MEDICAL GENETICS

Basic concepts for Medical Students

Aims:
Every medical student should be aware of the impact of hereditary, genetic and other related disorders as they constitute a big burden on health care providers. Although not all of them are common, as some are rare, collectively they cause a great deal of morbidity and mortality as well as difficulty in diagnosis in many occasions.

Learning objectives:
By the end of this chapter, the student must be able to:
1. Value the
2. Classify genetic disorders (including birth defects).
3. Discuss anatomy, physiology and pathology of chromosomes and genes on subcellular or molecular level.
4. Detect how each type produces the signs and symptoms of diseases or syndromes.
5. Define autosomal, sex-linked, recessive, dominant and how they are produced.
6. Classify birth defects and discuss its causes, types, and classification.
7. Recognize the basis of genetic counseling.
8. List the main tests available, with indications and interpretation of results.

BEFORE YOU START THIS CHAPTER, PLEASE REVIEW YOUR BIOLOGY, EMBRYOLOGY, AND BIOCHEMISTRY in relation to:

1. Mitosis and meiosis including all steps and stages.
2. RNA and DNA and chromosome structure, DNA transcription & translation, cell cycle, patterns and laws of inheritance (Mendel's Laws).
3. Symbols of family pedigree
4. Notes on DNA repair system.
5. Protein structure, synthesis, types (enzymes, hormones, etc.) and their functions, etc.

NOTE: What is found in BETWEEN BRACKETS AND IN GREEN is NICE TO KNOW, the rest is essential to know, i.e. not knowing it fails you.

IN THIS CHAPTER, YOU ARE GOING TO STUDY THE FOLLOWING TOPICS:
1. Introduction to genetic disorders "genetic vs. acquired disorders + Significance of genetic disorders "statistics about incidence and impact on health care providers" + what makes genetic and inherited disorders different from other disorders? "History, physical examination, family History, probabilities and recurrence".
2. Classification of genetic disorders.
3. Genetic basis of disease:
   a. Chromosomal disorders + examples
   b. DNA mutations + examples
   c. Multifactorial disorders + examples
   d. (Non-classical genetic disorders).
4. Features of AD, AR, X-linked (and mitochondrial inheritance) with their particular family pedigree.
5. **Teratogenesis & congenital malformation (birth defects)** with clinically important examples.

6. **Prenatal diagnosis & Genetic counseling.**

7. **Basics of genetic testing** (principles, indications, clinical significance, and interpretation of results):
   a. Chromosomal study,
   b. In-situ hybridization and
   c. Molecular study (PCR)

8. You may encounter the following topics (and more) in your future clinical study; they have a great deal of genetic basis and are good examples to understand and correlate with what you'd known in this chapter:
   a. Mental retardation
   b. Growth retardation
   c. Abnormal sexual differentiation / genital ambiguity
   d. Approach to a child with coarse features
   e. Approach to a child with a major or minor malformation.
   f. Antenatal care for mothers and unborn babies especially for high risk group.
   g. Radiological signs in prenatal Dx (US, MRI, CT, etc.)
   h. How to inform a family about their child with inherited disorders
   i. Genetics as an integral part of all medical specialties.

9. Advances in molecular biology testing and their clinical applications [gene therapy, DNA recombination, stem cells, and modern diagnostics: real-time PCR (RT-PCR), microarray, comparative genomic hybridization (CGH), etc.] → are nice to know topics that seem promising in the coming years.

References:

2. USMLE road map: Genetics. BY George H. Sack. 2008 (or later).
5. Other references & websites as referred to in place.
6. Just google your keyword(s) and find out what you'll get!! You'll find a lot of interesting information. I promise.

**My advices to you:**

- You may find this chapter non-orthodox. I hope you enjoy it as much as you benefit from it.
- The chapter may seem lengthy, but many paragraphs are explanatory and meant to make you understand an idea, and not intended to be memorized.
- Always return to your photos on the CD obtained from the dept. of pathology when studying this chapter.
- Do not hesitate to ask at any time (preferably after the lab. Time).
- Always return to your basic knowledge here "and go to other references" when you encounter a disease with a genetic basis in your clinical years and after graduation.
- Your questions & feedbacks are most welcome / send emails to (abmsadik@yahoo.com) Dr. Bassam Musa Sadik. I'll do my best to assist.
What is meant by Medical Genetics?

Medical genetics is the specialty of medicine that involves the diagnosis and management of hereditary disorders, i.e. it refers to the application of genetics to medical care. So, diagnosis, management, and counseling of individuals with genetic disorders as well as research on the causes and inheritance of genetic disorders would be considered part of medical genetics.

Various specialties within medical genetics are interrelated:

2. Cytogenetics.
4. Molecular Genetics
5. Human genetics, molecular biology, genetic engineering, or biotechnology.

Introduction:

Human diseases in general, whether medical or surgical, can roughly be classified into three categories:

1. Those that are genetically determined.
2. Those that are almost entirely environmentally determined.
3. And those to which both nature and nurture contribute.

However, progress in understanding the molecular basis of many so-called environmental disorders had tended to blur these distinctions. At one time, microbial infections were cited as examples of disorders arising wholly from environmental influences, but it is now clear that to a considerable extent, an individual's genetic makeup influences his or her immune response and susceptibility to microbiologic infections.

Despite the complexities of this nature-nurture interplay, there is little doubt that nature (i.e. the genetic component) plays a major, if not the determining, role in the occurrence and severity of many human diseases. Such disorders are far more frequent than is commonly appreciated (which represents only the tip of the iceberg).

- Around 20% of pediatric in-patients suffer from disorders of genetic origin.
- Up to 50% of spontaneous abortuses during the early months of gestation have a demonstrable chromosomal abnormality; there are, in addition, numerous smaller detectable errors and many others still beyond our range of identification.
- About 1% of all newborn infants possess a gross chromosomal abnormality.
- Approximately 5% of individuals under age 25 develop a serious disease with a significant genetic component.

Only those mutations compatible with independent existence constitute the reservoir of genetic diseases in the population.
Classification of Genetic disorders (important):

1. Classical Genetic Diseases:
   a. Chromosomal (Cytogenetic) disorders.
   b. Single gene (or unifactorial) disorders (Mendelian Disorders).
   c. Multifactorial disorders.

2. Non-Classical Diseases "or the single gene disorders with atypical pattern of inheritance":
   a. Diseases caused by mutations in mitochondrial genes.
   b. Triplet repeat mutations.
   c. Uniparental disomy.
   d. Genomic imprinting.
   e. Gonadal mosaism.

Added to that, is a large group of disorders "malformations" that manifest at birth, called congenital malformations, that many of them are caused by genetic disorders.

Genetic / Hereditary Disorders

1. Classical Genetic Diseases
   A. Chromosomal Disorders:
      Review of what you’ve already known:
      Normal human nucleated cells contain 46 chromosomes arranged in 22 homologous pairs of autosomal chromosomes in addition to one pair of sex chromosomes that could be similar (i.e. XX) or different (i.e. XY). This arrangement into pairs, based on the position of the centromere and on the length of the upper and lower arms of the chromosomes, is known as the Karyotype (Figures 6-1, 2 photos).

      It is estimated that about 1 of 200 newborn infants has some form of chromosomal abnormality. The figure is much higher in fetuses that do not survive to term. Cytogenetic (also called karyotypic) disorders may result from alterations in the number or structure of chromosomes and may affect autosomes or sex chromosomes. So, they could either be Numerical or Structural.

      A. Numerical abnormalities are defined as a gain or loss of one or more whole chromosome(s) (whether an autosome or a sex chromosome) or a whole set of chromosomes.

      The normal chromosome count is 46 (i.e. 2n = 46) as it is arranged in two sets of chromosomes. An exact multiple of the haploid number (n) is called euploid (normal state). Any number that is not an exact multiple of the haploid (n) is aneuploid, and it is one of the commonest changes that take place in malignant tissue.

      A gain of one or more set of chromosomes is known as polyploidy. This polyploidy may be triploidy when cells have (3n) or tetraploidy when the cells have (4n). Polyploidy generally results in spontaneous abortions. It results from a pathological condition known as endoreduplication i.e. when there is failure of cytoplasmic division that should follow the nuclear division and therefore the original duplicated two sets will remain in one cell.

      A gain of one chromosome is a state known as trisomy, e.g. trisomy of some autosomal chromosomes such as chromosome 21 is called Down’s syndrome, trisomy 18 is called Edward’s syndrome, trisomy 13 is called Patau’s syndrome. Other autosomal trisomies are not very frequent. Trisomy of the sex chromosome is exemplified by Klienfelter's syndrome in male (XXY) and triple X (XXX) syndrome.
in female, while a loss of one chromosome is called monosomy. All trisomies and monosomies are by definitions (aneuploidies).

A gain or loss in the sex chromosomes, especially the X-chromosome, is compatible with life and is relatively common; while the loss of an autosomal chromosome is usually non-compatible with life and a fertilized ovum carrying such a karyotype could not sustain pregnancy to full term and are usually lost early in pregnancy, i.e. abortion. Monosomy of sex chromosome in a female is called Turner’s syndrome (45 XO).

The chief cause of aneuploidy (and thus trisomy and monosomy) is non-disjunction of a homologous pair of chromosomes at the 1st meiotic division or a failure of sister chromatids to separate during the 2nd meiotic division. The latter may also occur during somatic cell division, leading to the production of two aneuploid cells. Instead of two homologous chromosomes separating and moving into opposite poles of the dividing cell, both move to the same pole so that one cell receives both and the other receives neither. The same may happen to the sister chromatids in mitosis.

(what is the other less common cause of aneuploidy? → find out)

Fertilization of such gametes by normal gametes from the other partner would result in two types of zygotes: trisomic, with an extra chromosome (2n+1), or monosomic (2n-1).

A post-zygotic mitotic non-disjunction would result in the production of a trisomic and a monosomic daughter cell; the descendants of these cells would then produce a mosaic. Mosaicism affecting sex chromosomes is common, whereas autosomal mosaicism is not.

(How can you define mosaicism?)

B. Structural abnormalities:

In this case, the cell has a normal number of 46 chromosomes but they are morphologically or structurally abnormal.

All chromosomes in a normal human karyotype have a long lower arm (known as q arm) and a shorter upper arm (known as the p arm), so that the centromere is located at different levels in between (Figure 2).

The human karyotype is divided into various groups of chromosomes according to the ISCN (International System for Chromosomal Nomenclature) (See Figure 1-1 photo 2).

X chromosome is included within group C, while Y chromosome is included within group G.

Group D & G chromosomes are called acrocentric chromosomes as they have a short arm with very small amount of DNA connected to the centromere by a very slender thread, giving it a satellite or antenna-like appearance. Even when this satellite is lost, no abnormality appears.

Structural abnormalities usually result from chromosomal breakage followed by loss or rearrangement of material.

They are of different types as follows:

1. DELETION: involves loss of a piece of a chromosome that could be either:

   i. Terminal, in which, there is one break in the chromosome and the portion distal to this break is lost. This lost piece could be carrying important genes, and its loss results in signs and symptoms related to the lost genes products (Figure 6-3 A).
ii. Interstitial, where the piece of a chromosome between two breaks is lost resulting in a chromosome that is shorter than the original with the same consequences e.g. Cri du chat (loss of short arm of chromosome 5) (Fig. 6-3 A & B).

Most of certain genes, known as oncogenes, are lost from the cells through deletion and their loss will result in development of malignancy of certain tissues, e.g. loss of retinoblastoma gene by interstitial deletion of the long arm of chromosome 13, i.e. 13q in which, the newborn carrying this deletion will develop retinoblastoma (Figures 6-4 A & B). Another gene is lost in deletion of short arm of chromosome 11, which results in development of Wilms’ tumor with aniridia (Figure 6-5).

Sometimes, the piece lost carries no gene and therefore it causes no abnormality. This is due to the fact that the genes are not situated on the chromosome one besides the other all along the chromosomes but there are parts of the chromosome carry no genes. In fact, only about 10% of the whole length of DNA from all chromosomes carries coding sequences.

If individuals, carrying a deleted chromosome, marry, their gametes formed would be abnormal and results in either normal children, repeated abortions or in the birth of a child with malformation.

2. INVERSION:

This abnormality results from two breaks in the chromosome and the piece between the two breaks will rotate 180° and is fixed back again in its rotated position.

The breaks may involve either the short arm or the long arm and the inversion is called paracentric (the breaks are on one side of the centromere), or the breaks may involve both arms and the inversion is called pericentric (the centromere is involved in the inversion), which is more severe (Figure 6-6).

Consequences of both types of inversion are the creation of unbalanced homologues of chromosomes, one carrying a duplicated gene(s) and the other is deficient in that gene(s). The individual carrying such inversions may, however, be without apparent signs; yet, his gametes are unbalanced resulting in the birth of an abnormal fetus or abortion.

3. TRANSLOCATION:

It is defined as an exchange of segments of chromosomes between non-homologous chromosomes.

Regular (reciprocal) translocation that takes place between any two chromosomes [other than the acrocentric group of chromosomes] is one of the main pathological changes seen in malignancies (Figure 6-7). It has recently been discovered that these translocations may translocate some oncogenes from their normal position to a new one where they are induced to function in an uncontrolled manner leading to override of the body's regulatory control mechanisms, ultimately leading to malignancy.

Such important genes are usually those coding for the production of a hormone that is usually needed only in a minute amount and is necessary for the growth regulating processes in the body. This is usually seen in leukemias and lymphomas, e.g. Philadelphia chromosome (the product of a translocation between chromosome 22 and 9) seen in chronic myeloid leukemia (Figure 6-8), or a translocation between 8 and 14 seen frequently in Burkitt's lymphoma.

Another type of translocation called Robertsonian (centric fusion) type that takes place between two acrocentric chromosomes, either one from each group or two chromosomes of the same group but different number or the pair of a homologue
where the two acrocentrics lose their short arm and both long arms of the chromosomes fuse together to form one chromosome (Figures 6-9).

An individual who carries such a translocation have multiple types of gametes. These gametes when fertilized by the normal gamete of the normal spouse will result in four possibilities (Figure 6-10):

1. Normal.
2. Normal count (i.e. 46) but carries the translocated chromosome. This individual or fetus will be trisomic for the acrocentric chromosome, i.e. it is the source of cases of translocation Down’s syndrome.
3. Balanced carrier like the original parent with 45 count of chromosome.
4. Monosomic (i.e. 45) and it is eliminated by abortion early in pregnancy as all autosomal monosomies.

So, an individual who carries a Robertsonian translocation will have three possibilities of producing a full term baby or a child with one of the 1st three categories listed above.

4. ISOCHROMOSOMES:

This abnormality results from aberrant division of the centromere which is the last part of the chromosome that divides in the mitosis to separate the two sister chromatids into individual chromosomes. This aberrant division takes place in a horizontal way rather than the perpendicular natural way. So, the resulting two chromosomes are imbalanced, one formed entirely of two short arms and the other of two long arms. Each of them is called an isochromosome (Figure 6-11).

These isochromosomes may be seen in some cases of Down's or Turner's syndromes.

5. RING CHROMOSOME:

It results from deletion of both ends of a chromosome and then the ends, because of the adhesive nature of the exposed DNA, will stick together forming a ring or a circle chromosome (Figure 6-12).

6. DUPLICATION OF PART OF A CHROMOSOME:

These abnormalities can produce some characteristic signs and symptoms called syndromes that can be confirmed by studying the number and structure of patient's chromosomes. This test is called chromosomal study or studying the karyotype.

(karyotype vs. phenotype)

The main indications for karyotype are:

1. Advanced maternal age (>35 years).
2. A family with a previous child known to have a chromosomal abnormality.
3. Clinical suspicion of a syndrome caused by a chromosomal abnormality.
4. Part of males or females infertility screening.
5. To determine sex (gender) of an individual.
6. Unexplained mental retardation.
7. Unexplained growth retardation
8. Some cases of cancer.
9. Miscellaneous conditions.
I. Disorders of Autosomal Chromosomes:

1. Cytogenetic types of Down’s syndrome (Karyotype writing):

   1. Regular Down’s, which constitutes to 95% of cases of born Down’s syndrome. It usually results from non-disjunction of the two sister chromatids of one homologue or non-disjunction of the ‘21’ homologue pair migrating to one pole, resulting in a gamete with double ‘21’ chromosomes (Figures 6-13, 2 photos).

   (Writing the karyotype is nice to know) but interpretation of results are essential

   How to write such a karyotype?

   Karyotype writing starts 1st by (a) mentioning the chromosome number, then (b) mentioning the sex of the patient (XX or XY), followed by (c) the sign of addition because there is an extrachromosome, then (d) the number of additional chromosome, i.e. 47XY, +21 (male with regular Down's) or 47 XX, +21 (female Down's)

   2. Translocation Down’s, they constitute to 3% of cases of born Down’s syndrome.

   Karyotype writing follows the 1st two steps above, then (c) after a semicolon write a letter ‘t’ (d) then opening an arc, put the number of the two chromosomes sharing in the translocation with a slash in between:

   i.e. 46XY, t(14/21) or t(15/21) or t(22/21) or t(21/21)

   3. Isochromosome ‘21’

   Isochromosome of the long arm of chromosome ‘21’ may take place resulting in Down’s syndrome.

   Karyotype writing: the same 1st two steps then (c) followed by letter ‘i’ after the semicolon with the number of chromosome causing the isochromosome, (d) followed by the symbol of the arm [the short arm ‘p’ or long arm ‘q’].

   i.e. 46XY, i(21q) or 46XX, i(21p)

   4. Mosaics: are individuals who have a mixture of cells in their body of different proportions of two or more different karyotypes.

   Karyotype writing is to write every line or type of karyotype on its own the same way as the above principles then separating them by a slash,

   i.e. mosaic Down of normal karyotype and another with translocation:

   46XY, / 46XY, t(14/21)

   46XX, / 46XX, t(14/21) and so on mixing any type of karyotype.

2. Cytogenetic types of Edward's syndrome (Karyotype writing):

   Edward's Syndrome (Figure 6-14) or trisomy of chromosome 18 is the most common type:

   - Regular Edward's S.: 47XX, +18
   - Mosaic type: 46XY / 47XY +18
3. Cytogenetic types of Patau syndrome (Karyotype writing):
   Patau's Syndrome (Figure 6-15) is in most cases a trisomy of chromosome 13:
   - Regular Patau S.: 47XX, +13
   - Translocation type: 46XY; t(13/21) or 46XX; t(13/22)
   - Isochromosome type: 46XX; i(13q)
   - Mosaic type: 46XY / 47XY +13 and so on …

4. Karyotype writing of a deletion syndrome:
   Writing the karyotype of a deletion again following the same 1” two steps, followed then by the number of chromosome being deleted followed by the sign of minus mentioning the sign of the arm affected,
   e.g. **Cri du Chat syndrome** (cat cry syndrome) is formed by partial deletion of the short arm of chromosome 5 in males or females:
   46XY, 5p- or 46XX, 5p-

5. Duplications of part of a chromosome:
   (Figure 6-16)

II. Abnormalities of Sex Chromosomes:

1. Cytogenetic types of Turner’s syndrome (Karyotype writing):
   By applying the same principles here, we may have:
   - Regular Turner’s syndrome 45XO
   - Or Turner's with long arm isochromosome X [46XX, i(Xq)]
   - Or a mosaic Turner's 46XX / 45XO
     (Figure 6-17)

2. Karyotype writing of other sex chromosome disorders:
   - Regular Klinefelter's Syndrome 47XXY or 48XXXY
   - Mosaic klinefelter's S. (seen in 15% of cases) 46XY / 47XXY
     or sometimes 46XY / 48XXXY

3. Karyotype writing of Y chromosome disorders:
   47XYY (rare disorder) → very tall with azoospermia (Figure 6-18)

N.B.: Needless to mention that **many other structural chromosomal abnormalities** are present, some of which are more complex than the examples depicted here, e.g. dicentric chromosome.

Or Just about everything you can imagine which can happen to that piece of string -- → does!

For a review and more examples, see Figures (6-19 → 9 photos).

Pay attention to the presentation in your labs.
B. Defects of Single Genes with Large Effect (Unifactorial or Mendelian Disorders):

Introduction:
The number of known Mendelian disorders has grown to more than 5000. Although individually many are rare, altogether they account for approximately 1% of all adult admissions to hospital and about 6-8% of all pediatric hospital admissions. They represent the most common purely genetic abnormality as compared to other causes.

They are caused by a mutation in a single gene. A mutation is a disturbance in the sequence of the nucleotide arrangement in the DNA molecule, or it is simply a permanent change in the DNA. Mutations may give rise to inherited disorders or may cause cancer or congenital malformation.

(When, Why and How?)

Review:
A gene is that part of the DNA that codes for a polypeptide chain. Only about 2% of the DNA codes directly for information, 24% is intronic sequence (non-coding sequences within the genes); the remaining 74% is a non-coding sequence outside the genes (Figure 6-20). The function of this huge amount of non-coding sequences has not been fully elucidated.

There are about 30,000 genes (in contrast to what was previously thought of about 100,000). Genes are not arranged one beside the other along the chromosome or the DNA. There is a variable length of those non-coding sequences in between and inside the genes. Those found inside the gene is called the intervening sequences (or the introns), while the coding sequences of the gene is called exon.

Some genes have few exons and some have many. Some genes are very small in size but some are very large with so many exons.

Some chromosomes are rich in genes but some have only few, which explains why some chromosome deletions or even duplication have no or minimal clinical effect.

Genes may behave as dominant, i.e. when only one of the alleles becomes mutated it results in a genetic disease, or they may behave as recessive, i.e. the diseases result only when both alleles (of both the maternal and paternal origin) are affected by the same mutation. A 3rd category of genes are those genes which determine an autosomal character but they are situated on the sex chromosome, called sex-linked genes. So we have autosomal dominant (AD) disorders, autosomal recessive (AR) disorders, and sex-linked disorders.

The question arises why some genes act in a dominant manner while others behave in a recessive one, i.e. the problem of dominance and recessiveness. To answer this question, one has to consider the following principles that must be remembered:

It was thought earlier that a single gene is responsible for the formation of a single type of protein, but since the protein is made up of units of polypeptides that could be the same or different in one molecule of protein, the principle becomes:

A single gene is responsible for the formation of a single type of polypeptide, and if we know that out body structures and functions from the moment of post-fertilization to the full maturity and later on are determined by proteins. The types of proteins are varied; they could be structural proteins, like fibrous tissue, elastic tissue; they could be immunoglobulins, signal transducing proteins, receptors, enzymes, hormones, etc. Therefore, the action of the gene being dominant or recessive is determined by the type of protein it produces and its function.

(Codominance means that both alleles of the same gene are fully expressed in the heterozygote e.g. HLA and ABO blood group antigens are good examples).
A single gene mutation may lead to multiple phenotypic effects, a phenomenon called **pleitropy** [e.g. Marfan's syndrome where there is skeletal, cardiovascular, and eye defects]; conversely, mutations at several genetic loci may produce the same trait [e.g. retinitis pigmentosa] in a phenomenon called **genetic heterogeneity**.

**Dominant** genes usually produce these types of proteins:
1. **Major structural** (or non-enzymatic) proteins, which form or are present in many parts of the body (e.g. collagen, spectrin, fibrillin, elastin, etc.); examples are cases of achondroplasia & Ehler Danlos syndrome (lax joints and skin).
2. A **key enzyme** in a complex metabolic pathway usually under feedback control (e.g. AD porphyria), or
3. A **membrane receptor** regulating a metabolic pathway (e.g. AD familial hypercholesterolemia disease) where the receptors for LDL are mutated. They are responsible for regulation of LDL in the cells and the circulation.
4. Or a **membrane transport protein**.

To explain the latter example and how the action of this pathway is executed, let us consider the pathway of circulating LDL.

LDL should enter the cells of the body for building cellular membrane and nuclear membrane from wear and tear. It could not enter the cells unless it is complexed with receptors on the cell membrane.

Once it is inside the cell, the complex will be degraded into free cholesterol and amino acid, which is the remnant of the proteinous coat of the lipoprotein. The free cholesterol in the cell constitutes the cholesterol pool of the cell, which its level is regulated by three systems of enzymes, the **HMG-CoA reductase** (**3-hydroxy-3-methylglutaryl coenzyme A reductase**), which forms cholesterol from fatty acids, **ACAT** (**acyl-CoA:cholesterol)** transferase, which hydrolyze cholesterol into ester rendering it inactive and **the number of the receptors on the surface of the cell**. If the pool concentration is low, messages are sent to activate the HMG-CoA reductase, inactivate ACAT and increase the number of receptors on the cell surface. Therefore, when one of the two alleles responsible for the formation of the receptors protein, half the number of receptors are formed only, so 50% of LDL which is used to be internalized inside the cell will remain in the circulation unable to enter the cells and a state of hypercholesterolemia results with reading of 400-500 iu/dl of cholesterol in the blood (Figure 6-20).

A defect of one single copy of a dominant gene produces a great deal of abnormalities that produce signs and symptoms of a disease related to the function of the deficient protein.

**As for recessive genes**, they produce enzymes which usually share in catabolic pathways (usually non-key enzymes); when both alleles are defective, there is no protein, i.e. no enzyme and therefore the catabolic pathway is obstructed with the accumulation of the biochemical substrate; while loss of one single copy is usually compensated for by the remaining active and normal gene (50% protein present).

Examples of those diseases are some of diseases of thalassemia, mucopolysaccharidosis, lipidosis, phenylketonuria (PKU), and many others.

(What happens if two copies of a dominant gene are defective?)
(What causes the appearance of signs / symptoms to appear in a recessive disorder?)
Sex-linked diseases: because most of the genes are carried on the “X” and very few are present on the “Y”, usually sex-linked is used for “X”-linked and we have both dominant and recessive disorders.

In this type of inheritance, there is a lot of deviation from what is expected from autosomal recessive pattern. The usual pattern, in clinical practice, is that only males who carry the mutated gene on their “X” chromosomes are clinically affected while females are usually silent carriers but they transfer the disease to their sons e.g. G6PD deficiency (favism), hemophilia A, … etc. as males have only one X-chromosome while females have two.

It happens that some females may present the disease clinically like males; this was explained by Lyon’s hypothesis, which states that in a female’s autosomal cells, and during early embryogenesis, all the “X” chromosomes will be inactivated except one which remains active during interphase.

- This process of inactivation takes place early in the post-fertilization period, 19-20 post-fertilization days.
- The process of inactivation is random concerning the origin of the inactivated “X”, i.e. paternal “X”, which comes from the father or maternal “X” that comes from the mother.
- In a cell, all the daughter cells that descend from it, the same “X” will remain inactive.
- All inactive X-chromosomes will be condensed as a (Barr Body) (Figure (6-21).

This means that around 50% of the “X” chromosomes are inactivated but this does not necessarily involve all the paternal “X” or all the maternal “X”, as in some areas, the paternal X is inactivated while in others, it is the maternal “X” that is inactivated. Therefore, the body of the female is a considered as mosaic concerning the active “X”. The female is considered heterozygote for the X chromosome (regarding origin of her X-chromosomes).

In a female who carries a mutated gene on “X” chromosome and present clinically (called manifesting carrier), the manifestations happen by chance that in most part of her body, the “X” that carries the abnormal “X” gene remain active (or the normal X is inactivated), which results in deficiency of the product of the gene → disease. This is because of the randomness of the inactivation. Some have more mutated Xs and some have more non-mutated Xs.

(What other causes of a manifesting carrier female can you count so far?)

Aetiology (Types of mutations in regard to the structural defect):
[[very important]]

All single gene diseases (dominant or recessive, autosomal or sex linked) are due to mutations, which are of different structural types:

1. **Point mutation:** in which one single base is changed (substituted), deleted, or added.
   - **Single point substitution** which is the commonest type. It usually results from a change in only one of the nucleotide bases that form the trios (three bases), each of which codes for a specific amino acid in the protein molecule.

The possible consequences are the following:
1. **Silent mutation:** A change in one base will result in a code which codes for the same amino acid in the protein. This is due to a fact known as the *redundancy* of the code, i.e. for each of the 20 amino acids; there is more than one code from the 64 codes formed by the four nucleotide bases. Therefore, the character of the protein would not change and nothing results but one can deduce from this fact that there are proteins formed by DNA sequence that are variable among individuals although the protein is the same. This variation forms the bases for DNA finger printing used to identify individuals like the original finger prints.

2. **Neutral mutation:** when one base is changed into another forming another code; the new code codes for another amino acid that is physically and chemically similar (not identical) to the normal one → the function of the new polypeptide chain is unaffected → no disease state.

3. **Missense mutation:** The change in the base will form a new code coding for a different amino acid in the protein changing its character and behaviour resulting in disease, e.g. all cases of thalassemia, sickle cell disease, PKU, etc.

4. If a mutation occurs in one of the three codons (called *termination or stop codons*) that usually stop the signal (e.g. termination of gene transcription) → we have 2 possible types:
   a. **Non-sense mutation:** which occurs when a codon is changed into a stop codon by changing one of its bases → the resulting polypeptide would be shorter than normal called truncated protein; e.g. some forms of β-thalassemia
   b. Or when a stop codon is converted into a coding one, so the gene transcription will continue behind the termination point adding more than normal number of amino acids until a second 'stop' codon is reached, resulting in a longer protein behaving abnormally → disease, e.g. Constant Spring type of haemoglobin (HbCS), where the termination codon of α-polypeptide of Hb is mutated resulting in addition of 31 amino acids to the original 14 amino acids of the normal chain.

b. & c. **Additional / deletion mutations:**
These mutations cause a gain or loss of one single nucleotide base → shift in the reading frame of the trios "codons" changing the whole codons from the point of mutation onwards creating a completely new type of protein or sometimes creates a termination code in the center of the molecule. These types of mutations are called **frame-shift mutations** (Figure 6-22).

2. The second type of mutations (beside the point mutations) are the **Addition or deletion** of more than one nucleotide bases:
   A. If 2 bases → frame shift mutation (less common cause than a point mutation).
   B. Addition / deletion of 3 bases or the multiple of 3, i.e. 6, 9, 12, 15 … etc. This will lead to addition / deletion of 1, 2, 3, 4, … etc. amino
acid(s) in the protein molecule leading to abnormal protein, i.e. Frieberg Hb, where 5 amino acids (i.e. 15 bases) is added between amino acids 78-79 sequence in β-Hb polypeptide.

C. Addition / deletion of a piece of DNA inside the gene (intragenic) or in between the gene (intergenic). Again this creates variability and may lead to a disease state but if not, it causes polymorphism in DNA sequences that can be used for genetic testing and DNA fingerprinting.

3. Unequal crossing over:

Usually during crossing over at meiosis, the chromosomes align themselves side by side. This process should be very precise that one allele would be in front of its brother allele and even the bases are matched to each other in number and type. So, when there is a crossing over and exchange of chromosomal segments, no disturbance occurs in the newly formed chromosomes or genes, but if the alignment is improper, then the alleles are pushed away for a certain degree. This case takes place in sites of the DNA where there are grouping of genes of similar DNA structure with very slight variation so one gene is mistaken for a different gene as being its allele and if crossing over occurs, a defect will result leading to the formation of two unbalanced homologues, one containing duplicated sequences and the other deleted sequences.

The more similarity of DNA within the sequences, the more likely unequal crossing over will occur. Crossing over is the process most responsible for creating regional gene duplications in the genome. Repeated rounds of unequal crossing over cause the homogenization of the two sequences. With the increase in the duplicates, unequal crossing over can lead to dosage imbalance in the genome and can be highly deleterious.

Summary:
Structurally, gene mutations can be in one of the following types:

1. Point mutations
   a. Single base substitution (most common type):
      i. Silent
      ii. Neutral
      iii. Missense
      iv. Non-sense
      v. Longer protein
   b. Single base addition / deletion → frame-shift mutation.
2. Addition / deletion of > 1 base:
   a. 2 bases → frameshift mutation
   b. 3 (or multiple of 3)
   c. A piece of DNA
3. Unequal crossing over
1. Classical genetic disorders
   A. Chromosomal abnormalities:
   B. Single gene mutations:
   C. Multifactorial Inheritance (MFI)

C. Multifactorial Inheritance (MFI)

Multifactorial (also called polygenic) inheritance is involved in many of the physiologic characteristics (e.g. weight, height, blood pressure, hair color, etc.). A multifactorial physiologic or pathologic trait may be defined as one governed by the additive effect of two or more genes of small effect but conditioned by environmental, non-genetic influences.

Even monozygotic twins reared separately may achieve different heights because of nutritional or other environmental influences. This form of inheritance is believed to underlie such common diseases as diabetes mellitus, hypertension, gout, schizophrenia, bipolar disorders and certain forms of congenital heart disease as well as some skeletal abnormalities.

In multifactorial inheritance, it is the additive effect of many genes of small effect PLUS a suitable environment that cause such disorders.

In single gene disorders, individuals in regard to the abnormal gene are one of 3 groups: a heterozygote (carrying one mutated and one normal gene and thus affected in AD and not affected in AR disorders), a homozygote for the mutated gene (and thus affected in all cases), or a homozygote normal. There is no gradient in between these 3 groups.

(What do we call a male carrying an x-linked recessive disorder? A heterozygous or a homozygous? Or something else?)

In MFI, we could group individuals in a community into many different grades, which have a normal distribution curve (Gaussian distribution) with a threshold point, which when exceeded, the disorder is expressed.

The facts that [MFI are affected by many genes (not just one) and that the additive effect of both genes and the environment determine the expression of MF disorder], are called Genetic Liability (or genetic predisposition) of the individual and this liability can be measured. So, MFI is only partially genetic (unlike other types of inheritance) and needs environmental factors to act for the disorder to appear.

You might imagine that the list of “multifactorial” disorders blends in with the entire list of human diseases. Hence are “all” diseases “genetic”?

- They are Multi-“FACTORIAL”, not just multi-GENIC
- If a disease or condition is scalable, rather than on or off, it is probably multigenic, or multifactorial, just part of the spectrum of HOMO-zygous diseases being HOMO-geneous, and HETERO-zygous diseases being VARIABLE.
- Common phenotypic expressions governed by “multifactorial” inheritance
  - Hair color
  - Eye color
  - Skin color
  - Height
  - Intelligence
  - Diabetes, type II

Mention (10) diseases with multifactorial type of inheritance
2. Single Gene Disorders with Atypical Patterns of Inheritance (or Non-Classical Genetic Disorders)

This group includes:

A. Diseases caused by mutations in mitochondrial genes.
B. Triplet repeat mutations.
C. Uniparental disomy / Genomic imprinting.
D. Gonadal mosaicism.

Specific Features of Single Gene Disorders:

Autosomal Dominant (AD) Disorders

Over 3000 conditions or traits shows this pattern of inheritance are identified. Some are very mild; others are lethal.

- AD disorders are manifested in the heterozygous state, so at least one parent of an index case is usually affected; both males and females are affected, and both can transmit the condition.
- When an affected person marries an unaffected one, every child has one chance in two of having the disease. In addition to these basic rules, autosomal dominant conditions are characterized by the following:
  - With every autosomal dominant disorder, some patients do not have affected parents. Such patients owe their disorder to new mutations involving either the egg or the sperm from which they were derived. Their siblings are neither affected nor at increased risk for developing the disease.
  - The proportion of patients who develop the disease as a result of a new mutation is related to the effect of the disease on reproductive capability.
  - If a disease markedly reduces reproductive fitness, most cases would be expected to result from new mutations. Many new mutations seem to occur in germ cells of relatively older fathers.
  - Clinical features can be modified by reduced penetrance and variable expressivity. Some individuals inherit the mutant gene but are phenotypically normal. This is referred to as reduced penetrance.
  - Penetrance is expressed in mathematical terms: Thus, 50% penetrance indicates that 50% of those who carry the gene express the trait.
  - In contrast to penetrance, if a trait is seen in all individuals carrying the mutant gene but is expressed differently among individuals, the phenomenon is called variable expressivity.
  - For example, manifestations of neurofibromatosis type 1 range from brownish spots on the skin to multiple skin tumors and skeletal deformities.
  - The mechanisms underlying reduced penetrance and variable expressivity are not fully understood, but they most likely result from effects of other genes or environmental factors that modify the phenotypic expression of the mutant allele. For example, the phenotype of a patient with sickle cell anemia (resulting from mutation at the β-globin locus) is influenced by the genotype at the α-globin locus because the latter influences the total amount of hemoglobin made.
  - The influence of environmental factors is exemplified by familial hypercholesterolemia. The expression of the disease in the form of atherosclerosis is conditioned by the dietary intake of lipids.
  - In many conditions, the age at onset is delayed: symptoms and signs do not appear until adulthood (as in Huntington disease).
Autosomal Recessive Disorders

Autosomal recessive inheritance is the single largest category of Mendelian disorders. Because autosomal recessive disorders result only when both alleles at a given gene locus are mutants, such disorders are characterized by the following features:

1. The trait does not usually affect the parents, but siblings may show the disease;
2. siblings have one chance in four of being affected (i.e., the recurrence risk is 25% for each birth); and
3. if the mutant gene occurs with a low frequency in the population, there is a strong likelihood that the proband is the product of a consanguineous marriage.

In contrast to those of autosomal dominant diseases, the following features generally apply to most autosomal recessive disorders:

- The expression of the defect tends to be more uniform than in AD disorders.
- Complete penetrance is common.
- Onset is frequently early in life.
- Although new mutations for recessive disorders do occur, they are rarely detected clinically. Since the individual with a new mutation is an asymptomatic heterozygote, several generations may pass before the descendants of such a person mate with other heterozygotes and produce affected offspring.
- In many cases, enzyme proteins are affected by a loss of function. In heterozygotes, equal amounts of normal and defective enzyme are synthesized. Usually the natural "margin of safety" ensures that cells with half their usual complement of the enzyme function normally.

X-Linked Disorders

1. All sex-linked disorders are X-linked, almost all X-linked recessive. Several genes are encoded in the "male-specific region of Y"; all of these are related to spermatogenesis.
2. Males with mutations affecting the Y-linked genes are usually infertile, and hence there is no Y-linked inheritance.
3. A few additional genes with homologues on the X chromosome have been mapped to the Y chromosome, but no disorders resulting from mutations in such genes have been described.
4. X-linked recessive inheritance accounts for a small number of well-defined clinical conditions.
5. The Y chromosome, for the most part, is not homologous to the X, and so mutant genes on the X are not paired with alleles on the Y. Thus, the male is said to be hemizygous for X-linked mutant genes, so these disorders are expressed in the male.

Other features that characterize these disorders are as follows:

- An affected male does not transmit the disorder to his sons, but all daughters are carriers.
- Sons of heterozygous women have, of course, one chance in two of receiving the mutant gene.
- The heterozygous female usually does not express the full phenotypic change because of the paired normal allele. Because of the random inactivation of one of the X chromosomes in the female, however, females have a variable proportion of cells in which the mutant X chromosome is active → skewed X-inactivation.
The word dysmorphology is derived by combining three Greek words (dys—bad or disordered; morph—shape or structure; and ology—the study or science of). Dysmorphology is a branch of clinical genetics concerned with the study of structural defects, especially congenital malformations.

- 20% neonatal deaths caused by these
- 3% live births have one or more congenital abnormality
- Even higher prevalence among still births
- 6% one year olds have one or more
- about 3 million fetuses and infants are born each year with major congenital malformations
- They account for nearly 500,000 deaths worldwide each year.
- However, individual congenital malformations are seen only infrequently by the individual practitioner.

**Introduction:**

Congenital malformations or birth defects are common among all races, cultures, and socioeconomic strata. Birth defects can be isolated abnormalities or part of a syndrome and continue to be an important cause of neonatal and infant morbidity and mortality.

Of all congenital malformations diagnosed by the end of first year of life, nearly 60% are identified in the first month and about 80% by the end of 3 months. With the introduction of prenatal ultrasound in obstetric care, many major congenital malformations are diagnosed prenatally, allowing parents to have the option of terminating the pregnancy.

Several pediatric disorders are of genetic origin. However, it must be borne in mind that not all genetic disorders are present in infancy and childhood, and conversely, many pediatric diseases are not of genetic origin (e.g. diseases resulting from immaturity of organ systems).

Three commonly used terms are to be understood: *hereditary, familial, and congenital*. Hereditary disorders, by definition, are derived from one's parents, are transmitted in the gametes through the generations, and therefore, are familial. The term 'congenital' simply implies "present at birth".

**Not all congenital diseases are of genetic origin** (e.g. congenital syphilis), and not all genetic disorders are congenital (e.g. Huntington's disease, is expressed only after the 3rd or 4th decade of life).

**Definition of CM:**

It is a deformation of structure or function of an organ that is present at birth.

- It may be on the surface of the body, e.g. cleft lip; or inside the body e.g. horseshoe kidney.
- It may be macrocellular, i.e. the defect affects large group of cells, tissue or organ, e.g. club foot, or microcellular, i.e. can be identified only by microscopic examination, e.g. sponge kidney where the defect is abnormal connection between the collecting tubules and the urineferous tubules leading to thin microscopical dilatation that gives the kidney a spongy feeling.
- It could be diagnosed by the naked eye or it may need special procedure for diagnosis, e.g. congenital heart defects.
• It may present at birth with signs and symptoms, e.g. duodenal atresia or may present later in life but the defect is present at birth, the signs and symptoms arises only later in life, e.g. adult polycystic kidney → chronic renal failure.
• It may be familial or non-familial, i.e. either there are multiple cases in one family or it is the only case in the family (sporadic);
• And lastly it could be of a genetic origin (cause), or could be a non-genetic, i.e. caused by environmental causes, e.g. some cases of microcephaly are of genetic origin while others are due to in utero infection (prenatal infection).

Causes:
Those agents with a potential to induce a structural anatomic anomaly and thus a congenital malformation in a developing fetus are termed teratogens (Greek: teratos [monster] and gen [producing]). Some teratogens act as a mutagen (when affects a growing fetus) and some as a carcinogen (when affects a mature adult cell type).

Pathological Action of Teratogens:
The effect of a teratogen is most effective on growing tissues with rapid division rate but not all teratogens affecting different foeti cause the same severity of action, i.e. the action of one teratogen is variable in different foeti.

This is due to the following factors:
  a. Nature of the teratogenic agent.
  b. The dose of the teratogen: larger doses of course cause more severe effects.
  c. Timing and duration of the exposure to the teratogen; the earlier and the more prolonged, the greater the effect would be (Figure 6-26).
  d. Genetic constitution (or host susceptibility): some foeti are more susceptible than others for the same dose and therefore they are more severely affected.
  e. Interaction with other factors, i.e. presence of concurrent exposures e.g. environment of the uterus, maternal metabolic state, and so on.

It is likely that the interactions between genes and environmental factors are responsible for most birth defects related to teratogenic exposures.

Potential Teratogenic mechanisms:
• mutational changes in DNA sequences
• chromosomal abnormalities leading to structural or quantitative changes in DNA
• alteration or inhibition of intracellular metabolism, e.g., metabolic blocks and lack of co-enzymes, precursors or substrates for biosynthesis
• interruption of DNA or RNA synthesis
• interference with mitosis
• interference with cell differentiation
• failure of cell-to-cell interactions
• failure of cell migrations
• cell death through direct cytotoxic effects
• effects on cell membrane permeability and osmolar changes
• physical disruption of cells or tissues.
Types of Teratogens, See Figure (6-27):

Genetic factors are responsible for one third of congenital malformations with known causes.

(A chromosomal abnormality occurs in 1 of 170 live born infants. Among chromosomally abnormal neonates, 1/3rd have an extra sex chromosome, 1/4th have trisomy of an autosome, and the remaining have an aberration of chromosomal structure such as a deletion or translocation. However, a significant majority of these infants have no phenotypic manifestations at birth. Nearly 10% of infants with lethal multiple congenital malformations have abnormal cytogenetic studies. However, this proportion is likely to be much higher today with advances in genetics.

With better understanding of the human genome and improved techniques in molecular cytogenetics, more and more structural chromosomal abnormalities are being identified as a cause of congenital anomalies previously considered to be of unknown etiology).

Environmental factors also play an important role in the etiopathogenesis of many congenital malformations. Maternal exposure to certain environmental agents can lead to disruption of the normal developmental process and result in both minor and major congenital anomalies.

Classification of Congenital Anomalies

A. Classification based on timing of insult.
Congenital anomalies can be placed into the following three categories on the basis of developmental stage during which the aberration in development took place.

1. Malformation. A malformation is a morphologic defect of an organ, part of an organ, or a region of the body due to an intrinsically abnormal developmental process. The examples include renal agenesis and neural tube defects (Figure 6-28).

2. Disruption. It results from the extrinsic breakdown of or an interference with an originally normal developmental process and the resulting anomaly can include an organ, part of an organ, or a larger region of the body. A disruption is never inherited but inherited factors can predispose to and influence the development of a disruption. An anomaly secondary to disruption can be caused by mechanical forces; ischemia, hemorrhage, or adhesions of denuded tissues and occur during or after organogenesis. An example of congenital anomaly caused by disruption is the amniotic band sequence (Figure 6-29).

3. Deformation. Deformational anomalies are produced by aberrant mechanical forces that distort otherwise normal structures. These anomalies occur after organogenesis, frequently involve musculoskeletal tissues and have no obligatory defects in organogenesis. Common causes of deformation are structural abnormalities of the uterus such as fibroids, bicornuate uterus, multiple gestation, and oligohydramnios (Figure 6-30). Deformations can be reversible after birth. These anomalies are unlikely to have a genetic basis, are often not associated with cognitive deficits, and have a low recurrence risk.

B. Classification of birth defects based on medical consequences.
Based on the medical consequences, a congenital malformation can be classified as either major or minor.

1. Major malformations. Major malformations are anatomic abnormalities which are severe enough to reduce life expectancy or compromise normal function such as neural tube defects, renal agenesis, etc. Major malformations can be further divided into lethal or severe malformations. A malformation is
considered lethal if it causes stillbirth or infant death in more than 50% of cases. The remaining major malformations are life-threatening without medical intervention and are considered severe.

2. **Minor malformations.** Minor malformations are structural alterations which either require no treatment or can be treated easily and have no permanent consequence for normal life expectancy. Minor malformations are most frequent in areas of complex and variable features such as the face and distal extremities. Minor malformations are relatively frequent and a higher incidence may be noted among premature infants and infants with intrauterine growth retardation. In general, minor malformations are more subtle, have low validity of diagnoses, and are not reported consistently. They are nevertheless significant as they may be an indication of the presence of a major malformation and may also provide critical clues to the diagnosis. The risk of having a major malformation increases with the number of associated minor malformations. For more examples, see Figures (6-34, 5 photos)

**Aetiology of birth defects:**

1. Genetic factors: > 35% including:
   a. Chromosomal anomalies: both numerical and structural.
   c. Multifactorial: are important cause of birth defects.

2. Environmental teratogens (45-50%):

   1. **In-utero infection:**
      Prenatal infection of the foetus with bacteria, viruses, or parasites may occur during pregnancy due to maternal infection that is transferred to the foetus through the placenta causing foetal infection. The most common is the viral infection with rubella virus (German measles), and influenza virus.
      
      I. Viral infections:
         a. Rubella → congenital rubella syndrome usually causes a more severe malformation the earlier it infects the fetus. The incidence of congenital malformation due to infection with rubella decreases as the infection is contracted later during pregnancy. The modern immunization against rubella in girls at reproductive age is one of the very successful methods to prevent this type of malformation. Rubella causes congenital heart defect, mainly septal defect, microcephaly as it invades the nervous system → mental retardation, or infection of the chambers of the eyes leading to blindness, cataract, in addition to causing deafness (Figure 6-35).
         b. Influenza virus usually causes cleft lip and palate.
         c. Mumps, measles, herpes simplex (type I & II) → neural tube defects, microcephaly, or hydrocephaly (Figure 6-36, 6-37).
         d. CMV: usually causes early fetal loss (abortion), but if not → severe malformation like microcephaly and mental retardation.
      
      II. Bacterial infection: The most important in this regard is *Treponyma pallidum* → congenital syphilis with multiple organ system effects ranging from severe to mild forms according to the timing of exposure (more severe in early infections and less severe later in pregnancy).
      
      III. Parasitic infestation:
         *Toxoplasma gondii* is an intracellular parasite, usually contracted from animals, mostly sheep or cats. It invades the CNS → microcephaly, mental retardation and jaundice (Toxoplasmosis). It has some similarities to rubella in terms of timing of infection or infestation.
2. Physical teratogens:
   These could be:
   a. Heat, whether sauna bathing, or from weather or fever (if it does not cause abortion) \( \rightarrow \) neural tube defects.
   b. Physical (mechanical) pressure that may be caused from inside the uterus or outside it pressing the growing fetus preventing its proper growth. These could be the following:
      i. Congenitally abnormal maternal uterus like unicornuate uterus or septate uterus.
      ii. Luminal fibroid (large leiomyoma) projecting into the lumen.
      iii. Corset wearing for a long period during pregnancy.
      iv. Decreased amount of liquor or absence of liquor (Potter's facies).
   These may cause abnormal positioning of the limbs or feet resulting in congenital hip dislocation or club foot, face compression, etc.
   v. Amniotic (fibrous) band, which are fibrosis of the amniotic membrane due to infection that may turn around a growing external part of the foetal body causing cut in its blood supply, ischemia and necrosis, like amputation of a finger or hand or foot.
   c. Ionizing radiation, whether diagnostic or therapeutic. Radiation or accidents usually causes malformation. Radiation causes embryonal or fetal death, microcephaly anophthalmia and spina bifida, and Congenital heart disease (CHD).
   The most sensitive time for radiation effect is between 2-4 weeks after fertilization.

3. Chemical teratogens, these could be:
   a. Non-medicinal:
      i. like pesticides, insecticide, and household chemical used in cleaning and industrial chemical. Some of those usually contain organic phosphorous, which is very toxic. These usually are ingested accidentally by contaminated food and water. They usually cause abnormality of the nervous system like tremor rigidity, and may cause spina bifida.
      ii. Alcohol and cigarette smoking are to be mentioned in this category, both cause intrauterine growth retardation and delayed mental development in later life after birth with small stature. Alcohol also results in a typical facial features and microcephaly (fetal alcohol syndrome).
   b. Medicinal chemicals: one should consider that all drugs are not safe during the early weeks post-fertilization and medication should be taken cautiously. The most properly documented drugs in causing congenital malformation are:
      i. Thalidomide: a drug used for sedation or treatment of hyperemesis gravidarum (severe nausea and vomiting during early months of pregnancy), usually causes amelia and phocomelia (short deformed limbs) (Figure 6-38).
      ii. Anti-convulsants, especially phenytoin causes ptosis, microcephaly, pilonidal sinus and subnormal mental
development, and camptodactyly (flexion contracture of some or all fingers).

iii. Anticoagulant, especially warfarin → neural tube defects.

iv. Cytotoxic drugs (anti-metabolites) as they depress cellular metabolism, especially the rapidly dividing ones → club foot (Figure 6-39), cleft palate, growth retardation.

v. Hormones: like progesterone (used to maintain pregnancy in threatened abortion), oestrogen and cortisone. The 1st causes ambiguous genitalia, the 2nd causes vaginal carcinoma of the growing female baby at an early age between 10-15 years, while cortisone causes cleft lip and palate and NTDs.

Paracelsus once said:
“Poison is in everything, and nothing is without poison.
The dosage makes it either a poison or a remedy.”

4. Maternal disorders:
   a. Diabetes mellitus, whether treated or not, and whether it is of type I (insulin-dependent) or type II (non-insulin dependant), usually causes caudal regression syndrome (one single fused limb) and femoral hypoplasia with agenesis of the sacrum (Figure 6-40).
   b. Maternal phenylketonuria (PKU), which is a genetic metabolic disease that could be treated. Females may reach reproduction with normal state but their high phenylalanine serum level results from their deficiency of phenylalanine hydroxylase enzyme will destroy the developing brain of the rowing embryo to cause brain atrophy and consequently mental retardation.
   c. Vitamin A maternal state both hypo and hyper: severe limitation of dietary intake results in hypo state as happen in Iraqi mothers during the embargo; hyper state results from treatment with the modern creams of vitamin A used for acne. Both result in microphthalmia (Figure 6-41). Deficiency states → NTDs and cataracts.
   d. Endemic goiter: a decrease in thyroid hormones production due to lack of a proper iodine intake especially during times of stress and increased T3 & T4 demand (pregnancy, puberty, rapid growth) → elevated TSH level and thus it may pass to the fetus and ultimately suppresses its own thyroid function → congenital hypothyroidism (cretinism).

A better understanding of the etiology and pathogenesis of these defects has led to several prevention strategies over the years. Rubella immunization and avoidance of teratogenic drugs in women of reproductive age, use of folic acid supplementation and maintenance of euglycemia in diabetic patients during the periconception period, premarital and preconception genetic counseling to couples at risk of certain genetic disorders, and screening for Down syndrome in presence of advanced maternal age are a few examples of very effective and successful strategies to prevent congenital malformations in a newborn.
Classification of medicines / chemicals in relation to their teratogenic effects  
(nice to know this year but essential to know in clinical practice)

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Medication has not shown an increased risk for birth defects in human studies.</td>
</tr>
<tr>
<td>B</td>
<td>Animal studies have not demonstrated a risk and there are no adequate studies in humans, OR animal studies have shown a risk, but the risk has not been seen in humans.</td>
</tr>
<tr>
<td>C</td>
<td>Animal studies have shown adverse effects, but no studies are available in humans, OR studies in humans and animals are not available.</td>
</tr>
<tr>
<td>D</td>
<td>Medications that are associated with birth defects in humans, however, there may be potential benefits in rare cases that outweigh their known risks.</td>
</tr>
<tr>
<td>X</td>
<td>Medications are contraindicated (should not be used) in human pregnancy, because of known fetal abnormalities that have been demonstrated in both human and animal studies.</td>
</tr>
</tbody>
</table>

**Drugs known to cause human birth defects**  
(nice to know this year but keep it as a reference for future use – essential)

- Diethylstilbestrol - Not on market
- Ergotamine - X
- Finasteride - X
- Fluconazole - C
- Folic acid antagonists - D (phenytoin) and X (methotrexate)
- Lithium - D
- Methimazole - D
- Methylene blue - C
- Mifepristone, RU-486 - D
- Minoxidil - C
- Misoprostol - X
- Mysoline - D
- Penicillamine - D
- Phenobarbital or methylphenobarbital - D
- Potassium iodine and medications that effect iodine levels (diatrizoate) - D
- Progestins - X (except megestrol and norethindrone - D)
- Raloxifene (Evista) - X
- Retinoic acid, isotretinoin (Accutane), acitretin (Soriatane), etretinate, topical tazarotene - X
- Statins (3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors) - X
- Tamoxifen - D
- Tetracycline - D
- Thalidomide - X
- Valproic acid - D
- Warfarin - X
- ACE inhibitors (eg, captopril, enalapril) - D
- Acetohydroxamic acid (AHA) - X
- Aminocaproic acid - D
- Androgens (eg, Danazol) - X
- Angiotensin II receptor antagonists (eg, losartan, valsartan) - D
- Antineoplastics (alkylating agents) - D
- Antineoplastics (antimetabolites) - X
  - 5-Fluorouracil
  - Methotrexate
  - Methylaminopterin
  - Cytarabine
  - Busulfan
  - Chlorambucil
  - Azathioprine
  - Cyclophosphamide
  - Mechlorethamine
  - Cisplatin
  - Bleomycin
- Aminoglycosides (eg, gentamicin, streptomycin) - D
- Aspirin - D
- Atenolol - D
- Benzodiazepines - D and X
  - Flurazepam (X)
  - Temazepam (X)
  - Triazolam (X)
- Bromides - D
- Carbamazepine - D
- Colchicine - D
- Corticosteroids - C
- Danazol - X
Diagnosis of Genetic Diseases

Diagnosis of genetic diseases requires the classical sequence of getting information about the patient like any medical disorder i.e. by history taking, clinical examination of the patient plus doing some additional laboratory (hematological, biochemical, serological, hormonal, etc.) or radiological (plain X-ray, CT-scan, MRI, Echocardiogram, etc.) tests when indicated. If a provisional diagnosis is made or a list of few differential diagnoses is thought of, a confirmatory test should be sought for to confirm or rule out that diagnosis.

Cytogenetics (Karyotyping):
A specific diagnosis for the genetic disorders may require a chromosomal study (a cytogenetic study to diagnose a numerical or structural chromosomal abnormality), which is the basic tool for any genetic laboratory, and can be used in a wide variety of gross chromosomal abnormalities (as discussed earlier), but many genetic diseases are caused by subtle changes in individual genes that cannot be detected by karyotyping.

Molecular cytogenetics:
Sometimes, the defect on the chromosome is more subtle (e.g. deletion of the largest gene in the whole human DNA i.e. the dystrophin gene of a size of 2.4 M.B., is beyond the capability of the light microscope to detect). In this case, in situ hybridization is used to detect the mutated chromosome.

Hybridization:
This is a procedure used in the diagnosis of genetic and other pathologies as well as in the diagnosis of cancer.

It is based on the fact that the two DNA strands are not identical but complementary.

The test is performed by adding a synthetic, single stranded DNA sequence (called a probe) [that is made complementary to a specific region of DNA under study and is being labeled with a specific dye] to the double stranded DNA from the patient (after making it single stranded by a process called denaturation). If the probe found its complementary region along the patient's DNA, it'll combine (hybridize) to it and starts emitting a color or "fluoresce". This emitted color can be detected using a UV-microscope.

Advantages:
This procedure can detect smaller chromosomal defects (mostly structural ones) and can also detects numerical chromosomal abnormalities during interphase as well as during metaphase (in contrast to karyotyping that requires the cells to be tested during metaphase only i.e. dividing cells). It also require less incubation time and fewer number of tested cells.

This procedure forms the basis of what is known as fluorescent in situ hybridization (FISH).

Nevertheless, this procedure cannot detect single point mutations or even addition / deletion of 2 or more nucleotide bases.

So, the technique used for detection of such smaller defects is usually DNA-based; the most representative and most commonly used one is polymerase chain reaction (PCR) that revolutionized the diagnostic ability of genetic testing. Most new techniques used nowadays are PCR-based.
Molecular Genetics

Traditionally the diagnosis of single-gene disorders has depended on the identification of abnormal gene products (e.g., mutant hemoglobin or enzymes) or their clinical effects, such as anemia or mental retardation (e.g., phenylketonuria). Now it is possible to identify mutations at the level of DNA and offer gene diagnosis for several mendelian disorders. The use of recombinant DNA technology for the diagnosis of inherited diseases has several distinct advantages over other techniques:

- It is remarkably sensitive. The amount of DNA required for diagnosis by molecular hybridization techniques can be readily obtained from 100,000 cells. Furthermore, the use of PCR allows several million-fold amplification of DNA or RNA, making it possible to use as few as 100 cells or 1 cell for analysis. Tiny amounts of whole blood or even dried blood can supply sufficient DNA for PCR amplification.
- DNA-based tests are not dependent on a gene product that may be produced only in certain specialized cells (e.g., brain) or expression of a gene that may occur late in life. Because virtually all cells of the body of an affected individual contain the same DNA, each postzygotic cell carries the mutant gene.

These two features have profound implications for the prenatal diagnosis of genetic diseases (giving a diagnosis of the yet unborn foetus).

There are two distinct approaches to the diagnosis of single-gene diseases by recombinant DNA technology: direct detection of mutations and indirect detection based on linkage of the disease gene with a harmless "marker gene."

DIRECT GENE DIAGNOSIS

It is also called the diagnostic biopsy of the human genome. It is indicated when the location of the gene being tested is known. Such diagnosis depends on the detection of an important qualitative change in the DNA. There are several methods of direct gene diagnosis; almost all are based on polymerase chain reaction (PCR) analysis, which involves exponential amplification of DNA from small quantities of starting material. If RNA is used as a substrate, it is first reverse transcribed to obtain cDNA (using an enzyme called reverse transcriptase) and then amplified by PCR. This method is often abbreviated as RT-PCR.

To detect the mutant gene, two primers (lengths of a single stranded DNA made complementary to a desirable length of the tested DNA 'part of the gene to be tested') that bind to the 3' (called three prime) and 5' (called five prime) ends of the normal sequence are designed. By using appropriate DNA polymerases (enzymes that build up DNA strand based on its complementary strand) and thermal cycling, the DNA between the primers is greatly amplified, producing millions of copies of the DNA between the two primer sites. The amplified normal DNA and patient's DNA are then digested with a restriction enzyme that cuts the amplified DNA into pieces of known sizes e.g. the normal DNA yields three fragments (67 base pairs, 37 base pairs, and 163 base pairs long); by contrast, the patient's DNA yields only two products, an abnormal fragment that is 200 base pairs (instead of two pairs of 37 and 163 b.p.) and a normal fragment that is 67 base pairs long. These DNA fragments can be readily resolved by gel electrophoresis (by which we can separate DNA bands or pieces according to their molecular weight) and then visualized after staining with ethidium bromide under ultraviolet light.

INDIRECT DNA DIAGNOSIS: LINKAGE ANALYSIS

Direct gene diagnosis is possible only if the mutant gene and its normal counterpart have been identified and cloned and their nucleotide sequences are known.
(its exact locus and size are well known). In a large number of genetic diseases, including some that are relatively common, information about the gene sequence is lacking. Therefore, alternative strategies must be employed to track the mutant gene on the basis of its linkage to detectable genetic markers. In essence, one has to determine whether a given fetus or family member has inherited the same relevant chromosomal region(s) as a previously affected family member. It follows therefore that the success of such a strategy depends on the ability to distinguish the chromosome that carries the mutation from its normal homologous counterpart. This is accomplished by exploiting naturally occurring variations or polymorphisms in DNA sequences.

Molecular diagnosis by linkage analysis has been useful in the antenatal or presymptomatic diagnosis of disorders such as Huntington disease, cystic fibrosis, and adult polycystic kidney disease. In general, when a disease gene is identified and cloned, direct gene diagnosis becomes the method of choice. If the disease is caused by one of several different mutations in a given gene, and direct gene diagnosis is not feasible, linkage analysis remains the preferred method.

**CLINICAL GENETICS**

It is the study of the possible genetic determinants affecting the occurrence of diseases and disorders.

**Role of The Geneticist**

- Diagnosis (History, Physical Examination, Investigations (including karyotyping or DNA diagnostic methods).
- Genetic counseling
  1. Discussing the problem with the family or the individual concerned
  2. Recurrence risk calculation
  3. (?) Treatment
  4. (?) Prenatal diagnosis
  5. (?) Prevention

**Who Might Benefit from Genetic Services?**

- Parents of a child born with a genetic disorder (AD, AR, XR), birth defect, or chromosomal abnormality.
- Parents concerned about certain finding in the family that is recurrent or severe (deafness, paralysis, short stature, mental retardation, developmental delay, childhood blindness, etc.)
- Children with developmental delay and unusual features (dysmorphic features)
- Individuals that fail to develop secondary sexual characteristics or children with ambiguous genitalia
- Couples with a history of difficulty becoming pregnant, repeated pregnancy losses, stillbirths, or early infant deaths
- Children with short stature that is unusual for the family
- Individuals with a family history of a birth defects or genetic disease.
- Multiple miscarriages, still births, or early infant deaths with congenital anomalies
- Couples who are first cousins or close blood relatives (consanguinity)
- Pregnant woman or their infants who have been exposed to a medication, drug, radiation, or other environmental agent
- Woman in their mid-30's and 40's who are pregnant or are planning a pregnancy
- Individuals of certain ethnic or geographic groups at increased risk for genetic disorders
To make a diagnosis:
• Information are collected – about family and medical history, family history sometimes including photographs of family members plus their physical examination.
• Consultation of other specialist(s)
• Selective laboratory investigations.
• Making a clinical diagnosis.
• Confirmation (whenever possible or feasible) by: chromosome testing, FISH, DNA testing, or other genetic testing, as needed.

What is Genetic Counseling?
It is a communication process that translates technical and complicated knowledge into practical information for individuals and families. Genetic Counseling can help an individual or a family with a condition or a disease to:
• Understand the cause of the condition
• Know the chance of having future children with the condition (recurrence risk)
• Learn about current research, testing procedures, prenatal diagnosis
• Contact community resources, and if possible, other families or patients with the same condition
• Adjust to the personal and family issues related to a genetic disorder

Prenatal Diagnosis
It employs a variety of techniques to determine the health and condition of an unborn fetus. Without knowledge gained by prenatal diagnosis, there could be an untoward outcome for the fetus or the mother or both. Congenital anomalies account for 20 to 25% of perinatal deaths.

Prenatal diagnosis is helpful for:
1. Managing the remaining weeks of the pregnancy
2. Determining the outcome of the pregnancy
3. Planning for possible complications with the birth process
4. Planning for problems that may occur in the newborn infant
5. Deciding whether or not to continue the pregnancy
6. Finding conditions that may affect future pregnancies

There are a variety of non-invasive and invasive techniques available for prenatal diagnosis. Each one can be applied only during specific time periods during the pregnancy for greatest utility. The techniques employed for prenatal diagnosis include:

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<td>Fetal blood cells in maternal blood</td>
<td>Karyotyping</td>
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<td>Maternal serum (alpha-fetoprotein, beta-HCG, and estriol)</td>
<td>FISH (on fresh tissue or paraffin blocks)</td>
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<td>Inhibin-A</td>
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Example: A commonly used screening test: "Triple" or "Quadruple" screen
Combining the maternal serum assays may aid in increasing the sensitivity and specificity of detection for fetal abnormalities. The classic test is the "triple screen" for maternal serum alpha-fetoprotein (MSAFP), beta-HCG, and estriol (uE3). The "quadruple screen" adds inhibin-A.