. In this case, it is not possible to predict the genotype from the clinical or biochemical phenotype. Hence, prenatal diagnosis or carrier detection tests are required for detection of the disorder. It is essential that the causative mutation in the family is identified first by analysing the DNA of the affected person or obligate carriers in the family.



(a)



(b)

Figs 3.6 a and **b** Inadequately treated child with thalassaemia major. Note the facial changes due to bone marrow hyperplasia.

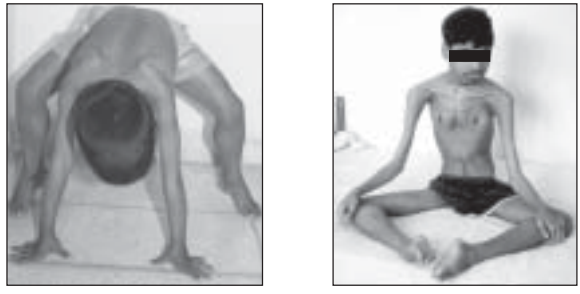
Fig. 3.7 Patients with thalassaemia major have to undergo transfusion of packed red blood cells every 2–3 weeks.

DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is a common genetic disorder inherited in an X-linked recessive fashion. It is caused by mutations in the dystrophin gene, which is the largest known human gene. Up to 65% of the mutations responsible for the disease are deletions and 5%–10% are duplications; the rest are caused by point mutations. Dystrophin is a cytoskeletal protein limited to the muscle cell membrane. The disease manifests before 5 years of age in boys with the complaint of difficulty in getting up from the sitting position and is associated with pseudohypertrophy of the calf muscles (Fig. 3.8). The muscle weakness progresses and the child becomes wheelchair-bound by 12 years of age and dies by twenty. A milder form of the disease is known as Becker muscular dystrophy (BMD) and is also caused by mutations in the dystrophin gene (Figs 3.9a and b). The onset of BMD is late but the extent of pseudohypertrophy and weakness of the calf muscles is similar to that in DMD.

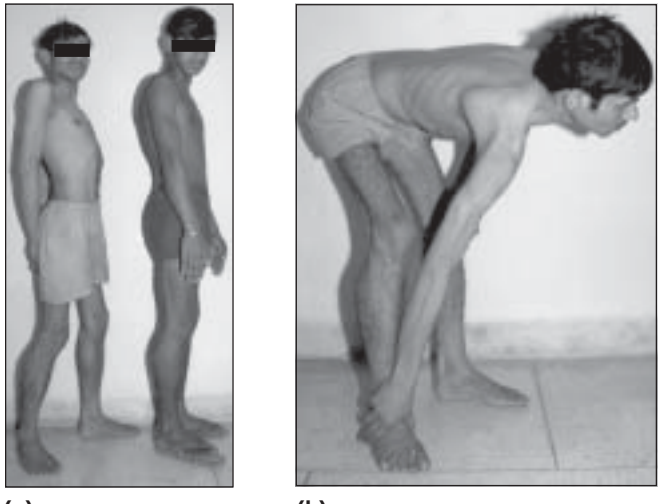
Being an X-linked recessive disease, the mother of the patient is a carrier of the disease in most of the cases. However, in a few cases, the mother is not a carrier and the disease occurs due to a *de novo* mutation. The risk of occurrence of the disease in the sons of a carrier woman is 50%, and 50% of her daughters are likely to be carriers.

Mutations that result in disruption of the reading frame prevent the production of a stable protein and are likely to be associated with DMD. Mutations that result in the production of abnormal proteins that are stable and partially functional are associated with the milder BMD phenotype. Deletions can occur anywhere in the gene but are usually



(a) (b)

Fig. 3.8 Duchenne muscular dystrophy. **a** A young child with difficulty in getting up from the sitting posture; needs support of the hands. **b** A young bedridden adult in the second decade of life. Note the severe wasting of all the muscles except those of the calf muscles.



(a) (b)

Fig. 3.9 Becker muscular dystrophy. **a** Young adult brothers with Becker muscular dystrophy. The onset of weakness was in the second decade of life. The elder brother (left) has more weakness and muscle atrophy than the younger one (right). **b** The elder brother in Figure 3.9a has difficulty in getting up from the sitting posture.

present in spots around the first 20 exons and around exons 45–55. These regions are described as hot spots.

There are several variations in the mutations giving rise to DMD. The clinical presentation cannot give any clue to the type of mutation. Hence, it is necessary to identify the mutation in each family by analysing the

DNA of the affected member before offering carrier detection and prenatal diagnosis. Multiplex PCR is used to detect a deletion.

Due of the lethal nature of the disorder and lack of treatment, carrier detection and prenatal diagnosis are of great help to families affected with DMD.

4 Patterns of inheritance

The first evidence for the control of inheritance of characters by factors, i.e. genes, came from the studies done by Gregor Mendel on plants. Mendel (1865), an Austrian monk, studied pairs of contrasting characters in pea plants and concluded that each character is governed by a factor (Box 4.1). Later, Garrod suggested that disorders such as alkaptonuria, albinism and cystinuria follow the recessive pattern of inheritance, in accordance with Mendel's laws. Soon, other examples followed and a number of single-gene (monogenic) disorders were described. Monogenic disorders follow the principles of inheritance described by Mendel and are also known as Mendelian disorders. Initial information about the patterns of inheritance of these disorders came from the analysis of data collected from affected families. It is only during the past few decades that it has become possible to study the causative genes by molecular methods. However, till date, the genes for only a few disorders have been mapped. Some single-gene disorders are listed in Table 3.4.

Box 4.1 Summary of Mendel's experiments

- Each character is governed by a factor (now known as 'gene'). Genes occur in pairs; one inherited from each parent.
- **Law of dominance:** Individual genes can have different forms, i.e. alleles, some of which (dominant) exert their effect over the others (recessive).
- **Law of segregation:** During meiosis, alleles segregate from each other with each gamete receiving one of the pair of alleles at a locus.
- **Law of independent assortment:** The segregation of different pairs of alleles (at different loci) is independent of each other.

As against these single-gene disorders, many characters such as height and many common familial diseases such as diabetes, hypertension, etc. do not usually follow such a simple pattern of inheritance. These traits and disorders are considered to be multifactorial in origin. In addition to multifactorial disorders, many other disorders that do not follow the Mendelian pattern of inheritance have been known for a long time. Recent advances in molecular biology have provided explanations for these disorders. The non-Mendelian or non-traditional modes of inheritance include the following:

1. Mitochondrial inheritance
2. Genomic imprinting
3. Uniparental disomy
4. Mosaicism: Somatic and germline
5. Dynamic mutations (triplet repeat disorders)
6. Oligogenic inheritance

These novel and interesting phenomena are discussed in detail in Chapter 5.

MENDELIAN INHERITANCE

Diseases inherited in a Mendelian fashion are categorized according to whether the gene is on an autosome or a sex chromosome and whether the trait is dominant or recessive (*see* Chapter 3).

Dominance and recessiveness

Before knowing about the dominant and recessive patterns of inheritance, it is necessary to understand the concept of dominance and recessiveness. 'Dominant' and 'recessive' are relative terms and are to be used only when one allele is compared with the other. At each locus, there can be many forms of a gene, which are known as alleles. If we consider two alleles, one dominant over the other, and assuming that both the alleles are present in the genotype, the phenotype will be determined by the dominant allele. For example, if there are two alleles, A and a, the phenotype of persons with genotypes AA and Aa are similar, but differ from that of a person with the genotype aa. It means that allele A is dominant and allele a is recessive. In relation to diseases, the disease-causing allele (i.e. allele with a mutation) may be dominant or recessive to the commonly occurring normal (wild-type) allele. If the disease-causing allele is dominant to the wild-type allele, the heterozygote with one wild-type and one mutated allele will have the disease. Such diseases

that manifest in the heterozygous state are known as dominant diseases.

In some cases, the disease manifests only when a person is homozygous for a mutated allele, and the heterozygotes are not affected. This is because the mutated allele is recessive to the wild-type allele. Such diseases are known as recessive diseases. In addition to dominant and recessive diseases, some diseases show semi-dominant inheritance. In these disorders, heterozygotes also manifest the disease, but the manifestations are less severe than that in homozygotes. This situation is seen in achondroplasia, which is a common form of skeletal dysplasia. Achondroplasia is considered an autosomal dominant disorder because heterozygotes for the disease-causing mutation have a short stature and generalized bone defects. If both the parents have achondroplasia, then they may have a child who is homozygous for the disease. Such homozygotes have a very severe form of the disease with marked shortening of the limb bones and an extremely narrow thorax, which lead to stillbirth or neonatal death. Thus, in the real sense, achondroplasia is a semi-dominant disease. However, for many autosomal dominant diseases such as Huntington chorea, the phenotype of heterozygotes is same as that of homozygotes.

Mechanism of dominance

There are several mechanisms by which a mutation can lead to disease manifestation even in the heterozygous state.

Loss of function mutation (haploinsufficiency)

Mutation in one copy of a gene may decrease the amount of protein produced. For many proteins, such a decrease may not give rise to any phenotypic effect. Phenotypic effects appear only when both the copies of the gene are mutated. Thus, most loss of function mutations exhibit recessive behaviour. However, when the total amount of protein produced is crucial or the protein is required in large amounts, the disease will manifest in the heterozygous state. The examples are acute intermittent porphyria and mutation in the gene producing C1 esterase inhibitor leading to angioneurotic oedema.

Gain of function mutations

1. Increased gene dosages: Duplication of the *PMP22* gene causes Charcot–Marie–Tooth disease, a type 1 hereditary sensory and motor neuropathy. The gene codes for peripheral myelin protein 22 and duplication of the gene increases production of the protein.
2. Ectopic or temporally altered mRNA production: Mutation in some

genes alters the time and place of their expression. Gamma globin (a part of foetal haemoglobin) is expressed only in foetal life. Mutations in the globin promoter region prevent normal shutting off of production of gamma globin chains after birth and lead to persistence of foetal haemoglobin.

3. Increased protein activity: Some mutations increase the life of a protein by providing it with resistance to proteolytic degradation. For example, hyperkalaemic periodic paralysis occurs due to the loss of regulatory inactivation of the sodium channel.
4. Dominant negative mutation: With this type of mutation, the mutant allele produces an abnormal protein which interferes with the function of the normal protein. An example is osteogenesis imperfecta. Point mutations (*see* Chapter 3) in the subunits of collagen give rise to abnormal protein and thus disrupt the assembly of the collagen molecule. In this case, missense mutations (which give rise to abnormal protein) are more harmful than null allele (which does not produce the protein).
5. Toxic protein alteration: These mutations cause structural alterations in the protein which accumulates and poisons the cell. An example is hereditary amyloidosis.
6. New protein functions: Some mutations alter the function of the gene product. For example, a missense mutation in the α_1-antitrypsin gene causes a bleeding disorder because the abnormal gene product is an inhibitor of thrombin.

Recessive mutations with a dominant effect

These mutations actually function as recessive at the cellular level but appear to be transmitted in an autosomal dominant fashion. Many of the hereditary cancers, e.g. retinoblastoma and hereditary polyposis coli are inherited in this way. In this case, a person inherits one mutation from a parent. This mutation is present in all the cells of the body. If he or she acquires the mutation in the other copy of the gene in a relavent somatic cell, that cell becomes cancerous.

AUTOSOMAL DOMINANT INHERITANCE

Disorders that manifest even in the heterozygous state and are caused by mutations in the genes on autosomes are known as autosomal dominant disorders. Figure 4.1 shows a pedigree of a disorder inherited in an autosomal dominant fashion.

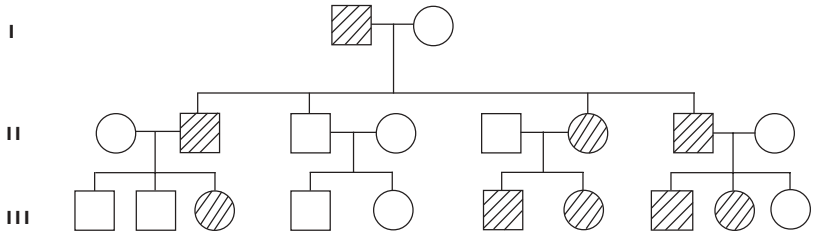


Fig. 4.1 A pedigree depicting the inheritance of an autosomal dominant disorder.

Characteristics of autosomal dominant disorders

1. Successive or multiple generations are affected.
2. The chance of the progeny being affected by an autosomal dominant disorder is independent of the sex, i.e. both males and females are affected in equal proportions.
3. Both males and females can transmit the disease to their offspring of either sex.

Risk of recurrence

Individuals with an autosomal dominant disease have one normal and one mutant allele. Thus, 50% of their gametes contain the normal and 50% the mutant allele (Figs 4.2a and b). Hence, the risk of transmission of the disease from an affected parent to his or her children is 50%. It should be noted that these figures of probability are rarely seen to be true in individual pedigrees. However, they hold true when data of various families are combined. For example, if we collect data from many families of Marfan syndrome, an autosomal dominant disorder, we see that 50% of the children of persons with Marfan syndrome are affected with the same disorder and the ratio of affected males to affected females is 1:1. However, if we take a single nuclear family, two, three or more children may be consecutively affected or unaffected.

Some characteristics of autosomal dominant disorders which affect the risk of their recurrence are as follows:

1. Penetrance: The penetrance of a disorder is the ratio of number of persons with disease manifestations to the number of persons with the mutation. Some individuals may have the mutant allele, but there may not be any manifestatithe. In Fig. 4.3, the normal individual (II-1) has the mother, a son and a daughter affected with an autosomal dominant disorder. This suggests that he is also carrying the

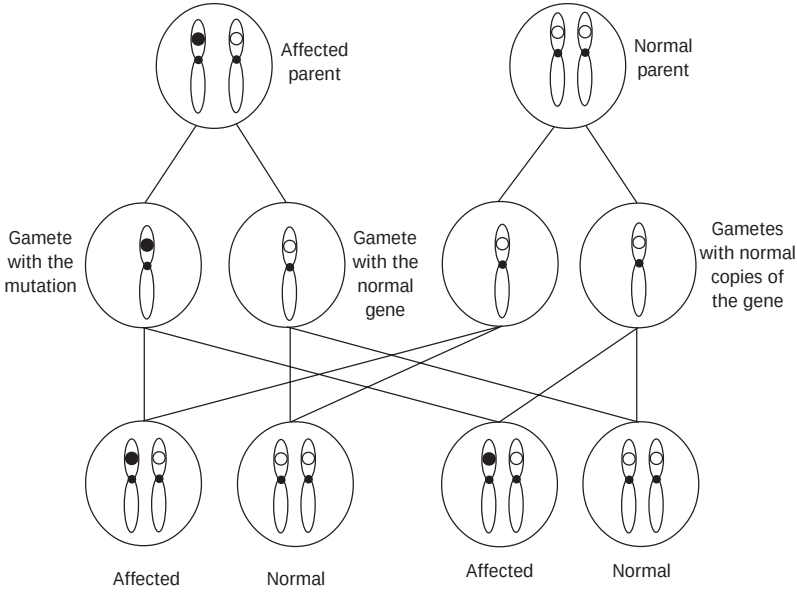


Fig. 4.2a Transmission of autosomal dominant disorders.

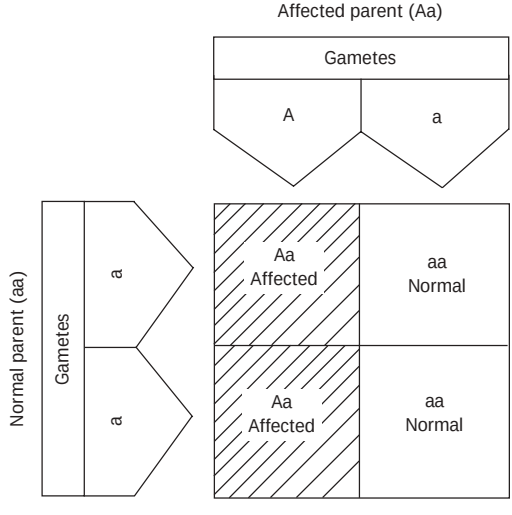


Fig. 4.2b A Punnett square showing the possible gamete combinations for an autosomal dominant disorder.

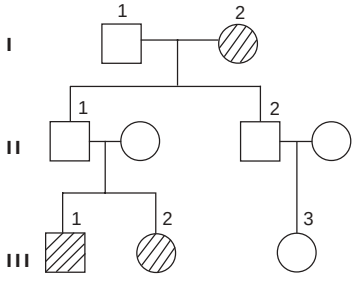


Fig. 4.3 A pedigree showing a 'skipped generation'. Individual II-1 is non-penetrant for the disease, i.e. a non-manifesting carrier of the mutant gene.

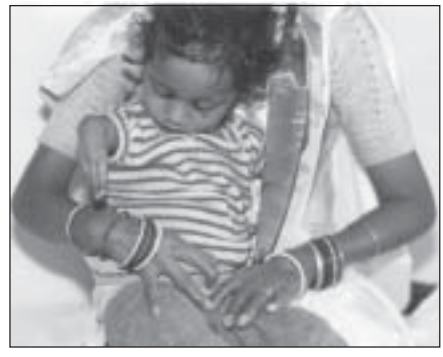


Fig. 4.4 A mother and her daughter with Holt–Oram syndrome. Note the variable severity of involvement of the upper limb in them.

mutated gene but has no manifestations of the disease. Such a situation, when not every person with the mutant allele shows features of the disorder, is known as incomplete penetrance. The reduced penetrance can become evident because of a 'skipped generation' in a pedigree or by identifying asymptomatic carriers of the mutant gene by DNA analysis. The penetrance of a disease has important implications in genetic counselling. For disorders with a reduced penetrance, the asymptomatic children of an affected parent may be carriers of the disease and hence, at risk for giving birth to affected children. The other implication is that though the risk of transmitting the mutant gene from an affected parent to the offspring is 50%, the risk of having clinically affected offspring will be less than 50%. The penetrance of some genes is dependent on the age of the individual as in Huntington chorea, myotonic dystrophy and spinocerebellar ataxia.

2. Variable expressivity: The severity of a disease may vary in affected persons of a family and is known as variable expressivity. It is a common phenomenon observed in autosomal dominant disorders, e.g. Holt–Oram syndrome (Fig. 4.4), neurofibromatosis type I and tuberous sclerosis. In neurofibromatosis, there is great variability in the number of neurofibromas, presence or absence of mental retardation and hypertension in various affected members of a family. Similarly, in tuberous sclerosis, one person may have a complete spectrum of manifestations including mental retardation, while another family member may have only a fine rash of angiokeratoma on the face.

The causes of variable expressivity and incomplete penetrance are interaction of the causative gene with other genes and environmental factors. Such genes, whose expression would modify the manifestation of a disease or its severity, are known as modifier genes. Information about modifier genes will help in predicting the phenotype from the genotype. With increasing knowledge of modifier genes, it is becoming obvious that even single-gene disorders are not truly governed by only one gene but are oligogenic. This appears logical as no gene functions in isolation. The phenomenon of interaction between various genes is described as epistasis. The difference between penetrance and variable expressivity is that penetrance is an all or none phenomenon, while expressivity shows varying grades of severity.

3. Anticipation: A disorder is said to demonstrate anticipation if the severity of the disease increases as it is passed down the generations. This is characteristically seen in myotonic dystrophy. The grandparent may have cataract, muscle weakness and myotonia in old age; the mother may have mild muscle weakness and myotonia and she may give birth to a neonate with hypotonia and mental retardation. For some disorders, increasing severity is manifested as an earlier age of onset of the disease.

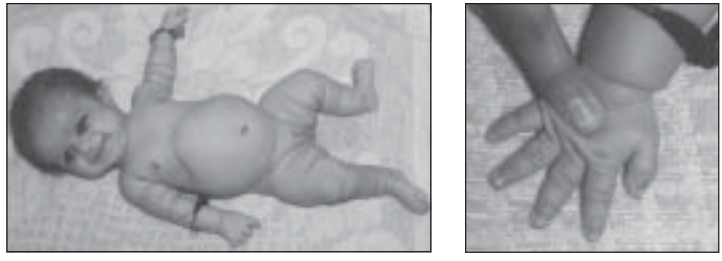
Counselling for autosomal dominant disorders

The risk of passing on an autosomal dominant disorder to the next generation by an affected person is 50%, but the risk may vary if the disease is known to have reduced penetrance. Information about the penetrance, variable expression and anticipation of the disease should be included while counselling.

The following two situations need special mention:

1. Sporadic case of an autosomal dominant disorder (Figs 4.5a and b): Commonly, a child with achondroplasia is born to normal parents (i.e. who do not have achondroplasia). Non-penetrance or variable expressivity is not known in achondroplasia.

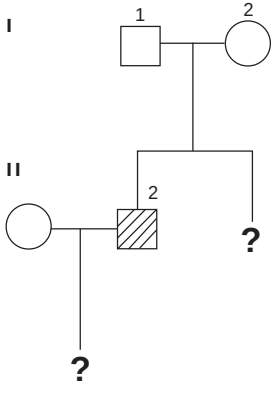
Figure 4.5c shows a pedigree with a sporadic case of achondroplasia. The risk of the disorder in the children of II-2 (proband) is 50% if he marries a normal female. What is the risk of his siblings having the disease? As the parents of the proband are normal, they do not harbour the mutation for achondroplasia. Hence, the mutation in the genes of the child has occurred *de novo* and is not inherited. Thus, the risk of his parents having another child with achondroplasia is nil or almost



(a) (b)

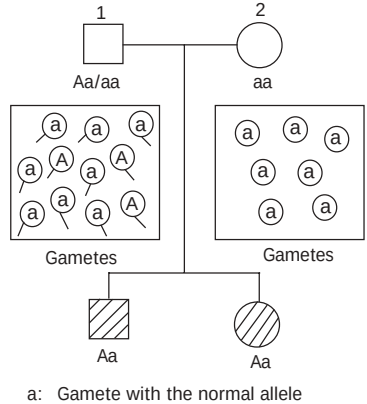
Fig. 4.5 a An infant with achondroplasia. Note the short stature with predominant shortening of proximal segments of the limbs. **b** Trident hand of the infant in Figure 4.5a characteristic of achondroplasia.

negligible. However, rarely, normal parents may give birth to two children affected with achondroplasia. Such cases indicate the possibility of germline mosaicism (Fig. 4.6), i.e. the gonads of the mother or father contain two types of cells, one with normal copy of the gene and the other with the achondroplasia mutation. Thus, although gonadal mosaicism cannot be tested for and detected, the possibility of its occurrence should be kept in mind. Hence, the risk of achondroplasia in the siblings of an affected child with normal parents is small (cannot be quantified) but not zero.



?: What is the risk of achondroplasia

Fig. 4.5c A pedigree showing a sporadic case of achondroplasia.



a: Gamete with the normal allele
A: Gamete with the mutant allele for achondroplasia

Fig. 4.6 Normal parents give birth to children with the autosomal dominant disorder achondroplasia. This suggests the possibility of gonadal mosaicism (shown in the father in this case).

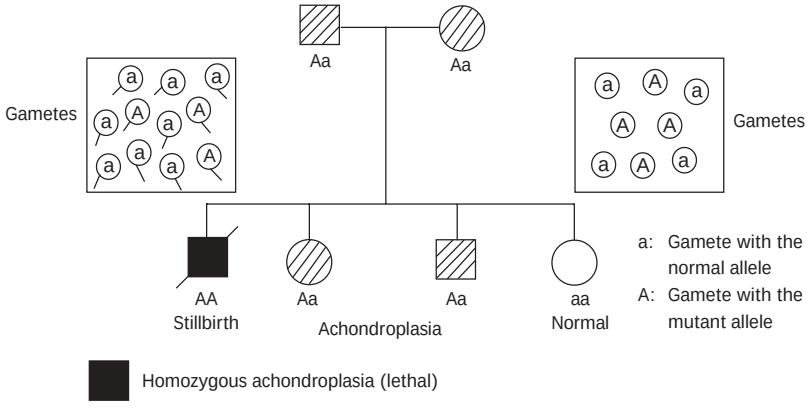


Fig. 4.7 Risks in the offspring of a couple when both of them are affected with achondroplasia.

2. If both the husband and wife have achondroplasia, the chance of having an affected child is 50% (Fig. 4.7). The possibility that they can have a normal child is 25% and there is a 25% possibility of the child inheriting both mutant alleles from the parents. Such a child who is homozygous for the achondroplasia allele will be affected with a severe form of skeletal dysplasia (homozygous achondroplasia) that is lethal in the neonatal period. It can be diagnosed ultrasonographically during early pregnancy.

AUTOSOMAL RECESSIVE INHERITANCE

Autosomal recessive inheritance is due to genes located on the autosomes, in which the mutant or disease-causing alleles are recessive to the wild-type ones and are therefore not clinically evident in the heterozygous state. The disorder manifests only in the homozygous state (Fig. 4.8). There may be many heterozygotes in the family, but they will be clinically normal and will not have offspring with the disease unless the spouse is also a carrier of the disease.

Characteristics of an autosomal recessive disorder

The following are the characteristics of an autosomal recessive disorder:

1. Both males and females are affected.
2. The disorder normally occurs in only one generation, usually within a single sibship.
3. The parents can be consanguineous, especially if the disorder is rare. However, consanguinity is not essential.

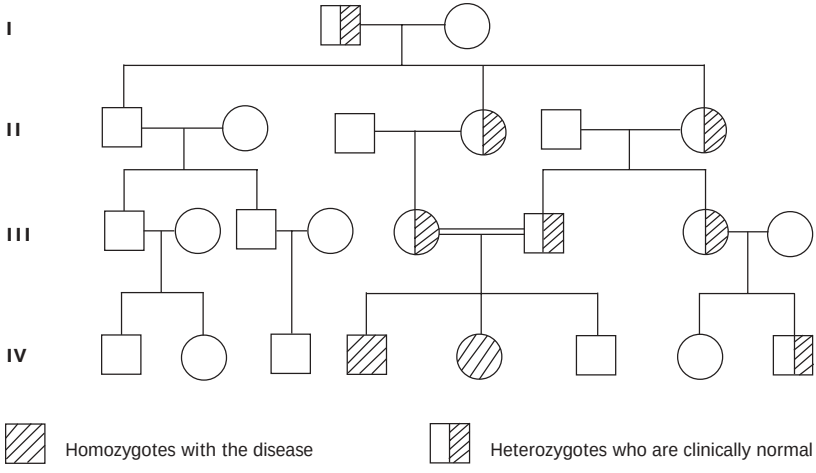


Fig. 4.8 Pedigree depicting an autosomal recessive disorder. Note that the affected persons are seen in only one generation. Consanguinity is not essential.

Risk of recurrence

When a child affected with an autosomal recessive disorder is identified in a family, it is obvious that the parents are (obligate) carriers of the disease (except in the rare situations of false paternity and uniparental disomy, which will be discussed later). Thus, it is not necessary to test for the carrier status of the parents. The risk of their second child being affected with the disorder is 25% (Fig. 4.9a).

If a heterozygote for an autosomal recessive disorder marries a normal person, 50% of his children will be heterozygotes like him, but there is no risk of having any affected child (Fig. 4.9b).

In a family, two, three or more consecutive children may be affected with the disorder (Fig.4.10). Similarly, two, three or more consecutive children may be normal though both the parents are heterozygous for an autosomal recessive disease.

Importance of carrier detection in the relatives of a person/child affected with an autosomal recessive disorder

If a person is affected with an autosomal recessive disorder, the chances of his/her siblings being carriers are 50%. Similary, there is a high chance that his/her relative is a carrier of the disorder. Thus, it is useful to identify the carriers by offering carrier detection tests for the relatives who are clinically normal. The carriers thus identified need to be counselled regarding carrier testing of the spouse. This is essential if the

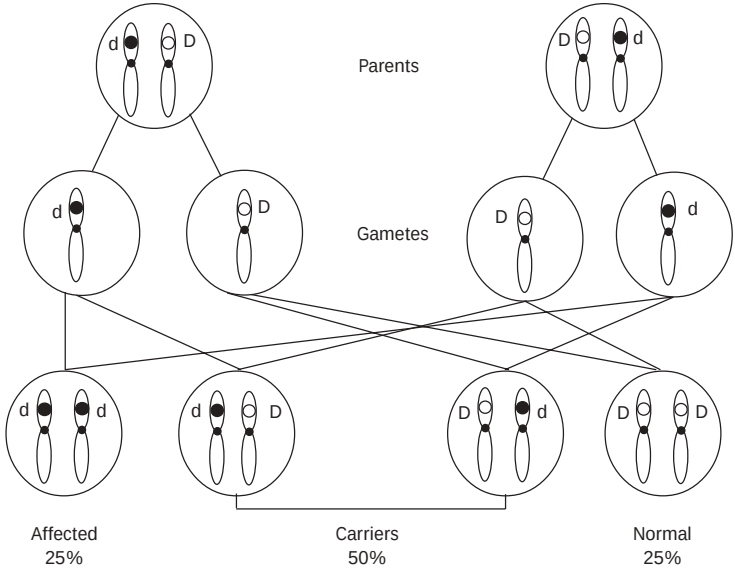


Fig. 4.9a Inheritance of an autosomal recessive disorder when both the parents are heterozygotes.

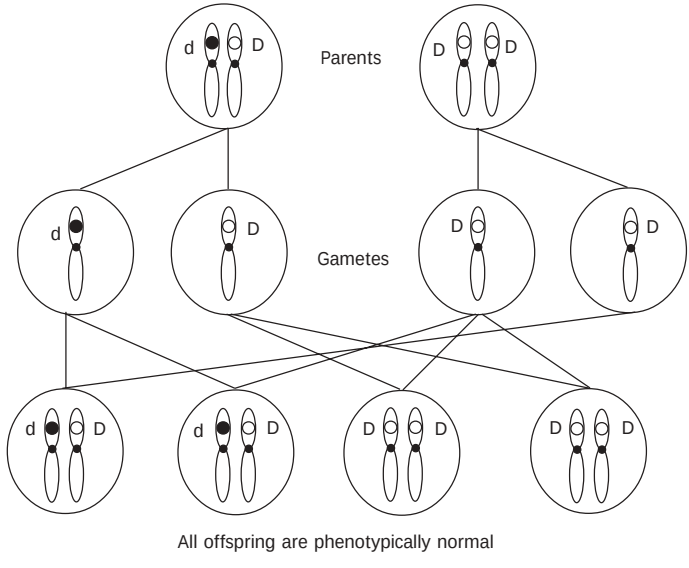


Fig. 4.9b Inheritance of an autosomal recessive disorder when one parent is a heterozygote and the other is normal.

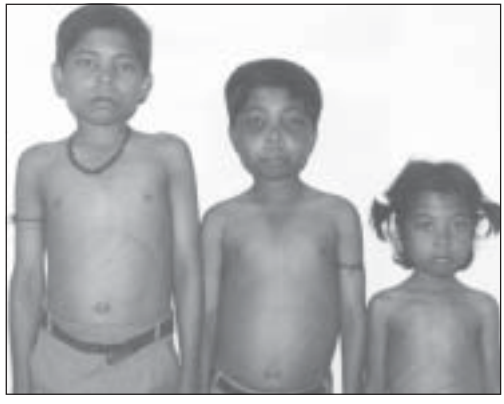


Fig. 4.10 Three siblings with thalassaemia intermedia.

prevalence of the disorder is high in the population, e.g. beta-thalassaemia and cystic fibrosis. The carrier frequency of these disorders in some populations is 2%–3% or even higher. In that case, even if the carrier relative of a person marries an unrelated person (someone from the general population), his or her chances of marrying a carrier and thus, being at risk of giving birth to an affected child, are significant.

Consanguinity and autosomal recessive inheritance

If a couple is consanguineous, i.e. having a common ancestor in the preceding five generations, the likelihood of their inheriting an identical allele (from their common ancestor) and passing it on to their child is high. Thus, the chance that the child of consanguineous parents is homozygous for an autosomal recessive disease increase. The rarer a particular disorder, the more the chances that the parents will be consanguineous. For example, in India, the percentage of consanguineous parents will be more in cases of the severe autosomal recessive form of osteopetrosis than in cases with thalassaemia major, which is a common disorder with a carrier frequency of 3%–17% in the general population.

Genetic counselling

As the carrier frequency of a rare autosomal disorder will be much higher in the relatives of an affected person than in the general population, genetic counselling of the relatives will be helpful in preventing recurrence of the disorder in successive generations. The other option, especially if carrier detection is not possible, is to avoid consanguineous marriages in that family.

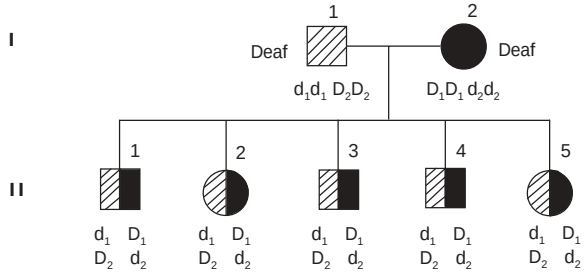


Fig. 4.11 A pedigree showing locus heterogeneity.

Genetic heterogeneity

It is not unusual for both the parents to have a similar clinical condition, e.g. deafness or albinism, but they may differ at the genetic level. Genetic heterogeneity is of two types: locus heterogeneity and allelic heterogeneity. In locus heterogeneity, the mutations at different loci may result in a similar clinical phenotype. Hence, even in two individuals with clinically similar disease, different genes at different loci may be mutated. Deafness is a good example of locus heterogeneity, i.e. the causative genes are numerous. Therefore, parents having deafness due to autosomal recessive genes atdifferent loci will have children with normal hearing. In addition, deafness can also be caused by non-genetic factors. In Figure 4.11, the father (I-1) is deaf due to homozygosity for an autosomal recessive deafness gene at locus 1 (d_1d_1) while his wife (I-2) is deaf due to homozygosity for a gene at locus 2 (d_2d_2). The father has normal alleles (D_2D_2) at locus 2 and the mother has normal alleles (D_1D_1) at locus 1. Therefore, all their children are heterozygous at both the loci, i.e. double heterozygotes and are thus not deaf.

The other type of genetic heterogeneity is allelic heterogeneity. This can be illustrated by the example of beta-thalassaemia. Both copies of the beta globin gene of patients with thalassaemia major are mutated, i.e. they are homozygous for mutant alleles. However, in reality, most of them are compound heterozygotes, i.e. both the mutant alleles are different, e.g. t_1t_2, t_2t_3, t_1t_3, etc. This is known as allelic heterogeneity. It is very common as hundreds of mutations are known for most of the disorders. Most of the patients with autosomal recessive disorders are compound heterozygotes (Fig. 4.12). In Fig. 4.12, the parents are carriers of an autosomal recessive disease. 'A' is the normal allele. The father is a carrier of the mutant allele 'a_1', caused by substitution of thymine (T) with cytosine (C) at a position in the gene. The mother is a carrier of mutation 'a_2' caused by deletion of a part of the gene. The individual II-4 is

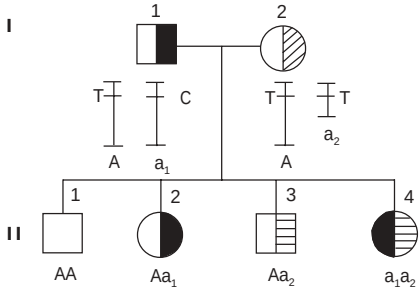


Fig. 4.12 A pedigree representing compound heterozygosity.

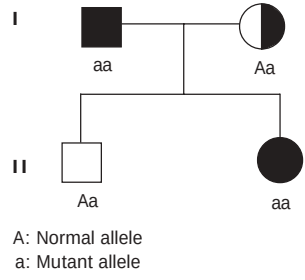


Fig. 4.13 A pedigree showing pseudodominance.

affected with the disease and is homozygous for the disease-causing allele. He is actually a compound heterozygote for the alleles a_1 and a_2.

If the parents are consanguineous, then the mutation carried by both of them will be the same at the molecular level.

Pseudodominance

If a carrier of an autosomal recessive disorder marries an affected person (Fig. 4.13), 50% of the children will be affected and the pedigree will resemble that of an autosomal dominant disorder.

SEX-INFLUENCED DISORDERS

Some diseases are more common in one sex than the other. An example is gout, which is more common in males than in females. After the menopause, there is no marked difference in the rate of occurrence in females, suggesting the protective influence of hormones.

SEX-LIMITED DISORDERS

Disorders of some organs, though caused by autosomal genes, can occur only in the sex that has that organ. For example, in familial testotoxicosis, precocious puberty occurs only in males though transmitted through females. The defect is not expressed in carrier females. For some disorders, distinguishing sex-limited autosomal disorders from X-linked disorders is difficult.

X-LINKED RECESSIVE INHERITANCE

This is conventionally referred to as sex-linked inheritance and is due to the presence of recessive genes on the X chromosome. Males have a single X chromosome and are therefore hemizygous for most of the genes

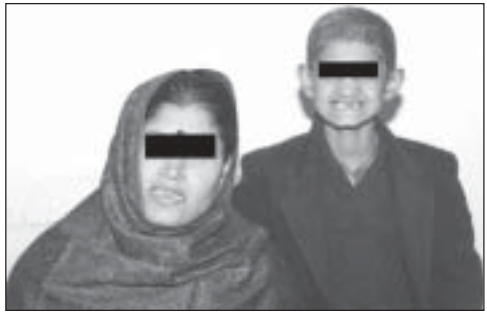


Fig. 4.14 A boy with X-linked anhidrotic ectodermal dysplasia with his mother. The boy has light-coloured sparse hair, depressed nose and hypodontia. His mother is normal except for some missing teeth.

on the X chromosome. Hence, if a male has a mutated gene on the X chromosome, he will have the disease. On the other hand, a female with one copy of the mutated gene and the other normal copy will usually not have manifestations of the disease (Fig. 4.14). She will have the disease only if she is homozygous for the mutation. As this is a rare possibility, females with X-linked recessive disorders are rare.

Characteristics of an X-linked recessive disorder

A pedigree showing the inheritance of an X-linked disorder is given in Fig. 4.15. The characteristics of this type of inheritance are as follows:

1. Males are affected almost exclusively.
2. All affected males are related through unaffected female (carrier) relatives.

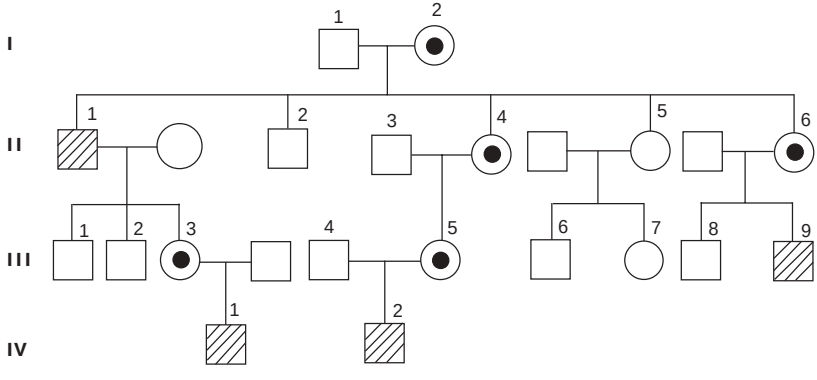


Fig. 4.15 A pedigree showing the inheritance of an X-linked disorder. Individual II-4 does not have an affected son but she and her daughter are obligate carriers.

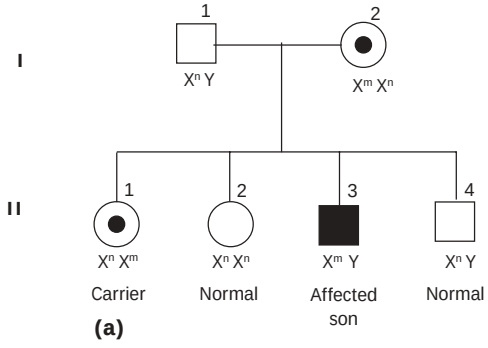


Fig. 4.16a Risk of recurrence of an X-linked disorder when the mother is a carrier and the father is not affected.

3. Male-to-male transmission is never observed because the X chromosome in a male is always contributed by the mother.
4. An affected male can pass on the disorder to his grandsons through his unaffected carrier daughter.

Risk of recurrence

The risk of recurrence is variable and depends on the genotypes of the parents. The most common case is when a woman is a carrier and marries a normal/unaffected man. Fifity per cent of her sons will be affected and the chances of her daughter being a carrier will be 50% (Fig. 4.16a).

The other situation can be a carrier female marrying an affected male (Fig. 4.16b). In this case, 50% of the daughters and 50% of the sons will be affected. The remaining 50% of the daughters will be carriers. In cases where the father is affected and the mother is normal (Fig. 4.16c), all the daughters will be carriers and all the sons will be normal.

Manifestations of an X-linked recessive disorder in a female

An X-linked disorder can manifest in a female in the following situations:

1. Non-random lyonization: The inactivation of one X chromosome in female cell makes it similar to the male cell as regards to the copy number of X chromosomes. Normally, lyonization occurs randomly in the early embryonic stage (Fig. 4.17a), i.e. in about 50% of the cells, one X chromosome is lyonized and in the rest, the other X chromosome. In some females, the normal X chromosome gets

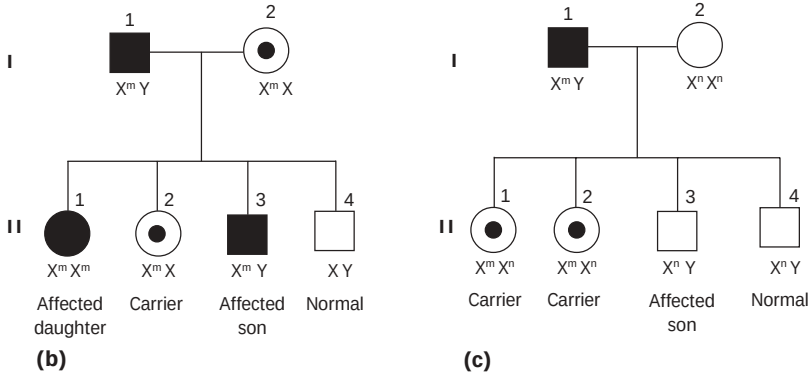
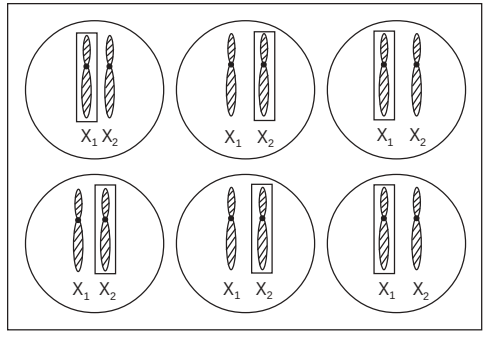


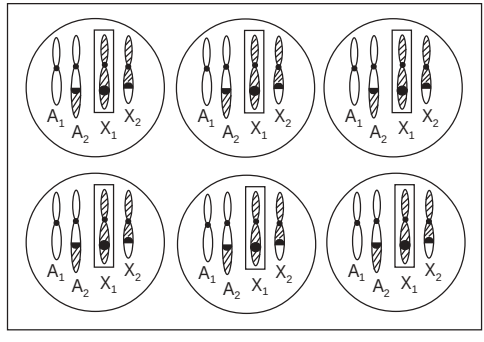
Fig. 4.16 b Risk of transmission of an X-linked disorder when the mother is a carrier and the father is affected. **c** Risk of transmission of an X-linked disorder when the mother is not a carrier and the father is affected.

lyonized preferentially in more cells, and thus the X chromosome with the mutation is active in a larger number of cells. This is known as non-random lyonization. It leads to manifestations (usually less severe than in an affected male) in the carrier female. The non-random lyonization may be a chance phenomenon or could be due to some factors that are presently not known.

2. Monosomy X: If a woman has only one X chromosome, i.e. she has 45 chromosomes, and the X chromosome has a mutated gene, then the disease will manifest in her.
3. Cytogenetic abnormality: If a woman is a carrier of an X–autosome translocation, i.e. a part of an autosome has translocated to an X chromosome, and the breakpoint (the point at which the break has occurred in the translocated chromosomes) on the X chromosome is disrupting a gene or preventing its expression, then the woman will manifest the disorder caused by non-expression of the disrupted gene. The process of inactivation of one X chromosome in a female is known as lyonization. However, lyonization of a translocated X chromosome will inactivate the part of the autosome attached to the X chromosome. This may not be compatible with the survival of the cell and is hence prevented. Whenever there is an X–autosome translocation, the normal X chromosome gets inactivated, preferentially in all the cells (Fig. 4.17b).
4. Homozygous female: If a woman is homozygous for an X-linked recessive disorder, then she will have the disorder (II-1 in Fig. 4.16b). This situation is commonly observed in disorders such as glucose-



(a)



(b)

Fig. 4.17 An X–autosome translocation can lead to manifestation of an X-linked recessive disorder in a female. A lyonized (inactivated) X chromosome is shown by enclosing it in a rectangle. **a** Normal lyonization. In half of the cells, X_1 is inactivated and in the rest, X_2 is inactivated. **b** Lyonization in a female with an X–autosome translocation. A_1 and A_2 are two homologous autosomes. X_1 and X_2 are the two X chromosomes. There is translocation between A_2 and X_2. The breakpoint on X_2 has disrupted a gene represented by a dark circle (•) on X_1. A part of the gene is on X_2 (◓) and a part on A_2 (◒). Thus, the copy of the gene on X_2 is now non-functional. If X_2 gets lyonized, the inactivation process will spread and inactivate the part of A_2 attached to X_2. To prevent lyonization of a part of A_2 on X_2, X_1 will get lyonized (or inactivated) in all cells of the body. Now, the intact copy of gene on X_1 is inactivated in all the cells of the body and the gene on X_2 is disrupted. Thus, no cell in the body will have a functional copy of the gene and this will lead to disease manifestation in the female.

6-phosphate dehydrogenase (G6PD) deficiency or colour blindness, which are not very severe.

Gonadal mosaicism

As disscussed in autosomal dominant disorders, gonadal mosaicism can occur in the case of an X-linked recessive disorder as well. Gonadal mosaicism is commonly seen in cases of Duchenne muscular dystrophy (DMD). Gonadal mosaicism cannot be predicted till a second affected son is born to a female who is not found to be a carrier by testing the DNA of her somatic cells (Chapter 16).

Genetic counselling of a family with a single affected male with an X-linked recessive disorder

When two or more males affected with an X-linked recessive disorder are seen in a pedigree, we know that the disease is inherited in that family. However, if a family has only one affected male without a family history of similarly affected persons, then the mother may or may not be a carrier of the disease. For diseases such as DMD and haemophilia A, one can identify carriers by testing the serum levels of creatinine phosphokinase or Factor VIII, respectively. However, these tests can identify only some of the carriers and may not provide definite answers in many. The definite test for carrier detection is mutation detection. Even if a woman is found to be a non-carrier by peripheral blood assay, the possibility of gonadal mosaicism cannot be ruled out and the family should be informed of this. Thus, to prevent recurrence of a disease, prenatal diagnosis must be offered to all families with one affected son.

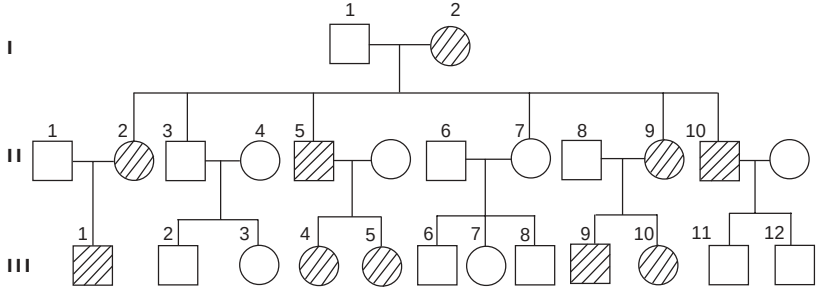


Fig. 4.18 A pedigree showing the inheritance of an X-linked dominant disorder.

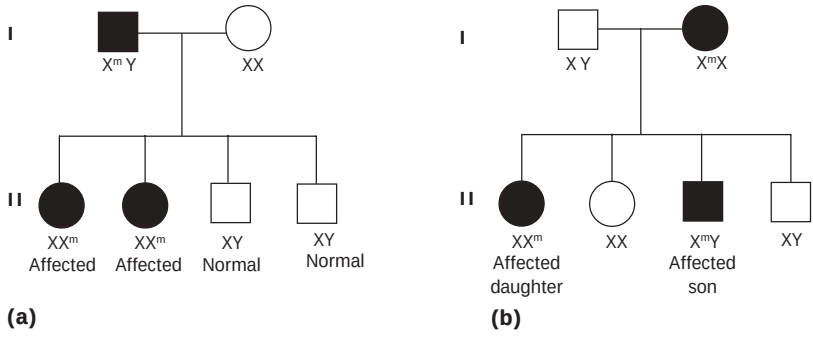


Fig. 4.19 Transmission of an X-linked dominant disorder when one parent is affected. **a** When the father is affected, all daughters are affected and all sons are normal. **b** When the mother is affected, 50% of the daughters and 50% of the sons are affected.

X-LINKED DOMINANT INHERITANCE

This is a less common form of inheritance and is caused by the presence of a dominant disease allele on the X chromosome. Figure 4.18 shows a pedigree consistent with an X-linked dominant disorder. It is superficially similar to an autosomal dominant disorder with transmission of the disease to the next generation.

Characteristics of X-linked dominant inheritance

1. Both males and females can be affected; females are less severely affected than males.
2. All daughters of affected males are affected (III-4 and III-5 in Fig. 4.18 and also in Fig. 4.19a).
3. There is no male-to-male transmission (II-10, III-11, III-12 in Fig. 4.18).
4. Affected females can transmit the disorder to both sons and daughters (Fig. 4.19b).
5. There is an excess of affected females as compared with affected males in a family.

An example of an X-linked dominant disorder is familial hypophosphataemic rickets. Fragile X mental retardation is another example where about 70% of female carriers have some manifestations of the disease and is described as X-linked semi-dominant or X-linked dominant with incomplete penetrance.

X-linked dominant disorders with lethality in males

Some X-linked dominant disorders are probably so severe in males that affected males are non-viable and such affected conceptuses are lost in early pregnancy as abortions. This hypothesis was thought of because for some disorders, the affected cases are all females. Affected males are not or are rarely seen. Examples of this form of inheritance are incontinentia pigmentosa and Aicardi syndrome.

Females with X-linked dominant lethal disorders are rarely able to reproduce because of the severity of the disease and hence, most of these cases are due to new mutations.

In X-linked dominant disorders, the normal X chromosome and those with the mutation get randomly inactivated. The cells of such females show a mosaic pattern. This mosaic pattern can be demonstrated in some diseases. Mosaic distribution of skin lesions and pigmentation that follows the developmental lines are seen in females heterozygous for the X-linked dominant disorder incontinentia pigmentosa.

Y-LINKED INHERITANCE AND PSEUDOAUTOSOMAL REGION GENES

There are important genes on the Y chromosome which are involved in spermatogenesis. Males with mutation of these genes are likely to be subfertile or infertile and thus less likely to transmit the mutation to the next generation in the normal manner of reproduction. However, with the technique of intracytoplasmic sperm injection (ICSI), these mutations will get transmitted from father to son. Other than these genes, no other genetic disorder is known to be linked to the Y chromosome.

Small regions at the tips of the X and Y chromosomes have similar base sequences. These regions are known as pseudoautosomal regions and recombinations take place between them. The pattern of inheritance of diseases caused by genes on these regions will be similar to those acquired by autosomal inheritance.

Leri–Weil dyschondrosteosis is a rare skeletal dysplasia associated with disproportionate short stature and Madelung deformity of the wrist. It is inherited in an autosomal dominant manner but is caused by mutation in the short stature homeobox gene (*SHOX*), which is in the pseudoautosomal region. Homozygosity for *SHOX* mutation results in the severe form known as Langer mesomelic dysplasia.

5 Non-Mendelian inheritance

Monogenic or single-gene disorders are known to follow a Mendelian pattern of inheritance. However, there are many disorders that do not follow this pattern. In the past few decades, many non-traditional or non-Mendelian modes of inheritance have been discovered. Advances in molecular biology have greatly improved the understanding of novel modes of inheritance; these are discussed in this chapter.

MITOCHONDRIAL INHERITANCE

Mitochondria contain several copies of circular DNA, which is a small molecule of 16 **kb** and contains 37 genes. The inheritance of mitochondrial genes differs from that of nuclear genes because only the mother contributes mitochondria to the zygote. Even though the sperm tail contains a few mitochondria, they do not enter the ovum. Thus, mitochondrial genes show a characteristic pattern of inheritance known as mitochondrial or maternal inheritance (Fig. 5.1). The mother can transmit the disease to all her children while the affected father will not transmit the disease to any of his children.

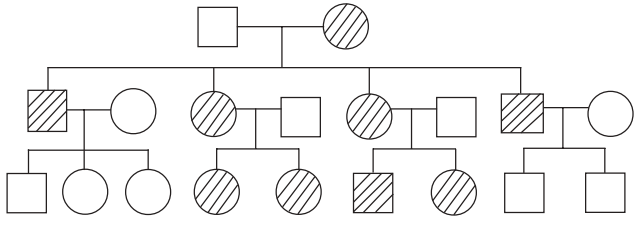


Fig. 5.1 A pedigree showing the distinctive transmission pattern of a mitochondrial disorder.

Mitochondria have their own protein-synthesizing system. Twenty-four mitochondrial genes code for rRNAs and tRNAs, which assist in translation. The other 13 mitochondrial genes encode proteins that function in cellular respiration. The products of these genes, along with many more proteins originating from nuclear genes, participate in oxidative phosphorylation. As these genes are vital for all organs, mitochondrial diseases have serious manifestations. These diseases involve the eyes, muscles, heart, brain, liver, and endocrine organs. Due to the great variability in presentation, they are difficult to diagnose (Table 5.1). These diseases should be suspected in a patient of any age with any (especially two or more) of the following symptoms: muscle weakness, ophthalmoplegia, loss of vision, encephalopathy, psychosis, deafness, stroke, seizures, ataxia, cardiomyopathy, diabetes, hypoparathyroidism, hypothyroidism, or renal tubular acidosis.

Mitochondrial mutations can be either inherited through the maternal lineage and manifest any time during life, or they can be acquired through the accumulation of somatic mutations aquired uing mitosis. Hence, many of these disorders have a delayed onset and are progressive. Mitochondrial DNA has a higher rate of spontaneous mutation than nuclear DNA and the accumulation of mutations in mitochondrial DNA has been proposed as being responsible for some of the effects of ageing.

Table 5.1 Some phenotypes of mitochondrial diseases

Disease	Phenotype	Homoplasmy/ Heteroplasmy	Inheritance
MELAS	Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes	Heteroplasmy	Maternal
MERRF	Myoclonic epilepsy, ragged red fibres in the muscles, ataxia, deafness	Heteroplasmy	Maternal
Leber hereditary optic neuropathy	Blindness	Homoplasmy	Maternal
Leigh disease	Neuropathy, mental retardation, lactic acidosis, retinitis pigmentosa	Heteroplasmy	Maternal
Kearns–Sayre syndrome	External ophthalmoplegia, heart block, retinitis pigmentosa, myopathy	Heteroplasmy	Sporadic, somatic mutation
Deafness	Deafness usually induced by aminoglycosides	Homoplasmy	Maternal

Heteroplasmy and homoplasmy

The variable number of mitochondrial DNA molecules with mutation in each cell is a unique feature of mitochondrial diseases.

Each cell contains more than 1000 mitochondrial DNA molecules. If one of them gets mutated, the mutation can be transmitted to the daughter cells. When the cell divides, each daughter cell will get some mitochondria with the mutation. There may be some cells having only normal mitochondria and others having only mitochondria with the mutated DNA. This condition is called homoplasmy (Fig. 5.2). On the other hand, there may be some cells that contain mitochondria with both mutated and normal DNA. This is known as heteroplasmy. The disease manifestation will depend on the number of mitochondria with mutation and their distribution in the various tissues. Most diseases are heteroplasmic and hence, there is great variability in the clinical severity of the disorders among siblings. In healthy individuals, about 99.9% of the mitochondrial DNA molecules are normal.

Another interesting aspect of mitochondrial diseases is the phenomenon of genetic bottleneck. If the mother has a mutation in her mitochondrial DNA, she will pass it to all her children. However, the

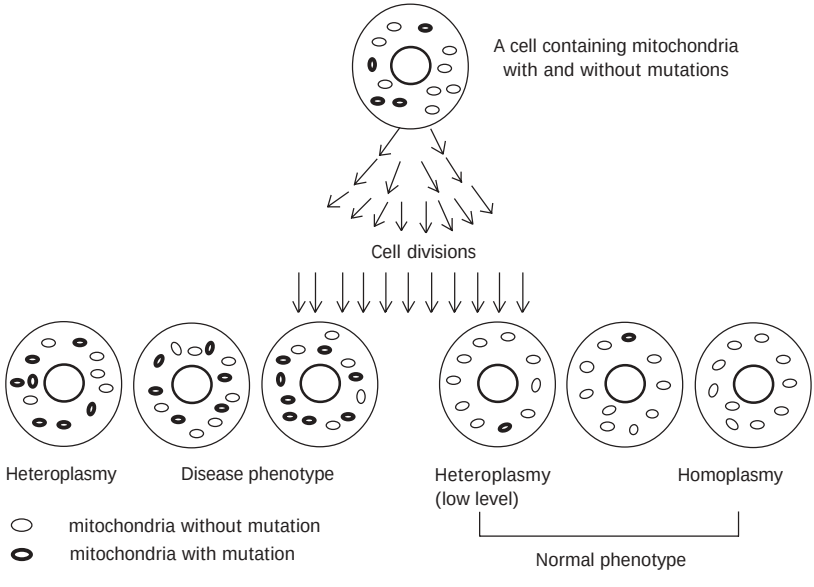


Fig. 5.2 Cell divisions give rise to two phenotypes. Manifestation of the disease will depend on the relative proportion of mitochondria with and without mutation.

number of mutated mitochondria will vary from ovum to ovum. This is because during oogenesis, the number of mitochondria is first greatly reduced and then the mitochondria multiply again before the oocyte stage. The reduction in the number provides an opportunity for a change in the relative proportion of normal and mutated mitochondria in the ovum. This is known as mitochondrial genetic bottleneck. Due to the variability at each stage, counselling for the risk of recurrence of the disease in the family and for severity of the phenotype is difficult in the case of a mitochondrial disorder.

GENOMIC IMPRINTING

In Mendelian disorders, there is no difference between the function of maternally and paternally inherited copies of the gene. However, for some genes, the behaviour of alleles inherited from the father differs from those inherited from the mother even if the DNA sequence of both the alleles is the same. This is known as genomic imprinting and occurs due to heritable but reversible modification of a gene during gametogenesis. If the maternal allele is inactive or suppressed, the gene is said to be maternally imprinted. If the paternal allele is inactive or suppressed, the gene is paternally imprinted. The evidence for this comes from pedigree analysis (Fig. 5.3).

The other evidence for genomic imprinting comes from uniparental disomy. In this condition, one of the parents contributes both the

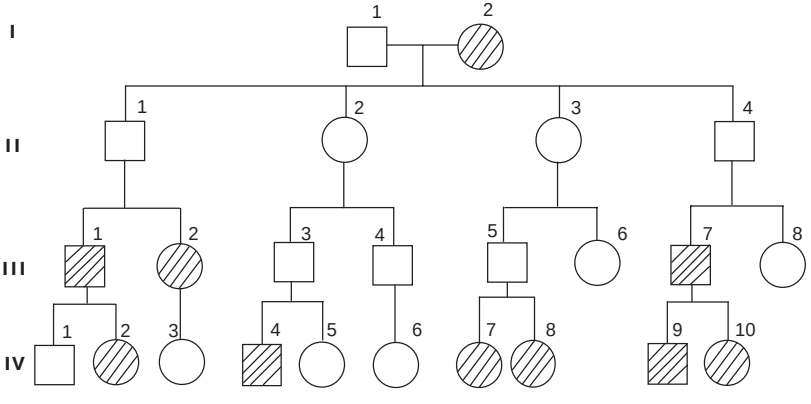


Fig. 5.3 A pedigree showing maternal imprinting.

Note: Although individuals I-2, II-1, II-2, II-3, II-4, III-3 and III-5 are carriers of the mutation (as they have affected offspring or grandchildren), they do not have the disease phenotype because they have inherited the disease allele from their mothers. In this case the paternal copy is functional and the maternal copy is inactive or imprinted.

homologous chromosomes. Thus, the chromosome from the other parent is absent. If there is an imprinted gene on that chromosome, then a genetic condition may result from the lack of genetic instruction from the relevant parent. For example, if a person has maternal isodisomy of a chromosome that has a maternally imprinted gene, he or she will not have any functioning copy of that gene, even though the DNA sequence of both the copies of the gene are normal. Example of diseases caused by uniparental disomy and imprinted genes are Prader–Willi and Angelman syndromes. They are discussed in the section on 'Diseases caused by uniparental disomy'. It should be noted that only a small proportion of the human genome is imprinted.

Mechanism of genomic imprinting

The mechanism of genomic imprinting is poorly understood. The mechanism of methylation of CpG dinucleotides is well understood (*see* Chapter 2)**.** CpG islands are clusters of dinucleotides of cytosine and guanine occurring together near the transcription initiation sites of many genes. Methylation of these CpG islands stops the transcription of the gene and thus silences it (*also see* Fig. 10.3a) . The methylation pattern of the gene can be transmitted during mitosis of the somatic cells. The imprinting is erased during gametogenesis and a new imprint is established before the gene is passed on to the next generation (Fig. 5.4).

UNIPARENTAL DISOMY

Inheritance of both the homologous chromosomes from one parent is known as uniparental disomy. It can involve the whole chromosome or a part of it. The mechanism for the latter could be an exchange between sister chromatids during mitosis of the somatic cells. Uniparental disomy can be isodisomy (Fig. 5.5a) or heterodisomy (Fig. 5.5b).

Mechanisms of uniparental disomy

Figures 5.5a and b show the possible mechanisms of uniparental disomy. Non-disjunction of chromosomes during meiosis I will lead to the formation of a gamete containing two copies of a chromosome instead of one. During fertilization, this gamete fuses with a normal gamete to form a trisomic zygote. Probably as a rescue mechanism, one of the three chromosomes is lost. If the remaining two are from one parent, then there will be uniparental disomy.

If non-disjunction of the homologous pair of a chromosome occurs in the first stage of meiosis, there will be heterodisomy and if it occurs

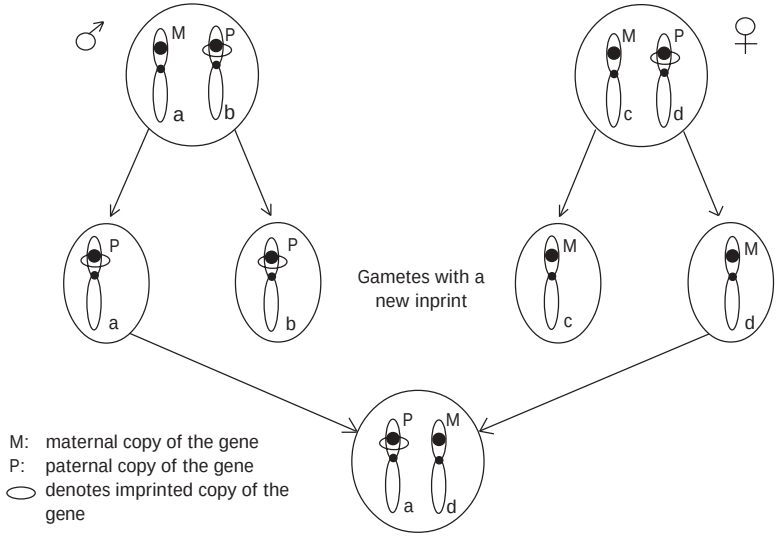


Fig. 5.4 The existing imprint is erased and a new imprint is established during gametogenesis. Chromosome 'a', which was of maternal origin in the father, is passed on as paternal to his offspring. Similarly, chromosome 'd', which was of paternal origin in the mother, is transmitted as maternal to her offspring.

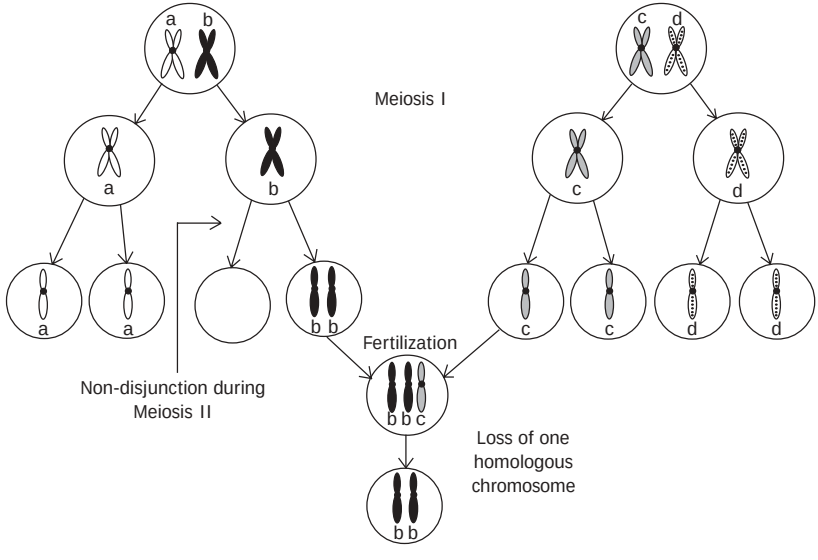


Fig. 5.5a Gametogenesis resulting in uniparental isodisomy; a and b are the two homologous chromosomes of one parent and c and d are the two homologous chromosomes of the other parent.

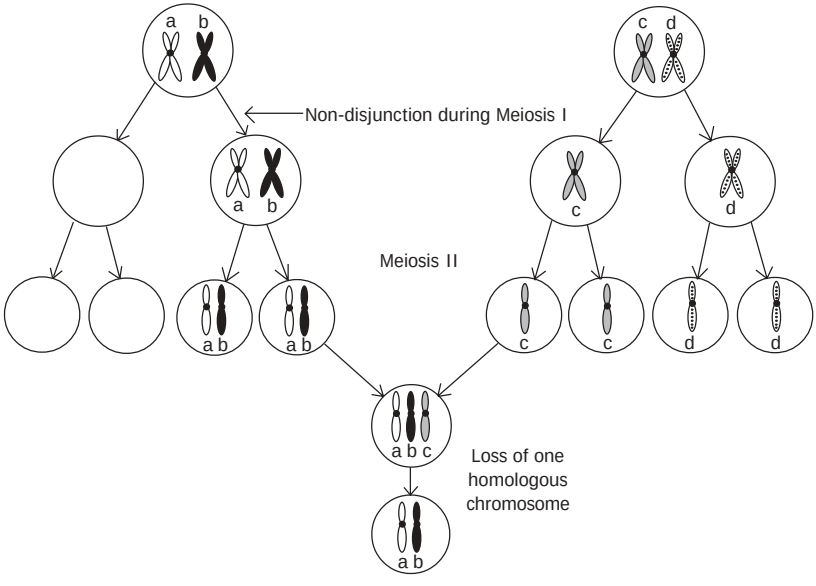


Fig. 5.5b Gametogenesis resulting in uniparental heterodisomy.

in the second stage, it will result in isodisomy. The other possible mechanism of isodisomy is fertilization of a disomic gamete with a nullisomic gamete (Fig. 5.6).

All the above mechanisms show that two errors of division have to occur either one after the other or in both the parents. Probably that is why uniparental disomy is not common.

Diseases caused by uniparental disomy

Uniparental disomy will lead to either bi-allelic expression of the imprinted gene or genes on the chromosome or no expression of the imprinted gene or genes on the chromosome. If there is maternal disomy of a chromosome that has a maternally imprinted gene, then there will be no expression of the gene. On the other hand, if there is maternal disomy of a chromosome that has a paternally imprinted gene, then there will be double expression of the gene. Diseases caused by uniparental disomy are Angelman syndrome, Prader–Willi syndrome and Beckwith–Wiedemann syndrome. The genes associated with Prader–Willi syndrome are maternally imprinted, i.e. only the paternal copies are expressed. Thus, maternal disomy or a deletion on the paternal chromosome, will cause Prader–Willi syndrome. Prader–Willi syndrome is characterized by mental retardation, obesity, behavioural problems, and hypogonadism

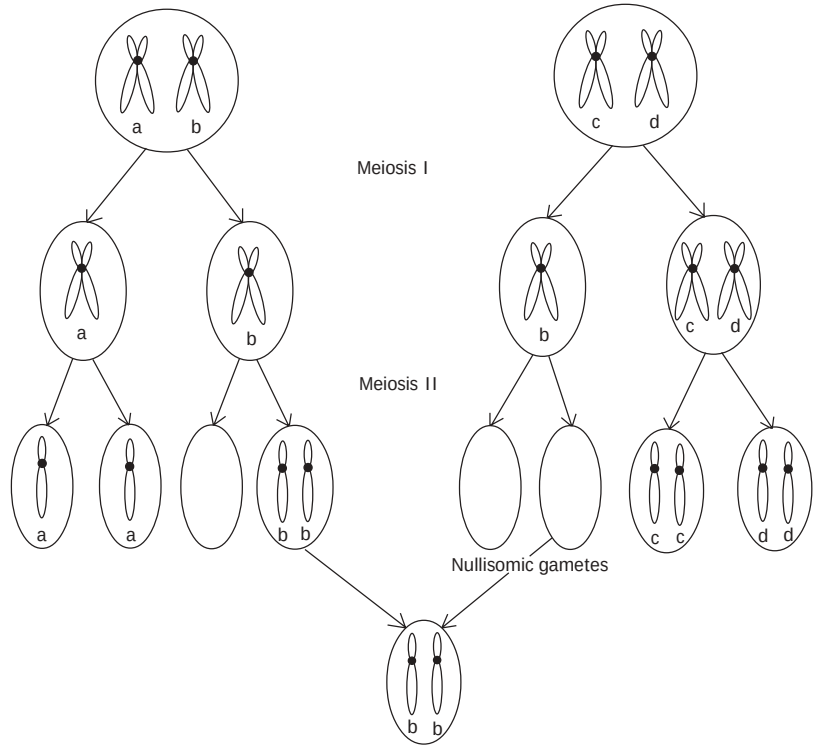
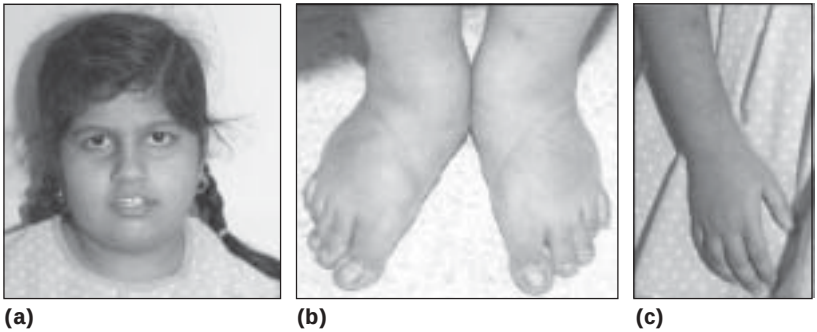


Fig. 5.6 Fusion of a disomic gamete with a nullisomic gamete leading to uniparental disomy.



(a) **(b)** **(c)**

Fig. 5.7 Prader–Willi syndrome. **a** Characteristic facies with almond-shaped eyes and narrow forehead. **b** Small feet. **c** Small hands and skin lesions due to 'skin picking' habit.

(Fig. 5.7). The gene associated with Angelman syndrome is paternally imprinted. Hence, deletion of the gene on maternal chromosome 15 or paternal disomy of chromosome 15 causes Angelman syndrome. Angelman syndrome is characterized by severe mental retardation, characteristic facies, gait disturbance, puppet-like movements of the hands, microcephaly, epilepsy and episodes of inappropriate laughter (Fig. 5.8). Both the diseases can be caused by deletion of a part of the long arm of chromosome 15 between bands q11 and q13, as well as by other mechanisms suppressing the expression of genes in the region of q11–13 (Table 5.2).

Fig. 5.8 Angelman syndrome.

Similarly, some of the genes on chromosome 11 express from the paternal chromosome only. Hence, when there is paternal disomy of the region, there is overexpression of these paternally expressed genes including insulin-like growth factor 2 (*IGF2*) gene. This alone, or in combination with loss of the maternal contribution of genes, is responsible for Beckwith–Wiedemann syndrome characterized by large size at birth, enlarged tongue and omphalocele. The uniparental disomy in Beckwith–Wiedemann syndrome involves only a part of the chromosome and may occur due to a post-zygotic mitotic event (Fig. 5.9).

Uniparental disomy involving almost all the chromosomes is known. Most individuals have no clinical defects. This indicates that only a very small part of the genome is imprinted. In addition to chromosome 15, chromosomes 7 and 14 show the imprinting phenomenon. This is

Table 5.2 Mechanisms causing Prader–Willi and Angelman syndromes

Mechanism	Prader–Willi syndrome	Angelman syndrome
Deletion as a cause	70% of cases	70% of cases
Origin of a chromosome with a deletion	Paternal	Maternal
Uniparental disomy	Maternal disomy in 30% of cases	Paternal disomy in 3%–5% of cases
Imprinting gene mutation	1%–2%	7%–9%
Single-gene mutations	Not known	Ubiquitin–protein ligase gene in 15% of cases
Others	Not detected	10%–20% of cases

because uniparental disomy of the chromosomes7 and 14 are associated with Russel–Silver syndrome and growth retardation, respectively.

Autosomal recessive diseases caused by uniparental disomy

There is another mechanism that can cause disease due to uniparental disomy. A child with cystic fibrosis was found to be homozygous for a cystic fibrosis mutation seen in his mother. His father was not a carrier of cystic fibrosis. Molecular study revealed that both of his chromosomes 7 were identical to the mother's chromosome 7, which had the cystic fibrosis mutation. Thus, even if the chromosome for which isodisomy takes place does not have any imprinted gene, the child can get an autosomal recessive disease if the disomic chromosome carries such a mutation. This can happen only with uniparental isodisomy and not with heterodisomy.

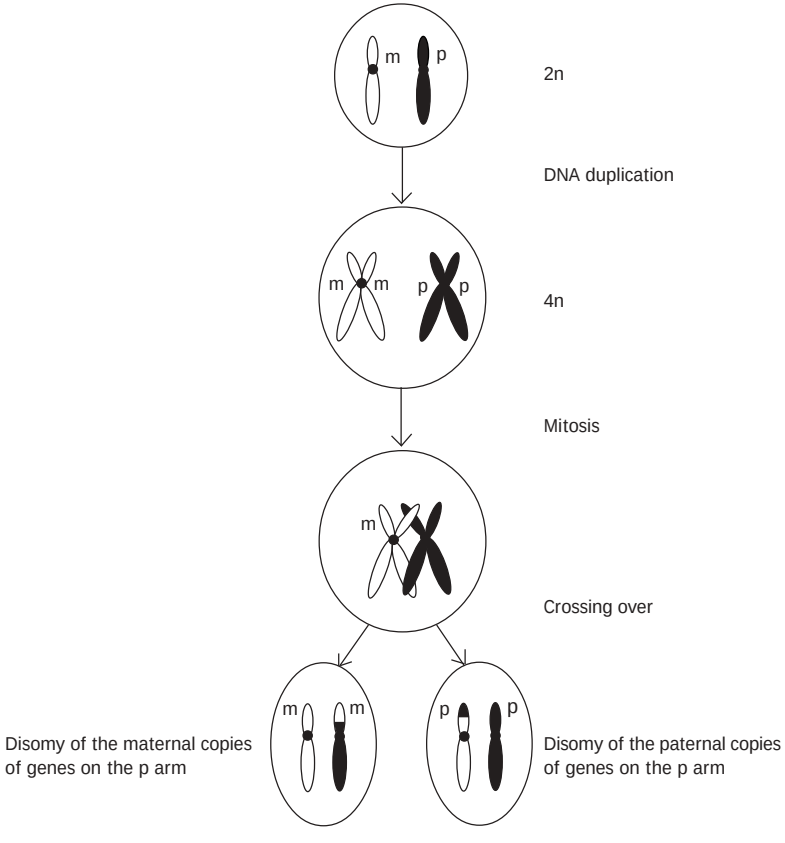


Fig. 5.9 Mechanism of partial uniparental disomy.

SOMATIC MOSAICISM

Mosaicism is defined as the presence of at least two cell lines that differ genetically but are derived from a single zygote or tissue. Mosaicism can involve a chromosome or a single gene. Chromosomal mosaicism is easier to document and has been observed more commonly than mosaicism involving a gene.

In McCune–Albright polyostotic dysplasia, there is fibrous dysplasia involving various parts of the bones, mosaic skin pigmentation anomalies and precocious puberty. It is caused by somatic mosaicism for mutation of the alpha 1 subunit of G protein. Diseases associated with the mosaic pattern of lesions are likely to be due to somatic mosaicism. Acquired somatic mutation leading to cancers is an another example. In many cases, somatic mosaicism may be associated with evidence of germline mosaicism.

GERMLINE OR GONADAL MOSAICISM

If a normal, non-carrier couple have an offspring with a dominant (autosomal or X-linked) disorder, then the offspring is most likely affected due to a new mutation which is not inherited from the parents. In such a case, there should be no similarly affected sibling, since occurrence of the same new mutation resulting in the same disorder again is very unlikely. However, such families, i.e. normal parents having two offspring with an autosomal dominant disorder, have been reported. This can only be explained by germline mosaicism, i.e. the presence of two populations of cells; one with and the other without a mutation in the gonad of a parent (*see* fig 4.6).

Germline mosaicism is documented in the lethal variety of osteogenesis imperfecta type II, haemophilia A, haemophilia B, Duchenne muscular dystrophy (DMD) and achondroplasia. Though an accurate estimation of the frequency of germline mosaicism cannot be given, the highest frequency reported has been about 15% for mothers of sporadic cases of DMD. With the possibility of germline mosaicism, the exact risk of recurrence in the siblings of a person having a sporadic autosomal dominant or X-linked disorder cannot be estimated. However, the possibility of recurrence cannot be denied and the family should be offered prenatal diagnosis, if available.

UNSTABLE DYNAMIC (TRIPLET REPEAT) MUTATIONS

Some disorders are caused by an increase in the number of triplet repeats within or near the gene (*see* Chapter 10). The triplet repeats are usually

stably transmitted from one generation to the next. In persons with higher number of repeats, the number changes during transmission from a parent to the offspring (usually increases). It has been seen that with an increasing number of triplets repeats, the severity of a disorder, especially the chances of an earlier age of onset, increases. Thus, the instability of triplet repeat mutation explains the phenomenon of anticipation, which was noted in autosomal dominant disorders. Table 5.3 lists some triplet repeat disorders, most of which are inherited in an autosomal dominant fashion.

DIGENIC/OLIGOGENIC INHERITANCE

It has now been proved that many genetic disorders that were previously described as monogenic are, in fact, caused by mutations at two or more loci. Digenic inheritance (Fig. 5.10) was first reported in retinitis pigmentosa, which was thought to be inherited in an autosomal dominant manner with reduced penetrance. Heterozygous mutations in both the retinal outer segment membrane protein 1 gene (*ROM1*) and the peripherin gene (*RDS*) are required to cause retinitis pigmentosa.

Another example of digenic inheritance is Bardet–Biedl syndrome, which was thought to be inherited in an autosomal recessive fashion (Fig. 5.11). At least six loci for Bardet–Biedl syndrome are known. Some pedigrees of Bardet–Biedl syndrome show that two *BBS2* gene mutations and one *BBS6* mutation are required for the disease to manifest. This suggests another variation of digenic inheritance—triallelic inheritance

Table 5.3 Disorders caused by dynamic mutations of triplet repeats

Disorder	Mode of inheritance	Triplet repeat and its location in relation to the gene
Fragile X mental retardation	X-linked	CGG 5' untranslated region
Myotonic dystrophy	AD	CTG 3' untranslated region
Huntington chorea	AD	CAG 5' untranslated region
Spinocerebellar ataxia type I	AD	CAG 5' untranslated region
Spinobulbar muscular atrophy	AD	CAG 5' untranslated region
Friedreich ataxia	AR	GAA first intron

AD: autosomal dominant; AR: autosomal recessive

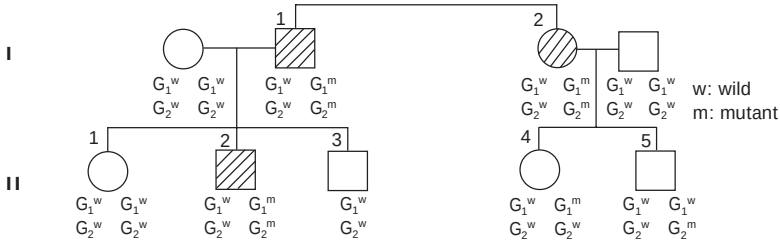


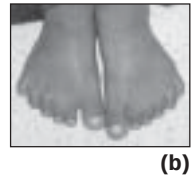
Fig. 5.10 A pedigree showing digenic inheritance. Mutations in two genes are necessary for the disease to manifest. I-1, I-2 and II-2 are double heterozygotes and manifest the disease. II-4 and II-5 carry one mutation only in G_1 and G_2 genes, respectively, and are normal.

(Fig. 5.12). Sometimes, mutations in more than two genes are necessary for the disease to manifest. Hirschsprung disease is an important example of oligogenic inheritance. A complicated interaction of genetic factors is involved in the pathogenesis of this disorder of the parasympathetic nervous system of the gut. Mutations in *the RET* and three more genes are found to cause the disease. Mutation in any of the genes alone does not cause the disease but, in association with mutation in two more genes, confers a susceptibility to Hirschsprung disease.

Monogenic to oligogenic to multifactorial disorders

It has become obvious that for many more so-called monogenic diseases, the synergistic effect of mutant alleles at other loci is necessary. Such modifier and synergistic loci will hopefully be identified soon. This will also explain phenomena such as incomplete penetrance and variable expressivity, which are still enigmas. On the other hand, the development of the concept of oligogenic inheritance will blur the distinction between Mendelian single-gene disorders and multifactorial complex inheritance. It shows that there is conceptual continuity of classical Mendelian and complex traits. The position of any given disorder along this continuum depends on whether the main contribution is of a single

(a)



(b)

Fig. 5.11 a A child with Bardet–Biedl syndrome. **b** Polydactyly.

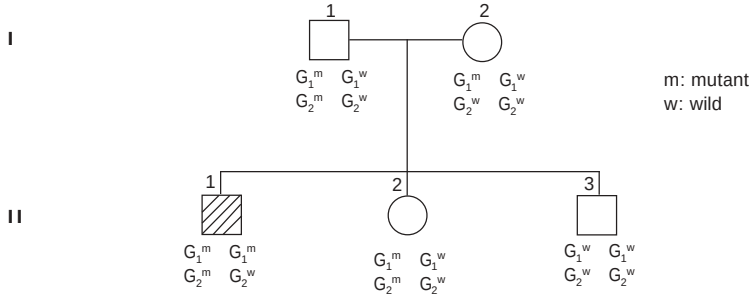


Fig. 5.12 A pedigree showing digenic triallelic inheritance. The affected person in this pedigree (II-1) has 3 mutations, one in gene G_2 and two in gene G_1. Carriers of one (I-2) and two (I-1, II-2) mutations are unaffected.

locus or a few loci or many loci, and also on the contribution of environmental factors. The more we are able to dissect out all causative and contributory genes, and environmental factors, the better will be the genotype–phenotype correlation. This will improve the ability to predict the phenotype from the genotype and also open up more therapeutic options.

Section II

Genetic Disorders

6 Chromosomes: Analysis and anomalies

STRUCTURE OF CHROMOSOMES

Chromosomes are made of DNA and are present inside the nucleus. They were first seen by Walther Flemming in 1882 and were termed 'chromosomes' by Waldeyer. The DNA is compactly arranged in the metaphase stage of cell division and can be seen in the form of chromosomes.The number and appearance of chromosomes varies from species to species. The correct number of chromosomes in humans was found to be 46 by Tjio and Levan in 1956.

A chromosome consists of two identical strands known as chromatids or sister chromatids, which are formed during the synthesis (S) phase of DNA replication. The sister chromatids are joined at a constriction site known as the centromere. Each centromere divides the chromosome into a short (p) and a long arm (q). The centromere has an important role in the movement of the chromosome during cell division.

Individual chromosomes differ in size and, depending on the position of the centromere, chromosomes are categorized as follows (Fig. 6.1):

Metacentric—centromere is at the centre of the chromosome

Submetacentric—centromere is at an intermediate position

Acrocentric—centromere is near the end of the chromosome

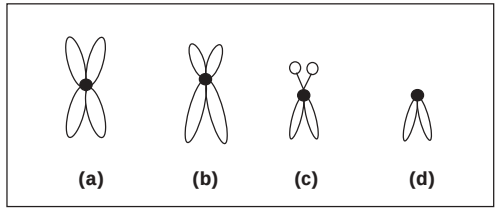


Fig. 6.1 Types of chromosomes based on their morphology. **a** Metacentric. **b** Submetacentric. **c** Acrocentric. **d** Telocentric.

Telocentric—centromere is at one end of the chromosome. In human beings, there is no telocentric chromosome.

CLASSIFICATION OF CHROMOSOMES

Based on the overall morphology, chromosomes are divided into seven groups (A to G), namely, A (1, 2, 3); B (4, 5); C (6, 7, 8, 9, 10, 11, 12, X); D (13, 14, 15); E (16, 17, 18); F (19, 20); and G (21, 22, Y). Chromosomes in groups D and G are acrocentric and their p arms do not contain important genes. Hence, deletions of the p arms of such chromosomes do not give rise to any phenotypic effect. Some of the acrocentric chromosomes have stalk-like appendages known as satellites which form the nucleolus during the interphase. A satellite contains multiple copies of ribosomal RNA (rRNA).

Twenty-two pairs of chromosomes are common to males and females and are known as autosomes. The 23rd pair consists of the sex chromosomes. There are two types of sex chromosomes, X and Y. A female has two X chromosomes and no Y chromosome, whereas a male has one X and one Y chromosome.

CELL DIVISION

Cell division is the basis of growth and reproduction. There are two types of cell division: mitosis and meiosis. Mitosis occurs in all cells of the body and at all times. Before mitosis, the DNA of a cell gets duplicated, to be distributed equally to the two daughter cells. Each daughter cell has a chromosome complement similar to the parent cells, i.e. 46 chromosomes in humans (Fig. 6.2). On the contrary,

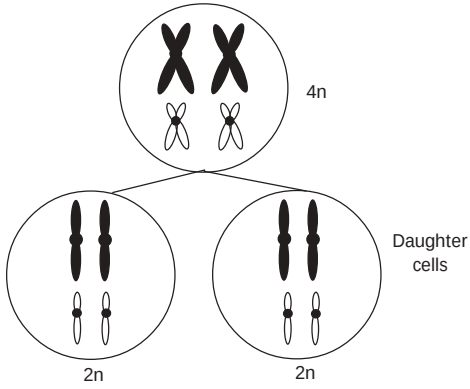


Fig. 6.2 Mitosis. Only two pairs (large and small) of homologous chromosomes are shown to explain cell division. The other chromosomes are also similarly distributed to the daughter chromosomes.

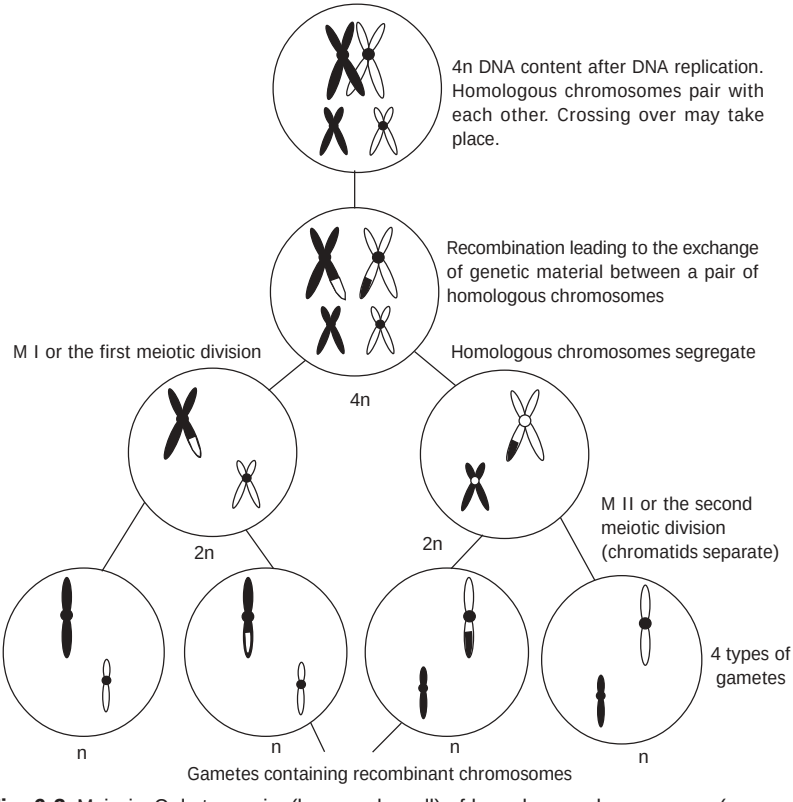


Fig. 6.3 Meiosis. Only two pairs (large and small) of homologous chromosomes (one black and its homologue white) are shown to explain cell division.

during the process of gametogenesis (meiosis or reduction division), the cell distributes half of its chromosomes (one of each of the pairs) to daughter cells, known as gametes. Thus, each gamete has 23 chromosomes (Fig. 6.3). Reduction division helps to maintain the normal diploid number of chromosomes in the zygote after fertilization. The other important phenomena of meiosis are random assortment of homologous chromosomes and recombinations. Random assortment of chromosomes means that for a pair of homologous chromosomes, if the paternal chromosome is being passed on to a gamete, then for the other pair, the maternal homologue may be passed on to the same gamete. Recombination allows the exchange of genetic material between chromatids of two homologous chromosomes. These characteristics of meiosis increase the genetic variability and maintain the uniqueness of an individual.

CLINICAL CYTOGENETICS

Clinical cytogenetics is the study of chromosomes, their structure and inheritance, as applied to the practice of medical genetics. Chromosomal disorders form a major category of genetic disorders. Chromosomal anomalies account for a large proportion of reproductive wastage, congenital malformations and mental retardation. Acquired chromosomal abnormalities in somatic cells play an important role in the pathogenesis of cancers.

Procedure for the study of chromosomes

Traditional cytogenetic study requires live, dividing cells. Such cells can be obtained from any tissue of the body, for example, blood, bone marrow, skin fibroblasts, tumour cells, liver, etc. For prenatal diagnosis, cells from the amniotic fluid, chorionic villi, placenta or foetal blood are used. Commonly used cells are T lymphocytes from the peripheral blood. The blood sample can be easily obtained and T lymphocytes can be induced to transform into blast cells capable of dividing by antigenic stimulation. There is no need to separate the lymphocytes from the blood sample. The commonly used antigen for chromosomal analysis is phytohaemagglutinin.

Half a millilitre of blood is added to a glass tube

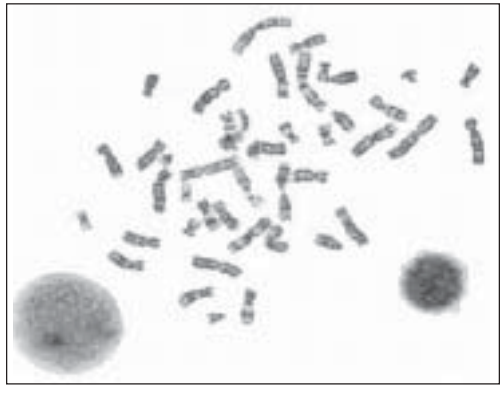


Fig. 6.4 A metaphase spread, along with two interphase nuclei.

containing 5 ml of the culture medium and phytohaemagglutinin. The cultures are grown under aseptic conditions in incubators at 37 °C for 3 days. After 72 hours, colchicine is added to the culture and kept for 1 to 2 hours. Colchicine arrests cell division during metaphase and condenses the chromosomes. This allows better visualization of the chromosomes. The cells are then treated with a hypotonic solution. As a result, the cells swell. This is followed by treatment with a fixative containing methanol and acetic acid. The solution-containing cells are then dropped on a slide. As soon as a cell touches the slide, it bursts and the chromosomes spread in a localized area on the slide. These chromosomes in metaphase (metaphase spread; Fig. 6.4) can then be analysed.

Before staining with Giemsa, the slide is treated with trypsin or heat. This gives rise to alternate dark and light areas on each chromosome. These areas are known as bands. Each chromosome is identified by its size, location of the centromere and specific banding pattern. The banding methods include G-,R-, Q- and C-banding.

G-banding with trypsin is the commonly used technique for chromosome analysis. The number of bands depends on the stage of cell division. Most metaphases have 400 to 500 dark and light bands on the haploid set of

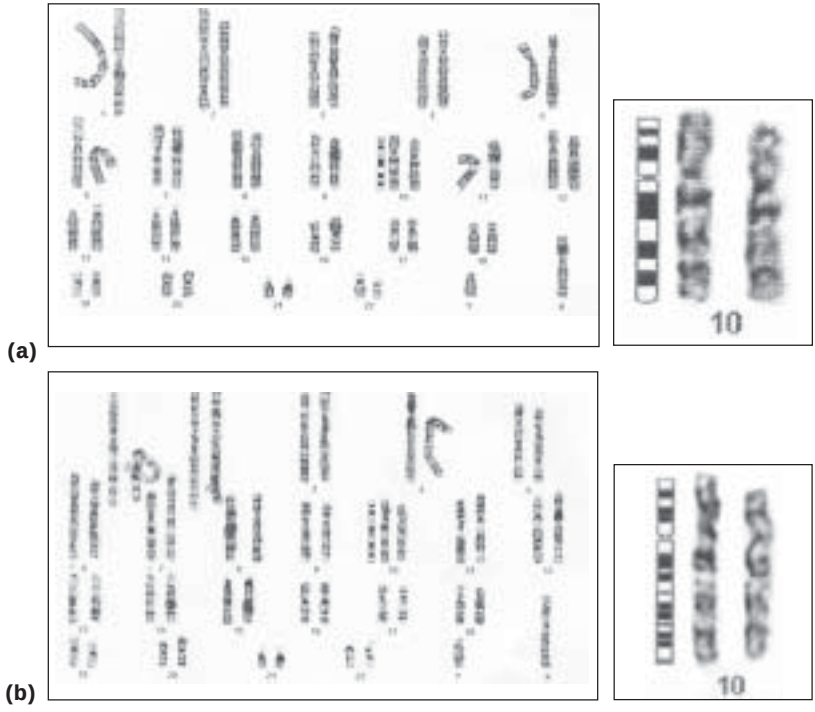


Fig. 6.5 Karyotypes showing 22 autosomes, and X and Y chromosomes. **a** 450 band level. **b** 550 band level. Note that each dark band on the q arm of chromosome 10 in Fig. 6.5a is split into two dark and one light bands in Fig. 6.5b.

chromosomes. Chromosomes in the early part of metaphase and prophase are longer and may have up to 850 bands per haploid set. Routinely, 500 to 550 bands are accepted as a satisfactory chromosome preparation for analysis. At this stage, each band corresponds to an average of 4 mb of DNA and thus, the loss or gain of even a single band leads to the loss or gain of a number of genes in the region.

The pattern of banding obtained by staining with the fluorescent dye quinacrine is called Q-banding. C-banding and silver staining of nuclear organizing regions (Ag NOR) are used to study two specific regions of chromosomes - heterochromatin and satellites, respectively. AgNOR staining identifies transcriptionally active rRNA genes on the p arms of acrocentric chromosomes. These are present on 6 to 8

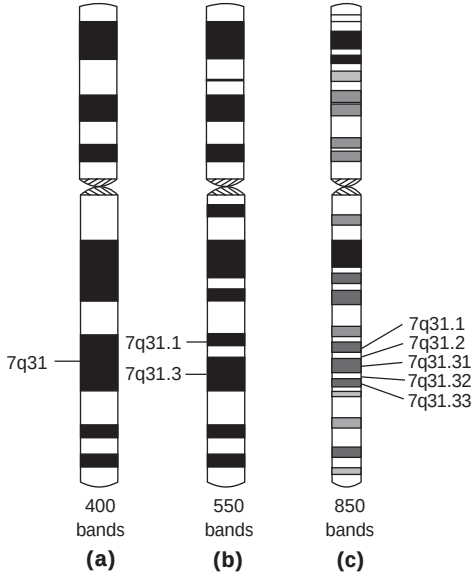


Fig. 6.6 Idiogram of G-banded chromosome 7. **a** At 400 band level. **b** At 550 band level. **c** At 850 band level. Note that band 7q31 (first band in the third region of the q arm of the chromosome) is split into three sub-bands, namely, 7q31.1, 7q31.2, 7q31.3. The band numbers are written by the side of the idiogram.

of the 10 acrocentric chromosomes.

Routinely, 10-20 metaphase spreads are analysed. Three to five metaphase spreads are photographed, the chromosomes are cut from the photograph and pasted in pairs in the ascending order of their number. Such an arrangement is known as a karyotype (Figs 6.5a and b). The word 'karyotype' is also used to denote the chromosome complement of a person. Nowadays, computers are used to prepare karyotypes. Software is available to capture digital images, and cut, paste and analyze chromosomes. The use of software for automated karyotyping has increased the speed of chromosomal analysis. The diagrammatic representation of the specific banding pattern of a chromosome is known as an idiogram (Fig. 6.6).

CHROMOSOME VARIATIONS OR POLYMORPHISMS

Chromosome analysis in normal individuals has revealed

some variations in the karyotype which are without phenotypic effects. The most striking of these is an increase in the size of the heterochromatic regions (which does not contain any coding sequences and stains darkly with G- and C-banding techniques) around the centromeres of chromosomes 1, 9 and 16 and at the terminal end of the q arm of the Y chromosome. These polymorphic changes (variations seen in normal people) in heterochromatin are known as heteromorphisms. Similar variations are seen in the terminal satellites of acrocentric chromosomes. These satellites contain genes for rRNAs and repetitive DNA. Variations in the size of the satellites and the degree of intensity to which they stain can be appreciated by Q-banding and Ag NOR staining. It is important to distinguish these polymorphisms from chromosomal abnormalities that are clinically significant.

CHROMOSOMAL ABNORMALITIES

Errors that occur during meiosis or mitosis can lead to *de novo* chromosomal abnormalities. On the other hand, chromosomal abnormalities can also be acquired or inherited from a parent. Unbalanced chromosomal abnormalities lead to alterations in hundreds to thousands of genes. They result in trisomy or monosomy of chromosome segments and have obvious clinical manifestations. Usually, monosomy of an autosome is not compatible with life. Duplications are considered to be less harmful than deletions. Mental subnormality almost always accompanies an imbalance of autosomes.

Numerical abnormalities

Inaccurate segregation of chromosomes during cell division leads to the gain or loss of a chromosome (numerical abnormalities), the classical examples of which are Down syndrome (47,+21) and Turner syndrome (45,X). Such chromosomal imbalances lead to serious clinical consequences (*see* Chapter 7). The underlying pathological mechanisms giving rise to such chromosomal abnormalities are unknown.

Numerical abnormalities can be euploidies or aneuploidies. A human cell with the full set of

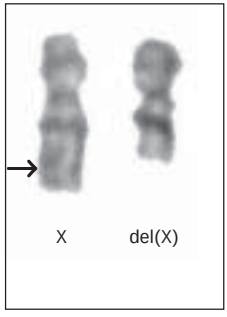
Table 6.1 Common structural abnormalities of chromosomes

Structural abnormalities	Description	Abbreviation according to the ISCN *
Deletion (deficiency)	Loss of a part of a chromosome; it may be terminal or interstitial	del
Duplication	Presence of two copies of a segment of a chromosome	dup
Inversion	There are two breaks on a chromosome and the segment between the breaks gets inverted through 180° and joins back	inv
Ring chromosome	Both ends of a chromosome break and join with each other to form a ring and there is deletion of the terminal segments of the p and q arms	r
Isochromosome	Loss of one arm of a chromosome and duplication of the other	i
Translocation	Transfer of chromosomal material between chromosomes	t
Robertsonian translocation	Special type of reciprocal trans-location between two acrocentric chromosomes in which the q arms of the two chromosomes join together and the p arms are lost	rob

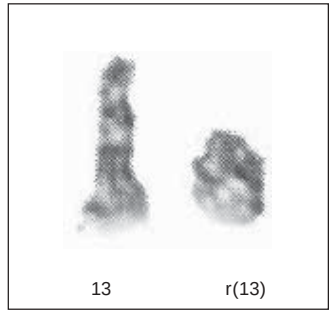
* International System of Human Cytogenetic Nomenclature

chromosomes is called euploid. For example, human cells with a single set of chromosomes (23) is haploid, a normal somatic cell is diploid while cells with 69 (triploid) and 92 (tetraploid) chromosomes are polyploids. Triploidy and tetraploidy usually result in miscarriage or stillbirth. Cells with missing or extra chromosomes are called aneuploids. The commonest type of aneuploidy is trisomy, i.e. presence of three homologous chromosomes instead of two in a somatic cell. The presence of only one of the homologous pair of chromosomes in a cell is called monosomy, e.g. 45,X.

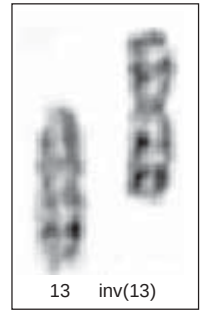
Structural abnormalities



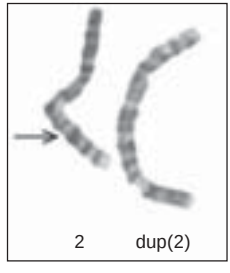
(a)



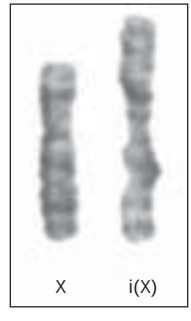
(b)



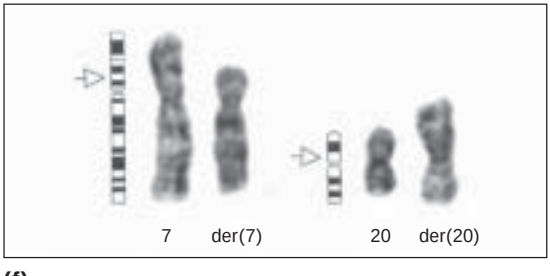
(c)



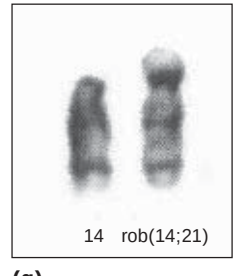
(d)



(e)

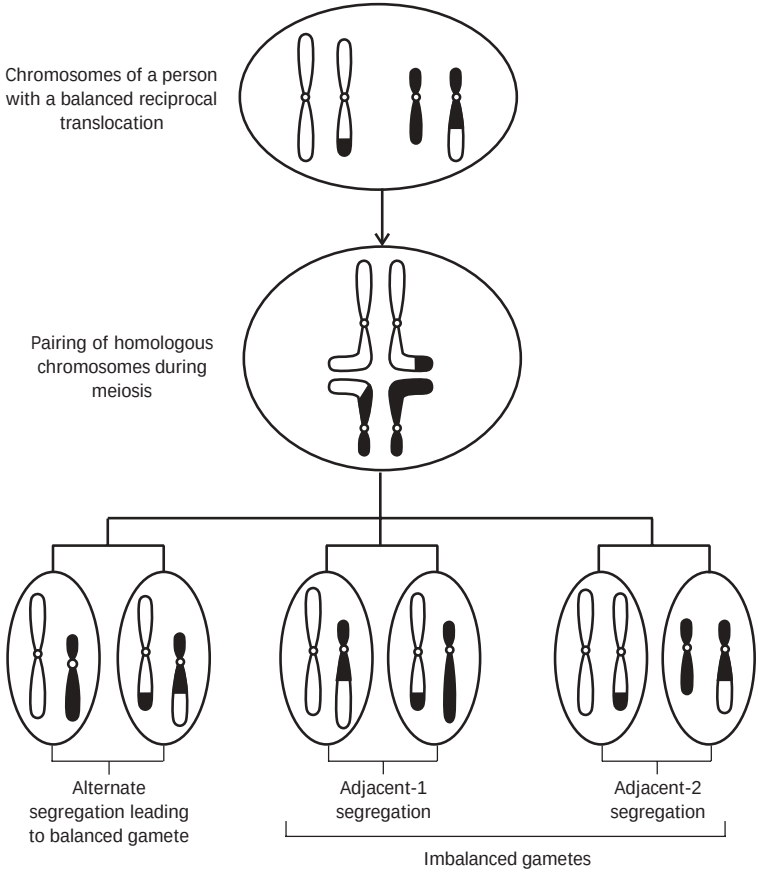


(f)



(g)

Fig. 6.7 Types of structural abnormalities (normal chromosomes are seen on the left of each picture). **a** Deletion of a part of the q arm of the X chromosome beyond the point shown by the arrow. **b** Ring chromosome of chromosome 13. **c** Pericentric inversion of chromosome 13. **d** Duplication (tandem) of a part of the q arm of chromosome 2 beyond 2q31 (→). **e** Isochromosome of the q arm of the X chromosome. **f** Reciprocal translocation between chromosomes 7 and 20. The breakpoints are shown by arrows on normal idiograms of chromosomes 7 and 20. **g** Robertsonian translocation between chromosomes 14 and 21.



Note: Segregation of three chromosomes to one gamete and one chromosome to another gamete will always lead to imbalanced gametes.

Fig. 6.8 Diagrammatic representation of pairing (quadrilateral formation) of translocated chromosomes and the types of segregations leading to balanced and unbalanced gametes. Adjacent-1 segregation: homologous chromosomes go to different daughter cells. Adjacent-2 segregation: homologous chromosomes go to the same daughter cell.

Chromosomal abnormalities can be structural also; these may be balanced or unbalanced. These are either inherited from a parent or arise *de novo* - caused by unrepaired or misrepaired DNA. Various types of structural abnormalities are listed in Table 6.1. Figure 6.7 illustrates some of these structural

abnormalities. These abnormalities result from breaks in one or more chromosomes. If, as a result of this arrangement, the total genetic material is neither lost or gained, the chromosomal rearrangement is known as balanced. Translocation or inversion can be balanced chromosomal arrangements. However, a chromosomal anomaly appearing cytogenetically balanced may have submicroscopic loss of a DNA segment leading to phenotypic abnormalities such as malformations or mental retardation. Other problems which can result from balanced rearrangements are infertility or recurrent reproductive losses (*see* Chapter 8).

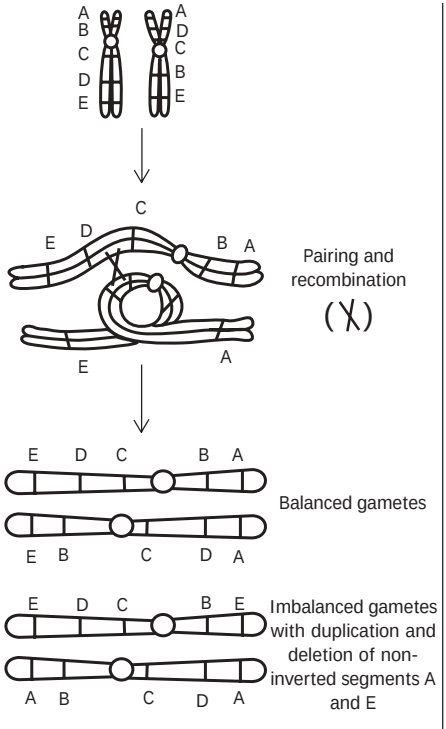


Fig. 6.9a Diagrammatic representation of the pairing of a normal chromosome with a chromosome with pericentric inversion and the mechanism of formation of imbalanced gametes due to recombination.

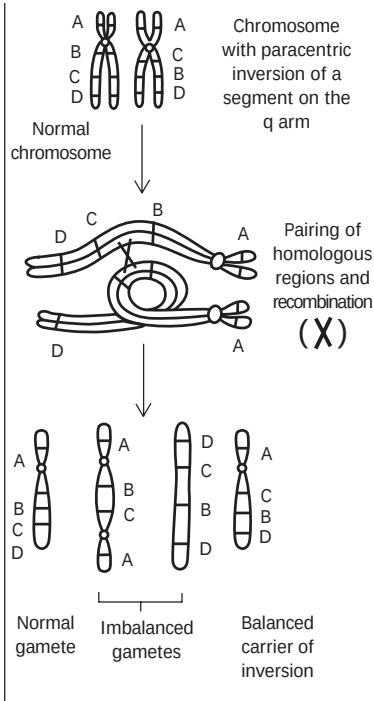


Fig. 6.9b Crossing over in an inversion loop of a carrier of paracentric inversion leading to a dicentric chromosome and an acentric chromosome.

Table 6.2 Symbols and abbreviations used for describing chromosomal aberrations

Symbol / Abbreviation	Definition
p	Short arm of a chromosome
q	Long arm of a chromosome
p ter	Terminal end of the short arm of a chromosome
q ter	Terminal end of the long arm of a chromosome
cen	Centromere
der	Derivative of a rearrangement
fra	Fragile site
mos	Mosaic
mar	Marker chromosome
ter	Terminal end of a chromosome
: [colon]	Break
:: [double colon]	Break and re-join
/ [slash]	Separates cell lines in describing mosaics or chimeras
+/– [plus/minus symbol before a chromosome]	Iindicates gain or loss of the chromosome
+/– [plus/minus symbol after a chromosome]	Indicates gain or loss of a part of the chromosome
; [semicolon]	Separates chromosomes and chromosome regions involved in rearrangements involving more than one chromosome
→[arrow]	Indicates from–to

* Adapted from the International System of Human Cytogenetic Nomenclature (ISCN)

Translocation

Translocation involves the exchange of chromosome segments between two chromosomes. It can be of three types. Reciprocal translocation requires a break on each of them and the exchange of a chromosome segment from one to another chromosome and vice versa. In a balanced translocation, 2:2 segregation of adjacent chromosomes leads to the production of imbalanced gametes; while alternate segregation leads to the production of one normal gamete and another with a balanced translocation (Fig. 6.8). In addition, 3:1 segregation of chromosomes with balanced translocation may occur, leading to the production of gametes with 22 or 24 chromosomes, which will lead to spontaneous abortion.

The second type of translocation is an insertional translocation. For an insertional translocation, three breaks are required on one or two chromosomes. Two breaks on a chromosome lead to separation of the segment between the breaks. This segment will then get inserted at another site on the same chromosome or another chromosome. Thus, there is one break on the chromosome in which the segment is inserted.

The third type of translocation is the centric fusion type. It is also known as Robertsonian translocation. It arises from breaks at or near the

Table 6.3 Chromosomal abnormalities and their karyotypes

Chromosomal abnormality	Description	Notes
mos47,XY,+21/46,XY or 47,XY,+21/46,XY	A male with Down syndrome due to mosaicism for trisomy 21 and normal cell line	
69,XXY	Triploidy (69 chromosomes, XXY sex chromosomes)	
45,X/46,XX	Mosaic Turner syndrome	The abnormal cell line is written first.
46,XX,inv(2)(p12p23)	Inversion of a segment on the p arm of chromosome 2 between bands p12 and p23	There is no semicolon between the breakpoints that are on the same chromosome.
45,XX,der(14;21)(q10;q10)	Carrier of a balanced translocation between two acrocentric chromosomes 14 and 21. As the p arms of chromosomes 14 and 21 are lost, the chromosome number is 45	Semicolon sepeates the two chromosomes involved in the translocation. Semicolon is also placed between the breakpoints.
46,XX,der(14;21)(q10;q10),+21	This case has three copies of chromosome 21; two normal copies of chromosomes 21 and one attached to chromosome 14. A normal copy of chromosome 14 is replaced by a derivative chromosome due to Robertsonian translocation between chromosomes 14 and 21	
46,X,t(X;14)(q28;q12)	Balanced translocation between chromosome X and chromosome 14	The X chromosome is written first.
46,XX,t(1;3)(p22;q13)	Balanced translocation between chromosomes 1 and 3. The breakpoint on chromosome 1 is 1p22 and that on chromosome 3 is 3q13	Chromosome with lower number is written first.
47,XY,+mar	47 chromosomes with one extra chromosome	

of unidentified origin

Table 6.3 (*cont.*)

Chromosomal abnormality	Description	Notes
46,XY,t(11;21)(p14;q11.2)	Balanced translocation between chromosomes 11 and 21. The breakpoint on chromosome 11 is p14 and that on chromosome 21 is q11.2	The first bracket shows the chromosomes involved in translocation, with the lower number written first. The second bracket represents breakpoints on the respective chromosomes. Note the semicolon separating chromosomes and breakpoints.
47,X,t(X;13)(q28;q12), inv(10)(p13q22),+21	Translocation between chromosomes X and 13, inversion of a segment on chromosome 10 between bands p13 and q22 with trisomy 21	Sex chromosomal abnormality is listed first. Structural abnormalities are listed before the numerical abnormalities.
46,XY,del(7)(p11) or 46,XY,del(7)(7q ter →7p11:)	Deletion of the q arm of chromosome 7 beyond the band p11. It implies that there is partial monosomy of a segment of chromosome 7; p11 to p terminal end. del (7) (7q ter →7p11:) describes the abnormal chromosome in long form	
46,XX,der(1)t(1;3)(p22;q13)	There are two normal copies of chromosome 3 and one normal copy of chromosome 1. One chromosome 1 is replaced by a derivative of chromosome 1 resulting from the translocation between chromosomes 1 and 3.	There is no need to write '–1' to show that one normal chromosome 1 is missing. This is an unbalanced chromosomal anomaly with partial monosomy of chromosome 1 and partial trisomy of 3. It can also be written 46,XX,der(1)(1qter ->1p22::3q13->3qter)

7 Chromosomal disorders: Clinical features and management

Chromosomal disorders result from the numerical or structural abnormalities of chromosome or chromosomes. The chromosomal abnormalities can be balanced or balanced and may involve the autosomes or sex chromosomes.

The spectrum of phenotypes due to chromosomal abnormalities include a normal phenotype, spontaneous abortion, multiple malformations, mental retardation, hypogonadism and poor reproductive outcome. The phenotype depends on whether the chromosomal abnormality leads to genetic imbalance or not. Numerical abnormalities of autosomes are usually associated with a poor outcome. This chapter describes some of the common chromosomal disorders and their management including genetic counselling.

PREVALENCE OF CHROMOSOMAL ABNORMALITIES

Forty to fifty per cent of spontaneously aborted conceptuses have chromosomal abnormalities (Table 7.1). With advancing gestational age, the overall prevalence of chromosomal abnormalities in the foetuses declines precipitously as abnormal foetuses unfit for survival have already been lost. Chromosomal abnormalities are seen in only 5%–10% of stillbirths and perinatal deaths (with or without obvious malformations). The prevalence of chromosomal abnormalities in livebirths is 0.625%, i.e. 1 in 160. This includes autosomal trisomies (0.12%), sex chromosome abnormalities (0.24%) and structural

Table 7.1 Chromosomal abnormalities seen in spontaneously aborted conceptuses

Chromosomal abnormalities	Spontaneously aborted conceptuses (%)
Autosomal trisomies (+16, +18, +21, +22)	50
45,X	19
Triploidy	16
Tetraploidy	6
Others (including structural abnormalities)	9

abnormalities (0.24%). One in 500 newborns is a carrier of a balanced rearrangement (Table 7.2). Such an individual usually does not have any phenotypic abnormality but may give birth to a child with chromosomal imbalances or may have reproductive problems such as infertility or recurrent spontaneous abortions.

Abnormalities of the sex chromosomes usually give rise to abnormal sexual development such as hypogonadism and infertility. Numerical and unbalanced abnormalities of autosomes lead to malformation/s and mental retardation. The prevalence of chromosomal abnormalities in foetuses with prenatally detected malformation is 5%–10%, and if there are two or more malformations, the prevalence is up to 20%–30%. Malformations that are more likely to be associated with chromosomal abnormalities include cardiac anomalies, duodenal atresia,

Table 7.2 Chromosomal disorders at birth

Disorder	Prevalence
Balanced translocation	1 in 500
Unbalanced translocation	1 in 2000
Pericentric inversion	1 in 100
Trisomy 21	1 in 800
Trisomy 18	1 in 8000
Trisomy 13	1 in 20,000
47,XXY	1 in 1000 males
47,XYY	1 in 1000 males
47,XXX	1 in 1000 females
45,X	1 in 5000 females

holoprosencephaly and omphalocele. Malformations such as anencephaly and meningo-myelocele, limb defects and arthrogryposis are less frequently associated with chromosomal anomalies (*see also* Chapter 8).

INDICATIONS FOR KARYOTYPING

The indications for chromosomal analysis or karyotyping are as follows:

1. Suspected or clinically obvious chromosomal syndromes such as Down syndrome and Turner syndrome
2. Unexplained mental retardation with or without malformations
3. Proportionate short stature in a prepubertal female (as some cases of Turner syndrome may present as isolated short stature)
4. Abnormal sexual development and differentiation
   —ambiguous genitalia
   —delayed or incomplete pubertal development in males and females
   —oligospermia or azoospermia
5. Infertility
6. Recurrent spontaneous abortions (three or more) or foetal losses
7. Unexplained stillbirth
8. Parents of a child with a structural chromosomal anomaly
9. Siblings and parents of a person with balanced structural rearrangement
10. Neoplastic conditions, particularly haematological malignancies, where identification of the chromosomal abnormality may be useful in diagnosis, prognosis and management
11. Multiple (two or more) monogenic disorders in a person
12. Known monogenic condition (of which mental retardation is not a feature) associated with mental retardation
13. A female with manifestations of an X-linked recessive disorder
14. Pregnancy at risk for chromosomal abnormality (*see* Chapter 8).

The 11th, 12th and 13th indications for karyotyping are interesting. Such cases are rare and are likely to be associated with structural abnormalities of chromosomes such as deletions or balanced translocations, and help in tracing the likely location of the gene causing the particular monogenic disease or diseases in a person with chromosomal abnormality. The causative gene / genes are likely to be located in the deleted region or at the breakpoints of a chromosomal rearrangement. Identification of such a case with phenotypic abnormality associated with small deletion of a chromosome or apparently balanced chromosomal rearrangement is an important step in gene

mapping. It reduces the effort of identifying the location of the disease-causing gene in the genome. Genes for many diseases such as Duchenne muscular dystrophy and anhidrotic ectodermal dysplasia have been mapped in this way.

DISORDERS CAUSED BY ABNORMALITIES OF AUTOSOMES

Down syndrome

Down syndrome phenotype was described by an English physician, John Langdon Hayden Down in 1866. In 1959, Lejeune discovered that trisomy 21 was the cause of Down syndrome. However, the pathophysiology of the disease is not known till date. Down syndrome is the commonest genetic cause of mental retardation accounting for 20%–30% of cases with mental retardation. The facial features of children with Down syndrome are characteristic (Fig. 7.1). However, in a newborn, the diagnosis may be somewhat difficult unless the physician is experienced.

Aetiology

Trisomy 21 is responsible for causing Down syndrome in 95% of the cases (Figs 7.2a and b). In more than 90% of these children, the extra chromosome comes from the mother due to non-disjunction (failure of separation) of chromosomes during meiosis I (Fig. 7.3). In 4% of cases, translocation of chromosome 21 to any of the D group chromosomes, i.e. 13, 14, 15, or G group chromosomes, i.e. 21 or 22 gives rise to Down syndrome (Figs 7.4a, b and c). Translocation with D and G group chromosomes accounts for 55% and 40% of Down syndrome cases (due to translocation), respectively. The remaining are due to translocation to chromosomes other than those of the D and G groups. One per cent of

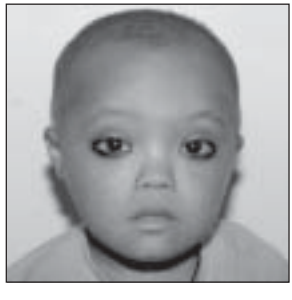


Fig. 7.1 A child with Down syndrome. Note the low-set ears, upward slant of eyes, flat bridge of nose and protruding tongue.

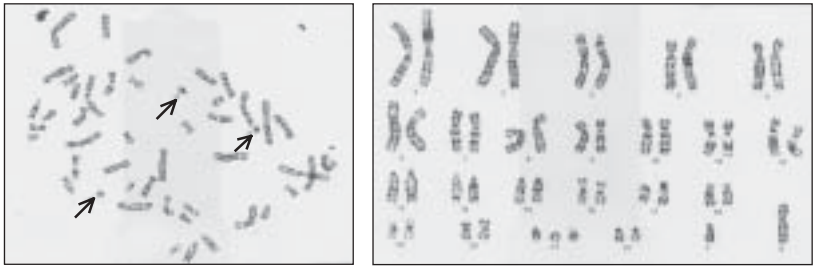


Fig. 7.2 **a** A metaphase spread showing trisomy 21. Chromosomes 21 are shown by arrows. **b** A karyotype showing trisomy 21.

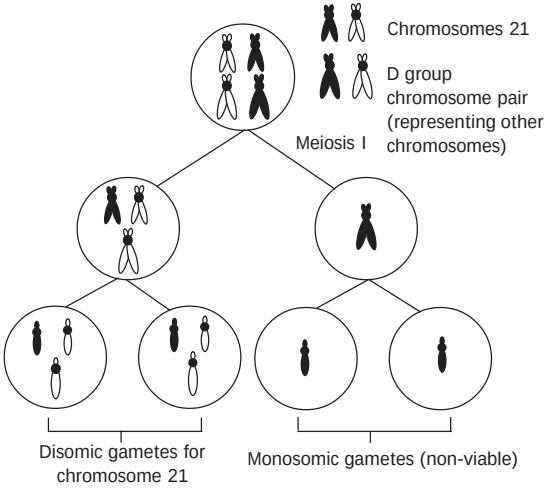


Fig. 7.3 Non-disjunction of chromosomes at meiosis I gives rise to a disomic ovum. Its fertilization will lead to trisomy 21 in the zygote.

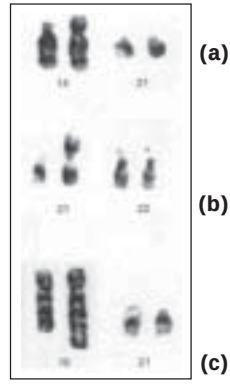


Fig. 7.4 Translocations involving chromosome 21 can cause Down syndrome. **a** t(14;21) **b** t(21;21) and **c** t(10;21)

patients with Down syndrome are mosaics; having one cell line with 47 chromosomes and a normal cell line with 46 chromosomes. Karyotyping the parents is necessary if the Down syndrome is caused due to translocation as one of the parents may be a carrier of a balanced translocation. Table 7.3 shows the risk of recurrence of translocation in Down syndrome when one of the parents is a carrier of a translocation.

Rarely, normal parents may have two or more children affected with trisomy 21. In such cases, gonadal mosaicism should be suspected as the cause for increased risk of recurrence of the disease in the family.

Table 7.3 Down syndrome due to translocation: Types, relative prevalence and risk of Down syndrome in the offspring of the carriers of translocation

Chromosomes involved in the translocation	Relative frequency (%) amongst the translocation Down syndrome cases	Prevalence of De novo* translocation	Prevalence of inherited translocation	Risk of Down syndrome in the offsprings of a translocation carrier(%)	
				Carrier mother	Carrier Father
Dq21q [t(14;21) or others]	54.2	55%	45%	10-15	1-5
21qGq t(21;21) t(21;22)	40.9	96%	4%	100 10-15	100 1-5
21 with chromosomes other than G & D group	4.9	Few	Most	10-15	1-5

*The risk of recurrence of Down syndrome in the siblings of a case with de novo translocation is 1%.

The risk of trisomy 21 in the offspring of women with trisomy 21 is about 50%. Males with trisomy 21 are usually infertile.

Clinical features

Table 7.4 lists the clinical features of patients with Down syndrome. Mental retardation is the most serious manifestation. The intelligence quotient (IQ) of children with the disease is usually between 40 and 50 but may be higher in those with mosaicism. Most of the affected children walk, talk in simple language and can be trained in self-care and a vocation that requires simple and repetitive tasks. Other important problems are epilepsy, hypothyroidism, deafness, atlantoaxial instability and increased risk of leukaemia. When serious malformations are present, death may occur during infancy, but otherwise life expectancy is not markedly reduced. In adults with Down syndrome, there is increased prevalence of dementia and neuropathological changes similar to those seen in Alzheimer disease.

Relation between maternal age and the prevalence of Down syndrome

The prevalence of Down syndrome increases with increasing maternal age (Table 7.5). In the West, a maternal age of 35 years at the time of delivery is taken as the cut-off, above which prenatal diagnosis by amniocentesis used to be offered to all women.

Depending on the type of chromosomal abnormality and carrier

Table 7.4 Clinical features of patients with Down syndrome

1. Brachycephaly	17. Narrow and high-arched palate
2. Flattened facial features	18. Dental abnormalities
3. Flat occiput	19. Broad hand and short fingers
4. Epicanthic folds	20. Short 5th middle phalanx
5. Oblique palpebral fissures	21. Transverse palmar crease
6. Hypertelorism	22. Short limbs
7. Brushfield spots	23. Clinodactyly of the 5th finger
8. Dysplastic ears	24. Increased gap between the 1st and 2nd toes
9. Low-set ears	
10. Small nose	25. Hyperextensibility of the joints
11. Depressed nasal bridge	26. Rough skin on the dorsum of the hands
12. Excess skin at the nape of the neck	27. Hypotonia
13. Short and broad neck	28. Mental retardation
14. Open mouth	29. Congenital heart disease
15. Protruding tongue	30. Duodenal atresia
16. Furrowed tongue	31. Hirschsprung disease

Table 7.5 Risk of giving birth to a child with Down syndrome with increasing maternal age

Age of the mother (years)	Risk of having a child with Down syndrome
20	1:1925
25	1:1205
30	1:885
35	1:365
40	1:110
45	1:32

status of the parents, the risk of recurrence may vary from 1% to 100%. Hence, chromosomal analysis of every child with Down syndrome is necessary, even if the diagnosis is clinically obvious.

Management

In a case of Down syndrome, identification of the associated malformations and their appropriate management are essential. The

diagnosis of the disease is usually obvious at birth and should be explained to the parents as early as possible. The prognosis regarding growth and development of the child needs to be told. However, rare complications such as increased risk for leukaemia, etc. need not be told immediately. Positive aspects of the progress such as the ability to attain self-care, to learn to walk and talk should be stressed. However, such children are not likely to undergo academic education and will need lifelong supervision and care. The importance of infant stimulation programmes and training facilities for improving the outcome must be explained.

Regular follow up (preferably yearly) to assess growth and development, ophthalmological examination and hearing assessment are indicated at least in the early years of life. There is a high incidence of hypothyroidism which increases with age. Yearly evaluation of thyroid function is required. Atlantoaxial instability is present in 2%–3% of children with Down syndrome. Hence, radiological investigations to look for it are essential, especially if the child wants to actively participate in sports and athletics, or when there is difficulty in walking or pain in the neck. Down syndrome persons can lead a happy and constructive life at home and at supervised workplaces (Fig. 7.5). All attempts must be made to provide them medical and supportive care and training.

Genetic counselling and prenatal diagnosis

Issues regarding the aetiology need to be clarified to the parents. Depending on the chromosomal abnormality in the child, the family should be counselled regarding the risk of recurrence of the disorder in future pregnancies. If the child has Down syndrome due to translocation, analysis of the karyotype of the parents is essential before counselling.

Fig. 7.5 Girls with Down syndrome dancing in a cultural programme.

The risk of recurrence of free trisomy 21 is 1%. Karyotyping of parents of a child with free trisomy 21 is not necessary. The parents should be informed about the availability of prenatal diagnosis during the next pregnancy. If the result of prenatal diagnosis show Down syndrome, the decision regarding continuation or termination of pregnancy should be left to the couple (*see* Chapters 19 and 20).

Other autosomal trisomies

Other autosomal trisomies are rare and are usually fatal during the neonatal period or infancy. The clinical features of some of the known autosomal trisomies and other syndromes (Fig. 7.6) are given in Table 7.6.

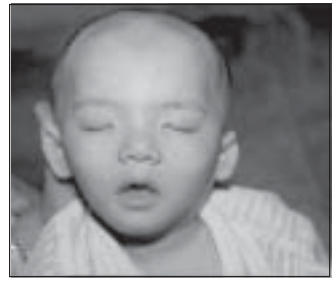


Fig. 7.6 A child with cri du chat syndrome.

Table 7.6 Clinical features of autosomal trisomies and other chromosomal syndromes

Syndrome	Phenotype
Edward syndrome (trisomy 18)	Growth retardation, clenched fists with overlapping digits, radial limb defects, heart defects, exomphalos, meningomyelocele, cryptorchidism, prominent occiput, hypertonia, death in infancy
Patau syndrome (trisomy 13)	Holoprosencephaly, cyclopia, anophthalmia, facial clefts, heart defects, renal abnormalities, polydactyly, death in neonatal life
Wolf Hirschhorn syndrome(4p-) (partial monosomy of 4p)	'Greek helmet' facies, hypertelorism, strabismus, low set ears, micrognathia, cleft lip and palate, iris coloboma, heart defects, mental retardationd
Cri du chat syndrome (5p-) (partial monosomy of 5p)	Characteristic cat-like cry, microcephaly, round face hypertelorism, broad nasal bridge, heart defects, mental retardation, hypotonia (Fig 7.6)
Partial monosomy of 18p	Brachycephaly, broad face, ptosis, strabismus, hypertelorism, broad nose, micrognathia, wide mouth, long protruding ears, varying degrees of holoprosencephaly, heart defects, mild-to-moderate mental retardation

DISORDERS CAUSED BY ABNORMALITIES OF THE SEX CHROMOSOMES

Turner syndrome

Turner syndrome is associated with monosomy or partial monosomy of the X chromosome, including mosaicism. Embryos with a 45,X karyotype are prone to spontaneous abortion. More than 99% of 45,X conceptuses abort spontaneously, accounting for one-fifth of all spontaneous abortions (Fig. 7.7). The features of Turner syndrome are given in Table 7.7.

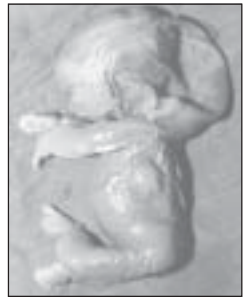


Fig. 7.7 A foetus with Turner phenotype. Note a large cystic hygroma behind the neck and generalized subcutaneous oedema.

Many patients (Fig. 7.8) with Turner syndrome have some or none of the somatic abnormalities, while 50% of cases have characteristic features. Non-pitting oedema of the hands and feet is present only in newborns. Most cases present as short stature and absence of secondary sexual characters at adolescence. As short stature may be the only manifestation, chromosomal analysis of prepubertal girls with short stature is indicated.

Gonads and sex hormone

Women with Turner syndrome have streak gonads by puberty. In untreated patients, menarche does not occur. Rarely, spontaneous menarche may be achieved with menstruation lasting for a few months

Table 7.7 Clinical features of Turner syndrome

1. Short stature
2. Short webbed neck
3. Cubitus valgus
4. Primary amenorrhoea
5. Lack of secondary sex characters
6. Presence of epicanthic folds
7. Hypertelorism
8. High-arched palate
9. Low posterior hair line
10. Shield chest with widely spaced nipples
11. Coarctation of the aorta
12. Horseshoe kidney and other renal anomalies
13. Multiple naevi
14. Lymphoedema of the hands and feet in newborns
15. Nail hypoplasia
16. Short 4th metacarpal and metatarsal

(a) (b) (c)

Fig. 7.8 a A patient with characteristic features of Turner syndrome. Note the webbed neck and shield chest. **b** A patient with Turner syndrome presenting with primary amenorrhoea. Note the increased carrying angle. **c** Multiple naevi in a girl with Turner syndrome.

or years and ending in early menopause. A few women with Turner syndrome become pregnant and are able to give birth. There is an increased risk of miscarriage and aneuploidy in the offspring of these women. In most of the patients, the production of oestrogen is low and that of gonadotrophins is high. Usually, there is lack of development of secondary sexual characters.

Other problems

Mental retardation is not a feature of Turner syndrome but the IQ may be lower than that in normal siblings. Specific deficits in perceptual and spatial thinking, mathematics and language skills may occur. There is an increased risk for autoimmune thyroiditis and essential hypertension by teenage in these patients.

Karyotype abnormalities

The karyotype 45,X is found in 40%–60% of patients with Turner syndrome. Some cases are mosaics (45,X/46,XX). The structural abnormalities responsible for Turner syndrome are the presence of an isochromosome of the q or p arm of the X chromosome, deletion of the p or q arm of the X chromosome and a ring X chromosome. Rarely, isochromosome of the q arm of the Y chromosome and X–autosome or X–Y translocation may present as Turner syndrome. These structural abnormalities may be associated with mosaicism for the 45,X cell line. Mosaics with 45,X and 46,XY cell lines may also present as Turner syndrome.

Management

The main problems associated with Turner syndrome are short stature and hypogonadism. Growth retardation is present since early childhood and persists into adulthood. The adolescent growth spurt is absent and the adult height is below –4 SD. No treatment can result in achieving a normal height; but recently, growth hormone therapy has been found to be effective in adding a few inches to the final height. Growth hormone therapy should be started in early childhood, as soon as the child starts falling below the 5th centile for normal, and must be continued till growth is complete.

There is no treatment that can induce ovarian function but treatment with oestrogen can give rise to adequate development of the breasts and pubic hair. Initially, conjugated oestrogen or ethinyloestradiol is started in a low dosage when a bone age of 15 years is achieved, or earlier if the patient is keen on the development of secondary sexual characters. The dose is increased every six months. After 1–2 years or whenever breakthrough bleeding occurs, cyclical progesterone is added to simulate normal cycles. Regular menstruation can be achieved with this treatment. Assisted reproduction using donor ova is possible.

Other associated problems such as visual defects, strabismus, congenital heart and renal defects, hypertension and hypothyroidism should be investigated and treated as required. Screening for diabetes mellitus should be carried out during adulthood, especially in obese patients.

Klinefelter syndrome

Klinefelter syndrome is defined as male hypogonadism with at least two X chromosomes and one Y chromosome.

Clinical features

Majority of the patients are identified because of delayed or diminished puberty, or because of gynaecomastia or infertility. There are no significant facial features except for the absence of facial hair growth. Prepubertal boys with Klinefelter syndrome appear normal. Initiation of puberty is usually normal but by midpuberty these boys become hyper-gonadotrophic. The stature is tall with long legs (eunuchoid habitus) (Fig. 7.9). There may be gynaecomastia and female distribution of body fat. Azoospermia, oligospermia and infertility are always present, except in some cases with 46,XY/47,XXY mosaicism. The size of the penis may be normal or reduced. The cardinal feature of Klinefelter syndrome is testicular

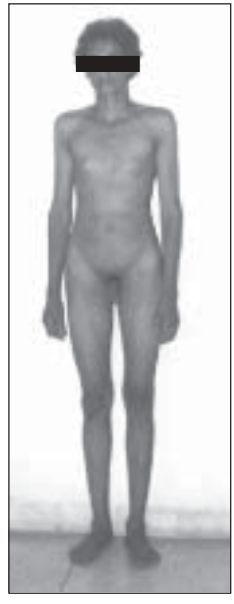


Fig. 7.9 A patient with Klinefelter syndrome.

hypoplasia. The testicles, besides being small, are usually fibrotic. Mental retardation (IQ < 70) is unusual but intelligence is often less than that of siblings. Learning disabilities are common.

Karyotype abnormalities

Eighty to eighty-five per cent of patients with Klinefelter syndrome have the 47,XXY karyotype. Fifteen per cent are mosaics for 46,XY/47,XXY. Other abnormalities include 46,XX/47,XXY; 46,XX/46,XY/47,XXY; 46,XY/48,XXXY; 45,X/46,XY/47,XXY and, rarely, structural abnormalities of the X chromosome.

Management

Treatment consists of testosterone supplementation starting with low doses of 50 mg every 3 weeks, which is gradually increased to 200 mg every 2–3 weeks. Surgery may be needed for gynaecomastia. Rarely, patients may request plastic surgery for the small testes. To improve the self-image, support from the family and counselling are needed.

Other abnormalities of the sex chromosomes

47,XXX and 47,XYY are common abnormalities. Individuals with a 47,XXX karyotype are females and 47,XYY are males. No definite phenotype is associated with these disorders and there are usually no medical or sexual problems. Previously, individuals with an XYY karyotype were considered to be prone to criminal tendencies but, with larger experience, this association has been found to be untrue. Prenatal diagnosis of these aneuploidies may create a dilemma for the counsellor and parents. After providing full information, the decision regarding continuation of the pregnancy should be left to the parents (*see also* Chapter 8).

MOSAICISM

Mosaicism for chromosomal anomalies is well demonstrated (*see* Chapter 8). One per cent of those with Down syndrome are mosaics. Depending on the relative preponderence of cells with normal chromosomes and those with chromosomal imbalance, the severity of the phenotype may vary. In some cases, the chromosomal anomaly may be limited to one type of tissue. In the Pallister–Killian syndrome, there is an extra chromosome which is an isochromosome of 12p. The abnormal cell line is limited to skin fibroblasts and is not detected in the peripheral blood lymphocytes (Fig. 7.10).

MICRODELETION SYNDROMES OR CONTIGUOUS GENE SYNDROMES

High-resolution banding and molecular cytogenetic techniques have led to the identification of deletions that are too small to be detected by conventional techniques. A number of microdeletion syndromes have been identified in recent years (*see* Chapters 5 and 12). This diagnosis can be easily confirmed by fluorescence *in situ* hybridization (FISH) techniques.

CYTOGENETICS OF TUMOURS

Chromosomal aberrations are common in malignant cells, which often show marked variation in the number and structure of chromosomes. Hyper- and hypodiploidy appear to be universal features of malignant cells. The best example of a unique marker for a neoplasm is that of the Philadelphia (Ph) chromosome, which is diagnostic of chronic myeloid

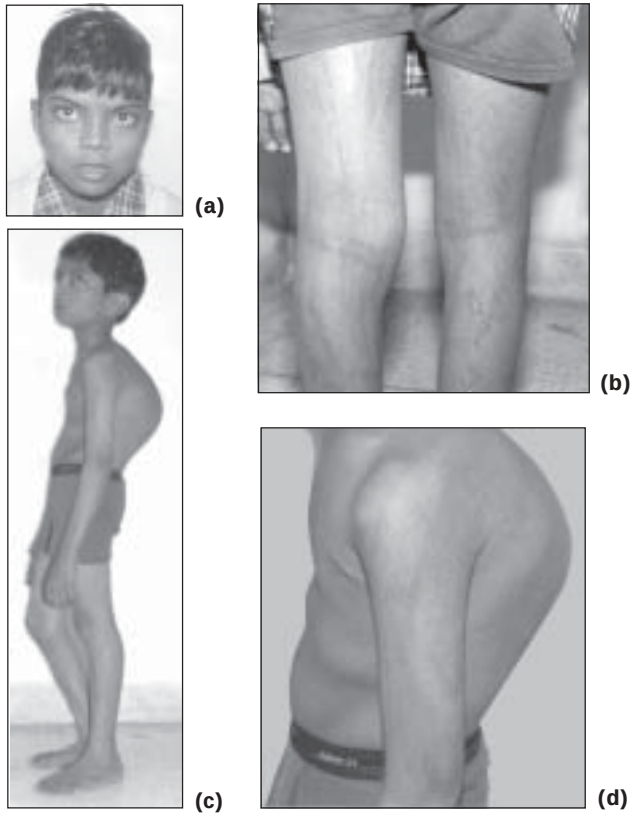


Fig. 7.10 A child with Pallister–Killian syndrome. The patchy pigmentary lesions suggest the possibility of mosaicism.

leukaemia (CML). Many chromosomal anomalies associated with cancers are not characteristic though the alterations are not totally random. Chromosomal alterations often lead to activation of protooncogenes or inactivation of tumour suppressor genes. In several leukaemias and lymphomas, the chromosomal rearrangement juxtaposes cellular proto-oncogenes to key regulators of immune response; for example, immunoglobulin and T-cell receptor (TCR) genes. Even though not specific, many of these alterations can serve as potential markers to monitor residual disease and its early relapse. (For more details, *see* Chapter 14.)

Philadelphia chromosome

In 1960, Hungerford and Novell from Philadelphia demonstrated the presence of an extra, small, abnormal chromosome in the blood and bone marrow cells of patients with CML, which was absent in the other tissues of the body. It was found in 90% of patients with CML and became a diagnostic marker of the disease. Subsequently, it was shown that the Ph chromosome arises as a result of chromosomal translocation between chromosomes 22 and 9. As a result, the *abl* (Abelson leukaemia virus) proto-oncogene from chromosome 9 is juxtaposed to the *bcr* (breakpoint cluster region) on chromosome 22. The product of the chimeric gene (known as the *abl–bcr* gene) has been shown to be present in leukaemia cells and plays an important role in causation of the disease.

Chromosomal breakage syndromes

A group of hereditary disorders are characterized by increased susceptibility to chromosomal breakages. These chromosome gaps and breaks may arise spontaneously or may be induced by DNA-damaging agents such as ultraviolet light, X-rays, alkylating agents, etc. (Fig. 7.11).

Besides increased susceptibility to tumours, each one of these conditions presents with a characteristic clinical feature of its own. Excess of chromosome breaks and gaps increases the susceptibility to neoplasia. These monogenic conditions are usually associated with an abnormality of DNA repair. A summary of some of the common syndromes is given in Table 7.8.

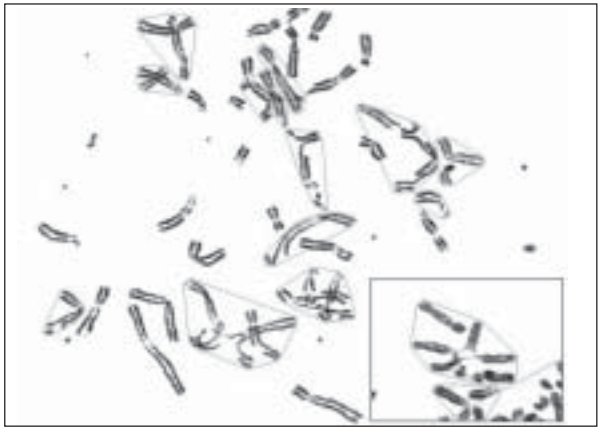


Fig. 7.11 Multiple chromosomal breakages; tri- and quadriradial (inset) formations in of a patient with Fanconi anaemia on adding mitomycin-C to the culture.

Table 7.8 Chromosome breakage syndromes

Condition	Features	Chromosomal breakage	Malignancies
Ataxia telangiectasia	Ataxia, oculocutaneous telangiectasia, repeated infections, low IgG, IgA	Increased spontaneous chromosomal breaks, enhanced by X-radiation	Leukaemias, lymphomas
Bloom syndrome	Light-sensitive facial rash, reduced IgA and IgM	Increased chromosomal breaks, especially on exposure to UV light	Lymphoreticular malignancies
Fanconi syndrome	Upper limb anomalies of the radius and thumb, short stature, pancytopenia	Multiple breaks on exposure to cross-linking agents such as mitomycin-C	Leukaemia, lymphomas, hepatic carcinoma
Xeroderma pigmentosum	Light-sensitive pigmented rash	Chromosomal abnormalities on exposure to UV light	Multiple basal cell/squamous cell cancers on sun-exposed areas before 20 years of age

All the above conditions follow the autosomal recessive mode of inheritance.

PRENATAL DIAGNOSIS

Families at high risk for giving birth to a child with a chromosomal disorder can be offered prenatal diagnosis by amniocentesis or CVS (*see* Chapter 8 and 19).

8 Chromosomal anomalies: Clinical presentations and prenatal diagnosis

Detection of a chromosomal anomaly calls for genetic counselling. The risk of recurrence in the siblings and offspring of a person with a chromosomal anomaly varies greatly, depending on the type of anomaly. Whatever may be the risk, the birth of chromosomally imbalanced babies can be prevented by prenatal diagnosis and counselling. This chapter discusses the common presentations of chromosomal abnormalities—mental retardation, malformation syndromes and poor reproductive outcome.

CHROMOSOMAL ANOMALIES IN MENTAL RETARDATION

Chromosomal abnormalities account for about one-third of the cases of moderate-to-severe mental retardation or developmental delay. More than 90% of these are due to trisomy 21. The rest include other trisomies and structural anomalies leading to partial deletions and duplications. Some structural abnormalities are inherited from the parents or are the result of recombinations involving a structurally abnormal chromosome in a parent. This implies that the parent can be a carrier of a balanced structural rearrangement. In this case, the risk of giving birth to an offspring with an unbalanced chromosomal abnormality associated with mental handicap/malformations is markedly increased. The recurrence of the chromosomal abnormality in subsequent pregnancies can be detected prenatally. Hence, chromosomal analysis should be carried out in all children with mental handicap [with or without dysmorphism] without any obvious cause. The following example illustrates the issues related to counselling for such a case.

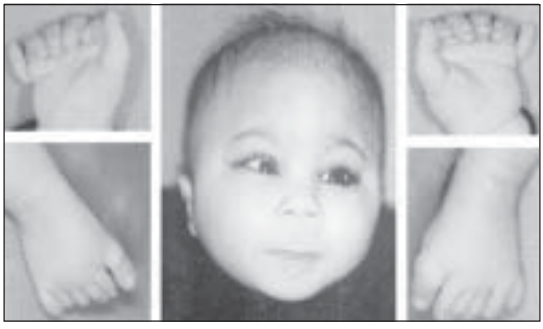
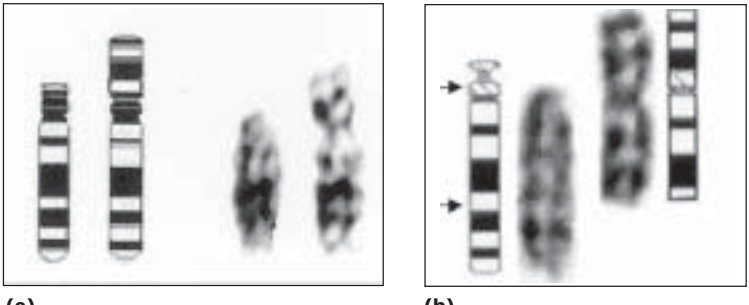


Fig. 8.1 A seven-month-old child with developmental delay, trigonocephaly, upslanting of the eyes and polydactyly. (Printed with the permission of 'Indian Pediatrics')

Figure 8.1 shows a seven-month-old child with developmental delay, trigonocephaly (prominent metopic suture), upslanting of the eyes and polydactyly in all the four limbs. His karyotype shows partial duplication of the q arm of chromosome 13 (Fig. 8.2a). The duplicated segment was translocated to the p arm of chromosome 13. Analysis of the karyotypes of his parents showed that the father was normal while the mother had an inversion of chromosome 13 (Fig. 8.2b).

In this family, there is a risk of up to 15% of giving birth to another child with a chromosomal abnormality. In addition, there is an increased risk for spontaneous abortion as some of the chromosomal imbalances may not be compatible with survival till birth. Figure 8.3 shows two of the possible recombinations during maternal meiosis. As predicted,



(a) **(b)**

Fig. 8.2 a Partial karyotype of the child shown in Figure 8.1 showing partial trisomy of 13q. The extra material on the p arm of the chromosome (on the right) is a part of 13q. **b** Partial karyotype of the mother of the child in Figure 8.1, showing pericentric inversion of chromosome 13. The normal chromosome is on the left side of the figure. The breakpoints are shown by arrows on the idiogram of the normal chromosome.

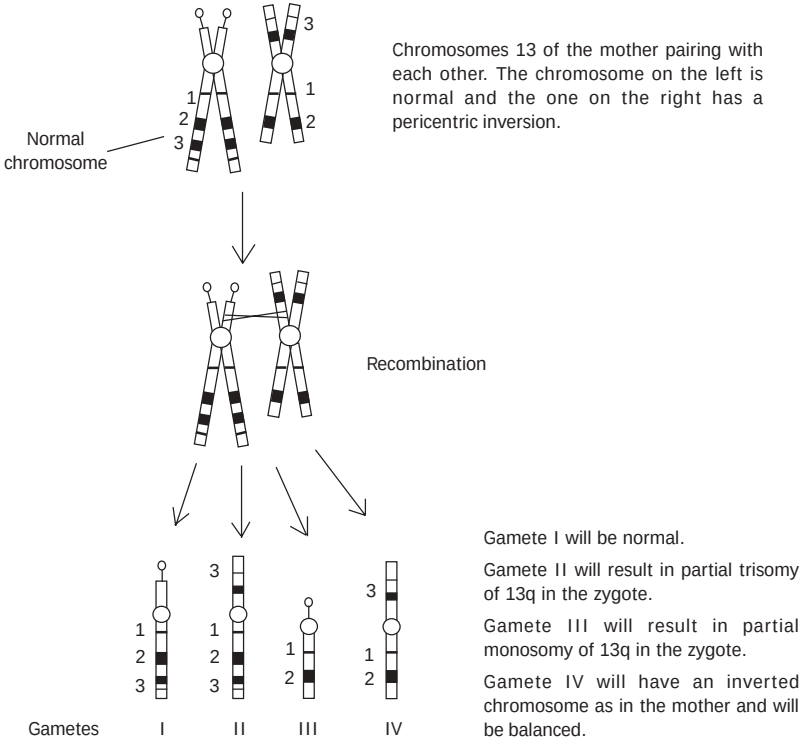
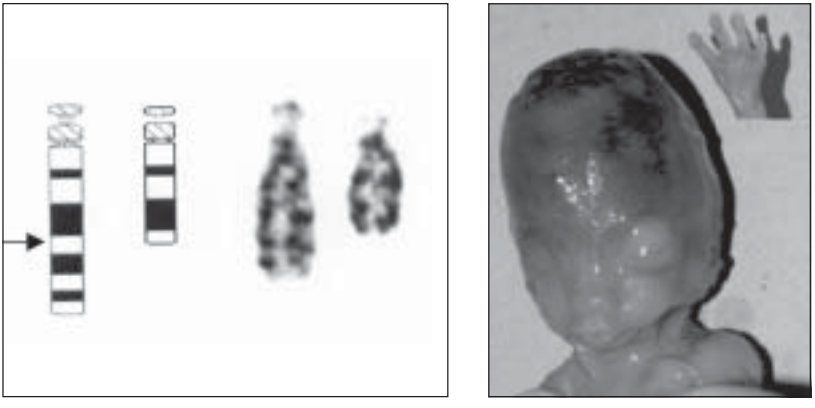


Fig. 8.3 Possible gametes due to recombination during meiosis of a carrier of pericentric inversion.

during the next pregnancy, the karyotype, analysed in the cells of the amniotic fluid, showed partial monosomy of chromosome 13 (13q) (Fig. 8.4a) and the foetus after termination showed malformation consistent with 13q monosomy (Fig. 8.4b).

In cases where the structural chromosomal anomaly arises *de novo*, i.e. karyotypes of the parents are normal, the risk of giving birth to another child with a chromosomal abnormality is considered to be less than 1%. However, most parents would like to be reassured by undergoing prenatal karyotyping in the subsequent pregnancies. The presence of malformation or facial dysmorphism with mental retardation is an indication for karyotyping. However, in some cases, chromosomal anomaly may not be associated with notable dysmorphism (Fig. 8.5). Hence, chromosomal analysis should be done in all cases with mental retardation without an obvious cause.

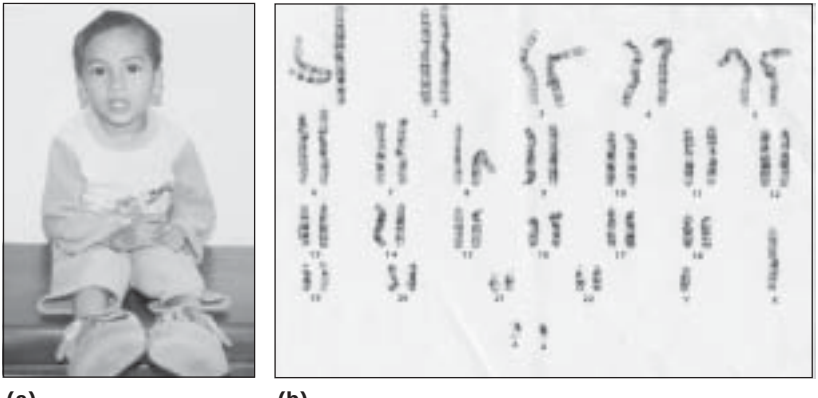


(a) (b)

Fig. 8.4 a Partial karyotype showing deletion of 13q. The chromosome with deletion is on the right side. **b** Foetus with deletion of 13q. Note the microphthalmia in one eye, micrognathia and oligodactyly (inset).

RECURRENT SPONTANEOUS ABORTIONS

Although the frequency of chromosomal abnormalities in newborns is 0.6%, it has been estimated that the prevalence of chromosomal abnormalities at conception is much higher. Most conceptuses having chromosomal abnormalities are lost as spontaneous abortions, while some are lost even before the pregnancy is clinically detected. Some die *in utero* (intrauterine foetal death) or are stillborn. Chromosomal



(a) (b)

Fig. 8.5 a A child with growth and developmental delay. There is no dysmorphism. **b** Karyotype of the child in Figure 8.5a showing two marker chromosomes. (A)

abnormalities account for approximately 50% of first-trimester abortions. The frequency of chromosomal anomalies in stillbirths is 5%–10%. Most of these chromosomal abnormalities are aneuploidies and hence do not occur in subsequent pregnancies. Though karyotyping of the products of conception after spontaneous abortion can provide information about the aetiology in a considerable number of cases, it is not essential in each case because the risk of recurrence of most of these abnormalities is low in subsequent pregnancies.

On the contrary, if there are recurrent (three or more consecutive) spontaneous abortions, chromosomal analysis of the couple is indicated. In about 3%–5% of these couples, one of the partners is a carrier of a balanced chromosomal rearrangement. The commonly seen chromosomal anomalies in these couples are balanced translocations (Fig. 8.6). Chromosomal inversions (pericentric and paracentric) are detected in a few of them. Recombinations due to inversions and abnormal segregation due to balanced translocations can lead to the formation of gameteswith trisomies and monosomies. Such imbalanced gametes may lead to non-viable conceptuses or malformed foetuses (*see also* Chapter 7). Common variants namely, inv (9), inv (Y) and long Y are not associated with recurrent abortions.

The risk of spontaneous abortion in translocation carriers is 20%–30%, and varies depending on the chromosomes involved and the breakpoints. The risk of giving birth to an abnormal child with chromosomal imbalance is 4%–6%. The risk is more if the balanced carrier is identified through the birth of a malformed child with a

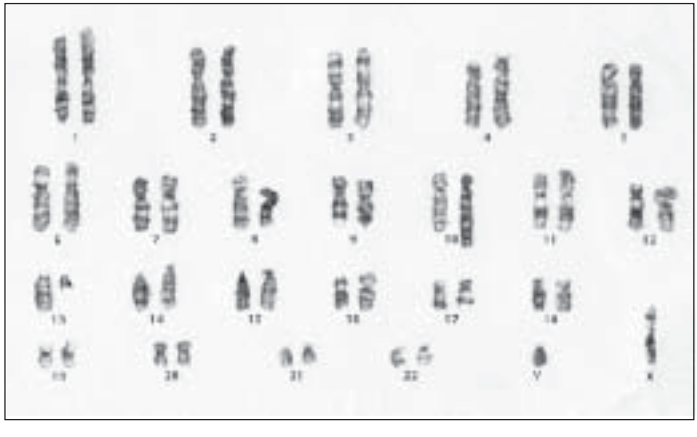


Fig. 8.6 A karyotype showing balanced translocation between chromosomes 10 and 13.

chromosomal abnormality. It means that a translocation in such a family can give rise to unbalanced zygotes that are able to survive beyond foetal life and lead to the birth of a child with an unbalanced karyotype, and malformations and mental retardation. Rearrangements in which both the translocated and the centromeric segments are large are unlikely to result in a viable child with chromosomal abnormality.

A carrier of a pericentric chromosomal inversion has a 40%–50% risk of abortion and 5%–10% risk of giving birth to a liveborn with chromosomal abnormality. For families identified by means other than through the birth of an abnormal child, the overall risk of giving birth to a child with a chromosomal abnormality is about 1%. The individual risk depends on the type of inversion. A carrier of an inversion associated with a large inverted segment and a small non-inverted segment is more likely to be at risk for giving birth to a malformed liveborn. If there is crossover in the case of paracentric inversion, it almost always results in non-viable gametes and the risk of giving birth to an abnormal offspring is extremely low.

The risk of birth of a malformed child also depends on the sex of the carrier. The risk is more when the female partner is a carrier of the chromosomal rearrangement. In some male carriers of translocation, there may be spermatogenic arrest that leads to infertility. Balanced chromosomal rearrangements rarely cause infertility in carrier females.

Genetic counselling for a carrier of a balanced rearrangement

It is important to inform the family that identification of the chromosomal abnormality in a partner while investigating for recurrent foetal loss may provide a cause that cannot be treated. However, it may also provide the hope of a normal liveborn child. Identification of a balanced chromosomal rearrangement in a couple indicates the need of explaining the result and its implications. The risk of abortion, a liveborn child with chromosomal imbalance [given above] and the chance of normal liveborn child should be told to the couple.

If the chromosomal imbalance is small, the foetus may be viable and result in the birth of a malformed child. The family should be informed about the risk of birth of a malformed child with a chromosomal imbalance, and they should be offered prenatal chromosomal analysis by amniocentesis, if the pregnancy continues beyond the first trimester. Families who have had the experience of multiple spontaneous abortions may not like to take even the minimum risk of abortion following

amniocentesis; they may prefer to avoid it and take the risk of having a malformed liveborn. In this situation normal ultrasonographic examination of the fetus decreases the risk of fetus with chromosomal abnormality.

Though the empiric data of live birth in balanced carriers create hope in their minds, one should not be too enthusiastic about a positive outcome while counselling, as some of the balanced rearrangements are more prone to segregation in a fashion that leads to the production of imbalanced gametes and repeated foetal losses.

The siblings of carriers of a balanced rearrangement should be offered karyotyping and genetic counselling before pregnancy.

Karyotyping of the products of conception

If there are three or more recurrent miscarriages, karyotyping of the products of conception may be carried out. The structural abnormality in the products of conception can give an idea about the possible chromosomal rearrangement in one of the parents. However, it should be noted that for identification of a chromosomal anomaly as a cause of recurrent spontaneous abortions, karyotyping of the couple is more important than that of the products of conception. If karyotyping of products of conception shows an imbalanced chromosomal abnormality involving the same chromosome as that in the balanced carrier partner, it proves that the chromosomal abnormality in the couple is likely to be the cause of recurrent spontaneous abortions or foetal losses. However, absence of a structural chromosomal imbalance in the products of conception does not rule out the possibility of a balanced rearrangement in the chromosomes of one of the partners.

PRENATAL CYTOGENETIC DIAGNOSIS

Since the early 1970s, prenatal diagnosis of chromosomal disorders has been carried out by culturing amniotic fluid cells obtained by amniocentesis at about 16 weeks of pregnancy. Chorionic villi or foetal blood cells can also be used for prenatal diagnosis. The details of the sampling procedures are given in Chapter 19. Rapid prenatal diagnosis of common chromosomal aneuploidies is possible by fluorescence *in situ* hybridization (FISH; *see* Chapter 17) on uncultured cells obtained from the amniotic fluid.

Indications for prenatal cytogenetic analysis

The indications for prenatal cytogenetic analysis are listed below:

1. A positive screening test (triple or quadruple test) on the maternal blood: Earlier, advanced maternal age was the commonest indication for prenatal cytogenetic analysis. However, now the commonest indication is an increased risk for Down syndrome detected by a screening test. With the availability of a screening test for Down syndrome (triple test), women of all ages are offered this or some other screening test, and amniocentesis is performed only if the screening test is positive (*see* Chapter 19).
2. A family with a child with Down syndrome or any other chromosomal anomaly
3. One of the parents is a carrier of a balanced chromosomal rearrangement
4. A family history of an X-linked recessive disorder for which a definitive DNA-based diagnosis is not available
5. Ultrasonographic diagnosis of foetal malformations: The possibility of chromosomal anomaly in a foetus with any single malformation is 5%–10%. If two or more malformations are detected, then the risk increases to 20% or more. Malformations more likely to be associated with chromosomal anomalies are cystic hygroma (45,X), duodenal atresia (Down syndrome), holoprosencephaly (trisomy 13), omphalocele and cardiac malformations.
6. Ultrasonographic abnormalities other than malformations: There are some ultrasonographic findings which may be present in normal foetuses also, but if present indicate an increased risk for chromosomal anomalies. The most important of these is increased nuchal translucency—more than 2.5 mm in the first trimester and 5 mm after that. The risk of a chromosomal anomaly in the presence of echogenic foci in the heart or an echogenic bowel is 1%. Other findings such as renal pyelectasis and cyst of the choroid plexus, if present in isolation, do not indicate the need for amniocentesis for karyotyping. But careful ultrasonography should be done to look for other malformations and counselling should be provided.
7. Prenatal diagnosis of non-chromosomal disorders: If chorionic villus sampling (CVS) or amniocentesis is being done for prenatal diagnosis of single-gene disorders by a DNA test, or of a metabolic disorder by a biochemical test, then the family needs to be offered prenatal cytogenetic diagnosis if the sample is adequate, i.e. chromosomal analysis can also be done on the sample in addition to the test for which the sample is primarily collected.
8. A relative, other than first degree, with a chromosomal anomaly or a child with a malformation: These are indications of a debatable

nature. Prenatal cytogenetic analysis in these situations is mainly to allay the anxiety of the family. It should be clarified to the family that if chromosomal analysis of a previous malformed child or stillborn was not done, then a normal amniotic fluid karyotype in the next pregnancy does not rule out other non-chromosomal causes of malformation or stillbirth.

PROBLEMS IN PRENATAL CHROMOSOMAL ANALYSIS

Prenatal cytogenetic analysis is performed to obtain a clear answer to the family's doubt about the possibility of occurrence of a chromosomal anomaly in the foetus. However, the presence of mosaicism or detection of an unexpected chromosomal abnormality may create new uncertainties rather than clarifying the situation. Maternal cell contamination and culture failure are other problems uncommonly encountered in prenatal cytogenetics.

Mosaicism

Mosaicism refers to the presence of two or more cell lines in an individual or a tissue sample. When mosaicism is found in cultured foetal cells, there may be problems in deciding whether the foetus is truly mosaic and whether the observation is of clinical significance.

A cell line with abnormal chromosomes may exist only in the extraembryonic tissues (chorion and amnion) and the embryo may be normal. This is known as confined placental mosaicism (CPM). This condition is more likely to be encountered during CVS than during amniocentesis. It is seen in 1% of chorionic villus samples, while true foetal mosaicism is seen in only 0.15% of the samples.

Cytogeneticists distinguish three levels of mosaicism in amniotic fluid or CVS cell cultures.

Level I: A single cell with a chromosomal abnormality. This is commonly seen in 2%–7% of amniotic fluid cultures. It can be disregarded if analysis of an adequate number of cells (20 or more) does not show the presence of a similar abnormality in any other cell.

Level II: Mosaicism involving two or more cells in a single primary culture only and not seen in slides prepared from other culture flasks. This is seen in 0.5%–1% of amniotic fluid cultures and 1%–2% of CVS cultures. It is difficult to interpret whether the mosaicism is true or has arisen in the culture. Level II mosaicism is almost always pseudomosaicism.

Level III: This is true mosaicism, i.e. present in the cells from which

the culture was derived. Abnormal cells are seen in two or more primary cultures. True mosaicism is seen in 0.1%–0.3% of amniotic fluid cultures and in about 1% of CVS cultures. Postnatal studies have confirmed that level III mosaicism in cultures is associated with about 20% risk of mosaicism in the foetus.

Mosaicism, especially that seen in CVS cultures, is mostly limited to some parts of the placenta (CPM) and may not reflect the foetal karyotype. The probability of the presence of a chromosomal abnormality in the foetus varies from case to case and depends on the chromosome involved. Interpreting mosaicism is difficult and conveying uncertainties in the prognosis is a challenging task for the counsellor. Further studies on another sample (amniocentesis, if CVS was done), careful ultrasonography to look for foetal malformation, data from the literature about the outcomes of mosaicism for the chromosome involved, can guide the counsellor to some extent. A normal study on a repeat sample and a normal ultrasonographic report indicate a low risk of phenotypic abnormality. However, definite prenatal confirmation of the presence or absence of mosaicism in the foetus is impossible. In the case of trisomy 20 and trisomy 12 mosaicism, the trisomic cell line is usually limited to the placenta. However, in some cases, foetuses with phenotypic abnormalities are reported.

The presence of a trisomic cell line in CVS cultures and a disomic cell line in the foetus indicates the possibility of trisomy rescue. This means that the extra chromosome was lost postzygotically, which allowed foetal viability. However, this situation raises the possibility that this 'rescue' might have been the result of uniparental disomy. Uniparental disomy of chromosome 15 leads to Angelman or Prader–Willi syndrome. Hence, if trisomy 15 is seen in the CVS culture and the amniotic fluid karyotype is normal, then molecular studies for uniparental disomy of chromosome 15 should be carried out.

Detection of unexpected chromosomal anomalies

Even if prenatal cytogenetic analysis is done for a high risk of Down syndrome, not only chromosome 21 but all the 23 pairs of chromosomes will be studied. Thus, in some cases, chromosomal anomalies of other chromosomes will also be detected. Some of these abnormalities, such as unbalanced numerical or structural abnormalities of autosomes, are associated with foetal malformations or other significant phenotypic abnormalities. However, there are other anomalies that may not have a poor outcome or wherein prediction of the outcome may not be possible.

Detection of such chromosomal anomalies poses a dilemma for the family and is a challenge for the counsellor. Some such chromosomal anomalies and counselling for these are discussed below.

Anomalies of the sex chromosomes

Sex chromosomal abnormalities such as 45,X and 47,XXY are associated with hypogonadism and infertility. Females with the 45,X karyotype can, in addition to hypogonadism, have a short stature, cardiac and renal malformations, and other phenotypic features of Turner syndrome. Both these chromosomal anomalies are not associated with mental retardation. Those with other sex chromosomal anomalies such as 47,XXX and 47,XYY are known to have a normal phenotype and reproductive life. Many of these are not identified during their lifetime. Prenatal detection may create unnecessary anxiety in the family. Other sex chromosomal abnormalities of uncertain prognosis are mosaicisms such as 45,X/46,XY; 45,X/46,XX; 46,XY/47,XXY. These individuals may have normal sexual development but the prognosis cannot be definitely predicted prenatally. Karyotype 45,X/46,XY may be associated with the Turner phenotype, ambiguous genitalia and may as well be seen in normal males. Postnatally detected cases with these chromosomal abnormalities are usually associated with phenotypic abnormalities. Hence, for the counselling of prenatally detected cases, data of outcome (along with follow up till puberty and adult life) of prenatally detected cases is necessary. At present, there is a paucity of such data. XY/XX is usually pseudomosaicism due to the growth of maternal cells. Ultrasonography for the morphology of the foetal external genitalia is helpful in deciding whether the XY/XX karyotype is likely to be due to maternal cell contamination.

Extra structurally abnormal (supernumerary marker) chromosome

Extra structurally abnormal chromosomes (ESACs) are marker chromosomes and are prevalent in about 1 in 2500 and frequent in mosaic forms with a normal cell line. If an ESAC is detected, urgent karyotyping of the parents is required. If one of the parents who is phenotypically normal also has a similar marker chromosome, the foetus can be expected to have a normal phenotype. However, if the parents' karyotypes are normal, further analysis of the marker chromosome is necessary. This includes the use of techniques such as C-banding and FISH (*see* Chapter 17) to know the origin of the marker and an ultrasound examination to look for malformations. The presence of a malformation

in the foetus, an euchromatic region (not stained by C-banding) and an ESAC originating from chromosome 18 or 12 indicate phenotypic abnormalities in the foetus. If the parents have normal karyotypes, the risk of abnormal phenotype in a fetus with an ESAC is 13%–15%.

Balanced structural rearrangement

The significance of such a prenatally detected finding cannot be assessed unless the karyotypes of the parents are studied. The presence of a similar, apparently balanced, rearrangement in a phenotypically normal parent indicates a possibly normal outcome for the foetus. However, a small (< 1%) risk of phenotypic abnormality may be present because of the possibility of a defect that is beyond the resolution of cytogenetics. A major difficulty is posed by an apparently balanced *de novo* (i.e. parents' karyotypes are normal) rearrangement. As the possibility of submicroscopic deletion, duplication or gene disruption at the breakpoints of rearrangement cannot be ruled out, there is some risk of an abnormal phenotype. An ultrasound examination may be partly reassuring but does not ensure a normal outcome. Available data show that there is a 6%–10% risk of phenotypic abnormalities in such cases of prenatally detected *de novo* balanced rearrangements. The risk is minimum (< 1%) in *de novo* Robertsonian translocation.

Polyploidy

Almost all cases of triploidy abort spontaneously. Tetraploidy is commonly seen in amniotic fluid cells and almost always arises in cultures. Hence, it can be ignored. Only ultrasound needs to be carried out to ensure that there is no growth retardation or malformation.

Maternal cell contamination

This can be identified with certainty only in male foetuses. It occurs in 0.5% of amniocentesis samples. Adequate dissection of the CVS and discarding the first 2 ml of amniotic fluid are useful ways of minimizing maternal cell contamination.

Culture failure

This is a rare but real event in most laboratories. Technical causes of culture failure can be minimized by processing a sample in duplicate, i.e. use of two sets of culture media, incubators, etc. Repeat sampling will be needed. It is important to clarify to the family that culture failure does not indicate an increased risk of foetal abnormality.

PRETEST AND POST-TEST COUNSELLING

Prenatal diagnosis should not be done without adequate pretest counselling. Pretest counselling should include informing the family about the technique, risks of sampling, reporting time and accuracy of the results. The accuracy of traditional prenatal cytogenetic analysis is >99.5%. The possibility of detection of chromosomal abnormalities other than the one for which prenatal diagnosis is being done should be mentioned before carrying out the prenatal diagnosis. While giving the report, the interpretation and implications of the report along with the error rate should be explained. The most important point is to get follow-up data of cases with normal and abnormal reports for laboratory audit as well as to generate data about the outcome of prenatally detected chromosomal abnormalities.

9 Genetic susceptibility to disease

Common disorders such as hypertension, psychiatric diseases, diabetes mellitus, congenital malformations, etc. have genetic components in their aetiology. These disorders result from complex interactions of various alleles at a number of loci and a variety of environmental factors. These disorders do not follow the simple Mendelian patterns of inheritance, but are known to occur at a higher frequency among the relatives of a patient as compared to the general population (Table 9.1). Identification of genes that increase the susceptibility to common disorders will modify the practice of medicine considerably as these disorders account for the majority of illnesses and deaths in humans. Research about the genetic basis of common disorders can lead to preventive strategies by identifying high-risk individuals and offering them prophylactic drug therapy or suggesting a change in lifestyle. Research will also lead to new drugs based on better understanding of the aetiopathogenesis and gene therapy.

Table 9.1 Characteristics of diseases inherited in a multifactorial pattern

- Diseases inherited in a multifactorial pattern occur more frequently among relatives of an affected person than in the general population.
- The risk of recurrence of the disease is more in first-degree relatives. The risk decreases as the relationship with the affected person becomes distant.
- If the affected person has a severe form of a disease then the risk of recurrence in his or her relatives is greater.
- If the affected person is of the sex that is known to be less commonly affected, the risk of recurrence of the disease in his/her relatives is greater.

GENETIC BASIS OF COMMON DISORDERS

A multifactorial pattern of inheritance is responsible for 'quantitative traits' or 'continuous traits' such as height, intelligence, blood pressure, blood sugar level, etc. These traits are measurable and also have a continuous distribution in the population. The inheritance of these multifactorial traits is controlled by the action of a number of genes and is also influenced by environmental factors. Unlike quantitative traits, traits of a genetic disorder are either present or absent and are referred to as 'discrete' or 'qualitative traits'. The multifactorial inheritance of qualitative traits is explained by postulating that these traits are controlled by the actions of genes and are also influenced by environmental factors. Disease occurs when the combined genetic and environmental liability exceeds a certain threshold. Studies are being carried out to identify the genetic factors responsible for common disorders using advanced molecular techniques and statistical methodologies.

GENETIC ANALYSIS OF COMMON DISORDERS

Multifactorial disorders cluster in families and thus indicate a genetic aetiology. However, family members also share a common environment. Hence, it is essential to analyse whether familial aggregation of a disease is an effect of the environment or has some common genetic factors and to dissect out the genetic contribution in the aetiology.

The following studies are carried out to prove that there is true familial aggregation:

1. Study of the relative risk: The relative risk is the ratio of prevalence of a disease in the relatives of an affected person to the prevalence of the disease in the general population. For example, the relative risk of schizophrenia in the siblings of a patient with schizophrenia is 12 and the relative risk of Crohn disease in the relatives of a patient with Crohn disease is 25.
2. Case–control studies: A patient with a disease is compared with a matched control without the disease, with respect to the family history of the disease. An increased prevalence of the disease in the relatives of the patient compared with the controls suggests familial aggregation.
3. Studies to dissect out the relative contribution of genes and the environment to complex disease traits: Twin studies provide important evidence for a genetic aetiology. Monozygotic twins have identical genomes (i.e. the alleles at all the loci are exactly similar)

while dizygotic twins and other siblings share 50% of their genes. Twins have an identical intrauterine environment. If both the twins have the same disease they are said to be 'concordant' while if one of them has a disease and the other does not, the twins are said to be 'discordant' for the disease. Thus, in case of genetic disorders, concordance increases as the degree of relatedness increases. Increased concordance rates for a disease in monozygotic twins compared with that in dizygotic twins indicate an important genetic component in the aetiology. A disease concordance rate of less than 100% in monozygotic twins is strong evidence that non-genetic factors play a role in the disease. The concordance rates in monozygotic and dizygotic twins for schizophrenia are 53% and 15%, respectively. For cleft lip the values are 30% and 5%. The study of monozygotic and dizygotic twins reared together or apart is also a useful way to measure the heritable component of the complex trait.

GENETIC MAPPING OF COMPLEX TRAITS

Studies are under way to identify genes involved in diseases in which the genetic component plays a major role. This task involves many difficulties. At present, the number of genes involved in a complex trait such as schizophrenia or ischaemic heart disease is not known. Also, genes contributing to complex traits may differ in different families and populations. Variations in these genes, i.e. polymorphisms, are present in many unaffected individuals as well.

Identifying genes in complex disorders

The various strategies to identify genes involved in complex disorders are discussed below.

Affected sib pair method

This method uses siblings concordant for the disease. Unaffected siblings are not used. This eliminates the problem of determining whether unaffected individuals are non-penetrant carriers of the alleles that predispose them to the disease or have not inherited the allele. In siblings, 50% of the genome is identical. If the study of an affected sibling pair shows that some loci or regions of the genome are shared more frequently by affected sibling pairs (i.e. more than the 50% expected by chance), then these loci or regions are likely to harbour the predisposing genes. This approach has not provided promising results as it cannot

identify the loci that make minor contributions to the disease. Theoretical calculations have shown that if the contribution of an allele is small, then many pairs of siblings need to be studied.

Association studies

This is a form of case–control study in which the frequency of occurrence of a particular allele at a locus in affected and unaffected individuals in a population is compared. The measure of association is the relative risk, which compares the risk of developing a disease when one carries a specific allele relative to the risk if one does not carry the allele. These studies are easy to carry out but association does not prove the role of the locus or allele in the causation of the disease. To minimize errors, it is necessary that the control be ethnically matched because the frequency of alleles varies in different populations.

Studies on candidate genes

Knowledge of the normal pathophysiology of a disease can suggest possible candidate genes. Hence, instead of searching the whole genome, studies focused on the candidate genes can be started. Examples are human leucocyte antigen *(HLA)* genes in type I diabetes mellitus, and angiotensin-converting enzyme (ACE) and angiotensin II receptor genes in hypertension. In other situations, mapping studies identify a region of the genome within which the locus for disease susceptibility is located and the genes in that region can be scrutinized to identify a possible candidate gene. The apolipoprotein E (apoE) locus as a cause of susceptibility to Alzheimer disease was identified in this way.

Large-scale population surveys based on single nucleotide polymorphism maps

Single nucleotide polymorphisms (SNPs) are variations of single base pairs spread all over the genome. These are observed in normal people and do not directly cause a disease. With the completion of the Human Genome Project, the locations of these SNPs are known. These can be studied in affected individuals to identify a common haplotype. The common haplotype indicates a commonly inherited chromosomal region that is likely to harbour the susceptibility gene/s.

COMMON MULTIFACTORIAL DISORDERS

Multifactorial disorders include childhood disorders and congenital malformations such as cleft lip with or without cleft palate, cardiac malformations, Hirschsprung disease, pyloric stenosis, etc. and adult-

onset disorders such as hypertension, diabetes mellitus, psychiatric diseases and ischaemic heart disease. Familial aggregation studies, relative risk ratios and twin studies have provided evidence of the genetic component in the aetiology of these disorders and have also quantified heritability. However, little is known about the exact number of loci involved and the causative alleles. Some complex disorders are discussed below with an aim to understand their multifactorial nature.

Cerebral venous thrombosis

If cerebral venous thrombosis occurs without any predisposing factor such as tumour or infection, the cause could be genes interacting with some environmental factor. The identified genetic factors are Factor V Leiden gene (mutant allele of clotting Factor V) and G to A mutation at position 20210 in the prothrombin gene. The risk of venous thrombosis increases 7 times and 80 times in heterozygotes and homozygotes for the Factor V Leiden allele, respectively. A mutation in the prothrombin gene raises the risk of venous thrombosis three- to six-fold. The environmental factor associated with an increased risk of thrombosis is the use of oral contraceptives. Presence of two or more factors increases the risk for a hypercoagulable state and life-threatening cerebral venous thrombosis.

Hirschsprung disease

This is characterized by the presence of an aganglionic segment in the colon which lacks peristalsis. This leads to constipation, symptoms of intestinal obstruction and massive dilatation of the colon proximal to the aganglionic segment. The length of the segment involved is usually small, but can vary. Hirschsprung disease occurs as an isolated defect but can also occur as a part of the type III Waardenburg syndrome characterized by deafness and patchy hypopigmentation. Linkage analysis of familial cases and mutation analysis of candidate genes have identified five genes, namely, the receptor tyrosine kinase (*RET)* gene, genes for glial cell line-derived neurotrophic factor (*gdnf*), endothelin B receptor (*EDNRB*), endothelin 3 (*EDN3)* and *SOX 10* gene. Affected families have mutations in any of these five genes. *RET* gene mutations are most commonly seen in Hirschsprung disease. Glial cell line-derived neurotrophic factor is a ligand that binds to the *RET* gene. EDNRB is the receptor for the *EDN3* gene-encoded ligand. The mechanism of action of these genes in the pathogenesis of Hirschsprung disease is not understood.

An important point to note is that in familial Hirschsprung disease

associated with *RET* gene mutations, there are normal individuals with the *RET* mutation. This indicates that the penetrance of *RET* mutations is not requirement for Hirchsprung disease. *RET* alleles confer susceptibility to Hirschsprung disease. The susceptibility increases if the individual has a mutation in the *gdnf* gene and other unidentified alleles at 19q31. The phenotype due to mutation in the *EDNRB* gene is also modified by the allele at *RET*. Thus, molecular data support the multifactorial nature of Hirschsprung disease, where the phenotype is the additive effect of susceptibility alleles at various loci.

Diabetes mellitus

Diabetes mellitus is a heterogeneous group of disorders. Some of the types are known to be inherited in a monogenic fashion. Though monogenic syndromes account for a small percentage of cases of diabetes, the genes involved in such cases may provide information, which can be applied to the whole spectrum of the disease.

Maturity-onset diabetes of the young (MODY) is non-insulin dependent diabetes mellitus (NIDDM) characterized by early onset and an autosomal dominant pattern of inheritance. Five loci are identified for MODY—MODY 1 (hepatocyte nuclear factor-4-α gene), MODY 2 (glucokinase gene), MODY 3 (hepatic transcription factor-1 gene), MODY 4 (insulin promotor factor gene) and MODY 5 (hepatic transcription factor-2 gene).

Insulin-dependent diabetes mellitus (IDDM) or diabetes type I is known to be associated with other autoimmune disorders such as thyroiditis, Addison disease and pernicious anaemia. This suggests the role of HLAs. Ninety-five per cent of persons with IDDM have DR3 and/or DR4 HLA. Siblings affected with IDDM have a similar HLA haplotype more commonly than would occur by chance. An individual with DR3 or DR4 HLA has a slightly increased risk of developing IDDM. Studies have shown that the HLA locus accounts for 30%–40% of the genetic predisposition to IDDM. Genomewide search has shown the possible involvement of at least 13 additional loci for susceptibility to IDDM. Viral infections are thought to be the triggering environmental event. Genetic susceptibility to long-term complications of diabetes on the retina and kidneys is also being investigated.

Hypertension

As in diabetes mellitus, a small percentage of cases with hypertension are due to single-gene mutations and are inherited in an autosomal dominant

fashion. These include Liddle syndrome, which is associated with mutations in the beta subunits of the epithelial sodium channel. Glucocorticoid-suppressible aldosteronism is another cause of early-onset hypertension associated with a variable degree of hyperaldosteronism and is inherited in an autosomal dominant manner. It is caused by fusion of the regulatory region and the first exons of the 11 β-hydroxylase gene and coding sequence of the aldosterone synthase gene. The gene for 11 β-hydroxylase is under the control of adrenocorticotrophic hormone (ACTH). As the regulator for 11 β-hydroxylase is fused with aldosterone synthase, the former controls the expression of the latter. Hence, exogenous glucocorticoid suppresses ACTH secretion which in turn downregulates the expression of aldosterone synthase and controls hypertension.

In most cases of hypertension, environmental factors such as the level of sodium in the diet, alcohol intake, exercise and obesity are probably of major importance. Various polymorphisms in genes for renin, angiotensinogen, ACE, angiotensin II receptor have been studied to identify the genetic susceptibility to hypertension. These studies have not revealed any significant contribution of these genes to hypertension in humans.

Coronary artery disease

The familial nature of coronary artery disease is well recognized, as are the environmental factors such as obesity, lack of exercise, dietary cholesterol and smoking. Monogenically inherited hyper-lipidaemia and hypercholesterolaemia are important genetic components of the aetiology. Polymorphisms in apoE and ACEs are shown to be associated with early-onset coronary artery disease.

Congenital malformations

Many of the isolated congenital malformations are known to recur in families and follow the characteristics of diseases inherited in a multifactorial pattern. Studies to identify genes for cardiac malformations, neural tube defects and cleft lip have been carried out (*see* Chapter 11). Experiments in knockout animals help to understand the characteristics of developmental genes.

Counselling for multifactorial disorders

At present, there is hardly any definitive information about the susceptibility loci for these disorders. Counselling depends on the observed risks in the data collected from families with affected

individuals. These are empiric risk estimates. The data from many families are combined, though the genes and their relative contribution to the disease phenotype may vary from family to family.

In the future, with the availability of definitive information regarding environmental factors and susceptibility genes, the approach to management may change from treatment of the disease to preventive measures.

PHARMACOGENETICS

The susceptibility to many diseases is genetically determined and so are the responses to drug therapy. The study of genetic influences on an individual's response to drugs is known as pharmacogenetics. A similar study of an individual's response to environmental agents is known as ecogenetics. Drug reactions are a major cause of morbidity and mortality. Thus, genetic analysis done to assess an individual's risk for drug reaction and the optimum dose required is an important technique that ushers in a new era of clinical genetics. Haemolysis induced by various drugs in glucose-6-phosphate dehydrogenase (G6PD) deficiency and malignant hyperthermia induced by anaesthetic agents have been known for a long time. G6PD deficiency and malignant hyperthermia are inherited in an X-linked and autosomal dominant fashion, respectively. The genes for these disorders and causative mutations have been identified.

The other important example of pharmacogenetics is slow and rapid acetylators of isoniazid, a drug used in the treatment of tuberculosis. Isoniazid is metabolized by the liver enzyme N-acetyltransferase. Mutation in the *NAT1* and *NAT2* genes leads to variations in the activity of N-acetyltransferase in the liver. Persons who are homozygous for the low enzyme activity allele of *NAT* are slow inactivators of isoniazid and are at an increased risk for side-effects of isoniazid such as polyneuritis, liver damage or a systemic lupus erythematosus-like reaction. On the other hand, rapid acetylators metabolize the drug rapidly and thus require larger doses of the drug to maintain an adequate blood level. The other drugs metabolized by N-acetyltransferase are hydralazine and sulphasalazine.

Mechanisms of pharmacogenetic effects

The various steps of drug metabolism include absorption, distribution in the body tissues, interaction with cells, breakdown and excretion. These processes involve many enzymes and proteins in the body. These proteins do not primarily metabolize drugs, but also various food

substances and metabolites in the body. The genes coding for these enzymes are polymorphic, and are responsible for individual variations to drug responses. Identification of single-gene polymorphisms that make a person susceptible to adverse drug reactions can help in identifying at-risk individuals before starting them on treatment. Thus, many effective drugs with serious adverse effects can be safely brought into use. One such example is the detection of a mutation in the mitochondrial DNA.This mutation makes a person susceptible to the ototoxic effects of aminoglycosides.

For the drugs discussed above, the adverse effects are due to polymorphism in one of the genes involved in the metabolism of the drug. However, the metabolism of drugs involves many genes and is multifactorial.

PHARMACOGENOMICS

The use of information from the human genome sequence to develop new and safe drugs is known as pharmacogenomics. A number of SNPs, spread all over the genome, have been identified. One SNP is present in every 500 to 2000 base pairs. SNPs are present in a substantial number of normal persons and do not cause disease by themselves. If present in the regulatory element or coding region of a gene, SNPs modify gene expression or alter the function or activity of the gene product and may thus be responsible for variations in the response to a drug. Automated analysis of SNPs spread all over the genome can generate SNP profiles for an individual or individuals. A comparison of the SNP profiles of individuals with and without adverse reactions to the drugs will identify the genetic variations responsible for causing the drug reaction. SNP-based information can be used to individualize drug dosages to achieve an optimum and safe drug level in an individual.

THE FUTURE OF MEDICINE

The discovery of new genes and SNPs will lead to a better understanding of the interactions of drugs and environmental agents with the genetic constitution and their role in carcinogenesis, mutagenesis, teratogenesis and etiopathogenesis of autoimmune diseases.

The post-human genome project developments in pharmacogenomics and genetics of common multifactorial disorders are likely to bring in a new era of ‘individualized medicine’ where identification of at-risk individuals and ‘tailor-made’ drug therapy will be based on the genetic constitution of an individual.

10 Triplet repeat disorders

INTRODUCTION

Trinucleotide repeats are involved in the causation of several neurological diseases. In contrast to other common types of mutations which are stably transmitted, triplet repeat mutations are highly unstable and undergo expansion as the gene is transmitted from one generation to the next. The mechanism by which triplet repeats expand is not clearly known. The number of trinucleotide repeats also varies from tissue to tissue in an affected person. Due to the unstable nature of these mutations, they are known as 'dynamic' mutations.

Trinucleotide repeat disorders are either dominantly inherited or X-linked, except Friedreich ataxia, which is inherited in an autosomal recessive fashion. The parental origin of the disease-causing allele is seen to influence age of onset and the severity of the disorder. In fragile X mental retardation and myotonic dystrophy, the number of trinucleotide repeats increases when the mutation is transmitted through the mother. On the other hand, in Huntington chorea and other triplet repeat disorders such as spinocerebellar ataxias, there is a greater risk of expansion and earlier age of onset of symptoms when the transmitting parent is the father.

LOCATION OF THE TRINUCLEOTIDE REPEAT SEQUENCES

The number and location of trinucleotide repeat sequences vary from disease to disease. The repeats may be present in either the non-coding or coding region of the gene. In many of the disorders caused by triplet repeats in the non-coding region, there is a group of patients in whom the number of repeats is between the normal and full mutation range. Such

Table 10.1 Common triplet repeat disorders and their characteristics

Triplet repeat disorder	Gene and its location	Trinucleotide repeat	Normal size of the repeats (bp)	Type of mutation		Location of the trinucleotide repeat
				Pre-mutation (bp)	Full mutation (bp)	
Fragile X syndrome	*FMR1* Xq27.3	CGG	6–52	50–200	>200	5' UTR
Fragile XE syndrome	*FMR2* Xq28	GCC	6–35	61–200	>200	5' UTR
Friedreich ataxia	*FRDA* 9q13–21.1	GAA	7–34	34–80	>100	Intron 1
Myotonic dystrophy	*DMPK* 19q13	CTG	5–37		50 to thousands	3' UTR
Huntington disease	*HD* 4p16.3	CAG	6–35	—	36–121	Coding
Spinobulbar muscular atrophy	*AR* Xq13–21	CAG	9–36	—	36–82	Coding
Spinocerebellar ataxia type 3	*SCA3* 14q32.1	CAG	12–40	—	55–84	Coding

UTR: untranslated region

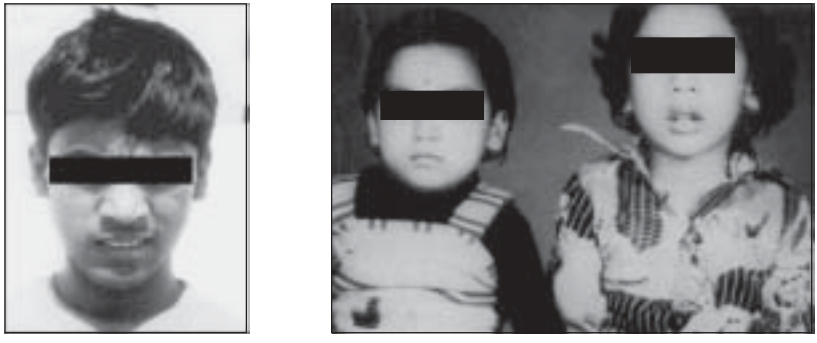
allels are known as premutations and are seen in non-penetrant (non-manifesting) carriers of triplet repeat disorders. Premutation carriers were supposed to be clinically asymptomatic, which may not be entirely true as fragile X premutation carriers are known to have premature ovarian failure and late-onset neurodegenerative disorders. Some common triplet repeat disorders and their characteristics are given in Table 10.1.

Three triplets repeat disorders are described below to illustrate the various peculiarities of this group of disorders.

FRAGILE X SYNDROME

Prevalence

The prevalence of fragile X syndrome in the general population is 1 in 4000 males. The syndrome accounts for 1%–5% of males with mental retardation in whom an obvious cause cannot be found. Clinical diagnosis is not possible and all children with mental retardation without



(a) (b)

Fig. 10.1 Fragile X syndrome. **a** Long face with a prominent mandible in a patient with fragile X syndrome. **b** The same patient in Figure 10.1a in childhood (right), with his younger brother (left) who is also affected with fragile X syndrome. Note that their faces are normal, and there is no dysmorphism.

any obvious cause should be tested for the fragile X syndrome.

Pattern of inheritance

Fragile X syndrome is a common cause of mental retardation, which is inherited in an X-linked semi-dominant fashion. One-third of carrier females show some manifestations. Females are less severely affected than males and some carrier females may be clinically normal. Affected males usually have moderate mental retardation.

Clinical features

The facial dysmorphism is subtle, but becomes more appreciable as the child grows (Figs 10.1a and b). The phenotype includes a long face and ears, prominent lower jaw, hyperactivity, perseverative speech, echolalia, poor eye contact and stereotypic hand movements. Some of these patients have autistic features. Cases with a phenotype similar to Prader–Willi syndrome have also been described. Macro-orchidism (testicular volume >30 ml) is a characteristic feature in post-pubertal boys. However, some affected males may have normal-sized testes, and not all males with macro-orchidism have fragile X syndrome.

Molecular genetics of fragile X syndrome

The name ‘fragile X’ comes from the cytogenetic abnormality observed

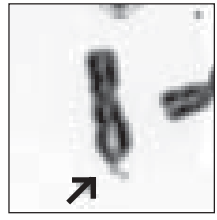
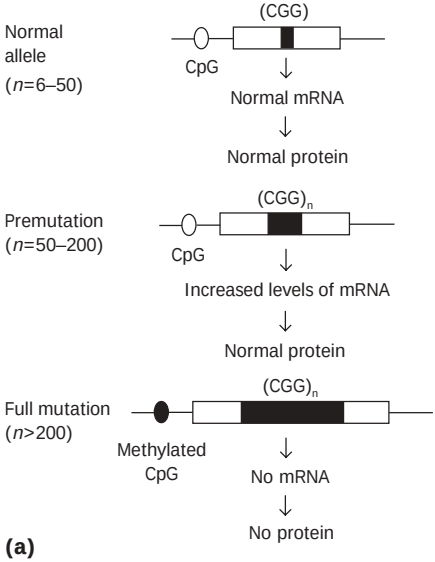


Fig. 10.2 Fragile X site at Xq27.3.

in persons with this syndrome. The cells of these patients when grown under conditions of folic acid deficiency show a gap at the terminal end of the 'q' arm of the X chromosome (Xq 27.3; Fig. 10.2). This is known as the fragile X A (FRAXA) site. Fragile X is expressed in only 10%–40% of cells of an affected male and less frequently in carrier females. Premutation carriers do not express fragile X. This is a phenotypic expression of mutation in the *FMR1* gene and thus fragile X syndrome is not a chromosomal but a single-gene disorder. Cytogenetic abnormality of the fragile X chromosome can be used as a diagnostic test but there are some chances of false-negative (due to the improper culture conditions) and false-positive results (due to the presence of other fragile sites distal to FRAXA such as FRAXE and FRAXF).

Fragile X syndrome is caused by the expansion of normally occurring trinucleotide repeats in the 5' untranslated region of the



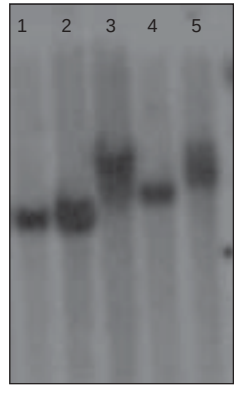


Fig. 10.3 a Normal allele, premutation and full mutation of the *FMR1* gene showing gradual increase in the size of CGG repeats. **b** Southern blot analysis of the *FMR1* gene in normal persons and patients with fragile X syndrome using EcoRI digestion enzyme and probe pP_2. Lanes 1 and 2 are normal and lanes 3, 4 and 5 show bands of larger size fragments due to expansion of CGG repeats in cases with fragile X mental retardation. (Courtesy: Dr B.K. Thelma, Department of Genetics, University of Delhi, South Campus)

FMR1 gene at Xq27.3. The number of CGG repeats in the *FMR1* gene in the normal population varies from 6 to 50 but when it is 50–200 (Figs 10.3a and b), it is a premutation. When the allele with a premutation is transmitted from the carrier mother to her offspring, the number of trinucleotide repeats further increases and, when it increases to more than 200 copies, there is hypermethylation of cytosines in the CpG island (*see* page 25) in the *FMR1* gene promoter region. As a result, the gene gets silenced, i.e. translation stops. The FMR1 protein is an RNA-binding protein and is expressed in foetal and adult tissues with high expression in the brain. Failure of synthesis of key proteins during synaptic development and maintenance may be the cause of mental retardation. However, the mechanism by which the repeats expand and why the expansion leads to methylation and silencing of the gene is not yet understood.

Mosaicism

Due to instability of the mutation, two types of mosaics are seen. In mutational mosaics, some cells of the body have the mutation while others have premutation. Those with mutational mosaicism may have a better intelligence quotient (IQ) than non-mosaics. The second type of mosaics are methylation mosaics in whom the number of CGG repeats is in the full mutation range but the gene is not methylated in all the cells.

Genetic counselling

The differences of inheritance of fragile X syndrome as compared to typical X-linked disorders have been noted for a long time. In comparison with X-linked recessive disorders, higher numbers (35%) of obligate carriers of fragile X syndrome clinically manifest variable mental subnormality and other dysmorphic features. Clinically asymptomatic males who are carriers are also seen in the fragile X syndrome pedigrees. Thses males are known as normal transmitting males (NTMs). Females with premutations and some females with full mutations do not manifest the syndrome. Hence, the mode of inheritance is described as X-linked semi-dominant. Another variation is the presence of clinically normal transmitting males. It has been noted that mothers of normal transmitting males have a fewer number of sons with mental retardation compared with daughters of such males. This was described as the Sherman paradox. This phenomenon was explained by the dynamic nature of the mutation, by which the number of repeats increases over generations. The number of affected

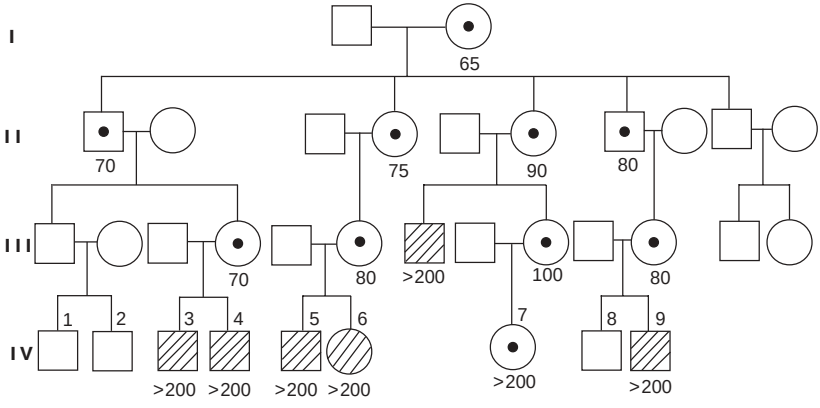


Fig. 10.4 A pedigree showing increase in the number of repeats in subsequent generations and conversion of premutation to mutation. Note that the number of affected cases is more in a later generation than the previous one. The number of CGG repeats for premutation and mutation carriers is also shown. Obligate carriers are shown by a dot (•) inside the symbols. Affected cases are shown by shaded symbols. The number of repeats has not increased when the allele is transmitted by the father.

cases increases in subsequent generations.

These characteristics of inheritance make genetic counselling for fragile X syndrome complex. Though the X chromosome with a premutation will be passed on to all female offspring of a carrier male, and a carrier female will transmit it to 50% male and 50% female offspring, not all of them will have manifestations. The chance that the premutation will get converted to a full mutation in the next generation increases with the increase in the number of repeats (Fig. 10.4 and Table 10.2). A full mutation will be transmitted as a full mutation only. The number of repeats increases over generations and contraction of repeats

Table 10.2 Risk of a premutation from the mother getting converted to a full mutation in the offspring

Number of triplet repeats in the mother	Risk of full mutation in the offspring (%)
55–59	4
60–69	5
70–79	31
80–89	58
90–99	80
100–200	98

is reported only occasionally. Even if the foetus is found to be a carrier of full mutation, the severity of mental retardation cannot be predicted because of factors such as mosaicism in males and lyonization in females. Variability of the phenotype is much more in females, and some carriers of full mutation do not have mental subnormality (Individual IV-7 in Fig. 10.4).

As fragile X syndrome is an X-linked disorder, screening of extended family members for carrier detection and genetic counselling helps in reducing the recurrence of the disorder in the family by prenatal diagnosis. Prevention of the disorder by screening all pregnant women for fragile X carrier status has also been shown to be feasible. The carrier frequency in the general population is 1 in 270.

The counselling of women with a total number of repeats in the grey zone of the alleles (i.e. between the higher limit of normal and lower limit of premutation [40–60 repeats]) is complex. The smallest number of repeats that got converted into a full mutation in one generation is 59.

Staining of hair root cells with antibody for FMR protein is found to be a reliable technique for the diagnosis of affected males. However, this method gives overlapping results in carrier and normal females. It cannot be used for the diagnosis of premutation carriers.

Identification of cases of fragile X syndrome by testing all males and females with mental retardation of unknown aetiology, and offering such families genetic counselling and prenatal diagnosis has become possible by the molecular diagnosis of triplet repeat mutations.

HUNTINGTON CHOREA

Huntington chorea is caused by the expansion of CAG repeats in exons and these repeats are translated into a long polyglutamine tract. Other triplet repeat disorders in which a similar polyglutamine tract is formed are spinobulbar muscular atrophy (Kennedy disease), spinocerebellar ataxias including types 1, 2, 3 (Machado–Joseph disease), 7, and dentato-rubropallidoluysian atrophy.

These disorders share many common features such as progressive neurodegeneration starting from mid-life, a gain of function with the expansion of triplet repeats and the presence of protein aggregates or nuclear inclusions. In the case of fragile X syndrome, the number of repeats in cases with full mutation is in hundreds. In contrast, the number of repeats in cases with full mutation of polyglutamine tract disorders is more than 35. The repeat expansion is more unstable when transmitted by the father. One important characteristic of these disorders

is that though these proteins are expressed throughout the brain and other tissues, only neurons in certain parts of the brain get affected and degenerate.

The CAG repeat in the first exon of the Huntington disease-causing gene is highly polymorphic and varies from 6 to 35 repeats in normal persons and from 36 to 121 in those affected by the disease. The disease is typically of adult onset when there are 40–50 repeats. Alleles with 36–39 repeats are associated with late-onset disease or complete absence of disease manifestations and, if the number of repeats is more than 70, the onset is in childhood. Huntington disease is an autosomal dominant disorder but loss of function of the disease-causing gene (haploinsufficiency) does not give rise to the disease. This is in contrast to fragile X syndrome where gene deletion gives rise to a phenotype similar to the one due to triplet repeat expansions. It appears that the mutant protein with expanded polyglutamine chains has a novel property that damages specific types of neurons by some toxic mechanism and causes neurodegeneration. However, the reasons for the late onset of neurodegeneration are not known.

Huntington chorea is associated with abnormalities of voluntary and involuntary movement such as chorea and dystonia. Initially, there are personality changes with gradual loss of cognition. These motor and mental disabilities lead to death. In the juvenile type of the disease, rigidity, dementia and epilepsy are seen. Usually, patients inherit the expanded repeats from their affected parents. Increase in the number of repeats over successive generations leads to an earlier age of manifestation of the disease. Occasionally, unaffected individuals carry alleles with repeat lengths at the upper limit of the normal range (29–35) which, however, can increase during meiosis to mutation and cause disease in the next generation.

Detection of mutation, especially in patients with atypical manifestations and without a family history, by molecular techniques provides an accurate diagnosis. DNA-based diagnosis is mainly useful for prenatal diagnosis and presymtomatic testing of at-risk individuals. Presymptomatic diagnosis of a late-onset disease without any prophylactic or curative treatment creates a lot of psychological stress on the person tested and the family. Prenatal diagnosis may indirectly lead to presymptomatic diagnosis of the disease in the parent. The presymptomatic diagnosis of late-onset disorders raises several medical, ethical, legal, psychological, professional and insurance problems for the

individual. All these issues need to be addressed to formulate proper guidelines for presymptomatic diagnosis. Adequate pretest counselling regarding the possible outcomes of the test and their implications on the person's life needs to be done. There should be facilities for follow up and psychological support.

MYOTONIC DYSTROPHY

Myotonic dystrophy is an autosomal dominant, multisystem disorder with highly variable manifestations and anticipation. It is characterized by myotonia, muscular dystrophy, cataract, hypogonadism and frontal balding. The age of onset is variable and many cases are very mild. The mutation in myotonic dystrophy is an expanded CTG trinucleotide repeat tract in the 3' UTR region of the protein kinase gene *DMPK*. Wild-type alleles have repeats in the range of 5–37 CTGs, whereas adult-onset cases have more than 50 repeats. In the severe congenital form, the number of repeats is in thousands. The underlying molecular mechanism of the disease is not known. The disease may be non-penetrant in some individuals while the severe congenital form causes mental retardation and may be life-threatening. The severe congenital form is always transmitted from an affected mother . Clinical features of congenital form are mental retardation and hypotonia and are different from that of adult-onset disease.

Electromyography is used for the diagnosis. Sometimes, it is difficult to differentiate it from myotonia due to other disorders. In such cases, mutation detection is diagnostic. Mutation detection is also used for prenatal and presymptomatic diagnoses. There is a close relationship between the size of the repeats and clinical severity of the disease.

Due to the lack of curative treatment, presymptomatic diagnosis is associated with problems similar to those in Huntington disease. Hence, presymptomatic diagnosis should not be offered without pretest counselling, adequate long-term support and medical care.

11 Congenital malformations

Structural anomalies of various parts of the body are the commonest congenital defects. Dysmorphology is the branch of clinical genetics that specializes in birth defects and combines a knowledge of principles of genetics, developmental mechanisms and the natural history of a variety of congenital abnormalities. The types of birth defects are: dysplasias, deformations, disruptions and malformations.

DYSPLASIAS

Dysplasias are morphological defects caused by abnormal maturation and organization of cells into tissues. In dysplasia, the defect is seen in all parts of the body where that particular tissue is present. For example, while in ectodermal dysplasia, hair, teeth, skin and nails manifest the abnormality, in skeletal dysplasia, bones at multiple sites will be involved (Fig. 11.1). These can involve one or multiple organs of the body.

Fig. 11.1 Spondyloepiphyseal dysplasia. Note that involvement of the bones has led to disproportionate short stature.

DEFORMATIONS

Abnormal development of an embryo due to an external mechanical force causing distortion of an otherwise normal structure is called deformation. Examples include dislocation of the hip or mild positional

talipes equinovarus (Fig. 11.2), which may occur because of intrauterine crowding. Limitation of movement inside the uterus, either because of a neurological defect in the foetus or limitation of space may also lead to joint deformities. Deformations usually carry a good prognosis if appropriate treatment is provided.

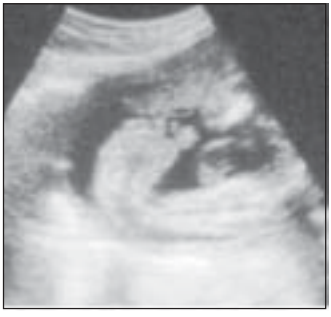


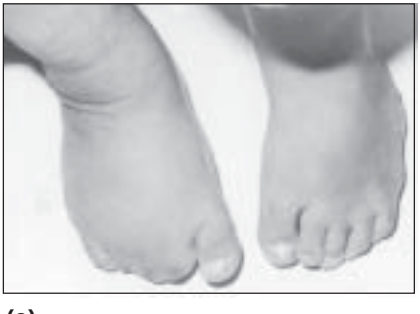
Fig. 11.2 Ultrasonographic picture of talipes equinovarus in a foetus.

DISRUPTIONS

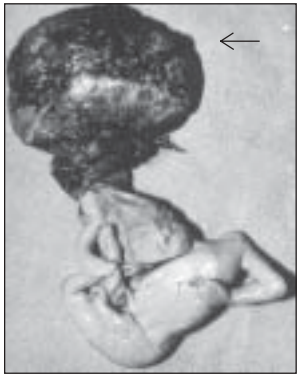
A disruption (Figs 11.3a and b) refers to damage/dissolution of a part following normal development, e.g. amputation of a digit or a limb due to an amniotic band or a thromboembolic episode.

MALFORMATIONS

Malformations are morphological defects that occur due to errors in the normal development and differentiation of an embryo. All malformations are thus congenital, although they may be diagnosed at a later stage. About 14% of newborns have a single minor malformation, 3% have two or more minor malformations, 3% have a single major malformation and 0.7% have multiple malformations. The frequency of malformations occurring during early gestation is probably even higher as many malformed foetuses are spontaneously aborted or are stillborn.



(a)



(b)

Fig. 11.3 Disruptions. **a** Amputation of the digits. **b** Massive disruption leading to damage of the brain and limbs. (The arrow indicates the placenta.)

Patterns of congenital malformations

A malformation may be isolated or associated with other major or minor malformations. On the basis of the underlying aetiopathology, multiple malformations are grouped as discussed below.

Sequence

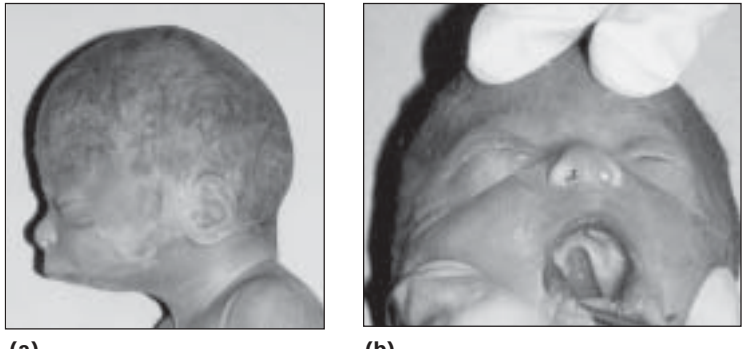
This is a chain of events resulting in multiple defects following a congenital anomaly. A well-known example is the 'Potter' sequence. It is caused by chronic leakage of the amniotic fluid or its deficiency due to decreased production. The result is oligohydramnios leading to foetal compression, presenting as squashed face, dislocation of the hips, talipes equinovarus and pulmonary hypoplasia. Another example is the Pierre–Robin sequence (Fig. 11.4) where the primary malformation is micrognathia. Secondary to micrognathia, the tongue falls back preventing closure of the palate and thus leads to a cleft palate.

Syndrome

The term 'syndrome', though often used loosely, indicates co-occurrence of distinct abnormalities that are definitely or presumably caused by a similar aetiological factor in all affected individuals. The examples include various chromosomal syndromes (Down syndrome) and single-gene disorders (Holt–Oram syndrome, oro–facio–digital syndrome, Treacher–Collins syndrome [Figs 11.5a and b], Carpenter syndrome [Fig. 11.5c], etc.).

Association

The co-occurrence of a group of malformations, occurring more frequently than expected by chance, which cannot be classified into a



(a) **(b)**

Fig. 11.4 A foetus with the Pierre–Robin sequence. **a** Retrognathia. **b** Cleft palate.

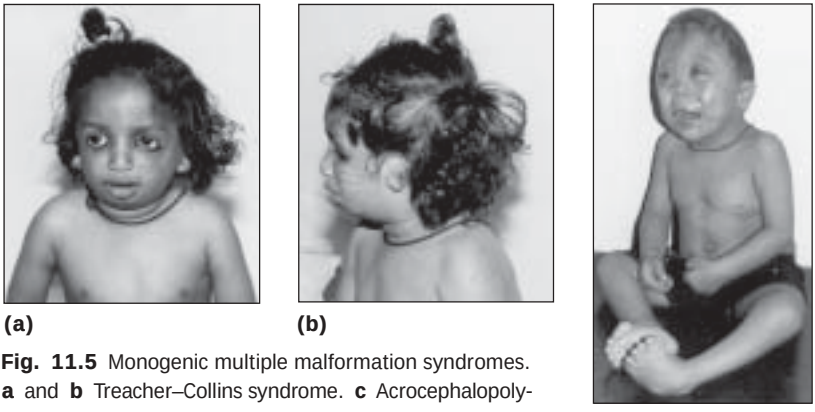


Fig. 11.5 Monogenic multiple malformation syndromes. **a** and **b** Treacher–Collins syndrome. **c** Acrocephalopolysyndactyly (Carpenter syndrome).

sequence or syndrome is called an association. The main difference between an association and a syndrome is the lack of consistency of the occurrence of abnormalities among different cases and absence of a satisfactory explanation for its causation. Examples are VATER (vertebral, anal, tracheal, oesophageal and renal abnormalities) and CHARGE (coloboma, heart disease, atresia choanae, retarded growth, genital and ear abnormalities) associations. The risk of recurrence of an association is usually low.

Types of malformations

Normal variant

This term is applied to those features that fall at the far end of the normal distribution. These may be manifestations of a syndrome but may also be present in an otherwise normal person. Other family members may also have similar features in varying degrees. The examination of first-degree relatives can help in deciding whether a particular feature is a normal variant or an abnormality. The examples are low anterior hairline, bulbous nose, abnormal shape or placement of the ears, etc.

Minor malformations

Malformations that do not cause any functional defects are called minor malformations. They are of cosmetic significance only. However, sometimes they may be part of a syndrome. Examples are cleft uvula, midline pseudocleft of the lip (Fig. 11.6a), preauricular tag, simian crease, polydactyly (Fig. 11.6b), etc. They have an important role in syndrome diagnosis.

(a) (b)

Fig. 11.6 Minor malformations. **a** Midline pseudocleft of the lip. **b** Polydactyly.

Major malformations

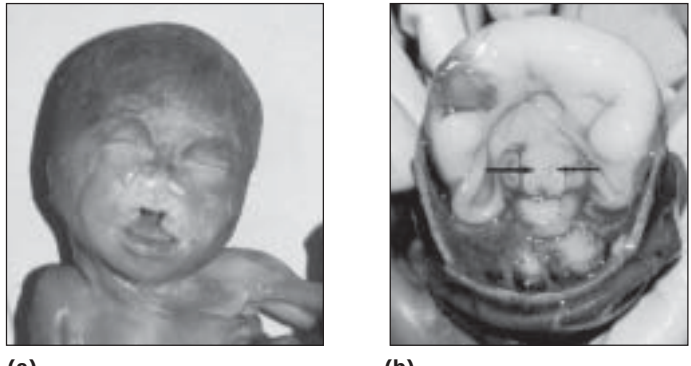
These abnormalities, if left uncorrected, may lead to significant impairment of body functions and may even reduce life expectancy. Cataract, congenital heart disease, meningomyelocele, holoprosencephaly (Fig. 11.7), etc. are examples of major malformations.

Aetiology of congenital malformations

There are many causes of congenital malformations, though in up to 50% cases no cause can be established.

Chromosomal abnormalities

These account for approximately 5% of all congenital malformations. Many chromosomal abnormalities are not compatible with life. They



(a) (b)

Fig. 11.7 **a** A foetus with holoprosencephaly. Note the facial features associated with holoprosencephaly: hypotelorism, absent nose and midline cleft lip. **b** Brain of the same foetus showing single, undivided ventricle and fused thalami (arrows).

end up as spontaneous abortions or late foetal deaths. Any imbalanced anomaly (numerical or structural) of an autosome is usually associated with several structural and developmental defects. Usually two or more malformations are seen.

Single-gene defects

These account for approximately 7.5% of all congenital malformations. They may be present either as an isolated defect or as multiple malformation syndromes. Examples of isolated malformations inherited in a monogenic pattern include hydrocephalus, holoprosencephaly, coloboma of the iris, microphthalmia, polydactyly, etc. There are many multiple malformation syndromes caused by single-gene defects. A few examples of single-gene determined multiple malformation syndromes are given in Table 11.1.

These disorders follow the Mendelian patterns of inheritance, confirming single-gene mutations as their aetiology. The disease-causing gene and the underlying biochemical defect has been identified for some of these disorders, e.g. Smith–Lemli–Opitz syndrome, Zellweger syndrome, Treacher–Collins syndrome, Greig cephalosyndactyly.

Multifactorial inheritance

This accounts for the majority of isolated congenital malformations involving the heart, central nervous system, urogenital system, lip, palate, etc. In these disorders, both genes (a few or many) and

Table 11.1 Single-gene determined multiple malformation syndromes

Syndrome	Mode of inheritance	Clinical features
Holt–Oram syndrome	AD	Thumb hypoplasia or absence, radial hypoplasia, congenital heart disease (commonly atrial septal defect)
Nail–patella syndrome	AD	Hypoplastic nails and patella, nephropathy
Robert syndrome	AR	Mesomelic short stature, oligodactyly, facial clefts
Smith–Lemli–Opitz syndrome	AR	Microcephaly, ptosis of eyelids, epicanthic folds, micrognathia, cryptorchidism, growth deficiency, syndactyly, hypospadias

AR: autosomal recessive; AD: autosomal dominant

environmental factors play a role in the causation of the disorder. A possible example is the association of an open neural tube defect with a mutation in the methyltetrahydrofolate reductase gene and maternal folic acid intake.

Environmental factors

An agent that can cause a birth defect by interfering with the normal embryonic or foetal development is known as a teratogen. Common examples of teratogens include drugs such as thalidomide and infective agents such as *Toxoplasma*. However, for low-grade teratogens (agents with little teratogenecity), the results of numerous human and animal studies may not be conclusive.

Table 11.2 lists some of the proven teratogenic drugs. It is important to note that the risk of the foetus being affected depends on the dosage and gestational age at which the drug is taken and probably also on the genetic background. It should also be noted that not all foetuses exposed to these teratogenic drugs develop congenital defects. While counselling for exposure to teratogens during pregnancy, information regarding the possible teratogenic effects and the probability of their occurrence should be given to the family to help them decide whether to continue or terminate the pregnancy. Infections known to be teratogenic in humans are those caused by cytomegalovirus, rubella virus, varicella zoster, *Treponema pallidum* (syphilis) and *Toxoplasma gondii*. Infection of the mother during pregnancy can lead to foetal infection and its effects

Table 11.2 Drugs and chemicals known to be teratogenic in humans

Drug/Teratogen	Effects on the foetus
Alcohol	Cardiac defect, short palpebral fissure, smooth philtrum, developmental delay, microcephaly
Chloroquine	Deafness, chorioretinitis
Diethylstilboestrol	Vaginal adenocarcinoma
Hydantoin	Hypertelorism, midface hypoplasia, distal digital and nail hypoplasia, growth and mental deficiency, heart and skeletal defects
Lithium	Cardiac defects
Retinoids	Ear abnormalities, hydrocephalus
Streptomycin	Deafness
Tetracycline	Dental enamel hypoplasia
Thalidomide	Phocomelia
Valproate	Neural tube defect
Warfarin	Nasal bone hypoplasia, punctuate calcification of bones

on the fetus. Re-infection and reactivation of latent viral infection are unlikely to cause foetal defects. Infection is unlikely to be associated with recurrent malformations.

Maternal disorders such as hyperthermia, uncontrolled diabetes and phenylketonuria (PKU) have also been shown to cause foetal defects. Untreated phenylketonuria in the mother causes mental retardation, microcephaly and heart defects in the child. Hence, expecting mothers with PKU should follow a phenylalanine-restricted diet during pregnancy.

Unknown causes

About half of all congenital malformations do not seem to have an obvious cause. This includes isolated malformations such as diaphragmatic hernia, anorectal malformations, posterior urethral valves, etc. and some combinations of malformations that cannot be categorized under the known syndromes. For some of the malformations or malformation syndromes, the cause may be a new mutation, microdeletion of chromosomes or uniparental disomy. Although the risk of recurrence can not be quantified, it is low for many of these isolated malformations.

Most of the major congenital malformations are detected at birth. About 50% of stillbirths are associated with malformations. With increasing use of ultrasonography, many malformations can be detected during pregnancy. If a malformation is detected in early foetal life and carries a significant burden, the parents may opt for termination of the pregnancy. Prenatal diagnosis may also assist in the planning of surgical management after the birth of the affected child.

Importance of aetiological diagnosis

Correct diagnosis is the most important prerequisite for genetic counselling. All attempts to arrive at an aetiological diagnosis of a congenital malformation must be made. Chromosomal analysis is indicated in cases with multiple malformations. It is useful in providing the aetiology and prognosis. For any malformed foetus, chromosomal analysis (prenatally or after termination) is of great help in providing accurate genetic counselling. Chromosomal analysis also assists in deciding the course of action in the case of prenatally detected treatable malformations. For example, 30% of foetuses with duodenal atresia have trisomy 21 (Down syndrome; Fig. 11.8).

Foetuses terminated after an antenatal diagnosis of malformation need to be studied carefully. The final diagnosis, based on radiological examination, detailed autopsy and chromosomal analysis, may be

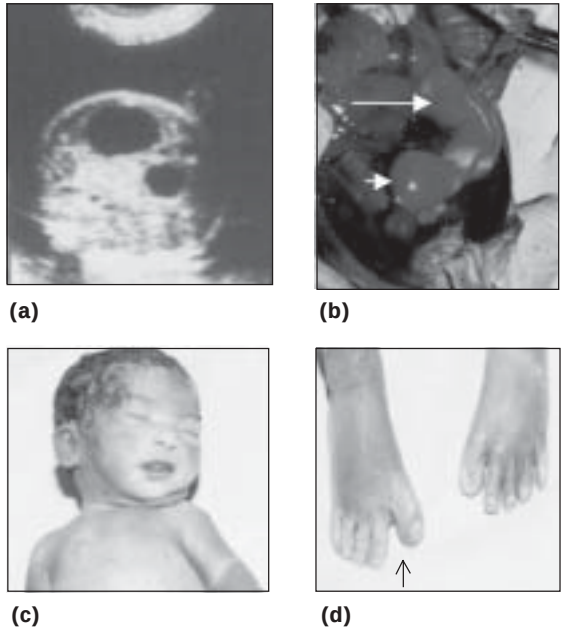


Fig. 11.8 Down syndrome in a foetus with prenatal diagnosis of duodenal atresia. **a** Prenatal ultrasonogram showing double-bubble appearance characteristic of duodenal atresia. **b** Autopsy specimen of the same foetus showing distended stomach (long arrow) and duodenum (short arrow). **c** The same foetus after termination. Note that facial dysmorphism is minimal. **d** Wide sandle gap. (Note the gap between the first and the second toes.)

different from the ultrasonographic diagnosis in as many as 30%–45% of cases. The risk of recurrence calculated on the basis of ultrasound-guided diagnosis alone may be erroneous in many cases. If facilities for autopsy are not available, or if the parents refuse it, then careful external examination and a photograph of the affected foetus can be of great help. Likewise, a whole body radiograph can also be informative, especially if there are short limbs and/or a narrow thorax.

All stillbirths with associated malformations and stillbirths without an obvious cause should be investigated in detail (*see* Chapter 20). Foetal autopsy and chromosomal analysis are essential for providing correct genetic counselling. The same holds true when the malformation is detected in a neonate. In the case of a neonate with a major malformation, the management of the affected child deserves priority. Also arriving at the correct diagnosis for predicting the prognosis and providing counselling for future pregnancies is equally important.

CLINICAL APPROACH

A prenatal history of exposure to teratogens, occurrence of pregnancy-related complications, foetal movements and findings of foetal ultrasonography, if done, should be recorded. A family history of consanguinity, similar or other malformations, genetic disease, history of foetal losses and parental age are equally important. The mode of delivery, foetal presentation, neonatal problems, and subsequent history of medical problems, growth and development should be noted.

Examination of a patient with malformation should be done without making the person uncomfortable. A photographic record of dysmorphic features is useful in following up the child and getting an opinion from a dysmorphologist. Anthropometric measurements (height, weight and head circumference) and, if feasible, measurements of the face and other parts of the body should be recorded. Similarly, assessment of the psychomotor development is also essential.

Special investigations such as computed axial tomography (CT) scan, ultrasonography, slit-lamp and fundus examination, and echocardiography, etc. may be needed for evaluation of the internal organs. Chromosomal analysis is indicated in suspected cases of chromosomal syndrome and in cases with malformations like congenital heart disease, omphalocoele, diaphragmatic hernia, etc.

Syndrome diagnosis

Access to texts on dysmorphology is essential for the diagnosis of multiple malformations. Computerized databases such as the London Dysmorphology Database (LDDB) and Pictures of Selected Syndromes and Undiagnosed Malformations (POSSUM) have made the task simple. However, even for using databases, it is necessary to identify and choose the correct features in the patient (diagnostic handles) to arrive at an accurate diagnosis.

The best 'handles' for computerized database searches are those that are rare, but well defined. For example, microcephaly is not a good handle as it is a feature of a number of syndromes while encephalocele or microphthalmia, being less common, is much more useful in arriving at a diagnosis. Similarly, low-set ears or a posteriorly rotated pinna, which may be normal variants, are much less useful compared with polydactyly or syndactyly.

Prenatal diagnosis

Many malformations can be diagnosed prenatally by ultrasonography, which is a non-invasive procedure. Malformations such as anencephaly can be diagnosed as early as 12 weeks while most other malformations

can be diagnosed in the second trimester after 15–16 weeks. Visualization is better at 18–20 weeks of gestation. The best time for foetal echocardiography is 18–20 weeks.

In addition to families with a history of malformations, ultrasound-guided screening for foetal abnormalities is increasingly becoming a part of routine antenatal care. The sensitivity of ultrasonography for the detection of foetal malformations varies from 40% to 8 5%. The incidence of false-positive diagnosis is low. The factors affecting the detection rate are the skills of the ultrasonologist, technical difficulties, absence of a sonographic sign associated with the abnormality, or late appearance of the abnormality. The issues related to prenatal diagnosis of malformations are discussed in Chapter 19.

Risk of recurrence

The risk of recurrence depends on the aetiological diagnosis. The risk of recurrence of hydrocephalus due to various causes (Table 11.3) illustrates this point. For isolated malformations, empiric risks depending on population studies are given (Table 11.4).

MANAGEMENT OF MALFORMATIONS

The treatment is mostly surgical. The results may vary from complete cure to some residual disability (mental or physical). In chromosomal and some other syndromes, associated mental and physical retardation cannot be cured. With increasing paediatric surgical facilities, the results are improving and the number of treatable conditions is increasing.

Table 11.3 Risk of recurrence of disorders associated with hydrocephalus

Causes of after	Features hydrocephalus	Risk of recurrence birth of an affected child
Meningomyelocele	—	5%
X-linked hydrocephalus	Adducted thumb	50% of boys
Retinoic acid teratogenicity	Microtia	Nil if retinoic acid is avoided
Meckel–Gruber syndrome	Polydactyly, polycystic kidneys, cleft lip, encephalocele	25%
Walker–Warburg syndrome	Microphthalmia, cleft lip, lissencephaly, encephalocele	25%
Trisomy 13	Cleft lip, holoprosencephaly, polydactyly, meningocele	1% or less

Table 11.4 Empiric risk of recurrence of isolated malformations

Malformation	Frequency per 1000 births	Risk of recurrence in siblings of an affected child with normal parents (%)
Anencephaly/spina bifida	4–5	5
Cardiac malformation	6–8	3–4
Cleft lip ± cleft palate	2	4–5
Cleft palate alone	0.5	2–6
Pyloric stenosis	2–3	3
Talipes equinovarus	3–4	2–8
Dislocation of the hip	3–4	3–4
Hirschsprung disease	0.1	6

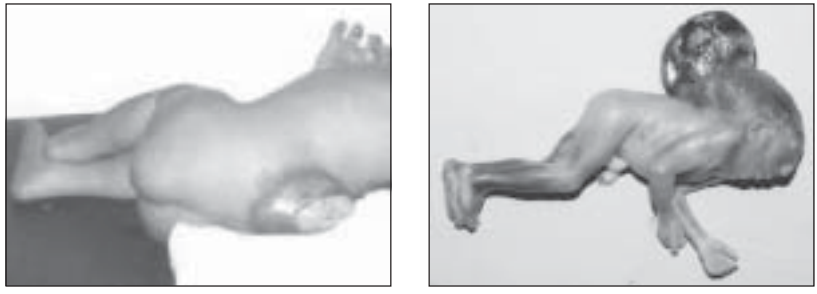
Foetal surgery and interventions such as shunt placement are now technically possible. They may be of high utility in selected cases of diaphragmatic hernia, urinary tract obstruction and hydrocephalus. However, prenatal intervention do not seem to change total outcome significantly.

Genetic counselling

In addition to surgical corrective treatment, genetic counselling of the family is an important part of the management of a child with malformation/s. Genetic counselling depends on an accurate aetiological diagnosis. If a family has a child with a congenital malformation, the parents should be provided with information regarding the risk of recurrence of the malformation in the siblings, measures to prevent recurrence and prenatal diagnosis. This should be done after providing medical care to the affected child. The family may be given time to adjust to the problem of the birth of a malformed child, but genetic counselling should be offered before the couple plans the next pregnancy. Even if the malformation is not treatable, the exercise of arriving at a correct diagnosis is essential to help parents plan for the next pregnancy.

NEURAL TUBE DEFECTS

Neural tube defects (NTDs) need special mention because they are the commonest congenital malformations in India and are preventable. The various types of NTDs are open spina bifida, meningomyelocele (Fig. 11.9a), encephalocele (Fig. 11.9b), anencephaly and iniencephaly (11.9b). The prevalence of NTDs in different parts of India ranges from 0.5 to 11 per 1000 births.



(a) **(b)**

Fig. 11.9 A child with **a** Meningomyelocoele. **b** Iniencephaly with encephalocoele.

The syndromes associated with NTDs are Meckel–Gruber syndrome, spondylocostal dysplasia, trisomy 13 and trisomy 18. Maternal valproate intake during pregnancy is also known to cause NTD.. These account for a small fraction of NTDs. Most of the NTDs are isolated and are thought to be multifactorial in origin. The risk of recurrence of NTD after the birth of an affected child is 3%–5% and increases to about 10% after the birth of two affected children.

Oral intake of 4 mg folic acid per day for at least 1 month before conception and 3 months after conception reduces the risk of recurrence of NTDs by 70%. The mechanism of action of folic acid is not known.

All women who have given birth to a child with an NTD should be advised periconceptional folic acid therapy to prevent recurrence. It is practically useful to start folic acid therapy immediately after the birth of a child with an NTD . The woman should be asked to take folic acid till she conceives again and to continue till 3 months after conception. To prevent NTD, supplementing women of childbearing age with 0.4 mg of folic acid is a good strategy. Prenatal diagnosis by ultrasonography at 16 and 20 weeks should be offered in the next pregnancy.

Estimation of the level of maternal serum alpha-fetoprotein (MsAFP) is another test for the antenatal diagnosis of an open NTD. It is a screening test and at a cut-off value greater than 2.5 MoM (multiples of median), more than 90% of cases with anencephaly and 80% of those with open spina bifida are detected. Estimation of the MsAFP is a good method for the prenatal diagnosis of an NTD in all low risk pregnant women without the family history of NTD.

DEVELOPMENTAL GENETICS

The genetic information derived from parents is stored in the zygote and,

Box 11.1 Important groups of developmental genes

Segmentation genes: The genes responsible for segmentation are known as segmentation genes. These genes are also involved in the control of left–right symmetry, and determination of polarity in the central nervous system and limbs. Sonic hedgehog is an important gene belonging to the group of segment polarity genes and plays an important role in the development of the ventral neural tube.

Homeobox genes: Homeotic genes contain a conserved 180 bp sequence known as the homeobox. Homeobox genes are involved in spatial pattern control and development. There are four homeobox gene clusters, each with a series of closely linked genes. *HOX9*, *HOX10* and *HOX13* are expressed in the limb buds and probably play an important role in limb development. Although 39 homeobox genes are known, only *HOX13* is identified as a cause of malformation. *HOX* 13 mutations cause a type of synpolydactyly.

Paired box (*PAX*) genes: The paired box is a highly conserved DNA sequence and codes for the DNA-binding transcription domain. Waardenburg syndrome and aniridia are caused by mutations in the *PAX3* and *PAX6* genes, respectively.

SRY-type HMG box (*SOX*) genes: *SRY* is a Y-linked gene that plays an important role in male sex determination. A series of genes known as *SOX* genes show homology with the *SRY* gene by sharing a domain known as high mobility group (HMG) box. The *SOX* genes are transcription regulators.

T-box (*TBX*) genes: T-box genes are dispersed throughout the human genome. *TBX1* gene is one of the genes deleted in DiGeorge/velocardiofacial syndrome. *TBX1* has an essential role in the growth and patterning of the pharyngeal apparatus.

Zinc finger genes: These are transcription factors containing a finger-like loop projection that forms a complex with zinc ions. Mutations in the zinc finger gene *WT1* on chromosome 11 causes Wilms tumour and Denys–Drash syndrome (a rare developmental disorder associated with ambiguous genitalia and progressive nephritis).

Signal transduction genes: Various growth factors regulate cell division and differentiation by the process of signal transduction, which involves a complex pathway of genetically determined intermediate steps. Some of them can cause cancers as well. The *RET* protooncogene is one example of a 'signalling' gene. Loss of function of the *RET* gene is responsible for some of the familial cases of Hirschsprung disease, while gain of function mutations are responsible for many of the inherited thyroid cancers.

Table 11.5 Malformation and causative genes

Malformation	Genes	Group to which the gene belongs
Holoprosencephaly	Sonic hedgehog	Segmentation gene
Aniridia	*PAX6*	Paired box genes
Waardenburg syndrome	*PAX3*	Paired box genes
Synpolydactyly	*HOXD13*	Homeobox genes
Hand–foot–genital syndrome	*HOXA13*	Homeobox genes
Campomelic dysplasia	*SOX9*	SRY-like gene
Holt–Oram syndrome	*TBX5*	T-box gene
Greig cephalopolysyndactyly	*GLI3*	Zinc finger genes
Palliester–Hall syndrome	*GLI3*	Zinc finger genes
Hirschsprung disease	*RET*	Signal transduction genes
Pfeiffer craniosynostosis syndrome	*FGFR1*	Signal transduction genes
Apert syndrome	*FGFR2*	Signal transduction genes
Crouzon syndrome	*FGFR2, FGFR3*	Signal transduction genes

along with environmental factors, is responsible for organogenesis, increase in size and appropriate functioning of each cell and tissue. Developmental genes play an important role in organogenesis,cell proliferation and differentiation [Box 11.1]. They also are important in adult life and play a part in carcinogenesis if expressed inappropriately. Though various developmental anomalies of genetic aetiology have been known to us for a long time, the genes for malformation syndromes have been identified only recently (Table 11.5). It has been seen that different phenotypes can be caused by mutations in one gene. The example is GLI3 gene; the mutations of which are known to cause thre different syndromes namely- Greig syndrome, Palliester Hall syndrome and autosomal dominent polydactyly. Simillarly, two or more genes are known to be a cause similar phenotypes. The identification of genes for malformation syndromes be helpful in understanding the complex nature of human development. and also will have clinical applications.

12 Mental retardation

INTRODUCTION

Mental retardation is an important and common clinical condition that calls for genetic counselling. Mental retardation or insufficient development of mental capacities is defined as 'significant subaverage intellectual function existing concurrently with deficits in adaptive behaviour and manifested during the developmental period'. A precise definition of mental retardation has proved problematic because the exact nature of 'intelligence' cannot be understood and its accurate measurement is impossible. The American Association of Mental Retardation defines mental retardation as a significant subaverage intellectual functioning (defined as an intelligence quotient [IQ] score below 70–75) existing concurrently with related limitations in two or more of the following applicable adaptive skill areas—communication, self-care, home living, social skills, community use, self-direction, health and safety, functional academics, leisure and work. In younger children in whom the IQ cannot be estimated, the term global developmental delay is commonly used. Developmental delay means a significant delay in two or more development domains (gross motor, fine motor, speech/language, personal/social or daily activities). The limitations of the definitions of mental retardation and developmental delay, and the estimation of IQ need to be kept in mind during diagnosis.

Taking care of a child or person with mental retardation is a tremendous lifelong burden on the family. To prevent the recurrence of mental retardation in future offspring, it is important for the family to know the risk of recurrence as there is no curative treatment. Mental retardation is a sign or symptom of various disorders of genetic, non-

genetic or unidentified aetiologies. The risk of recurrence of mental retardation varies from 0% to 50%, depending on the aetiology.

An aetiological diagnosis of mental retardation is essential to provide genetic counselling and the option of prenatal diagnosis to the family. Early identification of development delay in a child and investigations to arrive at an aetiological diagnosis of the problem are of utmost importance for the management of the child and the family.

AETIOLOGY

The prevalence of mental retardation in the general population is 2 to 3%. However, most of them are mild, i.e. an IQ of 50–70. Moderate to severe retardation (IQ <50) is uncommon and the prevalence at birth is 0.3%–0.5%. As intelligence is multifactorial in origin, many cases of mental retardation, especially mild and borderline ones, are due to assumed multifactorial aetiology and no definite aetiology may be found after examination and investigations. In those with severe retardation, the possibility of identifying a specific aetiological diagnosis is high. Cases in which the cause of mental retardation has been identified, the prognosis and accurate risk of recurrence can be provided. Prenatal diagnosis is available for some genetic causes of menatal retardation.

Several clinical series suggest that the cause of mental retardation can be identified in 40%–60% of patients undergoing evaluation. In many patients without an identifiable cause, mental retardation may be associated with abnormalities such as congenital malformation, neurological deficit, and risk factors such as consanguinity and a positive family history.

Table 12.1 lists various causes of mental retardation. In addition to group I, conditions in groups II and III also have an genetic component in their aetiologies. Some cases in group V are also likely to be genetic as recurrences are reported in some families of patients presenting as cerebral palsy.

Non-syndromic X-linked mental retardation

In the population with mental retardation, the proportion of males is higher because a number of causes of mental retardation are X-linked. The presence of associated features and biochemical anomalies helps in the diagnosis of syndromic causes of mental retardation. However, there are many cases of X-linked mental retardation without any specific features and these cases can not be categorized into any syndrome classification. More than 10 genes for non-syndromic X-linked mental

Table 12.1 Aetiology of mental retardation

Group I	Genetic	
	• Chromosomal	30%–35%
	—Down syndrome	
	—Other chromosomal syndromes	
	—Microdeletion syndromes	
	• Single-gene disorders	8%–10%
	—Multiple malformation syndromes	4%–5%
	—Fragile X and other X-linked mental retardation	1%–3%
	—Inborn errors of metabolism	4%
	—Presumed because of family history	
Group II	Multiple malformation syndromes not included in group I	15%
	• Known sporadic syndromes	
	• New or private syndromes	
Group III	CNS malformation	5%–7%
	• Neural tube defects	
	• Hydrocephalus	
	• Neuronal migration disorders	
	• Microencephaly	
	• Holoprosencephaly, Dandy–Walker malformation, etc.	
Group IV	CNS dysfunction due to identified perinatal, prenatal and postnatal causes	5%–10%
	• Infection—foetal infection, postnatal meningitis or encephalitis	
	• Teratogen exposure	
	• Asphyxia	
	• Haemorrhage or infarction	
Group V	Static insult presenting as cerebral palsy without substantial evidence of an environmental cause	5%–10%
Group VI	Unidentified	40%–60%

CNS: central nervous system

retardation have been mapped and more are being identified. This group of disorders appears to be a frequent cause of mental retardation. Some important genes belonging to this group are *MECP*$_2$ and *FGD1.* Mutations in the *MECP*$_2$ gene are known to be responsible for causing Rett syndrome, which is an X-linked dominant disorder presenting only in females. The mutations are usually lethal in males. It has been shown that some *MECP*$_2$ mutations are also responsible for non-syndromic X-linked mental retardation in males. Similarly, the *FGD1* gene associated with Aarskog syndrome—an X-linked syndrome—is also found mutated in some families with X-linked non-syndromic mental retardation. Each

of these X-linked genes are likely to be responsible for some cases of non-syndromic X-linked mental retardation as well as some sporadic cases mental retardation in males. The extent of contribution of these gene to mental retardation remains to be determined.

EVALUATION OF A CHILD WITH MENTAL RETARDATION

The aims of evaluating a child with mental retardation are to confirm the presence of mental retardation, assess its severity and identify the aetiology. Most of the severe cases present with developmental delay during infancy. Depending on the severity of mental retardation and observational capabilities of the parents, the developmental delay may be noted by 6–18 months of age. In the second half of the first year, an infant normally achieves important motor milestones. If the infant does not achieve these, the parents will notice the delay. In cases where mental retardation is severe or there is an affected sibling, the presence of the problem may be noted earlier. Similarly, in the presence of a malformation, facial dysmorphism, and neurological abnormalities such as hypotonia, hypertonia or seizures, the problem of associated mental retardation will be identified during the neonatal period or within the first 6 months of life.

Cases with mild mental retardation or borderline IQ may present with speech delay or scholastic failure at a later age. They need to be differentiated from those with isolated learning disabilities or a hearing defect.

For assessment of the levels of development and mental function, depending on the age of the patient, some development assessment tool like Denver Development Screeening test or the appropriate IQ tests should be used. In case of doubtful delays, i.e. delays limited to one or two fields only, or borderline IQ, the term mental retardation should not be used. However, adequate investigations to identify the cause need to be carried out.

History-taking

Family history, antenatal, perinatal and postnatal history play an important role in the diagnosis (Table 12.2). The history of developmental milestones helps in differentiating developmental delay from regression of milestones. Drawing a three-generation pedigree is a must. One should enquire about similar problems, stillbirths, malformations, seizures and abortions in the family. The family history of mental retardation should be carefully assessed. The exact nature of the problem in a similarly

Table 12.2 History-taking in a child with mental retardation

• Family history	Similar problems, stillbirths, malformations, seizures, abortions
• Antenatal history	Maternal illnesses such as fever, rash, pre-eclampsia, epilepsy, phenylketonuria; intake of drugs, teratogens, alcohol
• Perinatal history	Prematurity, asphyxia, birth weight, sepsis, hyperbilirubinaemia, resuscitation, neonatal illness, feeding problems, seizures
• Postnatal history	Feeding problems, seizures, jaundice, failure to thrive, abnormal odour, constipation, episodes of acute illness, hypotonia, hypertonia, scissoring, progressive deterioration, gain of milestones, psychomotor regression

affected relative should be confirmed by examination of the person and his/her medical records. A familial disorder suggests the possibilities of monogenic, chromosomal, multifactorial or even teratogenic aetiology.

A history of birth asphyxia should be objectively evaluated. Even if birth asphyxia is documented, it may not be the cause of mental retardation as neonates with brain dysfunction and malformations are also prone to respiratory problems at birth.

A history suggestive of a progressive course or loss of previously attained milestones strongly suggests the presence of genetic metabolic disorders (*see* Chapter 13). However, neurodegenerative disorders with an early onset such as Tay–Sachs disease or slowly progressive disorders such as Pelizaeus–Merzbacher disease may mimic static brain damage. Some metabolic disorders such as peroxisomal disorders are accompanied by facial dysmorphism.

Clinical examination

A complete neurological and developmental examination, accurate measurement of growth parameters, complete general and systemic examination are done as in the case of any other clinical problem. Malformations and dysmorphic features should be looked for from head to toe without making the person and family conscious of their looks or malformations.

Detailed ophthalmological examination should be carried out in each case. The presence of dysmorphism or specific abnormalities of the skin, eyes and hair can provide important diagnostic clues (Table 12.3). Some dysmorphic syndromes have a characteristic phenotype and common ones can be diagnosed at a glance by an experienced dysmorphologist

Table 12.3 Clinical clues to the aetiology of mental retardation

Feature	Disorders
• Microcephaly	Chromosomal malformation syndromes, lissencephaly, pachygyria, perinatal insult, foetal infections, primary microcephaly
• Macrocephaly	Ventriculomegaly, Canavan disease, Alexander disease, Sotos syndrome, Tay–Sachs disease
• Hair abnormalities	Menkes kinky hair disease, organic acidurias
• Café au lait spots	Neurofibromatosis, tuberous sclerosis
• Hypopigmented patches	Tuberous sclerosis
• Mosaic type of pigmentary abnormalities	Hypomelanosis of Ito, mosaicism for chromosomal abnormality
• Eczema, skin rashes	Phenylketonuria, biotinidase deficiency
• Cataract	Down syndrome, galactosaemia, Lowe syndrome, foetal rubella, Cockayne syndrome
• Cherry red spot	Gangliosidosis (Tay–Sachs disease), mucolipidosis, Niemann–Pick disease
• Dislocated lens	Homocystinuria
• Retinal abnormalities	Foetal rubella, foetal cytomegalovirus infection, Aircardi syndrome, tuberous sclerosis, neuronal ceroid lipofuscinosis, Walker–Warburg syndrome
• Nystagmus	Pelizaeus–Merzbacher disease, Joubert syndrome
• Coarse facies	Mucopolysaccharidosis, Coffin–Lowry syndrome, hypothyroidism
• Hepatosplenomegaly	Storage disorders

Table 12.4 Multiple malformation syndromes with characteristic phenotype

Syndrome	Features
• Cornelia de Lange syndrome	Synophrys, growth retardation, upturned nose, upper limb anomalies
• Rubinstein–Taybi syndrome	Overhanging columella, grimacing smile, broad thumbs and great toes
• Apert syndrome	Acrocephaly, proptosis, syndactyly
• Seckel syndrome	Microcephaly, growth retardation, beak-like nose
• Coffin–Siris syndrome	Increased body hair, fifth finger/nail hypoplasia
• Coffin–Lowry syndrome	Coarse facies, drumstick phalanges, kyphoscoliosis
• Smith–Lemli–Opitz syndrome	Ptosis, downslant of eyes, hypospadias, polydactyly, growth retardation

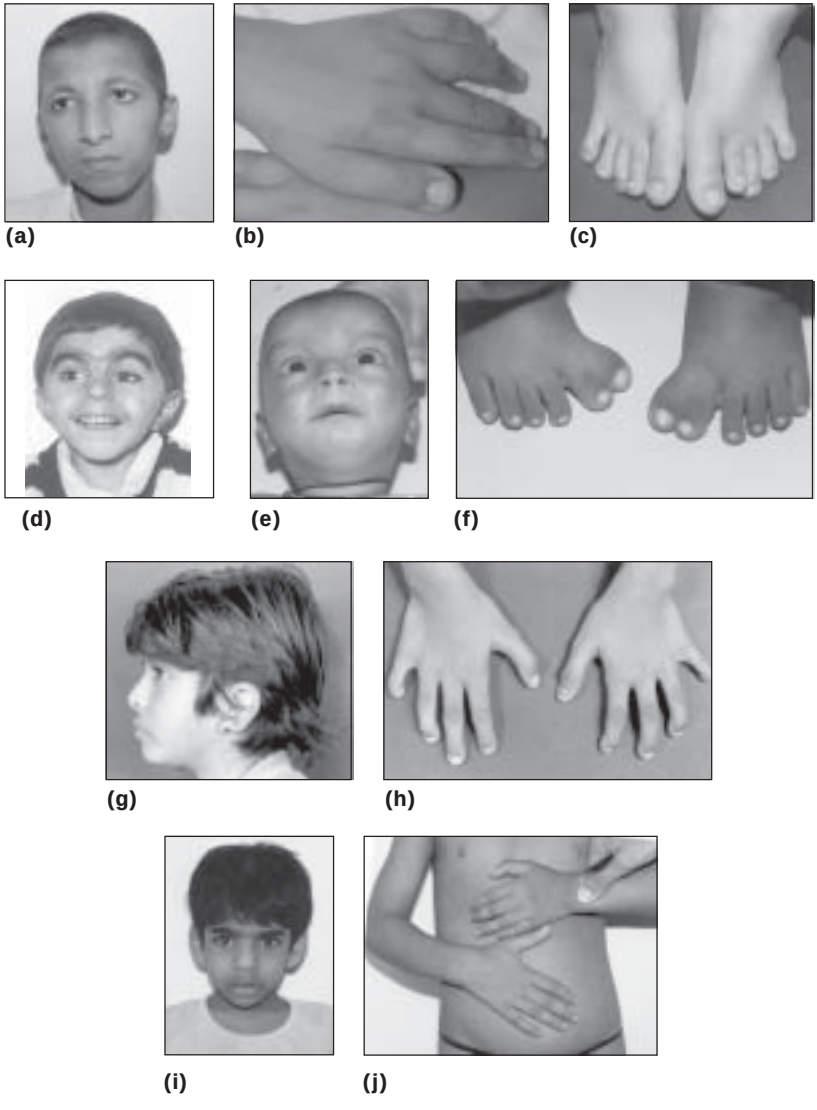


Fig. 12.1 Syndromes with mental retardation. **a, b, c** Rubinstein–Taybi syndrome: antimongoloid slant, overhanging columella, broad thumbs and great toes. **d** Cornelia de Lange syndrome: synophrys, and upturned nostrils. **e, f** Acrocallosal syndrome: Prominant forehead, hypertelorism, preaxial polydactyly of feet. Aplasia of corpus callosum is commonly present. **g, h** Coffin–Siris syndrome: increased body hair, aplasia of fifth finger nails, hypoplasia of terminal phalanges and nails. **i, j** Coffin–Lowry syndrome: coarse facies, thick lips, large hands with tapering fingers.

(a specialist dealing with malformations and malformation syndromes) (Table 12.4 and Fig. 12.1). For rare syndromes, the use of computerized databases such as the London Medical Databases, and Pictures of Selected Syndromes and Undiagnosed Malformations (POSSUM) are useful.

The two important points to be remembered while taking the history and conducting examination are: first, to establish whether there is an isolated motor delay due to neuromuscular diseases (spinal muscular atrophy, congenital myopathy or joint laxity) and second, to assess whether the disorder is of a progressive nature or whether there is any other evidence of a metabolic disorder as a cause of developmental delay.

Investigations

It should be noted that the presentations and aetiologies of mental retardation are too heterogeneous to fit into an algorithm. There cannot be a battery of tests applicable to all cases. Investigation of each case needs to be individualized, and based on the clinical findings (Fig. 12.2). Dysmorphic syndromes and metabolic disorders were considered exclusive of each other, but this is not absolute. The presence of dysmorphism in peroxisomal disorders such as Zellweger syndrome and identification of a metabolic abnormality in malformation syndromes such as Smith–Lemli–Opitz syndrome have shown the overlap between the two.

Frequently used investigations for evaluating a case of mental retardation are discussed below. In addition, various other investigations such as imaging studies; muscle biopsy; molecular studies for various disorders; and biochemical studies for amino acidopathies, lysosomal, peroxisomal and mitochondrial disorders are also extremely important. These need to be used in selected cases.

Chromosomal analysis

Chromosomal disorders account for about 30% of cases of mental retardation. The presence of malformations and microcephaly increases the likelihood of a chromosomal disorder. Down syndrome is the commonest cause of mental retardation. The diagnosis is possible at birth; long before the family notices developmental delay (*see* Chapter 7). A karyotype at the 550-band level is indicated in all cases of mental retardation without a obvious diagnosis.

Techniques such as fluorescence *in situ* hybridization (FISH) studies have made it possible to identify small chromosomal abnormalities that cannot be identified by routine analysis. The technique can be used to identify targeted microdeletions depending on clinical suspicion (Table

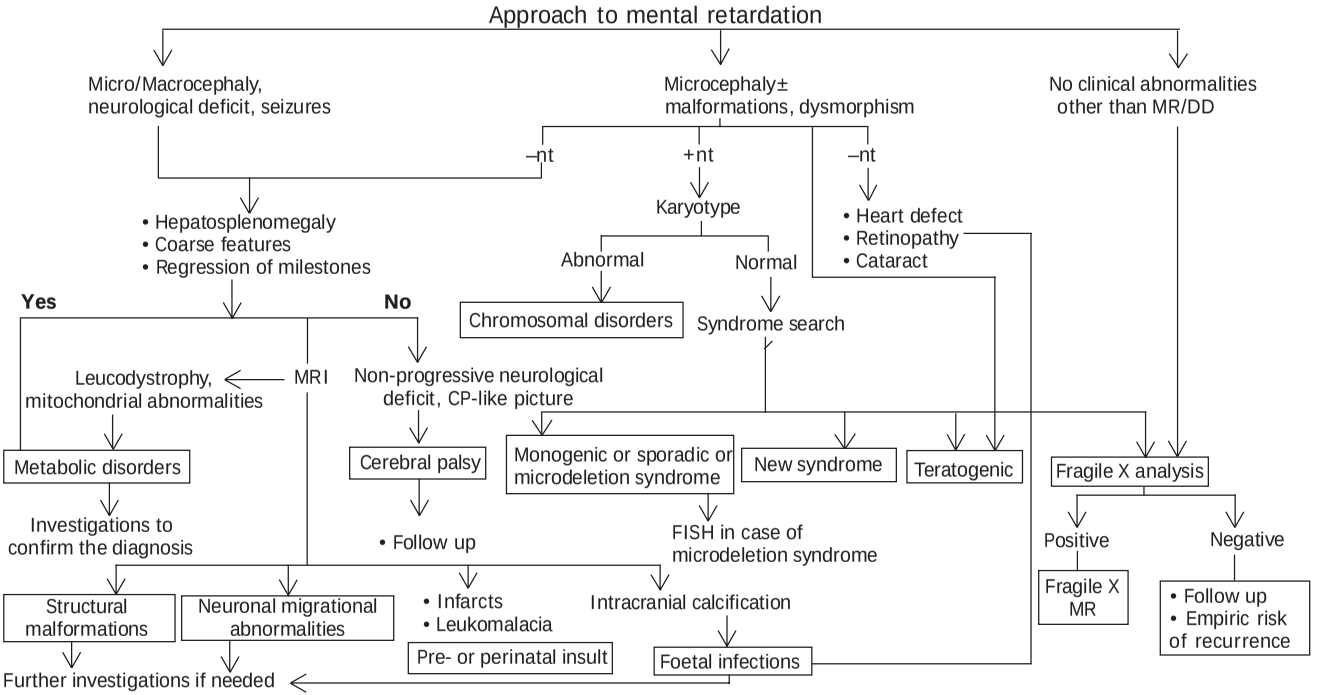


Fig. 12.2 Clinical approach to a case of mental retardation. This algorithm is a rough guide to plan the investigations. MR: mental retardation; DD: developmental delay; CP: cerebral palsy; MRI:magnetic resonance imaging; FISH: fluorescence *in situ* hybridization

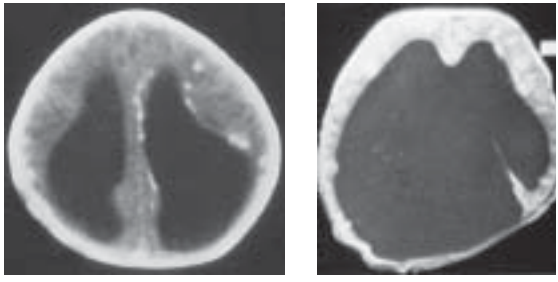
Table 12.5 Microdeletion syndromes

Syndrome	Chromosome region	Features
Miller–Dieker syndrome	17p13.3	Type 1 lissencephaly, characteristic face, bitemporal narrowing
Williams syndrome	7q11.23	Elfin facies, mandibular hypoplasia, fleshy lips, aortic and pulmonary stenosis, infantile hypocalcaemia, outgoing personality, impressive conversational and social skills
Prader–Willi syndrome	15q11q13	Hypotonia, micropenis, hypogonadism, hyperphagia and obesity after the second year of life, small hands and feet, behavioural problems, almond-shaped eyes
Angelman syndrome	15q11q13	Severe mental retardation, microcephaly, ataxic gait, hypotonia, epilepsy, prominent mandible, open mouth, happy disposition, inappropriate episodes of laughter
Velocardiofacial syndrome	22q11.2	Cardiac defect, pear-shaped nose, midface hypoplasia, hypoparathyroidism, thymic hypoplasia, growth deficiency, speech and learning disorders

12.5). In recent years, FISH has also been used for the detection of submicroscopic chromosomal deletions/duplications involving the ends of chromosomes, i.e. telomeres. The technique identifieschromosomal abnormalities in about 7% .of cases of mental retardation without obvious cause.

Testing for fragile X syndrome

Fragile X mental retardation is the commonest cause of inherited mental retardation. It is an X-linked semi-dominant condition caused by a mutation in the *FMR1* gene on the long arm of the X chromosome. It accounts for mental retardation in 1%–3% of males and 2%–4% of females without an obvious cause on clinical evaluation (*see* Chapter 10). The characteristic phenotype of the fragile X syndrome includes a long face, prognathism and macro-orchidism. It is subtle and evolves with age. The diagnostic tests can be cytogenetic or molecular. However, diagnosis of the fragile X syndrome has great implications for the family for carrier detection and prenatal diagnosis. Hence, *FMR1* testing by



(a) (b)

Fig. 12.3 **a** CT scan head showing brain damage due to fetal infection. **b** MRI brain showing semilobar holoprosencephaly.

molecular methods is indicated in all males and females without an obvious cause of mental retardation, with or without a positive family history.

Neuroimaging

An individual with mental retardation may or may not have a structurally or histologically abnormal brain. Neuroimaging has shown abnormalities in a considerable number of cases. It offers valuable information in many cases of mental retardation, especially in the presence of microcephaly (Fig. 12.3), macrocephaly, seizures (Fig. 12.4) and neurological signs such as hypertonia and hemiplegia. CT scan of the brain is a valuable test for suspected craniosynostosis or conditions associated with intracranial calcification. Magnetic resonance imaging (MRI)

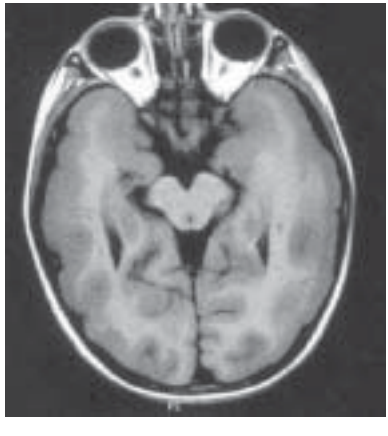


Fig. 12.4 MRI scan of the brain of a child with mental retardation and seizures showing broad and thick gyri—pachygyria.

Box 12.1 Abnormalities detected with neuroimaging in mental retardation

- Congenital morphogenetic abnormalities, e.g. holoprosencephaly, Dandy–Walker malformation, lissencephaly, schizencephaly
- White matter and metabolic disorder, e.g. leucodystrophies, Cockayne syndrome, Leigh disease
- Acquired defects, e.g. leucomalacia, infarcts, calcification, evidence of foetal infections
- Miscellaneous disorders, e.g. tuberous sclerosis

is more useful in delineating grey and white matter, and is the investigation of choice in most instances. Box 12.1 lists some of the abnormalities detected with neuroimaging. Magnetic resonance spectroscopy is presently being evaluated for its utility in identifying the causes of mental retardation.

Metabolic testing

Inborn errors of metabolism are an important group of disorders presenting as mental retardation or developmental delay. Screening for amino acid disorders, organic acidurias, mucopolysaccharidosis, long chain fatty acids in patients with idiopathic mental retardation gives a low yield but is individually useful for cases with positive results. Cases in which a metabolic disorder is suggested on a clinical basis, metabolic screening is effective in directing further confirmatory metabolic studies such as enzyme assays. Techniques such as gas chromatography mass spectrometry and tandem mass spectrometry can screen for a number of metabolic disorders.

Thyroid function tests

Thyroid function tests are indicated when clinical features of hypothyroidism are seen in an infant with developmental delay or in younger children where no cause can be found. It should also be carried out in all children with Down syndrome.

Other tests

Tests such as bone marrow examination, enzyme assays, skin biopsy, urine analysis for the presence of mucopolysaccharides, serology for Toxoplasma, rubella, cytomegalovirus and herpesvirus (TORCH) infections, etc. should be done as per the clinical suspicion. The serology of TORCH infections should be cautiously interpreted and the results correlated with the clinical findings before arriving at a definite conclusion regarding foetal infection as a cause of mental retardation.

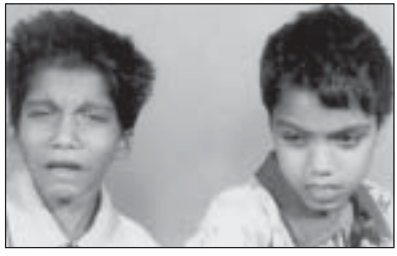


Fig. 12.5 Sisters with mental retardation and similar dysmorphic features such as upsweep of the hair and dysplastic ears.

MICROCEPHALY

Microcephaly is defined as occipitofrontal circumference that is less than 3SD below normal for a particular age and sex. Such a small brain is almost always associated with mental handicap. The causes may be genetic or non-genetic. Clinical clues can suggest the possibility of chromosomal anomalies, microdeletion syndromes, malformation syndromes or metabolic disorders. In appropriate situation a CT scan or MRI examination should be done to look for foetal infections, structural malformations, neuronal migrational abnormalities and evidence of perinatal insult.

Many cases of microcephaly are monogenic and associated with a 25% risk of recurrence. However, the diagnosis of autosomal recessive or primary microcephaly is a diagnosis of exclusion and is considered after ruling out causes of secondary microcephaly. The empiric risk of recurrence after the birth of one child with microcephaly varies from 1 in 6 to 1 in 8, as many of these cases could be autosomal recessive. If there are two similarly affected siblings with normal karyotype in the family or one affected child with consanguineous parents, then it is assumed that microcephaly is inherited in an autosomal recessive manner in that family and the risk of recurrence in the siblings of the proband is 25% (Fig. 12.5).

Primary microcephaly can occur due to mutations in a number of genes. At present, five loci for microcephaly have been mapped and these account for 68% of cases of primary microcephaly. In families where the causative gene is mapped, prenatal diagnosis can be provided using molecular techniques. For other cases, serial follow up ultrasonography is performed prenatally to monitor fetal head size. However, usually the suboptimal rate of growth of the head circumference does not become apparent before the late second or third trimester. Hence,

ultrasonography is not a useful technique for the prenatal diagnosis of microcephaly.

CEREBRAL PALSY

Cerebral palsy is a symptom complex rather than a specific disease. It covers a group of non-progressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain that arise in the early stages of development. About 30% of patients diagnosed with cerebral palsy have mental retardation.

Often, the history of delayed crying is given undue significance and mental retardation is attributed to birth asphyxia. Due to such simplistic evaluation and interpretation many cases of neurodegenerative disorders or other genetic causes of developmental delay are wrongly labeled as cerebral palsy, and a great opportunity to identify the real aetiology and prevent recurrence by prenatal diagnosis is lost. Unfortunately, many of these disorders presenting with abnormalities of muscle tone, seizures and developmental delay are autosomal recessive metabolic disorders with a high risk of recurrence. Hence, all such disorders need to be investigated completely and followed up before labeling them as cerebral

Table 12.6 Empiric risk of recurrence after one child with mental retardation

Accompanying feature	Risk of recurrence (approximate %)
• Microcephaly alone	1 in 6 to 1 in 8 (10–15)
• Microcephaly and other features	1 in 30 (3)
• Infantile spasms	1 in 30 to 1 in 100 (1–3)
• Non-specific dysmorphic feature	1 in 25 to 1 in 30 (4)
• None of the above	
—Male proband	1 in 13 (8)
—Female proband	1 in 20 (5)
• Holoprosencephaly with normal chromosomes and no forme fruste in the parents	1 in 20 (5)
• Lissencephaly type I	Very low, 1 in 4 (25)
• Lissencephaly type II	1 in 4 (25)
• Cerebellar hypoplasia	1 in 8 (12.5)
• Schizencephaly or asymmetric porencephaly	Very low
• Cerebral palsy	1 in 400 to 1 in 200
• Symmetrical spasticity	1 in 8 to 1 in 9 (10)
• Asymmetrical neurological signs	1 in 50 to 1 in 100 (1–2)
• Ataxic diplegia	1 in 24 (4)
• Congenital ataxia	1 in 8 (12.3)

palsy. Some slow progressive neurodegenerative disorders such as Pelizaeus–Merzbacher disease are difficult to distinguish from cerebral palsy without clinical suspicion and investigations. In case of cerebral palsy if there is a definite evidence of perinatall insult, then the risk of recurrence is nil or negligible. But in other cases of cerebral palsy, emperic risk figures are available (Table 12.6). The risk of recurrence for cerebral palsy with symmtrical spasticity or ataxic cerebral palsy is high as compared to that of diplegia or hemiplegia.

COUNSELLING FOR MENTAL RETARDATION

Based on the cause of mental retardation, the family should be provided information regarding the disorder, course, risk of recurrence of similar problem in the family and ways to prevent the recurrences. If the risk of recurrence is low or if prenatal diagnosis is available for the disorder, the parents may feel relieved to some extent. But even when the family is not interested in prenatal diagnosis, identification of the cause of mental retardation helps many families in accepting the problem and concentatrate their efforts in the direction of training and habilitation of the child rather than looking for cure. Identification of cause and knowing that the cause is beyond their control also lessens the guilt of many parents who may be considering themselves responsible for the problem.

If in spite of complete investigations the cause of mental retardation cannot be established, then empiric risk figures are used. When examinations or investigations show abnormal characteristics such as restricted growth (of the cranium /stature); specific dysmorphic features; a recognizable behavioural phenotype; congenital anomalies; neurological problems such as spasticity (symmetrical/asymmetrical); or a neuro-imaging abnormality such as lissencephaly, the risk of recurrence can be modified according to the associated feature (Table 12.6). The risk of giving birth to a mentally retarded child if both the parents are normal and one parent has one or more retarded siblings is 2.5%. The risk for a couple similar to the one above if they already have one retarded child is 12.5%

There is no curative treatment for mental retardation. All the above-described efforts are made to arrive at an aetiological diagnosis based on which, information regarding the correct prognosis, accurate risk of recurrence and options such as prenatal diagnosis to prevent recurrence can be provided to the family. This information is useful and necessary but most parents look for curative measures. A discussion with the parents of a child with mental retardation about the plan of investigations and utility of investigations helps in preventing them from fostering unrealistic expectations from the doctor and thus helps in successful counselling.

13 Genetic metabolic disorders

Metabolic processes in living organisms proceed in steps. Each step in a metabolic pathway is governed by an enzyme, which is the product of a gene. The concept of 'one gene, one enzyme' was developed by Beadle and Tatum. However, the first genetic metabolic disorder or inborn error of metabolism was described by Sir Archibald Garrod in 1902. The disorder was alkaptonuria, in which nappies of children suffering from the disease turn black on standing. Currently, about 400 inborn errors of metabolism are known. Various strategies have been successfully used to treat some of these disorders. Over the past few decades, many types of treatments have been developed for various metabolic disorders, but complete prevention of manifestations or normal outcome has been possible for only a few disorders. With improved modalities of diagnosis, new inborn errors of metabolism are being identified. The biochemical defects of many well-defined monogenic disorders are now known, e.g. Smith–Lemli–Opitz syndrome, a monogenic multiple malformation syndrome, is known to be due to a defect of cholesterol synthesis.

AETIOLOGY

Inborn errors of metabolism are caused by defective or altered functioning of proteins. Majority of these proteins are enzymes, but also include receptors, transporting and structural proteins, hormones, clotting factors, haemoglobin, etc. These disorders are monogenic, with most of them being inherited in an autosomal recessive or X-linked fashion. This is because the enzymes are required in small amounts and there is usually sufficient residual activity in the heterozygous state.

Metabolic defects that have been identified involve almost all known metabolic pathways, i.e. pathways of amino acids, proteins, carbohydrates, mucopolysaccharides, fatty acids, lipids, nucleic acids, metals, etc. Individually these disorders are rare. Correct and timely diagnosis can provide management to the affected individual and prevent its recurrence in the family by genetic counselling and prenatal diagnosis.

CLINICAL CLASSIFICATION OF INBORN ERRORS OF METABOLISM

Metabolic disorders are be divided into two main categories (Saundubray JM, Charpentier C. Clinical phenotypes: Diagnosis/ Algorythms. *In* The metabolic and molecular bases of inherited disease. Eighth edition Editors: Scriver, Beaudet, Valle, Sly. 2001 pp 1329-30).

Category I: Disorders that involve only one functional system (such as the endocrine system, immune system, coagulation factors) or affect only one organ or anatomical system (such as the intestine, erythrocytes, kidneys or connective tissue). The presenting symptoms are characteristic for the system involed (e.g. bleeding disorder in coagulation defect), making the diagnosis easy.

Category II: Diseases in which the basic biochemical lesions either affect one metabolic pathway that is common to a large number of cells

Table 13.1 Classification of Category II inborn errors of metabolism

Group	Clinical manifestations	Examples
1 Defects affecting the synthesis or catabolism of complex molecules; defects of intracellular trafficking and processing of proteins	Clinical manifestations are permanent, progressive and independent of intercurrent illness or food intake	Lysosomal storage disorders, peroxisomal disorders, alpha$_1$-antitrypsin deficiency
2 Defects of intermediary metabolism that lead to acute or progressive intoxication due to accumulation of toxic compounds proximal to the metabolic block	Acute or intermittent manifestations. The onset may be late. Central nervous system manifestations including coma are frequent. Treatment is dietary restriction or removal of toxins	Aminoacidurias, urea cycle defects, sugar intolerance
3 Energy deficiency disorders resulting from deficiency in energy production or utilization	Hypoglycaemia, lactic acidosis myopathy, cardiomyopathy, failure to thrive, hepatic and central nervous system involvement	Mitochondrial disorders Defects of gluconeogenesis

or organs, or are restricted to one organ but give rise to systemic consequences. The examples are lysosomal storage disorders, mitochondrial disorders, aminoacidopathies, etc. Depending on the pathophysiology, these disorders can be divided into three groups (Table 13.1). This chapter is limited to this category of disorders.

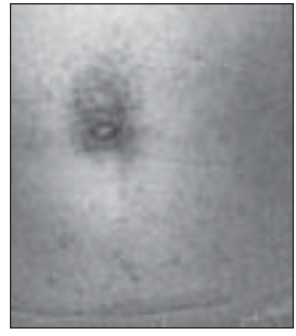


Fig. 13.1 Angiokeratomas around the umbilicus in a person with Fabry disease.

CLINICAL MANIFESTATIONS

The signs and symptoms of inborn errors of metabolism are many and diverse. Some manifestations such as cherry red spot, angiokeratomas (Fig. 13.1) and coarse facies may provide important diagnostic clues (Table 13.2). Many inborn errors are associated with manifestations that greatly overlap with non-genetic conditions such as septicaemia, hepatitis, stroke, etc. Therefore, a high level of suspicion and good

Table 13.2 Specific manifestations suggestive of inborn errors of metabolism

Manifestations	Disorders
• Cherry red spot in the eye	Tay–Sachs disease, Niemann–Pick disease type IA, galactosialidosis, generalized gangliosidosis
• Coarse facies	Mucopolysaccharidosis, fucosidosis, mucolipidosis, generalized gangliosidosis, mannosidosis
• Cataract	Lowe syndrome, Zellweger syndrome, galactosaemia, congenital defects of glycosylation
• Corneal opacities	Tyrosinosis type II, cystinosis, mucolipidosis, mucopolysaccharidosis, Fabry disease
• Angiokeratomas	Fabry disease, fucosidosis, galactosialidosis, beta-mannosidosis
• Steely, brittle, sparse hair	Menkes kinky hair disease
• Alopecia, skin rashes	Biotinidase deficiency
• Peculiar fat pads on buttocks	Congenital defects of glycosylation
• Macrocephaly	Tay–Sachs disease, Canavan disease, Alexander disease, Van der Knaap disease
• Cardiomyopathy	Pompe disease, fatty acid oxidation defects, respiratory chain disorders
• Retinitis pigmentosa	Kearns–Sayre syndrome, Refsum disease, Usher syndrome, abetalipoproteinaemia

Table 13.3 Clinical presentations of inborn errors of metabolism

- Acute neonatal illness: Neurological deterioration, vomiting, acidosis, seizures (septicaemia-like presentation)
- Acute or intermittent illness at any age with seizures, coma, acidosis
- Hypoglycaemia
- Seizures
- Psychomotor regression
- Developmental delay or mental retardation
- Ataxia
- Dystonia
- Psychiatric symptoms
- Neuropathy
- Deafness
- Acidosis
- Hepatic failure
- Hepatosplenomegaly

Coarse facies
- Cataract
- Corneal opacities
- Optic atrophy
- Cardiomyopathy
- Myoglobinuria
- Muscle pain
- Bone crisis [painful]

Diarrhoea
Vomiting
- Focal neurological signs
- Stroke
- Extrapyramidal signs
- Thromboembolic phenomena
- Peculiar odour of urine
- Siblings with similar illness
- Skin rash

investigative facilities are necessary for the diagnosis of such disorders. Table 13.3 lists the clinical situations where one should suspect inborn errors of metabolism.

From the possible manifestations, it is obvious that inborn errors of metabolism can present with clinical features involving any system of the body. The onset may be acute, chronic or intermittent. Most of the inborn errors of metabolism manifest in infancy or childhood, but many of them have late-onset variants. Congenital malformations were once thought to be distinct from metabolic disorders. However, metabolic disorders such as Smith–Lemli–Opitz syndrome and Zellweger syndrome are characterized by congenital malformations and facial dysmorphism. Clinical presentations of Category I disorders involving the liver, kidneys, immune system, blood, etc. are not discussed here as they are specific and limited to the system involved. The clinical presentations of category II of inborn errors of metabolism are listed below

1. *Acute illness with neurological deterioration in neonates and infants:* Many genetic metabolic disorders present in the neonatal period as soon as milk is introduced or during infancy. These include aminoacidurias, organic acidurias, galactosaemia, congenital lactic acidosis, glycogen storage disorders and fatty acid oxidation defects. Neonates have a limited pattern of manifestation and they respond to any type of insult in the same way. Therefore, it is essential to suspect

an inborn error of metabolism in an acutely sick neonate or infant. Baseline investigations for metabolic disturbances and septicaemia should be carried out in such cases.

2. *Developmental delay and mental retardation:* Delayed development with or without seizures may become obvious by 3–6 months of age. At this age, it is difficult to distinguish a progressive genetic neurometabolic disorder from static central nervous system insult or abnormalities. It is essential to investigate these infants for possible inborn errors of metabolism such as Tay–Sachs disease, Krabbe disease, Leigh disease, Pelizaeus–Merzbacher disease, etc. If such infants are clinically labelled to have cerebral palsy without proper investigations, then the family is deprived of accurate information regarding the risk of recurrence and prenatal diagnosis.

   For some metabolic disorders, the only manifestation may be developmental arrest and mental retardation without specific symptoms and the disorder may apparently appear to be non-progressive. The inborn errors of metabolism with this type of presentation include phenylketonuria, Rett syndrome, homocystinuria, creatine deficiency, etc. Though the diagnostic yield of investigations for genetic metabolic disorders in non-specific mental retardation is poor, the diagnosis of one such case has important implications for the family.

3. *Psychomotor regression:* Loss of previously acquired motor and mental developmental skills is a definite indication of a neurometabolic disorder of genetic origin. A classical example is metachromatic leucodystrophy; its infantile variety manifests at around one year of age (Fig. 13.2). As the child achieves many important milestones by that time, the regression is obvious. On the other hand, with earlier-onset disorders, the loss of milestones may be difficult to differentiate from developmental delay or arrest. Juvenile- or adult-onset disorders may have a slow onset with non-specific symptoms such as behavioural changes and slow intellectual deterioration. Unless associated with seizures, ataxia and other focal neurological signs, these patients may be misdiagnosed as

Fig. 13.2 An eighteen-month-old girl with metachromatic leuco-dystrophy. There is hypertonia in all the four limbs.

suffering from psychiatric disorders, especially early in the course of the disease. A history of a similarly affected sibling strongly suggests the possibility of a genetic cause, but is not always available. The possibility of a genetic metabolic disorder increases if the parents are consanguineous. At the same time, it is essential to look for environmental aetiologies such as subacute sclerosing panencephalitis, lead poisoning, etc. which can mimic neurometabolic disorders.

Apart from the clinical presentations discussed above, many inborn errors of metabolism manifest as cardiac failure, lactic acidosis, acute neonatal cholestasis, acute hepatic failure and hepatosplenomegaly. These presentations are also commonly caused by environmental aetiologies. Hepatosplenomegaly is an important clue to the possibility of storage disorders such as Niemann–Pick and Gaucher disease. If abnormal storage cells are present, bone marrow examination and liver biopsy can provide the diagnosis. The diagnosis should be confirmed by enzyme assays. Hepatosplenomegaly with coarse facies, skeletal changes of dysostosis multiplex in X-rays (Figs 13.3a and b) and joint contractures suggest the clinical diagnosis of mucopolysaccharidosis. Subtypes of mucopolysaccharidoses can be clinically distinguished by the presence or absence of mental retardation, corneal clouding, etc. However, as for other inborn errors of metabolism, confirmation of the diagnosis by an enzyme assay is a must for genetic counselling and prenatal diagnosis. It is equally important to distinguish mucopolysaccharidosis from other lysosomal storage disorders such as mucolipidosis, mannosidosis, fucosidosis and sialidosis. This can be done by analysis of the urine for mucopolysaccharides by simple tests such as the acid albumin and toluidine blue tests. The absence of mucopolysaccharidurias will direct the investigations towards testing for

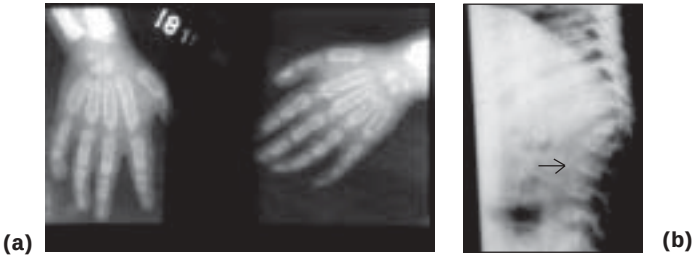


Fig. 13.3 Radiographs showing the changes of dysostosis multiplex in mucopolysaccharidosis. **a** Hands with modelling deformities of the metacarpals. The child also has contractures of the finger joints. **b** Spine showing platyspondyly and anterior beaking.

the presence of a group of disorders with similar clinical features.

One more group of disorders which needs mention due to its variability of presentation is the porphyrias. The presenting symptoms can be acute pain in the abdomen, muscle paralysis, dark-coloured urine, photosensitivity rash or neuropsychiatric symptoms. The diagnosis is difficult without a high level of suspicion, especially in the absence of a positive family history. An accurate diagnosis can be life-saving and helps to avoid unnecessary surgical intervention, associated complications and risks. Deafness, diabetes mellitus and other endocrinopathies can also be caused by genetic metabolic disorders, mainly of the respiratory chain.

DIAGNOSIS

The diagnosis of inborn errors of metabolism based only on clinical examination is usually not possible. However, detailed clinical examination gives clues to the diagnosis and helps to plan an investigative approach.

For some disorders mentioned in Table 13.1, a clinical examination can help in arriving at a differential diagnosis. However, a definite diagnosis will not be possible without detailed biochemical investigations. Nowadays, molecular defects have been identified as the cause for most of the inborn errors of metabolism. The role of DNA diagnosis is mainly limited to carrier detection and prenatal diagnosis.

INVESTIGATIONS

Investigations can be divided into three categories. The first category comprises of routine biochemical and haematological investigations

Table 13.4 Preliminary investigations for the evaluation of a suspected case with an inborn error of metabolism (coma, lethargy, seizures, ataxia)

- Blood pH and blood gases
- Blood sugar
- Serum sodium
- Serum potassium
- Serum creatinine
- Serum lactate
- Serum pyruvate
- Serum ammonia
- Serum alanine aminotransferase
- Serum calcium
- Serum phosphorus
- Serum creatinine phosphokinase (if hypotonia is present)
- Ketones in the blood and urine
- Serum osmolality
- Haemoglobin
- Total leucocyte count
- Differential leucocyte count
- Platelet count
- Red cell indices
- Peripheral smear examination

(Table 13.4). These level-one investigations are done in patients with acute illness. Haematological investigations, microbial cultures and imaging studies may be needed to look for non-genetic causes.

If there is no obvious non-genetic cause of illness, biochemical abnormalities like hypoglycaemia, acidosis, hyperammonaemia and hyperlactic acidaemia will give further clues to the diagnosis. Accordingly, investigations for aminoacidurias, organic acidurias, carbohydrate disorder, fatty acid oxidation disorders and urea cycle defects need to be ordered (Table 13.5).

The second level of investigations are directed more definitely towards a specific genetic metabolic disorder. These investigations provide a definitive diagnosis in certain disorders, though this is reached indirectly. Estimation of the levels of various metabolites (amino acids, mucopolysaccharides, etc.) in the urine and plasma, bone marrow examination, liver biopsy, nerve biopsy, muscle biopsy, electron microscopy, neuroimaging by computerized tomography or magnetic

Table 13.5 Investigative approach towards an acutely ill patient suspected to have an inborn error of metabolism

Recurrent vomiting, lethargy, seizures, coma without focal neurological signs

1. Acidosis
   - Ketosis (+)
     —Organic acidurias, maple syrup urine disease, respiratory chain disorders, glycogen storage disorder I, hereditary fructose intolerance
   - Ketosis (–)
     —Pyruvate dehydrogenase deficiency, fatty acid oxidation defects
2. Hyperammonaemia
   - Hypoglycaemia
     —Fatty acid oxidation defects
   - Normal sugar
     —Urea cycle disorders
3. Hypoglycaemia
   - Maple syrup urine disease, fatty acid oxidation defect, glycogen storage disorders
4. Hyperlactic acidaemia
   - Normal glucose
     —Respiratory chain disorders, carnitine palmitoyl transferase deficiency, multiple carboxylase deficiency
   - Hypoglycaemia
     —Hereditary fructose intolerance, fatty acid oxidation defects, glycogen storage disease I

Note: Causes such as encephalitis, lead poisoning, fasting, diabetic coma, drugs, poisoning or Addisonian crisis and other endocrine emergencies should be appropriately ruled out.

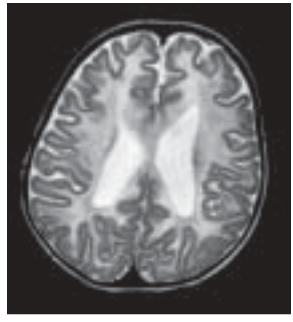


Fig. 13.4 T_2-weighted magnetic resonance imaging of the brain showing changes in the white matter suggestive of Canavan disease.

resonance imaging (Fig. 13.4) can be included in this group. The investigation of choice depends on the clinical findings and/or information obtained by the first level of investigations (Table 13.6). Tandem mass spectrometry and gas chromatography–mass spectrometry are investigations that estimate a number of metabolites and are used to screen a wide variety of inborn errors of metabolism.

Demonstration of an abnormally raised level of metabolite in the urine or serum is diagnostic in aminoacidurias, organic acidurias, Canavan disease, etc. The diagnosis of respiratory chain disorders is difficult. A battery of tests including lactate–pyruvate ratio, muscle biopsy with histochemical staining, tests for mitochondrial mutations in combination

Table 13.6 Investigations which are highly suggestive of inborn errors of metabolism

Investigation	Disorders
Bone marrow examination	Gaucher disease, Niemann–Pick disease
Liver biopsy	Glycogen storage disorders, alpha1-antitrypsin deficiency, neonatal haemochromatosis
Fibroblast microscopy	Mucolipidosis
Electron microscopy—rectal mucosa	Neuronal ceroid lipofuscinosis
MRI/CT scan of brain	Leucodystrophies, Leigh disease, Cockayne syndrome
Hair microscopy	Menkes kinky hair disease

MRI: magnetic resonance imaging; CT: computerized tomography

with the clinical picture are used. Often, the mitochondrial defects are tissue-limited, and biochemical and DNA tests have to be done on the tissue biopsy samples.

In addition to the second-level tests, for most disorders, estimation of the level of the protein involved—mostly an enzyme—is also essential. A low level of the enzyme would confirm the diagnosis. These enzyme assays can be done on leucocytes, fibroblasts and, in some cases, on the serum as well. Enzyme assays are also useful for carrier analysis, though carriers may sometimes have enzyme assay values overlapping with those of normal persons. Mutation analysis is available for many disorders and helps in prenatal diagnosis. For some neurometabolic disorders such as neuronal ceroid lipofuscinosis, a mutation-detection test is easier to perform compared to biochemical and electron microscopic tests.

For intermittently manifesting disorders, investigations during the attack are important. For disorders where dietary factors are the offending agents, the investigations may not show the diagnostic biochemical abnormality once oral intake of the offending agent is restricted and the patient is put on intravenous fluids. In cases with the possibility of inborn errors of metabolism, samples of the urine and serum, muscle and liver biopsy specimens, fibroblasts for culture and blood for DNA analysis should be collected and stored for further analysis. This is essential especially if the patient appears to be dying or has died undiagnosed.

Close collaboration among a clinician, biochemist and geneticist is essential for the diagnosis and management of inborn errors of metabolism. Referral to a specialized centre, if possible, is recommended. However, suspecting an inborn error of metabolism in the appropriate clinical situation is the responsibility of every clinician.

MANAGEMENT

The management of a patient with an inborn error of metabolism involves three aspects:

1. *Immediate supportive measures:* These are taken to stabilize the condition of the patient if he or she is seriously ill. This should be accompanied by laboratory investigations.
2. *Definitive management:* This will depend on the exact diagnosis. Complete curative management is available for a few disorders. Good palliative treatment based on various strategies such as dietary restriction, megavitamin therapy, enzyme replacement, etc. is available for many such disorders (*see* Chapter 18). Recombinant products and enzyme therapies are likely to have a major role in the

near future. Newer treatments are coming up and the number of disorders for which partial or complete improvement is possible is increasing.

3. *Genetic counselling:* This is important if a child is diagnosed to have a genetic metabolic disorder. The diagnosis is important even if a child is suffering from an untreatable disorder. Without accurate diagnosis of the proband, the exact risk of recurrence and prenatal diagnosis cannot be provided to the family. Sometimes, a family with a history of death of two offspring possibly due to a metabolic disorder comes for counselling and prenatal diagnosis. If the history and medical records of the affected children suggest some inborn error of metabolism, then the parents can be tested for their carrier status of the disorder. However, such investigations without confirming the disorder in the proband have limitations.

Most of the inborn errors of metabolism are autosomal recessive or X-linked and are usually of such a severe nature as to warrant prenatal diagnosis. The affected family needs to be told about the risk of recurrence in future offspring, possible treatment options and prognosis. If prenatal diagnosis is available, then the family needs to be informed about that option. All information about the time, procedure, error rate and availability of the prenatal diagnostic test should be given to the family.

Prenatal diagnosis can be done by biochemical tests such as enzyme assays and estimation of the defective or deficient protein in the foetal sample. Many enzymes can be reliably measured in chorionic villi or cultured amniotic fluid cells. Measurement of the level of a metabolite in the amniotic fluid can provide a reasonably reliable prenatal diagnosis. The examples are measurement of 17-hydroxyprogesterone for the diagnosis of congenital adrenal hyperplasia and N-acetyl aspartate (NAA) for the diagnosis of Canavan disease. Some enzymes are expressed only in liver cells. In such cases, a foetal liver biopsy may be needed for prenatal diagnosis.

DNA-based techniques are now available for the diagnosis of many metabolic disorders. These are useful especially when the enzyme is not expressed in the chorionic villi or when the biochemical basis of the disorder is not yet identified. These techniques are more accurate.

Genetic counselling should also provide information regarding the need for carrier screening of relatives of a family with an affected member. This is essential for X-linked disorders. If the family belongs to a community that allows consanguineous marriages, counselling regarding the avoidance of consanguinity, if possible, and investigations for carrier

detection among the relatives should be a part of genetic counselling.

SOME INBORN ERRORS OF METABOLISM

Disorders of amino acid metabolism

Phenylketonuria

Of the disorders of amino acid metabolism, phenylketonuria (PKU) is the best known example. It is caused by deficiency of the enzyme phenylalanine hydroxylase (PAH). The deficiency of PAH prevents the conversion of phenylalanine to tyrosine. Removal of phenylalanine from the diet is an effective treatment. If the dietary restriction is started immediately after birth, mental retardation can be prevented by controlling the blood phenylalanine levels. Untreated children are severely mentally retarded and have a fair complexion. Early instillation of a therapeutic diet needs presymptomatic diagnosis by neonatal screening. Classical PKU must be distinguished from benign hyperphenylalaninaemia (which does not need treatment) and hyperphenylalaninaemia due to defects in biopterin (cofactor for PAH) metabolism (dihydropteridine reductase or dihydrobiopteridine synthetase). The latter disorders lead to mental handicap despite the special diet therapy for PKU. Generally, patients with PKU are taken off a low phenylalanine diet after mid-childhood with the assumption that the function of the central nervous system will not be altered by hyperphenylalaninaemia. However, it has been seen that children born to mothers with PKU have microcephaly, mental retardation and cardiac defects, especially if the mother's blood phenylalanine levels are high during pregnancy. Thus, before planning a pregnancy, special diets for PKU should be re-started PKU women. The current recommendation is that all patients be kept on a phenylalanine-restricted diet for life.

Alkaptonuria

Alkaptonuria is the first identified inborn error of metabolism and is inherited in an autosomal recessive manner. The disease is caused by a block in the breakdown of homogentisic acid due to the deficiency of homogentisic acid oxidase, which accumulates and is excreted in the urine. The urine darkens on exposure to air. The same compound gets deposited in the cartilage of the ear and joints leading to early-onset osteoarthritis. Vitamin C in doses of 1–2 g per day is known to delay the

onset of joint problems.

Maple syrup urine disease

Maple syrup urine disease (MSUD) is one of the earliest and best known inborn errors of amino acid metabolism.. It is caused by the deficiency of branched chain a-ketoacid dehydrogenase. Branched chain dehydrogenase is a complex of multiple proteins and molecular defects in any of them can cause MSUD. The normal function of this enzyme is to oxidize alpha-ketoacids formed from leucine, isoleucine and valine. Neonates with the classical form of this disease develop severe acidosis and hypoglycaemia within a few days or weeks of birth. They have a high-pitched cry and hypertonia. There is a marked increase in the levels of leucine, isoleucine and valine in the blood. Some cases may have poor feeding ability, vomiting, lethargy, coma and seizures. Mild and intermittent forms have also been described. The classical form of MSUD can be diagnosed by a simple urine test. On addition of 2,4-dinitrophenylhydrazine (DNPH) to the urine, a yellow precipitate is formed. The therapy for an acute episode is the exclusion of proteins and a high intake of glucose, and intravenous administration of a high-calorie solution to minimize protein catabolism. Dialysis may be needed to remove high levels of leucine.

The results of management of the classical form are not encouraging. Most infants die or have significant neurological and intellectual impairment. However, mildly affected cases can be managed successfully.

Prenatal diagnosis is possible by DNA methods or by biochemical assays for dehydrogenase in cultured amniotic fluid cells or chorionic villi.

Disorders of carbohydrate metabolism

Galactosaemia

Galactosaemia is an autosomal recessive disorder. It is caused by deficiency of the enzyme galactose-1-phosphate uridyl transferase. Newborns present with vomiting, lethargy, failure to thrive, jaundice and anaemia in the second week of life. If not treated, they either die or develop mental retardation, cirrhosis and cataract. The diagnosis can be suspected by the presence of a reducing substance other than glucose in the urine and is confirmed by measurement of the enzyme in the red blood cells. Complications can be prevented by early diagnosis and feeding the child with milk substitutes. However, even well-treated patients have some deficits such as speech defects and some neurological sequelae. All affected females have ovarian failure.

Glycogen storage disorder I (von Gierke disease)

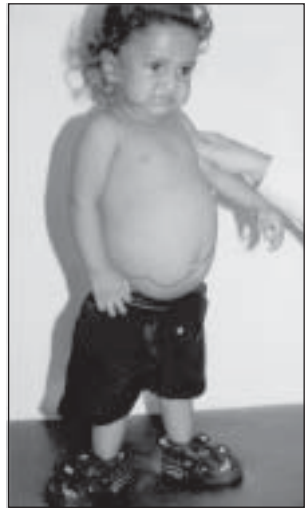


Fig. 13.5 An eighteen-month-old child with glycogen storage disorders type 1. A short stature, doll-like facies and hepatomegaly are characteristic features.

Glycogen storage disorder I (GSD I) or von Gierke disease is an autosomal recessive disease caused by the deficiency of glucose-6-phosphatase. Affected children present with hepatomegaly, and episodes of seizures, sweating and tachycardia due to hypoglycaemia. A doll-like facies is characteristic (Fig. 13.5). They may have severe lactic acidosis during episodes of minor infection. A liver biopsy specimen is used for enzyme assay. However, this is usually not necessary, as the clinical presentation with supportive investigations is diagnostic. The treatment is simple. Frequent feeds during the daytime and a diet rich in uncooked corn starch (to release glucose slowly) at night prevents hypoglycaemia. With this treatment, complications such as hepatic adenoma formation are reduced. Hyperuricaemia and hyperlipidaemia may need treatment.

Prenatal diagnosis by molecular methods is possible, as the gene and causative mutations are known.

Glycogen storage disorder II (Pompe disease)

Glycogen storage disease II (GSD II) or Pompe disease is also an autosomal recessive disorder. The deficient enzyme is acid alpha-glucosidase. This type of GSD primarily involves the muscles. The disease manifests in the first 6 months of life. There is developmental delay, hypotonia, macroglossia, hepatomegaly, cardiomegaly and congestive cardiac failure. Death occurs by 1–2 years of age. Late-onset disease is now being diagnosed frequently. The diagnosis is confirmed by an enzyme assay of the lymphocytes and fibroblasts. The enzyme can be assayed in the chorionic villi for prenatal diagnosis. The enzyme replacement therapy has been found successful in human trials..

Disorders of the urea cycle

The urea cycle is a five-step metabolic pathway that functions in the liver

cells for the removal of waste nitrogen from the amino acids arising due to the normal turnover of proteins. Two molecules of ammonia and one molecule of bicarbonate are converted to urea.

Ornithine transcarbamylase (OTC) deficiency

Ornithine transcarbamylase (OTC) is primarily found in the liver. Deficiency of OTC is an X-linked disorder. Male infants with OTC deficiency have an intractable and lethal neonatal course characterized by hyperammonaemia. However, males with mild defects survive. Most female carriers are asymptomatic but mild or intermittent manifestations are also seen. Treatment of an acute episode involves protein restriction with adequate caloric supplementation. Liver transplantation is the definitive therapy to prevent the inevitable brain damage.

Disorders of lipid metabolism

Familial hypercholesterolaemia is a common autosomal dominant single-gene disorder associated with high morbidity and mortality due to premature coronary artery disease. The disease is caused by mutations in the structural gene encoding the low-density lipoprotein (LDL) receptor, a cell surface protein responsible for binding LDL and delivering it to the interior of the cell. The disease is characterized by the elevation of plasma cholesterol carried by LDL, the principal cholesterol transport protein in plasma. Affected persons present in childhood or adolescence with subcutaneous deposits of lipid, known as xanthomas. More than 400 different mutations have been identified in the LDL receptor gene. Homozygotes have severe forms of the disease and may have myocardial infarction in childhood. Dietary restriction of cholesterol intake and the use of drugs that lower the cholesterol level decrease the risk of coronary artery disease.

Disorders of purine and pyrimidine metabolism

Lesch–Nyhan syndrome

This is an X-linked disorder caused by the deficiency of the enzyme hypoxanthine–guanine phosphoribosyl transferase, which results in an increased level of phosphoribosyl pyrophosphate. Motor delay is obvious by 4–6 months of age. Choreo-athetoid movements, mental retardation, spasticity and self-mutilation are the clinical features. Affected cases are detected by demonstrating a high ratio of uric acid to creatinine in the morning sample of urine. The treatment is mainly supportive as there is

no successful treatment for the neurological problems. For this reason, carrier detection and prenatal diagnosis are indicated.

Adenosine deaminase deficiency

About half of all children with the autosomal recessive form of severe combined immunodeficiency (SCID) have adenosine deaminase (ADA) deficiency. Bone marrow transplantation and ADA supplementation have been found to be successful in treating the disease. Gene therapy trials have also been done for ADA deficiency.

Organic acidaemias

Organic acidaemias are a group of inborn errors of (usually) amino acid metabolism in which the accumulated compounds are acids. Children affected with these disorders present with episodes of poor feeding, vomiting, lethargy, metabolic acidosis, hypoglycaemia, hyperammonaemia, neutropenia and thrombocytopenia. These episodes are often precipitated by intercurrent illness and high protein intake, and loss of development skills may follow. The organic acidaemias include autosomal recessive disorders such as methylmalonic acidaemia, propionic acidaemia, glutaric acidaemia, etc. Gas chromatography -mass spectrometry is the widely used methods for diagnosis. The treatment includes protein restriction and correction of dehydration and acidosis. Some cases respond to biotin or vitamin B_{12} therapy.

Lysosomal storage disorders

This group includes a number of disorders in which the deficiency of a lysosomal enzyme involved in the degradation of complex macromolecules leads to their accumulation. Children born with lysosomal storage disorders are usually normal at birth.

The age of onset of manifestations is variable in different disorders and may also be different in different varieties of the same disorder. However, the disorders are progressive and associated with a downhill course of variable duration. In most disorders which have an onset in infancy or early childhood, death occurs in the first or second decade of life.

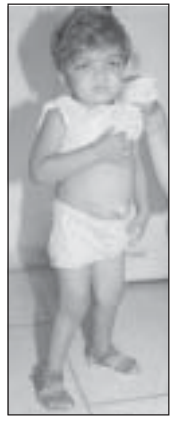


Fig. 13.6 Mucopolysaccharidosis type I (Hurler syndrome).

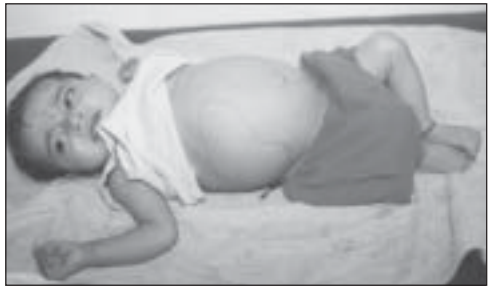


Fig. 13.7 Hepatosplenomegaly in an infant with Niemann–Pick disease type B.

Fig. 13.8 Two siblings with Gaucher disease type III.

Lysosomal storage disorders include three groups: (i) mucopolysaccharidoses, (ii) gangliosidosis and sphingolipidosis, and (iii) oligosaccharidosis. These are briefly described below.

Mucopolysaccharidoses

Mucopolysaccharidoses (MPS), as the name suggests, are progressive disorders caused by excessive intralysosomal accumulation of glycosaminoglycans (acid mucopolysaccharides) in various tissues. This results in clinical manifestations such as coarse facies, thick skin, corneal clouding, organomegaly, mental retardation, growth retardation, skeletal changes and joint contractures (Fig. 13.6). There are of many types of mucopolysaccharidoses with variable combinations of these manifestations. Except Hunter syndrome (MPS II), all other types of MPS are inherited in an autosomal recessive fashion. Bone marrow transplantation, if done early in the course of the disease, arrests the progression in some types of MPS. Enzyme therapy has recently become available for MPS I (Hurler syndrome).

Gangliosidosis and sphingolipidosis

This includes storage disorders such as GM_1 gangliosidosis, GM_2 gangliosidosis (Tay–Sachs disease), Niemann–Pick disease (Fig. 13.7), Gaucher disease (Fig. 13.8), Krabbe disease and metachromatic leucodystrophy (Fig. 13.2); the latter two predominantly involve the white matter of the brain. All these disorders involve the central nervous system with or without organomegaly; the peripheral nervous system is also involved. Enzyme replacement therapy for Gaucher disease type I is successful.

Oligosaccharidosis

Table 13.6 Clinical presentations of some lysosomal storage disorders

Disorder	Deficient enzyme	Age of onset	Mental retardation	Neurological deficit	Coarse facies	Hepatosplenomegaly	Joint contractures	Dysostosis multiplex	Others
GM_1 gangliosidosis	Ganglioside beta-galactosidase	Infancy to adulthood	+ (variable with age of onset)	+	+	+	+	+	Cherry red spot in the macula, gingival hypertrophy, macrocephaly
GM_2 type I Tay–Sachs disease	Beta-hexosaminidase	Infancy (juvenile chronic forms known)	+	+	–	–	–	–	Cherry red spot in the macula, hyperacusis, ataxia
Niemann–Pick A	Sphingomyelinase	Infancy to adulthood	+	+	–	+	–	–	Cherry red spot in the macula, failure to thrive
Niemann–Pick B	Sphingomyelinase	Infancy to adulthood	–	–	–	++	–	–	Pulmonary infiltrates
Gaucher disease type I	Glucosylceramide beta-glucosidase	Childhood to adolescence	–	–	–	+++	–	–	Expansion of the bone marrow, bone pains, fractures, hypersplenism
Gaucher disease type II	Glucosylceramide beta-glucosidase	Infancy	+	+	–	++	–	–	**[Au?]**
Gaucher disease type III	Glucosylceramide beta-glucosidase	Childhood	+ (slow progression)	+	–	+	–	–	Oculomotor apraxia, gaze palsy
Metachromatic leucodystrophy	Aryl sulphatase A	Infancy to childhood	+ (dementia in adult onset)	+	–	–	–	–	Peripheral neuropathy, ataxia

(*…cont.*)

Table 13.6 (*cont.*) Clinical presentations of some lysosomal storage disorders

Disorder	Deficient enzyme	Age of onset	Mental retardation	Neurological deficit	Coarse facies	Hepatosplenomegaly	Joint contractures	Dysostosis multiplex	Others
Mucopolysaccharidosis I (Hurler syndrome)	Alpha-L-iduronidase	1–2 years	+	+	++	++	++	++	Hydrocephalus, hernias, clouding, corneal short stature, cardiac valvular disease
Mucopolysaccharidosis II (Hunter syndrome)	1–duranate 2–sulphatase	2–4 years	+	+	++	++	++	++	No corneal clouding, nodular skin, cardiac involvement
Mucopolysaccharidosis IV-A (Morquio syndrome)	N-acetylgalactosamine	2–3 years	–	–	–	+	–	++ (spondyloepiphyseal dysplasia)	Disproportionate short stature, kyphosis, odontoid hypoplasia
Mucopolysaccharidosis VI (Maroteaux Lammy syndrome)	Aryl sulphatase B	Early childhood	–	–	++	++	++	++	Corneal opacities, cardiac valvular disease, short stature
I cell disease	GlaNac phosphotransferase	At birth	++	++	++	++	++	++	Corneal haziness
Fucosidosis	Alpha-L-fucosidase	6–12 months	++	+	+	+	++	++	Angiokeratomas, short stature, cardiomegaly
Sialidosis	Glycoprotein foetal sialidase	Neonatal, infancy and childhood	±	+	+	+	±	+	Cataract, myoclonic jerks, deafness

These disorders, on the one hand, share some common features with the MPS (coarse facies, organomegaly and skeletal changes of dysostosis multiplex) and, on the other, with sphingolipidosis (cherry red spot in the macula and demyelination of the peripheral nerves). Fucosidosis, sialidosis, I cell disease, pseudo-Hurler polydystrophy and mannosidosis belong to this group of disorders.

The clinical presentations of some lysosomal storage disorders are given in Table 13.6. The biochemical and metabolic bases of most of these disorders are well understood. Carrier detection and prenatal diagnosis is possible.

Neuronal ceroid lipofuscinosis

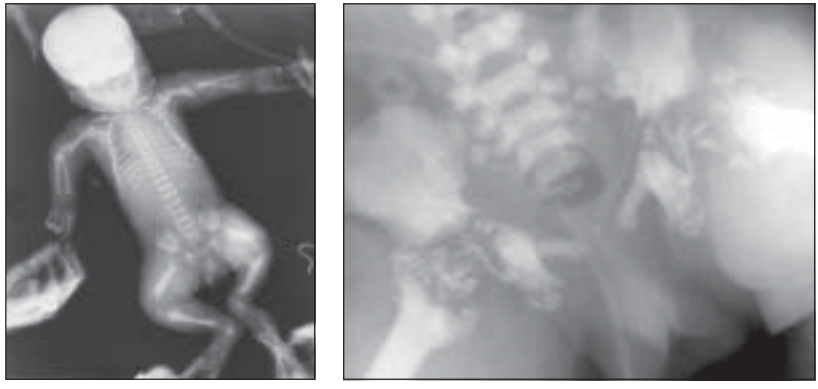
A separate group of central nervous system storage diseases, neuronal ceroid lipofuscinosis, is clinically well described but their pathophysiology is poorly understood. It is a common neurodegenerative disorder characterized by motor and mental retardation, blindness and seizures. Three major types of the disorder are known—infantile, classical late infantile and juvenile—caused by autosomal recessive mutations in the *CLN1, CLN2* and *CLN3* genes, respectively. The protein products of the *CLN1* and *CLN2* genes are lysosomal enzymes and that of the *CLN 3* gene is a lysosomal membrane protein. Eight CLN genes are known.

Peroxisomal disorders

Peroxisomes are subcellular organelles bound by a single trilayer lipid membrane. They are present in all cells and contain many enzymes which carry out the reactions of fatty acid oxidation and cholesterol biosynthesis. The term peroxisomal disorders was introduced by Goldfischer and Reddy (1984) to describe the newly recognized group of genetic diseases resulting from either a generalized or a more limited

Table 13.7 Classification of peroxisomal disorders

Group I	Peroxisome deficiency disorder	Zellweger syndrome, infantile Refsum disease, neonatal adrenoleucodystrophy
Group II	Deficiencies of multiple peroxisomal enzymes	Rhizomelic chondrodysplasia, Zellweger-like syndrome
Group III	Deficiency of a single peroxisomal enzyme and normal peroxisome structure	X-linked adrenoleucodystrophy, adult Refsum disease



(a) **(b)**

Fig. 13.9 A radiograph of a neonate with the peroxisomal disorder- rhizomelic chondrodysplasia punctata. **a** Whole baby **b** Pelvis and hips

impairment of peroxisomal functions. Peroxisomal disorders are classified into three groups. Group I disorders result from a general impairment of peroxisomal functions with a severely reduced number or complete absence of functional peroxisomes. In the other two groups, peroxisomal enzymes are deficient (Table 13.7).

Zellweger syndrome is a prototype of Group I and Group II disorders. It is characterized by dysmorphism, severe hypotonia, developmental delay, hepatosplenomegaly and cataract. The presence of neuronal migration anomalies, renal cysts, punctate calcification of the epiphyses are other features which, in association with facial dysmorphism, may mislead to the diagnosis of chromosomal anomaly. Neonatal adrenoleucodystrophy and infantile Refsum disease have many similarities with Zellweger syndrome but vary in the age of onset and have mild dysmorphism. Rhizomelic chondrodysplasia punctata is characterized by a disproportionately short stature with predominant shortening of the femora and humeri. There is punctate ossification of the epiphyses (Fig. 13.9). Very long-chain fatty acids are elevated in peroxisomal disorders c except rhizomelic chondrodysplasia punctata for the diagnosis of which estimation of plasmalogens is required. At present, no treatment is available for these disorders.

Disorders of respiratory chain deficiency

Oxidative phosphorylation, i.e. ATP synthesis through the respiratory chain, is a ubiquitous metabolic pathway that supplies energy to most

Box 13.1 Clinical features of respiratory chain deficiency disorders

- Hypotonia
- Developmental delay
- Psychomotor regression
- Spasticity
- Ataxia
- Leucodystrophy
- Seizures
- Myopathy
- Myoglobinuria
- Cardiomyopathy
- Nephrotic syndrome
- Proximal renal tubulopathy
- Recurrent vomiting
- Visual problems
- Diabetes
- Other endocrinopathies
- Deafness
- Ptosis
- Ophthalmoplegia
- Microcephaly
- Ketoacidosis
- Hypoglycaemia
- Hepatomegaly
- Hepatocellular dysfunction
- Anaemia

organs and tissues. Hence, a deficiency in the respiratory chain can affect any organ system, at any age. The oxidation reaction takes place in the mitochondria. The respiratory chain enzymes are coded by nuclear as well as mitochondrial genes. Any mode of inheritance can be observed in a mitochondrial disorder.

The clinical presentation of these disorders is extremely variable (Box 13.1) and similar to many genetic and non-genetic disorders, as well as infectious diseases. The diagnosis at the appearance of the first symptom of the disease may be difficult. A respiratory chain disorder should be considered if there are symptoms related to the neuromuscular system, especially in combination with non-neuromuscular symptoms and a progressive course. The disease can manifest at any age. The syndromes of a (mitochondrial) respiratory chain disorder are delineated on the basis of clinical features. The classification of some cases is difficult due to overlaping features.

Diagnostic evaluation should start with biochemical tests including estimation of plasma lactate, lactate–pyruvate ratio, blood glucose, free fatty acids, and tests for screening multiple organ functions. The diagnostic tests include specific enzyme assays on muscle biopsies and other tissues, and mutation detection by DNA studies. Demonstration of the presence of ragged red fibres (RRF) by trichrome Gomori staining is a hallmark of mitochondrial myopathy. However, absence of RRF does not rule out the diagnosis of a mitochondrial disorder. The results of the tests should be interpreted in combination with the clinical presentation.

The diagnostic tests for the investigation of oxidative phosphorylation

are difficult and have several pitfalls. The most important limitation is variability in the different tissues. The treatment is symptomatic and does not alter the course of disease.

Fatty acid oxidation disorders

Beta-oxidation of fatty acids is an important function of the mitochondria. It plays a major role in energy production, especially during periods of fasting. Plasma membrane carnitine transport defect and medium chain acetyl-CoA dehydrogenase (MCAD) defect are examples of fatty acid oxidation disorders.

Carnitine transport defect results in carnitine deficiency. The clinical presentations are hypoglycaemia, cardiomyopathy and skeletal muscle involvement. These patients respond dramatically to carnitine therapy. Deficiency of MCAD is the most common defect in the pathway. The most frequent presentation is episodic hypoketotic hypoglycaemia provoked by fasting. This may lead to sudden death. The treatment of an acute episode is administration of intravenous glucose.

Miscellaneous

In addition to the inborn errors of metabolism decribed above, there are many others such as Wilson disease (copper metabolism), haemochromatosis (defect in iron metabolism), alpha 1 antitrypsin deficiency which predominantly affect specific metabolic pathways and organs.

Inborn errors of metabolism have varied manifestations that present from birth to adulthood. Many manifestations, especially an acute presentation, mimic infections and acquired metabolic disorders. Awareness of the inborn errors of metabolism among clinicians of all specialties is essential for the early identification of cases that need further investigations.

14 Genetics of cancer

Cancer is a genetic disease caused by alterations in the genes and/or expression of genes. Genetic alterations in most of the cancers arise in the somatic cells and are transmitted to the successive generations of cancer cells but are usually not transmitted to the offspring of the patient. In about 5%–10% of cancers, the disease is transmitted from a parent to the offspring. Such types of inherited cancers are rare. Approximately 50 heritable cancer syndromes are known. These syndromes provide an opportunity to understand the genetic basis of cancers and the role of various genes in their pathogenesis.

GENETIC BASIS OF CANCER

Unregulated proliferation of cells is the basic pathology of cancers. Cell division and cell death are governed by several genes known to cause cancers(Box 14.1). Although at present many such genes are known, little is known about the complex interaction between genes responsible for the cancer phenotype.

Cancer is the result of mutations in a number of genes. The mutation takes place in one cell, which proliferates and the mutation gets transmitted to the daughter cells. Occurrence of more mutations gives these cells a growth advantage. As the cell divides and re-divides, more

Box 14.1 Functions of proteins coded by genes implicated in cancers

- Signalling pathway for cell proliferation
- Cytoskeletal components involved in the maintenance of contact inhibition
- Regulation of cell cycle
- Programmed cell death (apoptosis)
- DNA repair mechanism

and more genes get mutated, thus providing the cell with the potential to become more malignant. Thus, development of cancer is a multistage process (Fig. 14.1).

Several hypotheses have been put forward for over a century to explain the genetic basis of cancers in humans and other animals.

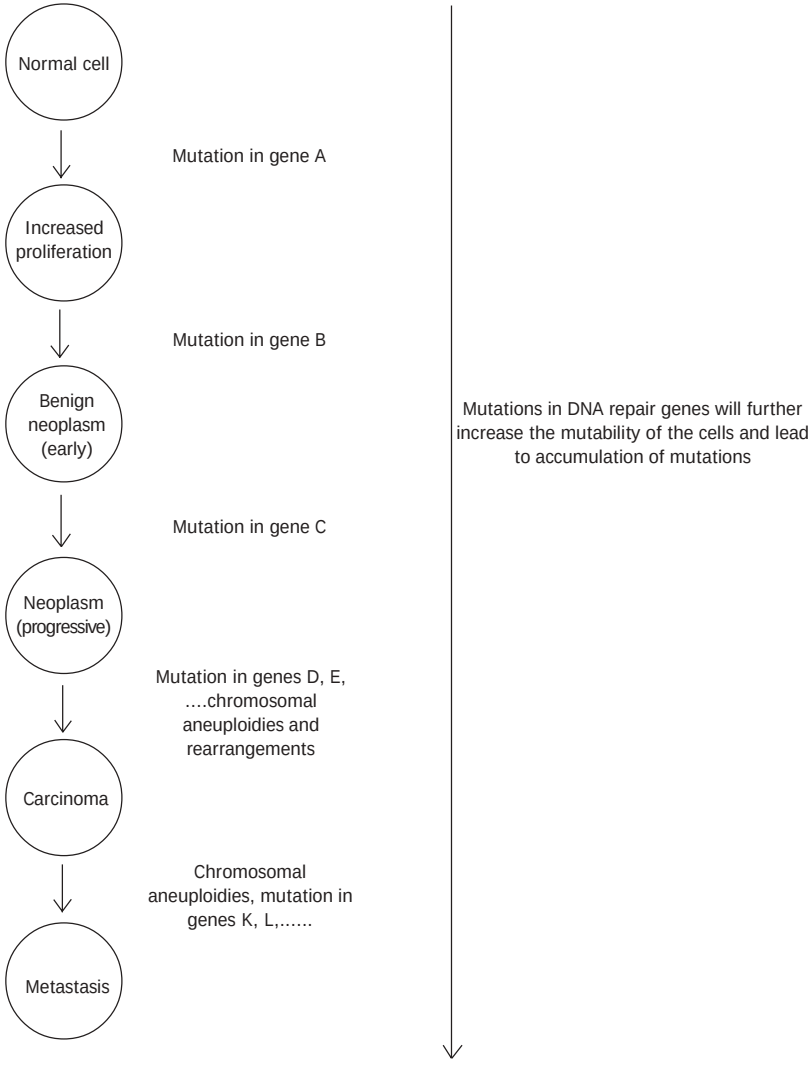


Fig. 14.1 Cancer development and its further evolution to an increasingly malignant nature is a multistep process.

Families with many members affected with cancer were reported in the past. However, there could be non-genetic reasons for familial clustering of cancers such as exposure to environmental agents or dietary factors. In 1911, experiments by Rouse gave insights into the pathogenesis of cancers. Rouse demonstrated that sarcomas could be induced in chicken following inoculation with cell-free filtrate obtained from an independent chicken sarcoma. Identification of an abnormal chromosome 22—Philadelphia chromosome—in the bone marrow cells of patients with chronic myeloid leukaemia (CML) led to the identification of the *abl* and *bcr* genes on chromosomes 9 and 22, respectively. Philadelphia chromosome is derived from the translocation between chromosomes 9 and 22. Cellular Abelson proto-oncogene (*abl*) is transferred from chromosome 9 into a region of chromosome 22 known as breakage cluster region (bcr), resulting in a chimerical transcript derived from the *abl* and *bcr* genes. This leads to the formation of a fusion protein consisting of bcr protein at the amino end and abl protein at the carboxyl end, which is associated with the alteration leading to leukaemia. The development of imatinib, a drug that blocks the action of the bcr–abl fusion protein and controls CML, is the first example of a success story in cancer research. Identification of more cancer-related genes and understanding their role in pathogenesis is likely to introduce newer therapeutic strategies and improve the outcome of cancer therapy.

Chromosomal aberrations are common in malignant cells. Some chromosomal abnormalities are more common in some types of cancers and it is known that they are the cause rather than the effect of cancer. Many such specific chromosomal abnormalities have led to the identification of cancer-related genes. One such example is translocation of the c-*myc* gene from chromosome 8 to chromosome 14 in Burkitt lymphoma. Leukaemias and lymphomas have been extensively studied for chromosomal anomalies because of the ease of cytogenetic analysis on blood and bone marrow specimens. New improved cell culture techniques and newer cytogenetic techniques such as comparative genomic hybridization have made the identification of genetic abnormalities in solid tumours easy. Cytogenetic analysis of cancers has become an important tool in cancer research as well as in the diagnosis and management of cancer.

Other than cytogenetic analysis, techniques such as DNA transfection and linkage analysis of families with cancer are used to identify cancer-related genes. In DNA transfection studies, DNA from primary human tumours is transferred into non-tumor cells and these cells acquire the growth potential of the tumour. Using this approach, the first oncogenic

gene—a mutated *H-RAS* gene—was identified in the human bladder cancer cell line. Linkage analysis in families with breast carcinoma led to identification of the *BRCA1* and *BRCA2* genes.

CANCER-CAUSING GENES

Cancer-causing genes are categorized into three broad classes—oncogenes, tumour suppressor genes and DNA repair genes.

Oncogenes

An oncogene is a mutated form of a normally occurring gene known as a proto-oncogene. Proto-oncogenes have important functions in normal growth and development. When they mutate to oncogenes, they gain new functions that facilitate malignant transformation by stimulating proliferation, increasing blood supply to the tumour, or inhibiting apoptosis.

Although a protooncogene is a normal gene and a cellular oncogene (c-*onc*) its mutated copy, these terms are used interchangeably. The identification of oncogenes began with studies of viruses that can transform normal cells into tumour cells. These retroviruses are not oncogenic in humans but can rapidly induce tumours in animals and transform cells *in vitro*. The genome of these viruses contains nucleic acid sequences that were acquired or transduced from host cells during genetic recombination. These sequences are termed viral oncogenes (v-*onc*).

Oncogenes have a dominant effect at the cellular level, i.e. mutation in a single copy of the gene leads to its activation and results in a malignant change in the cell. Examples of oncogenes are given in Table 14.1.

Table 14.1 Cancer-causing genes

Oncogene	Properties of the protein coded by the oncogene	Type of tumour
K-RAS	p21 GTPase	Colorectal, pancreatic cancers
H-RAS	p21 GTPase	Urinary bladder cancer
MYC	Transcription factor	Burkitt lymphoma
bcr–abl	Tyrosine kinase	Chronic myeloid leukaemia
NEU	Growth factor receptor	Breast, ovarian cancer
EGFR	Growth factor receptor	Gliomas, carcinomas

Tumour suppressor genes

The normal function of tumour suppressor genes is to block the development of tumours by regulating cell growth. Loss of function of the gene leads to uncontrolled cell division, abnormal cell growth, or defective apoptosis. In contrast to oncogenes, tumour suppressor genes act in a recessive manner at the cellular level, i.e. mutations in both the alleles of a gene is needed to cause cancer.

The study of inherited cancers provides information on tumour suppressor genes. In inherited cancer syndromes, i.e. cancer running in families, one of the two copies of the tumour suppressor gene is mutated and is passed on from one generation to the next. The mutated gene is present in all the cells of an individual. This type of mutation is known as germline mutation. When the second copy of this gene gets mutated in one or more cells of the body, the cell develops into a tumour. This sequence of events, known as 'two hits', explains the autosomal dominant pattern of inheritance of some cancers. The 'two-hit hypothesis' was put forward by Knudson in 1971 (Fig. 14.2) based on an epidemiological study of a large number of cases of sporadic and familial retinoblastomas. Identification of the retinoblastoma gene and germline mutations in familial retinoblastoma has proved the 'two-hit hypothesis'. In inherited retinoblastomas, the mechanism of somatic mutation of the second copy of the gene can be gene deletion, point mutation, loss of the chromosome, etc. In sporadic retinoblastomas, both mutations occur in somatic cells and hence, the disease is unifocal and of late onset as against multifocal, bilateral retinoblastomas of early onset in the inherited variety.

The gene *p53* is an important tumour suppressor gene found to be mutated in 25% cases of breast cancer and more than 50% of bladder, colon and lung cancers. The p53 protein was first identified as a host cell protein bound to T antigen, the dominant transforming oncogene of the DNA tumour virus SV40. The p53 protein functions as a check-point in the cell cycle at the G_1 phase. It interacts with other factors and prevents damaged DNA from being replicated. Mutant p53 protein is stable and can form complexes with the p53 protein acting in a dominant negative manner.

Table 14.2 summarizes some familial cancer syndromes and the genes causing them

DNA repair genes

The third group of genes involved in cancers are the DNA repair genes. These genes do not affect cell growth but a mutation in these genes

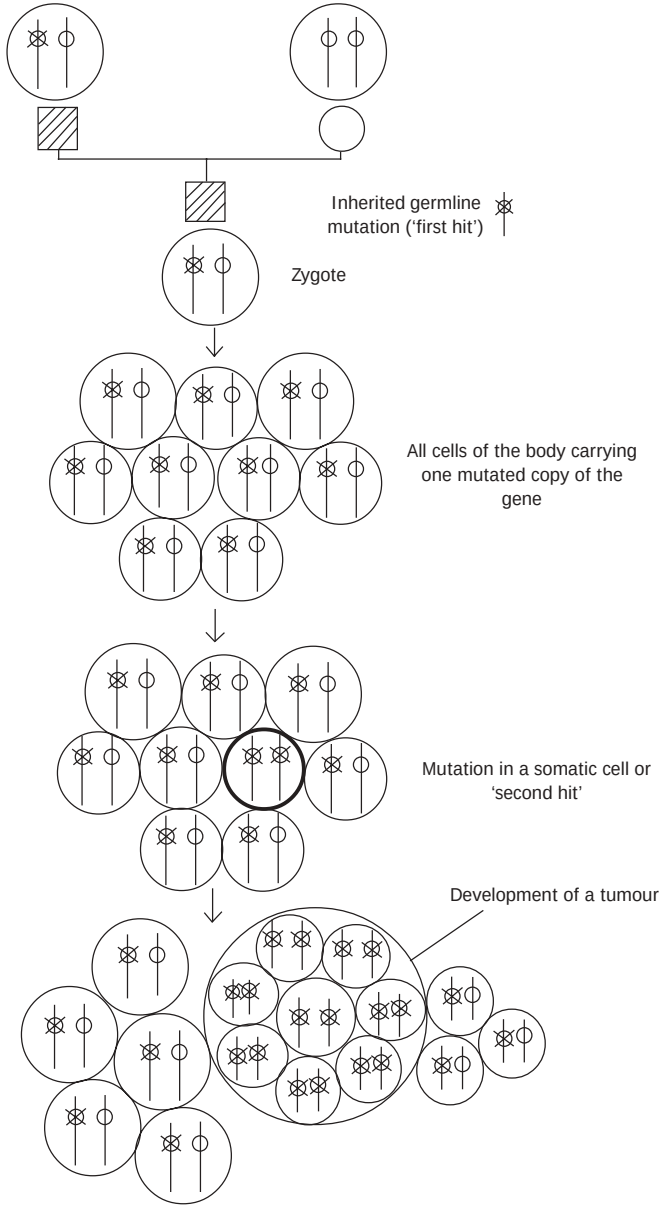


Fig. 14.2 'Two-hit hypothesis'. In inherited retinoblastomas, one copy of the mutated tumour suppressor gene is present in all cells of the body. If one or more cells of the apprepriate tissue acquire a somatic mutation, that cell with both non-functioning copies of the tumour suppressor gene will develop into a tumour.

Table 14.2 Summary of some familial cancer syndromes

Syndrome	Tumour	Other cancers/traits	Gene	Gene function
Familial retinoblastoma	Retinoblastoma	Osteosarcoma	*RB1* *	Regulates the cell cycle and transcription
Familial adenomatous polyposis	Colorectal cancer	Intestinal polyposis, jaw osteomas, desmoid tumour	*APC* *	Regulates the level of the transcriptional activator beta-catenine
Li–Fraumeni syndrome	Sarcomas, breast cancer	Brain tumour, leukaemia, lymphoma	*p53* * (*Tp53*)	Regulates transcription, responds to DNA damage, controls the cell cycle and apoptosis
Neurofibromatosis II	Acoustic neuromas	Meningioma, gliomas	NF_2 *	Links cell surface molecule to cytoskeleton
Multiple endocrine neoplasia type II	Medullary thyroid cancer	Phaeochromocytoma, parathyroid hyperplasia	*RET* †	Receptor tyrosine kinase
Familial breast cancer	Breast cancer	Ovarian cancer	*BRCA1* ‡	Break repair of DNA

* Tumour suppressor gene; † Oncogene; ‡ DNA repair gene

increases the rate of mutation in various other genes in the cell, including oncogenes and tumour suppressor genes. The resulting accumulation of mutations leads to the development of tumour. This group of genes includes those responsible for cancer syndromes inherited in an autosomal dominant fashion such as Li–Fraumeni syndrome, breast carcinoma and hereditary non-polyposis colon cancer (HNPCC). The autosomal recessive syndromes belonging to this group are ataxia telangiectasia, Bloom syndrome, xeroderma pigmentosum and Fanconi anaemia.

FAMILIAL CANCERS

Some cancers occur more frequently in relatives of patients than in the general population. These cancer syndromes follow the Mendelian pattern of inheritance, indicating that mutation in a single gene is the

Table 14.3 Factors suggestive of inherited cancer susceptibility in a family

- Several first- or second-degree relatives with a common cancer
- Several close relatives with related cancers, e.g. breast and ovarian cancer
- Two family members with the same rare cancer
- An unusually early age of onset
- Bilateral or multifocal tumours
- Tumours in two different systems in the same individual
- Associated features suggestive of a cancer syndrome. For example, mucosal pigmentation in Peutz–Jeghers syndrome, café au lait spots in neurofibromatosis

predominant contributory factor to disease causation. Inherited cancers account for only 5% or less of all patients with cancers. However, it is important that at-risk individuals in such families be identified through family history and by mutation detection if possible, and offered regular surveillance for the early detection of cancers. This can greatly improve the prognosis. Study of these families has also contributed considerably to the knowledge of genes involved in cancers. It should be noted that cancers occuring in these familial cancer syndromes also occur sporadically because of aquired mutations in somatic cells. In fact, more often cancers are not inherited. For example, HNPCC accounts for only 5% of all colon cancers. Hence, the most important step in counselling for cancers is to identify families who are at higher risk for cancers (Table 14.3). The genetics of some cancers is discussed below to illustrate the principles of genetic counselling for familial cancers.

Breast and ovarian carcinoma

Carcinoma of the breast is a common cancer occurring in women. Approximately 1 in 12 women develop breast cancer. In some families, it appears to follow an autosomal dominant pattern of inheritance. These account for less than 10% of cases with breast cancer. The two genes responsible for causing breast carcinoma are *BRCA1* and *BRCA2*. Mutations of the *BRCA1* and *BRCA2* genes accounts for about 50% and 30% of familial breast cancer, respectively. These mutations also increase the risk for ovarian and male breast cancer. If a woman is found to be a carrier of *BRCA1* or *BRCA2* mutation, her lifetime risk of developing carcinoma of the breast is 80%–85%. The risk for ovarian cancer is more in *BRCA1* mutation carriers than in *BRCA2* mutation carriers. In men, only 6%–10% of patients with breast cancer have mutations in the *BRCA* genes, irrespective of the family history. Mutation-detection tests are available but are technically demanding and costly. Hence, mutation-

Table 14.4 Guidelines for offering a mutation detection test for *BRCA1* and *BRCA2* genes

- ≥3 first- or second-degree relatives with breast cancer (all on one side of the family), regardless of the age
- ≥2 affected relatives, but at least one diagnosed at <45 years of age
- ≥1 case of ovarian cancer at any age and ≥1 case of breast cancer at any age
- Multiple primary or bilateral breast cancers
- Male breast cancer

detection tests need to be offered only to high-risk families (Table 14.4), i.e. to those who have at least 10% probability of being a mutation carrier based on the family history.

Women with a low or moderate risk of breast carcinoma need to be offered mammographical monitoring. Families at high risk need closer surveillance for carcinoma of the breast and ovaries. Mutation detection in these families helps in the definitive identification of carriers and thus, identification of persons who need close follow up and those who do not. Thus, mutation analysis of at-risk individuals removes uncertainty regarding the mutation carrier status. It should be noted that mutation detection needs to be done first in the affected family member. If a mutation is identified in the affected person; then the same mutation can be looked for in the relatives to identify the carriers. If a mutation is not detected in the affected individual then carrier detection test cannot be offered to the relatives. In such a case, the risk estimated through family history remains the same and does not decrease. Carriers of mutation may be offered prophylactic drug therapy, prophylactic mastectomy and oophorectomy. These procedures markedly decrease the risk of cancer and death, but may not be acceptable as they are radical.

Mutation detection and identification of carriers involves complex issues regarding the sensitivity of the tests, implication of positive and negative tests, marked increases in the risk of cancer in the future, information regarding surveillance protocols and prophylactic measures. Hence, good counselling before and after the test is essential. Other issues such as insurance, psychological reaction and confidentiality are also important. The families at high risk for breast carcinoma not only need mutation-detection tests but also education, counselling and psychological support.

Retinoblastoma

Retinoblastoma, a tumour of the retina occurring in children, is a

prototype of tumours associated with tumour suppressor genes. The incidence is 1 in 20,000 births. Forty per cent of the cases of retinoblastoma are familial, while the rest are sporadic. Retinoblastoma is detected at any time from birth to about 7 years of age, but commonly occurs in children less than 3 years of age.

Retinoblastoma is caused by a mutation in the *RB1* gene located on the long arm of chromosome 13 (13q14). The location was found out because of detection of a microscopic deletion in 5% of patients with retinoblastoma. The *RB1* gene product, p110-RB1, is a cell cycle regulator and also plays an important role in embryogenesis.

In most cases of inherited retinoblastoma, the germline mutations are deletions, major structural rearrangements or point mutations. These can be detected only by molecular techniques. Detection of mutation in an affected person provides an accurate estimate of the risk of occurrence of the disease in siblings and offspring, and opens up options for prenatal diagnosis. Mutation detection is also useful as carriers of germline mutation are at a 2000 times higher risk than the general population for developing osteosarcoma. The risk is more if the child has received radiotherapy. Pretreatment screening for mutations is also useful in unilateral sporadic cases as 15% of them have germline mutations.

In familial cases, the patient has one germline mutation and the other is an acquired somatic mutation. Thus, heterozygous carriers of germline mutation often have multiple tumours that may affect both the eyes. Heterozygotes usually acquire a secondary somatic mutation, which gives rise to a tumour, but the chance is not 100% and some carriers of germline mutation will not develop tumour at all. Thus, the penetrance of the retinoblastoma gene is high but not complete, and although bilateral tumours are common in familial cases, 15% of unilateral tumours are also inherited. In familial cases, mutation detection is not necessary for genetic counselling regarding the risk of recurrence of the disease in the family, but will be necessary to provide prenatal or presymptomatic diagnosis to an at-risk relative. The risk of recurrence can be given to the family based on the clinical data (Table 14.5).

Sporadic cases with bilateral tumours have a germline mutation that is usually ‘new’ and is not inherited from the parents. Hence, the risk of occurrence in the siblings is low. But the chance that the mutation will be transmitted to the offspring is 50%. Unilateral sporadic cases usually do not have a germline mutation and thus have a low risk of occurrence in the offspring and siblings. Mutation detection in sporadic cases can thus help in identifying cases with germline mutations and in management and counselling, but these are technically difficult and may

Table 14.5 Risk of recurrence of retinoblastoma depending on clinical information

Family history	Tumour		Type of mutation		Risk of recurrence of the disease	
	Unilateral	Bilateral	Somatic	Germline	For siblings (%)	For offspring (%)
Sporadic	✓	–	85%	15% (new)	1 *	1
Sporadic	–	✓	–	New	2 *	45
Familial	✓	–	–	Inherited	≤45	≤45
Familial	–	✓	–	Inherited	45	45

* Approximate value, The penetrance of the disease is 90%.

not be logistically and financially possible for all patients.

Genetic counselling of a family with retinoblastoma is important. Familial cases will be aware of the inherited nature of the disease but adequate care should be taken while conveying the genetic nature of the disease and the possibilities of recurrence in sporadic cases. The investigative work-up, treatment plan and probable outcome of retinoblastoma should be discussed with the family before taking up the issue of recurrence. Examination of siblings or other at-risk relatives should also be carried out.

Familial colon cancer

Cancers of the colon and rectum are the commonest of the cancers. A small proportion of them are transmitted in an autosomal dominant fashion. There are two forms of familial colon cancer. One is associated with numerous adenomatous polyps in the colon (familial adenomatous polyposis [FAP]), while the other- HNPCC is not associated with polyps. FAP and HNPCC account for 1% and 5%–8% of all cancers of the colon and rectum, respectively. The *APC* gene is responsible for causing FAP. Carriers of mutation in the *APC* gene develop numerous benign polyps in the colon during the first two decades of life. About 80% of these cases develop malignant tumours. The normal function of the *APC* gene is to inhibit abnormal cell proliferation. A mutation in one allele of the *APC* gene is present in all cells of the body of mutation carriers, while both alleles are mutated in those with adenomas and carcinomas.

Individuals who have one or more first-degree relatives with FAP are at high risk and should be screened by flexible sigmoidoscopy by the age of 10–12 years. Patients with colonic polyps or a mutation in the *APC* gene or both should undergo annual endoscopic examination and removal of the polyps. Usually by the age of 20 years, there are too many

polyps and at that time, prophylactic subtotal colectomy followed by annual endoscopy of the remaining rectum are recommended.

Patients with HNPCC do not have any phenotypic presentation, such as adenomas, which can help in the early diagnosis of mutation carriers. The possibility of HNPCC should be thought of if there is a clustering of colon cancer and other HNPCC-associated cancers such as cancers of the rectum, endometrium and small bowel or ureter. The diagnostic criteria for HNPCC are given in Table 14.6.

The genes responsible for causing HNPCC are DNA mismatch repair genes *hMLH1, hMSH2, hMSH6, hPMS1* and *hPMS2*. These genes recognize and repair mispaired bases arising from errors in DNA replication. Mutations in the *hMLH1* and *hMSH2* genes are more common. The presence of one normal allele of these genes is adequate for normal functioning. However, when both the alleles are mutated, the cell acquires hundreds of mutations, some of which are in the oncogenes and tumour suppressor genes, thus leading to cancer.

The lifetime risk for colon cancer in heterozygous carriers of HNPCC genes is 90% in males and 70% in females. Females, in addition, have a 40% risk of developing endometrial cancer. The Amsterdam criteria are used to identify families with HNPCC so that they can be offered genetic testing and counselling. Younger patients without any family history are also candidates for genetic testing. The possibility of mutation of DNA mismatch repair genes can be identified by testing tumour DNA for microsatellite instability. Tumours with mutated mismatch repair genes will show accumulation of different sizes of microsatellite markers due to replication errors. This is known as a replication error-positive phenotype and is used to identify patients for analysis of HNPCC gene mutation.

Regular screening by colonoscopy every 3–5 years and ultrasonography every year starting from 35 years of age is suggested for HNPCC family members to ensure an early diagnosis of cancers. If gene mutations are identified in an affected person in a family, genetic testing of the relatives for identification of carriers becomes possible.

Table 14.6 Amsterdam criteria II for the diagnosis of hereditary non-polyposis colon cancer

1. Exclusion of familial polypopsis in cases with colorectal cancer and verification of the tumour type by pathological examination
2. Diagnosis of hereditary non-polyposis colon cancer-associated cancer in at least three relatives, one of them being the first-degree relative of the other two
3. Two or more successive generations are affected
4. At least one of the patients should be less than 50 years of age at the time of diagnosis

von Hippel–Lindau disease

von Hippel–Lindau (VHL) disease is a rare cancer-predisposing syndrome associated with the involvement of multiple systems. The commonest tumours are retinal and cerebellar haemangioblastomas. Pheochromocytoma, renal carcinoma, haemangioblastomas of the spinal cord, and cysts in the kidneys, pancreas and epididymis are other associations. The presence of two haemangioblastomas or one haemangioblastoma and other visceral lesions, or a family history is sufficient for the diagnosis of VHL disease. A mutation in the *VHL* gene causes the disease. The carriers of gene mutation, identified through family screening, need to be screened regularly for the detection of central nervous system tumours, kidney tumours and pheochromocytoma by investigations such as ophthalmological examination, brain magnetic resonance imaging, abdominal computed tomography scan or ultrasonography and estimation of urinary vanillyl mandelic acid (VMA; for pheochromocytoma). If mutation analysis is not available, the same screening protocol should be followed for all probable heterozygote carriers in the family.

Low penetrance genes

Many families do not follow the characteristic autosomal dominant transmission of cancers but show an increased risk of cancers in relatives compared with the general population. Such types of familial aggregation of cancers can be due to genetic mutations or polymorphisms, which slightly increase the risk of cancers. In other words, these can be described as low penetrance genes. An example is polymorphism in the *APC* gene, the T and A transversion at nucleotide 3920, which is possibly associated with some increase in the risk for colorectal cancer. Heterozygotes for ataxia telangiectasia are also known to be at an increased risk for carcinoma of the breast.

SPORADIC CANCERS

Though a lot is known about familial cancers, these account for a small percentage of cancers. Some genes responsible for familial cancers may be responsible for sporadic cancers by way of acquiring somatic mutations. Studies on the common form of bowel cancer, which is not inherited, has shown deletion of the *FAP* gene in 40% of adenomas and 70% of carcinomas of the colon. On the contrary, *BRCA1* and *BRCA2* gene mutations are not identified in sporadic breast cancers. Germline mutation of the *TP53* gene is responsible for causing a rare familial cancer

syndrome known as the Li–Fraumeni syndrome. Somatic mutations of *TP53* causing loss of function of both the *TP53* alleles is one of the commonest genetic alterations in sporadic cancers including those of the breast, ovary, bladder, cervix, oesophagus, lung and skin. Research on cytogenetic abnormalities in cancers is providing more information about cancer-related genes and their role in the aetiopathogenesis of cancers. An epigenetic phenomenon such as methylation has been found to be responsible for the loss of function of many cancer-related genes (*see* chapter2 page 25). Thus, even if there is no alteration in the sequence of a gene, methylation of the gene can result in silencing of the gene leading to cancer. The increasing information about aetiopathogenesis of cancers is opening the newer avenues of treatment.

GENETIC TESTING FOR CANCER SUSCEPTIBILITY

Increasing knowledge of the genetics of familial cancers has made genetic testing for cancer predisposition possible. However, it must be noted that familial cancer syndromes account for a small number of all cancers. Even for those cancer syndromes for which testing is available, there are some limitations of the techniques and availability of data. The tests involve a long-term undertaking for the individual at risk and his/her physician. The physician involved in genetic counselling of such patients should have a good understanding of the tests, their indications, interpretation, limitations and utilities. The guidelines for genetic testing of cancer are listed below:

1. Only individuals with a definite family history suggestive of a genetic cancer syndrome should be offered genetic testing.
2. The test should be correctly interpreted.
3. The result of the test should either aid in the diagnosis or modify the management of the patient or family member.
4. Children should be tested only if the malignancy develops in childhood and if the testing helps in improving the outcome by early diagnosis or modification of management.
5. Physicians should inform at-risk individuals about the risks and benefits of carrier detection. Information about early detection of cancers, and preventive and management modalities should be provided during pre- and post-test counselling. The limitations of the tests should be explained before they are carried out.

Genetic testing for cancer susceptibility is a continuously evolving field based on intense research efforts. Hence, physicians involved in testing and counselling should have up-to-date knowledge of the disease

and the genetic tests available. Physicians should be well trained and capable of dealing with psychological and social issues associated with the identification of individuals at high risk for cancer. All efforts should be made to protect the confidentiality of the genetic information. However, the patient should be reminded of the importance of communicating the results of the test to family members who may benefit from the information. None of the cancer susceptibility tests currently available are as yet appropriate for screening asymptomatic individuals in the general population.

Section III

Techniques in medical genetics

15 Recombinant DNA technology

INTRODUCTION

The ability to manipulate genetic material is the basis of recombinant DNA technology. The term 'genetic engineering' describes the technology appropriately. With this technology, DNA can be artificially cut, joined and synthesized. This has made it possible to analyse normal and disease-causing genes, study the functions of a gene, augment or suppress gene expression and insert new DNA into a cell. All these possibilities have improved the understanding of biology, health and diseases, and have found many applications in medicine (Table 15.1). However, the ultimate goal of genetic engineering, i.e. the replacement of a defective gene with a normal one, is yet to be achieved.

Table 15.1 Applications of recombinant DNA technology in medicine

- DNA-based diagnostic tests for
  - —carrier detection
  - —prenatal diagnosis
  - —presymptomatic diagnosis of late-onset disorders such as familial cancer of the breast and Huntington chorea
  - —infectious diseases
  - —cancers
- Study of gene functions for drug designing
- Pharmacogenomics
- Synthesis of recombinant products for the treatment of various diseases, e.g. antihaemophilic Factor VIII for haemophilia A and erythropoietin for chronic renal failure
- Recombinant vaccines: Hepatitis B vaccine
- Gene therapy for cancers and monogenic diseases

TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction enzymes

Restriction enzymes are endonucleases of bacterial origin. The names of these enzymes are derived from the bacteria from which the enzyme is extracted, e.g. *Eco* RI is the name of the restriction enzyme obtained from *Escherichia coli*. The function of these enzymes is to degrade the foreign DNA of viruses infecting the bacteria. They cut the foreign DNA at specific sites determined by the DNA sequences. These sites are small, palindromic sequences containing 4–8 base pairs. Depending on whether the cleavage points fall on the symmetry axis or not, the cut ends will be 'blunt' or 'sticky', respectively (Figs 15.1a and b).

After an enzyme cuts the target DNA at two different sites, it can be joined to DNA from a different source, which is also cut by the same restriction enzyme. The enzyme used for joining the two DNA fragments is called DNA ligase. This results in the formation of a recombinant DNA. Restriction enzymes are thus an important component of genetic engineering. They are used in cloning, Southern blot analysis and for detecting a change in the base sequence. The latter is possible only if a change in the nucleotide sequence (disease-causing mutation or polymorphism) either leads to the abolition of the restriction site normally present or creates a new restriction site.

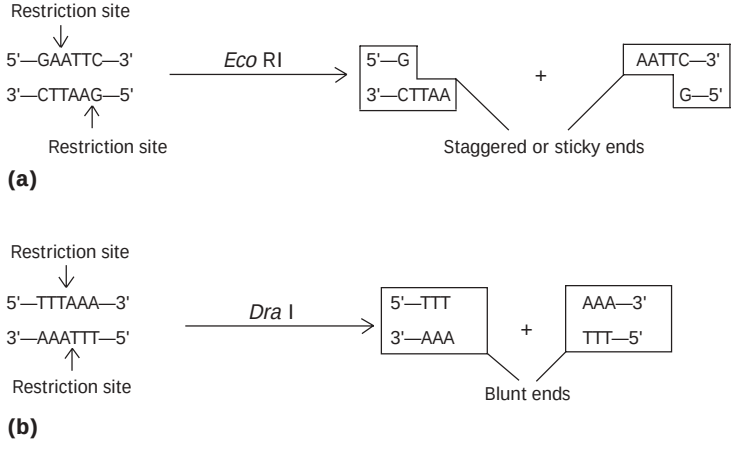


Fig. 15.1 Action of restriction endonucleases on DNA. **a** Production of staggered or sticky ends. **b** Production of blunt ends.

Table 15.2 Enzymes used in recombinant DNA techniques and their functions

Enzyme	Function
DNA ligase	Joins DNA molecules
DNA polymerase	Synthesizes double-stranded DNA using a previously available DNA strand as a template
Taq DNA polymerase (heat stable)	Used in polymerase chain reaction
Reverse transcriptase	Transcribes messenger RNA (mRNA) into complementary DNA (cDNA)
DNase I	Produces single-stranded cuts in the DNA

Other enzymes

Many other enzymes of bacterial origin are used in recombinant DNA technology (Table 15.2).

Probes

A probe is a small fragment of DNA or RNA that detects the DNA of interest in a sample. Its sequence is complementary to that of the DNA segment of interest. It can be single- or double-stranded. A probe can be obtained from cloned genes or can be synthesized. It has to be labelled with radioactive isotopes or fluorescent dyes.

The two strands of the probe and DNA sample are separated by heating. On reannealing, the probe hybridizes with the complementary DNA sequences, which can be identified because the probe is labelled with a detectable label such as radioactive phosphorous or a fluorescent dye. The probe will hybridize only with the exact complementary nucleotide sequence or partial complementary sequence of the DNA sample, depending on how stringent the hybridization conditions are (temperature, salt concentration or presence of denaturants such as formamide).

Vectors

A gene or DNA fragment to be studied has to be isolated and made into multiple copies before it can be tested further by hybridization, sequencing, etc. A process to get multiple copies of a DNA segment by selective amplification is known as cloning. Cell-based cloning method involves attaching a foreign DNA fragment to the DNA (carrier DNA) of a system which is capable of independent replication.

Table 15.3 Commonly used vectors

Vectors	Size of the DNA fragment they can carry (kilobases)
Plasmids	Up to 15
Bacteriophages	Up to 20
Cosmids	Up to 45
Bacterial artificial chromosomes (BACs)	100–300
Yeast artificial chromosomes (YACs)	100–2000

The term 'vector' denotes a carrier DNA molecule used in cloning the DNA segment of interest. When a vector containing the target DNA (recombinant vector) is introduced into the host cell, it replicates with the DNA of the host cell resulting in production of multiple copies of the target DNA. For naturally occurring vectors to be used for DNA cloning, they need to be modified to ensure that the foreign DNA is inserted at a specific location and the recombinant vector containing the foreign DNA can be identified. The common types of vectors used and the size of the DNA fragment they can carry are listed in Table 15.3.

Plasmids

Plasmids are circular, double-stranded DNA molecules that occur naturally in bacteria and replicate extrachromosomally in bacteria or yeast. They usually carry antibiotic resistance genes and can easily pass from one bacterium to another. Plasmids are also distributed to daughter cells during cell division.

Bacteriophages

Bacteriophages or phages are viruses that infect bacteria. A DNA fragment can be introduced into a virus which then infects a bacterium and multiplies. During replication, the inserted DNA fragment also multiplies and gets incorporated into the daughter cells.

Cosmids

Cosmids are modified plasmids and contain cos sequences found in λ phage. They can incorporate DNA fragments of up to 45 kilobases (kb).

Bacterial artificial chromosomes

Many vectors used for DNA cloning in bacteria contain a high-to-medium number of replicons. The inserted DNA fragments in these vectors are unstable and there may be deletion or rearrangement of fragments. Modified vectors such as bacterial artificial chromosomes (BACs) can carry large foreign DNA fragments of up to 300 kb in size.

Yeast artificial chromosomes

These chromosomes contain telomeres, centromeres and the autonomous replicating sequence of the yeast chromosome. Yeast artificial chromosomes (YACs) can carry large fragments of DNA (up to 2000 kb). Cloning of large fragments of DNA is necessary for gene mapping.

MOLECULAR METHODS

Recombinant DNA technology can be split into two main areas:

1. Amplification of the DNA of interest (target DNA)
2. Methods of DNA analysis

DNA cloning and polymerase chain reaction (PCR) are used for amplification. The methods of DNA analysis include restriction fragment length polymorphism (RFLP), Southern blot hybridization and various methodologies based on PCR.

DNA isolation

The first step of any molecular technique is to isolate DNA in pure form; free from proteins, RNA and other cellular components. Although the study of DNA is possible *in situ* by methods such as *in situ* PCR, fluorescent *in situ* hybridization (FISH), most experiments need DNA in pure form. DNA can be isolated from peripheral leucocytes, skin fibroblasts, solid tissues, tumour cells or even cells from the urine or mouth washings. The cells are dissolved in detergent solution and the lysate is treated with protein- and RNA-degrading enzymes (proteinase and RNase, respectively) to purify the DNA. Alcohol is then added to precipitate the genomic DNA as a fibrous thread (Fig. 15.2).

The DNA fibre is spooled and then suspended in a mildly alkaline buffer for storage. Purified DNA can be easily transported at room temperature and stored for long periods in liquid nitrogen or at –80°C. Thus, DNA samples of patients, their families, or populations can be stored for a long time. Lymphocytes from the peripheral blood can be transformed with the help of Epstein–Barr virus into immortalized cell lines which can then serve as an unlimited source of DNA. DNA samples and cell lines collected from patients and families with genetic disorders (DNA banks) are important resources for gene mapping.

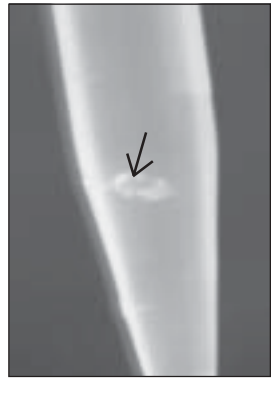


Fig. 15.2 Thread-like DNA in an Eppendorf tube.

AMPLIFICATION OF THE TARGET DNA

Cloning

DNA cloning is the selective amplification of a specific DNA segment or sequence so that it can be produced in relatively large amounts, permitting the analysis or manipulation of its structure and investigation of its functions.

Figure 15.3 shows the important steps in cloning. The DNA of interest is incorporated into a plasmid by cutting both of them with the same restriction endonuclease. The two DNA segments are joined at the similar cut ends, generating a recombinant molecule. The recombinant plasmid is then introduced into a bacterial cell. The bacterial cell is cultured either in broth or on agar plates.

Plasmids contain an antibiotic resistance gene. This is used to select the bacteria containing the recombinant plasmid. If the recombinant plasmid contains the ampicillin resistance gene, then the bacteria are

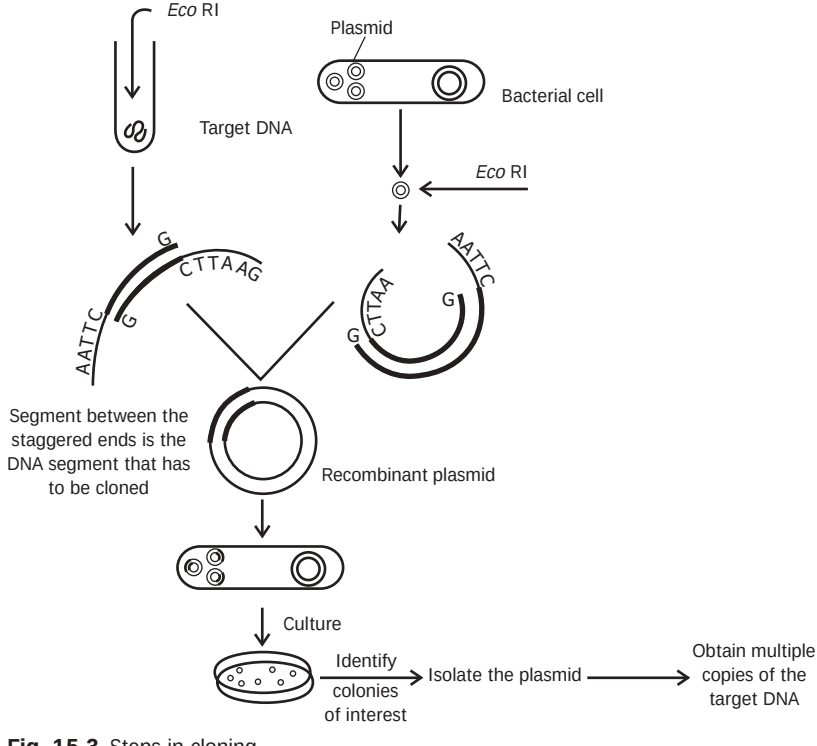


Fig. 15.3 Steps in cloning.

grown in the presence of ampicillin, so that only the bacteria containing the ampicillin resistance plasmid (and also the DNA segment to be cloned) will multiply. As the bacteria multiply, the plasmid and inserted DNA fragment will also replicate and a large amount of the target DNA will be available.

Cloning is used for gene mapping, gene sequencing, identification of mutations, construction of libraries, production of proteins in bacterial systems and for studying gene functions.

Construction of libraries

Copies of the amplified DNA segment are available in the form of a clone of bacteria containing the particular DNA segment. A collection of such clones, each containing a different DNA segment, is known as a DNA library. A DNA library usually contains all DNA fragments of the original source of the cloned DNA. If the original source of DNA is the total nuclear DNA, the library will contain all the genomic DNA in fragments. Such a library is known as a genomic library. For constructing a genomic library, vectors capable of carrying large-size DNA fragments such as YACs are necessary.

Another common type of library used to identify and isolate genes is a cDNA library, which represents complementary DNA copies of the mRNA population present within a particular tissue. cDNA libraries contain only the coding sequence, as the original source is mRNA and non-coding DNA is not transcribed in mRNA. The other advantage of cDNA libraries is that they represent a specific set of genes which are predominantly expressed in that tissue from which the library is made. For example, a cDNA library made from reticulocytes will predominantly show beta globin mRNA, as the main function of reticulocytes is to synthesize haemoglobin.

Polymerase chain reaction

Polymerase chain reaction is an alternative to cloning for generation of large amounts of target DNA. It was first used by Saiki in 1985 to detect the mutation causing sickle cell disease. The technique was formally described by Mullis *et al.* in 1986. It has revolutionized the diagnosis of genetic and infectious disorders, as well as research in gene mapping. A comparison of PCR and cloning is given in Table 15.4.

Requirements for PCR

1. *Template DNA:* It contains the gene sequence to be amplified (target DNA).

Table 15.4 Comparison of polymerase chain reaction (PCR) and gene cloning

Parameter	PCR	Cloning
Manipulation	*In vitro*	*In vitro* and *in vivo*
Selection of target DNA	First step	Last step
Biological reagents required	DNA sample, *Taq* polymerase, deoxynucleotide triphosphates, primers	DNA sample, restriction enzyme, vector, ligase, bacteria and their culture system
Concentration of the starting material	Nanograms (ng)	Micrograms (μg)
Automation	Yes, thermocycler machine	No
Time taken	Few hours	1–2 days
Applications	More	Less

2. *Primers:* For carrying out PCR, it is necessary to know the sequences on either side of the target DNA. Primers are single-stranded, 18–20 bp-long DNA oligonucleotides which are complementary to the 3' ends of the target DNA sequence (Fig. 15.4). As the 3' end of each primer points towards the target sequence and flanks it, they initiate the synthesis of copies of the target sequence.
3. *Taq DNA polymerase:* This enzyme is essential for the replication of DNA. Initially available DNA polymerase was thermolabile and had to be re-added after each cycle of PCR as the raised temperature during the denaturation step destroyed its activity. Discovery of thermostable *Taq* DNA polymerase from *Thermus aquaticus* made automation of PCR possible.
4. *Deoxynucleotide triphosphates (dNTPs):* The basic components of DNA are the nucleotides. Deoxynucleotides of each base, i.e. dATP, dGTP, dTTP and dCTP, are added to the reaction mixture for primer-aided synthesis of complementary DNA.
5. *Equipment:* The reactions are carried out in small plastic Eppendorf tubes (Fig. 15.5a). A thermocycler or PCR machine is used to carry out automated cycles of heating and cooling (Fig. 15.5b).

Steps in PCR

PCR is done in three steps—denaturation, annealing and extension. The sample DNA is denatured by heating at 93–95 ^{o}C so that the two strands separate. As the reaction mixture cools (50–70 ^{o}C), the primers anneal with the complementary sequences in the sample DNA. *Taq* DNA

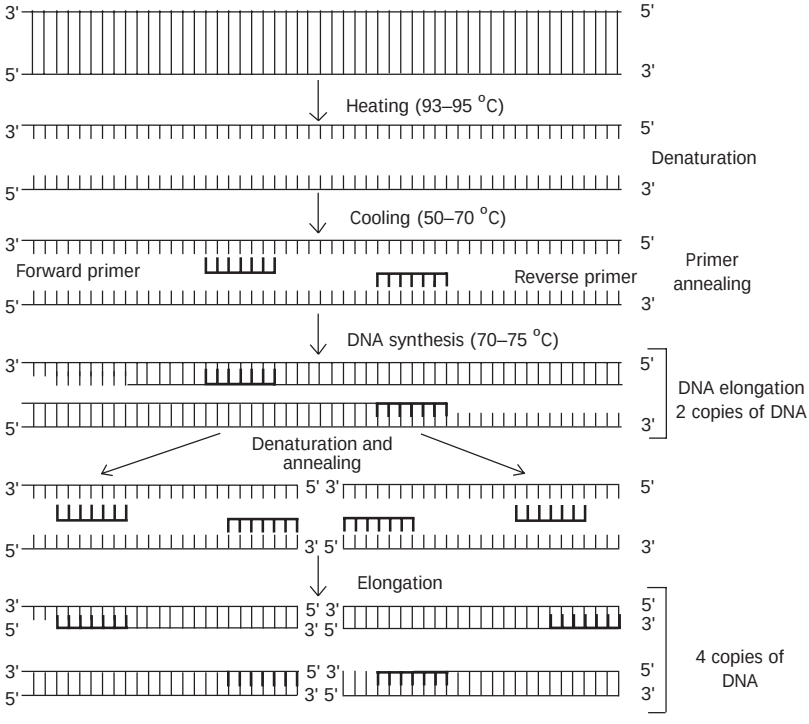


Fig. 15.4 Principle of polymerase chain reaction (PCR).

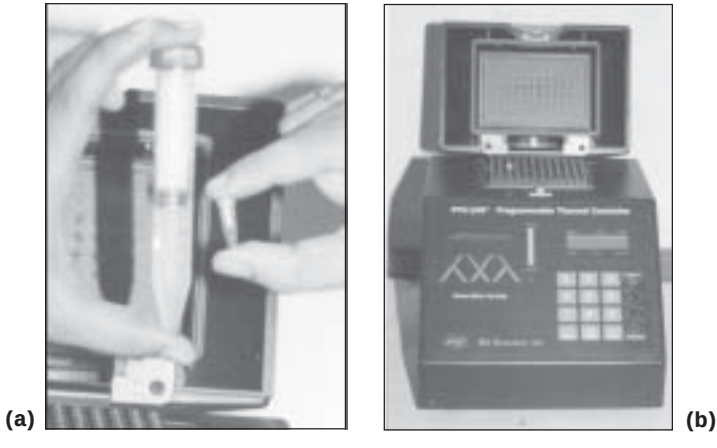


Fig. 15.5 **a** Eppendorf tube used for polymerase chain reaction. Note the 15 ml centrifuge tube on the left for comparison of sizes. **b** A thermocycler containing space for a number of tubes.

polymerase in the test mixture will catalyse the addition of nucleotides (present in the tube along with sample DNA, primers and buffer) to the primers, and new DNA strands will get synthesized. The temperature for DNA synthesis is 70–75°C. The new strands will be complementary to the template strands. Thus, from a single molecule of double-stranded DNA, two molecules of the target DNA sequence are produced at the end of the first cycle. Each round of amplification takes only a few minutes. Repeated cycles of denaturation, annealing and extension multiply the DNA copies exponentially and even with as little as 30 cycles, large amounts of DNA are produced.

To confirm amplification, electrophoresis of the PCR product can be carried out on an agarose or polyacrylamide gel (Fig. 15.6a) along with DNA markers of standard molecular sizes (ladder). DNA stains with ethidium bromide and after electrophoresis, bands can be seen under ultraviolet (UV) light (Fig. 15.6b). In Figure 15.6b, chorionic villus sampling (CVS) of the foetal DNA shows that bands in the CVS lane correspond to one band each of the mother and father. This implies that the foetus has inherited one allele from each parent.

The PCR product can be used for further studies such as sequencing, hybridization with allele-specific probes. restriction fragment length polymorphism (RFLP) analysis, etc.

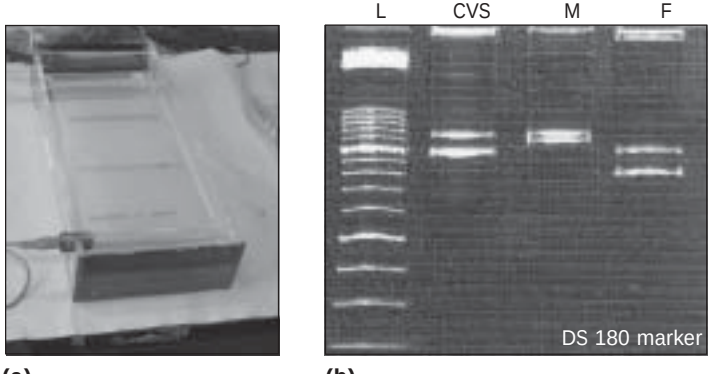


Fig. 15.6 a Electrophoresis apparatus used for separation of a polymerase chain reaction product. **b** Visualization of the PCR product after gel electrophoresis. CVS: chorionic villus sample; M: mother; F: father; L: 50 bp DNA ladder. PCR was done for a VNTR marker.

Applications of PCR

The advantages of PCR are rapid turnaround time, extreme sensitivity and the need for a small quantity of the sample DNA—the DNA from even one cell can be amplified. Due of its versatility and ease of performance, PCR is used routinely in molecular diagnosis and in all spheres of research. It has replaced Southern blot hybridization in many DNA-based diagnostic tests. It can be modified in many ways to suit one's purpose. The uses of PCR include the following:

1. Genetic disorders caused by mutations involving a single base change, or a small deletion or insertion can be identified using PCR for the following:
   (i) For identifying patients, carriers of mutation.
   (ii) For carrying out prenatal and preimplantation diagnosis
2. Diagnosis of infectious diseases
3. Diagnosis and follow up of cancer
4. Study of bone marrow engraftment
5. DNA fingerprinting used for forensic purposes
6. Paternity testing
7. Study of samples from histology slides or paraffin blocks
8. Human leucocyte antigen (HLA) typing for kidney and bone marrow transplantation
9. Study of gene linkage and gene mapping
10. Study of single nucleotide polymorphism—for identifying genes responsible for complex disorders, genetics of drug toxicity (pharmacogenetics)
11. Study of evolution

Limitations of PCR

PCR is a sensitive technique and can amplify sequences from a minute amount of target DNA and even from degraded DNA or DNA embedded in a medium such as paraffin, formalin, etc. However, the technique has some limitations:

1. At least a part of the sequence of the target DNA should be known.
2. With PCR, it is possible to sequence only small segments of DNA, usually a few hundred base pairs in size, whereas cloning can amplify segments of large sizes varying from 10–20 kb to 2 million bases (Mb).
3. The error rate due to incorporation of a wrong base is higher in PCR than in cloning. This is because *Taq* DNA polymerase, which is used

for PCR, does not have a proofreading function. Thus, at the end of PCR, many newly synthesized DNA fragments will have one or few errors and hence will be *similar* but not *identical* to the target DNA.

TECHNIQUES FOR DNA ANALYSIS

Nucleic acid hybridization assay

Numerous applications of molecular genetics involve identification of the target DNA in genomic DNA, cloned DNA, PCR-amplified products, in chromosomes and even in DNA in the interphase nuclei. This can be carried out by hybridization with labelled probes in a hybridization chamber (Fig. 15.7). The probe and the target DNA are first denatured by heating and then allowed to hybridize. One of them, usually the target DNA, is immobilized on a nitrocellulose or nylon membrane. If the target DNA contains sequences complementary to the probe, the probe will hybridize with that DNA segment. The excess probe is then removed by washing. The absence or presence of a signal can be detected due to its label. This will give information about the absence or presence of the target DNA sequence in the sample. The location of the DNA sequence of interest in the genome and its number of copies can also be determined.

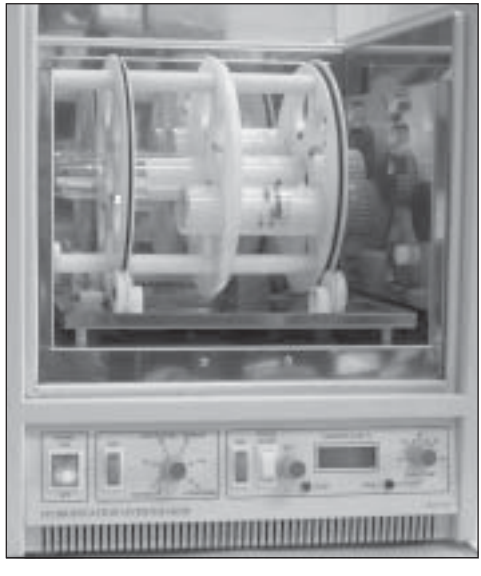


Fig. 15.7 A hybridization chamber.

Southern blot hybridization

The Southern blot hybridization test, a method based on nucleic acid hybridization, was described by Edward Southern in 1975. It is a commonly used method for analysing DNA segments. Nucleic acid hybridization is used for the identification of specific DNA segments within genomic DNA. This technique involves mixing denatured DNA from two sources and then, under appropriate conditions, allowing complementary base pairing of homologous sequences. If DNA from one of the sources is labelled, i.e. a DNA probe, then identification of the target DNA from the other source is possible.

Figure 15.8 illustrates the principle of Southern blot hybridization. The genomic DNA is digested with a restriction enzyme that cuts it into fragments of various sizes. These fragments are subjected to electrophoresis on agarose gel. The fragments separate according to their size, with smaller fragments running faster and ahead of longer ones. On staining the gel with ethidium bromide, a smear is visualized. This is because bands of various sizes merge with each other. The DNA fragments in the gel are then denatured with alkali, making them single stranded. The gel containing the DNA fragments (from the whole genome) is sandwiched between a capillary wick and filter paper in a way that fluid will pass upward through the gel and take along with it the denatured DNA, transferring it onto the nitrocellulose filter paper/ nylon membrane which binds the DNA permanently. This is called a Southern blot. This stabilized imprint is then placed in a hybridization solution containing a DNA probe (radioactively labelled) for the gene of interest. The probe hybridizes with the complementary DNA fragment on the Southern blot. This can be visualized after washing off the excess of probe, drying and placing the filter/membrane against an X-ray film for autoradiography. Bands on the autoradiogram represent those DNA fragments that contain sequences complementary to the probe sequence. The size (length) of the DNA fragments can be estimated by using reference standards (DNA markers) that are run on the gel along the side of the sample DNA.

A similar method can be used to study RNA (Northern blot) or proteins (Western blot) when appropriate probes are available.

The Southern blot test helps in finding out whether a particular gene (or a part of it) is present in the sample DNA or not, and whether the size of the DNA fragment carrying the gene is normal, has increased or decreased. Thus, it can identify the presence or absence of a gene, large deletions or duplications, or large gene rearrangements. However, it cannot identify substitution of a base or a few base pairs, or small deletions, or insertions of a few nucleotides.

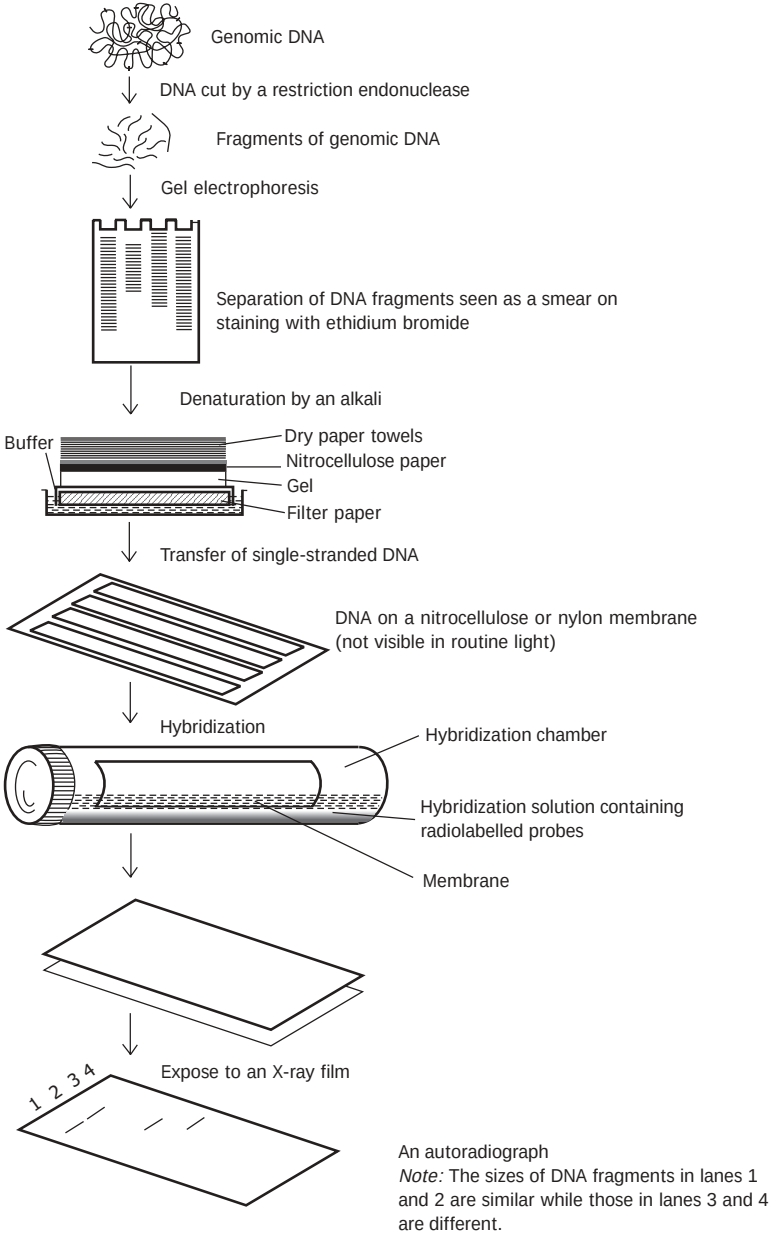


Fig. 15.8 Southern blot hybridization.

Restriction mapping

Restriction mapping is used to identify the sites for various restriction enzymes. In this technique, DNA from a particular source is digested by a restriction enzyme and hybridized with a specific DNA probe. Depending on the presence or absence of a restriction site, DNA fragments of different sizes will be formed. This method allows identification of the restriction sites of various enzymes in the region of the gene of interest. These restriction sites constitute a restriction map. The presence or absence of a restriction site at a particular location (RFLP) in the genome is used as a marker for linkage analysis.

DNA sequencing

Knowing the exact sequence of nucleotides is essential for understanding molecular biology. The target DNA is cloned or amplified before sequencing.

Manual sequencing

Before sophisticated, automatic sequencing machines were invented, sequencing was done manually. The commonly used technique for manual sequencing is the 2,3-dideoxy chain termination method, also known as the Sanger method.

An aliquot of single-stranded DNA templates obtained from the DNA to be studied is added to four tubes, each containing DNA polymerase, primers and all the four deoxynucleotides (dATP, dCTP, dTTP and dGTP), some of which are radioactively labelled. In addition, one type of dideoxynucleotide triphosphate (ddNTP) is added to each tube. DNA replication starts in the incubation mixture and stops when a ddNTP is incorporated into the newly synthesized strand. This occurs because ddNTPs lack a hydroxyl group at the 3' carbon position and thus prevent the addition of further nucleotides. For example, in a tube containing dideoxycytosine triphosphate (ddCTP), there will be various DNA fragments terminating at different positions of cytosine in the chain. Polyacrylamide gel electrophoresis (PAGE) of the amplified products of each tube is carried out and then autoradiographed (Figs 15.9a and b). The sequence of the synthesized DNA, which is complementary to that of the original DNA strand, is determined by reading the band pattern on the autoradiogram from the bottom of the gel towards the top.

Automated sequencing

Automated sequencing uses primers or nucleotides labelled with fluorescent dyes. These can be detected by a computerized laser detection

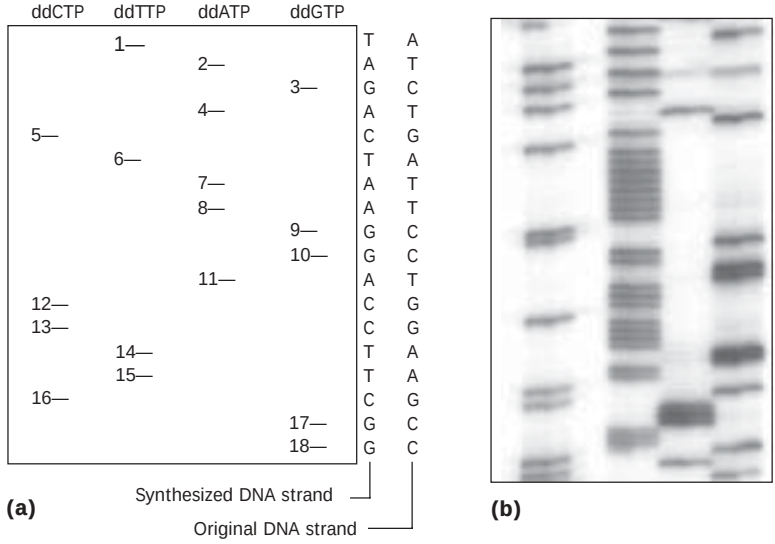


Fig. 15.9 a Autoradiogram of manual sequencing (diagrammatic representation). **b** Autoradiogram of sequencing gel (Courtesy: Dr B. Mittal, Department of Medical Genetics, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow).

system, allowing rapid, accurate and automated sequencing. Since different fluorescent dyes are used in each of the four base-specific reactions, all the four reactions can be loaded in a single lane. The output is in the form of intensity profiles for each of the differently coloured fluorochromes (Fig. 15.10). Use of automated sequencing has markedly increased the speed of human genome sequencing. The technique is also used for the detection of mutation in single-gene disorders. In mutationally heterogeneous disorders, i.e. when a number of mutations give rise to a phenotype, no single or few tests can be done to identify the mutation. In such cases, sequencing becomes essential.

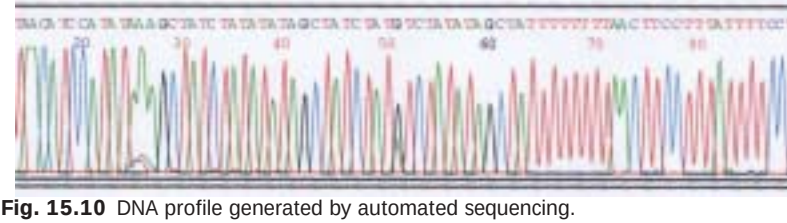


Fig. 15.10 DNA profile generated by automated sequencing.

Mutation-screening techniques

Genes vary from several hundred thousand to million base pairs in length. Hundreds of mutations are described in most of the genes and there are no clinical or investigational clues to decide which mutation should be tested first. Sequencing the whole gene is difficult and expensive. Hence, mutation-screening techniques are used as the first step in detecting a mutation. Once a mutation is detected in one member of a family, other affected members of the family or possible carriers need to be tested for that particular mutation only. There are a number of techniques that can be used to screen for mutations. These techniques differ in their ease of use and reliability. Sequencing is done to confirm the mutation detected by screening techniques. Various mutation-screening methods and their principles are discussed below.

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is based on the principle that if a double-stranded DNA fragment is run on a gel containing a denaturing agent, the strands will separate easily if there is a mismatch between the two strands. The separated single strands will lag behind the double-stranded DNA on the gel. The denatured DNA of a patient and denatured probe (labelled) are mixed and electrophoresis is done on a denaturing gel. The probe, which is complementary to the normal sequence of the gene, will hybridize with the complementary strands from the sample DNA. In case of a mismatch, the resulting heteroduplex will melt and branch as it runs on the denaturing gel. This will give an extra band lagging behind the band for the homoduplex. In the case of a disease inherited in an autosomal dominant fashion, the patient's normal copy of the gene will be used for homoduplex formation. For autosomal recessive disorders, both copies of the gene of the patient will have mutations. Therefore, the DNA from a normal individual needs to be added to the mixture (Fig. 15.11).

Single strand conformational polymorphism

If the radiolabelled PCR products of double-stranded DNA are made single stranded, they fold up to form a three-dimentional structure. An alteration in the DNA sequence can result in a different conformational pattern which, under appropriate gel conditions, results in a different electrophoretic mobility, the so-called single strand conformational polymorphism (SSCP). The technique can detect up to 85% sequence changes in short (200–300 bp) segments of the DNA (Fig. 15.12).

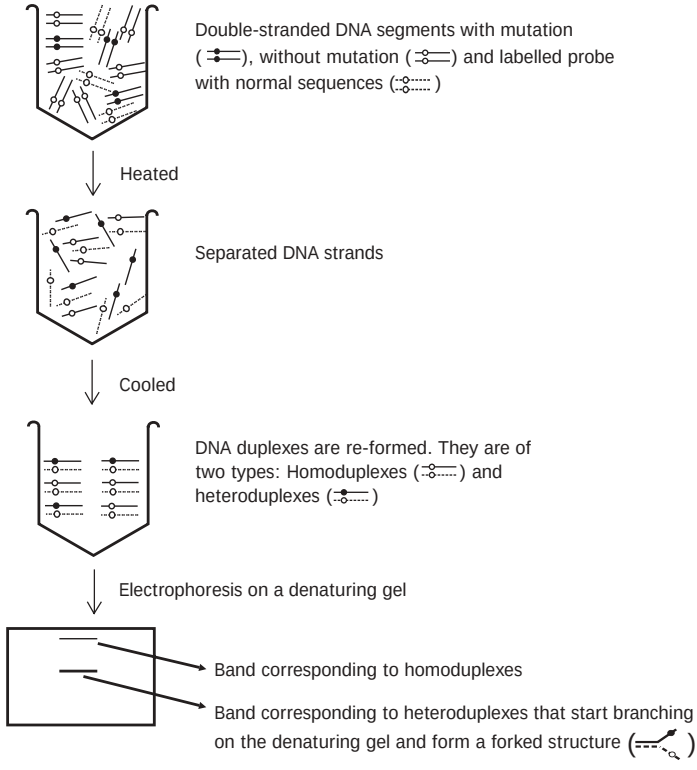


Fig. 15.11 Denaturing gradient gel electrophoresis.

In addition to the above two methods, chemical cleavage mismatch (CCM) and the protein truncation test are also used to screen for mutations.

Sequencing for confirmation of mutation

Mutation picked up by screening method needs to be confirmed by sequencing. However, as automated fluorescent sequences are easily available, sequencing is being used for mutation screening. Sequencing of cDNA obtained from mRNA is increasingly being used to screen for mutations. Messenger RNA contains all coding sequences of the gene and its sequencing is a highly sensitive method for the detection of mutation.

Mutation-detection systems

There are various PCR-based methods such as RFLP, amplification refractory mutation system (ARMS), dot blot analysis, etc. which are used to identify known mutations. These are discussed in detail in Chapter 16.

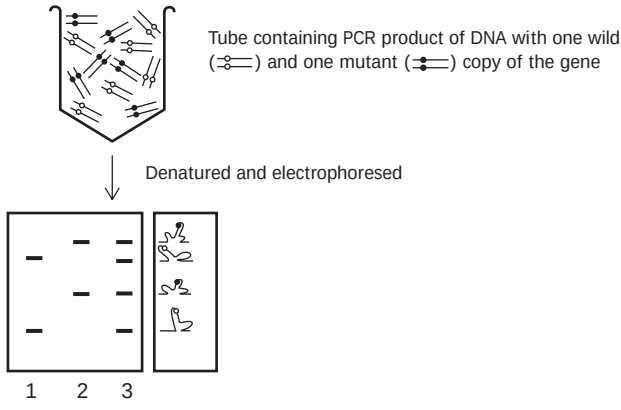


Fig. 15.12 Principle of single strand conformational polymorphism (SSCP): The mobility of a molecule depends not only on the size of the molecule but on its shape as well. *Note:* The box by the side of the electrophoresis picture represents different types of conformation patterns corresponding to each strand of wild and mutant alleles. If the sequences of the strands are different, each strand will have a different conformation and hence, the band pattern would differ. Hence, two bands each for the mutant and the wild allele, are seen.

Fluorescence *in situ* hybridization

As in Southern blot analysis, a probe can also be hybridized to DNA within interphase nuclei or chromosomes in metaphase fixed on a slide. This technique is called fluorescence *in situ* hybridization (FISH), as it uses fluorescent labels. It combines molecular genetics with cytogenetics and is widely used in clinical genetics (*see* Chapter 17).

DNA microarray technology

In any disorder, or at any point in time, in a particular physiological or pathological state, there is continuous interaction between a number of genes. The expression of many genes is being upregulated or downregulated. Studying various genes at a time has become a necessity in this era of genomics. The development of DNA microarray technology has greatly helped in overcoming these problems. This technology is commonly used to study the expression of many genes at a time and that too, on a single slide known as a DNA chip (Fig. 15.13).

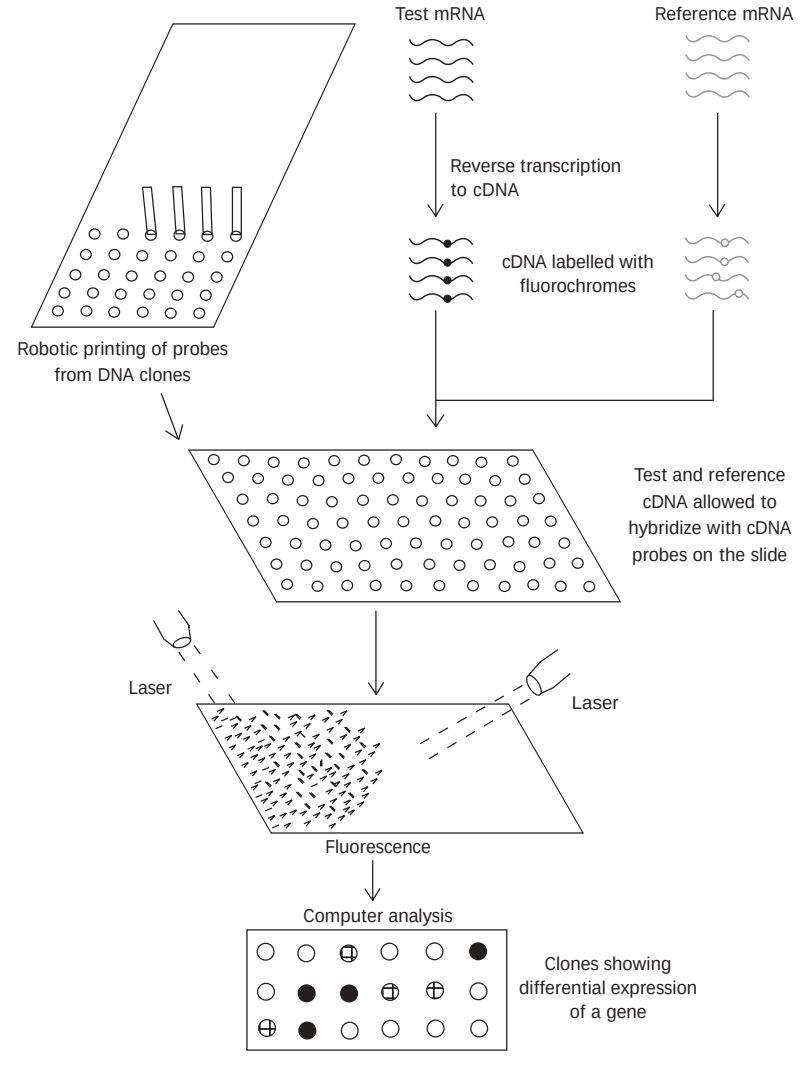


Fig. 15.13 The concept of a microarray.

A microarray or DNA chip utilizes solid surfaces (such as glass) and fluorescent labelling for detection. Probes comprising oligonucleotides from various clones are robotically placed on a small slide. A 1 cm^2 area of a slide can contain thousands of different nucleotides. These are attached to the 'chip' in a structured arrangement in what is known as a microarray. Messenger RNA is extracted from the sample to be tested. It is converted to cDNA which is then labelled with a fluorochrome. Messenger RNA from normal cells is also extracted and converted to cDNA. The cDNA of normal tissue is labelled with another fluorochrome. Both labelled cDNAs are mixed and hybridized to probes in the microarray. Lasers are used to excite the fluorochromes. The excitation pattern of each spot will depend upon the relative ratio of the corresponding cDNA segment in the test and normal cDNA. Analysis of the colour pattern of the microarray after hybridization is done by capturing the digital images and analysing them with the help of a computer. These gene expression studies are of great importance in drug designing, pharmacogenetics and cancer research.

Microarrays are also used to analyse a sample for hundreds of mutations at a time on a slide. This is very useful for DNA diagnosis of single gene disorders associated with hundred of mutations. In a manner similar to PCR, microarrays have a multitude of applications; many more will develop and evolve over time.

16 DNA-based diagnosis of single-gene disorders

INTRODUCTION

The most important clinical application of DNA technology is its use in the prenatal diagnosis of genetic disorders. A change in the nucleotide sequence of a gene (mutation) is the basic defect in single-gene or monogenic disorders. Detection of this change is the confirmatory diagnostic test for these disorders. The mutation may be inherited or may arise spontaneously (*de novo*) in the germ cell of a parent during gametogenesis. As DNA in all cells of the body is similar, DNA from peripheral leucocytes can be examined to identify a defect in a gene even if the pathogenesis of the disease is in the brain. Similarly, DNA obtained from the cells of chorionic villi or amniotic fluid is similar to that of the foetus and a diagnosis of abnormalities in the foetus can be made with great accuracy using this DNA sample.

In the past two to three decades, causal genes for about 400 disorders have been identified and cloned. DNA technology can be used for the diagnosis of these disorders. Clinicians who order these DNA-based diagnostic tests have to know the principles and limitations of these tests. For most disorders, the number of mutations associated with the disease is in hundreds and it is usually not possible to test for each and every mutation. Similarly, mutations in non-coding regions such as introns or promoters may not be looked for and hence the mutation-detection test may be negative even if the patient has the disease. Further, even if a person has a mutation, he may or may not develop the disease (non-penetrance) or the severity of the disease may be different from that in other members of the family with the same disease. These implications of positive and negative test results should be clear to the clinician and must also be explained to the patient before the test is ordered.

Over the next few decades, the DNA tests may also become available for the identification of individuals at risk for common multifactorial disorders such as hypertension and diabetes mellitus. This will demand a good understanding of the DNA tests and their uses in clinical practice. Table 16.1 lists some monogenic disorders for which DNA-based diagnostic tests are available. For a rare disorder, the geneticist will need to find out from the latest literature and databases whether a DNA-based test is available and which centre offers the test. The appropriate sample can then be transported to that particular laboratory.

Table 16.1 Some monogenic disorders for which DNA-based diagnostic tests are available

Predominant system involved	Disorders
Blood	Beta-thalassaemia and alpha-thalassaemia Abnormal haemoglobin such as Hb E, Hb S, etc. Haemophilia A and haemophilia B Glanzmann thrombasthenia Immunodeficiency syndromes
Nervous system	Neuronal ceroid lipofuscinoses Spinocerebellar ataxia Friedreich ataxia Spinal muscular atrophy X-linked hydrocephalus Menkes kinky hair disease
Liver	Alpha 1-antitrypsin (AAT) deficiency
Liver and nervous system	Wilson disease
Muscle	Duchenne muscular dystrophy Congenital muscular dystrophy Limb-girdle muscular dystrophy Facioscapulohumeral muscular dystrophy Myotonic dystrophy
Cardiac	Cardiomyopathies
Bone	Osteogenesis imperfecta Achondroplasia Craniosynostosis syndromes
Skin	X-linked ectodermal dysplasia Various types of epidermolysis bullosa
Connective tissue	Marfan syndrome
Renal	Autosomal dominant polycystic kidney disease
Eyes	Retinitis pigmentosa

INDICATIONS FOR DNA TESTS

The word 'genetic test' is loosely used to refer to a test on genetic material and/or for a genetic disorder and may imply a DNA test or chromosomal analysis. The indications for chromosomal analysis are distinct (*see* Chapter 7) and different from those for DNA analysis and the use of the term 'genetic test' is not recommended.

A DNA test is used to detect a mutation to confirm the presence of a disease caused by that mutation. DNA tests are of two types: (i) those for direct detection of a mutation and (ii) those for tracking a mutation in a family. Detection of a direct mutation in a patient confirms the diagnosis. All members of a family affected by a genetic disorder will have the same mutation or mutations as present in the proband, and the test for the detection of that particular mutation can be used for the diagnosis of other affected cases—presymptomatic, prenatal and less severely affected members in the family, and for the detection of carriers.

Not all genetic disorders need to be confirmed by a DNA test. The diagnosis may be confirmed by clinical examination alone or by specific biochemical, haematological, histological or imaging investigations similar to those used for non-genetic disorders (Table 16.2). For example, although DNA tests for the diagnosis of thalassaemia major and sickle cell disease are available, it is not mandatory to carry them out for the diagnosis of these disorders. Haemoglobin electrophoresis is used to diagnose thalassaemia major and quantification of abnormal haemoglobin for sickle cell anaemia.

On the other hand, there are some diseases, especially neurological, where the clinical presentation may be suggestive of the disease but there is no diagnostic test other than mutation detection. Monogenic diseases for which DNA-based diagnostic tests are available can be classified according to the role of DNA test in patient management. The various indications for carrying out DNA tests are given below.

Disorders diagnosed by mutation-detection tests

This group includes disorders for which a non-genetic diagnostic test is not available.

Fragile X syndrome

The cytogenetic demonstration of the presence of a fragile site at the Xq27.3 locus is diagnostic. However, false-positive (fragile site at the Xq28 locus) and false-negative (due to inappropriate cell culture conditions) results are possible. The other diagnostic difficulty in fragile

Table 16.2 Diagnosis of single-gene disorders

Basis of diagnosis	Disorders	Diagnostic test/s
Clinical examination	• Duchenne muscular dystrophy • Crouzon syndrome • X-linked ectodermal dysplasia • Neurofibromatosis I	Not essential
Definite biochemical or haematological test based on clinical clues	• Thalassemia major • Haemophilia A • Metachromatic leucodystrophy	Foetal haemoglobin estimation Factor VIII assay Aryl sulphatase A assay
Imaging and other investigations along with examination and clinical features	• Marfan syndrome • Tuberous sclerosis • Epidermolysis bullosa	Echocardiogram and ophthalmological examination Computed tomography scan of the head, ophthalmological examination and ultrasonography of the kidneys Skin biopsy, electron microscopy and histochemistry
Mutation detection in suspected cases	• Fragile X mental retardation • Myotonic dystrophy • Spinal muscular atrophy • Cystic fibrosis • Charcot–Marie–Tooth disease • Huntington chorea	DNA tests to detect mutations in the gene causing the disorder

X syndrome is that the clinical features are subtle, e.g. characteristic features such as macro-orchidism and facial dysmorphism appear as the child grows. Hence, it is necessary to conduct DNA tests for the diagnosis fragile X syndrome in all males and females with idiopathic mental retardation.

Spinal muscular atrophy

There is no diagnostic test for spinal muscular atrophy (SMA) other than the supportive evidence of neurogenic changes on electromyography. Detection of a deletion in the *SMN1* gene on chromosome 5 confirms the diagnosis of SMA. There may be some cases of SMA caused by mutations

in genes other than *SMN1* and also due to point mutations in the *SMN1* gene. These cases will not be detected by the test used for detecting a deletion in the *SMN1* gene.

Cystic fibrosis

In some cases of cystic fibrosis (CF), the chloride content in sweat is normal. In such cases, detection of a mutation in the *CFTR* gene is important for the diagnosis of the disease. Although such a test will confirm the diagnosis in suspected cases, a negative result will not rule it out. This is because, as mentioned previously, some mutations may not be identified by the methodology used for detection of mutation.

Other disorders

There are many neurological disorders such as Huntington chorea, hereditary motor and sensory neuropathy, spinocerebellar ataxia and myotonic dystrophy, where the clinical features are characteristic and clinical diagnosis is reliable. Electrophysiological studies such as electromyography and nerve conduction analysis also provide important supportive evidence. Detection of mutation may not be absolutely necessary in typical cases of these disorders, especially in the presence of a positive family history. However, in cases without a positive family history, or with a mild or atypical presentation, mutation detection is the only diagnostic test.

In this group of disorders, the need for a DNA test to confirm the diagnosis will vary from one disorder to another. For example, a mutation-detection test may not be necessary for the diagnosis of typical familial cases of spinocerebellar ataxia or CF, but plays a major role in the diagnosis of fragile X syndrome and SMA.

DNA tests for prenatal diagnosis or carrier detection

Many genetic disorders have well-characterized haematological, biochemical or anatomical abnormalities. For such disorders, diagnostic tests other than mutation detection are available. This group includes diseases such as the haemoglobinopathies; haemophilia A; metabolic disorders, e.g. metachromatic leucodystrophy, Wilson disease, mucopolysaccharidosis; autosomal dominant polycystic kidney disease; and various types of epidermolysis bullosa. However, for many of these disorders, prenatal diagnosis is possible only by DNA analysis.

For disorders such as the thalassaemias, haemophilia A and storage disorders, prenatal diagnosis is possible by non-DNA methods also, but DNA analysis makes the prenatal diagnosis or carrier detection easy and accurate. Thus, molecular tests for these disorders need not be ordered unless the family needs or is likely to need prenatal diagnosis in the future. In such a situation, mutation detection in the affected member of the family or storing his/her blood sample for future analysis will be essential. This is necessary as identification of mutations in the proband or carrier parent/s is a prerequisite for doing DNA based prenatal diagnosis.

Mutation-detection tests can also be used for presymptomatic diagnosis of late-onset diseases such as Huntington chorea and autosomal dominant polycystic kidney disease. For diseases such as familial breast carcinoma, identification of at-risk individuals by mutation-detection tests can improve the prognosis by close surveillance and early diagnosis. For many of these disorders, even after presymptomatic diagnosis, nothing can be done to alter the course of the disease. However, it can help in taking reproductive decisions.

DNA tests required for population screening

Population screening can be used for two purposes. One is for preventing the birth of affected children by identifying carriers and offering them prenatal diagnosis; for example, to prevent CF and fragile X mental retardation. Detecting carriers of these disorders is possible only by conducting a mutation-detection test. The other purpose is to identify individuals at risk for a disease presymptomatically and offer them preventive options. Diseases chosen for population screening should have a high prevalence in the general population or in a particular ethnic group. In some countries mass screening for identification of carriers by mutation detection has already commenced for cystic fibrosis and its feasibility is being evaluated for fragile X mental retardation in some countries. Mutation-detection tests can be used to identify individuals at risk for diseases such as hereditary haemochromatosis and thrombophilia, as well as those with a predisposition for familial cancer and pharmacogenetic risk factors. However, at present, the implementation of such screening programmes at the population level is impeded by the inability to predict the phenotype and its severity in an asymptomatic patient. This limitation is due to the lack of a clear understanding of the complex interaction of a particular gene defect with other genetic and non-genetic factors determining the phenotype.

TECHNIQUES FOR DNA-BASED DIAGNOSIS

If one wishes to confirm the diagnosis of a monogenic disorder in a patient by a DNA-based test, then the mutation in the causative gene in that patient needs to be identified. Linkage analysis is not useful for this purpose. However, once the diagnosis is confirmed in the proband by mutation analysis or any other diagnostic method, then linkage analysis can be used in the relatives of the proband for carrier detection, prenatal diagnosis, or presymptomatic diagnosis. DNA diagnostic tests use techniques such as polymerase chain reaction (PCR) and Southern blot analysis (*see* Chapter 15). Usually, PCR-based techniques are used because they are easy and fast. Commonly used PCR-based methods do not involve radioactive labelling and the DNA bands obtained after electrophoresis of PCR products can be seen by ethidium bromide staining. Mutation-detection tests for carrier detection and prenatal diagnosis of two common monogenic disorders—beta-thalassaemia , Duchenne muscular dystrophy (DMD) and hemophilia A—are described below. These illustrate the principles of DNA-based diagnosis. Similar methodologies are used for many other monogenic disorders like Wilson diease, oculocutaneous albinism, spinal muscular atophy, etc.

Diagnosis of beta-thalassaemia

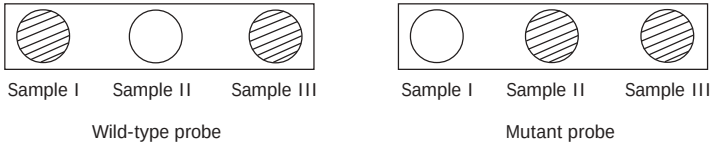
High level of foetal haemoglobin in a case of severe anaemia with splenomegaly is diagnostic of thalassaemia major and mutation detection is rarely needed for diagnosis. However, mutation detection is the test used for prenatal diagnosis.

As all affected members of a family will have the same mutation, it is necessary to identify the thalassaemia-causing mutation before opting for prenatal diagnosis. The identification of mutation/s can be done by analysing the DNA of an affected member or the parents, as they are obligate carriers.

PCR-based approaches are reported to be accurate and reliable for the prenatal diagnosis of beta-thalassaemia. Two commonly used methods are dot blot analysis and amplification refractory mutation system (ARMS).

Dot blot analysis

Part of the beta globin gene adjacent to a mutation is amplified by PCR (*see* Chapter 15). The product is immobilized on two separate membranes and hybridized with allele-specific oligonucleotide (ASO) probes complementary to the wild and mutant sequences (Fig. 16.1). The DNA of normal individuals will hybridize with the wild-type probe only, while the DNA of those homozygous for the mutation will hybridize with the mutant probe only, and the DNA of those heterozygous for the mutation



Sample I Normal DNA
Sample II Homozygous for the mutation
Sample III Heterozygous for the mutation

Fig. 16.1 Hybridization of the PCR product with wild-type and mutant probes. The shaded dots show a signal due to hybridization. Plain circles represent the absence of hybridization.

will hybridize with both the probes.

Reverse dot blot analysis

Tests based on the principle of dot blot analysis can also be done in the reverse manner. The normal and mutant ASO probes serve as hybridization targets for PCR-amplified DNA. The probes are dotted and immobilized on the membrane. The PCR-amplified product is hybridized with both the probes. This is known as reverse dot blot analysis.

Allele-specific amplification or amplification refractory mutation system

The target DNA is amplified using two sets of primers each in two separate tubes. Each tube has two different sets of primers. One primer is common (downstream primer) to both the tubes. However, the other primer has a sequence specific to the mutation (mutant allele) in the first tube. The other tube contains the primer specific for the normal (wild-type) allele.

Two separate reactions are carried out, each with the wild-type and mutant primer, together with the common downstream primer. The reactions will give rise to four possible situations (Fig. 16.2a). Annealing of the primer to the sample DNA will occur only in the presence of a perfect complementary primer sequence. Electrophoresis of the PCR products is then done (Fig. 16.2b). A band will be seen only in the reaction with the wild-type primer if the person is homozygous for the normal allele (Sample I, Fig. 16.2b). If the person is homozygous for the mutant allele, then there will be a band in the reaction with the mutant primer only (Sample III, Fig. 16.2b). A heterozygous person will show

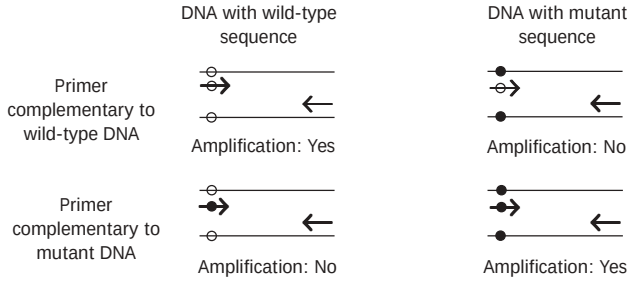
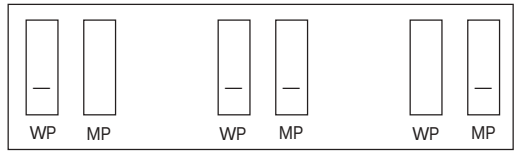


Fig. 16.2a Amplification refractory mutation system. Amplification occurs only if the sequence of the wild-type or mutant primer matches with the sample DNA.



Sample I Homozygous for the normal allele
Sample II Heterozygous
Sample III Homozygous for the mutant allele

WP Wild-type primer
MP Mutant primer

Fig. 16.2b Electrophoresis of the products of polymerase chain reaction.

amplification with both the sets of primers (Sample II, Fig. 16.2b).

PCR for the detection of deletions

Deletions are rare in beta-thalassaemia. However, deletion of the 619 base pair in the beta globin gene is a common mutation in Indian patients. It can be tested by amplifying the DNA segment using primers on either side of the deleted region. On electrophoresis, the normal gene will give a band having a size of 861 bp and the gene with the deletion of the 619 bp will give a band that is 242 bp in size.

The set of primers for the 619 bp deletion can be used with any ARMS–PCR. It tests for the 619 bp deletion and simultaneously, acts as a positive control for ARMS–PCR to confirm that the PCR is working satisfactorily. Such a positive control is necessary because in ARMS–PCR, the absence of amplification suggests the absence of that particular allele. Lack of amplification due to the failure of PCR will give incorrect results.

It is obvious from the above discussion that a separate PCR-based test is needed for each mutation. Hence, it is necessary that the information about common mutations in each population is available to decide the

strategy for mutation detection. If DNA analysis of a patient does not reveal any of the common mutations, then the rarer mutations need to be looked for, or other techniques for screening of mutations have to be used to identify rare or new mutations.

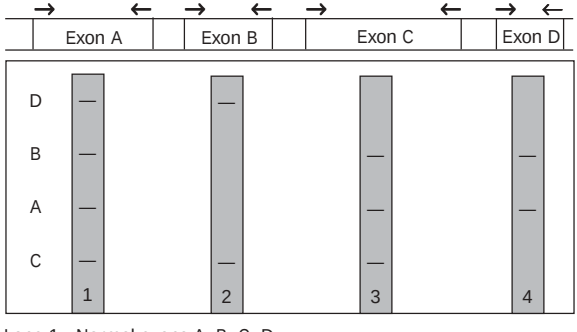
If mutations are not detected in the proband, then linkage analysis using restriction fragment length polymorphism (RFLP) markers linked to the beta globin gene locus can be used for prenatal diagnosis.

Diagnosis of Duchenne muscular dystrophy and haemophiliaA

Multiplex polymerase chain reaction analysis

In X-linked disorders, deletion of a part of a gene in affected male can be easily detected by the absence of the band due to non-amplification of the part of the gene corresponding to the deleted region.

Multiplex PCR utilizes this principle and groups of 6 or 9 exons are amplified simultaneously in a tube. The products are separated by gel electrophoresis. Each band in the gel represents an exon. In the presence of a deletion in an exon, the corresponding band will be absent (Fig. 16.3a). However, in females heterozygous for an X-linked disorder, amplification of the corresponding exon from the normal chromosome would mask the deletion on the mutated chromosome, i.e. electrophoresis of the PCR products will show the band corresponding to the deleted exon though the exon is deleted on one of the two X chromosomes of the carrier. Carrier females can be identified by quantitative PCR as the band corresponding to the deleted exon will have



Lane 1 Normal exons A, B, C, D
Lane 2 Deletion spanning exons A and B
Lane 3 Deletion of exon D
Lane 4 Deletion spanning exons C and D

Fig. 16.3a Multiplex polymerase chain reaction: Electrophoresis pattern of exons.

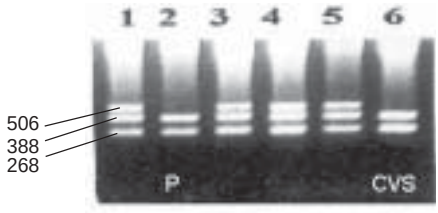


Fig. 16.3b Use of multiplex polymerase chain reaction (PCR) for the prenatal diagnosis of Duchenne muscular dystrophy (DMD). Photograph of electrophoresed PCR products of exons of the dystrophin gene. Lane 2 (P-proband) shows the absence of the 506 bp band corresponding to exon 48. In lane 6, DNA from a chorionic villus sample (CVS) shows the same deletion indicating that the foetus is affected with DMD.

a decreased density as compared to those for non-deleted exons.

Ninety-eight per cent of deletions can be identified using 25 pairs of primers for hot spots in the dystrophin gene. Once a deletion is identified in the proband, the amplification of that particular exon can be done using the DNA extracted from the foetal sample for prenatal diagnosis (Fig. 16.3b).

If deletion is not detected in a patient with DMD, various methods for screening point mutations such as single strand conformational polymorphism (SSCP), heteroduplex analysis, etc. can be used. However, the success of these techniques is limited due to the large size of the dystrophin gene. Hence, for cases where the mutation is not detected, it is convenient to use linkage analysis for prenatal diagnosis rather than attempting to identify point mutations.

Linkage analysis

If the disease-causing mutation cannot be identified in a patient or is difficult to test, DNA markers situated in or near the gene can be used to trace the mutated gene in the family. This method, known as linkage analysis, can be used for prenatal diagnosis and carrier detection. The use of linked markers is based on the fact that closely related genes or DNA segments are transmitted together (without recombination) to the next generation. This method is also useful for the detection of carriers of X-linked disorders such as DMD and haemophilia.

Linkage analysis uses polymorphic markers in or near the causative gene. Polymorphic markers are DNA sequences that vary (i.e. have different allaes) in the general population but do not cause disease. In the case of an X-linked recessive disorder, there must be at least two affected members in the family to confirm that the disease is inherited in the family. Linkage analysis can be used to track down the disease-

causing gene in such a family. However, if there is only one affected member in the family, then he could be affected due to a *de novo* mutation (occurring only in him) and his mother may not be a carrier. Thus, the use of linkage analysis in a family with only one affected member has its limitations. Before using linkage analysis, the diagnosis in the proband should be confirmed by mutation detection or other appropriate means.The other limitation of linkage analysis is error due to recombination (*see* page 248). The possibility of errors due to recombination can be minimized by using markers close to or in the gene and by using multiple markers simultaneously.

Figure 16.4 illustrates the concept of linkage for the diagnosis of an X-linked disorder. A, B and C are the three alleles at a locus near the disease-causing gene. M (∗) is the mutation in the gene. A, B and C can be identified by PCR, while M is not identified or is difficult to test. As the affected sons has the B allele, we know that the allele is linked to the disease-causing copy of the gene in this family. Thus, all females with the allele B will be carriers of the disease and all males with the allele B will be affected by the disease. Thus, the basic assumption in linkage analysis is that the mutated gene and the linked marker are transmitted

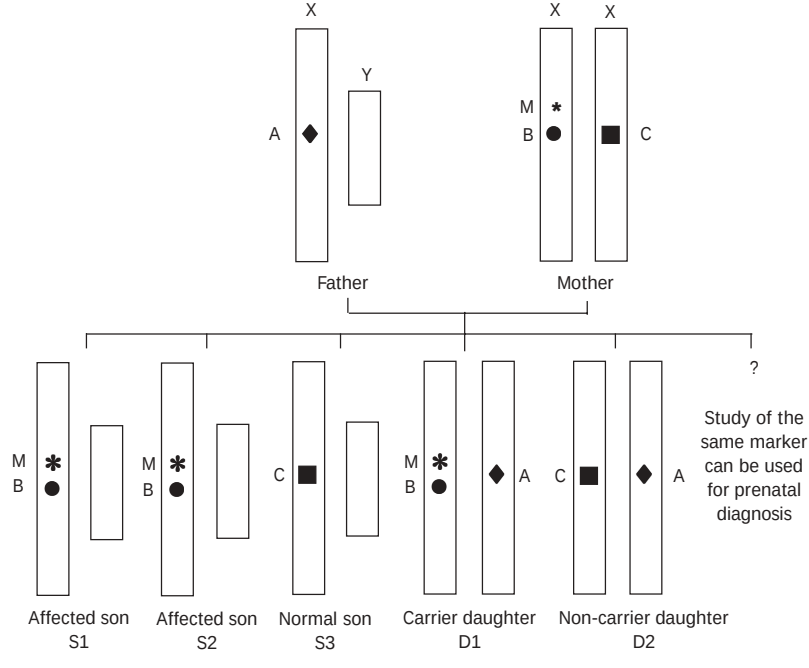


Fig. 16.4 Linkage analysis for the diagnosis of X-linked disorders.

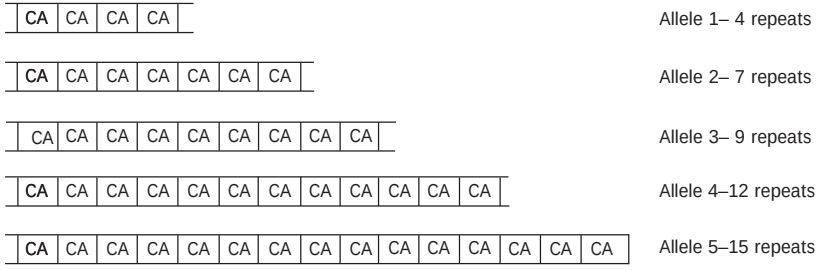


Fig. 16.5 Microsatellite marker (CA repeats). A number of alleles are possible. When the DNA segment containing CA repeats is amplified by PCR and the product is electrophoresed, the size of the band will vary depending on the number of CA repeats.

together to the next generation. This is true most of the times as the markers close to the disease causing gene are chosen for linkage analysis. However, sometimes there can be recombination in between the disease causing gene / mutation and the linked marker studied. This will lead to false positive or false negative diagnosis. Whenever linkage analysis is used for prenatal diagnosis, the family should be well aware of the errors occurring due to recombination. Linkage analysis can also be used in the case of autosomal recessive or autosomal dominant disorders.

Markers used for linkage analysis are RFLP and repetitive markers such as microsatellites and minisatellites. Microsatellite markers are short tandem repeats (STR) of di-, tri- or tetranucleotides. A number of alleles of STRs are possible. Hence, the chance that a person has two different alleles on homologous chromosomes is high (Fig. 16.5).

A marker has to be present in the heterozygous state (informative marker) to be useful for linkage analysis. Such marker has two different alleles in the mother and can be used to distinguish between her two X chromosomes, i.e. the X chromosome transmitted to her son with the disorder and the other X chromosome which is not present in the affected son. If a marker is homozygous, i.e. has the same allele on both the X chromosomes of the mother of the patient, it cannot be used for carrier detection or prenatal diagnosis of X-linked disorders (non-informative marker). The data of multiple markers can be used to create haplotypes. Linkage analysis cannot be used for carrier detection and prenatal diagnosis in the case of recombination between markers.

Figure 16.6 shows the use of CA repeat markers in a family with DMD. The markers for which the mother is homozygous, i.e. STRs 44, 45, 49 and 50, are not informative and cannot be used for linkage analysis. The family analysis shows that both 5' DYS 11 and 3' DYS CA are informative markers in this family as the mother is heterozygous for both of them.

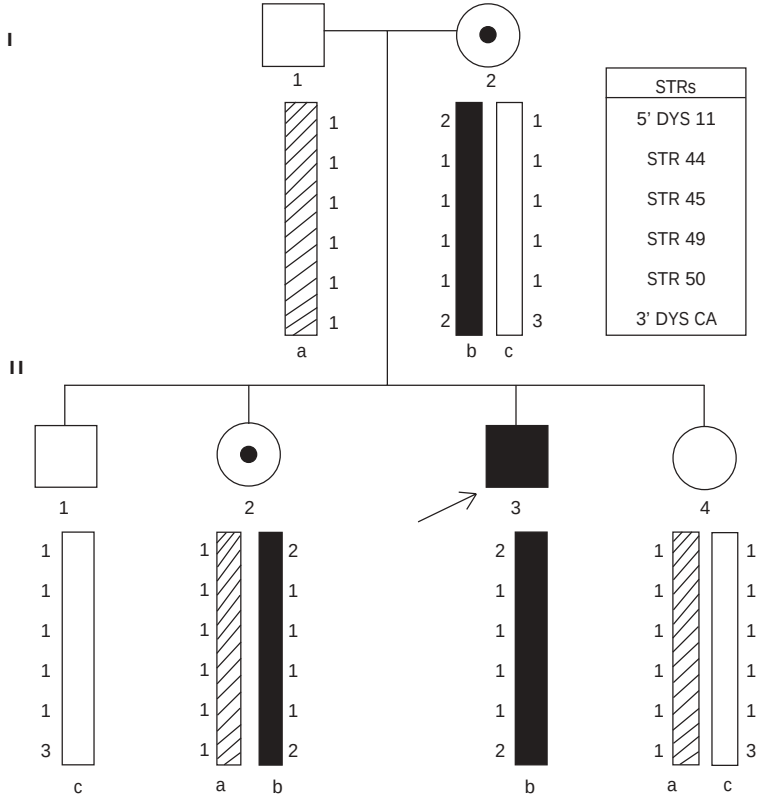


Fig. 16.6 Linkage analysis of markers in the Duchenne muscular dystrophy (DMD) gene. Analysis of the family shows that 5' DYS 11 and 3' DYS CA are informative markers in this family as the mother is heterozygous for both of them. The use of the same marker shows that sister II-2 of the proband is a carrier of DMD and sister II-4 is not a carrier of DMD. Alleles of each maker are given numbers on 1, 2, & 3.

In Figure 16.7 the mother is heterozygous for three CA repeat markers, 5' DYS 11, STR 50 and STR 62/63. Using these markers, it is seen that the affected sons have inherited the 'b' haplotype (combined allele pattern of all markers). Thus, sister II-4 is not a carrier and sister II-5 is a carrier. The carrier status of sister II-2 cannot be determined as she has inherited a mixed maternal haplotype (c/b) suggesting recombination between 5' DYS 11 and STR 50.

Instead of, or in addition to, microsatellite markers, RFLP markers can be used for linkage analysis. In the case of RFLP markers, only two alleles are possible; while in the case of microsatellites markers, the alleles can be

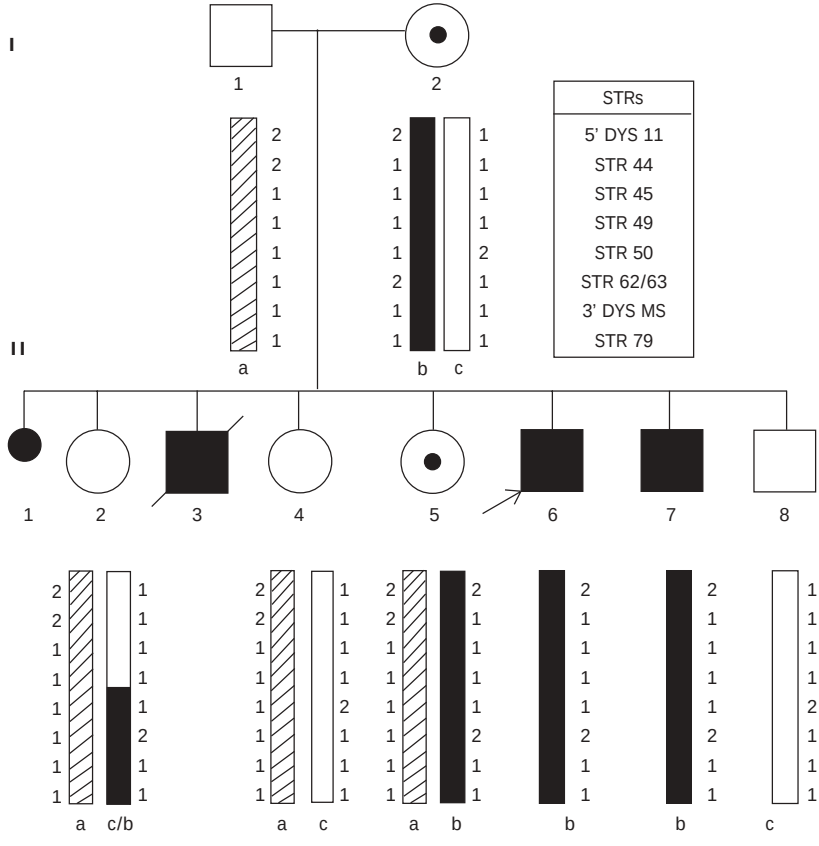


Fig. 16.7 Analysis of a family affected with Duchenne muscular dystrophy. Note the recombination in sister II-2.

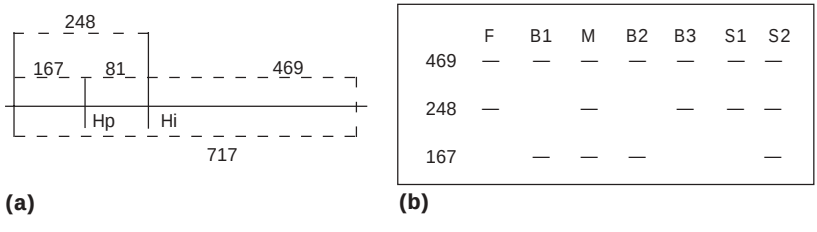


Fig. 16.8 **a** A map of Hind III restriction site in the Factor VIII gene. **b** Diagrammatic representation of the electrophoresis pattern after Hind III digestion of PCR products.

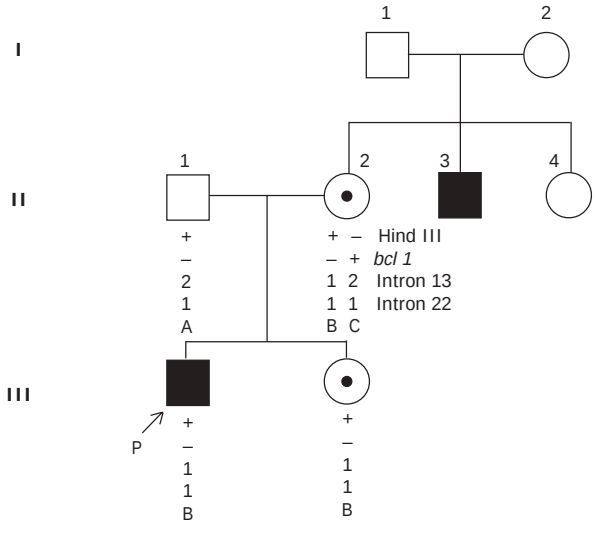


Fig. 16.9 The use of various markers in carrier detection of haemophilia A. The woman (II-2) is an obligate carrier of haemophilia A as her son (III-1) and brother (II-3) have haemophilia. A, B and C denote haplotypes generated by combining data of alleles of four markers.

many. Therefore, a person is more likely to be heterozygous for STRs than for RFLP. In the case of RFLP markers, the two possible alleles are presence (+) or absence (–) of a restriction enzyme cutting site (Fig. 16.8a).

Figure 16.8a shows a map of Hind III restriction site in the Factor VIII gene. The Hi site is present in all X chromosomes whereas Hp is the Hind III polymorphic site present on some X chromosomes and absent on others. If a 717 bp DNA segment of a chromosome is amplified by PCR and treated with the Hind III enzyme, a 469 bp segment will be produced in all. If the Hp site is present (+), then there will be two additional fragments of 167 bp and 81 bp. If the Hp site is absent (–), then there will be only one fragment of 248 bp.

Figure 16.8b shows the electrophoresis pattern of Hind III polymorphism in a family with haemophilia A. The brothers B1 and B2 are affected with haemophilia A and have an Hp site. Therefore, they show the 167 bp band. B3 is the normal brother. F and M are the father and mother, respectively. Sister S2 is a carrier of haemophilia A and sister

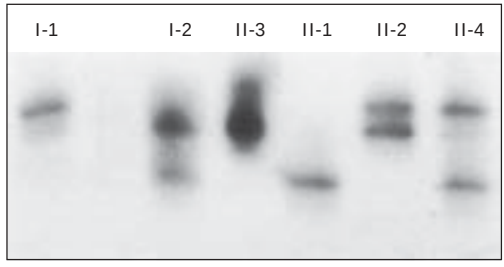


Fig. 16.10 Electrophoresis pattern of 3' DYS CA marker in the dystrophin gene in a family.

S1 is not. If out of the two X chromosomes of the mother, the Hp site is present in one and absent in the other, Hind III polymorphism can be used for prenatal diagnosis and carrier detection.

Figure 16.9 shows a pedigree with data on the Hind III restriction site and other markers such as the *bcl* restriction site and CA repeats in introns 13 and 22 in factor VIII gene. The mother (II-2) is heterozygous for all markers except intron 22. The affected son (P) has inherited haplotype B from the mother. His sister has also inherited the B haplotype from the mother and hence, is a carrier of haemophilia.

Figure 16.10 shows the use of dinucleotide (CA) repeat markers for carrier detection in the case of DMD. I-2 is the mother whose two bands represent two different alleles, i.e. two copies of the dystrophin gene on two different chromosomes. Father (I-1) and sons (II-1 and II-3) have only one band because males have only one X chromosome. The allele in the affected son (II-3) corresponds to the upper allele of the mother and hence, the upper allele is linked to mutated dystrophin gene in the family. The same allele is inherited by one sister (II-2), who is thus a carrier. Sister II-4 has inherited the lower allele from her mother and hence, is not likely to be a carrier of haemophilia A.

Linkage analysis can also be used for the prenatal diagnosis of autosomal disorders. However, samples from affected and unaffected family members are necessary for interpretation of the results.

DNA-based diagnostic tests have great utility in clinical practice, but they cannot be used in isolation. Clinical and laboratory data, and accurate diagnosis of affected cases have to be provided to the laboratory doing the DNA test for correct interpretation of the results. The utility and limitations of the test need to be discussed with the patient and/or the family beforehand.

17 Fluorescence *in situ* hybridization in clinical practice

INTRODUCTION

During the past four decades, there have been many developments in cytogenetics and much is now known about chromosomal anomalies. Pre- and postnatal diagnosis of chromosomal abnormalities has become routine. The study of chromosomes from cancer cells for diagnostic and research purposes is a common practice. Routine cytogenetics involves cell culture, which is tedious. Analysis of chromosomes needs expertise in identifying banding patterns that are specific for different chromosomes. As the smallest visible deletion or duplication consists of 4 million bases (Mb) (4×10^6 base pairs [bp]), routine cytogenetics cannot resolve genetic abnormalities of sizes smaller than this. These limitations of cytogenetics were overcome by a technical advancement called fluorescence *in situ* hybridization (FISH). Although information obtained through conventional cytogenetics is enormous and cannot be completely replaced by any other technique at present, FISH can provide much more information and can be used in situations where routine techniques cannot be employed.

PRINCIPLE AND PROCEDURE

FISH is based on the principle of hybridization, i.e when double-stranded DNA is heated, the two strands separate into single-stranded DNA (denaturation) and on cooling, the single-stranded DNA hybridizes with its complementary sequence to form double-stranded DNA (reannealing). When hybridization is done using inact DNA in chromosomes or interphase nuclei, it is known as '*in situ* hybridization' (ISH). The technique of ISH was first described in 1969.

In ISH, the DNA sequence of interest is labelled with a radioactive substance or a fluorescent dye (Fig. 17.1). The labelled DNA sequence is called a probe. This probe is denatured by heating and is added to a slide that has chromosomes or interphase nuclei spread on it. The DNA of the chromosomes or interphase nuclei is also denatured. The probe DNA anneals with the complementary DNA on the slide. As the probe is labelled, the presence or absence of the sequence of interest in the sample on the slide can be detected. It will also show the location of the sequence on the chromosomes (hence called *in situ*) and the number of copies present in the cells.

In the early days, DNA probes were labelled with radioisotopes such as tritium (^{3}H) or iodine (^{125}I) and were detected by autoradiography. In addition to the hazards of radioactivity, other disadvantages of the method were poor resolution and weak signals. Further developments led to the use of non-radioactive labels such as fluorochromes (fluorescent dyes), and FISH became a popular technique with researchers and laboratories.

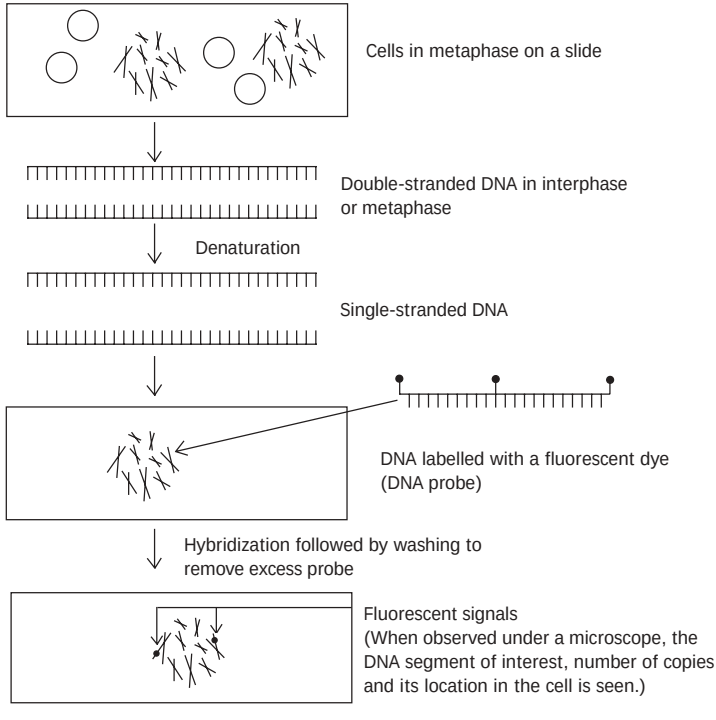


Fig. 17.1 Principle of fluorescence *in situ* hybridization.

Biotin and digoxigenin have proved to be the most useful detection systems. Probes labelled with biotin are detected by fluorochromes coupled to avidin, streptavidin or antibiotin antibody. Digoxigenin-labelled probes are detected by fluorochrome-labelled antidigoxigenin. The most commonly used fluorochromes are fluorescein isothiocyanate (FITC), tetramethyl rhodamine isothiocyanate (TRIC) and Texas red. With these reagents coupled to avidin, streptavidin and various antibodies including antibiotin, antidigoxigenin and antiavidin, it is possible to build a strong fluorescence signal using several layers of fluorochrome-coupled antibodies.

In recent years, for some applications, indirect detection systems using antibodies are being replaced by direct labelling methods in which fluorochromes such as FITC are coupled directly to the nucleotides that are used as DNA probes.

DNA PROBES AND THEIR APPLICATIONS

Total genome probes

Total genome probes are prepared from DNA extracted from blood samples or cell cultures. Chromosomes hybridized with these probes show an evenly distributed signal along their length and can be used to identify sections of human chromosomes in human–rodent somatic cell hybrids.

Chromosome-specific paint probes

Probes specific for each chromosome can be prepared from chromosome-specific genomic libraries or from flow-sorted chromosomes (i.e. by separating the chromosome of interest from a number of cells in metaphase by a flow sorter). These paint probes are commercially available. The probe from a chromosome, e.g. chromosome 15 probe can paint the whole chromosome 15 from one end to the other and also any part of chromosome 15 attached to some other chromosome, or if present as a marker chromosome (Fig. 17.2). Thus, these probes can be used to identify interchromosomal rearrangements and marker chromosomes of unidentified origin. These probes cannot identify intrachromosomal rearrangements such as inversion and tandem duplication. Areas containing repetitive elements such as centromeres and telomeres are not painted.

Chromosome-specific centromeric probes

They are cloned from the repetitive DNA that is located close to the

centromeres (Fig.17.3). Centromeric probes are available for all human chromosomes except chromosomes 13, 14, 21 and 22, which share indistinguishable sequences. The centromeric DNA remains condensed even during interphase and these probes give a dense signal for each copy of the chromosome tested.

Centromeric probes are mainly used for the determination of the number of copies of chromosomes present in the interphase nuclei. They are used for the detection of aneuploidies in uncultured amniotic fluid cells, for preimplantation diagnosis from a single cell of a blastocyst and for the analysis of non-disjunctional abnormalities in sperms. For such applications, probes specific for sequences on q arms are used for chromosomes 13 and 21, as the centromeric probes for these two chromosomes are not specific and show cross hybridization.

Locus-specific DNA probes

Probes for small regions of the chromosomes are available. Each of these probes can identify sequences specific for a small region, which cannot be resolved by the techniques of conventional cytogenetics. These probes are used for the diagnosis of microdeletion syndromes such as Prader–Willi syndrome (Fig. 17.4), Williams syndrome, retinoblastoma, etc. In these disorders, the deleted segment is very small and the deletion may not be visible cytogenetically. They can also be used to delineate the exact breakpoints of a chromosomal rearrange-ment in cancer. Chromosomal rearrangements involving telomeres or ends of chromosomes are difficult to study by routine cytogenetics. Probes specific for telomeres are available and they are likely to play an important role in the evaluation of patients with mental retardation.

METHOD

- Cultured or uncultured cells from the blood, amniotic fluid, chorionic villi, fibroblasts, solid tumours or mouth washings are dissociated, if necessary with collagenase, and then treated with a hypotonic solution on a slide. The slides are then air-dried.
- The slides prepared by routine cytogenetics can be used for FISH analysis of metaphase chromosomes.
- The DNA on the slides is denatured by heating in formamide at 65–72 ^{0}C for 2–5 minutes before placing in ice-cold 70% ethanol. This is followed by dehydration through an ethanol series and air-drying. The rapid cooling ensures that the denatured single-stranded DNA in the preparation does not reanneal.

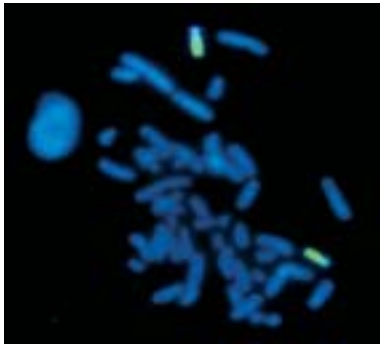


Fig. 17.2 Fluorescence *in situ* hybridization with paint probe for chromosome 15. Both the chromosomes 15 are seen as green and no other chromosome shows a green colour. This indicates that chromosome 15 has not been translocated to any other chromosome and no part of any other chromosome is translocated to chromosome 15.
(*Source of probes:* Vysis Inc. Courtesy: Dr Ashutosh Halder, All India Institute of Medical Sciences, New Delhi).

Fig. 17.3 Fluorescence *in situ* hybridization on a metaphase spread with 3 probes labelled with different colours; the probe for the heterochromatic region of chromosome 1q is yellow; (labelling ratio = 50:50 Texas red and fluorescein isothiocyanate [FITC]), the probe for the X centromere is green (FITC) and the probe for heterochromatin for the q arm of Y chromosome is red (Texas red). The metaphase shows two copies of chromosome 1, and one X and Y chromosome each.
(*Source of probes:* Galton Laboratory, UK). Courtesy: Dr Ashutosh Halder, All India Institute of Medical Sciences, New Delhi)

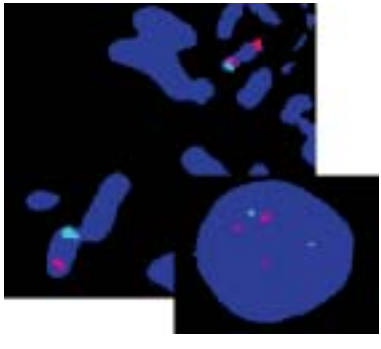


Fig. 17.4 Fluorescence *in situ* hybridization for the detection of microdeletion using a locus-specific probe. The probes are for chromosome 15 centromere (green), distal 15q-PML region (red) and proximal 15q (SNRPN locus for Prader–Willi syndrome) (red). One green signal is seen for each chromosome 15 centromere. Distal red signal (for colour probe-PML) is present on both chromosomes. One chromosome does not show a signal for SNRPN and confirms deletion of SNRPN region. Interphase nucleus is also showing 2 green and only 3 red signals.

- A directly labelled probe (a probe that has a fluorochrome attached to its nucleotides) can be used. If the probe is not labelled previously, it is labelled by nick translation, random primer extension, or by amplification in the presence of labelled nucleotides using polymerase chain reaction (PCR). These methods incorporate nucleotides labelled with biotin or digoxigenin into the probe DNA. The probes usually contain many repetitive sequences, which are distributed all over the genome. These, if not removed, will hybridize with all the chromosomes non-specifically and give rise to background fluorescence. To avoid background fluorescence, the probe, after denaturing, is prehybridized with human Cot-1 DNA for a brief period. The repetitive DNA anneals rapidly and later cannot hybridize with the DNA on the slide. This step is very important.
- Next, labelled DNA probe in a hybridization buffer is added onto the denatured slide preparation, covered with a coverslip, sealed and incubated at 37 °C overnight.
- After hybridization, the coverslip is removed and the slide is washed to remove the unhybridized probe. If the probe is directly labelled with a fluorochrome, slides can be immediately studied under a fluorescent microscope. If the probe is labelled with a non-fluorescent label such as digoxigenin or biotin, further steps to detect the label are necessary. The method of detection will depend on the hapten used in labelling the probe. If a biotinylated probe is used, the slide is treated with fluoresceinated avidin and incubated in the detection buffer followed by washing. The slide is stained with a fluorescent dye known as 4,6-diamidino-2-phenylindole (DAPI), which stains all chromosomes and is used to identify each chromosome. The slide is then mounted in an antifade solution (to prevent the fading of fluorescence).

The slides are viewed under a fluorescence microscope. Different fluorochromes need lights of different wavelengths and, accordingly, appropriate excitation and emission filters are chosen. Multiple excitation bandpass filters can be used in the microscope to observe several fluorochromes simultaneously. This means that the blue counterstain (DAPI) can be used, for example in conjunction with FITC (green) and TRIC (red) probe signals. Combining different fluorochromes in different proportions can increase the number of colours used. Digital fluorescence microscopy and image-processing systems are also available. They are necessary for multicolour FISH. In addition, these systems allow image enhancement, colour adjustment, image reversal and a number of processes that help in analysis.

APPLICATIONS

The advantages of FISH, such as the ability to identify the origin of a small part of a chromosome, to identify microdeletions and low levels of mosaicism, and to study chromosomes in the interphase, have made this a versatile and useful technique for clinical cytogenetics as well as research purposes. The applications of FISH are as follows:

1. To identify or confirm the origin of chromosomes involved in rearrangements: Chromosomal rearrangements, especially those involving small parts of chromosomes, may be difficult to resolve by conventional cytogenetics. In such cases, FISH using chromosome paint of a suspected chromosome or multiple chromosomes could help in confirming the chromosomes involved in the rearrangement.
2. To identify the origin of a marker chromosome: A small chromosome, the origin of which cannot be identified by traditional cytogenetic techniques, can be easily identified using whole chromosome paint probes. This is especially important in prenatal cases where a *de novo* marker is seen in the amniotic fluid karyotype. FISH is useful for patients with Turner syndrome who has a small marker chromosome in addition to one X chromosome. It needs to be know whether the marker is from the X or Y chromosome as presence of Y indicates removal of gonads.
3. To delineate breakpoints of a chromosomal rearrangement: By using closely placed probes, the breakpoints of a translocation can be delineated in finer detail. FISH also helps in identifying cryptic deletions.
4. To detect low levels of mosaicism: The difficulties in studying a large number of cells by traditional cytogenetic techniques is overcome by using FISH. Such study is useful when low level of mosaicism is suspected.
5. Rapid analysis: The short time needed for FISH is an advantage. This becomes important in prenatal diagnosis, especially when the test is done during late pregnancy. For example, in a prenatally diagnosed duodenal atresia in late gestation, one may need to know whether the foetus has trisomy 21 or not. In such cases, FISH can provide the report in a day compared with a minimum of 10 days with amniotic fluid culture and karyotyping.
6. Microdeletion syndromes: Deletions in contiguous gene syndromes are small and not detectable by routine karyotyping. FISH probes for a number of syndromes such as Cri du chat syndrome (5p15.2), Williams syndrome (7q11q23), Prader–Willi and Angelman

syndromes (15q11q13), Miller–Dieker syndrome (17p13.3), DiGeorge syndrome (22q11.2) are commercially available and can be used for pre- and postnatal diagnosis.

7. Single cell diagnosis: FISH has made preimplantation diagnosis and analysis of foetal cells from maternal blood a reality. A single cell from a blastocyst can be analysed for common chromosomal aneuploidies before implantation. This improves the *in vitro* fertilization results. Similarly, foetal cells isolated from maternal blood are few in number and can also be analysed by interphase FISH for the detection of aneuploidies.
8. Study of non-dividing cells: Cells that are in the non-dividing stage or difficult to grow can only be studied by FISH. Thus, the study of sperms for aneuploidies or chromosomal analysis of solid tumours can be easily done by FISH.
9. Chromosomal aberrations in cancers: In addition to being a useful tool for research in cancer genetics, FISH is also used for identifying common chromosomal aberrations that are specific for particular cancers. This is clinically useful in diagnosis, prognosis and follow up of cancer patients to detect relapse. The commonest chromosomal abnormality in cancers—presence of the Philadelphia chromosome in chronic myeloid leukaemia—can now be studied using probes for the *bcr* and *abl* genes, which correctly identify rearrangement even if it is present in only a few cells.
10. Study of telomeric regions: Chromosomal rearrangements involving telomeric regions are difficult to identify. The probes for these regions can be used to identify chromosomal abnormalities in mental retardation syndromes and reproductive losses.
11. Follow up of bone marrow transplantation (BMT): In sex-mismatched BMT, the engraftment can be evaluated with 'X' and 'Y' probes.

FISH on uncultured amniocytes

The speed of FISH has made it an important technique for prenatal diagnosis. However, it is necessary to remember that though high levels of sensitivity and specificity have been achieved in FISH, it cannot be used to study all chromosomes at a time, and unsuspected structural anomalies of chromosomes cannot be detected by this method. Thus, FISH on interphase cells should not be used in preference to routine cytogenetic prenatal diagnosis but should presently remain limited to the appropriate situations demanding quick results. For rapid prenatal

diagnosis, a panel of 5 probes specific for chromosomes 13, 18, 21, X and Y is used. With this panel of probes, FISH can detect common chromosomal aneuploidies, it cannot detect aneuploidies of chromosomes other than these five and structural abnormalities of chromosomes. When FISH with five chromosome probes is used for prenatal diagnosis, the risk of other chromosomal abnormalities, which cannot be detected by this technique, needs to be explained to the patient. With a normal FISH report, there is about 0.6% risk that the foetus still has a chromosomal anomaly. Only 89% of chromosomal abnormalities associated with a bad prognosis are detected by interphase FISH. The sensitivity is better when the indication for prenatal diagnosis is advanced maternal age (95%), but does not go beyond 85% for other indications such as foetal structural malformation.

Research applications

In addition to the above applications, FISH is widely used for research purposes such as chromosomal localization of genes and DNA sequences, preparation of a physical map, characterization of somatic cell hybrids, study of the organization of chromosomal domain in interphase nuclei and in studies on genetic toxicology.

MODIFICATIONS OF FISH

Comparative genomic hybridization

This method is an important tool in cancer research and for the mapping of various genes involved in tumorigenesis. Comparative genomic hybridization (CGH) uses a mixture of fluorescein-labelled tumour DNA (green) and Texas red-labelled normal genomic DNA (red). This mixture is hybridized to normal DNA in metaphase. The relative amounts of tumour and normal DNA that anneal to a particular region of a chromosome depend on the number of copies of DNA complementary to that region in the test sample. If the test sample contains relatively larger quantities (multiple copies) of a particular DNA sequence than the reference normal sample, there will be an increased green/red fluorescence ratio in the complementary region; while in the case of deletion of a particular region in the tumour tissue DNA, the ratio will be reversed. The abnormalities thus detected in the tumour genome are used for identifying cancer-related genes. This method is also a useful adjunct for confirming or further characterizing unbalanced chromosomal abnormalities and screening for deletions and duplications involving any

part of the genome.

Spectral karyotyping

This technique is a recent development in FISH and can be used to study the whole genome. Earlier, only a part or parts of a chromosome or one or two chromosomes could be studied at a time by FISH. Spectral karyotyping (SKY) is a multicolour genome-wide FISH. It uses probes for all chromosomes simultaneously. The probe for each chromosome is labelled with one or a combination of two or more fluorochromes. Hence, each chromosome emits a different fluorescence. Digital imaging systems, with their high spectral resolutions, can identify and display all the pixels of the image in a selectively defined pseudocolour. This technique enables the screening of metaphase speads for the presence of numerical and structural aberrations of all chromosomes.

The technique combines the resolution a power of FISH with the advantages of conventional cytogenetic methods to simultaneously scan all human chromosomes in a single image. At present, the use of this technique is limited to research laboratories.

Fibre FISH

DNA in chromosomes is in a condensed form; therefore, two cosmid probes hybridized to the same chromosome can be resolved only if they are more than 2–3 Mb apart. Chromatin fibres in the interphase are less condensed, making the resolution in interphase better. The chromatin can be further stretched with the help of melting agarose. The use of FISH on these extended fibres can be applied to map and to know the sequential order of contiguous gene sequences.

Fluorescence *in situ* hybridization—a combination of cytogenetics and molecular genetics—is a revolution in the study of chromosomes and genes. In addition to its increasing applications, the technique itself is growing fast. It may soon be possible to directly visualize various molecular events in the cell using this technique.

Section IV

Diagnosis, treatment and prevention of genetic disorders

18 Treatment of genetic disorders: Conventional Management and gene therapy

INTRODUCTION

Many genetic disorders still do not have a curative or effective treatment. Many are associated with early death, physical or mental disability or chronic ill health. This makes prevention by genetic counselling and prenatal diagnosis an important part of the management of families at risk of genetic disorders. On the other hand, due to the lack of treatment and rarity (or low prevalence) of these disorders, genetic disorders are not considered in the differential diagnosis of most diseases. Even if suspected, cases with possible genetic disorders are often not investigated, especially if it is obvious that the prognosis is poor. In the previous chapters, the importance of aetiological diagnosis for providing genetic counselling to families with or at risk for genetic disorders was stressed upon. This chapter deals with various conventional as well as novel methods of treatment for genetic disorders. Radical cure by either replacing the defective gene or repairing the mutation is still not possible. However, successful interventions are available at various levels, which provide cure, prevent illness or disability, or ameliorate symptoms. Different strategies to treat single-gene disorders are discussed below along with examples. The success of new therapies such as enzyme replacement therapy and bone marrow transplantation holds promise for many more disorders. With a better understanding of the metabolic and molecular bases of genetic disorders, new strategies and drugs for treatment may come up in the near future. Gene therapy, as an ultimate cure for all monogenic disorders, has been shown to be feasible in initial studies, but safe and effective gene therapy for genetic disorders is not yet available.

CURRENT TREATMENT MODALITIES

Developmental disorders like genetic syndromes, mental retardation, and progressive neurodegenerative disorders are largely uncurable, currently. However, many malformations are surgically treatable. In spite of knowing the defective enzyme and having a good understanding of the metabolic pathways, only a few genetic metabolic disorders can be cured or ameliorated. In 1983, only about 10% of genetic metabolic disorders showed complete response to treatment, 40% showed partial response and there was no effective treatment for the rest. Over the past 20 years, there has been a steady increase in the number of disorders with partial response to treatment but the number of curable genetic disorders has not changed substantially.

STRATEGIES FOR CONVENTIONAL TREATMENT OF MONOGENIC DISORDERS

The aims of conventional treatment are to replace the defective protein, enhance its production, improve its function and minimize the consequences of deficiency by dietary management, remove the toxic product or replace/remove the diseased organ.

Augmentation of low levels of protein

This is possible in certain diseases where the patient has a small but inadequate amount of protein. Crigler–Najjar syndrome is an autosomal recessive disorder caused by a defect in the conjugation of bilirubin which makes it water soluble before it is excreted in the bile. The enzyme is absent in Crigler–Najjar syndrome type I and deficient in Crigler–Najjar syndrome type II. In Crigler–Najjar syndrome type II, production of the enzyme can be induced by phenobarbitone. The increase in enzyme activity after phenobarbitone treatment is sufficient to decrease the serum level of bilirubin by 25% and improve the prognosis. Similarly, in mild haemophilia A, the infusion of desmopressin (dideamino vasopressin [DDAVP]), will double or triple the level of Factor VIII in the blood and this may be sufficient to take care of minor bleeding episodes.

Replacement of the deficient protein or enzyme

A commonly seen example of replacement of the deficient protein is the infusion of antihaemophilic Factor VIII and Factor IX in haemophilia A and haemophilia B, respectively. The defective enzyme or protein can be replaced by blood transfusion as in severe combined immunodeficiency

(SCID), due to adenosine deaminase (ADA) deficiency and thalassaemia major. For most of the enzyme deficiency disorders, replacement of the enzyme is not possible due to lack of adequate quantities of the enzyme and inefficiency of the transport system to deliver the enzyme intracellularly. These technical difficulties have been overcome and enzyme therapy is being successfully carried out in more than 2000 cases of Gaucher disease type I. This success story is being repeated for mucopolysaccharidosis type I and Fabry disease.

Enhancement of the function of a deficient protein

Vitamins are the co-factors for enzymes in various metabolic reactions. High doses of vitamins (which can be given safely) correct or bypass the metabolic defects of some genetic disorders and are used successfully for treatment. The examples are biotin in biotinidase deficiency, vitamin B_6 (pyridoxine) in homocystinuria and vitamin D in vitamin D-resistant rickets. The mechanism of action of the vitamins varies in each disease. If mutation has reduced the affinity of the enzyme for the co-factor, large amounts of the vitamin may partially restore enzyme activity and prevent manifestation of the disease.

Dietary restrictions

This strategy has been used successfully in preventing mental retardation in patients with phenylketonuria and has also been applied to many other genetic metabolic disorders such as galactosaemia, maple syrup urine disease, etc. Complete elimination or restriction of the offending compound (mostly an amino acid) from the diet is necessary, depending on whether the offending agent is non-essential or essential, respectively. Cholesterol restriction in familial hypercholesterolaemia is an example where diet can help in reducing the risk of ischaemic heart disease.

Lifelong compliance is a major problem with diet restriction. In spite of strict adherence to diet, some residual deficits are seen in many disorders. Patients with phenylketonuria and galactosaemia who have undergone good treatment have a normal intelligence quotient (IQ) but still have some neuropsychological deficit.

Drug therapy

Various drugs are available for the treatment of genetic disorders with known pathogenesis. The examples are cholestyramine in familial hypercholesterolaemia and penicillamine in Wilson disease. Drugs that do not directly effect the pathogenesis of the disease mechanism but act

by modifying the factors affecting the clinical manifestations are also used in treating some genetic disorders. Examples include the use of hydroxyurea in sickle cell disease and some cases of thalassaemia intermedia. Hydroxyurea acts by increasing the concentration of foetal haemoglobin, thus modifying the course of the disease.

Drug avoidance

This strategy is a part of pharmacogenetics. As more and more information about genetic factors causing adverse drug effects becomes available, the list of these types of genetic disorders will increase. Clinical examples are avoiding the use of primaquine in glucose-6-phosphate dehydrogenase deficiency and phenobarbitone in acute intermittent porphyria.

Treatment of the phenotype

Even if the aetiology cannot be addressed, the phenotypic effects of some diseases can be modified. For example, some malformations are surgically treatable and the oxygen carrying capacity of blood cells can be increased by blood transfusion in sickle cell disease.

Organ transplantation

In some cases, the defective organ can be replaced, for example, kidney transplantation in autosomal dominant polycystic kidney disease. Bone marrow transplantation provides a cure for many genetic immunodeficiency syndromes, osteopetrosis, thalassaemias, etc. Transplantation has been found useful in many lysosomal storage disorders such as mucopolysaccharidosis. Instead of bone marrow transplantation, stem cells from the placental blood can be used. Easy availability and less stringent matching requirements of human leucocyte antigen (HLA) are advantages with the use of haematopoietic stem cells from placental blood.

Organ removal

Colectomy in familial adenomatous polyposis (FAP) is a successful way of preventing colon cancer. Splenectomy cures anaemia in hereditary spherocytosis.

The above discussion illustrates various successful treatment modalities for genetic disorders. These disorders can be managed without

modifying the genetic defects at the DNA level. The number of disorders for which good prognosis is possible with treatment is limited. Many other genetic disorders do not have successful treatments and only palliative or supportive therapy can be provided. Hope lies with advances in gene therapy for these disorders.

GENE THERAPY: THE FUTURE

Correction of a defective gene or its replacement with a normally functioning gene appears to be the logical cure for genetic disorders. Recombinant DNA technology has made this possible. Gene therapy is described as the introduction of a gene into a cell to achieve a therapeutic effect. A large number of gene therapy trials for the treatment of genetic disorders and cancers are going on. The initial success was reported in the immunodeficiency disorder caused by ADA deficiency. However, for continued effect, the treatment had to be repeated every 6 weeks. Long term success was recently reported for SCID-X1. However, some of these patients developed malignancy, substantiating the theoretical possibility of the development of cancer as a result of gene therapy. Thus, safe and effective gene therapy is yet to be developed.

Gene therapy can be used to correct genetic defects in fertilized eggs (germline gene therapy) or somatic cells (somatic gene therapy). Due to the possible adverse effects on the next generation and many other ethical reasons, germline gene therapy is not accepted. For somatic gene therapy also, there are guidelines for protocols, and permission of ethics committees of hospitals and regulatory bodies of the country is essential. The strategy for different diseases will vary, depending upon the pathogenesis of the disease. Various strategies for gene therapy are discussed below.

Gene augmentation therapy

If a disease is caused by a mutation causing loss of function, introduction of a functional copy of the gene into a cell will restore the normal function of the gene. In this case, the location of integration of the gene will not matter. Long-term expression of a gene and control of its expression are important limiting factors. At present, diseases requiring a large or an exact amount of the gene product to prevent the disease phenotype cannot be treated by gene therapy. Deficiency of ADA is a disorder where a small amount of a protein is required to prevent symptoms of the disease. Similarly, haemophilia is another good candidate for gene therapy because achieving an antihaemophilic factor level of over 1% will

substantially modify the course of the disease. On the other hand, diseases such as beta-thalassaemia need greater and controlled expression of the beta globin gene, and imbalance between the ratio of alpha and beta globins leads to haemolysis. Such diseases are not good candidates for gene therapy.

Targeted mutation correction

The fundamental way to correct a gene defect is to correct the mutation, i.e. change the mutated nucleotide sequence to normal. This is practically difficult, but in principle it can be done by homologous recombination. This is necessary if the mutation produces a dominant negative effect, i.e. the product of the mutated copy of the gene interferes with the function of the product of its normal copy. In such situation, the introduction of a normal copy of the gene without taking care of the mutated one in the cell will not be useful.

Inhibition of gene expression

In case of mutations that have a negative dominant effect as described above, expression of the mutated gene can be blocked at the DNA, RNA or protein level. This strategy is also useful for cancers due to either expression of a novel gene or inappropriate expression of a gene.

Gene therapy to achieve pharmacological effects

This therapy is commonly used for cancers by way of introducing a toxic gene, the expression of which kills the cells or by introducing genes that make the cancer cells susceptible to anticancer drugs. Genes of cytokines can be introduced in non-diseased cells such as those of the immune system to enhance their potential to kill diseased cells.

Gene therapy can be done *in vivo* or *ex vivo* depending upon the approach used for gene transfer (Fig. 18.1).

Ex vivo gene transfer

In *ex vivo* gene transfer, cells from the patient are collected and grown *in vitro*. The gene is then introduced into the cultured cells. These cells are re-introduced into the same individual. To use this approach, it is necessary that the tissue to be used can be removed from the body and cultured *in vitro*. Examples of these types of cells are haematopoietic cells and fibroblasts.

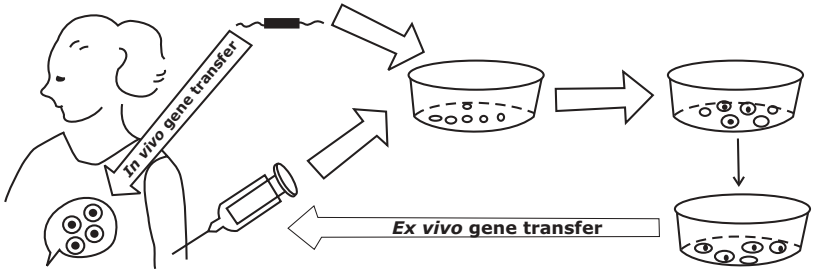


Fig. 18.1 *In vivo* and *ex vivo* gene transfer. (~▬~ gene to be transfered)

In vivo gene transfer

In this method, cloned genes are transferred directly into the tissues of the patient. This method is used in situations where the tissue in which the gene is to be inserted cannot be removed from the body. Examples are the brain and the lungs.

Following gene transfer, the gene may either integrate into the chromosomes (Fig. 18.2; Case 1) or may not integrate and remain extrachromosomal (episomes) (Fig. 18.2; Case 2). The gene integrated into the chromosome will replicate during cell division and be stably passed on to all daughter cells. The episomal gene replicates extrachromosomally and will not be transmitted to all daughter cells, and will get lost after subsequent cell divisions. This type of approach is applied in gene therapies where the target tissue consists of non-dividing cells, or transient gene expression is sufficient for therapeutic purposes.

The most important factor for gene transfer is the vector used. The gene can be transferred by injecting it into the cell and tissue or by bombarding the cells with gold particles coated with DNA. These approaches are less efficient. Commonly used vectors are viruses and non-viral vectors such as liposomes (Table 18.1). The commonly used viral vectors are derived from retroviruses, adenoviruses and adeno-associated viruses. Retroviruses used in gene therapy are rendered incapable of independent replication to prevent the side-effects associated with infectivity. Retroviruses can infect only dividing cells. Since all viral genes are removed from the vector, viruses cannot replicate by themselves. Retrovirus vectors are used only in *ex vivo* gene therapy protocols.

Adenoviruses can be produced at high titres in cultures and are the second most commonly used delivery system in gene therapy, including

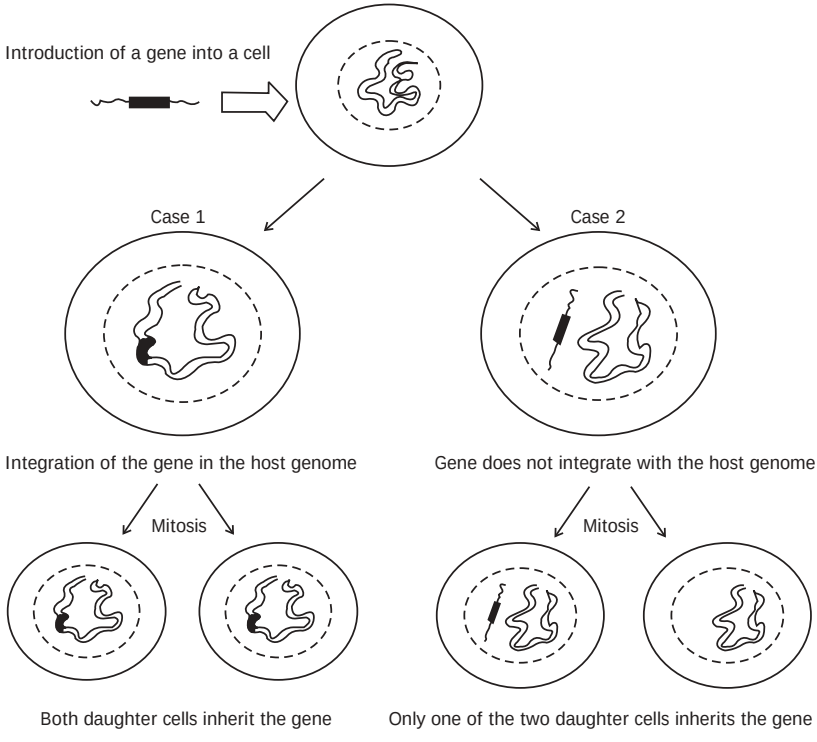


Fig. 18.2 Fate of a gene after gene transfer.

gene therapy for cystic fibrosis. They can infect non-dividing cells. The disadvantage, however, is episomal integration of the gene and hence, transient expression.

Safety is a matter of concern while using viral vectors. Viral vectors used in gene therapy are replication incompetent. However, there is a remote possibility of producing recombinant viruses that can lead to infection. Repeated injections of adenoviruses may produce severe inflammatory response. These concerns have led to the use of liposomes, which are lipid bilayers surrounding an aqueous vesicle. These can be used to introduce foreign DNA into a target cell. Large size DNA can be packaged inside the liposomes, but the efficiency of transfer is less. The search for a safe and efficient vector is still on.

Risks of gene therapy

The risks thought to be associated with gene therapy are the following:

Table 18.1 Comparison of vectors used for gene therapy

Vectors	Advantages	Disadvantages
Retroviruses	• Chromosomal integration and stable modification of the target cell • Can infect only dividing cells • Suitable for *ex vivo* treatment	• Uncontrolled integration; may be oncogenic • Cannot infect non-dividing cells
Adenoviruses	• Can infect non-dividing cells, thus suitable for gene therapy of cystic fibrosis, Duchenne muscular dystrophy • Non-integration; avoids the risk of uncontrolled integration • Efficient gene transfer	• Transient expression of gene • Provokes immune response
Liposomes	• Safe • Can carry large DNA molecules	• Inefficient transfer • Transient expression

(i) adverse reaction to the virus or gene; (ii) activation of a proto-oncogene resulting in the formation of an oncogene; and (iii) introduction of the dominant disease-causing mutation in the next generation. These risks were considered to be theoretical and rare. However, death due to reaction to adenovirus injection (for gene therapy) in a patient with ornithine transcarbamylase deficiency, and development of leukaemia in some cases of immunodeficiency syndrome successfully treated with gene therapy have proved that these possibilities are not so rare. These events have emphasized the need for strict control over gene therapy research and informed consent.

Some results of gene therapy

More than 600 clinical gene therapy trials involving about 3500 patients were identified worldwide in 2002. Trials are being carried out for various types of cancers. Other disorders for which gene therapy has been tried are monogenic, infectious, autoimmune and vascular disorders. Gene therapy trials of some monogenic disorders are briefly discussed below.

Adenosine deaminase deficiency

The first attempt at gene therapy was made in two girls with ADA deficiency. Along with gene therapy, ADA enzyme was also given. The treatment was repeated every 6 weeks. There was clinical improvement, but no ADA expression was detected; thus the role of gene therapy in clinical improvement was doubtful.

Severe combined immunodeficiency

Patients with SCID-X1 were successfully treated with gene therapy in the year 2000 and they continued to show a long period of gene expression and improved immune function. This suggests that these patients were possibly cured. However, 2 of the 10 treated cases developed acute lymphoblastic leukaemia due to the activation of an oncogene.

This success story of gene therapy also confirmed that the risk of development of cancer as a result of gene therapy is real and significant.

Haemophilia A and haemophilia B

Following the experiments in mice and canine models of haemophilia, there have been two phase I clinical trials. In the haemophilia A trial, the gene was introduced in fibroblasts *ex vivo*. The fibroblasts were re-introduced into the peritoneal cavity by laparoscopy. Increase in the level of Factor VIII by about 1% in some cases, and a significant decrease in bleeding episodes and requirement of Factor VIII infusion was documented. The improvement lasted for only a few months.

Duchenne muscular dystrophy

Gene therapy for Duchenne muscular dystrophy (DMD) has been successfully carried out in transgenic mice but human trials could not be attempted. The gene for DMD—dystrophin—is a large one. A small part of the gene—minigene—derived from a milder form of the disease (Becker muscular dystrophy) was chosen for gene transfer into myoblasts. However, gene expression was found to be transient. A major difficulty in DMD is that the gene needs to be introduced into a significant fraction of cells of the skeletal and cardiac muscles.

Cystic fibrosis

Trials with *in vivo* cystic fibrosis (transmembrane conductance regulator -CFTR) gene therapy have shown limited success. Expression of the gene was transient and not sufficient to cause clinical improvement.

Non-heritable disorders

Various strategies of gene therapy are also being tried for many non-heritable disorders such as cancers, and infectious and autoimmune diseases.

OTHER TREATMENT MODALITIES BASED ON RECOMBINANT DNA TECHNOLOGY

Though gene therapy may take time to become clinically applicable, research in recombinant DNA technology has been instrumental in developing newer

Table 18.2 Disorders for which foetal therapy is available

Disorder in the foetus	Treatment
Arrhythmias	Antiarrhythmic drugs
Anaemia	Foetal blood transfusion
Goitre/hypothyroidism	Thyroxine
Congenital adrenal hyperplasia	Dexamethasone
Hydrocephalus	Ventriculoamniotic shunt
Hydronephrosis	Shunt or fulgurization of the valve
Diaphragmatic hernia	Repair or tracheal ligation
Meningomyelocele	Repair

treatments for genetic and non-genetic disorders through recombinant proteins, genetically engineered antibodies and vaccines.

FOETAL THERAPY

Foetal diagnosis has led to foetal treatment. It is now possible to offer foetal therapy for many medical and surgical disorders (Table 18.2).

Prenatal management of congenital adrenal hyperplasia and prevention of the development of genital ambiguity is an example of successful foetal therapy of a genetic disorder. Surgical management of malformation by foetal surgery or interventional procedures such as shunt placement have shown high levels of technical feasibility. For conditions such as foetal hydrocephalus, the identification of patients who would be benefited by foetal intervention instead of neonatal management, is difficult. In a series of cases, foetal intervention has not shown better results when compared with cases treated postnatally.

STEM CELL TRANSPLANTATION

Undifferentiated cells which are capable of proliferation and differentiation into various types of differentiated cells are known as stem cells. Stem cells derived from an embryo have such capabilities. Haemopoetic stem cells and stem cells derived from umbilical cord blood are also useful for transplantation.Although the use of embryonic stem cells is considered ethically debatable, current research indicates a great potential for the efficacy of stem cells in the treatment of diseases like Parkinson disease, diabetes mellitus, ischaemic heart disease, cardiomyopathy, etc. Transplantation of stem cells *in utero* has been tried in SCID and thalassemia.

19 Prenatal diagnosis

The diagnosis of foetal disorders *in utero* is an important milestone in clinical genetics. With the increasing availability of prenatal diagnosis, many families at high risk for the occurrence or recurrence of a genetic disorder, who might otherwise forego having children, can now plan pregnancies. Usually, the purpose of prenatal diagnosis is to detect whether the foetus is affected with a serious disorder in early pregnancy. This provides the option of termination of pregnancy and thus the birth of an affected child can be prevented. However, in most cases, the results indicate an unaffected foetus and the anxiety of the parents is relieved. In some cases, especially in the later half of pregnancy, prenatal diagnosis provides an opportunity to organize appropriate obstetric and neonatal management. Professionals involved in such tests are often confronted with many ethical and psychological issues surrounding prenatal diagnosis.

INDICATIONS FOR PRENATAL DIAGNOSIS

Prenatal diagnosis is done in families at high risk for serious disorder in the foetus which may be associated with lethality, difficult treatment, serious physical or mental handicap, or significant mortality. Prenatal diagnosis is also being done in the low-risk population for disorders that have a high frequency, e.g. neural tube defects (NTDs) and other malformations, Down syndrome. In these situations, prenatal screening tests precede the diagnostic tests. The disorders for which prenatal diagnosis is done include chromosomal disorders (*see* Chapter 8), single-

gene disorders (*see* Chapter 3), congenital malformations (*see* Chapter 11), foetal infections and, rarely, other non-genetic disorders such as immune hydrops, maternal idiopathic thrombocytopenia, maternal diabetes mellitus and exposure to teratogens. Table 19.1 lists some disorders for which prenatal diagnosis is available. Prenatal diagnosis can be done by non-invasive techniques such as ultrasonography (USG), or by molecular, biochemical or other specialized techniques after obtaining a foetal sample by invasive techniques.

TECHNIQUES FOR OBTAINING FOETAL SAMPLES

Prenatal diagnostic techniques to obtain foetal tissue samples are called invasive methods of prenatal diagnosis. Chorionic villi and amniotic fluid are the commonly used samples for prenatal diagnosis.

Samples obtained from these tissues can be used for various DNA-based tests. The DNA of these tissues is similar to that of the foetus and defective DNA present in the cells of the chorionic villi or amniotic fluid will also be present in the foetus. These tissues can also be used for various biochemical tests such as enzyme assays for the prenatal diagnosis of metabolic disorders. Most enzymes which are expressed in fibroblasts are also produced by cells of the chorionic villi or cultured amniotic fluid. Chorionic villus sampling (CVS) is the technique of choice for enzyme-based prenatal diagnosis. An enzyme assay done directly on uncultured cells of the chorionic villi gives accurate results. Chorionic villi are also used for prenatal diagnosis based on DNA tests. CVS can be done in the early stages of pregnancy (first trimester) and provides enough cells (without culturing) for analysis. Amniotic fluid, on the other hand, contains fewer cells and may need to be cultured for 8–10 days before the biochemical test can be conducted on amniotic fluid cells.

Some biochemical tests such as 17-hydroxyprogesterone assay for 21-hydroxylase deficiency, mucopolysaccharide analysis for various types of mucopolysaccharidoses and alpha-fetoprotein (AFP) estimation for the prenatal diagnosis of NTD can be carried out only on amniotic fluid. However, with the availability of DNA-based tests for 21-hydroxylase deficiency, enzyme assays for mucopolysaccharidosis and high-resolution USG for NTD, there is no need to perform amniocentesis for these disorders. Amniocentesis is commonly used for the prenatal diagnosis of chromosomal disorders. This is because in most cases, the risk of chromosomal anomalies is small (usually about 1%) and CVS has a slightly higher risk of miscarriage (about 2%) than amniocentesis. On the other hand, CVS is the technique of choice for monogenic disorders that have a 25%–

Table 19.1 Some disorders for which prenatal diagnosis is available

1. Congenital malformations
2. Chromosomal disorders
3. Single-gene disorders
   - Multiple malformation syndromes
     —Holt–Oram syndrome *
     —Meckel–Gruber syndrome *
     —Oral–facial–digital syndromes *
     —Craniosynostosis syndromes *†
   - Haematological disorders
     —Thalassaemias †
     —Haemoglobinopathies †
     —Haemophilia A and haemophilia B †
   - Metabolic disorders
     —Tay–Sachs disease †§
     —Metachromatic leucodystrophy †§
     —Wilson disease †§
     —Ornithine transcarbamylase deficiency †§
     —Mucopolysaccharidoses (types) †§
     —Smith–Lemli–Opitz syndrome †§
     —Congenital adrenal hyperplasia †§
   - Neuromuscular disorders
     —Huntington chorea †
     —Myotonic dystrophy †
     —Duchenne muscular dystrophy †
     —Fragile X syndrome †
   - Renal disorders
     —Autosomal dominant †/recessive †* polycystic kidney disease
   - Connective tissue disorders/skeletal dysplasias
     —Osteogenesis imperfecta †
     —Ehlers–Danlos syndrome †
     —Achondroplasia †
     —Achondrogenesis *
     —Thanatophoric dysplasia *†
     —Marfan syndrome †
   - Skin disorders
     —Epidermolysis bullosa (various types) †‡
     —Ichthyosis †‡
     —Ectodermal dysplasia †‡
4. Non-genetic foetal disorders
   - Foetal infections §
   - Immune hydrops §
   - Foetal effects of maternal diseases
     —Idiopathic thrombocytopenic purpura §
     —Diabetes mellitus *

Mode of prenatal diagnosis: * ultrasonography, † molecular techniques, ‡ skin biopsy, § other tests

50% risk of occurrence in families with an affected member or carrier parents.

Foetal blood sampling, or biopsy of the skin, liver or muscle can be done for the diagnosis of disorders for which DNA-based or biochemical tests are not available.

Chorionic villus sampling

Following fertilization, the zygote first differentiates into a blastocyst, which contains an inner mass of cells that develops into the foetus and an outer trophoblastic layer that develops into non-foetal structures such as the amnion, chorion and placenta. Biochemical, molecular and cytogenetic analyses of the chorionic villi or placental biopsy material are commonly used for prenatal diagnosis. To avoid errors in diagnosis because of contamination of the sample with maternal cells the maternal decidua must be removed from the chorionic villi under a dissecting microscope.

Chorionic villus sampling can be done by the transcervical or transabdominal route. Data suggest that carrying out CVS in early pregnancy can result in limb reduction defects in the foetus. Thus, this test should be avoided during the initial 10 weeks of gestation. Usually, CVS is performed at 10–12 weeks of gestation. Placental biopsy can be obtained in the later part of gestation as well.

The transcervical approach

The transcervical procedure is done under ultrasound guidance. First, the foetal heart rate and growth is assessed. With the patient in lithotomy position, a Portex catheter is inserted aseptically in the cervix. The plastic cannula encloses a metal obturator that can be bent at the angle required to reach the placenta. The movement of the cannula is monitored by perabdominal USG (Fig. 19.1). Once the catheter is inside the placenta, the obturator is withdrawn and a 20 ml syringe containing 2–3 ml of transport medium (any medium used for cell culture) is attached to the catheter. Chorionic villi are then aspirated by multiple, rapid to-and-fro movements of the catheter at 20 ml of negative pressure. The catheter is withdrawn under continuous maximum negative pressure. About 5–10 mg of chorionic villi are adequate for DNA studies, while enzyme assays need 25–30 mg of the sample.

Absolute contraindications for transcervical CVS include active cervical or vaginal pathology. Relative contraindications include leiomyoma obstructing the cervical canal and history of bleeding from the vagina in the previous two weeks.

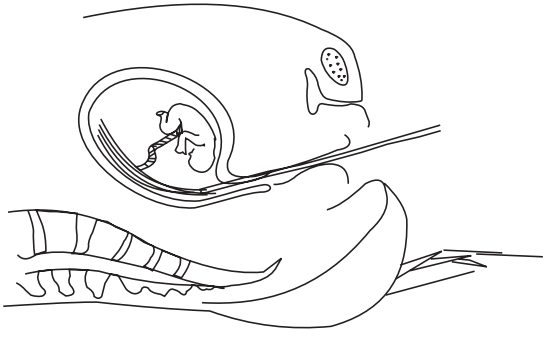


Fig. 19.1 Transcervical chorionic villus sampling.

The transabdominal approach

The placenta can usually be approached transabdominally irrespective of its location, except in the case of a posterior placenta in a retroflexed uterus, where it may be difficult to approach. Transabdominal CVS is more acceptable to patients and can be done when the transcervical approach is contraindicated. It is said that the learning curve with transabdominal CVS is shorter than that for transcervical CVS. After assessing foetal well-being by USG, the location of the placenta and needle insertion site are identified. Local asepsis should be strict as in any invasive procedure. The needle insertion site is infiltrated with a local anaesthetic.

An 18 or 19 gauge spinal needle is inserted under ultrasonographic visualization (Fig. 19.2). Some operators prefer to use a thinner needle such as a 20 gauge one. To obtain an adequate sample, the needle should traverse the long axis of the placenta. The needle stylet is withdrawn and a 20 ml syringe containing 3 ml of transport medium is attached. The needle is moved to-and-fro under suction. It is then withdrawn under continuous negative pressure. The cells of the chorionic villi in the syringe and needle are aseptically transferred to a screw-capped tube. The villi can be transported at room temperature with a maximum transport time of up to 24 hours if used for culture purposes or DNA tests. For special tests the transport conditions need to be confirmed from the laboratory to which the sample is to be sent.

Amniocentesis

Traditionally, amniocentesis is performed at 15–17 weeks of gestation. The procedure, previously used to estimate AFP, is now commonly used

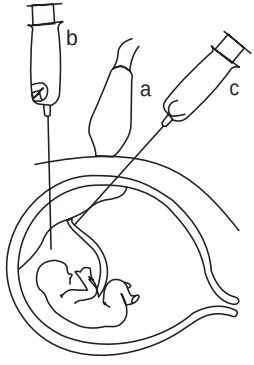


Fig. 19.2 Perabdominal sampling. USG probe (a), Needle placement for amniocentesis (b), for cord blood sampling (c).

for cytogenetic analysis in cases at an increased risk of Down syndrome, which is identified by the triple test (a screening test for Down syndrome on maternal serum). It is also a commonly chosen procedure for other indications of cytogenetic prenatal diagnosis. As discussed above, amniotic fluid can also be used for various DNA-based and biochemical tests. Traditional chromosomal analysis requires 10–15 days and results can be available before 20 weeks of pregnancy, the period before which termination of pregnancy is legally possible in most countries.

Amniocentesis is easier than CVS as in most cases amniotic fluid pockets of a reasonably good size are available. The procedure should be preceded by careful ultrasonographic examination to assess foetal growth and to look for malformations. Identification of a major malformation may change the need for amniocentesis.

A 20 or 22 gauge spinal needle is used. Local anaesthesia is not required. The needle should be inserted under ultrasonographic guidance. Pockets near the midline and fundus are preferred. If the placenta is anterior, the needle can traverse it. First, 2–3 ml of fluid is aspirated in a 5 ml syringe and the fluid along with the syringe is discarded. This is done because the first few millilitres of fluid are likely to contain maternal blood cells, muscle cells or fibroblasts. In the second trimester, 20–30 ml fluid can be aspirated using 10 ml syringes. Asepsis is important both for the patient as well as for the sample that has to be cultured. Amniotic fluid can also be transported at room temperature in leak-proof tubes. Sometimes a needle prick may lead to blood-stained amniotic fluid. It does not affect cell culture or increase the possibility of miscarriage. Similarly, green-coloured fluid, presumably due to meconium staining, is also not associated with a poor outcome of

pregnancy. However, the presence of brownish fluid due to a previous haemorrhage suggests a high possibility of foetal loss due to miscarriage.

The mother's urinary bladder should be emptied beforehand, to avoid aspiration of urine instead of the amniotic fluid. In twin pregnancies, fluid from two separate sacs can be aspirated satisfactorily with one or two pricks. Following aspiration of the amniotic fluid from one of the sacs, 2–3 ml of the coloured dye indigo carmine can be injected into it to prevent repeat aspiration from the same sac.

Foetal blood sampling

Ultrasound-guided foetal blood sampling (FBS) can be done by inserting a needle into the umbilical cord at its placental location or at the site of entry of the umbilical vein into the liver. It is usually done at the end of 18 weeks of gestation. Due to the availability of DNA-based diagnostic tests for most disorders diagnosed by FBS, the indications for this technique are decreasing. Foetal blood can be used to diagnose haemophilia A, haemophilia B, various types of thalassaemias, severe combined immunodeficiency (SCID) and foetal infections. FBS is also indicated for rapid chromosomal analysis when a malformation is detected late in pregnancy. Chromosomal analysis from foetal blood is possible in a day by karyotyping naturally dividing cells in the blood or within 72 hours using traditional culture methods. Nowadays, with the availability of fluorescence *in situ* hybridization (FISH; *see* Chapter 17), rapid tests for common aneuploidies can be done on uncultured amniotic fluid cells and the report is available within a day. This obviates the need for FBS.

The umbilical cord is also used for foetal blood transfusions and drug delivery.

SAFETY OF PRENATAL SAMPLING PROCEDURES

All the above procedures can be performed in the outpatient department. The risk of spontaneous abortion following these invasive procedures is 0.5% in the case of amniocentesis and 1%–2% in the case of CVS. These figures are over and above the risk of spontaneous abortion at the corresponding gestation period. FBS is a more invasive technique and needs greater expertise. The risk of foetal loss following FBS is 2%–3%. Other than the risk of miscarriage, there are no long-term effects on the growth and development of children born after prenatal diagnostic procedures.

Early amniocentesis, i.e. amniocentesis at 14 weeks or earlier, is considered an option for prenatal diagnosis. Studies have shown that the rates of success and foetal loss following the procedure are

Table 19.2 Comparison of three procedures for prenatal diagnosis

	CVS	Amniocentesis	FBS
Gestational age	11–12 weeks	15–16 weeks or later	18 weeks onwards
Sample success rate	96%–99%	99.5%	90% (1st attempt)
Risk of spontaneous abortion	1%–2%	0.5%	2%
Reporting time*	3–15 days	10–15 days	1 week
Culture failure	<1%	<1%	Nil

CVS: chorionic villus sampling; FBS: foetal blood sampling; *Cytogenetic analysis

comparable with those of traditional amniocentesis. But the number of early amniocenteses reported till date is small and some studies have reported an increased risk of spontaneous abortion following the procedure. An increased incidence of talipes equinovarus in children born after early amniocentesis is also reported.

Following the procedure the risk to the mother is minimal. Minor maternal complications such as transient vaginal spotting and minimal amniotic fluid leakage occur in ≤1% of cases, but are almost always self-limiting. Rarely, amnionitis can occur and septicaemia in the mother has been reported but serious maternal complications are said to be 'remote' risks. Table 19.2 summarizes the relative risks and accuracy of the three procedures.

ULTRASONOGRAPHY

Ultrasonography is a good modality for visualization of the foetus, placenta, and other normal and abnormal intrauterine structures. Being dynamic, it allows good delineation of the foetal anatomy irrespective of the foetal position and movements. The method is non-invasive and is not associated with any known risk to the mother or the foetus. The required equipment is nowadays available in most hospitals and the technique has become an integral part of prenatal obstetric care. However, specialized USG for foetal malformations requires a skilled operator with special knowledge and expertise of malformations and multiple malformation syndromes.

There are three situations where foetal USG is carried out:

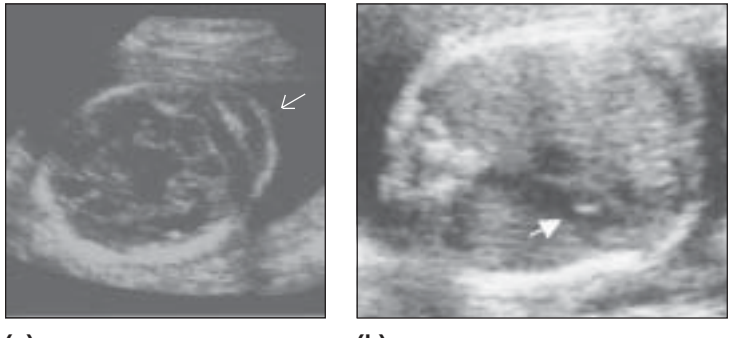
1. A previous child in the family with a malformation or a malformation syndrome.
2. The foetus in the present pregnancy is detected to have a malformation and then referred to an expert for confirmation or better delineation of

the malformation and to look for associated malformations.

3. In low risk pregnancies it is advisable to get USG done at about 18 weeks of gestation to screen for malformations. Routine ultrasound scanning may not improve the outcome of pregnancy in terms of increased number of livebirths or decrease in perinatal morbidity, but is a useful tool to screen for malformations and reduce perinatal mortality due to lethal malformations. Sensitivity of USG in low risk population varies greatly depending on the skill of the ultrasonographer.
4. In addition, supported by the data regarding ultrasonographic markers for chromosomal aneuploidies, first-trimester 'genetic' ultrasound examination is being incorporated into routine practice.

Prenatal ultrasonography for chromosomal anomalies

A number of foetal abnormalities that can be detected by USG are associated with chromosomal aneuploidy. These include major malformations as well as other abnormalities which by themselves are not likely to cause any problem. The latter group includes increased nuchal thickening, echogenic bowel, echogenic foci in the heart (Figs 19.3a and b), and absence of the nasal bone. The presence of nuchal thickening or increased nuchal translucency (3 mm or more in the first trimester and more than 5 mm in later pregnancy) and the absence of the nasal bone are the most sensitive markers and help in diagnosing up to 80% of foetuses with Down syndrome. Absence of the nasal bone at 11–14 weeks of gestation is a sensitive marker for Down syndrome, but a trained ultrasonographer is required to identify it.. In the presence of these ultrasonographic findings, determination of the foetal karyotype is



(a) **(b)**

Figs 19.3 Ultrasonograms showing **a** increased nuchal thickening (arrow) and **b** echogenic focus in the heart (arrow).

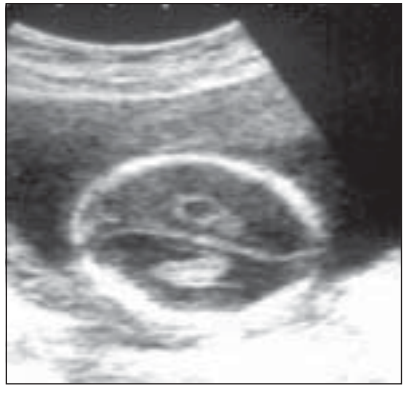


Fig. 19.4 Ultrasonogram showing a cyst of the choroid plexus.

indicated. These, along with the first-trimester maternal serum markers, are likely to replace the triple test done in the second trimester for the screening of Down syndrome. In addition, there are other ultrasonographic markers such as a cyst of the choroid plexus (Fig. 19.4) and renal pyelectasis which by themselves do not indicate the need for foetal karyotyping, but call for a careful search for the presence of other risk factors for aneuploidy.

Prenatally detected malformations and counselling

Malformations may be detected on routine USG in families without any risk of malformation. Diagnosis of a malformation in the foetus is an unexpected shock to the family. They need adequate information regarding the malformation, its prognosis, availability of surgical treatment and its outcome. Based on this information, the family will take a decision regarding continuation or termination of the pregnancy. Of course, psychological, social, religious beliefs and family issues will also be important factors influencing such a decision. If the malformation is detected late in pregnancy and the family decides to continue the pregnancy, appropriate management during delivery and of the neonate can be planned.

Major malformations that can be detected prenatally are listed in Table 19.3. Some malformations are difficult to miss while others are difficult to diagnose. The sensitivity of USG to detect malformations also depends on the malformation, expertise of the ultrasonographer and whether the examination was done in a high- or low-risk case. In addition to major malformations, minor malformations such as polydactyly, clinodactyly, increased gap between the first and second toes, small ears, etc. can also be visualized. These are to be looked for, especially in the

Table 19.3 Prenatally detected malformations and the sensitivity of ultrasonography in their detection (Chitty LS, Prenatal Diagnosis 1995;15:1241-1257)

Malformation	Detection rate in pregnancies at low risk for malformation (%)
Anencephaly	100
Spina bifida	80
Encephalocele	100
Hydrocephalus	40
Holoprosencephaly	60
Diaphragmatic hernia	60
Cardiac malformations	20
Omphalocele and gastroschisis	100
Tracheo-oesophageal fistula	7.7
Urinary tract abnormalities	66
Limb reduction defects	43
Spinal abnormalities	57
Cystic hygroma	100
Facial clefts	20
Talipes equinovarus	22
Hydrops	20
Skeletal dysplasias	50

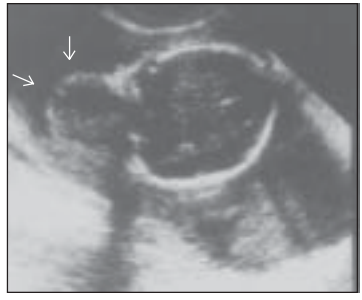
presence of a single malformation, and help in syndrome diagnosis.

Some malformations such as hydrocephalus and hydronephrosis may manifest in the later part of pregnancy and may not be detected in early pregnancy. Counselling for malformations with a universally lethal prognosis (stillbirth or neonatal death) such as anencephaly, acrania, iniencephaly, lethal skeletal dysplasias and large encephaloceles is simple (Figs 19.5a and b). Though the decision to terminate the pregnancy is difficult for the family, a lethal outcome is definite. Thus, after the detection of any such malformation in early pregnancy, choosing the option of termination is acceptable to most of the families. If diagnosed in the later part of pregnancy, i.e. after the period of viability, it may pose a problem in those countries where late termination of pregnancy is not legal.

In many malformations, a definite prognosis cannot be predicted. Detection of a malformation with an uncertain prognosis (Box 19.1) poses a great dilemma for the family and is a challenge for the counsellor. The prognosis for such malformations may vary from stillbirth to complete surgical cure with a normal outcome for life and function. The prognosis depends on associated malformations, chromosomal anomalies, severity of the malformation and gestational age at diagnosis.

The example of prenatally diagnosed ventriculomegaly is discussed

(a)



(b)

Fig. 19.5 a A foetus with a large encephalocele. **b** Ultrasonogram showing a large encephalocele.

Box 19.1 Prenatally detected malformations with uncertain prognosis

- Ventriculomegaly
- Hydronephrosis—unilateral or bilateral
- Duodenal atresia
- Diaphragmatic hernia
- Congenital heart defects
- Omphalocele
- Cleft lip
- Obstruction of the urinary bladder
- Meningomyelocele

below to illustrate the problems related to counselling.

Fetal ventriculomegaly

Fetal ventriculomegaly can be due to various obstructive and non-obstructive causes (Box 19.2). If ventriculomegaly isassociated with a chromosomal anomaly or severe brain malformation such as holoprosencephaly, or is a part of the multiple malformation syndrome, then surgical treatment of obstruction may not be possible and relieving the obstruction will not improve the poor mental and neurological function. Hence, prenatal diagnosis of ventriculomegaly calls for foetal

Box 19.2 Some causes of ventriculomegaly

- Meningomyelocele
- Aqueductal stenosis
- Dandy–Walker malformation
- Foetal cytomegalo virus infection
- Chromosomal syndrome
- Hydrolethalus syndrome
- Lissencephaly
- Walker–Warburg syndrome
- Vein of Galen malformation
  Holoprosencephaly

chromosomal analysis by amniocentesis or FBS and search or associated malformations such as those of the ear anomalies, microphthalmia, polydactyly, NTD and cleft lip. The presence of even minor malformations suggests a syndromic aetiology of the ventriculomegaly and indicates a poor prognosis. On the other hand, the absence of other malformations on USG does not rule out a syndromic aetiology as some associated malformations/dysmorphic features may not be detectable ultrasonographically or may be missed. In the absence of any associated malformation and chromosomal anomaly, the prognosis of foetal ventriculomegaly is uncertain. The size of the ventricles and thickness of the cerebral mantle are not good predictors of prognosis. The prognosis of foetal ventriculomegaly is different from that detected in neonates. In this situation of uncertainty, data from the literature can give some information about the likelihood of various possibilities. Most pregnancies with foetal ventriculomegaly are terminated. Out of those pregnancies which continue, 50%–80% end in stillbirth and the neurological function is normal in about 50%–70% of the survivors following surgery. In some foetuses with borderline ventriculomegaly (ventricular–atrial diameter of 10–14 mm) and rarely, mild ventriculomegaly, the condition may regress and will not need any surgery after birth. Such cases should be evaluated for chromosomal anomalies and followed up for the size of the ventricles.

Such uncertain prognosis makes decision-making difficult for the concerned family. Counselling should be a collaborative effort by the ultrasonographer, obstetrician, clinical geneticist, paediatric surgeon or surgeon from the required specialty. Many families, especially those with precious pregnancies, would like to continue the pregnancy even after detection of a malformation and try to provide the best treatment to the baby—pre- and postnatally.

Omphalocele is a malformation that is also associated with an uncertain prognosis. If isolated, it is surgically treatable, but if it is big or is a part of multiple malformation syndromes such as chromosomal syndrome, limb–body wall complex, pentalogy of Cantrell, OEIS complex (omphalocele, exstrophy of the cloaca, spinal defect) the prognosis is poor. Duodenal atresia, holoprosencephaly, omphalocele, cardiac malformation and diaphragmatic hernia are malformations associated with a high risk of chromosomal anomalies.

Thus, detection of a malformation in a foetus calls for (i) a careful search for associated anomalies including that of the heart, (ii) chromosomal analysis of the foetus, and (iii) counselling the family about the possible outcome/s and their likelihood. All these may need referral to a centre with expertise in foetal medicine and clinical genetics.

FOETAL CELLS IN MATERNAL BLOOD

It is well known that maternal blood contains foetal cells, though in very small numbers, from the current and sometimes even previous pregnancies. The foetal cells present in maternal blood are trophoblasts, lymphocytes and nucleated red blood cells. It has now become possible to isolate foetal cells from the maternal blood and use them for prenatal diagnosis of single-gene and chromosomal disorders. A variety of techniques and monoclonal antibodies are used to isolate foetal cells. Though this non-invasive mode of prenatal diagnosis has been successfully carried out in research settings, the technology is at present in a stage of development and not available for clinical purposes.

PREIMPLANTATION GENETIC DIAGNOSIS

Genetic analysis of an embryo before implantation is possible and has been successfully used for the diagnosis of various monogenic and chromosomal disorders. This needs *in vitro* fertilization. Biopsy samples of one or two embryonic cells (blastomeres) are taken from a 6–10-cell stage embryo. These blastomeres can be tested by polymerase chain reaction or FISH for the diagnosis of monogenic or chromosomal disorders, respectively. In addition to blastomeres, polar bodies or trophoectoderm cells from the blastocyst can also be used for this procedure. Embryos without a genetic abnormality are implanted in the uterus.

Preimplantation genetic diagnosis avoids the need for carrying out prenatal diagnosis and subsequent termination of pregnancy if the fetus is found to be affected. Families which are against the termination of pregnancy or have already undergone repeated terminations because of prenatal diagnosis of affected foetuses may opt for this technique. Preimplantation genetic diagnosis for chromosomal anomalies is also known to improve the success rate of *in vitro* fertilization as implantation fails if the embryo is aneuploid. However, diagnosis using a single cell is technically challenging and is associated with problems leading to a false-positive or false-negative diagnosis. Thus, the technique appears to be an attractive option but there are many scientific and ethical concerns related to it.

20 Perinatal evaluation: A prerequisite for genetic counselling

FOETAL LOSS AND NEONATAL DEATH

Genetic abnormalities can be severe enough to cause early or late foetal loss or neonatal death. Many non-genetic conditions are also responsible for the adverse outcome of a pregnancy. One such mishap creates anxiety in the couple as they fear recurrence of a similar event. As the aetiologies of pregnancy loss are many and varied, no counselling can be given unless the cause of foetal or neonatal death is ascertained. Usually, detailed examination and investigation of the stillborn are not carried out. The only available information may be an ultrasound scan report and findings of the gross external examination of the stillborn noted by an obstetrician. Such information is inadequate to provide any meaningful counselling or prenatal diagnosis for the next pregnancy.

Chromosomal anomalies are a cause of pregnancy loss but only a few of them are inherited. Thus, the chance that karyotyping of a couple with pregnancy loss can provide some information regarding the risk of recurrence is less. A normal karyotype reports of the couple may provide false assurance if they are not told that many other genetic causes (especially monogenic disorders) cannot be ruled out even if the karyotype is normal.

In the case of perinatal loss, complete examination and investigation of the foetus or neonate should be carried out. All efforts should be made to find the cause of perinatal death and then, depending on the aetiological diagnosis, appropriate counselling can be provided.

THE NEED FOR EXAMINING A FOETUS, STILLBORN OR NEONATE

A pregnant woman and her family always expect a normal child at birth.

However, 15% of conceptuses abort spontaneously, 1% are stillborn, 3% of neonates have a major congenital malformation and 0.7% of neonates have multiple congenital malformations. When any such mishap occurs in a family, in addition to the grief of the loss, there is a question: 'Why did this happen?' A physician or an obstetrician must be ready to face the next question at a later date, i.e. 'Will it happen again?' The number of people asking this question and their anxiety increases as the small family norm gets accepted. With couples planning only one or two pregnancies, families expect normal children and one or two of such mishaps cannot be easily forgotten or brushed away.

What is true for a stillbirth is also true for a prenatally diagnosed malformed foetus or malformed newborn. If a malformation is untreatable and severe enough to call for termination of the pregnancy, no further investigations are carried out. However, the information that is sufficient to take a decision of termination may be inadequate to provide genetic counselling for the next pregnancy. Grieving family members may not be interested in knowing about the cause of such a mishap at that moment but may ask whether such an event would recur in the next pregnancy. To be able to answer this question, obstetricians and paediatricians should carry out appropriate investigations and an autopsy when the foetus or neonate is available for investigations (Fig. 20.1 and Figs 20.2 a and b).

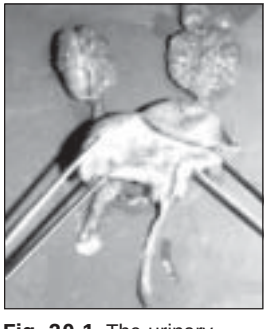
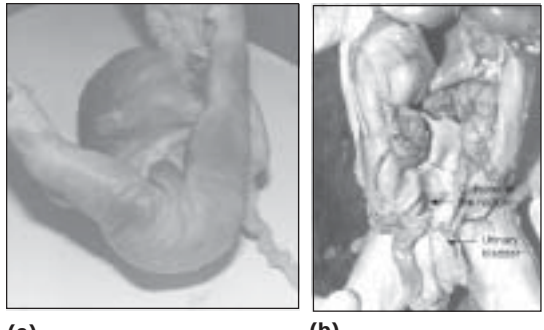


Fig. 20.1 The urinary bladder with thickened walls, dilated posterior urethra and minimal bilateral hydronephrosis in a foetus terminated for obstruction of the urinary bladder. Autopsy confirmed the diagnosis of a posterior urethral valve.



(a) **(b)**

Fig. 20.2 Foetus terminated for dilated gut loops and severe oligohydramnios. Autopsy diagnosis was a urorectal malformation sequence. **a** Knob-like external genitalia with no urethral or anal opening. **b** Dilated rectum due to rectovesical fistula. A catheter is shown to pass from the urinary bladder to the rectum through the fistula.

The role of a paediatrician, obstetrician and clinical geneticist

In the above-mentioned situation, the proband is the dead foetus or neonate. In modern medical care, with the advent of ultrasonography, other prenatal diagnostic techniques and foetal therapy, obstetricians have a new and additional role to play in foetal medicine. Paediatricians provide neonatal care. Thus, perinatal death is an interphase between the expertise of the obstetrician and paediatrician. A paediatrician, especially one interested in dysmorphology, can play a pivotal role in carrying out a post-mortem examination and the necessary investigations in the foetus or neonate. Foetal or neonatal autopsy is a multidisciplinary effort involving an obstetrician, a pathologist, a radiologist, and a clinical geneticist.

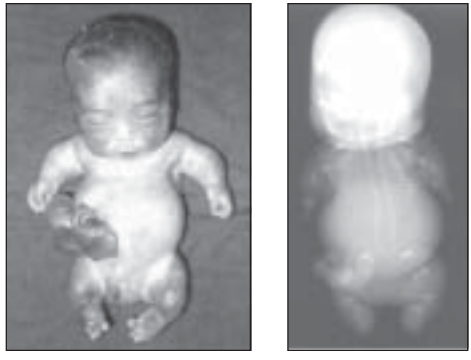
It is difficult to get such trained persons but the necessary expertise can be developed by learning about normal and abnormal development, dysmorphology, basic genetics and through experience. Good observational skills and systematic functioning are as important as in any other medical specialty.

Geneticists and paediatricians have a primary role to play because of their familiarity with congenital malformations and dysmorphology. In addition they are the best persons to explain the diagnosis and risk of recurrence of such an event to the family, and to offer continued support to the couple.

WHICH FOETUSES SHOULD BE STUDIED?

Each reproductive loss occurring during late gestation, i.e. stillbirths, neonatal deaths and pregnancies terminated for congenital malformations, need to be studied to the best possible extent. It is neither necessary nor practically feasible to study every spontaneous abortion unless such abortions are repeated. The presence of the following problems indicates the necessity for detailed investigations and autopsy:

- External malformations such as cleft lip, polydactyly, microphthalmia, syndactyly, omphalocele, etc.
- Short limbs and/or narrow thorax: These are the features of skeletal dysplasias. Radiological examination can give the diagnosis and exact risk of recurrence (Figs 20.3a and b). Prenatal diagnosis can be provided during the next pregnancy.
- Growth retardation: Growth-retarded foetuses are likely to have chromosomal abnormalities, malformation syndromes or placental abnormalities. The risk of recurrence varies with the condition.
- Macerated foetus: Although examination and investigation of a macerated foetus may be difficult and the chances of failure of tests



(a) (b)

Fig. 20.3 a Foetus with achondrogenesis. Note the short limbs and the short and narrow thorax. **b** Radiograph of the foetus in Figure 20.3a. Note the absence of ossification of vertabral bodies.

such as chromosomal analysis are high, such foetuses have a relatively high risk for chromosomal abnormalities or congenital malformations.
- Families in which previous unexplained foetal or neonatal loss have occurred.
- Cases wherein the cause of death is not obvious.

Nowadays, an important and major group consists of pregnancies terminated because of a prenatal ultrasonographic diagnosis of malformations. In every pregnancy terminated for this reason the foetus needs to be examined after termination. This is essential not only for auditing the ultrasonographer's skills, but also for providing correct genetic counselling to the family. This is because even with the best ultrasonographic set-up, autopsy shows additional findings in about 45%–50% of cases. These findings may be missed or may not be detectable by ultrasonography. These additional findings change the aetiological diagnosis and risk of recurrence in about 25%–35% of cases. Thus, genetic counselling based only on ultrasonographic diagnosis may be erroneous.

CAUSES OF FOETAL LOSS

The above-mentioned indications for foetal autopsy give an idea about the genetic causes of foetal loss. Table 20.1 lists the various causes of perinatal death. Fifty per cent of foetal losses are attributed to genetic causes. This includes a number of isolated congenital malformations of different organ systems of the body. Most of the congenital malformations

Table 20.1 Causes of perinatal death

Genetic

- Chromosomal abnormalities: Trisomy, triploidy, tetraploidy, monosomy X, deletion, duplication
- Skeletal dysplasias: Achondrogenesis, short rib polydactyly syndromes, thanatophoric dysplasia, osteogenesis imperfecta type II, etc.
- Recognizable syndromes: Smith–Lemli–Opitz syndrome, Meckel–Gruber syndrome, Walker–Warburg syndrome, etc.
- Alpha-thalassaemia and some metabolic errors such as mucolipidosis I, Gaucher disease
- Congenital malformations: Neural tube defects (NTDs); abnormalities of the central nervous system such as holoprosencephaly and hydrocephalus; renal abnormalities such as renal agenesis, cystic and obstructive diseases of the kidney; cardiac defects such as transposition of the great vessels, hypoplastic left heart; laryngeal and tracheo-oesphageal abnormalities; abdominal wall defects such as omphalocele and gastroschisis; and multiple malformations of unknown aetiology

Non-genetic

- Placental causes such as decreased placental blood flow, chorioangioma
- Foetal infections such as syphilis, or those caused by cytomegalovirus and parvovirus B-19
- Problems related to the umbilical cord such as true knots
- Obstetric problems such as obstructed labour, antepartum haemorrhage, etc.
- Maternal illness such as diabetes mellitus, cardiac failure, hypertension
- Twins: Twin–twin transfusion

have a multifactorial aetiology. The risk of recurrence of these malformations in a family varies from nil to 5%. Before counselling for such isolated malformations, it is essential to make sure that the malformation is not a part of a chromosomal or monogenic syndrome as the risk of recurrence will vary according to the aetiology.

This point is stressed by the list of aetiologies for common malformations such as neural tube defects (Table 20.2). The exact aetiological diagnosis also helps in providing better prenatal diagnosis during the next pregnancy.

Most of the conceptuses with chromosomal abnormalities are lost in early pregnancy as spontaneous abortions. Five per cent of stillbirths and neonatal deaths are due to chromosomal abnormalities. The most common of these are autosomal trisomies and 45,X. Ninety-eight per cent of 45,X conceptuses are lost during pregnancy. A typical presentation is the second trimester ultrasonographic diagnosis of a large cystic hygroma with massive subcutaneous oedema. The cause of such a phenotype is 45,X in most of the cases and trisomies in some. Rarely, the karyotype may be normal.

Table 20.2 Important syndromes associated with neural tube defects

Syndrome	Inheritance	Important clinical features
Meckel–Gruber syndrome	AR	Encephalocele, anencephaly, polydactyly, polycystic kidneys, cleft lip
Fronto–facio–nasal dysplasia	AR	Coloboma of the iris, hypertelorism, frontal encephalocele
Jarcho–Levine syndrome	AR	Vertebral and rib anomalies, thoracic deformity
Walker–Warburg syndrome	AR	Lissencephaly, microphthalmia, muscular dystrophy, cleft lip, occipital encephalocele
Silverman dyssegmental dysplasia	AR	Encephalocele, short trunk, anisospondyly
Trisomy 18 (Edward syndrome)	Chromosomal	Cardiac defect, hypertonia, growth retardation, clenched fists, exomphalos, meningomyelocele, prominent occiput
Isolated neural tube defect (NTD)	Multifactorial	Hydrocephalus and talipes eqinovarus are secondary to NTD

AR: autosomal recessive
Note: Uncontrolled diabetes and treatment with antiepileptics (valproic acid, carbamazapine) can also cause NTD.

Many chromosomal abnormalities can be detected prenatally if karyotyping is done using amniotic fluid or foetal blood after ultrasonographic diagnosis of a malformation. About 10% of foetuses with a single malformation and 30%–35% of foetuses with multiple malformations have chromosomal abnormalities. Malformations such as omphalocele, congenital heart disease, multicystic kidney and holoprosencephaly are more likely to be associated with chromosomal anomalies while anencephaly, polydactyly and cleft lip are less likely to be associated with an abnormal karyotype. Prenatal chromosomal analysis becomes more important if the malformation is treatable, e.g. duodenal atresia.

Prenatal diagnosis of lethal skeletal dysplasias by ultrasonography or clinical examination at birth is obvious in most cases. They account for 2%–3% of stillbirths. Radiography is essential for the diagnosis of a specific type of dysplasia.

CLINICAL APPROACH TO PERINATAL DEATH

The basic approach to perinatal death is the same as in any clinical situation and includes history-taking, examination and investigations.

Details of a prenatal history of maternal illness, drug intake, onset of foetal activity and the volume of amniotic fluid are important. Equally important are the foetal position, foetal monitoring, type of delivery and history of neonatal problems, especially in the case of a fresh stillbirth or neonatal death. A careful family history about spontaneous abortions, stillbirths, neonatal deaths, malformations and consanguinity needs to be taken by asking direct questions, though any such positive history need not always be related to the present pregnancy loss.

External examination

External examination includes careful observation and measurements. This is of paramount importance as is its documentation in the form of a photograph. Minor dysmorphic features may be difficult to appreciate in small foetuses. Photographs are much better than lengthy descriptions and also provide an opportunity to get a second opinion, especially from a clinical geneticist. Crown–heel length, crown–rump length, head circumference, weight and foot length are useful for assessing the gestational age. More detailed anthropometry can be done if skeletal dysplasia is suspected. The eyes, ears, face, limbs, digits, nails, spine, joints, external genitalia, anal opening and skin should be carefully examined. The use of a magnifying lens can help in the examination of small foetuses and the dermatoglyphics.

Internal examination

Examination of the internal organs for malformations is necessary. The protocol for foetal autopsy is available in the literature. Even in a macerated foetus, the major malformation can be detected. If it is not possible to conduct an autopsy immediately, the foetus can be stored in 10% formalin after the collection of samples for karyotyping.

Study of chromosomes

Chromosomal study is indicated in the presence of malformations, foetal hydrops, intrauterine growth retardation, oligohydramnios, maceration or previous foetal loss. Blood is collected from the umbilical cord or foetal heart in a heparinized syringe or vacutainer under aseptic conditions, or a piece of skin obtained after cleaning with alcohol can serve as the sample. The heparinized blood or pieces of skin are kept in a sterile tube containing saline or preferably tissue culture medium. The samples can be transported at room temperature for a period of up to 24 hours.

The placenta is also a good source of samples for cytogenetic studies but contamination with maternal cells has to be taken care of and the

possibility of confined placental mosacism needs to be considered while interpreting the results. The problem of poor cell growth, and contamination of sample can be overcome by carrying out amniocentesis or placental biopsy before termination of the pregnancy.

Radiographic studies

Anteroposterior and lateral radiographs are mandatory, especially if skeletal dysplasia is suspected. Rarely, other imaging techniques such as ultrasonography or a computed tomography (CT) scan may be useful for documenting anomalies of the central nervous system.

Histopathology

Histopathological examination of fetuses with cystic disease of kidneys, renal agenesis, neuronal migrational abnormalities and suspected infectious causes should be performed for confirming the diagnosis. Lung histology may be useful in confirming hyaline membrane disease. Routine histopathological examination of all organs is not indicated unless done for research purposes. The need for histopathological evaluation is evident at gross autopsy.

The placenta and umbilical cord

Foetal death can be attributed to abnormalities of the placenta or umibilical cord in 15% of cases. In cases of intrauterine growth retardation and infection, the placental histology may provide important information.

Haematological and serological investigations are indicated if infection is suspected (*Toxoplasma*, rubella, cytomegalovirus and herpesvirus [TORCH], or parvovirus) or in the case of foetal hydrops. Table 20.3 lists the investigational approach in the case of foetal hydrops. Some metabolic disorders such as peroxisomal disorders, Smith–Lemli–Opitz syndrome and sialidosis can result in perinatal death. Biochemical tests and enzyme analysis using blood or fibroblasts may need transportation of samples to appropriate centres.

Utility of foetal autopsy

In a good research set-up with the availability of complete investigations, accurate diagnosis of foetal death is possible in 90% of cases. However, the diagnostic yield may vary depending on the availability of investigations. If no abnormality is detected on autopsy, especially in cases with normal growth and death at the time of labour, a number of

Table 20.3 Investigations of a foetus or neonate with non-immune hydrops

- Haemoglobin
- Total leucocyte count
- Platelet count
- Haemoglobin electrophoresis for diagnosis of alpha-thalassaemia
- Level II ultrasonography/investigation for malformations
- Doppler studies of umbilical blood vessels
- Echocardiology
- Karyotype
- Serology for syphilis, *Toxoplasma*, cytomegalovirus, parvovirus B-19
- Serum proteins, albumin

possible causes of perinatal death are ruled out. In cases where a diagnosis is reached, the exact risk of recurrence of the disease in the family can be estimated. The risk of recurrence may vary from negligible to usually up to 25%; negligible risk definitely relieves the tension in the family. If prenatal diagnosis is available for the condition, it may solve the problem to a great extent. Prenatal diagnosis can be more accurate at earlier periods of gestation when one is sure about what to look for.

Thus, the importance of examination and investigations for a perinatal death cannot be overemphasized. These facilities need the coordinated efforts of an obstetrician, paediatrician, pathologist and, if available, a clinical geneticist. Perinatal autopsy also provides a perinatal mortality audit and plays an important role in understanding normal and abnormal development. Multiple malformation syndromes provide an important substrate for the study of the genetics of development.

Acceptance of foetal autopsy

Autopsy facilities are not yet routine in Indian medical practice. Lay persons are not aware of the utility of autopsy. Autopsy of perinatal deaths may not be of immediate help but is important in the positive planning for future pregnancies. This needs to be explained to the families by obstetricians and paediatricians for better acceptance of an autopsy. Irrespective of the gestational age, the sense of loss and grief may be intense. At such a moment, sympathetic discussion is needed to convince the families of the need for foetal autopsy, which they may consider mutilation. Rarely, when autopsy is not possible for some reason, careful external examination, a photograph and radiograph may provide diagnostic information. The use of perinatal autopsy services depends totally on the awareness among obstetricians and paediatricians regarding its need and utility.

21 Genetic counselling

Rapid advances in molecular genetics over the past few decades have led to a tremendous increase in knowledge regarding genetic diseases. This information is being applied to the clinical management of diseases in the form of genetic counselling, carrier detection, and presymptomatic and prenatal diagnosis. With better control of infections and nutritional diseases, genetic diseases are emerging as a significant cause of morbidity and mortality. As genetic diseases can present at any age and can involve any system of the body, primary physicians and specialists must realize the importance of diagnosing genetic disorders and offering genetic counselling, and need to develop the necessary expertise in the field.

DEFINITION OF GENETIC COUNSELLING

The American Society of Human Genetics in 1975 defined genetic counselling as 'a communicative process which deals with human problems associated with the occurrence and/or recurrence of a genetic disorder in a family'. This process involves an attempt by one or more appropriately trained persons to help an individual or a family (i) to comprehend the medical facts, including the diagnosis, probable course of the disorder and available management; (ii) to understand the manner in which heredity contributes to the disorder, and the risk of recurrence in the family; (iii) to understand the alternatives for dealing with the risk of recurrence; (iv) to choose a course of action which seems to them appropriate in view of this risk, their family goals, ethical and religious standards, and to act in accordance with that decision; and (v) to make the best possible adjustment to the disorder in an affected family member

and/or to the risk of recurrence of that disorder. To this list one should also add the goal of providing social and psychological support to the affected family.

Genetic counselling helps the families to use genetic information in a personally meaningful way that minimizes psychological distress and increases personal control. This helps them to cope with the genetic disorder and to take appropriate reproductive decision. Counselling sessions help to decide about a test, adjust to a diagnosis or risk, reduce suffering due to loss and to explore options.

The person who seeks genetic counselling is called the *c*onsultand or counsellee and the one who gives it is the counsellor. In addition to medical specialists, trained persons with various backgrounds such as nursing, social work and psychology can also function as genetic counsellors.

INDICATIONS FOR GENETIC COUNSELLING

The clinical presentation of various genetic disorders are listed below:

1. Congenital malformations—lethal or non-lethal, e.g. anencephaly, congenital heart disease, skeletal abnormalities, etc.
2. Stillbirth/s with or without congenital malformation
3. Mental retardation (including developmental delay) with or without malformation/s, facial dysmorphism and neurological deficit
4. Neurodegenerative diseases presenting as neuroregression, focal neurological deficit, ataxia, spasticity, hypotonia, epilepsy, etc.
5. Myopathies and muscular dystrophies
6. A neonate or infant who is acutely sick, shows failure to thrive, or has recurrent episodes of vomiting, acidosis and convulsions
7. Ambiguous genitalia or abnormalities in sexual development, e.g. primary amenorrhoea, infertility, etc.
8. Skeletal dysplasias presenting as disproportionate short stature and syndromes of primordial dwarfism presenting as proportionate short stature
9. Any unusual disease of the skin, eyes, bones, or unusual facial features
10. Childhood deafness
11. Known genetic diseases such as thalassaemia, Wilson disease, haemophilia A and mucopolysaccharidosis
12. Down syndrome or other chromosomal disorders
13. Relatives of an individual having a chromosomal rearrangement
14. Any disease if it appears familial
15. Familial cancer or cancer-prone diseases (*see* Chapter 14)

16. Exposure to a known or suspected teratogen during pregnancy
17. Consanguineous marriages
18. Advanced maternal age
19. Positive screening test for a genetic disorder.

The presence of any of the above-mentioned features suggests that the disorder is, or probably is, genetic in aetiology and needs evaluation by a clinical geneticist and genetic counselling.

STEPS IN GENETIC COUNSELLING

Genetic counselling is a multistep process and involves clinical expertise similar to that required for any other medical specialty. Accurate diagnosis of the affected person in the family is of paramount importance. The steps involved include (i) history-taking with particular emphasis on pedigree construction, (ii) clinical examination, (iii) arriving at a diagnosis and disease management, (iv) genetic counselling with strong emphasis on the risk of recurrence of such a disorder and prenatal diagnosis. A standard medical history of the proband (one who draws attention to the family) and other affected persons in the family is required.

Construction of a pedigree chart

Pedigree analysis is an important step in genetic counselling. It is used to get more information on the family's history of a particular disorder or trait (for details *see* Chapter 1, pp. 8–12).

Clinical examination

Complete physical examination and recording of relevant anthropometric measurements are necessary. In the case of a malformation, an accurate description of the facial features, minor malformations and even normal variants should be noted. Whenever possible, a photograph must be taken. A photographic record of a dysmorphic child is very informative. Examination of the parents is often necessary to verify whether a dysmorphic feature (such as the shape of the ears) is a common familial characteristic or has any diagnostic value.

Investigations and diagnosis

There may be disorders with apparently similar symptoms caused by different genes (genocopies, genetic heterogeneity) or even by environmental factors (phenocopies). Various haematological, biochemical and imaging investigations are needed for the diagnosis of

the proband. In some cases, specialized tests such as chromosomal analysis, enzyme assays, metabolite measurement and DNA analysis may be essential to arrive at the final diagnosis.

DNA diagnosis

As more and more disease-related genes are being mapped and sequenced, many new DNA-based diagnostic tests are becoming available for single-gene disorders (*see* Chapter 16). Currently, DNA-based tests for the diagnosis of beta-thalassaemia, haemoglobinopathies, haemophilia, Duchenne muscular dystrophy (DMD), fragile X syndrome, spinal muscular atrophy, etc. are available in India. For most of these disorders, the number of mutations associated with the disease is large. For example, in the case of beta-thalassaemia, more than 200 mutations have been identified in the beta globin gene and even today, new mutations are being identified. Likewise, more than 300 mutations in the Factor VIII gene are known to cause haemophilia A; almost each family appears to have a unique genetic change. It is for these reasons that a centre involved in research of a particular disease is able to provide more reliable tests for that disease. Due to the rarity of most genetic diseases, it is better to send samples to specialized centres rather than developing facilities for every tests at each centre.

For most disorders, a DNA-based diagnostic test is not required for confirmation of the diagnosis. However, molecular tests are extremely important for prenatal diagnosis and carrier detection. Unless the type of mutation/s in the proband or carrier parent/s is identified, prenatal diagnosis is not feasible. This should preferably be done before the next pregnancy.

Examination of family members

Careful clinical examination and investigation of the parents and family members are integral parts of the evaluation of a child with suspected genetic disease, particularly in the case of autosomal dominant disorders. The presence of even a minor stigmata (forme fruste) in a family member may change the risk of recurrence as in the case of tuberous sclerosis and neurofibromatosis. If a child of apparently normal parents has tuberous sclerosis then careful examination and investigation of the parents for manifestations of tuberous sclerosis are necessary before counselling. If one parent has some feature of tuberous sclerosis such as adenoma sebaceum, renal cysts or subungual fibroma, the risk of recurrence in the siblings of the proband can be up to 50%. If neither of the parents is found to have any feature of tuberous sclerosis on

investigation, then the risk of recurrence in the siblings of the proband will be negligible. However, there may be considerable variation in the penetrance of the disease. There may be no manifestation in the parents even while he/she is a carrier of the disease (skipped generation). These issues must be kept in mind while counselling for an isolated case of an autosomal dominant disorder.

Syndrome diagnosis

Patients having multiple malformation syndromes, with or without mental retardation, constitute an important group needing genetic counselling. With an ever-growing list of syndromes, the exact diagnosis may be difficult. This situation is greatly helped by various computerized databases, namely, London Dysmorphology Database and Pictures of Selected Syndromes and Undiagnosed Malformations (POSSUM). Books which give detailed descriptions of syndromes and diagnostic approaches to these are also of great help.

Counselling

Besides accurate diagnosis, skilful communication is of great importance in genetic counselling. Other prerequisites for counselling are a quiet and comfortable room and adequate time. Counselling should be carried out in strict privacy. It is advisable that both the husband and the wife be present when the reproductive options are discussed. Counsellors should be sympathetic listeners who should allow the parents (consultands) to vent their pent-up feelings and emotions. They should provide the information in simple, non-technical and local language. The depth of explanation should be commensurate with the educational background of the couple. If the consultands do not have personal experience of the disease under consideration, they may have considerable difficulty in understanding the burden of the disease. However, it is necessary for them to understand the disease properly so that they can take appropriate decisions. The risk of recurrence of the disease in the family should be explained, if necessary with the help of diagrams. A risk of 25% should be explained both ways, i.e. 1 in 4 and 25%. It should also be explained that a risk of 1 in 4 implies that the chances of the child being unaffected is 75%. The concept of probability may be explained by the example of tossing a coin or dealing with a deck of cards, etc. It should be made clear that chance has no memory and as such there may be 2, 3 or even more consecutively affected children in a family even when the risk is only 5%, 25% or 50%.

In the case of polygenic multifactorial disorders such as birth defects, it is often useful to compare the risk of recurrence of the disease in the family against the risk of occurrence of the disease in the general population. It should be explained that any family could have a child with a genetic disease or congenital malformation, even if there is no positive family history, and parenting of an affected child or being a carrier of a genetic disease is not a social stigma. It should not be considered as a discriminating factor. Common misconceptions about heredity should be dispelled.

Last but not the least, one of the most important aspects of genetic counselling is the discussion of reproductive options. Depending upon the consultand's judgement of the risk of recurrence of the genetic disorder in the family and that of the burden of the disease, the couple may opt for contraception, adoption, *in vitro* fertilization or further pregnancy, with or without prenatal diagnosis. The accuracy and limitations of prenatal diagnostic tests and issues related to termination of pregnancy also need to be discussed. The family may be referred to a genetic centre for the latest information about the disease and availability of facilities for prenatal diagnosis. A written summery of the counselling is useful and should be given to the consultand.

NON-DIRECTIVENESS IN GENETIC COUNSELLING

Counselling is the art of presenting facts without influencing decision (non-directiveness). On the other hand, directiveness is a form of persuasive communication involving various combinations of deception, coercion and threat. Counselling should always be non-directive. Medical doctors find the non-directiveness of counselling to be contrary to the traditional doctor–patient relationship, which assumes that medical advice is the doctor's responsibility. However, decisions in genetic counselling are more personal than medical. The couple should be given all the available information about the disease and the reproductive options should be open to them, but the decision has to be taken by them.

The goal of genetic counselling is the client's psychological well-being; a goal that will help clients to take good decisions for themselves, which are consistent with their own values. The counsellor should never make decisions for the client regarding marriage, children, abortion and other personal issues. These are the clients' responsibilities and rights. Although a couple may seek help in decision-making by asking what the counsellor or other people would do in a similar situation, it is unwise

to be drawn into expressing a personal opinion. It is, however, reasonable to advise that the couple must consider the consequences of each decision so that the decision is taken after careful deliberation and not in haste.

In addition to the perceived risk of recurrence, various other factors such as the desire to have children, severity and burden of the disease, personal experience with the disease, social and religious views, and availability of prenatal diagnosis may affect the reproductive decision of the couple. The couple's decision (even if it is different from the counsellor's personal views) should be respected and supported. A follow-up session may be needed to reinforce the information provided to the couple in the earlier session (usually forgotten or wrongly remembered by consultands), to answer new queries, to provide an update and psychological support to the family during the process of coping till acceptance and adjustment take place.

PSYCHOLOGICAL ASPECTS OF GENETIC COUNSELLING

Psychological needs and issues associated with the process of genetic counselling are many and varied. The four phases of the coping process, which are common in all situations of grief, are experienced in genetic counselling as well: (i) the initial phase of shock and denial; (ii) phase of anger or guilt; (iii) phase of anxiety or depression; and (iv) the phase of acceptance and adjustment. Counsellors have to be aware of these events and should plan their approach accordingly for the successful outcome of genetic counselling. Overt and occult queries and misconceptions regarding cause and blame should be addressed to minimize tension among the family members. Assessing the consultand's expectations beforehand and tailoring counselling accordingly are likely to improve client satisfaction.

PRENATAL DIAGNOSIS

The availability of prenatal diagnosis for a number of genetic disorders has made counselling and decision-making much easier (*see* Chapter 19). A variety of tissues can be collected from the foetus for chromosomal and DNA analysis. These include chorionic villi (at 11–12 weeks), and amniotic fluid (16–20 weeks). Rarely, foetal skin, muscle or liver, or a foetal blood sample (at 18–20 weeks) may be collected for biochemical or histological analysis. Attempts are being made to isolate foetal cells from the maternal circulation, but this is still in the experimental stages. Besides chromosomal and DNA analyses, these tissues can also be used for biochemical tests and enzyme assays.

High-resolution ultrasonography can detect a number of structural malformations such as those of the central nervous system, gut, kidneys, limbs and heart. However, associated malformations may be missed. After termination, as many as 50% of the foetuses are found to have additional malformations. Genetic counselling based on ultrasonographic diagnosis alone is erroneous in 30%–40% of cases. Chromosomal analysis of malformed foetuses is advised before deciding about the continuation of pregnancy in surgically treatable congenital malformations such as duodenal atresia. As a policy, all foetuses aborted following antenatal diagnosis should be sent for foetal autopsy for purposes of medical audit and definitive diagnosis.

SPECIAL CONSIDERATIONS IN GENETIC COUNSELLING

A precise diagnosis, up-to-date knowledge and good communication skills are essential for successful genetic counselling. As the genetic counsellor dispenses words and not tablets, it is difficult to judge the outcome of genetic counselling and at present, no adequate scheme to assess clients' satisfaction has been fully developed. Many a time, the family does not know what to expect from genetic counselling. Most families come with the expectation of a curative treatment for the disorder. The knowledge that no specific treatment is available for the disorder (which is often the case) is frustrating for the family. In such a situation, genetic counselling may not be successful because of the disparity between the expectations of the family and the counselling delivered. Hence, the family should be given an idea of what to expect from genetic counselling for their child and how this information will benefit them. Relevant points in counselling for common genetic problems are discussed below.

Chromosomal disorders

The risk of recurrence of aneuploidy after one conception with aneuploidy in a family is usually 1% or less. Karyotyping of the parents of the hild with aneuploidy is not required unless the child has some structural abnormality of the chromosome such as a translocation, partial duplication or deletion. Extended family counselling is indicated if a parent is found to be a carrier of a balanced structural rearrangement. Exact counselling will depend on the type of chromosomal abnormality detected. Prenatal diagnosis is available for chromosomal disorders (see chapters 7 and 8).

Congenital malformations

Isolated congenital malformations are usually multifactorial in origin (*see* Chapter 11). Empiric risk figures are available for many of them. The empiric risk of recurrence is the observed frequency of recurrence in a population of affected families. The empiric risk varies according to the number of affected children, severity of the disease in the affected individuals and sex of the individual who is transmitting or is at risk for the disease. Empiric risks may vary in different geoethnic communities. It is essential to confirm that the malformation is an isolated one before using these figures. Sometimes it may be a part of a syndrome where other manifestations are minimal and, therefore, not easily discernible. The risk in the latter case is quite different. For example, in the case of an isolated neural tube defect, the risk of recurrence is 5%, while the risk of recurrence increases to 25% if this defect is a part of an autosomal recessive disorder such as Meckel–Gruber syndrome or spondylocostal dysplasia.

Mental retardation

The prevalence of severe mental retardation in the general population is about 0.5% and of mild mental retardation about 2–2.5%. Pathological causes of severe mental retardation may be more common than those for mild. Genetic causes are responsible for about 40% of mental retardation. Associated malformations and/or dysmorphism indicate a chromosomal or non-chromosomal syndromic aetiology. In its absence, a biochemical cause such as phenylketonuria or homocystinuria needs to be investigated. If associated with neurological manifestations and/or seizures, the possibility of various metabolic neurodegenerative diseases needs to be investigated in detail. Easy labels such as cerebral palsy may lead to false reassurance. Neuroimaging with computed tomography scan and magnetic resonance imaging identifies a number of structural malformations of the brain, neuronal migration anomalies, degenerative diseases and previous vascular, hypoxic or infective insults, and provides an aetiology in many cases. It is mainly indicated in cases with micro- or macrocephaly, or in cases with an associated neurological deficit.

Fragile X syndrome is the most common cause of inherited mental retardation. As the phenotype is subtle, clinical diagnosis is not possible, especially in young children. Thus, all patients with mental retardation without any obvious cause need to be investigated for fragile X mental retardation. If no cause is found, then the empiric risk of reccurrence of mental retardation in a sibling is 5%–7%(see chapter 12).

Perinatal death

In 50% of stillbirths the cause is genetic. The aetiologies are diverse and hence the genetic counselld on the aetiological diagnosis. Chromosomal analysis, autopsy, whole body radiography and clinical photography help in arriving at the final diagnosis in about 80% of cases. As a bare minimum, photographs and radiographs should be taken, which may provide a definite diagnosis in many cases. The availability of an aetiological diagnosis of perinatal death helps in estimating an appropriate risk of recurrence and provides better prenatal diagnosis during the next pregnancy.

22 Community genetics

PREVENTION OF GENETIC DISORDERS

Prevention is better than cure. In medicine, prevention can be at various levels and includes measures such as adopting a good diet and lifestyle, immunization, or early diagnosis and treatment. In the case of genetic disorders, an additional preventive approach is to prevent the birth of an affected child through genetic counselling and prenatal diagnosis. This approach has been widely utilized over the past few decades, but is mainly limited to families that have a member affected with a serious genetic disorder.

The first step of this approach is to identify a family at high risk for giving birth to a child with a genetic disorder. This is done by taking a detailed three-generation family history. Although such a preventive approach is of great importance for the concerned family, it has little impact on the society. Preventive strategies at the population level in the form of genetic screening programmes are available for some common disorders.

POPULATION SCREENING

The branch of medical genetics concerned with the screening and prevention of genetic diseases at the population level is known as community genetics. Screening for hereditary disorders and genetic predisposition will have a great impact on the health and well-being of the population at large, especially when more information about the genetic predisposition to common disorders becomes available. Diseases that need to be included in population-screening programmes should be common enough to influence the health of society. The diseases chosen for screening may vary from population to population.

Population screening offers genetic testing to all relevant individuals of a defined population. The individuals selected for testing will vary

depending on the preventive strategies, which can be divided into the following two categories:

1. Early diagnosis and treatment: This strategy is used both for neonatal screening programmes and for the identification of a genetic predisposition to adult-onset diseases.
2. Prevention of the birth of a child with a genetic disorder: This strategy utilizes antenatal screening to identify foetuses affected with a genetic disorder or people who are carriers of genetic disorders. Carriers can be provided information on the risk of birth of a child with a genetic disorder and offered prenatal diagnosis.

To select a disease for population screening, the following criteria should be fulfilled:

1. The disease should have a high incidence rate in the population, cause serious effects on health and should be treatable or preventable.
2. Easy, sensitive and inexpensive screening tests should be available.
3. Tests for confirmation of the diagnosis should be available.

Equally important requisites for the success of population-screening programmes are awareness among the population, acceptability and feasibility at the population level. The programme should be cost-effective. The cost calculations also need to consider the psychological burden of the disease, associated mortality, morbidity and handicap.

Neonatal screening

Neonatal screening is the first population-based screening programme for genetic disorders. Its success and widespread implementation has proved the feasibility and utility of population screening. Demonstration of the efficiency of diet management in preventing mental retardation in phenylketonuria and galactosaemia led to the initiation of neonatal screening. Screening for these two disorders is implemented by law in most of the developed countries. Other diseases that can be managed effectively after neonatal diagnosis are biotinidase deficiency, hypothyroidism and congenital adrenal hyperplasia. Some countries advocate screening for disorders that are not completely treatable even after neonatal diagnosis. This group of disorders include cystic fibrosis, sickle cell disease and maple syrup urine disease. In the case of these diseases, there is partial improvement in disease manifestation and the outcome is better following diagnosis by neonatal screening.

It has been shown that early administration of penicillin prophylaxis to patients with sickle cell anaemia decreases the mortality due to

pneumococcal infection. For diseases such as Duchenne muscular dystrophy (DMD), neonatal diagnosis is possible but, at present, no treatment is available to change the course of the disease. The only advantage of early diagnosis is to offer genetic counselling and prevent recurrence of the disease in the family. Otherwise, by the time a child manifests the disease, the next sibling is already born. However, in the absence of any treatment, neonatal screening for DMD is not justified.

At present, neonatal screening tests are done on a blood sample collected by heel prick on a filter paper on the second or third day of life. With the present trend of early discharge from hospital after childbirth, the sample may be collected just after 24 hours of delivery which may lead to false negative results. Hence, repeat sampling before day 7 is advocated for cases in which the sample was collected at or before 24 hours. There are other reasons for false-positive and false-negative results such as blood transfusion, illness in the neonate, administration of intravenous fluids, transient abnormalities, incorrect method of sample collection and sample contamination.

Traditionally, the Guthrie test based on a bacterial inhibition assay was used. The test is now being replaced by more sensitive and automated enzyme-linked immunosorbent assay (ELISA). Tandem mass spectrometry is a technique that can simultaneously screen for the presence of many metabolic disorders including aminoacidopathies, organic acidurias and fatty acid oxidation defects. This promising technique is in its experimental stage.

An important part of neonatal screening is to follow up positive screening test results and conduct confirmatory tests. Quick results of the confirmatory tests are essential for management of the neonate and to reduce the anxiety of the parents. Each screening test has some false-negative results although the rate is low. Even after normal results of screening, the disease needs to be suspected in appropriate clinical situations.

Prenatal screening

Prenatal diagnosis is the most important landmark in the field of clinical genetics. Detection of foetal disorders with a poor outcome early in pregnancy offers the option of termination of pregnancy and prevention of the birth of a child with a handicap or lethal disorder. Neural tube defects (NTDs) and Down syndrome are two such common disorders for which screening tests are available.

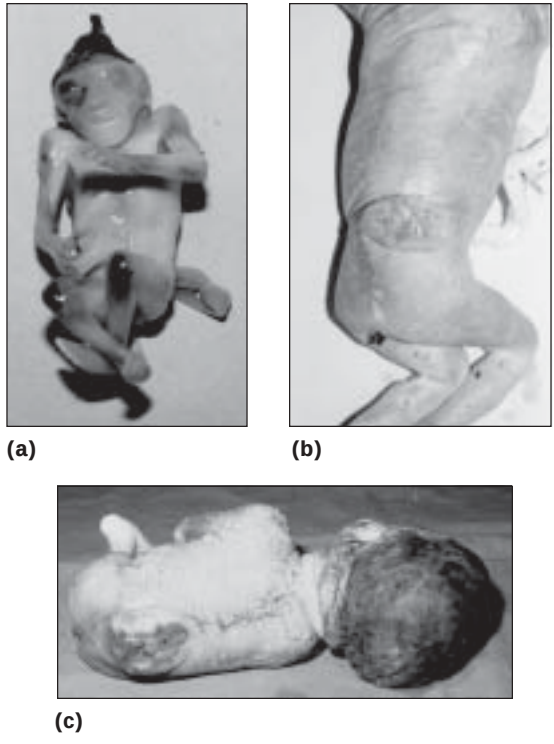


Fig. 22.1 **a** Foetus with anencephaly. **b** Foetus with meningomyelocele. **c** Foetus with open spina bifida.

Screening tests for neural tube defects

Neural tube defects include anencephaly, meningomyelocele, encephalocoele, open spina bifida (Figs 22.1a, b and c) and milder forms of spinal defects such as spina bifida occulta with or without spinal dysraphism. Anencephaly is always lethal and major spinal defects have a poor prognosis for survival and function. The survivors usually have disabling neurological defects such as mental retardation, paralysis of the lower limbs and lack of bladder–bowel control. Anencephaly and most of the spinal defects are open, i.e. without any skin covering.

Open defects are associated with the presence of high levels of alpha-fetoprotein (AFP) in the mother's serum. AFP is an albumin equivalent in the foetus and it leaks into the amniotic fluid whenever there is an opening in the skin covering, e.g. in the case of open spinal defects and omphalocele. Along with the amniotic fluid, the level of AFP rises in

the mother's serum as well. Thus, the level of maternal serum AFP has been used as a screening test for NTDs for more than two decades. Levels of AFP are expressed as multiples of the median (MoM). The median is derived for each week of gestation, by using laboratory samples from the pregnant women. The level of AFP in maternal serum has to be tested from the 15th to the 20th week of gestation. The test has maximum sensitivity at the 17th completed week of gestation and will detect 91% and 85% of foetuses with NTDs using 2 MoM and 2.5 MoM as cut-offs, respectively. A high value of AFP in the maternal serum is not a definitive finding for the diagnosis of NTD. With the present improved resolution of ultrasonography (USG), invasive tests such as amniocentesis are not needed in most cases. In expert hands, the sensitivity of USG for the diagnosis of NTD is more than 95%. Hence, testing the level of AFP in the maternal serum, followed by USG in those with a high AFP level is a good strategy for the prevention of NTDs in the general population. Testing the level of AFP in the amniotic fluid acts as a complementary technique to USG and may be reserved for cases with unsatisfactory ultrasonographic evaluation of the foetus. Skin covered defects can not be detected by AFP screening.

Screening tests for Down syndrome

Tests on the mother's blood are available for the screening of Down syndrome, which is the most common cause of mental retardation. Offering amniocentesis to pregnant women above 35 years of age is one way of preventing the birth of children with this syndrome. However, many children with Down syndrome are born to younger mothers and an invasive test such as amniocentesis cannot be offered to all pregnant women.

The markers commonly tested for the screening of Down syndrome are AFP, unconjugated oestriol (uE_3) and the beta subunit of human chorionic gonadotrophin (β-hCG). The levels of AFP and uE_3 are low and that of β-hCG is increased in the serum of the mother carrying a foetus with Down syndrome. The three markers together are offered as a triple test to all pregnant women irrespective of their age. Depending on the results and mother's age, the risk for Down syndrome in that particular pregnancy is calculated using a software. If the risk is 1 in 250 or more, the test is said to be positive, and the woman is offered amniocentesis and chromosomal analysis from the amniotic fluid cells. A risk of less than 1 in 250 is not considered high enough to indicate the invasive test. The risk of spontaneous abortion following amniocentesis is 1 in 200. The triple test followed by amniocentesis detects up to 65%

of foetuses with Down syndrome. False-positive results are seen in 5% of women tested. Ultrasonographic estimation of the foetal gestational age is required for better sensitivity of the triple test. The triple test also identifies foetuses with trisomy 18. The other marker used to increase the rate of detection of Down syndrome is the level of inhibin-A in the mother's serum. It increases the sensitivity of the screening test to 75% at the false-positive rate of 5%. Markers such as hCG and pregnancy-associated placental protein-A (PAPP-A) are being used for screening in the first trimester.

In addition to biochemical markers, ultrasonographic detection of malformations such as duodenal atresia and cardiac defects increases the chances of the foetus having Down syndrome. Other USG findings such as increased nuchal thickness (more than 2.5 mm in the first trimester), absence of the nasal bone, presence of an echogenic focus in the heart and mild dilatation of the renal pelvis in the foetus are also markers for Down syndrome. Of these, increased nuchal thickness and absence of the nasal bone have high sensitivity and may replace biochemical markers in future. Other USG markers cannot be used *per se* for the screening of Down syndrome, but their presence in routine USG should alert the ultrasonographer to the requirement for detailed evaluation and counselling.

Counselling for the screening test

The above-mentioned screening tests are offered to all pregnant women and the woman and her family have to decide whether to undergo the test or not. As these tests are offered to women at low risk for Down syndrome and NTDs, the families usually do not know anything about the disorder for which the screening test is being done. It is essential that the woman be properly informed about the disorder for which she is to be tested, and the implications of positive or negative results (Table 22.1). Equally important is to tell her that the test is voluntary and she will have to decide whether to go ahead with it or not. It is difficult for most women to understand the concept of a screening test and its voluntary nature. For the success of the screening programme, awareness about these disorders and the preventive measures need to be created in the general population. Obstetricians need to be adequately trained to provide counselling for these common problems.

Population screening for carrier detection

For many monogenic disorders, the detection of carriers is possible. The

Table 22.1 Pretest counselling for Down syndrome

The following information should be conveyed to a pregnant woman before she is offered the triple test * for screening of Down syndrome:

- Down syndrome is the most common cause of mental retardation.
- A child with Down syndrome can be born in any family. In 800 births, there is one child with this syndrome.
- Children with Down syndrome are moderately retarded. They can learn self-care, speak and undertake repetitive, simple occupations. However, even in adult life they need the support and supervision of a normal adult.
- Triple test is done on the mother's blood to identify whether there is a high risk (1 in 250 or more) of her child having Down syndrome.
- If the test is positive, an invasive test (amniocentesis) will have to be done to confirm whether the foetus has Down syndrome.
- The risk of spontaneous abortion following amniocentesis is 1 in 200 or 0.5%.
- If the foetus is found to be affected with Down syndrome, there is no curative treatment before or after birth. The only way to avoid the birth of an affected child is termination of pregnancy.
- A positive triple test does not imply that the foetus has Down syndrome or is definitely abnormal.
- The triple test cannot identify all foetuses with Down syndrome and rarely a child with Down syndrome is born to a woman with a negative triple test.
- The triple test can be done from the 15th to the 20th week of pregnancy, but should preferably be done during the 15th to 18th week.
- The women should be informed about the cost and availability of the triple test and amniocentesis.
- It should be explained that a negative triple test does not mean that the foetus will be free from all genetic disorders and malformations.

* The test is voluntary and the woman herself will have to decide whether to take the test or not.

carrier of an autosomal recessive disorder will be at high risk for giving birth to a homozygous affected child if he or she marries a carrier of the same disorder. Identification of such couples and offering them genetic counselling and prenatal diagnosis will help in preventing the birth of an affected child. Population screening for carrier detection of commonly occurring genetic disorders is an effective strategy to decrease the prevalence of these disorders. If there is no population screening programme then such carrier couples are identified only after they give birth to an affected child. The aim of population screening is to prevent

Table 22.2 Diseases of importance for population screening in various ethnic groups

Disease	Tests	Population
Tay–Sachs disease	Biochemical enzyme assay	Ashkenazi Jews
Beta-thalassaemia and haemoglobinopathies	Red cell indices, HbA_2 estimation and haemoglobin electrophoresis	Inhabitants of Mediterranean countries, Indians
Alpha-thalassaemia	Red cell indices and haemoglobin electrophoresis	Chinese and those in eastern Asia
Cystic fibrosis	Detection of common mutation by molecular methods	Caucasians
Sickle cell disease	Sickling test	Afro-Caribbeans, some tribes in India

the birth of the first child with a genetic disorder in the family. The disease chosen for population screening should be common in the population to be tested. The diseases of importance may vary in different ethnic groups (Table 22.2).

The feasibility of such population-screening programmes has been demonstrated in Mediterranean countries where it was possible to reduce the incidence of thalassaemia major by 90%. Before starting population-based programmes, it is essential to collect data regarding the prevalence of the disease, spectrum of mutations, available healthcare system, etc. The appropriate time for population screening is a matter of debate. Screening of couples in the early part of pregnancy soon after conception (first half of the first trimester) or pre-pregnancy appears to be the best time. For better acceptability of carrier detection, a high level of public awareness is essential. Public education can be done through school curricula, and the print and electronic media. Carriers should not be at any medical or insurance risk because of their carrier status. Inadequate public education will lead to the association of social stigma with carriers and thus prove detrimental to the concept of population screening.

X-linked disorder namely; fragile X mental retardation also appear to be good candate for population screening. Preliminary studies have shown that a screening programme for this disorder is feasible.

Population screening for common disorders

Screening for common late-onset genetic disorders can be done at the population level to identify at-risk individuals. These individuals can be offered preventive medicine or a change in lifestyle can be suggested.

At present, this appears to be feasible for haemochromatosis, a relatively common autosomal recessive disorder. However, it has been seen that many homozygotes do not manifest the disease. Low penetrance and lack of knowledge of factors affecting the development of clinical manifestations prevent population-based screening for haemochromatosis.

Factor V Leiden mutation is another candidate for population screening. This mutation predisposes the carrier to high risk for venous thrombosis, especially in the presence of external factors such as oral contraceptives, immobilization, etc. At present, mutation testing is limited to patients with clinical evidence of venous thrombosis and relatives of known carriers of the mutation.

Genes associated with cancers of breast, ovary, colon, etc. have been identified. But the tests are available only for those with familial cancers and are not useful for population screening.

If future research identifies definite genetic risk factors for common disorders such as diabetes, hypertension, ischaemic heart disease, psychiatric diseases, etc. genetic screening will become an important part of preventive medicine. However, it will also lead to many complex psychosocial and legal issues, which will pose many difficult decision-making situations for doctors and as well as patients. The implementation of population screening programmes will put more demands on primary care physicians and obstetricians who will have to be adequately trained in genetic counselling.

23 The era of genomic medicine

INTRODUCTION

At the beginning of the twenty-first century, the sequence of the the human genome became known to mankind. Genes responsible for causing many single-gene disorders have been identified and many more genes are being mapped. Genomics—the study of the genome, its organization and function—is a rapidly developing branch of science. It has a broader and more ambitious reach than genetics. Genomic medicine is defined as the use of genotype analysis (DNA testing) to enhance the quality of medical care. The present applications of genetics are mainly useful for monogenic disorders which, being rare, involve only a small part of the population. Genomic medicine will deal with common disorders such as diabetes, hypertension, cancers, etc. and will thus have an impact on the health of the general population. The various ways in which genomics can influence medicine are as follows:

1. Identification of genes causing various single-gene disorders, thus making diagnosis and prevention possible for these disorders
2. Increasing the availability of recombinant products and DNA vaccines
3. Development of new drugs and therapeutic strategies based on the knowledge of molecular pathology and pharmacogenomics
4. Identification of genes and polymorphisms responsible for causing common multifactorial disorders. This will lead to presymptomatic identification of individuals susceptible to a disease and offer him/her preventive therapy.
5. Use of stem cell research and stem cell transplantation for many haematological and neurodegenerative disorders, and testing their potential in producing organs for transplantation.

Embryonic stem cells hold a great potential in the management of hitherto untreatable disorders. The use of embryonic stem cells, however, is a subject of ethical debate. These ethical dilemmas need to be addressed to for further progress in this direction.

Results of research on the genetics of multifactorial disorders will guide the influence of genomics on medicine over the next few decades. Though a great deal of work is going on in the field of complex multifactorial disorders at present, the issues involved appear too complex to resolve. Only time will tell how clearly the genetic components of the aetiologies of diabetes, hypertension and ischaemic heart disease are identified.

THE HUMAN GENOME PROJECT

The Human Genome Project (HGP) began in 1990 as a coordinated effort of the Department of Energy and the US National Institutes of Health (NIH). The project was originally planned to be completed in 15 years, but effective resources and technological advances led to the completion of the project in 2003. A working draft of the entire sequence of the human genome was announced in June 2000, with publication of analyses in January 2001. In addition to the United States of America, other nations, notably France, the United Kingdom, Japan, Germany and China also contributed to the HGP. Completion of the Human Genome Project is a landmark on the road of genomic medicine. The objectives of this project are listed in Table 23.1.

OUTCOME OF THE HUMAN GENOME PROJECT

The human genome reference sequence is available to researchers worldwide. It will serve as an information resource that will be the basis for research, discovery of genes and will have many practical applications. The human genome sequence is the same (almost 99.9%) in

Table 23.1 Objectives of the Human Genome Project

• Determination of the sequence of the 3 billion base pairs that make up the human genome
• Identification of all the approximately 30,000 genes in the human genome
• Development of new DNA technologies such as automated DNA sequencing
• Development of bioinformatics, i.e. the use of computer-based facilities for collecting, storing, organizing, interpreting and communicating data from HGP
• Study of model organisms such as *Escherichia coli*, worms, fruit fly, mouse, etc.
• Study of the ethical, legal and social issues related to the Human Genome Project

all individuals. The 0.1% variation is responsible for the enormous variability of phenotypes and susceptibility to disease seen in human beings.

Single-nucleotide polymorphisms (SNPs) are frequently occurring variations in individuals and are being studied for their associations with susceptibility to common disorders and drug reactions. The available map of closely spaced SNPs is of great help in the research of multifactorial disorders. Only 2% of the genome encodes information for protein synthesis and the functions of more than 50% of discovered genes are unknown. The repetitive sequences that do not code for proteins account for at least 50% of the human genome. These sequences are thought to have no direct functions, but they shed light on chromosome structure and dynamics. Genes appear to be concentrated in random areas along the genome, interspersed with vast expanses of non-coding DNA.

Over 40% of human proteins share a similarity with those found in fruit flies and worms. The similarity between genes in humans and model organisms helps greatly in studying the functions of genes by creating knockout animals and developing animal models of various diseases for research.

FUNCTIONAL GENOMICS

After sequencing the genome, the next step is to study the functions of genes in normal development and physiology, and their dysfunction in disease states. This is known as functional genomics. This not only involves the identification of gene products and their functions but also helps in understanding gene–gene interactions. It is obvious that no gene functions in isolation. Other genes and environmental factors keep on modifying the expression of a gene. Mutating specific genes in animals is an important part of functional genomics and animal models of diseases created by such targeted mutagenesis serve as test systems for the study of various treatment modalities.

Microarrays are the newest tools for the analysis of patterns of gene expression in health and disease. A comparison of these patterns provides information about the role of various genes in the pathophysiology of a disease. An important use of this technique is to study gene expression in various cancer cells to understand the molecular pathology of cancers.

Proteomics is the identification of proteins and elucidation of their involvement in the biological pathways, as well as their interaction in

development, differentiation, health and disease. Proteomics is an important component of functional genomics as proteins carry out the functions of the cell and are dynamically changing at every point in time.

ISSUES IN CLINICAL GENETICS

Advances in medical genetics are associated with many ethical, legal and social issues related to research and application of knowledge in clinical situations. A part of the HGP involves research and education in the ethical, legal and social implications (ELSI) of the project. The four basic principles of an ethical approach to a dilemma in medicine are autonomy, beneficence (doing good to a patient), non-maleficence (avoiding harm) and justice (ensuring that all individuals are treated equally and fairly). These principles are applied to all ethical dilemmas in medical genetics. Most of the ethical dilemmas pose conflicting issues and there may not be satisfactory solutions to many problems. Along with rapid developments in genetics and their applications in medicine, newer dilemmas keep arising before researchers, patients, doctors and society. Some ethical dilemmas in genetic testing are listed below:

- Prenatal diagnosis of diseases and malformations that are treatable and associated with minimum disability. For example, deafness and minor malformations of cosmetic significance only.
- Prenatal diagnosis for sex determination
- Testing for late-onset diseases
- Testing of children for carrier detection
- Access of genetic information to an employer or insurance company
- Privacy of genetic information
- Informed consent: How much information should be given to a patient?
- Privacy versus implication of testing for immediate family members. For example, if a son is found to be a carrier of Huntington chorea, it implies that his unaffected father is also a carrier of the mutation as his paternal grandfather was affected. Even if the father does not want to know about his disease status, he would get to know his status if his son tests positive.
- Confidentiality of information for a carrier versus counselling of possible carriers among the relatives. For example, the relatives of a person who is a carrier of an X-linked recessive disorder need to be informed about their risk of giving birth to a child with the disorder. However, carriers should grant permission to disclose the information about their carrier status to relatives.

In addition to these issues, there are many other ethical issues in genetic research such as gene therapy, ownership of genetic sequences, control over genetic information, embryonic stem cell research, etc. The use of advances in genetics for the betterment of society with responsibility and justice is the challenge before us.